

EFFECT OF SUPPLEMENTING ESSENTIAL FATTY ACIDS TO PREPARTUM
HOLSTEIN COWS AND PREWEANED CALVES ON CALF PERFORMANCE,
METABOLISM, IMMUNITY, HEALTH AND HEPATIC GENE EXPRESSION

By

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To my loved parents: Maxi and Pascual, for their endless love and for all they taught me, not only with words but by examples. All I am and all I have achieved, have their hallmark.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACC	Acetyl CoA carboxylase
ADF	Acid detergent fiber
ADG	Average daily gain
ALA	α -linolenic acid
APO	Apolipoproteins
APT	appropriate passive transfer
ASP	Acid soluble protein
BCS	Body condition score
BP	Biological process
BVD	Bovine viral diarrhea
BW	Body weight
CCO	Coconut oil
CD18	β -integrin, adhesion molecule
CD62L	L-selectin, adhesion molecule
ChREBP	Carbohydrate regulatory element binding protein
CLA	Conjugated linoleic acid
CO	Corn oil
CYP	Cytochrome P450
CYP7A1	Cholesterol 7- α hydroxylase
DEG	Differentially expressed genes
DHA	Docosahexaenoic acid
DM	Dry matter
DMI	Dry matter intake

DPA	Docosapentaenoic acid
ES	Enrichment score
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FA	Fatty acid
FABP	Fatty acid binding protein
FAME	Fatty acid methyl esters
FASN	Fatty acid synthase
FcRn	Neonatal Fc receptors for IgG
FE	Feed efficiency (gain/intake)
FO	Fish oil
FXR	Farsenoid X receptor
GK	Glucokinase
GLA	γ -linolenic acid
GO	Gene ontology
HNF-4 α	Hepatonuclear factor 4 α
Hp	Haptoglobin
IFN- γ	Interferon - γ
Ig	Immunoglobulin
IGF	Insulin-like growth factor
IGFBP	IGF binding protein
IL	Interleukin
KEGG	Kyoto encyclopedia of genes and genomes
LCFA	Long chain fatty acids
LDL	Low density lipoprotein

LPS	lipopolysaccharide
LXR	Liver X receptor
MCFA	Medium chain fatty acids
MDH	Malate dehydrogenase
MF	Molecular function
MHC	Major histocompatibility complex
MLX	Max like protein X
MR	Milk replacer
MUFA	Monounsaturated fatty acids
n-3	Family of ω -3 fatty acids
n-6	Family of ω -6 fatty acids
NDF	Neutral detergent fiber
NEFA	Nonsterified fatty acids
NFkB	Nuclear factor kB
NRC	The National Research Council
OA	Oleic acid
OVA	Ovalbumin
PBMC	Peripheral blood mononuclear cells
PHA	Phytohaemagglutinin
PI3	Parainfluenza 3
PK	Piruvate kinase
PPAR	Peroxisome proliferator receptor
PUFA	Polyunsaturated fatty acids
rBST	recombinant bovine somatotropin
RXR	Retinol X receptor

SAO	Safflower oil
SCFA	Short chain fatty acids
SFA	Saturated fatty acids
SO	Soybean oil
SREBP	Sterol regulatory element binding protein
STP	Serum total protein
TCR	T- cell receptor
Th	T- helper cell
TNF- α	Tumor necrosis factor - α
VLDL	Very low density lipoprotein

Abstract of Dissertation Presented to the Graduate School
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Major: Animal Sciences

A series of experiments were conducted to examine the effect of supplementing essential fatty acids (FA) to prepartum Holstein cattle and newborn calves. The overall objective was to evaluate the effect of increasing intake of linoleic acid (LA) during the preweaning period on overall calf performance. In the first study prepartum cattle were fed one of three supplements, namely control (no fat), hydrogenated FA (SFA) or Ca containing essential FA (EFA). Colostrum FA profile of dams fed EFA reflected the concentration of LA in the fat supplement. Colostrum from nulliparous heifers was a better source of n-3 FA. Calves born from dams fed SFA had greater serum concentrations of total Immunoglobulin G (IgG), but efficiency of IgG absorption did not differ. In same study, 96 Holstein cattle were fed prepartum the same supplements as in experiment 1 and newborn calves were fed milk replacer (MR) of low LA (LLA) or high LA (HLA). Feeding SFA prepartum increased grain intake and average daily gain (ADG) without improving feed efficiency (FE) of calves born from fat-fed dams. Feeding HLA increased ADG, FE, plasma glucose and IGF-I, LA and its derivatives in liver, blood lymphocytes, phagocytosis by neutrophils and interferon- γ from mononuclear cells.

Expression of liver genes was strongly affected by the combination of prepartum diets and MR. Upregulated pathways included the PPAR signaling pathway, glycolysis/gluconeogenesis and oxidative phosphorylation whereas downregulated pathways included genes involved in inflammatory processes and ubiquitin-mediated proteolysis. Cardiomyopathy and tight junction pathways were upregulated in calves fed HLA-MR, but were downregulated if calves born from SFA- or EFA-fed dams. Calves born from fat-fed dams prepartum produced more milk at first lactation, possibly mediated by fetal programming. The last study aimed to determine the requirement of LA for preweaned calves. Heifers gained more BW in the first 30 d of life as intake of LA increased. Wither and hip growth was greater in calves consuming LA exceeding 0.206 g/kg of BW^{0.75} during the 60-d study. Several markers of immunity were increased when LA was fed between 0.206 and 0.333 g/kg of BW^{0.75}.

CHAPTER 1 INTRODUCTION

Reaching an appropriate growth rate and health performance of dairy calves before weaning that would allow to double the birth weight by weaning period and minimize the incidence of diseases is one of the primary goals of dairy herd management. After birth dairy calves are immediately removed from their dams and transferred to a different unit to initiate the preweaning period in which they spend six to eight weeks consuming milk or milk replacer. The preweaning period, which requires APT of immunity, is often considered as the most critical period of early life (Beam et al., 2009; Furman-Fratczac, 2011).

The newborn calf is completely dependent of immunoglobulin (Ig) supplied by colostrum consumption because the epitheliochorial placenta of cows prevents transfer of Ig during the fetal period (Kehoe and Heinrichs, 2007). Establishment of APT is crucial to reduce neonatal morbidity, mortality, and strengthen calf immunity (Quigley and Drewry, 1998; Donovan et al., 1998). Moreover APT has been associated with improved weaning and postweaning body weight (BW; Robison et al., 1988) and with greater milk production (DeNise et al., 1989).

Several studies have evaluated different nutritional strategies to improve calf performance. Feeding high-energy diets for rapid growth during the pre-weaning period have reduced both the age to reach the target breeding weight and costs associated with raising replacement heifers (Radcliff et al., 2000; Raeth-Knight et al., 2009). In addition, the optimized feeding management of heifers during the preweaning period can have a positive impact on future milk production. One kg increase in average daily gain increased milk at first lactation by 850 kg (Soberon et al., 2012).

The role of essential fatty acids (EFA) in the growth and health of preweaned dairy calves is poorly understood. Pioneer studies (Jenkins et al., 1985; Jenkins et al., 1986; Jenkins and Kramer, 1986) supplemented the milk replacer (MR) of newborn calves with different sources of fat and reported that concentration of EFA in liver and plasma reflected the composition of FA in the MR but classical symptoms of EFA could not be reproduced. Other studies used preruminant calf hepatocytes, cultured with different FA to evaluate oxidative and gluconeogenic activity of the liver (Mashek et al., 2002; Mashek et al., 2003, Mashek and Grummer, 2004). The type of FA used to incubate liver of preweaned calves did not affect propionic acid metabolism to produce glucose and cellular glycogen. However, regardless the type of FA, the formation of both glucose and glycogen were decreased when FA concentrations increased from 0.1 to 1.0 mM. Limited information has been generated regarding the role that dietary EFA might have in modifying the expression of genes in liver.

Strategic feeding of pregnant cows during late gestation has been documented as having a tremendous impact on the future life of their offspring (Osgerby et al., 2002; Dwyer et al. 2003; Hess 2003). The “programming” of the future outcome of calves during the fetal period could be due to epigenetic regulation as a consequence of maternal nutrition during fetal development or nutrition during the first year of life (Funston et al., 2010; Singh et al., 2010). Few studies have evaluated the strategic supplementation of prepartum diets with EFA on the future life of their offspring.

The present dissertation begins with an overview of the roles of fatty acids (FA) in calf metabolism and the calf immune system, including information from the most relevant and/or available studies evaluating the effect of fat supplementation on calf

immunity and liver metabolism. Chapter 3 describes an experiment that was aimed to evaluate the effect of supplementing calcium salts of FA enriched in EFA on colostrum FA profile and production of total IgG and how that colostrum affected APT of calves born from those dams. The objective of Chapter 4 was to evaluate the effect of feeding EFA to dams during late gestation and to calves in their preweaning diets on calf growth, health, and immune responses. In Chapter 5, the liver FA profile and global gene expression of calves from Chapter 4 was evaluated. Chapter 6 is a second in vivo study that aimed to determine the requirement of linoleic acid (LA) of Holstein calves during the preweaning period. Calves were fed a milk replacer with increasing concentrations of linoleic acid and the potential LA requirement was evaluated in terms of growth, health, and immune responses. The final chapter is a general conclusion and discussion of the major findings of the aforementioned studies.

CHAPTER 2 LITERATURE REVIEW

Overview of Fatty Acids

Lipids are more than just high-energy-provider molecules. Their composition is as complex as proteins which are building blocks of amino acids. The building blocks of the most common structure of lipids, triglycerides, are individually different fatty acids (FA) that are attached to a glycerol backbone. The lipid FA composition varies according to different sources such as animal or vegetable origin. Although lipids of animal origin tend to have greater proportions of saturated FA (SFA) and those from vegetable origin tend to have a greater proportion of unsaturated FA, there are some fat that break this rule. For many years, lipids were considered simple inert molecules, with the single function of being a source of energy. However, classical pioneer studies found that specific FA were actually essential for animal health, reproduction and survival (Burr and Burr, 1929, 1930). Recent studies are focusing on identifying the different functions of the essential FA (EFA) and the mechanisms by which those FA perform. Some classical and new knowledge related to chemistry, sources, metabolism, and essentiality of FA will be detailed in the following sections.

Nomenclature and Classification

This section will introduce basic concepts of the most common nomenclature systems (IUPAC nomenclature, common or trivial names, and short-hand (ω) terminology) used to classify FA according their chain length, unsaturation number, and isomeric configuration. O'Keefe (2002) discussed the difficulties in defining lipids such as insolubility in water but solubility in nonpolar solvents. However O'Keefe (2002) argued that even this definition is not an exact one because very short chain FA (SCFA,

C1-C4) are soluble in water. The author concluded that a more precise working definition is difficult given the complexity and heterogeneity of lipids.

The IUPAC nomenclature is one of the systematic nomenclatures regulated by internationally accepted rules agreed on by chemists and biochemists (Gunstone, 1996). Under this nomenclature, the FA is named after the parent hydrocarbon, for example an 18-carbon FA is named as octadecanoic (Figure 2.1). Double bonds are described using Δ configuration, which represents distance from the carboxyl carbon, considered as carbon number 1. A FA of 18 carbons with one double bond is named octadecenoic acid and one with 2 double bonds as octadecadienoic acid and so on. The double bond position is described with numbers before the FA name (Δ 9-octadecenoic acid or simply 9-octadecenoic acid). The *cis/trans* terms are used to describe the geometric positions of double bonds. In the *cis* configuration adjacent hydrogen atoms are located on the same side of the double bond whereas in the *trans* configuration they are located on opposite sides (Gurr et al., 2002).

Common (trivial) names were originally given before the chemical structure of the FA were elucidated and often were chosen to indicate the source of FA and are still used widely (Gunstone, 1996). Some examples of those names are palmitic acid (from palm oil), oleic acid (OA, from olive oil), linoleic acid (LA), and α -linolenic acid (ALA, from linseed oil) and arachidonic acid (AA, from groundnut oil, *Arachis hypogea*). Trivial names are not indicative of structure and can result in confusion when a name is assigned to a particular FA such as bovidic acid. The more carbons and double bonds a FA possesses, the more difficult a trivial name becomes, and more preferred are the IUPAC names. Good examples are eicosapentaenoic acid (EPA) and docosahexaenoic

acid (DHA) since they can have different isomers. However for convenience, EPA refers to the c-5,c-8,c-11,c-14,c-17 isomer whereas DHA refers to the all-cis 4,7,10,13,16,19-isomer (O'Keefe, 2002).

Systematic names for FA are too cumbersome for general use and shorter alternatives are used widely (Scrimgeour, 2005). One way to shorten the names are by using numbers in an abbreviated form such as 18:2 for octadienoic acid. But to better describe the isomeric form, other descriptors have to be added such as 18:2 (9, 12), 18:2 (9c, 12c), and 18:2 (n - 6). All of these refer to the same FA. The first number indicates the position of the double bonds in the C18 chain with reference to the carboxyl end counted as C1. The second formula confirms the *cis* configuration of the double bonds and the third one describes the FA in Greek terminology (Figure 2.1), which starts counting the carbon from the methyl group and describing this carbon as an ω -carbon (or n-carbon) thus n-6 means that the first double bond is at carbon six counting from the methyl group (Gunstone, 1996). The (n) abbreviation or symbol is the most popular because of its simplicity and because most of the FA of nutritional importance can be named, but holds some limitations such as: cannot be used for FA with *trans* configuration, all double bonds can only be in the methylene-interrupted position, and FA cannot have additional functional groups or have double-bond systems (O'Keefe, 2002, Table 2-1).

Sources

A vast variety of vegetable and animal fat sources are available (table 2.2) for feeding ruminants such as oilseeds, rendered fats, purified vegetable oils, marine oils, and ruminally protected fats (e.g. hydrogenated FA, calcium salts of FA), with the latter being modified to prevent ruminal microbe metabolism. In preweaned calves, fat is an

important source of energy. For economic reasons, milk fat is rarely used in commercial milk replacers (MR). Alternatively vegetable and animal fats are used commonly. Animal fat sources are tallow, lard, and white grease. Vegetable oils such as coconut and palm also are used but vegetable and marine oils that provide long chain polyunsaturated FA (PUFA) are of minimal inclusion.

The most common form of lipid in fats and oils is glycerolipids, which are essentially triglycerides (TG), accompanied by small amounts of phospholipids, mono-glycerides, di-glycerides, and sterols or sterol esters. The FA commonly found in TG are SFA (of varied length chain), monounsaturated FA (MUFA, mostly > 12 carbons), or PUFA (> 17 carbons) (FAO, 2010). All naturally occurring PUFA are in the *cis* configuration and are primarily identified by their Greek terminology ($\omega = n$), with n-3 and n-6 being the most important families in terms of commonality of occurrence and animal health and nutrition. Specifically LA (n-6) and ALA (n-3) are the only 2 recognized EFA whose functions will be discussed in detail later. Those 2 FA are parents of other FA which can be synthesized by elongation and desaturation enzymatic processes to generate family members of the same n- group (FAO, 2010; Eastridge, 2002). The richest sources of LA are oil-containing seeds such as safflowers, sunflowers, cotton, corn, and soybeans whereas the richest sources of ALA are canola and linseed oil; marine fats are rich sources of very long chain PUFA such as EPA and DHA.

Metabolism

For dietary fats to be used by the body they must be metabolized in the lumen of the small intestine (or in prior compartments in ruminants). The digestion products should pass through the gut wall and be resynthesized in the intestinal epithelial cells

and packaged for transport in the blood stream (Gurr et al., 2002). The composition of lipids entering the duodenum in ruminant cattle differs from the composition in nonruminant calves. This difference is due to the initiation of lipid metabolism in the forestomach of ruminant cattle whereas in nonruminant calves very little metabolism occurs before lipids enter the small intestine. Salivary lipase can begin to act on dietary TG upon ingestion (Bauchart, 1993).

Lipid metabolism in ruminants is unique because the rumen compartment holds feeds for extended periods of time for microbial digestion prior to delivery of feeds further down the digestive tract where mammalian digestion occurs. However in newborn calves, lipid metabolism takes place just as it happens in nonruminants because their rumen has not developed a microbial population nor anatomical maturity. Metabolism of TG by lipases of ruminal anaerobic microbes involves an extensive hydrolyzation leading to the formation of free FA (FFA) which are subjected to partial hydrogenation by microbial hydrogenases. Stearic acid is the final product of a complete hydrogenation of 18-carbon FA. However what most commonly occurs is an incomplete hydrogenation resulting in the formation of intermediate products of hydrogenation such as *cis* and *trans* isomers of monoenoic FFA (ie. C18:1 n-9 and C18:1 n-7) and isomers of PUFA such as conjugated LA (CLA) (Hocquette and Bauchart, 1999). Hence the final product of microbial hydrolysis and biohydrogenation is a pool of FFA that are far more saturated than that of the dietary FA. In addition to long chain FFA arriving at the small intestine, fats may also include dietary TG escaping microbial hydrolysis, microbial phospholipids (containing odd and branched-chain FA), and phospholipids from bile and sloughed intestinal endothelial cells flowing toward the duodenum (Hocquette and

Bauchart, 1999; Drackley and Andersen, 2006). Feeding large quantities of fat can have detrimental effects on microbial activity and animal productivity due to an inhibitory effect on cellulolytic microorganism that can depress fiber digestion (Eastridge, 2002). Various techniques of lipid protection such as lipid encapsulation and saponification of long chain FA have been developed to limit the extent of ruminal lipid hydrogenation and possible disturbances in fermentation (Hocquette and Bauchart, 1999).

In preruminant calves, lipid digestion starts with pregastric lipases secreted by the mouth. O'Connor and coworkers (1996) reported that pregastric lipase in lambs is specific for the 3 position of the glycerol carbon chain holding the FA which then releases only 1 FA per TG whereas Villeneuve and coworkers (1996) reported a nearly similar pregastric lipase activity at positions 1 and 3 with preferential release of SCFA and medium chain FA (MCFA) from ingested TG. All dietary fat, including products of pregastric lipase digestion, arrive to the abomasum where they are emulsified by physical agitation and mixture with HCl (Drackley, 2008). The coagulation of milk casein in the abomasum is critical to slow down the movement of milk from the abomasum and increase the efficiency of the digestive process in the small intestine of suckling calves. This results in a greater retention time of dietary TG which delays the postprandial increase of lipids in circulation (Hocquette and Bauchart, 1999; Guilloteau et al., 2009). Milk fat delivered to the abomasum undergoes some digestion by pregastric lipase which remains active in the acid conditions of the abomasum (Drackley, 2008). After leaving the abomasum, the end products of gastric digestion pass into the duodenum, coming in contact with gall bladder and pancreatic secretions.

During the first month of life, pancreatic enzyme activities increase by 50 to 160% for most enzymes. Pancreatic lipase activity increases with age but this enzyme cannot express its full activity in older preruminants since colipase is a limiting factor. This explains why lipids are sometimes poorly utilized in older preruminant calves (Guilloteau et al., 2009). Pancreatic lipase, in the presence of colipase and bile salts, hydrolyzes diglycerides and the remaining TG to 2-monoglycerides and FFA. Bile salts and 2-monoglycerols aid in the emulsifications of lipids and micelle formation. Micelles migrate to the brush border of the small intestine and facilitate absorption of FFA and monoglycerides through specific FA binding proteins (FABP) located in the membrane of the enterocytes (Drackley, 2008; Hayashi et al., 2012).

The transportation routes that a FFA can take will depend on its chain length. The MCFA (≤ 12 C) are preferentially secreted as FFA bound to albumin, specifically in the portal vein, by which MCFA arrive to liver and are first metabolized through β -oxidation. Their products are transferred to the Krebs cycle or utilized for synthesis of ketone bodies (Sato, 1994). On the other hand, long chain FA (LCFA) are reconverted to TG and packaged into lipoproteins, primarily chylomicrons, which are secreted from the cells into the extracellular space, where they are picked up by the lymphatic system and delivered to the vena cava. In this way, dietary FA are delivered to peripheral tissues for their use (Drackley, 2008). Apolipoproteins of chylomicrons are characterized by the prevalence of apolipoprotein-B48 (APO-B48), with minor peptides of the APO-C family and with variable amounts of APO-A (Bauchart, 1993). Small amounts of very low density lipoproteins (VLDL) are synthesized in the small intestine but rather synthesized by the liver. The VLDL account for ~5% of total lipoproteins in preruminant calves, with

APO-B100 as the main APO constituent (Wang et al. 2012). Lipoprotein lipase present on the surface of endothelial cells of capillary vessels hydrolyze TG in the chylomicron and VLDL, facilitating the release of FFA and monoglycerides. Hence each of these compounds can enter the peripheral tissues. Tissues with high lipoprotein lipase activity in growing ruminants include adipose tissue, skeletal muscle, and heart whereas the remnant is preferentially taken by hepatocytes (Drackley, 2005).

The liver secretes metabolized lipids in various forms such as acetate, ketone bodies, and lipoproteins containing TG, but the secretion of TG in VLDL is limited. As a consequence, young or adult ruminants are more prone to develop steatosis. This condition is primarily due to the processing and storage of TG rather than to de novo synthesis of TG which are actually lower in ruminant hepatocytes (Hocquette and Bauchart, 1999). Metabolism of FA in liver is finely regulated by transcription factors such as peroxisome proliferator activator receptor- α (PPAR- α), retinol X receptor (RXR), liver X receptor (LXR), and sterol regulatory element binding protein (SREBP). Their role in synthesis, transport, and β -oxidation of lipids will be discussed in detail in coming sections.

Essentiality

Using rats, Burr and Burr (1929) aimed to identify the effect of fat-free diets on rats. Rats fed fat-free diets developed a condition characterized by skin and tail lesions accompanied by hair loss, impaired reproduction, increased trans-epidermal water loss, and weight loss. All these conditions were reversed when rats were fed diets of 2% lard (~10% LA). In a follow-up study, Burr and Burr (1930) purified the source of FA provided to rats in order to identify factors potentially responsible for recovery from “fat deficiency.” Rats supplemented with pure LA or with oils rich in LA recovered from signs

of fat deficiency. Better recovery was documented when rats were fed oils rich in LA rather than when fed purified LA. At that time, authors postulated that LA was a dietary essential, however the better responses obtained when linseed oil was fed already was shedding light on the essentiality of ALA present in the aforementioned oil. Two years later Burr and coworkers (1932) reevaluate the supplementation of other individual FA and concluded that LA and ALA were similarly effective in enhancing recovery of rats fed free-fat diets and that the mixture of LA and ALA was more effective than each individual FA per se.

A study from Cunningham and Loosli (1954) did not support the essentiality of LA in the early life of calves fed fat-free synthetic milk as observed in rats by Burr and Burr (1929, 1930). Calves developed leg weakness and muscular twitches within 1 to 5 wk of age and died if a source of fat was not supplied. Lard or coconut oil fed at 2% (wet basis) of milk prevented the appearance of symptoms. Hence authors concluded that in the first 5 wk of life, body reserves for EFA were enough to prevent deficiency of LA and ALA but that a source of fat was still needed during the first wk of life. On the other hand, Lambert et al. (1954), aiming to replicate studies by Burr and Burr (1929, 1930), fed fat-free MR to dairy calves. Authors reported that calves fed fat-free milk had a marked retardation in BW gain, signs of scaly dandruff, dry hair, excessive loss of hair on the back, shoulders and tail, and diarrhea. Among the lipids that prevent development of or promoted prompt recovery from all the mentioned signs of EFA deficiency were butter oil, hydrogenated soybean oil plus lecithin, and mixed methyl esters of OA and LA. Differences among the various dietary groups in the plasma concentrations of LA and AA were small and the values for LA were low in all instances.

Early studies reported that rats fed diets lacking ALA apparently grew and reproduced normally. Therefore it was controversial to assign the label of “essentiality” to ALA (Sander, 1988). Tinoco et al. (1978) fed diets free of ALA to rats and did not observe any signs of fat deficiency or disease but concentrations of ALA derivatives in certain tissues were decreased markedly. One year later in a review paper, Tinoco et al. (1979) concluded that if ALA was essential, its role was focalized at the retina and brain level where ALA was found in greater concentrations. Later studies reviewed by Innis (1991) clearly demonstrated that ALA derivatives have a critical role in brain development during the fetal period and in early life.

In an attempt to determine the requirement of LA in rats, using a requirement criteria for organisms to maintain constant concentrations of AA in different organs, Bourre et al. (1990) fed female rats increasing amounts of LA (from 0.15 to 3.2 g of LA/100 g of diet). They reported that dietary requirements for LA varied from 0.15 to 1.2 g of LA/100 g of diet, depending on the organ and the nature of the tissue FA (ie. constant concentrations of AA were found in nerve structures when at least 0.15 g of LA/100 g of diet was fed, in testes and muscle with LA fed at 0.3 g/100 g of diet, in kidney with LA fed at 0.8 g/100 g of diet, and in liver, lung, and heart with LA fed at 1.2 g/100 g of diet). Authors concluded that the minimum dietary requirement ensuring constant concentrations of AA in all tissues and organs was estimated to be about 1.2 g of LA/100 g of diet.

The essentiality of LA and ALA is clearly accepted currently. Although several studies have tried to determine potential requirements, so far, only laboratory animals such as mice and rats have determined requirements for LA but not for ALA (NRC, 1995).

For other species, particularly humans, some recommendations have been released, but these recommendations most of the time do not focus on the actual role of individual FA but consider them as a group (e.g. n-6 FA) or even consider the EFA as a proportion of one to another (e.g. LA:ALA or n-6:n-3), a generalization that has led to a vast debate and controversy in recent years.

Czernichow et al. (2010) reviewed studies reporting different effects of n-6 FA, particularly those reducing the risk of cardiac diseases. Authors considered that intakes of n-6 ideally above 10% of the total energy appear justified. However Ramsden et al. (2010) evaluated the same studies reviewed by Czernichow et al. (2010) but analyzed them in a different manner. They concluded that recommendations of Czernichow et al. (2010) for a beneficial effect of increasing LA was done considering the feeding of diets rich in n-6 FA but that also included n-3 FA. Hence, it is unlikely that increased intake of n-6 per se will reduce cardiac disease but may actually increase the risk by exacerbating the inflammatory process during cardiac and heart disease. Calder and Deckelbaum (2011) discussed the two previous contradictory reviews and concluded that Ramsden et al. (2010) made a better assessment of assigning FA to their parent n-group. However authors criticized both studies saying that they used FA terminology too loosely, such as considering PUFA, n-6 PUFA and LA as common interchangeable terms. The lack of clear distinction among terms could lead to inaccurate statements and wrong advice. Authors recommended that advice should be given in terms of specific FA, based on the consideration that every FA has its own role and functionality.

In an attempt to evaluate the ratio n-6:n-3 as a valid measurement of EFA requirement, Harris (2006) reviewed available literature and concluded that there is no

evidence that lowering intake of n-6 FA (which will reduce the ratio) will result in a reduced risk of cardiac diseases, suggesting rather to focus on increasing the intake of n-3 without considering the n-6:n-3 ratio. Similarly a report by Stanley et al. (2007) summarizing the conclusions of a workshop gathered by the United Kingdom Food Standards Agency concluded that the n-6:n-3 ratio is not a useful concept. Calder and Deckelbaum (2011) recommended to avoid discussing requirements or biological function of FA in terms of the ratio n6:n3 under the consideration that every individual FA within the n-6 and n-3 groups is not biologically equivalent to the others, and also that both groups of FA do not have complete opposite functions.

Regarding a differential requirement of LA due to gender, Greenberg et al. (1950) concluded that male rats had a daily requirement of LA between 50 to 100 mg whereas that for females was 10 to 20 mg/d. Authors based their conclusion on the growth rate of rats fed different amounts of LA as compared to the growth of rats fed a fat-free diet. Authors also pointed out that LA intake over 50 mg/d in female rats led to a decrease in BW gain. Some years later, Pudelnkewicz et al. (1968) evaluated the potential requirement of LA in rats by measuring BW gain, skin health, and the triene:tetraene ratio (< 4) to determine EFA status. The main factor leading to the recommended LA requirement for male or female rats (1.3 and 0.5% of dietary calories, respectively) was the C20:3 n-9 to AA ratio. In all evaluated tissues (liver, heart and erythrocytes) and in plasma, the intake of LA that allowed a C20:3 n-9 to AA ratio of 0.4 or less was about 2.5 greater in males than females, with liver having the greater requirement. Authors also reported that feeding female rats with LA over 1.2% of calories increased the incidence of skin lesions and BW gain was reduced. For male rats, all amounts tested

(up to 4.7% of calories) did not have negative impacts. The findings of Greenberg et al. (1950) and Pudelnkewicz et al. (1968) were the basis for the current recommendation of LA intake in growing rats by the NRC (1995).

Similarly Nikkari et al. (1995) reported greater proportions of LA in cholesterol ester but a lower proportion in its derivatives in cholesterol ester and phospholipids fractions. On the other hand, other authors working with humans have not found an effect of gender when evaluating the proportion of FA in different fractions of serum phospholipids (Antar et al., 1967); in fractions of serum cholesterol ester, phospholipids, and TG (Holman et al. 1979); or in plasma TG, FFA, different phospholipids fractions, or total phospholipids fraction in red blood cells (Manku et al., 1983). Moreno et al. (2006) reported that the FA profile of fat depots in pre- and postweaning beef heifers had greater proportions of MUFA (primarily OA) but that proportions of SFA and PUFA did not differ when compared to males. Recently Dervishi et al. (2012) aimed to evaluate the FA profile of intramuscular fat in suckling lambs from ewes fed one of two diets with different FA profiles. Authors reported a differential effect of diet on some FA but gender did not affect the proportion of any FA in intramuscular fat.

The question of essentiality of LA and ALA has been answered in different studies in the last 80 years. However specific requirements of EFA of all livestock species still need to be determined. Some studies performed in rats have pointed out a differential requirement due to gender but more studies are needed to confirm this statement, particularly if the observed differential effect of gender seen in rats held true for preruminant calves.

Overview of Newborn Calf Immunity

The immune system is a versatile defense system that has evolved to protect multicellular organisms from invading foreign pathogens. It is composed of different cells and molecules capable of recognizing and eliminating an unlimited variety of foreign invaders acting together in a dynamic network. The immune molecules and cells are grouped into two systems, namely, innate and adaptive (acquired) immunity, which interact and collaborate to protect the body (Kind et al., 2007).

Invader pathogens must first overcome numerous surface barriers, such as skin, enzymes and mucus that can have direct antimicrobial activity or physical barriers to prevent attachment of the microbe. The keratinized surfaces of the skin or the mucus-lined body cavities are ideal habitats for most organisms; hence microbes must breach the ectoderm. Any organism that breaks through this first barrier encounters molecules and cells of the two immune defense systems, the innate and acquired immune responses (Delves and Roitt, 2000).

The newborn calf is born into an environment populated by a vast amount of pathogens such as bacteria, viruses, and parasites with the capacity to overtake the calf's body and end its life. The calf has a system of immunity capable of resisting pathogenic invasion, because it has the ability to recognize and eliminate different pathogens. However, the immune response is immature in early life and must "learn" through interaction with infectious agents. Different factors such as antibodies obtained from intake of colostrum provide assistance during the naive phase of calf immunity, as a calf ages its interaction with pathogens through vaccination or natural encounters lead to a mature response (Woolums, 2010).

Innate Immunity

The innate immune system developed early in evolution consists of all immune defenses that lack memory. Thus a common feature of innate responses is that they remain unchanged, however, often the antigen is encountered (Delves and Roitt, 2000). Given the limited exposure to antigens in utero and the naive adaptive system, newborns must rely on their innate immune system for protection to a significant extent. The tasks of this system are to shield the body from microbial invasion, reducing the number and virulence of microorganisms, and to coordinate and instruct the acquired immune responses (Levy, 2007).

Responses to pathogen invasion initiates within minutes to hours with the activation of the innate immunity system. Most components of this system are present before the onset of infection and constitute a set of disease-resistance mechanisms that are not specific to a given pathogen. Innate immunity is composed of cellular and molecular components (soluble factors) that recognize classes of molecules peculiar to frequently encountered pathogens (Kindt et al., 2007). The main innate immune response is an acute inflammation as a response to infection with pathogens in which cells and molecules of the immune system move into the affected tissue (Delves and Roitt, 2000).

The cellular components of the innate immune system originate in the bone marrow. These include granulocytes (neutrophils, eosinophils, and basophils), macrophages, dendritic cells, natural killer cells, and $\gamma\delta$ T lymphocytes. Macrophages possess receptors for carbohydrates that are not normally exposed on the cells of vertebrates, such as mannose, and therefore can discriminate between foreign and self molecules (Delves and Roitt, 2000). Macrophages and neutrophils have receptors for

antibodies and complement, which coat the microorganism and lead to enhanced engulfment (phagocytosis) of these microorganisms. Dendritic cells are localized in different tissues and constantly sample the environment to identify infectious agents when they first encounter the host (Woolums, 2010). Dendritic cells have surface receptors capable of distinguishing pathogen-associated molecular patterns on the surface of microorganisms that activate them, and once activated, they become antigen-presenting-cells, migrating to the local draining lymph node, where they present antigens to T cells (Delves and Roitt, 2000).

A principal role of eosinophils is to fight against parasites. Eosinophilia has been related to an increased response against parasites (Ganheim et al., 2007). Recent studies indicate that basophils, in addition to releasing histamine as an allergic response, also can produce a vast array of effector molecules such as cytokines and might aid the maintenance of a Th2 cytokine-dependent immunity (Siracusa et al., 2010). Natural killer cells are especially important in fighting viral infection and cancer; $\gamma\delta$ T lymphocytes can secrete cytokines that modify the function of other immune cells and can kill host cells infected with viruses, bacteria, or parasites (Woolums, 2000).

Soluble factors of the innate immunity include the many proteins of the complement system, enzymes such as lysozyme and proteins such as lectins and defensins, which bind non specifically to various classes of molecules typical of infectious agents. Once bound, the soluble factors may impair or kill the infectious agent (Woolums, 2000). Moreover, activation of the complement cascade generates highly reactive and powerful activation products with chemotactic, inflammatory, and cytotoxic activities which are key features for initiation of the inflammatory process

(Zipfel, 2009). Acute phase proteins are other soluble factors of the innate immunity system. Their concentrations in plasma increase rapidly in response to infection, inflammation, and tissue injury and are commonly used as markers of inflammation (Delves and Roitt, 2000). Cytokines constitute another group of soluble mediators, acting as messengers both within the immune system and between the immune system and other systems of the body, forming and integrated networks highly involved in regulation of immune responses (Delves and Roitt, 2000).

The main feature of innate immunity after pathogen invasion is to trigger an acute inflammatory response. Complement proteins coat the surface of pathogens and also serve as chemoattractants of neutrophils. Vascular permeability is increased due to release of histamine and complement proteins. In parallel, substances released by pathogens and damaged tissues upregulate expression of adhesion molecules on inflamed endothelium, alerting cells, such as neutrophils, of the presence of infection. Circulating neutrophils recognize adhesion molecules expressed on the endothelium surface through its receptor L-selectin (CD62L) initiating the process of rolling along the vessel wall and becoming activated. Once activated by chemoattractants and chemokine, they rapidly shed CD62L from their surface and replace them with another adhesion molecule named β -integrin (CD18). This one primarily binds to intracellular adhesion molecule-1 expressed on inflamed endothelium under the influence of inflammatory mediators. The activated neutrophils pass through the vessel walls, moving up the chemotactic gradient to accumulate at the site of infection, where they are well placed to phagocyte pathogens and kill them by production of toxic intracellular molecules including reactive oxygen species, hydroxyl radicals, hypochlorous acid, nitric

oxide, antimicrobial cationic proteins and peptides, and lysozyme (Delves and Roitt, 2000; Ley et al., 2007).

Chase et al. (2008) reviewing the competence of cells and soluble factors of innate immunity in newborn calves, reported the following: a) complement activity at birth is approximately 50% of that in adult cows, increasing gradually and rising to ~50% of adult levels by 4 wk of age; b) the number of circulating neutrophils around birth is approximately 4 times higher than in 3 wk-old calves; c) neonatal neutrophils and macrophages have reduced phagocytic ability but their capacity is increased after the ingestion of colostrum; d) by 1 wk of age, neutrophils are functional and able to mount an effective response, improving gradually to adult levels by 5 mon of age; and e) the number of circulating natural killer cells is also lower at 1 wk of age (3% of total lymphocytes), increasing to 10% by 6 wk of age.

Passive Acquired Immunity

The transfer of Ig from the dam to the neonate is termed passive transfer in the majority of species and the transfer of Ig starts occurring during the fetal period (Weaver et al., 2000). The exception is ruminant animals which deliver no Ig to the neonate prepartum. Therefore the newborn calf is completely dependent on the Ig supply from colostrum because the epitheliochorial placenta of cows prevents transfer of Ig during the fetal period (Kehoe and Heinrichs, 2007). Colostrum is the first secretion of the mammary gland and the first feed offered to newborn calves. In addition to providing the needed amount of Ig to ensure APT, it also provides other essential nutrients that have passed across the placenta in minimal proportions, as well as other nutrients that are needed to satisfy the nutritional requirement of calves during first hours of life (Kehoe and Heinrichs, 2007).

The most important component of colostrum for the life of the calf are the Ig. Colostrum contains 5 types of Ig (IgG, IgA, IgM, and IgE, IgD). However IgG accounts for 85 to 90% of the total Ig. Specifically IgG is divided in two subclasses, IgG1 and IgG2, which are found in similar proportions in a cow's peripheral blood (Sasaki et al., 1976). However in colostrum, the proportion of IgG1 was about 5 times greater than that of IgG2 (Sasaki et al., 1976). Concentrations of IgG subclasses in serum of calves fed colostrum reflect the greater proportion of IgG1 found in colostrum. Studies have reported values of 7 to 9 times greater concentrations of IgG1 compared to IgG2 (Hidioglou et al., 1995; Godden et al., 2009).

The pool of Ig reaching the intestine and able to be transported across the intestinal epithelium was initially assumed to occur by non-selective pinocytosis (Klaus et al., 1969; Jones and Waltman, 1972). However later studies discovered the existence of specific Ig receptors known as neonatal Fc receptors (FcRn) present in intestinal epithelium, initially identified in human epithelial cells of the intestine, suggesting its involvement in IgG binding and transfer of passive immunity (Israel et al., 1997). A potential protective mechanism of FcRn in favor of circulating IgG that prevents premature degradation and clearance from circulation has been recently hypothesized (Goebel et al., 2008).

Establishment of APT is crucial to reduce neonatal morbidity and mortality, strengthen calf immunity, and increase calf life span (Robison et al. 1988, Quigley and Drewry, 1998; Donovan et al., 1998). Calves are considered to have an APT if they have a serum total IgG ≥ 1 g/dL after 24 h of colostrum feeding (Tyler et al., 1996; Weaver et al., 2000). Other authors consider serum total protein (STP) as a good

measure of APT. Calves having ≥ 5.0 g/dL of circulating STP are considered to have APT (Donovan et al., 1998, Calloway et al., 2002).

Appropriate passive transfer has been associated with improved weaning and postweaning weight. A correlation analysis including ~900 heifers from birth to 180 d of age indicated a positive relationship of serum IgG concentrations at 24 to 48 h of age with average daily gain (ADG) and weaning weight. Authors also reported an increased mortality in heifers having < 1.2 g/dL of serum IgG at 24 to 48 h of age (6.8 vs. 3.3%) when compared with heifers with greater concentrations of IgG (Robison et al. 1988). Concentrations of serum IgG at 24 to 48 h also were positively associated with mature equivalent milk and fat production during the first lactation, although no effect was found for age at first calving. Regression analyses of mature equivalent milk on IgG concentration was 8.5 kg of milk per 1 g/L of IgG. The regression of mature equivalent fat on serum IgG concentrations was 0.28 kg per 1 g/L of IgG (DeNise et al., 1989).

Different factors could prevent dairy calves from reaching APT. The time of colostrum feeding, the quality (in terms of IgG concentration of colostrum), and the quantity of colostrum fed are reported as the most critical factors determining an APT. Neonatal calves have an ability to absorb complete proteins such as Ig, however this capacity is lost within a few hours after birth before gut closure occurs. It is recommended feeding calves as soon as they are born. Stott et al. (1979, a, b, c) in a series of studies demonstrated that the rate of Ig absorption depended primarily on the amount of colostrum fed and how soon after birth the ingestion occurred. Final gut closure was not dependent on the amount of colostrum provided but it depended on the timing of colostrum feeding; in non fed calves, their ability to absorb entire Ig through their intestine

will last until 24 h of life, with a marked decrease after 12 h of age. However in calves fed right after birth, the gut closure occurs earlier (Stott et al., 1979a). At a fixed time of feeding, amount of colostrum fed is the main factor affecting rate of Ig absorption. However if first feeding is delayed, it would negatively affect the rate of Ig absorption (Stott et al., 1979b). When feeding colostrum in ranging amounts from 0.5 to 2 L, Stott et al. (1979c) reported a positive linear correlation of amount of colostrum fed and time of feeding on serum total IgG concentration. Neither BW (33 to 52 kg) at birth nor colostrum IgG concentrations (28.4 to 46 g/L) were correlated with the maximum absorption observed when feeding at an early age and with greater amounts.

Concentration of IgG in colostrum, in spite of Stott et al. (1979c) reporting no effect of colostrum IgG concentrations on serum IgG, has been reported to have a positive correlation with serum IgG concentrations after colostrum feeding. Morin et al. (1997) found that, in fact, as Stott and coworkers confirmed, the most important factors associated with serum IgG concentrations were volume of colostrum fed and timing of administration when colostrum IgG1 was low (23.9 g/L). However when colostrum had of greater IgG1 concentration (60 g/L) and fed at the same volume and time, Holstein calves had more IgG1 in serum. In addition, they also reported that feeding 4 L of high quality colostrum at 0 h resulted in greater IgG in serum than those of calves fed only 2 L of the same high quality colostrum, concluding that feeding this amount did not saturate the absorptive mechanisms. On contrary, Jaster (2005) evaluated the best option to feed colostrum to Jersey calves. Author reported that provision of high quality colostrum (IgG1, 84 vs. 31 g/L) in 2 feedings (2 L at 0 and 12 h) resulted in greater serum IgG concentrations than calves fed same 4 L in a single feeding.

Active Acquired Immunity

A general concept is that it is not until the innate immune system is overcome by pathogens or infection that the adaptive immune system is activated. However, in recent years, close interactions between components of both innate and adaptive immunity indicate that both function as a highly interactive and cooperative system, producing a combined response more effective than either system could produce by itself. The advantages of the acquired immune response are the following: 1) specificity, the ability to maximize the efficacy of the immune response while minimizing unnecessary collateral damage and 2) memory, which provides protection from future infection with the same pathogen (Palm and Medzhitov, 2009).

The main soluble factor in the acquired immune response is the antibody, also defined as Ig, produced by B lymphocytes and found in different parts of the body including fluids. Antibodies bind to molecules on pathogens and prevent them from infecting the host or target them for destruction by immune cells. Different types of antibodies are produced by B cells, including IgM, IgG, IgA, IgE, and IgD. These Ig have different functional characteristics and exist in variable concentrations in different parts of the body. Levels of antibodies increase slowly the first time a pathogen is encountered, but in subsequent encounters with the same or similar pathogens, antibody levels can increase very rapidly (Woolums, 2010).

Like B lymphocytes, T lymphocytes also arise in the bone marrow but migrate to the thymus glands to mature. Maturing T cells express a unique antigen-binding-molecule called T-cell receptor (TCR) on their membranes. Two types of T cells are differentiated based on the membrane glycoproteins present on their surfaces, namely T helper (Th) expressing CD4 and T cytotoxic expressing CD8. Most TCR can

recognize only antigens that are bound to cell membrane proteins called major histocompatibility complex (MHC) molecules. The MHC molecules are grouped into 2 classes, namely MHC-I, expressed by nearly all nucleated cells and class MHC-II, expressed only by antigen-presenting-cells. After encountering naive T cells with antigen-presenting-cells, the T cell proliferates and differentiates into memory T cells and various effector T cells (Kindt et al. 2007).

Helper T cells assist a wide variety of other cells to respond optimally to infection. They do this through production of cytokines and expression of surface molecules that can stimulate other cells to improve their activity. They are further subdivided into groups called Th1, Th2, Th0, and Th17 which are based on the combination of cytokines expressed by a given Th cell. The Th1 type cytokines [interferon- γ (IFN- γ) and interleukin (IL-2)] play a key role in initiating early resistance to pathogens and induction of cell-mediated immunity, enhancing macrophage stimulation and phagocytic activity of viruses and other pathogens that live inside host cells (Marodi, 2002). The Th2 cytokines (IL-4 and IL-5) are particularly important in the induction of antibody production, especially on mucosal surfaces (Woolums, 2010). A polarization of Th cells is measured as the ratio of IFN- γ :IL-4, a lower ratio indicates a bias for a Th2 response, which has been associated with impaired cell mediated activity (Mizota et al., 2009).

Cytotoxic T cells may kill target cells by one of at least three distinct pathways. Two involve direct cell-to-cell contacts between effector and target cells. The third is mediated by cytokines, such as IFN- γ and tumor necrosis alpha (TNF- α), which are produced and secreted as long as TCR stimulation continues. These cytokines affect the opposed target cell. The TNF- α engages its receptor on the target cell and triggers

the caspase cascade leading to target-cell apoptosis, whereas IFN- γ induces transcriptional activation of the MHC-I antigen presentation pathway, leading to enhanced presentation of endogenous peptides by MHC-I (Andersen et al., 2006).

Chase et al. (2008), reviewing the particularities of B and T lymphocyte function in newborn calves, reported that the following: a) the number of circulating B cells is greatly reduced in the first wk of age (~4% of the total lymphocytes) compared to ~20% to 30% in adults, which is reached by 6 to 8 wk of age; b) the low number of B cells coupled with the calves' endogenous corticosteroids and absorbed maternal hormones result in a prolonged lack of endogenous antibody response, even in the face of an apparent Th2 cytokine bias in neonates; c) circulating IgA, IgG1, and IgG2 do not reach appreciable concentrations until 16 to 32 d after birth; d) T-cell subsets have an adult-like ratio (CD4:CD8) in neonates with ~20% for Th cells and ~10% for T cytotoxic cells; and e) mitogen activation of T lymphocytes is slightly depressed at birth and remains constant through 28 d after birth.

In preruminant calves, the FA profile of the phospholipids fraction of cells, including immune cells, is expected to reflect the FA profile of the diet due to the lack of rumen functionality. Thus modifying the dietary FA profile is expected to also modify that of the immune cells. Chapkin et al (1988) fed mice four different diets, CCO (0% LA and ALA); safflower oil (SAO, 78.2% LA), borage oil (BO, 36.6% LA and 25.2% GLA), fish oil (FO, 1.2% of LA, 16.9% EPA and 12% DHA). The FA profile of PBMC- phospholipids fractions differed with the diet fed to mice. Mice fed CCO or BO had PBMC- phospholipids fractions with the lowest proportion of LA. Concentration of mead acid, an indicator of EFA deficiency was higher in mice fed CCO. Interestingly, concentration of

AA was quite constant except in the PBMC- phospholipids fractions of mice fed FO which was lower compared to other diets (6.3 vs. 14.7% of total fat). Regarding n-3 FA, ALA was not detected in either PBMC- phospholipids fractions whereas EPA, DPA, and DHA were only detected in mice fed FO diet.

Insulin and Growth Factors in Colostrum

By the beginning of the 1980's it was discovered that cow's colostrum was enriched in different growth factors; however they were not fully investigated (Georgiev, 2008b). In cow's colostrum, the growth factor present in greatest proportion is IGF whereas in humans, it is epidermal growth. Epidermal growth concentrations remain high during the lactation period whereas IGF are high only in colostrum (Georgiev, 2008b; Blum and Baumrucker, 2008). In general, regardless of the type of diet, concentrations of IGF and insulin are higher in colostrum than in blood (Oda et al., 1989).

Among the postulated physiological characteristics of IGF and insulin, the most critical in newborn calves is enhancing the growth and development of the gastrointestinal tract by affecting cellular proliferation and differentiation (Roffler et al., 2003; Georgiev et al., 2003). Calves fed colostrum compared to those deprived of colostrum exhibited an enhanced epithelial cell proliferation as evidenced by greater circumference, area, and height of the villus. Authors assumed this response to be due to the presence of growth promoting factors in colostrum (Buhler et al., 1998). Later studies verified the positive effect on development of the intestinal tract if IGF-I was present in colostrum but not if IGF-I was provided orally or by parenteral administration (Roffler et al., 2003; Georgiev et al., 2003). However, studies evaluating the effect of

diet manipulation on concentration of growth factors in colostrum and their transfer to the newborn are scarce.

Sparks et al. (2003) arbitrarily grouped newborn calves as having low (< 10 ng/mL) or high (> 10 ng/mL) concentrations of IGF-I in serum before colostrum feeding. After 48 h of colostrum feeding, no differences were reported between groups for IGF-I and IGF binding protein (IGFBP) types -2, -3, -4, and -5. Authors found a negative correlation of IGF-I at 0 h to the difference between serum IGF-I at 48 and 0 h ($r = -0.82$) because calves born with greater concentrations of IGF-I had a significant decrease at 48 h after colostrum feeding, whereas a positive correlation of concentrations of IGF-I in colostrum and IGF-I in serum at 48 h was detected ($r = 0.45$). In an attempt to evaluate the effect of bovine somatotropin (rBST) on IGF-I concentrations in colostrum and calves, Pauletti et al. (2007) found that prepartum supplementation with rBST increased concentrations of IGF-I in colostrum but concentrations of IGF-I in serum were lower at the first or second day of colostrum feeding regardless of supplementation with rBST. Hammon et al. (2000) delayed the intake of colostrum in calves and reported that calves fed colostrum within the first 2 to 3 h of birth, their IGF-I concentrations were greater and were maintained during the first 36 h after colostrum feeding compared to delays of more than 12 h, however feeding colostrum did not increase IGF-I concentrations. On the other hand, insulin concentrations increased after first colostrum feeding but not if the delay in colostrum feeding was over 24 h. All previous studies have hypothesized that reduced serum concentrations of IGF-I, although in greater concentrations in colostrum, might be due to colostrum IGF having a local effect rather than being absorbed into circulation.

Effect of Supplemental Fatty Acids on Passive Transfer

Among the main factors contributing to ensure an APT are timing, quality (in terms of Ig concentration), and quantity of colostrum fed. These factors have been extensively evaluated. However a limited number of studies have involved the effect of supplementing EFA prepartum. Rajaraman et al. (1997) fed newborn calves with skim colostrum and CCO as a replacer of the milk fat or normal colostrum. Authors did not find differences in AP (IgG1 > 15 g/L) or in the activity of PBMC during the first week of life. However concentrations of fat-soluble vitamins were lower in the treatment group deprived of milk fat.

Dietz et al. (2003) supplemented pregnant beef cows with no oil source, safflower seeds (6.4% of dietary DM), or whole cottonseeds (14.3% of dietary DM). Both seeds are rich in LA. Authors reported no difference in colostrum concentrations of IgG (85, 96, and 83 g/L, respectively) and calf birth weight. Only serum of calves born from cows fed control or whole cottonseed diets were measured for IgG concentration after 36 h of birth and no difference was reported (38.6 and 37.1 g/L of IgG, respectively) between calves born at ambient temperatures > 6°C. Later, Lake et al. (2006c) classified late gestation beef cows as in BCS 4 or 6 and measured the serum IgG concentration in their calves after 18 h of birth finding no difference in calves born from dams have low vs. high BCS (15.6 vs. 13.4 g/L of IgG).

Novak et al. (2012b) restricted the intake of energy by 13% (by increasing intake of NDF) in late gestation dairy cows and reported no difference in DM intake. Colostrum IgA was greater from cows of lower energy intake; however total Ig, IgG (17.3 vs. 16.2 g/L, high and low energy respectively), IgA, and IgM did not differ after colostrum feeding at 3 d of age. Similarly birth weight did not differ. Serum IGF-I was not affected

by the prepartum diets when measured at 3 d of age (130.6 vs. 99.3 ng/mL for high and low energy intake treatments, respectively).

Limited studies have evaluated the effect of feeding fat supplements to cows on fatty acid (FA) composition of colostrum and most of them did not include the effect of parity. However, few studies using dairy cows and ewes supplemented with CLA have reported not effect of parity in total CLA (Kelsey et al., 2003; Tsiplakou et al., 2006). However, Mierlita et al. (2011) when comparing effect of 3 ewes' breeds, reported that primiparous ewes produced greater proportion of LA, GLA, ALA, EPA and total CLA. Moreover the few studies performed with cows, regardless parity consideration have focused on supplementation of n-3 or CLA FA instead of n-6 FA.

Effect of Supplemental Fatty Acids on Total Fat and Fatty Acid Profile

Colostrum

A limited number of studies have evaluated the effect of supplementing EFA prepartum on colostrum FA profile of ruminants. Most were focused on supplementation of n-3 FA. An early study conducted by Noble and coworkers (1978) supplemented pregnant ewes with a protected PUFA supplement (70% sunflower oil + 30% SO). Intake of fat was increased from 8 to 1 wk prior to calving from 3.4 to 37.6 g/d and 9.4 to 113 g/d for the control and supplemented group, respectively but caloric intake remained the same. The major FA present in colostrum was OA. Linoleic acid accounted for less than 1% in colostrum of ewes fed control diets but was 8% in colostrum of ewes supplemented with PUFA. The presence of elongated FA derived from LA or ALA were not detected.

Capper et al. (2006) fed pregnant ewes Ca salt of palm oil (Megalac, 4.1% of C16:0 and 2.0% of LA, % of concentrate DM basis) or FO (1.5% of LA, 0.4% of EPA

and 0.04% of DHA, % of concentrate, DM basis). Ewes fed Megalac during the prepartum period produced colostrum with greater proportions of LA, C18:0, C18:1 *trans*, and OA but proportions of C16:0 and AA did not differ with fat supplement. Supplementing ewes with FO increased the proportions of CLA c9 t11, ALA, EPA, and DHA in colostrum. In addition, supplementation of FO reduced colostrum yield and total fat concentration.

Aiming to evaluate the effect of prepartum supplementation of FA on colostrum FA profile, Santschi et al. (2009) fed prepartum cows a control diet (90.6% C16:0, 4.7% LA, and 0.5% ALA, % of total FA) or a linseed supplement (6.6% C16:0, 19.3% LA, and 53.6% ALA, % of total FA). Authors reported that colostrum from linseed-supplemented cows had lower proportions of C16:0 but greater proportions of C18:0, CLA c9 t11, and ALA. Proportions of OA, LA, AA, EPA, and DPA did not differ due to fat supplement. Leiber et al. (2011) supplemented prepartum cows (n = 6) with seeds rich in LA (safflower) or ALA (linseed) and reported that only prepartum cows fed seeds rich in ALA increased the proportion of this FA in colostrum but did not influence the proportions of EPA and DHA. Authors concluded that those physiologically necessary FA were maintained to avoid deficiency regardless of the type of FA fed prepartum.

Studies have reported that supplementation of FA during the prepartum period modifies the FA profile of colostrum. A common feature among studies is that the dietary FA profile tended to be reflected in colostrum FA. However efficiency of transfer is poor particularly for PUFA with LA and ALA derivatives appearing to have a preferential synthesis in mammary gland to ensure proper proportions of AA, EPA and DHA regardless of dietary FA.

Plasma

Early studies using pregnant ewes focused on assessing the dietary transfer of LA and AA to the offspring in utero (Noble et al. 1978, Soares, 1986). However those studies did not report concentrations of any of the n-3 FA. In order to test the effect of differences in maternal intake of LA in late gestation, Elmes et al. (2004) fed late gestation ewes diets differing in LA concentration, namely a control diet (3.8% total fat, DM basis; 29% LA and 22% ALA, % of total FA) or high LA diet (4.5% total fat, DM basis; 41% LA and 16% ALA, % of total FA). They reported that plasma phosphatidylcholine fraction of fetus (138 gestational d), had increased proportions of LA, GLA, AA, DPA and DHA were higher in fetus from high LA supplemented ewes; ALA was undetectable, authors hypothesized that the high availability of LA in dams tissue might increased the enzymatic activity of desaturases and elongases increasing the proportion of LA and ALA derived FA.

Lake et al. (2006b) supplemented lactating beef cows with no fat or safflower seeds rich in LA or OA. Diets did not affect the total fat concentration in plasma, but calves suckling dams fed safflower seeds rich in LA or OA had greater plasma concentrations of those respective FA. Concentration of C18:0 was greater in plasma of calves suckling cows supplemented with safflower seed regardless the type of FA enriched in the seed, whereas EPA was greater in calves suckling no fat- supplemented cows and fewer in calves suckling cows supplemented with safflower seed rich in LA.

Moallem and Zachut (2012) supplemented cows during the last 22 d of gestation with encapsulated fat containing 240 g/d of SFA, 300 g/d of linseed oil (15 g of LA and 56 g of ALA daily), or 300 g/d of FO (5.8 g of EPA and 4.3 g of DHA daily). No differences in plasma concentrations of LA, ALA, AA, EPA and DPA in calves were

detected before colostrum feeding. However, plasma concentrations of GLA and DHA were 1.3 and 1.7 times greater in the FO-group than the other groups, respectively. Authors concluded that DHA supplementation, and not its precursor ALA, was needed in the diet to increase concentration of this critical FA in fetal development.

In an attempt to evaluate the rise in plasma cholesterol concentrations, Wrenn et al. (1973) fed preweaned calves with an increase proportion of LA (14.1 vs. 2.5% of total fat) in milk. Growth was not affected by differential intake of LA, similarly cholesterol did not differ. Jenkins et al. (1985) reported that using tallow (3.8% LA), CCO (3.2% LA) or CO (52.7% LA) as sources of fat in MR resulted in calves fed CCO having greater concentrations of plasma free cholesterol but not cholesterol ester (50 to 57% of lipid fraction), which was the main lipid fraction in plasma followed by phosphatidylcholine (25 to 30% of lipid fraction). Interestingly, LA in plasma cholesterol ester and phosphatidylcholine fractions was only greater when calves were fed CO. Concentration of AA did not differ in the cholesterol ester fraction, but was lowest in the phosphatidylcholine fraction of calves fed CO. In a follow up study, Jenkins and coworkers (1986) evaluated the use of tallow (2.0% LA), CAO (20.4% LA) or 1:1 mixture of tallow + CAO on plasma lipid fractions of calves. The cholesterol ester (41 to 46% of total fat) and phosphatidylcholine (35 -40% of total fat) fractions were in greater concentrations. However concentration of LA in both lipid fractions was greater when tallow + CAO were fed, whereas concentration of AA was greater when feeding tallow. In a following study, Jenkins and Kramer (1986) fed MR with 4 different FA sources: CCO (0.1% LA), CCO + CO (95% CCO + 5% CO, 2.8% LA), CCO + CAO (92.5% CCO + 7.5% CAO, 1.6% LA), or tallow (5% LA). Authors reported that fat source did not

change the total concentration of fat in cholesterol ester or phosphatidylcholine fractions. However cholesterol ester reflected better the dietary FA profile with fewer concentrations of C12:0 and C14:0 and greater concentrations of LA in calves fed tallow. In calves fed CCO + CO, concentrations of C12:0 and C14:0 were greater and LA was similar as in calves fed tallow. Concentrations of AA in cholesterol ester were greater in calves fed CCO + CAO, followed by similar concentrations when CCO + CO or tallow was fed.

Jenkins and Kramer (1990) evaluated the inclusion of FO in MR containing primarily tallow and vegetable fats. Tallow and CCO served as fat sources in the control MR resulting in a n-6:n-3 of 7.1. In the other MR, half of the control was replaced with either CO alone (n-6:n-3 = 36.5) or with a mix of CO and FO in the two following ratios: 2/3 CO and 1/3 FO (n-6:n-3 = 3.1) and 1/3 CO and 2/3 FO (n-6:n-3 = 1.0). No difference was detected in ADG and FE due to MR. Total fat in plasma was lowest when FO was added to the MR. Cholesterol ester and phosphatidylcholine were the most abundant lipid fractions in plasma. Regardless the lipid fraction, concentration of LA was greater in calves fed C + CO but that of AA was greater in calves fed the highest proportion of FO. Feeding any proportion of FO increased EPA concentration in cholesterol ester and phosphatidylcholine fractions, but only that of DHA in phosphatidylcholine, whereas the highest proportion of FO was needed to increase the concentration of DHA in cholesterol ester fraction.

Liver

Fatty liver is a critical condition that leads to impaired liver function. Several studies in humans (Reddy and Rao, 2006; Cave et al. 2007; Semple et al. 2009; Thomson and Knolle, 2010) have documented very well the effect of hepatic steatosis in

liver function and the multiple etiologies of this disorder. Dairy cows in the transition period face a high risk for fatty liver, due to the high demand of nutrients for milk production, accompanied by a limited intake that forces the cow to mobilize corporal tissue and generate intermediates of energy such as FFA. When these FFA are taken up by the liver in high quantities, the oxidative and secretive capacity of lipids by liver is exceeded. Hence, the arriving FFA are only partially oxidized forming ketone bodies or reesterified to TG, which end up accumulating in liver decreasing the metabolic function of liver. Bobe et al. (2004) wrote a comprehensive review of the pathology and etiology of fatty liver in dairy cows. The authors concluded that fatty liver is a multifactorial, multifaceted disease with nutritional factors as the main drivers of this condition.

Jenkins and Kramer (1986) fed MR with 4 different FA sources: CCO (0.1% LA), CCO + CO (95% CCO + 5% CO, 2.8% LA), CCO + CAO (92.5% CCO + 7.5% CAO, 1.6% LA), or tallow (5% LA). Feeding tallow reduced the total fat in liver and this was due to a lower proportion of TG (5%), which was the greatest lipid fraction in the CCO MR (48.55), whereas, proportion of phosphatidylcholine was the highest in the other diets (23.3% CCO, 34.8% CO, 32.4% CAO, and 43.% tallow). The FA profile of TG was the only one containing significant proportions of C12:0 and C14:0 and they were in greater proportions in calves fed CCO MR. Proportions of LA and ALA in the TG fraction also better reflected the dietary FA profile. On the other hand AA was not present in the TG fraction but in the phosphatidylcholine fraction and was greater in liver of calves fed CO or CAO.

Jenkins and Kramer (1990) evaluated the inclusion of FO in MR containing primarily tallow and vegetable fats. Tallow and CCO served as fat sources in the control

MR resulting in a n-6:n-3 of 7.1. In the other MR, half of the control was replaced with either CO alone (n-6:n-3 = 36.5) or with a mix of CO and FO in the two following ratios: 2:3 CO and 1:3 FO (n-6:n-3 = 3.1) and 1:3 CO and 2:3 FO (n-6:n-3 = 1.0).

Phospholipids were the greater fraction in calf liver. Individual phospholipid fractions [phosphatidylcholine (52%), sphingomyelin (1.2%), and phosphatidyl ethanolamine(21%)] as well as total fat did not differ due to MR. Concentration of LA was greater in calves fed CO, whereas AA concentration was greater in calves fed the control and high FO MR.

Jambrenghi et al. (2007) supplemented lambs with a control diet (3.3% fat, 39.8% of LA as % of total fat) or a high fat diet enriched with LA (7.9% fat, 45.5% of LA as % of total fat) for a 45 d finishing period. Feed intake and final BW were not changed. However, the FA profile of the liver was influenced by the diet. Concentrations of C16:0, C16:1, and C18:0, ALA, and EPA were greater for control calves, whereas OA, LA, AA, and DHA were greater for the group fed more fat and LA.

Effect of Supplemental Fatty Acids on Preweaned Calves Performance

Obtaining good growth and health performance of dairy calves before weaning is one of the primary goals of a dairy herd management. Dairy herd managers have to deal with challenging circumstances once the calf is born, such as to ensure appropriate passive transfer of IgG from colostrum (Beam et al., 2009) and prevention and treatment of diseases such as diarrhea, omphalitis, septicemia, and pneumonia which are among the most commonly diagnosed diseases leading to morbidity and mortality in calves (Donovan et al., 1998). To prevent a high incidence of calf diseases and profitability of the herd, care should be taken not only during the preweaning period but

also during the gestation period, particularly during the last trimester of gestation, during which time the fetus has its greatest development.

Effect of Supplemental Fatty Acids during Pregnancy on Growth Performance and Hormonal and Metabolic Profile of Prewaned Calves

Early studies using human subjects have reported a direct effect of nutritional status in late pregnancy on fetal growth and birth weight. Naeye et al. (1973) evaluated 467 gestating women and reported that low-calorie intake during late gestation was highly and negative correlated with fetal growth and weight. Kramer (1987) reviewed 895 publications related to potential causal reasons of intrauterine growth retardation in human subjects and reported that regardless of racial origin and economic status, poor gestational nutrition was a common cause of lighter birth weight. One of the most evaluated nutrients to produce adverse effects on the offspring was protein. Anthony et al. (1986) and Carstens et al. (1987) fed protein levels below the requirement for maintenance of cows during late gestation and although BW and BCS of cows at calving was lower for undernourished cows, the birth weight of their calves did not differ.

More recent studies using ruminants found contradictory effect of undernutrition during late gestation. Osgerby et al. (2002) fed pregnant lambs a diet meeting only 70% of total nutrient requirements and reported that undernourished fetuses at 135 d had lighter heart, pancreas, thymus, gut, and kidney weights; bone growth also was affected; Dwyer et al. (2003) reduced the nutritional intake of pregnant lambs by 35% and reported a 9.3% reduction in birth weight and a reduced ability of offspring to suckle their dams. On the other hand, Hess (2003) evaluated 18 studies that supplemented late gestation beef cows with fat and results were not consistent; that is, calf birth weight was decreased (n=2), increased (n=3), or unchanged (n=12). Hess (2003) therefore

concluded that fat supplementation of dams in late gestation did not affect birth weight. Similarly, Banta et al. (2006) aimed to evaluate the effect of LA supplementation in middle and late gestation cows by feeding 0.68 kg of soybean meal, 3.01 kg of soybean hull or 1.66 kg of sunflower seed rich in LA. All diets provided same intakes of CP and RDP but soybean hull and sunflower supplements provided 2.34 more Mcal/d. Authors reported no effect of supplements on birth and weaning weights of calves. Later, the same authors (Banta et al., 2011) adjusted the supplements to provide same intakes of N and energy by feeding 0.23 kg of soybean hull, 0.68 kg of sunflower seed rich in LA plus 0.23 kg of soybean hull, or 0.64 kg of mid-oleic sunflower seed plus 0.23 kg of soybean hull and reported similar response as in their previous study.

In a review article by Barker (1997) he stated that “many human fetuses have to adapt to a limited supply of nutrients and in doing so they permanently change their physiology and metabolism. These “programmed” changes may be the origins of a number of diseases later in life.” One of the common diseases associated with this programming event is diabetes. Pettitt et al. (1987) reported that offspring of diabetic women had twice the risk of developing diabetes than offspring of non diabetic women; even though when the incidence of this condition was adjusted using maternal weight and birth weight as covariate.

Fowden et al. (2006), based in previous studies, identified the most probable periods in which fetal programming occur. These potential periods start pre-conceptual and pre-implantation, in which either under- or overnutrition can affect birth weight and incidence of disease later in the offspring. The majority of fetal maturation occurs during late gestation, where many tissues undergo structural and functional changes in

preparation for extrauterine life (Funston et al., 2010). In fact, several studies have evaluated the effect of undernourishment in late gestation on the metabolism of offspring. Although most studies have reported a low birth weight with concomitant effects on metabolic response of offspring (Barker et al., 1993; Barker, 1997; Ozanne and Hales, 2002; Drake and Walker, 2004, 2005; Gicquel et al., 2008) some studies have reported that metabolic response of offspring can be programmed in uterus without a change in birth weight (Pettitt et al., 1987; Ferezou-Viala et al., 2007).

Funston et al. (2010) reviewed how maternal nutrition affects conceptus growth and postnatal responses in beef cattle. The most common negative effects reported when pregnant cows were undernourished were on birth weight, health, growth, reproduction, carcass weight and carcass quality. Singh et al. (2010) reviewed the factors that account for phenotypic variation in milk production by dairy cows. They concluded that a substantial proportion of the unexplained phenotypical variations were due to epigenetic regulation (change in gene expression without modifying DNA sequence) as a consequence of maternal nutrition during fetal life or nutrition during the first year of life. Recently Soberon et al. (2012) reported a positive correlation of preweaning ADG with first-lactation yield; for every 1 kg of preweaning ADG, heifers, on average, produced 850 kg more milk during first lactation. They concluded that increased growth before weaning results in some form of epigenetic programming resulting in a positive effect on milk yield.

Few studies have evaluated the effect of supplementing FA during late gestation or early lactation on their effect on overall calf performance. Early studies reported better growth rate of calves by providing extra calories using fat through a concentrate

source (Espinoza et al., 1995). Bottger et al. (2002) supplemented beef cows from 3 d through 90 d post partum with isonitrogenous and isocaloric supplements, a control, a safflower seed rich in LA (76% LA) or rich in OA (72% OA). They reported that calf BW gain during the supplementation period was not influenced by supplement fed; neither did 205-d adjusted weaning weights. Encinias and coworkers (2001, 2004) supplementing pregnant beef cows or ewes with fat rich in LA versus a control diet of low fat, did not find any effect of additional prepartum fat in birth and weaning weight of their offspring.

Lake et al. (2005) fed lactating beef cows with isocaloric and isonitrogenous diets of low (1.2% of DM) or high (5% of DM) fat, by providing a supplement rich in LA or in OA. They reported no effect of diets in BW gain of suckling calves. In a companion paper Lake and coworkers (2006a) reported increased concentrations of plasma glucose in calves suckling cows supplemented with LA compared to those fed control diets, but no change in insulin, IGF-I or NEFA was reported due to dam diets. Greater plasma glucose accompanied with no change in insulin concentrations might indicate reduced sensitivity of peripheral tissue for uptake of glucose.

Chechi and Chema (2006) fed pregnant rats and their pups with diets of 20% fat rich in SFA (15% LA) or PUFA (70% LA). They reported that pups fed SFA pre- and postweaning had the highest concentration of plasma total cholesterol, whereas the PUFA/PUFA fed group had the lowest, but plasma triglyceride concentration did not change among groups. The cholesterolemic effect of SFA/SFA diets might be due to increased proportions of LDL-cholesterol.

Undernutrition during late gestation in women results in reduced birth weight of the offspring. However, in beef cows, supplementation of fat during prepartum has yielded contradictory results, with most of the studies reporting no effect of fat supplementation on calf birth weight and preweaning BW. At the best of our knowledge, no study has evaluated the metabolic and immune response of preweaned dairy calves born from EFA supplemented cows, this topic warrants further investigation, considering the recent discover of potential fetal programming effect of nutrition during early life in future offspring productivity.

Effect of Feeding Supplemental Fatty Acids to Preweaned Calves on their Growth Performance and Metabolic Profile

Few studies are available in which preruminant dairy calves were fed increased amounts of LA. The first studies were done in an attempt to replace milk fat in skim milk with vegetable sources of fat in order to reduce the cost of raising calves. Later, studies have focus in the supplementation of specific sources of FA.

Early work of Jacobson et al. (1949) intended the evaluated the use of different types of SO in total replacement of milk fat (3% wet basis) in calf performance. They reported that crude expeller SO produced poor growth, severe scours and high mortality, whereas performance of calves fed hydrogenated SO equaled that of calves fed whole milk. In a second companion study (Murley et al., 1949) totally replaced milk fat with hydrogenated, refined or crude SO (3% wet basis) and reported, similar results but that feeding refined SO resulted in fewer incidences of scours than crude SO. Calves fed either refined or crude SO had the poorest growth. From the same lab, Richard et al. (1980) replaced milk fat with 2% (web basis) of SO, CO or tallow and did not find any effect on ADG but feeding vegetable oils increased plasma cholesterol

concentrations. Authors indicated that fat globule size after reconstitution of milk by homogenization was comparable to that of cows' milk.

Some years later, the laboratory of K.J. Jenkins in Ontario, Canada, evaluated the supplementation of specific FA by reconstituting skim milk and sweet whey with different fat sources as the only feed of calves. In one study, Jenkins and coworkers (1985) reported the use of CCO (3.2% LA), tallow (3.8% LA) or CO (52.7% LA) as total sources of fat in MR (~20% fat DM basis). Calves fed CCO or tallow had greater ADG and feed efficiency (FE) than calves fed CO. This likely occurred as a result of severe scouring by calves fed CO. In a follow-up study, Jenkins and coworkers (1986) evaluated tallow (2.0% LA), CAO (canola oil, 20.4% LA), CO (53.2% LA), and a 1:1 mix of tallow + CAO or tallow + CO as only fat sources of reconstituted skim milk (~20% fat DM basis) for calves. Again authors reported that reconstituted milk with CO promoted scours and poor calf gains, which was not reversed when tallow + CO. Feeding tallow + CAO or tallow AL did not produce scours and resulted in calves with better ADG and FE. In other study, Jenkins and Kramer (1986) replaced fat in skim milk with 4 different fat sources: CCO, CCO + CO (95% CCO + 5% CO), CCO + CAO (92.5% CCO + 7.5% CAO), or tallow. All calves, regardless of the fat source fed, were free of diarrhea. Increasing intake of EFA by including CO or CAO did not affect BW gain and FE; however feeding tallow increased ADG, DMI and FE when compared to calves fed milk containing just CCO but not when CCO was combined with CO or CAO.

Leplaix-Charlat et al. (1996) fed 5 wk old calves for 17 d, with a 23% fat MR (DM basis) containing either tallow (3.7% LA) or SO (51.2% LA) with or without additional dietary cholesterol (1% of MR, DM basis) aiming to evaluate the plasmatic distribution of

lipoproteins. Calves fed SO had greater concentrations of total fat in liver and total cholesterol in plasma compared with calves fed tallow. The effect of high LA diets was due to increased concentrations of high density lipoprotein without modification of LDL or VLDL. Plasmatic concentrations of NEFA were reduced dramatically when SO was fed. The inclusion of dietary cholesterol had no effect on NEFA when SO was fed but reduced NEFA when tallow was fed. Plasmatic levels of APO B were similar in calves fed either source of fat but increased about 3 fold when cholesterol was included in the diet.

Piot et al. (1999) fed 2 wk old calves for 19 d, a MR formulated with either CCO (42% C12; 3% LA) or tallow (22% C16:0; 38% C18:1; 2.4% LA) and reported no difference in ADG, MR intake and plasma concentrations of BHBA. However, calves fed CCO had decreased plasma concentrations of glucose and insulin. Whether these decreased plasma concentrations were due to reduced secretion of insulin by MCFA or an enhanced ability of peripheral tissue for glucose uptake could not be defined since calves had no difference in ADG.

In an attempt to evaluate the effects of feeding isocaloric, isonitrogenous MR that varied in the amount and type of FA, Mills et al. (2010) fed calves MR with 23% fat and varied proportions of MCFA as the only feed. MR contained 2% MCFA (control) or 32% MCFA supplied by either CCO (23% C8:0) or caprilate (23.6% C12:0). After insulin challenge calves fed caprilate had a greater decrease in plasma glucose concentration. Empty BW gain was better for control calves. Liver of calves consuming CCO was heavier and contained 15% more fat (as is basis) than the other two groups. Authors stated that it was unclear why CCO induced lipid accumulation in the liver, but

increased capacity of initial FA oxidation and subsequent preferential de novo synthesis or chain elongation of MCFA may have occurred.

In order to prove whether vegetable fat mixtures could be used instead of lard (15.2 % DM basis), Huuskonen et al. (2005) fed calves with MR containing 3 different fat sources, namely mixture-1 [palm oil (75%) + CCO (25%); 7% LA, 0.1% ALA], mixture-2 [palm oil (75%) + CCO (20%) + rapeseed oil (5%); 8.0% LA, 0.7% ALA], or lard (12.1% LA, 1.2% ALA). During the preweaning period, ADG or number of days with diarrhea did not differ, but calves fed lard had a reduced and poor FE. Post weaning ADG also was not affected by the type of fat fed preweaning. Calves fed MR with mixture-2 tended to have lower starter intake during the preweaning period, but total DMI did not differ among treatments.

Berr et al. (1993) aimed to study excretion of cholesterol by n-3 and n-6 PUFA. Authors fed rats with 3 different sources of fat (~9 % wet basis). Feeding FO reduced plasma concentrations of total cholesterol but not when CCO or SAO were fed. This decrease was due primarily to the decrease in high density lipoprotein - cholesterol concentrations, which is one of the main mechanism by which feeding of n-6 FA reduced the concentration of circulating cholesterol.

Recently, the few studies evaluating dietary inclusion of EFA or its derivatives to newborn calves have focused in the supplementation of n-3 FA from vegetable (ALA) or animal (EPA and DHA) origin. Ballou and DePeters (2008) evaluated the inclusion of FO in calf MR to replace 1 or 2% of the fat of a control MR (20% fat, DM basis). They reported no effect on growth, ADG, FE or serum concentrations of glucose, insulin, urea nitrogen, NEFA, and TG. However at day 20, calves fed FO only had lower NEFA and

TG concentrations, no clear pattern in metabolite concentrations was evident and interpretation of these temporal results was difficult.

Hill et al. (2009) fed calves a grain mix containing 0, 0.125 or 0.25 5% Ca salt of linseed oil or 0.25% Ca salt of FO (DM basis). No effect of oils was detected during the first 28 d of life. When the first 56 d of life was evaluated, ADG and hip width increased linearly as flax oil increased in the grain mix whereas serum concentrations of urea N and glucose decreased. The better ADG and lower serum concentrations of glucose in calves supplemented with flax oil might indicate a better sensitivity of tissue for glucose to be utilized for protein synthesis. Hill et al. (2011) fed newborn calves with MR (16% fat DM basis) containing only fat sources to which NeoTec 4 (blend of butyric acid, CCO and flax oil) replaced 0 or 1% of the animal fat. The NeoTec 4 contained 7 times more butyrate and MCFA and 2 times more ALA. Intake of MR as percentage of BW and grain mix intake did not differ, but calves fed NeoTec 4 had 10% better ADG and FE tended to be greater, possible as a result of improved immune response, as suggested by reduction of diarrhea incidence in calves fed NeoTec 4.

Early studies reported that vegetable oils rich in long chain FA in replace of fat in MR have resulted in detrimental effects in calf performance, whereas CCO had resulted in improved performance similar to that of tallow. Recently, studies have not revealed clear effect of n-3 or n-6 supplementation on calf performance, making this area in need for more research.

Effect of Supplemental Fatty Acids Fed During Pregnancy on Offspring Health and Immunity

A very limited number of studies have evaluated the effects of supplementing FA during late gestation on immune response of cattle offspring. Most of the studies

supplementing fat prepartum were conducted using beef cattle. Since calves stay with their dams after birth, during the preweaning period, more variables are encountered when assessing on calf performance.

Das (2003) proposed that the negative correlation between breast-feeding and insulin resistance and diabetes mellitus can be related to the presence of significant amount of PUFA in human breast milk, and that the provision of PUFA during late pregnancy and lactation can prevent diabetes mellitus from developing. In a review article, Enke et al. (2008) indicated that dietary PUFA and their derivatives consumed during mid to late gestation had a programming effect on early immune development and immune maturation by regulating numerous metabolic processes as well as by modifying gene expression. Recently, Klemens et al. (2011) evaluated the odds ratio of incidence of allergic diseases and production of inflammatory cytokines due to fat supplementation using 5 randomized controlled trials. They concluded that supplementation during pregnancy but not during lactation reduced the risk of allergic diseases and production of inflammatory cytokines.

Petit and Berthiaume (2006) fed beef cows isonitrogenous and isocaloric diets starting in late gestation. Diets contained either Megalac® (14% of concentrate, DM basis), linseed (33.2% of concentrate, DM basis), or no fat supplement. Calves born from dams fed fat had lower rate of mortality both at birth and at weaning although birth weight and ADG preweaning were not different.

During the last 55 d of gestation, Lammoglia et al. (1999) fed beef cows isocaloric and isonitrogenous diets of 1.7 or 4.7% fat (safflower seeds fed at 0 and 6.7% of dietary DM). Calves were fed standard colostrum and challenged to cold-stress conditions (0°C

for 140 min). Calves born from cows fed safflower seeds kept their body temperature throughout the stress period, whereas control calves decreased their body temperature after 70 min. Calves fed safflower seed also had lower cholesterol concentrations in plasma after 60 min of cold exposure, whereas glucose concentration in plasma was ~40% greater the whole 140 min of cold stress, suggesting that increased glucose availability resulted in better heat production. In contrast, Dietz et al. (2003) reported that the body temperature and plasma concentrations of glucose were not affected in calves born from cows fed no supplemented diet, safflower seeds, or whole cottonseed.

Encinias et al. (2004) reported that lambs had lower incidence of mortality and a greater number of lambs were weaned per ewe fed isocaloric diets of 10 vs. 0% safflower seeds. However, neither birth weight nor weaning weight were affected by feeding of safflower seeds. Similarly, plasmatic concentrations of NEFA and glucose did not differ within 48 h post birth. Similar intake of energy by pregnant ewes was proposed as a cause for lack of diet effect.

Effect of Supplemental Fatty Acids Fed to Preweaned Calves on Their Health and Immunity

As indicated earlier, initial studies of supplementation of LA to calves were done by partially replacing milk fat. Those initial studies had in common a greater incidence of diarrhea by calves fed additional LA. Authors concluded that the likely causes of increased diarrhea were type of oil and poor quality process during homogenization or dispersion of fat supplements into dry skim milk. (Jacobson et al., 1949; Murley et al., 1949).

Jenkins et al. (1985, 1986) reported that calves fed CO alone or a 1:1 mixture of CO + tallow had greater incidence of diarrhea than calves fed tallow, CAO or a mixture

of tallow + CAO. However when Jenkins and Kramer (1986) replaced 5% of CCO with CO, calves did not suffer from diarrhea. Later, Jenkins (1988) fed calves with MR containing tallow, CO, or CO with aspirin (to inhibit potential role of prostaglandin promoting scours). Feeding CO, regardless the inclusion of aspirin, produced more, less ADG, and worse FE than when tallow was fed. However, compared with Jenkins et al. (1985), the incidence of diarrhea was the lesser in later study although the same diets were used in both studies. Low pressure dispersion of CO was used in the 1985 study whereas a homogenizer was used in 1988 study, which resulted in smaller globules of fat (<1 um vs. 1 to 20 um).

A vast amount of in vitro and in vivo studies have evaluated the potential of FA to modify different markers of immune response. However, a limited number of them have evaluated the effect of feeding LA specifically. Moreover, most of those studies have been performed using humans or rodents.

Kelley et al. (1989, 1990) fed adult human subjects diets of low or high LA concentrations by reducing or increasing the proportion of fat respectively. In both studies they were unable to detect any dietary effect on number of circulating T and B lymphocytes and on in vitro proliferation of PBMC to different mitogens and production of complement proteins. Total number of circulating leukocytes also was unchanged. Actual concentrations of LA in tissues or blood were not measured. A potential reason of lack of LA effect might be low differential concentrations of LA in tissues between subjects on test diets. Barone et al. (1989) evaluated the reduction of LA intake on immunity of young men by reducing total fat (< 30% of dietary calories) intake. They reported that activity of blood isolated natural killer cells was increased. However,

subjects were not directly controlled so actual intake of LA could not be determined. In a later study, Heber et al. (1990) fed young men with a low fat diet (< 20% of calories) supplemented or no with CCO or SAO. Activity of natural killer cells was increased when men were fed low fat diet compared to the baseline measure but oil supplementation did not affect the activity of natural killer cells. These results indicate that amount of fat unluckily source of FA modify the activity of natural killer cells.

Yaqoob et al. (2000) supplemented the diet of adult human subjects with 9 g/d of SAO for 12 wk and was unable to effect in lymphocyte proliferation, natural killer cells activity, or production of cytokines (TNF- α , IL-1 α , IL-2 or IFN- γ) by PBMC. However, a potential reason for lack of effect was that the FA profile of plasma phospholipids or PBMC were not altered by the feeding of SAO, thus reducing the chance for LA to modify activity of immune cells.

In vitro and animal controlled studies (better experimental control) have more marked effects of supplemental LA than studies using humans supplemented with LA. Calder et al. (1990) cultured murine macrophages in presence of a variety of FA. Those FA were rapidly taken up by the cells enriching the neutral and phospholipid fractions with the FA from the medium. Macrophages enriched with C14:0 or C16:0 showed a decreased ability to phagocyte unopsonized zymosan particles whereas those enriched with LA, ALA, AA, EPA or DHA had an enhanced phagocytic ability with AA having the greatest effect on rate of uptake. A change in FA profile of phospholipid fraction of lymphocytes affecting the membrane fluidity was proposed as the mechanism of improved phagocytic activity in calves supplemented with PUFA. Thanasak et al. (2005) cultured bovine PBMC with 2 doses (125 and 250 μ M) of LA or ALA and reported that

the greater concentration of LA inhibited proliferative response of PBCM to mitogens whereas ALA had no effect on proliferation. Increased concentrations of ALA decreased the concentrations of leukotriene B4 whereas LA had no effect. However prostaglandin E2, a prostaglandin with immunomodulatory effect, was increased in ALA media. A potential contrasting effect of leukotriene B4 and prostaglandin E2 functions might be the reason for the inhibitory effect of LA on lymphocyte proliferation. Later Gorjao et al. (2007) evaluated the proliferative response of human lymphocytes to IL-2 stimulation when cells were incubated with different doses of various FA. Oleic acid and LA stimulated proliferation at non toxic concentrations (<75uM) that could induce apoptosis and necrosis whereas other FA decreased proliferation by causing cell death (C16:0 and C18:0) or cell-cycle arrest and apoptosis (EPA, DHA) if concentration were >25uM.

Wallace et al. (2001) fed mice a low fat control diet or diets supplemented with CCO, SAO or FO; the FA profile of phospholipids fraction of spleen lymphocytes reflected the diet. Thymidine incorporation into Concanavalin-A stimulated lymphocytes and IL-2 production were greater after CCO feeding whereas IFN- γ production was decreased when feeding SAO or FO. The ratio of IFN- γ :IL-4 was used as the ratio of production of Th1:Th2 type cytokines. This ratio was lower for mice fed SAO or FO; whereas, mRNA expression of cytokines at 4 and 8 h indicated that the production of cytokines affected by the feeding of specific FA was regulated at the level of gene expression.

Rodrigues et al. (2010) fed rats doses (0, 0.11, 0.22, 0.44 g/kg BW) of OA or LA. Neutrophil migration was greater in mice fed the 2 greater doses of OA but only the lower dose (0.11 g/d) of LA was needed to enhance neutrophil migration in response to

intraperitoneal injection of glycogen. Enhanced migration may be possible due to an increase in expression of CD62L, production of the chemoattractant CINC-2 $\alpha\beta$, and enhanced rolling of neutrophils, all were enhanced with both FA but no FA was found to increase expression of CD18, an important integrin in the process of neutrophil extravasation. In the presence of lipopolysaccharide (LPS), only LA reduced the production of CINC-2 $\alpha\beta$ after 4 h and OA only inhibited IL-1 β after 18 h.

The ratio of Th1:Th2 cells or of their derived cytokines (IFN- γ :IL-4) are measured to evaluate the polarization of the immune system toward antibody-mediated- (Th1 < Th2) or cell-mediated- immunity (Th1 > Th2). For 4 wk Mizota et al. (2009) fed liquid sources of fat to mice subjects to change the dietary ratio of n-6:n-3 (0.25, 2.27 or 42.9) due changes in LA and ALA. Production of IFN- γ by mononuclear cells from splenocytes declined when LA rich diets were fed relative to greater ALA diets. Whereas interleukin-4 was reduced when either lower or greater LA rich diets were fed. Thus the ratio of IFN- γ :IL-4 was greater in mice fed the high ALA diet, indicating, contrary to the common antiinflammatory definition of n-3 FA, that n-3 enriched diets at the level evaluated here, had inflammatory properties.

In a later in vivo study, Diwakar et al. (2011) evaluated the impact of feeding rats with different proportions of LA and ALA. The 4 experimental diets were: diet-1:53.6% LA and 0.45% ALA, diet-2: 40% LA and 8.8% ALA, diet-3: 32.2% LA and 16.7% ALA, and diet-4: 9.9% LA and 32.2% ALA. Supplementation of diets rich in ALA (greater than D1), increased the proportions of ALA, EPA, and DHA in the membrane of splenocytes and peritoneal macrophages. Proliferation of splenocytes stimulated with concanavalin-A and phytohaemagglutinin (PHA) decreased when any of the 4 diets was fed. A similar

effect happened with the production of nitrite at 12 h post stimulation of peritoneal macrophages. Production of leukotriene B₄ by peritoneal macrophages was only decreased by diet 3 and 4 but TNF- α and IL-2 concentrations did not differ. These responses agree with others reporting antiinflammatory effect of diets rich in ALA. In this study this effect was executed by the decreased proliferation of lymphocytes and potential reduction of the phagocytic activity of immune cells.

In an attempt to evaluate the effect of n-6 FA to alter immune function, Thanasak et al. (2004) fed castrated goats either olive oil (10% LA) or CO (55% LA) for 3 wk. Goats in the CO group had greater LA concentrations in both plasma and erythrocyte at 21 d after supplementation. Goats fed CO experienced a reduction in the percentage of blood lymphocytes expressing α -4 integrin (CD49d) at day 21. However no change were observed in lymphocyte proliferation after concanavalin-A or PHA stimulation, in total white blood cell count, or in lymphocytes expressing CD2, CD4, CD8, CD21 or MHC-II. Authors could not give a conclusive mechanism for these immune responses but stressed that a combination of all mechanism by which FA perform their action such as changes in membrane fluidity might affect intracellular interaction, receptor expression, nutrient transport, signal transduction, regulation of gene expression, protein acylation or calcium release might be potential factors.

Beef calves undergo stress during long distance shipping and arrival in new environment which induce inflammatory response. Feeding of soybean seed with high LA might exacerbate that inflammatory response which might lead to undesirable animal performance. Farran et al. (2008) transported crossbreed heifer calves (~200 kg initial BW) from Kansans to use in 35 d receiving diet experiments. Heifers were fed

diets containing tallow (2.3% LA and 0.3% ALA), linseed (15.9 LA and 54.2% ALA) or soybean seed (54% LA and 8% ALA). Changes in plasma FA profile were in parallel to the diets. Calves fed soybean seed had the lower ADG and FE, but percentage of calves treated for bovine respiratory disease did not differ due to fat supplements. A group of calves were challenged with LPS and resulting rectal temperatures were lower for soybean seed and linseed fed groups whereas concentrations of plasma TNF- α were greater for heifers fed soybean seeds when compared to those fed tallow. Diets did not affect plasma haptoglobin, fibrinogen, or total white blood cell count after LPS challenge.

In a study performed at the University of Florida, Araujo and coworkers (2010) in a first trial, evaluated the effect of supplementing rumen inert SFA (2.1% of dietary DM, 1.7% LA; Energy Booster 100) or PUFA (2.5% dietary DM, 28.5% LA, Megalac-R) and a control non fat supplemented diet for 30 d after transportation and feedyard entry of Bradford steers (218 kg BW). Steers fed rumen inert SFA had decreased DMI and tended to gain less BW compared with control steers but no effect was detected for plasma concentrations of fibrinogen and ceruloplasmin. In a second trial, Brahman crossbreed heifers (276 kg BW) were fed diets of 0 or 5.7% of Megalac R starting 30d before transportation to ensure adaptation to diets. No effect of diets was detected on DMI and ADG post transport. Also no difference in plasma concentrations of ceruloplasmin was detected; however, plasma concentrations of haptoglobin were lower during the first week post transportation for heifers fed Megalac-R. A raise in circulating concentrations of haptoglobin is a liver response to proinflammatory cytokines. Megalac-R primarily contains LA but also minimal concentrations of ALA. It could be

that the production of cytokines might change in response to a specific FA (LA or ALA) or a combination of both FA (LA + ALA) that in turn modified the synthesis of haptoglobin. However a reduced inflammatory response that could detrimentally affect performance could not be ruled out, in fact DMI, ADG, and/or FE, post transport, was not improved by the feeding of Megalac R.

Other University of Florida study, Silvestre and coworkers (2011) fed transition dairy cows Ca salts of palm oil or SAO. Neutrophils of cows fed SAO from 30 d prepartum to 35 d postpartum had greater concentrations of vaccenic acid (0.45 vs. 0.96%), LA did not differ (20.6 vs. 23.2%), lower concentrations of *c*9, *t*11 CLA (1.69 vs. 0.85%) and ALA (1.43 vs. 1.02%). Cows fed SAO had increased plasma concentrations of haptoglobin and fibrinogen. The percentage of blood neutrophils with phagocytic and oxidative burst activities were not affected by diets but mean number of *E. coli* phagocytized per neutrophil and mean intensity of H₂O₂ produced per neutrophil were increased in cows fed SAO at 4, and 4 and 7 d postpartum respectively. Percentage of neutrophils positive to CD62L and CD18 were greater in cows supplemented with SAO at 4 and 7 d postpartum. Throughout the evaluation period, mean number of CD62L expressed per neutrophil was greater in cows fed SAO but number of circulating neutrophils expressing CD62L was lower whereas mean number of CD18 did not change with diets. Concentration of TNF- α in incubated isolated neutrophils at 35 d postpartum was greater in cows fed SAO diets both with and without LPS stimulation, but total mass increase did not differ with diets. Concentration of IL-1 β was greater in neutrophils when cells of cows fed SAO were stimulated with LPS and mass change was also greater. Supplementation of LA during the transition period enhanced the

inflammatory and acute responses of dairy cows to better cope during immunosuppressed period prone to exacerbate incidence of diseases.

Lake et al. (2006c) conducted two experiments to determine the effect of maternal lipid supplementation on calf response to an antigenic challenge, in trial 1 a control low fat diet and a diet containing high LA safflower seeds (9.5% of diet DM), both isocaloric and isonitrogenous, were fed to primiparous beef cows from 1 to 40 d of lactation. Calves born from cows fed safflower seeds had a lower and delayed response of antibody production in response to an ovalbumin (OVA) challenge. In a second trial cows were blocked by BCS at birth and were supplemented with no oils seeds, high linoleate safflower seeds (8.1% of dietary DM), or high oleate safflower seeds (7.6% of dietary DM). Calves born from cows supplemented with LA or OA had lower serum concentrations of anti-OVA IgG but cell mediated immune response were not affected. Potential change in FA profile of lymphocyte affecting membrane fluidity and/or lymphocyte proliferation was proposed as the potential cause of impaired production of antibodies in calves suckling from LA supplemented cows.

To the best of our knowledge there is no study evaluating the inclusion of LA in MR to modify activity of different markers of immune response in newborn calves, however, some work were recently developed to evaluate the effect of n-3 FA from animal or vegetal origin. Ballou and DePeters (2008) evaluated the inclusion of FO in calf MR to replace 1 or 2% of the fat of the control MR (20% fat, DM basis. Authors reported no differences in fecal score, concentrations of white blood cells, hematocrit, total protein, and phagocytic activity of polymorphonuclear leukocytes in blood. However, production of anti-OVA IgG was attenuated after the second OVA injection in

calves fed 1% but not 2% of FO. Differential effect on germinal center affinity maturation of Ig class switching might be the mechanism by which a quadratic response in IgG production was observed when supplementing FO.

Hill et al. (2011) fed newborn dairy calves with a control MR containing 15% of animal fat or a 15% fat-MR containing 1% of NeoTec -4 (blend of butyric acid, CCO and LSO). Calves fed NeoTec -4 had fewer numbers of days with an abnormal fecal score and also the average fecal score tended to be lower. After *pasteurella* vaccination, the relative mRNA abundance (respect to non vaccinated and non NeoTec-4 supplemented calves) of TNF- α , IL-4, IL-6, and IL-10 did not differ pre- or post vaccination, but the change in relative mRNA abundance from pre to post vaccination them was negative for TNF- α whereas tended to be positive for IL-4. These effects coincided with lower rectal temperatures and less refusal of MR after vaccinations in calves fed NeoTec-4. Additionally, calves fed NeoTec-4 had greater antibody titer post vaccination with parainfluenza 3 (PI3) and bovine virus diarrhea type I. Results indicate that butyric acid and ALA can cause a reduced inflammatory response by potentially reducing the synthesis of proinflammatory cytokines and changing the immune response over an antibody-type instead to a cell-mediate response.

A very limited amount of in vivo studies have focused in the effect of supplementing LA to evaluate the modification of immune response in dairy calves. From the available studies, including other species or adult cattle, LA seems to modify the activity of different cells of the immune system. The primary effect of LA by itself or its derivatives appears to be the induction of inflammatory response by increasing the production of proinflammatory eicosanoids, synthesis of proinflammatory cytokines, and

enhancing the migration and activity of leukocytes on injured tissues. Contrary, ALA could have an opposite effect on inflammation through increasing the production of anti-inflammatory eicosanoids and cytokines and by reducing the migration and activity of leukocytes. However, some invitro studies have demonstrated that LA could reduce the activity of immune cells and production of proinflammatory cytokines when cells were cultured with greater concentrations of LA. All these effects might indicate that under in vivo circumstances, the physiological status of preweaned calf could modify the need of LA and their effect on immune cells. Future research should focus in the effect of increased intakes of LA modifying different parameters of immune response.

Effect of Supplemental Fatty Acids on Hepatic Gene Expression

The liver plays a critical role in the systemic circulation; its strategic position in blood circulation (connected to systemic circulation by vena cava and hepatic artery and to intestines through portal vein and bile duct) allows the liver to carry out all its different metabolic functions. Among the metabolic functions are lipid, carbohydrate, and protein metabolism, including protein generation and metabolism of toxic or waste products (Thomson and Knolle, 2010).

Liver disorders or diseases, primarily fatty liver, can lead to impairment of liver function. Hence it is of high importance to provide a balanced diet to avoid excessive accumulation of FA in liver, primarily because of excessive weight loss in cattle. Different dietary strategies have been evaluated in humans to reduce the risk of hepatic steatosis and many of these studies have been replicated recently in cattle, primarily in transition cows, which are the group with higher risk of fatty liver. Grummer (2008) divided the nutritional strategies to prevent fatty liver in cows into two groups: a) diet formulation to increase energy density and b) inclusion of feed additives with different

modes of action such as reduction of adipose lipolysis, enhanced hepatic VLDL secretion or increased hepatic FA oxidation. Supplementation of n-6 and n-3 PUFA have become an important dietary strategy in humans and rodents, similar to including supplementation of PPAR agonists (Clarke, 2001; Sekiya et al., 2003; Guo et al., 2006a,b; Rakhshandehroo, et al., 2009) with even more recent evaluation in cattle (Litherland et al., 2010; White et al., 2011a, b; Bionaz et al., 2012).

Polyunsaturated FA elicit their effects by coordinating suppression of lipid synthesis and upregulating FA oxidation in liver. Clarke et al. (2001) and Sampath and Ntambi (2005) reviewed different studies supplementing n-3 and n-6 FA. Authors concluded that n-3 FA had a more potent activity than n-6 FA and that suppression of lipid synthesis in liver is a more sensitive pathway regulated by PUFA than the lipid oxidation pathway. Before the discovery of nuclear receptors capable of binding FA and establishment of a direct role of FA in gene regulation, it was established that FA can affect cell signaling and gene expression by affecting membrane phospholipid content or through the production of eicosanoids (Sampath and Ntambi, 2005).

Regarding livestock animals, more studies on PUFA regulation of gene expression have been carried out using pigs and chickens but scarcely any using ruminants. Definitely more studies are needed to elucidate the important mechanisms by which FA can exert their regulatory function on gene expression in dairy cattle.

Regulation of Hepatic Peroxisome Proliferator Receptor- α

A well described ligand-activated nuclear transcription factor is PPAR- α , which plays important roles in lipid and carbohydrate metabolism. Upon activation PPAR- α forms a heterodimer with a FA and RXR- α . This heterodimer binds to PPAR responsive elements in the regulatory regions of target genes to influence gene expression

(Schmitz and Ecker, 2008). Two isoforms, PPAR- α and PPAR- γ are the most well understood isoforms. The PPAR- α isoform is primarily expressed in the liver where it is involved in promoting gluconeogenesis and stimulating the transcription of genes that are critical for peroxisomal and mitochondrial oxidation of FA, as well as a regulator of other transcription factors (Weickert and Pfeiffer, 2006; Calder, 2012).

Among all transcription factors, PPAR has a wide effect on expression of genes for different processes of lipid metabolism as well as on other pathways such as glucose and amino acid metabolism, and inflammation. For each of those processes PPAR affects the expression of several genes. A good proportion of these genes were already identified due to the presence of a PPAR response element in the promoter region (Rakhshandehroo et al., 2010).

Effect of PUFA on PPAR- α activity. Fatty acids, and more specifically PUFA, are natural ligands of PPAR- α . Forman et al. (1997) used monkey kidney fibroblast cells (CV-1 cells) and analyzed the binding ability of different FA to PPAR- α . Authors reported that MCFA (C12:0 to C16:0) were weak activators of PPAR- α whereas the best activators were ALA, AA, and LA. They reported also that derivatives of lipoxygenase metabolism such as 8(s)- hydroxyeicosatetraenoic acid was a potent ligand of PPAR- α , whereas leukotriene B4 was a weak one.

Hostetler et al. (2005) evaluated the affinity of different forms of FA for PPAR- α using a direct fluorescence ligand binding assay in *E. coli* strains expressing the recombinant mouse PPAR protein. They concluded that saturated or unsaturated LCFA-acyl CoA and certain PUFA (*cis* and *trans* parinaric acid and C18:4) were able to bind PPAR- α with higher affinity whereas, regardless of chain length, SFA were not

significant binders. Recently Bionaz et al. (2012) used bovine kidney cells cultured with a PPAR agonist and individual 12 carbon FA to evaluate the differential expression of 30 genes involved in lipid metabolism and inflammation. They reported that out of 15 genes, well known to be target genes of PPAR- α in nonruminants, 10 were upregulated by the PPAR- α agonist in bovine kidney cells. Interestingly, the stronger activation effect was induced by C16:0, C18:0, and EPA followed by C20:0 and CLA *c9 t1*. Authors concluded that the preference for SFA in bovine may be due to adaptation of PPAR in ruminants to cope with greater availability of SFA in their diets.

Regulation of Hepatic Sterol Regulatory Element Binding Protein

Three isoforms of SREBP have thus far been identified; SREBP-1a and SREBP-1c. The SREBP-1 form is an important regulator of genes involved in lipid synthesis whereas SREBP-2 has been shown to control genes important to cholesterol homeostasis. The SREBP-1c is the major isoform in rodent and human liver (Sampath and Ntambi, 2005). The SREBP are initially synthesized as large proteins and have to undergo a maturation process that includes reduction in size and further transit to the nucleus. Once in the nucleus, it binds to *cis* elements, termed sterol regulatory elements, in the promoters of target genes and induces the transcription of a variety of genes involved in cholesterol, TG, and FA synthesis (Sampath and Ntambi, 2005).

A fasting-refeeding experiment in rodents (Horton et al., 1998) indicated that refeeding enhanced expression of FA biosynthetic enzymes compared to the prefasting condition but expression of cholesterol synthetic enzymes only returned to the prefasting level. The expression of SREBP-1 and SREBP-2 followed exactly the same pattern of expression after the refeeding state. These findings led to the hypothesis that the inhibitory effect of PUFA on lipogenic gene expression could occur via either

repression of SREBP mRNA or inhibition of SREBP maturation (Horton et al., 1998; Sampath and Ntambi, 2005).

Effect of PUFA on SREBP activity. Yahagi et al. (1999) supplemented wild and transgenic mice that over-expressed a mature form of SREBP-1 in liver, with different sources of FA. In wild mice, SFA and MUFA sources did not reduce the expression of mature SREBP-1 whereas EPA and DHA were more potent depressors of SREBP-1 expression followed by LA. The rate of decrease in mature SREBP-1 paralleled those in mRNA for lipogenic enzymes such as FA synthase (FASN) and acetyl-CoA carboxylase (ACC). In the transgenic mice, dietary PUFA did not reduce the amount of SREBP-1 protein. This result excluded the possibility that PUFA accelerated the degradation of mature SREBP-1. These results demonstrated that the suppressive effect of PUFA on lipogenic enzyme genes in the liver is caused by a decrease in the mature form of SREBP-1 protein, which is presumably due to the reduced cleavage of SREBP-1 precursor protein.

In an attempt to evaluate the effect of cholesterol supplementation on regulation of transcription factors, Kim et al. (2002) fed mice 3 sources of fat (5% diet) namely triolein, SO (rich on LA), and FO (rich in EPA and DHA) supplement with or without 2% of cholesterol (cholesterol is a potent inducer of stearoyl CoA desaturases expression) . Authors reported that when a high cholesterol diet was combined with either SO or FO, maturation of SREBP-1 mRNA was repressed whereas levels of mRNA, protein synthesis, and enzymatic activity of stearoyl CoA desaturase -1 were increased. Interestingly, mice of the same dietary group had increased levels of SREBP-1 mRNA, however the mRNA levels of SREBP-1 target genes such as FASN and LDL receptor

were decreased. Results indicated that the main control of PUFA-mediated suppression of SREBP-1 target genes is through repressing SREBP-1 maturation and demonstrated that cholesterol regulates stearoyl CoA desaturase -1 gene expression through a mechanism independent of SREBP-1 maturation.

The mechanisms by which PUFA affect SREBP is still unclear. One recent study (Di Nunzio et al., 2010) evaluated the effect of different FA to suppress SREBP activity and regulate the flow of nonesterified cholesterol using hepatic hepG2 cells. The supplementation of FA reduced SREBP activity in the order of EPA = LA = AA > ALA = DHA = DPA > OA. Likewise, the incorporation of PUFA increased nonesterified cholesterol flow from the plasma membrane to intracellular membranes. Suppression of SREBP activity by PUFA may depend on the degree of incorporation into cellular lipids, and it may be associated with increased flow of nonesterified cholesterol between the plasma membrane and intracellular membranes.

Regulation of Hepatic liver X Receptor

The LXR are transcription factors belonging to the nuclear receptor super family. Two isoforms exist, LXR- α and LXR- β , with LXR- α being primarily expressed in liver. These receptors are recognized as important regulators of cholesterol metabolism, lipid biosynthesis, and glucose homeostasis as well as regulators of the storage and oxidation of dietary fat (Weickert and Pfeiffer, 2006). This receptor is activated by binding to oxysterols, which are derived from the cholesterol oxidative process. After binding, LXR needs to form an obligated heterodimer with RXR before binding the DNA on the LXR responsive element (Ducheix et al., 2011). This receptor plays a crucial role in regulation of FA metabolism by activating the expression of SREBP-1c (Yoshikawa et al., 2003) and carbohydrate regulatory element binding protein (ChREBP) (Cha and

Repa, 2007) when binding to its promoter region in each of these two transcription factors. Independently, LXR can also bind LXR response elements on the promoter region of FASN, ACC, and stearoyl CoA desaturase-1 (Ducheix et al., 2011).

Effect of PUFA on LXR activity. In an attempt to investigate the molecular mechanism by which dietary PUFA decrease hepatic SREBP-1c expression, Yoshikawa et al., (2002) established mouse SREBP-1c promoter luciferase reporter assays in HepG2 cells and HEK293 cells. Supplementation of EPA in the medium with HepG2 or HEK293 cells, both co-transfected with LXR α or LXR- β , decreased the SREBP-1c promoter activity when the heterodimer LXR/RXR was activated. Deletion of the two liver LXR responsive elements present in the SREBP-1c promoter region eliminated the suppressive effect of PUFA. Authors evaluated the effect of different FA on their ability to decrease SREBP-1c promoter activity resulting in the order: AA > EPA > DHA > LA, whereas SFA had no effect and oleic acid had minimal effect. These results indicate that both LXR responsive elements are important PUFA suppressive elements suggesting that PUFA could be deeply involved in nutritional regulation of cellular FA concentrations by inhibiting the LXR-SREBP-1c system, which enhances lipogenesis. The same group, Yoshikawa et al. (2003), using similar methodology demonstrated that PPAR- α inhibited SREBP-1c promoter activity induced by LXR, concluding that deletion of the two LXR response elements in the SREBP-1c promoter region were responsible for this inhibitory effect of PPAR- α .

In contrast to the regulation of LXR activity by PUFA reported by Yoshikawa et al. (2002, 2003), Pawar et al. (2003) concluded that PUFA suppressed SREBP-1 and its target genes by other mechanisms than by LXR. They used primary hepatocytes and

FTO-2B hepatoma cells supplemented with different PUFA. Authors reported a similar response in both cells when rats were fed diets of 10% FO. They reported that EPA in primary hepatocytes or FO in in vivo conditions suppressed hepatic SREBP-1c regulated genes (FASN, S14, glycerol-3-phosphate acyltransferase and liver pyruvate kinase), and induced PPAR- α regulated genes [cytochrome P450 (CYP)- 4A, mitochondrial HMG-CoA synthase, acyl-CoA synthetase-1, and acyl-CoA oxidase] but the feeding of FO did not affect the LXR α regulated transcripts that do not require SREBP-1c for their activation (CYP7Aq, ATP- binding cassette subfamily G5 and G8), concluding that the PUFA suppression of SREBP-1 and its target lipogenic genes is independent of LXR α .

A more recent study (Howell et al, 2009) indicated that hepatic cells transfected with LXR responsive element had an increased activity of full-length SREBP-1c when treated with an LXR agonist. However this activity was reduced when cells were treated with DHA. Moreover, DHA blunted the LXR α dependent activation of a CAL4-LXR α chimeric protein. These results did not favor the idea of competitive antagonism of ligand binding, but they demonstrated that n-3 PUFA effectively mitigated the induction of SREBP-1 via reduced trans-activation capacity of LXR.

Regulation of Other Hepatic Receptors

Hepatocyte nuclear factor 4 α (HNF-4 α). It is a highly conserved nuclear receptor that binds to direct repeated elements as a homodimer. This receptor seems to be indispensable for hepatocyte differentiation and hepatic functions, such as cholesterol and lipoprotein secretion. It is expressed mainly in liver, kidney, intestine, and pancreas and is capable of activating target genes even in the absence of a ligand (Sampath and Ntambi, 2005).

Hertz et al. (1998) studied the binding of recombinant HNF-4 α dimer to its cognate C3P promoter as a function of the degree of unsaturation and chain length of fatty acyl-CoA. Binding was activated by different C14 to C16 saturated fatty acyl-CoA but was inhibited by (C18:0)-CoA and (C18:3, n-3)-CoA. When amounts of HNF-4 α were limited, C3P binding was dependent on concentrations of C14:0-CoA within the range of concentrations required for ligand binding to HNF-4 α . Both activation of C3P binding by C14:0 –CoA and inhibition by C18:3 -CoA were observed using mammalian HNF-4 α .

Inhibitor- κ B and necrosis factor κ B (NF κ B). These factors are present in the cytoplasm of cells, in their inactive form, as a heterodimer. Phosphorylation of inhibitor- κ B causes its degradation. Upon degradation NF κ B is separated from inhibitor- κ B and translocate to the nucleus. In nucleus, NF κ B modifies the transcription of a variety of genes involved in inflammation, including cytokines, adhesion molecules, acyl-CoA oxidase-2, and inducible NO synthase (Calder, 2012).

The translocation of NF κ B can be both positively and negatively regulated by various PUFA. While AA is a more potent stimulator of NF κ B translocation and thus has a positive effect on the transcription of its target genes, EPA more potently inhibits NF κ B translocation, resulting in lower transcription of NF κ B target genes (Camandola et al., 1996). Products of the AA metabolism through the activity of P450 epoxygenases, such as different AA- derived. One derivate group is epoxyeicosatrienoic acids, which have vasodilatory properties and can prevent the nuclear accumulation of NF κ B through the prevention of inhibitor- κ B phosphorylation, which mark it for subsequent degradation (Node et al., 1999).

Retinol x Receptor (RXR). This receptor has already been mentioned as the heterodimer that PPAR- α needs for its nuclear translocation. However, RXR first needs to be activated by its natural ligand 9-cis retinoic acid. Once bound to its ligand, RXR is indirectly involved in different cellular processes such as transduction of the retinoid signaling pathway and lipid anabolism and catabolism (Schmitz and Ecker, 2008).

Lengqvist et al. (2004) evaluated the activation of RXR by different FA in transfected cells with an RXR α expression vector by direct addition of the tested FA. Whereas DHA, EPA, and AA were robust activators of RXR α , C16:0 and C18:0 were not. It was also demonstrated that the activation of RXR was not due to presence of PPAR or any other ligand from other receptor factors.

Farnexoid X receptor (FXR). Farnexoid X receptor is a nuclear receptor controlling the expression of genes whose products are critically important in bile acid and cholesterol homeostasis. Stimulation of FXR enhances the expression of a short heterodimer protein, which has a negative feedback effect on LXR activity (Schmitz and Ecker, 2008). Zhao et al. (2004) evaluated transfected HepG2 with FXR fusion protein for its ability to bind FA and reported a positive binding affinity of FA for FXR in the order ALA > AA > DHA, whereas C16:0 and C18:0 had no binding activity on FXR. The expression of the FXR target genes, bile salt export pump and kininogen, were differentially regulated by PUFA supplementation. Bile salt export pump was induced with PUFA supplementation but kinonegin expression was depressed. Through this selective mechanism of regulation of target FXR genes, PUFA may contribute to the beneficial effect on lipid metabolism by preventing the accumulation of cholesterol in liver and circulation, enhancing its transport as part of bile acids.

ChREBP and max like protein X (MLX). The ChREBP is a transcription factor involved in mediating glucose-responsive gene activation. It is most abundantly expressed in tissues in which lipogenesis is highly active, such as the liver and its activity is enhanced in diets rich in carbohydrates. ChREBP was recognized initially by its ability to bind the carbohydrate response element within the promoter region of the PK gene (Yamashita et al., 2001). Later other studies determined that ChREBP additionally induces positive transcriptional effects on lipogenic enzymes such as ACC and FASN (Dentin et al., 2004). Additionally, Stoeckman et al. (2004) utilized human embryonic kidney 293 cells to identify if MLX was a heterodimer partner of ChREBP regulating the expression of glucose responsive genes. The cotransfection of plasmids expressing either ChREBP or MLX with a carbohydrate response element - containing reporter plasmid into human embryonic kidney 293 cells did not activate the promoter containing ChRE on target lipogenic genes; however the expression of both ChREBP and MLX significantly enhanced promoter activity for reporters containing carbohydrate response element from several lipogenic enzymes.

The role of ChREBP in lipogenesis has led researchers to evaluate its potential role in the pathophysiology of hepatic steatosis, which in humans has been highly correlated with further diseases such as obesity, insulin resistance, and type-2 diabetes (Postic et al., 2007). To prevent the occurrence of these diseases, the feeding of PUFA has been evaluated to prevent the negative impact of high carbohydrate and high SFA diets through PUFA capacity to reduce the activation of ChREBP. Dentin et al. (2005) fed mice a 10% fat diet containing either C18:0, C18:1, or a mix of PUFA containing 45% LA, 5% EPA, and 3.5% DHA. Mice supplemented with PUFA but not with C18:0 or

C18:1 suppressed ChREBP activity by increasing ChREBP mRNA decay and by altering ChREBP translocation from the cytosol to the nucleus, independently of an activation of the AMP-activated protein kinase. Inhibition of translocation was accompanied by an inhibition of liver pyruvate kinase and FASN, key lipogenic genes.

Regulation of Hepatic Uptake and Binding of Fatty Acids

Dietary FA esterified in chylomicron- TG or in VLDL-TG are derived both dietary and endogenous biosynthesis. Triglycerides are hydrolyzed into FA by the action of lipoprotein lipase. Upon hydrolysis, dietary NEFA enter into the cell, similar to albumin-bound NEFA mobilized from storage depots. The mechanism by which NEFA enter the cell are still unclear (Bordoni et al, 2006). Pownall and Hamilton (2003) discussed the controversies regarding the contribution of passive diffusion of FA versus protein-mediated FA transport and concluded that both models have their validity and would lead to a common rationalized model.

Some studies evaluated the expression of FA transport protein genes (also known as SCL27 family and composed by 6 subfamilies). Motojima et al. (1998) discovered a genes coding for FA transport proteins being upregulated by PPAR- α . A direct effect of PPAR- α in this upregulation was verified when PPAR- α null mice were used and no change in FA transport protein was detected. Rakhshandehroo et al. (2009) comparing the differential co-regulation of genes by PPAR- α in human and mouse hepatocytes, reported that the solute carrier family 27 (fatty acid transporter), member 2, was co-upregulated in both species. The mammalian fatty acid binding protein (FABP) family binds long chain FA with high affinity; this family comprises a group of high-affinity intracellular FA binding proteins with both unique and overlapping functions. The FABP family modulates intracellular lipid homeostasis by regulating FA transport in the nuclear

and extra-nuclear compartment of the cell, impacting systemic energy homeostasis and other unique functions depending on the cell type. Liver FABP have been hypothesized to be involved in lipid absorption by the enterocyte and in hepatocyte lipid transport and lipoprotein metabolism (Storch and McDermott, 2009).

Liver FABP was postulated to be responsible to aid PPAR- α targets such as FA to reach the nuclear receptor. Wolfrum et al. (2001) reported that liver FABP and PPAR- α are co-localized in nucleus of mouse primary hepatocytes and that liver FABP has the ability to directly interact with PPAR- α and PPAR- γ but not with RXR α or PPAR- β . The interaction of liver-FABP and PPAR- α was independent of the ligand binding, but activation of PPAR- α was positively correlated with concentration of liver FABP for all ligands tested. Among the ligands tested to enhance activation of PPAR- α , C18:0 was found to have the shallowest slope, with the steepest slope in decreased order of: ALA > OA > AA.

In an attempt to evaluate the molecular mechanisms responsible for the pleiotropic effects of PPAR- α agonists, Guo et al. (2006a) treated mouse hepatocytes with 3 different PPAR- α agonists. Authors documented that all agonists enhanced PPAR- α transactivation. Among the differentially expressed genes (DEG) the most prominent group was that of lipid metabolism with FABP1 increasing about 20 to 30 fold with all agonists. However, duck hepatocytes supplemented with LA or EPA caused an upregulation of PPAR- α and their target genes acyl-CoA oxidase and lipoprotein lipase, but no change was reported for liver FABP (Liu et al., 2011).

Regulation of Hepatic Fatty Acid Oxidation

Regulation of lipid metabolism is coordinated mainly by the liver, which actively metabolizes FA as fuel and continuously produces VLDL particles to provide a constant

supply of FA to peripheral tissues. Oxidation of FA in liver occurs through the 3 main following pathways: peroxisomal β -oxidation, mitochondrial β -oxidation, and microsomal ω -hydroxylation, with most of the enzymes of these pathways being tightly regulated by PPAR- α . Disturbances in these pathways are the basis for hepatic steatosis and alterations in plasma lipoprotein concentrations (Rakhshandehroo et al., 2010).

Peroxisomal β -oxidation

Peroxisomes are known to be involved in many aspects of lipid metabolism, including synthesis of bile acids and plasmalogens, synthesis of cholesterol and isoprenoids, alpha-oxidation, glyoxylate and H_2O_2 metabolism, and β -oxidation of very-long-straight-chain or branched chain acyl-CoA (Rakhshandehroo et al., 2010). The role of PUFA in peroxisomal β -oxidation is through the activation of PPAR. The activation not only enhances the proliferation and size of peroxisomes but also upregulates different key enzymes involved in the oxidative process.

At present, three different types of FA are known to fully rely on peroxisomes for β -oxidation. These include the following: 1) very long chain FA such as C24:0 and C26:0; 2) the 2-methyl branched-chain FA pristanic acid (2, 6, 10, 14 - tetramethylpentadecanoic acid); and 3) the bile acid synthesis intermediates dihydroxycholestanic acid and trihydroxycholestanic acid. In addition, LCFA can be β -oxidized in peroxisomes but are preferentially oxidized in mitochondria (Wanders and Waterham, 2006; Wanders et al., 2010).

Peroxisomes contain the full enzymatic machinery to β -oxidize FA, although oxidation does not go to completion. In general, the architecture of the peroxisomal β -oxidation system is comparable to that of mitochondria and consists of subsequent

steps of: dehydrogenation, hydration, dehydrogenation again, and thiolytic cleavage. Among the enzymes involved in peroxisomal β -oxidation and found to be upregulated by PPAR- α in liver of humans and/or rats are acyl-CoA oxidase-1, enoyl-CoA, and hydratase 3-hydroxyacyl CoA dehydrogenase that have PPAR response elements in their promoter regions (Rakhshandehroo et al., 2010). The end products of peroxisomal β -oxidation are shuttled to mitochondria, either as carnitine-esters and/or as free FA for final β -oxidation (Wanders et al., 2010).

Mitochondrial β -Oxidation

This process provides energy, as ATP yield for every oxidation cycle, to different cellular processes, with SCFA (< C8), MCFA (C8 to C12), and LCFA (C12 to C20) as principal targets. Mitochondrial β -oxidation results in progressive shortening of FA into acetyl-CoA subunits, which either condenses into ketone bodies or enters into the tricarboxylic acid cycle for further oxidation to water and carbon dioxide (Reedy and Rao, 2006). Mitochondrial β -oxidation is primarily regulated by control of its key gene carnitine palmitoyltransferase -1. Among the regulators of carnitine palmitoyltransferase -1 are: carnitine concentrations, malonyl-CoA, FA, fatty- acyl CoA, and different peroxisome proliferators (Reddy and Rao, 2006).

Genes that control the import of FA into the mitochondria are upregulated by PPAR- α . Similarly, PPAR- α activates the major enzymes within the β -oxidation pathway including various acyl CoA dehydrogenases, mitochondrial trifunctional enzyme, and genes involved in β -oxidation of unsaturated FA. In addition PPAR- α governs the synthesis of ketone bodies via mitochondrial HMG-CoA synthase and HMG-CoA lyase (Rakhshandehroo et al., 2010).

Microsomal ω -hydroxylation

The mammalian CYP4 family of P450 enzymes catalyzes the preferential ω -hydroxylation of FA (e.g., CYP450 ω -hydroxylases of the CYP4 family are known to convert AA to its metabolite 20-hydroxyeicosatetraenoic acid). The enzymes of this family differ in their substrate specificities in terms of FA chain length and degree of unsaturation. In some instances, these enzymes exhibit preferential affinities for prostaglandins and leukotrienes, but almost invariably preferentially catalyze ω -over ω -1 hydroxylation of their substrates (Johnston et al., 2011).

Expression of CYP4A genes is extremely sensitive to PPAR- α ligand activation, indicating that CYP4A genes may serve as PPAR- α marker genes. Microarray data performed in human hepatocytes have revealed significant induction of CYP4A11 by the PPAR- α agonist Wy14643 (Rakhshandehroo et al., 2009). The ω -hydroxylation of SFA and unsaturated FA may lead to the generation of high affinity PPAR- α ligands, including 20- hydroxyeicosatetraenoic acid or 20-OH-EPA from EPA and 20-OH-DHA from DHA, and 20- hydroxyeicosatetraenoic acid from AA, with a potential inhibition of synthesis of the former ligand by the n-3 derivate ω -oxidases (Harmon et al., 2006). Leukotrien B4 is degraded by microsomal ω -oxidation and peroxisomal β -oxidation in myeloid cells and hepatocytes. Degradation is accompanied by loss of biological activity. Interestingly, the degradative process of leukotriene B4 with subsequent loss of its biological proinflammatory function, takes place at microsomal ω - and peroxisomal β -oxidation in hepatocytes by the activity of degradative enzymes. The activity of these enzymes is increased by the proliferation of PPAR- α , which in turn can also be activated by binding to leukotriene B4 (Crooks and Stockley, 1998).

Regulation of Lipogenesis and Hepatic Steatosis

Whereas several factors contribute to enhance lipogenesis such as LXR, SREBP, and ChREBP, PPAR- α through its function to regulate the activity of genes involved in any of the 3 FA oxidation systems discussed above has a key role in lipid homeostasis and prevention of hepatic steatosis (Reddy and Rao, 2006). Early studies supplemented different FA sources to rats fed fat-free diets (Clarke et al., 1977). Rats supplemented with LA (3% of diet, as-fed basis) for 7 d reported a decreased activity of FASN and ACC as well as a reduction in the deposit of total FA in liver. Toussant et al. (1981) fed rats a fat-free diet or diets supplemented with SAO at 5 or 10% of diet (as-fed basis). Authors did not find a reduction in FASN activity when rats were fed diets of 5% SAO. However, when rats were fed diets of 10% of SAO, the enzymatic activity of ACC was reduced as was the synthesis of FA in liver. Berger et al. (2002) evaluated the effect of increasing dietary concentration of PUFA relative to a control diet (10% fat, 0% AA, and DHA) on mice global hepatic gene expression. The diets were: 0% AA + 0% DHA, 0.5% AA, 0.5% DHA, or 0.5% AA + 0.5% DHA. Supplementation of 0.5% of DHA or a mixture of AA + DHA decreased the expression of SREBP with respect to mice fed the control diet whereas supplementation of AA did not. Regardless of the type of fat fed, expression of PPAR- α was not affected, although most of its target genes were, particularly those containing PPAR response elements. Among the PPAR- α target that were downregulated in hepatocytes of mice fed diets containing FA were: acetyl CoA synthetase 1 and ATP citrate lyase, whereas only FASN was downregulated when AA or AA + DHA were supplemented. The rate of downregulation was stronger with the combination of FA rather than with single FA which was unexpected.

Piot et al. (1999) reported that calves fed CCO compared with tallow had a greater oxidation rate of C12:0 in liver, and the liver contained more fat. They concluded that the incomplete oxidation of C12:0 led to the synthesis and elongation of FA to be finally deposited in the liver. Gruffat-Mouty et al. (1999), when comparing the rate of secretion of VLDL in rat and calf liver, reported no reduction in the rate of synthesis of APO-B100 between species. They concluded that there may be a defect in VLDL assembly and/or secretion which could affect the export of VLDL-TG from calf liver. Later the same group (2001) reported that the feeding of CCO to calves increased the infiltration of FA into liver tissue by reducing the synthesis of APOB. Sato et al. (2005) fed chickens with sources of fat with different lengths of FA and reported that C12:0 was the most potent FA in reducing the synthesis of mRNA APOB at the transcriptional level.

Jambrenghi et al. (2007) supplemented lambs with a control diet (3.3% fat, 39.8% LA as % of total fat) or a LA diet (7.9% fat, 45.5% LA as % of total fat) for a 45-d finishing period. The expression of cytosolic ACC and FASN were reduced in the LA group even though the intake of total fat was more than twice that compared to lambs fed the control diet. However, microsomal and mitochondrial acyl chain elongation activity were increased in lambs fed LA, with a concomitant increase in Δ^9 desaturase activity in liver microsomes.

One of the roles of PPAR- α is to reduce the plasmatic concentration of TG. The mechanism by which this happens is probably through reducing the synthesis of VLDL. Newly discovered roles of PPAR- α in intracellular lipid trafficking and metabolism may be responsible to enhance reduction of plasma lipids. Nevertheless, the actual target

genes underlying the suppressive effect of PPAR- α on hepatic VLDL production remain to be elucidated (Rakhshandehroo et al., 2010).

Activation of PPAR- α by an agonist can also increase the clearance of TG- rich lipoproteins VLDL and chylomicrons by enhancing the activity of the lipoprotein lipase through activation of APOA5 which is a positive regulator of lipoprotein lipase or through downregulation of APOC3 which is an inhibitor of lipoprotein lipase activity. On the other hand, PPAR α activation can also downregulate the activity of lipoprotein lipase by upregulating the activity of ANGPTL4, which inhibits the clearance of TG-rich proteins by stimulating the inactivation of lipoprotein lipase (Kersten, 2008). These different regulatory mechanisms of lipoprotein lipase indicate that PPAR α can induce both pro- and anti-lipolytic pathways with predominately prolipolytic activity under continued PPAR- α activation.

Regulation of Glucose and Carbohydrate Metabolism

Important players in glycolysis are: transporters for glucose entry and the key glycolytic enzymes, phosphofructokinase and PK (Peeters and Baes, 2010). Among the transcription factors having a direct role in carbohydrate metabolism are PPAR- α and ChREBP. Notable changes in carbohydrate gene expression due to PPAR- α activation are only observed in mouse hepatocytes rather than human hepatocytes (Peeters and Baes, 2010). Hence for the effect of PPAR α regulation of expression of genes in carbohydrate metabolism, only studies with rodents will be presented. Yamada and Noguchi (1999) summarized the nutrient and hormonal regulation of PK gene expression and indicated that most *in vitro* studies done with rats reported that feeding PUFA (LA, EPA, and DHA) reduced the expression of PK in hepatocytes by up to 70%.

Jump et al. (1994) evaluated the effect of 300 μ M of GLA, ALA, AA, or EPA on PK expression in rat hepatocytes. These FA inhibited the expression of PK gene to a similar extent as did triolein. In the same study, feeding FO (10% of diet) enhanced the rate of reduction of glycolytic enzymes GK, PK, and MDH in hepatocytes in the pre-meal and post-meal states compared to hepatocytes from rats fed triolein. Enzymatic concentration of PK in rat hepatocytes decreased 25% when fed LA (3% of dietary DM) for 7 d compared to that from rats fed a fat-free diet, while a non-significant reduction of GK enzymatic activity was detected (Clarke et al., 1977). On the other hand, Toussant et al. (1981) fed rats a fat-free diet or diets supplemented with SAO (5% as-fed basis), tallow (5% as-fed basis), or C18:0 (10% as-fed basis). The feeding of SAO reduced GK activity, but the other treatments did not change in respect to the control diet. A further evaluation of the fat-free diet and the LA-supplemented diet (5% of LA, as-fed basis) did not change the enzymatic activity of glycolytic enzymes GK, phosphofructokinase, and PK.

Berger et al. (2002) evaluated the effect of increasing the dietary concentration of PUFA relative to a control diet (10% fat, 0% AA and DHA) on global hepatic gene expression. The diets were: 0% AA + DHA, 0.5% AA, 0.5% DHA, or 0.5% AA + 0.5% DHA. Supplementation of either PUFA diet resulted in the upregulation of the key gluconeogenic enzyme phosphoenolpyruvate carboxykinase in rat liver. Although expression of PPAR- α and c-AMP signaling were not modified by feeding PUFA, authors speculated that the higher expression of phosphoenolpyruvate carboxykinase may be mediated with an overall effect on limiting fat accumulation and shunting metabolic flux to gluconeogenesis.

Unlike the demonstrated effect of PUFA to enhance gluconeogenesis in rats, other studies have documented PUFA to have a negative effect on gluconeogenesis in cultured bovine hepatocytes. Gluconeogenesis activity was measured through the synthesis of glucose using propionate as a precursor. Mashek et al. (2002) measured glucose production in hepatocytes from weaned ruminating calves treated first with 1mM of C16:0 and then additionally added either 1mM of C16:0, C18:1, C18:2, C18:3, C20:5, or C22:6. Hepatocytes treated with C18:1 produced more glucose from added propionate than those produced by adding C20:5 or C22:6, even though all three LCFA were reported as inducing greater oxidation. Later Mashek and Grummer (2003) tested the same set of FA but used hepatocytes from preruminant calves. At this time, only C22:5 affected gluconeogenesis from propionate and that was to decrease it. Finally Mashek and Grummer (2004) used monolayer cultures of hepatocytes from preruminant calves treated with 1 mM of C16:0 and supplemented them with 0.1 or 1 mM of LA, CLA α 9 β 11, or CLA α 10 β 12. Regardless of FA concentrations, the type of FA did not affect propionic acid metabolism to produce glucose, cellular glycogen or the combination of both. Regardless of the type of FA, the formation of both glucose and glycogen were decreased when FA concentrations increased from 0.1 to 1.0 mM.

Regulation of Bile and Hepatic Cholesterol

Bile acids are amphipathic molecules derived from cholesterol in the liver. Its synthesis generates bile flow from the liver to the intestine. Bile acids facilitate biliary excretion of cholesterol, endogenous metabolites, and xenobiotics in addition to their function in intestinal absorption of lipids and nutrients. The liver has a critical role in maintaining cholesterol homeostasis by balancing multiple pathways such as *de novo* cholesterol and bile acid synthesis, dietary cholesterol uptake, biliary cholesterol

excretion, lipoprotein synthesis, and reverse cholesterol transport (Li and Chiang, 2009). Transcription factors closely related with bile and cholesterol metabolism in liver are SREBP, HNF-4 α , FXR, and PPAR- α . The latter is the most diversified target gene of PUFA. Since PPAR have a regulatory effect on the former transcription factors, so do the PUFA have a regulatory effect on bile and cholesterol metabolism.

The HNF-4 α is known for its activity in stimulating cholesterol 7 α -hydroxylase (CYP7A1), which is a rate-limiting enzyme in the conversion of cholesterol to bile acids in liver. PPAR agonists were evaluated for their potential to reduce the activation of CYP7A1 using HepG2 cells through luciferase reporter activities (Marrapodi and Chiang, 2000). The heterodimer PPAR- α /RXR α did not prevent the binding of HNF-4 α to CYP7A1. However, it significantly reduced the expression of HNF-4 α by binding the HNF-4 α to a conserved sequence in the PPAR- α response element, which is the binding site for HNF-4 α . This prevented the transactivation of CYP7A1 by HNF-4 α (Marrapodi and Chiang, 2000).

Lower levels of sterols are sensed by the SREBP- cleavage activating protein (SCAP). This protein aids to the maturation of the SREBP, which upon translocation to the nucleus, bind to promoters of SREBP in target genes related to synthesis and metabolism of cholesterol. When levels of cholesterol are increased, the SREBP cleavage-activating protein complex is retained in the endoplasm reticulum to stop the maturation/activation of SREBP (Bengoechea-Alonso and Ericsson, 2007). Bile acids are physiological ligands for FXR as are PUFA. A study has revealed that the downregulation of CYP7A1 by FXR did not require binding to DNA, suggesting a potential indirect effect (Castillo Olivares and Gil, 2000). FXR also inhibits the entry of

intestinal bile acids into hepatocytes by repressing the expression of hepatic bile acid uptake transporters (Niu et al., 2011).

Regulation of Inflammation and Immune Response

The role of FA in regulation of gene expression within the immune cells can be done through different mechanisms that include effects on receptor activity, on intracellular signaling process, or on transcription factor activation (Calder, 2008). Changes in FA profile of membrane phospholipids might be expected to influence immune cell function in a variety of ways such as 1) alteration of the physical property of the membrane such as membrane fluidity and lipid raft conformation, 2) effects on cell signaling pathways either through modifying the expression, activity, or avidity of membrane receptors, modifying intracellular signaling transduction mechanisms, modifying transcription factor activation and then gene expression, 3) alteration in the production pattern of lipid mediators that have different biological functions (Calder, 2008).

Bouwens et al. (2009) evaluated the supplementation of FA to human subjects fed one of three diets: 1) 1.8 g of EPA + DHA, 2) 0.4 g of EPA + DHA, or 3) SAO (79% OA, % of total FA). The oils (900 mg of oil/d) were fed in capsules on a daily basis for 26 wk. Microarray data from PBMC RNA (pretreatment baseline was the reference for each treatment group) resulted in PBMC from subjects fed the highest dose of EPA+DHA having significant decreases in the expression of genes involved in inflammatory pathways such as eicosanoid synthesis, interleukin signaling, mitogens activated protein kinase signaling, NFkB toll like receptor signaling, oxidative stress, cell adhesion, PPAR signaling, LXR/RXR activation and hypoxia signaling. Interestingly, the group fed SAO (rich in OA) also had downregulated genes involved in different

pathways of inflammation (80% of overlapping pathways as in the high EPA + DHA diet) as well as all the same pathways related to cell adhesion. Unexpectedly, expression of PPAR- α and some of its target genes were also downregulated in PBMC of humans fed the high EPA + DHA diet.

Effect on Oxidative Phosphorylation

Oxidative phosphorylation is the culmination of the energy-yielding metabolism in aerobic organisms. All oxidative steps in the degradation of carbohydrates, fats and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP (Nelson and Cox, 2008). The major components of the mammalian system of oxidative phosphorylation are the four complexes of the respiratory chain, NADH:ubiquinone reductase (complex I), succinate:ubiquinone reductase (complex II), ubiquinol:cytochrome c reductase (complex III), cytochrome c oxidase (complex IV), and F_1F_0 -ATP synthase (complex V) (Schagger and Pfeiffer, 2001). This mechanism is critical to provide of ATP for different metabolic processes.

Summary

The first strategic feeding of FA was to increase the energetic density of diets. However, the studies of Burr and Burr (1929, 1930) determined the essentiality of LA and ALA. Strategic feeding during prepartum and preweaning period are the most influential periods affecting future animal performance. The newborn calf is born deprived of Ig, with a naive immune system, hence ensuring APT is critical for the newborn calf to cope with environmental pathogens as it starts “building up” the capacity of calf’ adaptive system after first and subsequent encounter with different pathogens. Future research should be oriented to optimize calf nutrition by strategic

supplementation of critical nutrients to boost animal immune response, preventing risk of disease, hence optimizing growth and overall efficiency.

Table 2-1. Common fatty acids terminology [Adapted from O’Keefe, 2002. Nomenclature and classification of lipids. Chemistry and properties. Chapter 1 in: Foods Lipids: Chemistry, Nutrition and Biotechnology Marcel Dekker (Pages 21 and 24, tables 4 and 5). Inc., New York, USA].

Systematic name ^a	Common Name	Shorthand ^b
Saturated Fatty Acids		
Dodecanoic	Lauric	12:0
Tridecanoic	—	13:0
Tetradecanoic	Myristic	14:0
Pentadecanoic	—	15:0
Hexadecanoic	Palmitic	16:0
Heptadecanoic	Margaric	17:0
Octadecanoic	Stearic	18:0
Nonadecanoic	—	19:0
Eicosanoic	Arachidic	20:0
Docosanoic	Behenic	22:0
Unsaturated Fatty Acids		
c-9-Hexadecenoic	Palmitoleic	16:1 n-7
c-9-Octadecenoic	Oleic	18:1 n-9
c-9,c-12-Octadecadienoic	Linoleic	18:2 n-6
c-9,c-12,c-15-Octadecatrienoic	Linolenic	18:3 n-3
c-6,c-9,c-12-Octadecatrienoic	alpha –Linolenic	18:3 n-6
c-8,c-11,c-14-Eicosatrienoic	Dihomo-gamma-linolenic	20:3 n-6
c-5,c-8,c-11,c-14-Eicosatrienoic	Arachidonic	20:4 n-6
c-5,c-8,c-11,c-14,c-17-Eicosapentaenoic	EPA	20:5 n-3
c-7,c-10,c-13,c-16,c-19-Docosapentaenoic	DPA	22:5 n-3
c-4,c-7,c-10,c-13,c-16,c-19-Docosahexaenoic	DHA	22:6 n-3

^a c-x is the double bounded carbon atom in *cis* configuration and x is the number of that carbon atom counting from the carboxyl end.

^b Number of carbon atoms : number of double bonds. For unsaturated fatty acids, n-x indicates the first double bonded carbon counting from the methyl end.

Table 2-2. Fatty acid composition (% of total fatty acids) of major sources of fatty acids in dairy cattle

sources	Total FA ¹	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4	C20:5	C22:5	C22:6
Vegetable oils ²												
Palm	88.4	0.4	1.1	43.8	4.4	39.1	10.2	0.3	-	-	-	-
Coconut	85.0	48.2	18.5	8.7	2.7	6.0	1.5	0.1	-	-	-	-
Safflower	88.9	-	-	6.1	2.3	13.4	76	0.3	0.5	0.5	-	-
Canola	88.9	-	0.1	5.1	1.7	60.1	21.5	9.9	-	-	-	-
Linseed oil	88.8	-	0.1	5.5	3.7	19.3	16.2	53.4	-	-	-	-
Cottonseed	88.7	-	0.8	24.2	2.3	17.4	53.2	0.2	-	-	-	-
Corn	88.8	-	-	12.3	1.9	27.7	56.1	1.0	-	-	-	-
Soybean	88.8	-	0.1	10.8	3.9	23.9	52.1	7.8	-	-	-	-
Sunflower	88.9	0.5	0.1	6.4	4.5	22.1	65.6	0.5	-	-	-	-
Animal fats and blends												
Tallow ³	88.7	-	3.0	25.1	19.7	42.1	3.0	0.3	-	-	-	-
Yellow grease ⁴	88.6	0.2	1.0	21.3	6.1	41.5	21.4	1.4	-	-	-	-
Fish oil ⁵	90.5	-	8.3	16.9	3.2	10.3	1.5	2.1	0.9	13.2	2.4	12.5
Lard ⁶	-	-	1.7	30.2	22.6	26.1	12.1	1.2	-	-	-	-
Commercial fats												
Megalac ⁷	82.5	1.4	3.1	47.4	4.6	34.7	5.5	0.2	-	-	-	-
Megalac R ⁷	82.5	1.0	1.9	32.4	5.0	23.4	30.5	3.1	-	-	-	-
Energy booster 100 ⁵	98.0	-	2.9	29.1	55.3	6.3	0.3	-	-	-	-	-

¹ Calculated with the corresponding fatty acid composition, except for commercial fats (manufacturer claims).

² Dubois et al., 2007, except for linseed oil (Sterk et al., 2010).

³ Onetti et al., 2002.

⁴ Avila et al., 2000.

⁵ Ballou et al., 2009.

⁶ Huuskonen et al., 2005.

⁷ Theurer et al., 2009.

CHAPTER 3
EFFECT OF SUPPLEMENTAL ESSENTIAL FATTY ACIDS TO PREGNANT HOLSTEIN
COWS ON COLOSTRUM FATTY ACID PROFILE AND CALF PASSIVE IMMUNITY

Background

Attaining an appropriate growth rate and health performance of dairy calves before weaning that would allow to double the birth weight by weaning period and minimize the incidence of diseases is one of the primary goals of dairy herd management. Dairy farmers must manage health challenges once the calf is born (Beam et al., 2009; Donovan et al., 1998). Therefore to minimize the outbreak of calf diseases and not jeopardize the profitability of the herd, immediate and effective care of the newborn calf should occur right after birth by effective feeding of colostrum of good concentration of immunoglobulin G (IgG > 50 g/L) in order to ensure APT.

The transfer of immunoglobulins (Ig) from the dam to the neonate is termed passive transfer. With the exception of ruminants, transfer of Ig begins in the fetal period (Weaver et al., 2000). Therefore the newborn calf is completely dependent on the supply of Ig from colostrum because the epitheliochorial placenta of cows prevents transfer of Ig during the fetal period (Kehoe and Heinrichs, 2007). Establishment of APT is crucial to reduce neonatal morbidity and mortality, and strengthen calf immunity (Quigley and Drewry, 1998; Donovan et al., 1998). Moreover APT has been associated with improved weaning and postweaning body weight (BW; Robison et al., 1988) and with greater milk production (DeNise et al., 1989).

Colostrum is rich in Ig, particularly IgG which accounts for 85 to 90% of total Ig. Transportation of the pool of IgG reaching the intestine across intestinal epithelium initially was assumed to occur by non-selective pinocytosis (Klaus et al., 1969; Jones and Waltman, 1972). However later studies discovered the existence of specific Ig

receptors known as neonatal Fc receptor (FcRn) present in intestinal epithelium (Israel et al., 1997). The FcRn was initially identified in human epithelial cells of intestine, suggesting its involvement in IgG binding and transfer of passive immunity (Israel et al., 1997). A potential protective mechanism of FcRn in favor of circulating IgG that prevents its premature degradation and clearance from circulation has been recently hypothesized (Goebel et al., 2008). Fatty acid profile of enterocyte cell membrane tends to reflect that of the diet; hence greater supplementation of PUFA might change the fluidity of membrane and expression of receptors.

In addition to Ig, colostrum has been documented to contain significant concentrations of different growth factors (Georgiev, 2008b; Blum and Baumrucker, 2008). Compared to colostrum-deprived calves, calves fed colostrum exhibited an enhanced epithelial cell proliferation as evidenced by greater villous circumference, area, and height (Buhler et al., 1998). Later studies verified the positive benefits of insulin-like growth factor-I (IGF-I) present in colostrum on development of the intestinal tract but the benefit was lacking when IGF-I was administered orally or parenterally (Roffler et al., 2003; Georgiev et al., 2003). However, studies evaluating the effect of maternal diet manipulation on concentration of growth factors in colostrum and their transfer to the newborn are scarce.

Limited studies have evaluated the effect of feeding fat supplements to cows on fatty acid (FA) composition of colostrum and most of them did not include the effect of parity. However, few studies using dairy cows and ewes supplemented with CLA have reported not effect of parity in total CLA (Kelsey et al., 2003; Tsiplakou et al., 2006). However, Mierlita et al. (2011) when comparing effect of 3 ewes' breeds reported that

primiparous ewes produced greater proportion of LA, GLA, ALA, EPA and total CLA. Moreover the few studies performed with cows, regardless parity consideration have focused on supplementation of n-3 or CLA FA instead of n-6 FA.

The hypothesis of this study was that supplementing dam diets with LA modifies the FA profile of colostrum and really improves efficiency of IgG absorption. Therefore the objective was to evaluate the effect of supplementing Ca salts of FA enriched with LA and ALA to Holstein cattle in late gestation on colostrum FA profile and production and transfer of total and specific IgG. An additional goal was to evaluate the change in serum concentrations of insulin and IGF-I in calves after colostrum feeding.

Materials and Methods

Experimental Design and Dietary Treatments

The experiment was conducted at the University of Florida's dairy farm (Hague, FL) from October 2008 to June 2009. All procedures for animal handle and care were approved by the University of Florida's Animal Research Committee. Pregnant nulliparous (n = 28) and previously parous (n = 50) Holstein cattle were sorted according to calving date, parity, BW, and body condition score (BCS) and assigned to one of three treatments at 8 wk before their expected calving date.

Dietary treatments were the following: no fat supplementation (Control), 1.7% of dietary dry matter (DM) of mostly free saturated FA (SFA, "Energy Booster 100", Milk Specialties, Dundee, IL), and 2.0% of dietary DM as Ca salts of FA enriched with EFA, "Megalac R", Church and Dwight, Princeton, NJ). The control diet was formulated to have low concentrations of total FA and EFA, whereas SFA and EFA diets were isoenergetic and all diets were isonitrogenous (Table 3-1). Proportions of unsaturated FA were minimal in the SFA supplement compared to the EFA supplement (Table 4-2).

During the first 4 wk of the experimental period (-8 to -4 d relative to calving), cows were housed in a sod-based pen and fed as groups according to the dietary treatments. At 4 wk before the expected calving date, cows were moved to a sod-based pen equipped with Calan gates (American Calan Inc., Northwood, NH) and daily DM intake (DMI) was measured. Cows were weighed using a digital scale at 8 and 4 wk before the expected calving date and at calving. At the same time, BCS was determined using a 5-point scale (from 1 meaning extremely skinny to 5 meaning obese) divided into 0.25 points using the Elanco Animal Health BCS chart (Elanco, 1996).

Prepartum Body Weight, Feed Intake and Analyses

Prepartum diets were prepared as a total mixed ration and offered once daily (1000 h). Feed offered was adjusted daily to achieve 5 to 10%orts. Orts were collected and weighed daily. A bermudagrass silage sample was collected once a week and analyzed for DM by drying in a forced-air convection oven (American Scientific, LLC, Model DN-41) at 55°C for 48 h or until constant weight, in order to maintain the formulated DM ratio of forage to concentrate (56:44, DM basis). Dried silage and hay samples (collected once weekly) were ground to pass through a 1-mm screen using a Wiley Mill (Arthur H. Thomas, Co, Philadelphia, PA). Samples of concentrate mixtures were collected once weekly and composited monthly. Forages and concentrates were analyzed for ash (600°C for 2 h, AOAC, 2000), and neutral (NDF) and acid detergent fiber (ADF) according Van Soest (1991) using an ANKOM 200 Fiber Analyzer (ANKOM, Macedon, NY). Heat stable α -amylase and sulfite were used in the NDF assay. Nitrogen concentration was determined using a Vario MAX CN Macro Elementar Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) by the Dumas combustion method (AOAC, 2000) and protein concentration was calculated as N x 6.25.

Concentrations of FA in prepartum diets were estimated based on available composition of FA in individual ingredients whereas estimated intake of LA per cow was estimated using the CPM dairy FA submodel. Energy intake during prepartum was calculated based on the DMI and estimation of the energy concentration of diets by the NRC (2001) model. The last 14 d before calving were used for calculation of DMI.

Prepartum Ovalbumin Challenge and Assay for Bovine Anti-OVA IgG

Cows were injected subcutaneously (s.c.) with 1 mg of OVA (Sigma Aldrich, Saint Louis, MO) diluted in Quil A adjuvant solution (0.5 mg of Quil A in 1 mL of PBS – Accurate Chemical & Scientific Corp., Westbury, NY) using sterile procedures upon study enrollment (-60 d relative to expected calving date), and again 30 d after the first injection. A blood sample (10 mL) was collected just prior to each vaccination with OVA and at calving. Blood samples were collected in a tube without anti-coagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and serum was separated at room temperature, followed by 15 min of centrifugation (2095 x *g*, Allegra X-15R centrifuge, Beckman Coulter, Inc).

Serum concentration of bovine anti-OVA IgG was measured by an enzyme-linked immunosorbent assay (ELISA) as described by Mallard et al. (1997). Positive and negative control sera to bovine anti-OVA IgG were obtained from a pool of sera of known high (sera of cows 1 wk after second OVA injection) and low (sera of cows never exposed to OVA) concentrations of OVA, respectively. All samples from the same cow or calf were analyzed in the same plate. All plates contained a balanced number of animals from each diet. Results were corrected by dividing the experimental sample by the positive control at the same specific dilution. Results of each dilution were averaged

and the average of 2 dilutions was reported. Intra- and inter-assay coefficients of variation were 9.2 and 9.7%, respectively.

Calving Management

Calves were born from December 24th, 2008 through April 5th, 2009. Pregnant cattle gave birth to calves in a sod-based pen. All cows were monitored for signs of calving initiation every 30 min between 0530 to 1530 h and then every 2 h between 1530 and 0530 h. Ease of calving was scored according to Sewallem et al. (2008) as unassisted (1), easy pull (2), hard pull (3), and surgery (4). Within 2 h of birth calves were weighed, ear-tagged, and the umbilical cord was disinfected with 10% Betadine solution (Purdue Frederick Co., Norwalk, CT). Calves were temporarily housed in individual hutches (1 x 1 m) equipped with a heat lamp and finally moved to individual wire hutches (1 x 1.5 m) when they were between 6 to 16 h of age.

Colostrum Feeding and Analyses

Within 2 h of birth, cows were milked with a cow-side vacuum pump. Colostrum quality was recorded using a colostrometer. Immediately after weighing, calves were given 4 L of colostrum from their own dam regardless of IgG concentration using an esophageal feeding tube. When an animal did not produce sufficient colostrum for her calf, colostrum from another animal fed the same treatment was used to feed that calf. Remnant colostrum (> 1 L having IgG concentration > 50 g/L) after calf feeding was stored (-4°C).

A sample of colostrum (10 mL) from each dam was collected to determine concentration of bovine total IgG by single radial immunodiffusion (VMRD Inc., Pullman WA). Colostrum samples were diluted 1:5 with double distilled water. Diluted samples (3 µL) were applied to serial radial immunodiffusion plates containing agarose gel with

anti-bovine IgG. Plates containing the samples were left undisturbed for 23 h at room temperature. Resulting ring diameters were measured with a monocular comparator (VMRD Inc., Pullman WA). A standard curve was plotted with reference sera (4, 8, 16 and 32 g/L of IgG) supplied by the manufacturer. Concentrations of IgG in diluted samples were read from the standard curve and correction for the dilution factor was applied afterwards. Intra- and inter-assay variations were 3.0 and 3.3%, respectively.

A colostrum sample from each dam (~100 mL) was freeze dried (Labconco Kansas City, MO) and delivered to Michigan State University for analysis of FA. Briefly total FA from freeze-dried colostrum samples were extracted using the method of Hara and Radin (1978). Fatty acid methyl esters (FAME) were prepared by base-catalyzed transmethylation (Christie, 1989). The FAME were quantified using a GC-2110 Plus gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector (1:100 split ratio) and a flame ionization detector using a CP-Sil 88 WCOT fused silica column (100 m × 0.25-mm i.d. × 0.2- μ m film thickness; Varian Inc., Lake Forest, CA). Gas chromatographic conditions were described by Kramer et al. (2001). The FAME were identified by comparison of retention times with known FAME standards (Supelco 37 component FAME mix, cis/trans FAME mix, bacterial acid methyl ester mix, and polyunsaturated FA No. 3 mix from Supelco Inc., Bellefonte, PA; GLC reference standard 463 and conjugated LA (CLA) mixture #UC-59 M from Nu-Chek Prep, Elysian, MN). Short-chain FAME were corrected for mass discrepancy using the correction factors published by Ulberth and Schrammel (1995).

Blood Collection for Measures of Immunoglobulin and Protein Concentration

Calf blood was collected via jugular venipuncture before colostrum feeding and again between 24 to 30 h after colostrum feeding. Blood samples were collected in a

tube without anti-coagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), and serum was separated at room temperature followed by 15 min of centrifugation at 2095 x g (Allegra X-15R centrifuge, Beckman Coulter, Inc). Serum total protein (STP) concentrations were determined using an automatic temperature-compensated hand refractometer (Reichert Jung; Cambridge Instruments Inc. Buffalo, NY). Serum total IgG concentrations were measured in serum diluted 3:4 with distilled water. Final concentrations of IgG were obtained from the curve plotted with the standards provided by the manufacturer as described in the previous section for colostrum IgG analysis.

In order to test the maternal transference of a specific IgG by feeding of colostrum, serum of calves at 0 h (before feeding colostrum) and at 2 d of age were analyzed for bovine anti-OVA IgG using an ELISA procedure as described by Mallard et al. (1997). Details of the procedure were described in a previous section for prepartum cattle. Intra- and inter-assay coefficients of variation were 8.8 and 11.7%, respectively.

Concentrations of insulin and IGF-I were analyzed in sera samples at 0 and 24 to 30 h to verify their transfer from colostrum feeding. Concentration of IGF-I was analyzed following the manufacturer's protocol (Active nonextraction IGF-I ELISA, Diagnostic Systems Laboratory, Inc.) with some modifications in sample pre-treatment. Releasing IGF-I from their binding proteins was done with half of the indicated volumes for sample pre-treatment reagents to maintain the final suggested dilution of samples (1:30). A control sample was run in duplicate in each plate. The intra-plate variation for IGF-1 of control samples was 2.4%, whereas the inter-plate variation was 3.2%. Insulin concentrations were analyzed using a double antibody radioimmunoassay (Badinga et

al., 1991) in serum samples collected at 0 and 24 h of life. Intra- and inter-assay variations were 7.3 and 14.6%, respectively.

Estimation of Appropriate Passive Transfer and Efficiency of IgG Absorption

Calves were considered to have an APT if serum concentration of total IgG was ≥ 1 g/dL after 24 h of colostrum feeding (Tyler et al., 1996; Weaver et al., 2000). The apparent efficiency of IgG absorption (AEA, %) was calculated according to Quigley and Drewry (1998) assuming that serum volume was 9.9% of calf BW (Quigley et al., 1998) using the following equation: (IgG concentration in serum at 24 h of life in g/L \times [0.099 \times BW (kg) at birth]) \div IgG intake in grams. Additionally, STP concentrations ≥ 5.0 g/dL was used as an indicator of APT (Donovan et al., 1998; Calloway et al., 2002).

Statistical Analysis

The experiment had a block randomized design. On a weekly basis, a cohort of cows at 8 wk before the expected calving date was blocked by parity (nulliparous and parous) and BCS and, within each block, randomly assigned to one of three treatments. Test of block in the model was not significant and thus was deleted. Dependent variables with more than one observation within experimental unit were analyzed as repeated measures using the mixed procedure of SAS 9.2 (SAS Institute, 2009). Repeated measure data were tested to determine the structure of best fit, namely compound symmetry, compound symmetry heterogeneous, autoregressive-1, and autoregressive-1 heterogeneous as indicated by a Schwartz Bayesian information criteria value closest to zero (Littell et al., 1996). For the analysis of serum bovine anti-OVA IgG in cows, the measurement determined at 8 wk before expected calving day was used as a covariate. Cow nested within treatment and parity was used as a random term. The following model was used:

$$Y_{ijkl} = \mu + T_i + P_j + (TP)_{ij} + CL_{(ij)} + D_l + (TD)_{il} + (PD)_{jl} + (TPD)_{ijl} + E_{ijkl}$$

Where: Y_{ijkl} = dependant variable; μ = overall mean; T_i = fixed effect of treatment i (control, SFA, and EFA); P_j = effect of parity j (nulliparous and parous); $(TP)_{ij}$ = effect of treatment by parity interaction; $CL_{(ij)}$ = random effect of cow nested within treatment and parity ($k = 1, 2, 3, \dots, n$); D_l = effect of day relative to calving ($l = -60, -59, \dots, 0$); $(TD)_{il}$ = effect of treatment by day interaction; $(PD)_{jl}$ = effect of parity by day interaction; $(TPD)_{ijl}$ = effect of treatment by parity by day interaction; E_{ijkl} = residual error.

For nonrepeated measures regarding dams, the preceding model was used after removing day and interactions with day. Calf variables were analyzed using nonrepeated measures analysis using the mixed procedure of SAS 9.2 (SAS Institute, 2009). Calf nested within treatment and parity was a random term. The statistical model for the analysis was the following:

$$Y_{ijkl} = \mu + T_i + P_j + (TP)_{ij} + CL_{(ij)} G_k + (TG)_{ik} + (PG)_{jk} + (TPG)_{ijk} + E_{ijkl}$$

Where: Y_{ijkl} = dependant variable; μ = overall mean; T_i = fixed effect of treatment i (control, SFA, and EFA); P_j = effect of parity j (nulliparous and parous); $(TP)_{ij}$ = effect of treatment by parity interaction; $CL_{(ij)}$ = random effect of calf nested within treatment and parity ($k = 1, 2, 3, \dots, n$); G_k = effect of gender (male and female); $(TG)_{ik}$ = effect of treatment by gender interaction; $(PG)_{jk}$ = effect of parity by gender interaction; $(TPG)_{ijk}$ = effect of treatment by parity by gender interaction; and E_{ijkl} = residual error.

All variables were tested for normality of residuals using the Shapiro-Wilk test (SAS version 9.2, SAS Inst. Inc., Cary, NC). Non-normally distributed data were transformed as suggested using the guided data analysis of SAS and back transformed using the LINK and ILINK function of GLIMMIX procedure respectively. Temporal

responses to treatments were further examined using the SLICE option of the MIXED or GLIMMIX procedure.

Appropriate orthogonal contrasts were performed for dam variables [1) fat supplement = FAT (SFA + EFA) vs. no fat, 2) FA supplement = FA (EFA vs.SFA), 3) effect of parity, 4) contrast 1 by parity interaction, and 5) contrast 2 by parity interaction]. Additional contrasts for calf variables included gender interactions with each of the above contrasts. If any 3-way interaction or the interaction of gender by parity were not significant ($P > 0.25$), the interaction was dropped from the model and the new model was rerun (Bancroft, 1968). Coefficients of correlation were estimated using the CORR procedure of SAS (SAS Institute 2009) to describe the relationships between and within cow and calf variables. Differences discussed in the text were significant at $P \leq 0.05$ and tended to be significant at $0.05 < P \leq 0.10$.

Results

Prepartum Cow Performance

Seventeen of the enrolled dams did not have sufficient days in Calan gates so intake data is provided for 61 cattle. Intake was stable until the last 1 to 3 d at which time DMI decreased markedly (effect of day, $P < 0.01$, Figure 3-1). As expected both DMI (11.8 vs. 10.0 kg/d) and net energy of lactation intake (17.3 vs. 14.7 Mcal/d) were greater in parous cows compared to nulliparous heifers (effect of parity, $P < 0.01$, Table 3-3). Neither feeding fat prepartum nor the type of fat affected DMI. Intake of DM prepartum was correlated positively with gestation length ($r = 0.31$, $P = 0.01$, Table 3-7) and with BW change during last 8 wk prepartum ($r = 0.58$, $P < 0.01$).

Concentrations of serum anti-OVA IgG increased with increased number of injections of OVA as expected (effect of day, $P < 0.01$, Figure 3-2). Parities responded

in a like manner to OVA injections. Throughout the prepartum period, cattle fed SFA had greater mean concentration of serum anti-OVA IgG than cattle fed EFA (0.65 vs. 0.45 OD, $P = 0.02$, Table 3-3).

Holstein cattle ($n = 78$) consumed their assigned diets for a mean of 56 d and this did not differ among dietary treatments or parities (Table 3-3). Body weight and BCS at enrollment were similar for cattle on all diets with means of 616 kg and 3.41, 610 kg and 3.31, and 616 kg and 3.31 for BW and BCS for cattle fed control, SFA, and EFA, respectively (Table 3-3). As expected, at enrollment nulliparous heifers weighed less than parous cows (527 vs. 701 kg, $P < 0.01$) but BCS did not differ (3.36 vs. 3.35). However at calving, parous cows fed the control diet tended to have a greater mean BCS than parous cows fed fat (3.51 vs. 3.40) whereas BCS of nulliparous heifers fed fat tended to have a greater BCS compared to those not supplemented with fat (3.40 vs. 3.31, FAT by parity interaction, $P = 0.10$, Table 3-3). However BW gain between enrollment and calving was not affected by dietary treatment and did not differ between parities (mean of 54.3 kg). Length of gestation was shorter for nulliparous heifers compared to parous cows (275 vs. 278 d, $P < 0.01$) but was not affected by feeding fat. In general, mean value for calving score was low because cattle that had calving scores greater than 2 were not enrolled in order to avoid confounding effects of prepartum diets with stress at calving on calf measures. Nevertheless nulliparous heifers fed the control diet had a greater mean calving score compared to those fed fat (1.25 vs. 1.00) whereas calving score of parous cows did not differ due to fat feeding (1.06 vs. 1.09, FAT by parity interaction, $P = 0.04$).

Immunoglobulin G Concentration and Fatty Acid Profile of Colostrum

Of the 78 enrolled Holstein cattle, only 70 cows produced colostrum. Volume of colostrum produced was not affected by diets but nulliparous heifers produced less colostrum (3.6 vs. 7.0 kg, $P < 0.01$, Table 3-3). Total IgG concentration in colostrum were greater in nulliparous heifers fed the control diet vs. fat supplemented diets (102 vs. 83 g/L) but the dietary effect was the opposite in colostrum from parous cows (96 vs. 115 g/L, FAT by parity interaction, $P = 0.05$).

Total concentration of FA in colostrum was not affected by fat source or parity and averaged 6.9 g/100 g of DM (Table 3-4). Parity had a marked effect on proportion of individual and groups of FA in total colostrum FA. Proportions of FA $<$ or $>$ C16:0 were greater in nulliparous heifers (20.3 vs. 17.7% and 43.6 vs. 39.4% of total FA for $<$ and $>$ C16:0, respectively, $P \leq 0.01$). On the other hand, proportion of C16 (C16:0 and C16:1) was greater for parous cows compared to nulliparous heifers (42.6 vs. 35.8% of total FA, $P < 0.01$). The proportion of total SFA, monounsaturated FA (MUFA), and n-6 FA were not different between parities. However total polyunsaturated FA (PUFA, 4.61 vs. 4.02% of total FA, $P < 0.01$), total CLA (0.32 vs. 0.19% of total FA, $P < 0.01$), total branched FA (1.36 vs. 0.97% of total FA, $P < 0.01$), total C18:1 *trans* FA (2.22 vs. 1.46% of total FA, $P < 0.01$), and total n-3 FA (1.00 vs. 0.54% of total FA, $P < 0.01$) were all greater in nulliparous heifers compared to parous cows. Although many FA tested significant for the effect of FAT, the effect was mainly due to the feeding of EFA vs. SFA; hence feeding fat prepartum had minimal effects on proportions of FA in colostrum. Proportions of C14:1 (0.54 vs. 0.41%, % of total FA, $P = 0.01$) and C16:1 (1.88 vs. 1.63%, % of total FA, $P < 0.01$) were decreased whereas that of C18:0 was increased (8.4 vs. 9.6%, % of total FA, $P < 0.01$) by fat feeding.

Both parities fed EFA as compared with those fed SFA produced colostrum with greater proportions of LA (3.35 vs. 2.31% of total FA, $P < 0.01$) and C20:2 n-6 (0.04 vs. 0.02% of total FA, $P < 0.01$). The other n-6 FA were increased by supplementing EFA only in colostrum from nulliparous heifers (0.61 vs. 0.54% for AA, 0.33 vs. 0.28% for C20:3 n-6, and 0.13 vs. 0.10% for C22:4) but not from parous cows (0.39 vs. 0.43% for AA, 0.24 vs. 0.27% for C20:3 n-6; 0.08 vs. 0.08% for C22:4; FA by parity interaction, $P \leq 0.03$). Total proportions of n-6 FA were greater in colostrum from cattle fed EFA compared to those from cattle fed SFA (4.31 vs. 3.21% of total FA, $P < 0.01$) with LA accounting for approximately 75% of the total n-6 FA.

Proportions of individual n-3 FA were affected minimally by diets. Specifically, ALA, C20:3 n-3, and DHA did not differ. Cattle fed EFA had lower proportions of eicosapentaenoic acid (EPA) than those fed SFA (0.08 vs. 0.10 % of total FA, $P < 0.01$). All seven identified C18:1 *trans* FA were greater or tended to be greater in colostrum from cattle fed EFA compared to those fed SFA. Hence, sum of all individual C18:1 *trans* FA were greater in colostrum from cattle fed EFA compared to those fed SFA (2.06 vs. 1.58% of total FA, $P < 0.01$). Similarly, both of the identified CLA (c9, t11 CLA and t10 c12 CLA) were also greater in EFA-fed cattle (0.33 vs. 0.21% sum of CLA of total FA, $P < 0.01$).

Transfer of IgG and Hormones by Feeding of Colostrum

Calves born from parous cows were heavier than those born from nulliparous heifers (42.4 vs. 36.8 kg, $P < 0.01$, Table 3-5). Also, as expected, males were heavier than females at birth (41.0 vs. 38.2 kg, $P = 0.02$, data not shown). Males born from cattle fed SFA tended to be heavier than males born from cattle fed EFA (43.2 vs. 39.6 kg) whereas birth weight of females did not differ (38.3 vs. 39.7 kg; FA by gender

interaction, $P = 0.06$, Figure 3-3). Calves were fed the same amount of colostrum (4 L). Hence intake of IgG by calves reflects the concentration of IgG in the colostrum they consumed.

Calves born from nulliparous heifers fed the control diet consumed more IgG than calves born from nulliparous heifers fed fat (410 vs. 340 g of IgG) whereas calves born from parous cows fed fat consumed more IgG than calves born from parous cows fed the control diet (459 vs. 383 g of IgG; FAT by parity interaction, $P = 0.04$). Serum total protein at birth (mean of 4.77 g/dL) and after colostrum feeding (mean of 5.81 g/dL) did not differ due to diet fed prepartum nor to parity. Concentration of IgG in colostrum was correlated positively with STP measured in serum of calves at 24 to 30 h after colostrum feeding ($r = 0.50$, $P < 0.01$).

Serum concentration of total IgG at birth was low but, tended to be greater in males born from dams fed the control diet than in males born from dams fed fat whereas females showed the opposite effect (Figure 3-4 A, FAT by gender interaction, $P = 0.09$). Contrary, serum concentration of total IgG at 24 to 30 h after feeding of colostrum was greater in males born from cows fed fat as compared to those males born from cattle fed control diet (2.78 vs. 2.03 g/dL) whereas that of females did not differ due to diet (FAT by gender interaction, $P = 0.03$, Figure 3-4 B). Concentration of IgG in colostrum was not correlated with calf serum concentration of IgG at birth ($r = 0.02$, $P = 0.89$, Table 3-7) but was positively correlated with serum IgG after colostrum feeding ($r = 0.54$, $P < 0.01$). In addition, a strong positive correlation existed between serum concentrations of total IgG and STP measured in calves 24 to 30 d after feeding of colostrum ($r = 0.81$, $P < 0.01$).

Regardless of gender, calves born from dams fed SFA tended to have greater concentrations of serum total IgG, after 24 to 30 h of colostrum feeding, than those born from dams fed EFA (2.83 vs. 2.44 g/dL, $P = 0.07$, Table 3-5). This trend became significant when total serum IgG was expressed as a proportion of STP (43.5 vs. 38.2%, $P = 0.05$). Concentrations of a specific IgG (i.e. anti-OVA IgG at 24 to 30 h after colostrum feeding) followed the same pattern; that is, calves born from dams fed SFA had greater serum concentrations of anti-OVA IgG compared to dams fed EFA (1.13 vs. 0.90 OD, $P = 0.01$). The AEA of IgG consumed did not differ between calves born from dams fed SFA or EFA but these calves, as a group, had a better AEA than calves born from dams fed the control diet (27.9 vs. 23.4 %, $P = 0.03$, Table 3-5). Males were more efficient in absorbing IgG than females (28.6 vs. 24.1%, $P = 0.02$, data not shown). The AEA was correlated positively with serum concentrations of total IgG ($r = 0.42$, $P < 0.01$) and STP ($r = 0.24$, $P = 0.03$, Table 3-7) in calves at 24 to 30 h after colostrum feeding whereas AEA was correlated negatively with the concentration of IgG in colostrum ($r = -0.39$, $P < 0.01$).

Serum concentrations of insulin and IGF-I differed according to sampling day. Insulin increased from 1.01 ng/mL at birth to 1.69 ng/mL ($P = 0.01$, Table 3-6) at 24 to 30 h after feeding of colostrum whereas IGF-I concentrations showed an opposite response with means of 90.7 and 69.8 ng/mL for birth and 24 to 30 h after colostrum feeding, respectively (Figure 3-5; effect of day, $P < 0.01$). Neither diet, parity, nor gender affected serum concentrations of insulin at birth (Table 3-6). However at 24 to 30 h after feeding of colostrum, female calves tended to have greater circulating concentrations of insulin than male calves (1.98 vs. 1.36 ng/mL, Figure 3-5 A, effect of gender, $P = 0.10$).

Fat feeding during prepartum increased serum IGF-1 concentrations of female calves at (104.7 vs. 83.7 ng/mL) but decreased of that of males (82.5 vs. 104.7 ng/mL, Figure 3-5 B, FAT by gender interaction, $P = 0.04$). After 24 to 30 h of colostrum feeding, feeding fat prepartum continued to have a negative impact on serum IGF-1 of male calves (59.0 vs. 77.3 ng/mL) but prepartum diet did not affect serum IGF-1 of females (81.5 vs. 77.7 ng/mL, Figure 3-5 B, FAT by gender interaction, $P = 0.09$). Serum concentrations of insulin and IGF-1 at birth were correlated positively with birth weight ($r = 0.24$, $P = 0.03$ for insulin and $r = 0.27$, $P = 0.01$ for IGF-1, Table 3-7). At 24 to 30 h after feeding of clostrum, serum insulin was correlated positively with AEA ($r = 0.23$, $P = 0.04$).

Discussion

Although not in this study, reduction in DMI during the prepartum period of dairy cows supplemented with diets of similar density but different FA composition was reported by others (Douglas et al., 2004; Moallen et al., 2007; Duske et al., 2009). On the contrary, Petit et al. (2007) did not report a difference in DMI when isocaloric diets formulated with linseed or energy booster were fed (12.9 vs. 12.1 kg/d, respectively). Similarly, Caldari-Torres et al. (2011) did not detect differences in DMI of prepartum cows fed isocaloric diets containing SFA ("Rumen Bypass Fat", Cargill, Minneapolis, MN, fed at 1.5% of dietary DM) or unsaturated FA ("Prequel-21", Virtus Nutrition, Fairlawn, OH, 63.6% of LA 1.8% of dietary DM). Greater reduction of DMI by supplemental fats has been associated with the feeding of more unsaturated fats (Allen, 2000). A possible mechanism by which unsaturated FA reduce DMI could be its function as a signal of satiety and energy status (Bradford et al., 2008). Recently, Allen and Bradford (2012) listed a series of observations from previous studies as evidences

favoring oxidation of fuels in liver as the most likely mechanism involved in regulation of intake in dairy cows fed energy dense diets.

In a recent published meta-analysis, Rabiee et al. (2012) evaluated the effect of fat supplements grouped as tallow, Megalac (rich in C16:0 and C18:1 FA), seed oils (rich in LA), hydrolyzed FA, or n-3 FA-rich Ca salts, each compared to their respective control diets. Authors reported that all fat supplements decreased DMI by an estimated mean of 0.88 kg/d per cow. However, ALA-rich Ca salts induced the most dramatic reduction in DMI (2.1 kg/d per cow). Milk yield tended to improve in cows supplemented with Megalac and ALA-rich Ca salts. The combined effect of Ca salts of FA on DMI and milk production indicate that this supplement could improve efficiency of milk production. In the present study production of colostrum was not affected by fat supplementation nor source of FA. The current finding contrasts to that of Banchemo et al. (2004) and Hashemi et al. (2008) who reported greater production of colostrum by ewes supplemented with more energetic diets.

The EFA supplement used in our current study is partially protected from hydrolysis and hydrogenation in the rumen because it is in the Ca salt form, hence a greater proportion of LA and ALA in Megalac-R can reach the intestine for further absorption and utilization. Consequently, greater concentrations of LA and ALA and their derivate FA might have been found in peripheral tissues and in fluids such as colostrum of cows. Studies have reported that when different ruminally-protected sources of FA such as Megalac (rich in C16:0), Ca salts of FO (rich in EPA and DHA), safflower seed oil (rich in LA), or linseed oil (rich in ALA) were fed to pregnant cows, an

increased proportion of the enriched FA was found in colostrum (Noble et al., 1978; Capper et al.; 2006; Santschi et al., 2009; Leiber et al., 2011).

Calculated intake of LA based upon actual DMI was 53.7, 58.8, and 98.6 g/d for cattle fed control, SFA, and EFA diets, respectively. Linoleic acid accounted for 75% of total n-6 FA in colostrum and was in greater concentrations when cattle were fed EFA, as was C20:2 n-6 and C22:4 n-6. Additionally, greater proportions of total and individual CLA as well as total C18:1 *trans* FA were detected in colostrum of cattle fed EFA which agree with others who measured FA profile of colostrum of cows supplemented with FO or linseed oil during the prepartum period (Capper et al.; 2006; Santschi et al., 2009). Plasma FA profile of prepartum cattle in the current study were not analyzed, but in agreement to the findings in colostrum FA profile, Lessard et al. (2004) found greater proportions of LA and C18:1 *trans* FA in plasma of transition cows supplemented with micronized soybeans compared to those supplemented with linseed or only greater proportions of C18:1 *trans* when compared to those cows supplemented with Megalac. The fact that increased concentrations of *trans* isomers of mono- and di-unsaturated FA were detected in colostrum of dams fed EFA indicates that the Ca salt form was not fully protecting the LA. The metabolism of LA by ruminal microorganisms will result in the formation of CLA and C18:1 *trans* FA (Lundy et al., 2004).

Based on these results, the enzymatic elongase/desaturase activity in the mammary gland was prioritizing the synthesis of LA derivatives to the detriment of the synthesis of ALA derivatives. This is suggested because cattle fed EFA had lower proportions of EPA in colostrum, although proportions of ALA, DHA, and total n-3 FA did not differ between cattle fed the two sources of FA. Studies using humans reported that

increased supplementation of LA or ALA increased the proportions of their corresponding derivatives in plasma (Chan et al., 1993; Goyens et al., 2006; Liou et al., 2007).

In the current study, colostrum fat from nulliparous heifers had greater proportions of ALA, AA, EPA, DPA, and DHA whereas LA was greater in colostrum FA of parous cows. Additionally total C18:1 *trans* and CLA c9, t11 were greater in colostrum FA of nulliparous heifers. In chapter 4 it is reported that calves born from parous cows had lower proportions of EPA, DPA and DHA in plasma before colostrum feeding than that of nulliparous heifers, which matches with the proportions detected in colostrum in this study.

Previous studies using human subjects found a negative relationship between parity and DHA concentrations in dams and in their neonates (Al MD et al., 1997). In contrast, Van Gool et al. (2004) failed to match the parity effect detected in dam serum DHA with DHA in the offspring. A potential mechanism of “dilution of FA concentration” due to greater production of colostrum by multiparous cows can be ruled out since the total FA concentration in colostrum remained unchanged due to parity. Limited research exists on the colostrum FA profile and this makes it hard to hypothesize about preferential synthesis of EFA derivatives in nulliparous heifers. However, considering that nulliparous heifers were raised in sod-base pens, with some access to pasture whereas parous cows were kept in free-stall barns, it can be possible that nulliparous heifers were mobilizing fat with greater proportions of PUFA obtained from previous access to pasture than that of parous cows. A recent study from Liu et al. (2011) reported that multiparous yak had greater proportions of total MUFA, total PUFA, CLA

c9- t11, ALA, and DHA compared to primiparous yak fed the same diet. These results contradict to findings of the current study. Authors attributed the greater proportions of these FA in multiparous yak to a greater growth and development of the mammary gland in the older animals; however total short chain and medium chain FA were not constantly greater in multiparous yak.

Some studies have evaluated the parity effect on FA composition of milk. Mierlita et al. (2011) evaluated the effect of parity on milk FA from sheep and reported increased proportions of ALA, EPA, CLA c9, C18:1 *trans*11, and total C18:1 *trans* FA in nulliparous sheep which is in agreement with the findings of the current study. However parity effects on DPA and DHA were not detected as was found in the current study. The major individual CLA detected in the current study was CLA c9, t11, whereas CLA t10, c12 was detected only in cows fed EFA but in limited proportions. Contrary to results in the current study, studies that evaluated milk of ewes reported no effect of parity on total CLA (Kelsey et al., 2003; Tsiplakou et al., 2006). Mierlita et al. (2011) also reported lower proportions of C18:0 in primiparous cows, hypothesizing that an incomplete biohydrogenation and/or a rapid passage of digesta was occurring in primiparous cows that prevented complete biohydrogenation, hence allowing the increase in CLA c9, t11 and total C18:1 *trans* FA delivered to the lower tract. However, in the current study, C18:0 proportions were greater in nulliparous heifers, disagrees with their hypothesis.

Mallard et al. (1997) evaluated the responses of prepartum cows to OVA challenge and classified them as high or low responders. Cows with greater serum concentrations of anti-OVA IgG had a lower incidence of diseases. Some researchers

have hypothesized that reduction in serum Ig concentrations around calving could be due to greater sequestration by the mammary gland (Detilleux et al., 1995). In the current study we did not measure concentrations of anti-OVA IgG in colostrum but total IgG in colostrum was greater in cattle fed SFA and the transfer of this specific antibody to the serum of calves also was greater if they were born from cattle fed SFA. In agreement with our findings, Mallard et al. (1997) and Watger et al. (2000) reported that cows with a greater response to prepartum OVA injections supplied greater concentrations of antibody to the mammary gland, therefore to the calf through feeding of the colostrum. Linoleic acid is commonly seen as an inducer of inflammatory responses. However some in vitro studies have reported that moderate amounts of LA could partially inhibit lymphocyte proliferation (Karsten et al., 1994; Gorjao et al., 2007), which assumes an antiinflammatory effect of LA. In the current study cattle fed EFA had lower concentrations of anti-OVA IgG in serum and total IgG in colostrum, which might indicate an antiinflammatory property of LA. Nevertheless all calves fed 4 L of good quality colostrum within 2 h of birth had > 2.2 g of total IgG per L of serum which is about 100% more than the minimum needed to ensure APT.

Only a few studies have evaluated the effect of additional fat with greater proportions of LA in isocaloric prepartum diets on measures of passive immunity and those were primarily done using beef cows. Dietz et al. (2003) fed cows isocaloric diets differing in concentrations of LA. Authors did not report differences in concentrations of colostrum IgG or in serum IgG of calves after colostrum feeding.

Lake et al. (2006c) aimed to evaluate the effect of prepartum energy balance on passive transfer of Ig. Prepartum beef cows were nutritionally managed to achieve

different BCS at partition (4 vs. 6). Prepartum cows targeted to have greater body condition were fed a more energy dense diet. No differences in IgG concentration of serum collected 48 h after birth was detected due to BCS of dams (15.6 vs. 13.4 g/L of IgG). This result contrasts with the current study, in which neither intake of energy prepartum nor BCS at calving differed from dams fed SFA or EFA but concentrations of serum total IgG and anti-OVA IgG were greater for calves born from dams fed SFA. Studies done with beef cows as those indicated above, are different from studies done with dairy cows. Beef calves are allowed to suckle their dams, whereas dairy calves are removed from their dams and normally force-fed colostrum. Hence, concentration of serum IgG after feeding of colostrum in beef calves can be a combination of different factors including willingness of calf to drink colostrum, and timing of intake whereas in dairy calves under the current experimental conditions, volume and timing of colostrum feeding were standardized along calves which prevented these variables from affecting serum IgG and AEA. Considering studies done with dairy cows, our results are in contrast to those of Novak et al. (2012b) who did not find any effect of lower intake of energy (88 vs. 100% of required energy) by prepartum Holstein cows on total concentrations of Ig and IgG in colostrum and serum of calves at 3 days of age (1.62 vs. 1.73 g/L of serum IgG).

Adequate management of time of colostrum feeding and total intake of IgG are important factors influencing APT (Heinrichs and Elizondo-Salazar, 2009). Calves in our current study were fed within 2 h of birth. Therefore, the only factor left to potentially affect APT is intake of IgG. Because all calves were offered the same volume of colostrum, the concentration of IgG in the colostrum was of primary importance. Calves

born from nulliparous heifers fed the control diet had greater intake of IgG than calves born from nulliparous heifers fed either source of fat. However this greater intake was not reflected in a greater AEA or a greater serum concentration of total IgG in this group of calves. The improved AEA in calves born from cattle fed either SFA or EFA, which was accompanied by a trend for greater serum concentrations of IgG, included calves born from nulliparous heifers. Hence, the improved AEA in calves born from nulliparous heifers fed fat, that also consumed less IgG compared to calves born from nulliparous heifers fed the control diet, might simply reflect the inverse relationship of IgG intake and AEA as reported by others (Quigley et al., 1994; Garry et al., 1996) and also identified in our present study ($r = -0.40$, $P < 0.01$, data not shown). However we hypothesize that reduced AEA was not only due to a simple effect of greater intake of IgG saturating the receptors for IgG in the enterocyte and therefore limiting the absorption of available IgG. In the current study, calves born from parous cows fed any source of fat had greater intake of IgG but also had a greater AEA as compared to calves born from parous cows fed the control diet. Hence the improved AEA of calves born from cattle fed fat might indicate that the feeding of fat to the dam may allow the calf to more efficiently absorb IgG. Lessard et al. (2006) challenged prepartum dairy cows with an OVA injection at -6 and -3 wk prepartum and measured transfer of anti-OVA IgG into the colostrum. Multiparous cows supplemented with micronized soybeans (20.3% of dietary DM) had a greater increase in concentration of anti-OVA IgG in colostrum than cows fed either a low fat or a high ALA diet. They concluded that dietary PUFA may influence the secretory function of mammary epithelial cells of multiparous

cows by modifying the FA profile of those epithelial cells and therefore modulating the transfer of blood IgG to the mammary gland.

The most recent mechanism discovered by which Ig are transported across the intestinal epithelium is with the assistance of FcRn, which in humans was identified in epithelial cells of the intestine, suggesting its involvement in binding of IgG and transfer of passive immunity (Israel et al., 1997). Later FcRn was not only associated with enhanced transport of IgG but also with protecting circulating IgG from degradation (Goebel et al., 2008). Composition of FA in cell membranes has been associated with a modified response of cells to expression of receptors such as those of the immune cell. Therefore it is valid to hypothesize that dams supplemented with fat (SFA or EFA) can pass those FA to the calf in utero through the placenta. Those FA become part of the enterocytes of the calf which influence the activity of FcRn resulting in improved efficiency of absorption of IgG. , However, based on our results, we cannot assign the benefit in AEA to a specific type of FA since no difference in AEA was identified between calves born from cattle fed SFA vs. EFA.

Oda et al. (1989) reported that regardless of prepartum diet type, concentrations of IGF-I, and insulin were greater in colostrum than in plasma of prepartum cows. In the current study, the concentrations of IGF-I and insulin in colostrum and in serum of parturient cows were not measured. However, the lack of effect of diets on IGF-I and insulin concentrations in serum of calves before and after colostrum feeding would not necessarily mean that concentration of these growth factors did not differ in colostrum due to prepartum diets. The beneficial effect of increased concentrations of IGF-I found in colostrum has been associated with an improved local effect on gastro intestinal tract

development (Hammon et al., 2000; Georgiev, 2008b; Blum and Baumrucker, 2008). However with the current findings we cannot rule out that calves born from dams fed diets with different FA profile might have differential development of their gastrointestinal tract, in disregard of no differences in serum IGF-I after colostrum feeding.

Sparks et al. (2003) reported a negative correlation between IGF-I at 0 h and the difference between serum IGF-I at 48 and 0 h ($r = -0.82$), which was confirmed in the present study for insulin ($r = -0.54$) and IGF-I ($r = -0.65$). Sparks et al. (2003) also reported a positive correlation of IGF-I in colostrum with IGF-I in serum of calves after 48 h of colostrums intake ($r = 0.45$). These results might suggest that colostrum with greater IGF-I concentrations allow calves to maintain greater concentrations of serum IGF-I after colostrum intake, even though actual mean values of serum IgG are decreased from birth to that measured 1 to 2 d after colostrum feeding. Lack of effect of prepartum diets on serum IGF-I after colostrum feeding might suggest that colostrum IGF-I concentrations did not differ among prepartum diets.

Summary

The FA profile of colostrum of cattle fed EFA reflected the concentration of LA in the fat supplement and its metabolism in the rumen of the pregnant cattle. Increased proportions of LA and its n-6 derivatives indicate that elongase/ desaturase activities in the mammary gland were active. However, increased proportions of total and individual CLA as well as total C18:1 *trans* FA in colostrum of cattle fed EFA indicate that the Ca salt of EFA was not completely effective in preventing the processes of biohydrogenation by ruminal microbes. Interestingly, colostrum of nulliparous heifers appeared to be a better source of n-3 FA (ALA, EPA, DPA, and DHA) than that of parous cows.

Intake of IgG did not differ due to dietary treatments but serum concentrations of total IgG and anti-OVA IgG after colostrum feeding were greater in calves born from cattle supplemented with SFA vs. EFA. Hence feeding of newborn calves with colostrum of prepartum Holstein cattle fed SFA instead of EFA would enhance APT. Feeding of fat prepartum improved AEA across parities from 23.3 to 27.9% regardless of type of fat supplemented. It is possible that cattle fed fat gave birth to calves that had a more efficient mechanism to transfer IgG into circulation, possibly by modifying the activity of FcRn receptors in the intestinal tract due to the likely differential composition of FA in the cell membrane. Concentrations of serum IGF-I in calves did not increase but were reduced with the feeding of colostrum and were not affected by the type of diet. This might indicate that IGF-I is poorly absorbed into circulation or that IGF-1 is used to enhance proliferation and differentiation epithelial intestinal cells.

Table 3-1. Ingredient composition of experimental diets fed to pregnant Holstein cattle starting at 8 weeks from expected calving date.

Ingredient, % of DM	Prepartum diets ¹		
	Control	SFA	EFA
Bermuda silage	56.0	56.0	56.0
Ground barley	8.0	8.0	8.0
Peanut meal	10.0	10.0	10.0
Citrus pulp	21.9	20.2	19.9
Saturated fatty acids ²	-	1.7	-
Ca salts of fatty acids ³	-	-	2.0
Mineral mix ⁴	4.1	4.1	4.1
Nutrient composition, (DM basis)			
NE _L ⁵ , Mcal/kg	1.42	1.49	1.5
CP, %	14.0	14.0	14.0
NDF, %	47.4	47.4	47.4
ADF, %	25.3	25.3	25.3
Fatty acids, %	1.68	3.37	3.35
Linoleic acid ⁶ , g/d	57	62	116

¹ Control = no fat supplement ed; SFA = saturated fatty acids; EFA =essential fatty acids.

² Energy Booster 100 (Milk Specialties, Dundee, IL).

³ Megalac-R (Church & Dwight, Princeton, NJ).

⁴ Contains (DM basis) 34.5% corn meal, 5.0% dicalcium phosphate, 16.0 calcium carbonate, 10% calcium sulfate, 5% magnesium oxide, 10% magnesium sulfate, 4% sodium chloride, 1.7% Zinpro 4-plex (Zinpro, Minneapolis, MN), 0.4% Rumensin 80 (Elanco Animal Health, IN), 0.35% Sel-Plex 2000 (Alltech Biotechnology, Nicholasville, KY), 0.002% Ca iodate, and a vitamin premix. Each kg contains 24.5% CP, 9.8% Ca, 1.5% P, 4.2% Mg, 3.2% S, 1.7% Na, 10.7 % Cl, 475 mg of Zn, 160 mg of Cu, 456 mg of Mn, 7.4 mg of Se, 37.4 mg of Co, 13.2 mg of I, 118,000 IU of vitamin A, 27,500 IU of vitamin D, 2,600 IU of vitamin E, and 770 mg of monensin.

⁵ Calculated from the estimation of energetic values of individual ingredients using the NRC software (2001) and considering intake at 3X of maintenance.

⁶ Considering 12 kg of DMI (CPM dairy fatty acid submodel).

Table 3-2. Fatty acid (FA) profile of fat supplements fed to pregnant Holstein cattle starting at 8 weeks from expected calving date.

FA	SFA ¹	EFA ²
	% of identified FA	
C14:0	3.3	1.0
C14:1	ND ³	ND
C15:0	0.4	ND
C16:0	35.1	34.3
C16:1	0.4	0.1
C17:0	1.5	0.1
C18:0	51.6	4.5
C18:1	3.1	27.1
C18:2	ND	27.4
C18:3 α	0.7	2.3
Other FA	3.8	3.2

¹ SFA = Energy Booster (Milk Specialties, Dundee, IL).

² EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² ND = Not detected.

Table 3-3. Performance of nulliparous and parous Holstein cattle fed diets supplemented without fat (control), with saturated fatty acids (SFA), or with essential fatty acids (EFA) the last 8 weeks of pregnancy.

Measure	Dam Diet ¹						SEM	P values ³				
	Control		SFA		EFA			FAT	FA	P	FAT x P	FA x P
	Parity ²											
	Prim	Mult	Prim	Mult	Prim	Mult						
N ^o of cows ⁴	4	16	8	13	6	14						
DMI ⁵ , kg	10.6	11.6	10.2	12.4	9.3	11.5	0.7	0.67	0.16	<0.01	0.41	0.98
NE _L Intake ⁶ , Mcal/d	15.0	16.5	15.2	18.4	13.8	17.0	1.0	0.73	0.16	<0.01	0.38	0.93
Serum anti-OVA IgG ⁷ , OD	0.34	0.56	0.68	0.63	0.43	0.48	0.09	0.22	0.02	0.37	0.19	0.56
N ^o of cows ⁸	8	17	11	16	9	17						
Days in diets	54.6	54.4	54.8	56.7	53.7	57.2	1.59	0.44	0.85	0.20	0.30	0.59
BW enrollment, kg	538	694	511	709	532	701	23.7	0.91	0.80	<0.01	0.52	0.54
BCS enrollment	3.34	3.47	3.36	3.27	3.31	3.32	0.09	0.26	1.00	0.84	0.31	0.52
BW calving, kg	587	752	569	777	583	743	21.9	0.93	0.65	<0.01	0.62	0.28
BCS calving	3.31	3.51	3.36	3.41	3.44	3.38	0.07	0.82	0.68	0.30	0.10	0.45
BW change, kg	49.4	58.4	57.2	67.6	50.7	42.4	12.7	0.96	0.21	0.72	0.73	0.46
Gestation length, d	275	276	275	278	273	279	1.35	0.25	0.62	<0.01	0.19	0.28
Calving ease Score ⁹	1.25	1.06	1.00	1.13	1.00	1.06	0.08	0.12	0.66	0.97	0.04	0.66
Colostrum ¹⁰ , Kg	4.13	7.71	3.14	6.59	3.44	6.65	1.09	0.33	0.87	<0.01	0.90	0.91
IgG colostrum ¹⁰ , g/L	102	96	83	122	83	109	11.1	0.99	0.59	0.04	0.05	0.56

¹ Control = no fat supplemented; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² Null = nulliparous.

³ P values for orthogonal contrasts and interactions. FAT= (SFA + EFA) vs. Control, FA = EFA vs. SFA, P = parity.

⁴ Total of 61 cattle that were allocated to the Calan gate system.

⁵ Day effect, $P < 0.01$.

⁶ Day effect, $P < 0.01$.

⁷ Day effect, $P < 0.01$; parity by day interaction effect, $P = 0.03$.

⁸ Scoring system: unassisted (1), easy pull (2), hard pull (3), and surgery (4).

⁹ Total of 70 cows after removing 8 cows that did not produce colostrum collected.

Table 3-4. Mean concentrations of total fatty acids (FA, % of colostrum DM), individual, and group of FA (g of FA/100 g of total FA) in colostrum of Holstein cattle fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

Measure	Dam diets ¹						SEM	P values ²				
	Control		SFA		EFA			FAT	FA	Parity (P)	FAT x P	FA x P
	Null	Parous	Null	Parous	Null	Parous						
FA												
Total FA, %	7.82	7.05	5.65	6.33	7.84	6.58	1.08	0.37	0.28	0.62	0.80	0.39
C4:0	2.11	1.72	2.25	1.80	2.21	1.79	0.11	0.31	0.80	<0.01	0.84	0.89
C6:0	1.17	0.93	1.24	0.91	1.18	0.93	0.05	0.76	0.71	<0.01	0.58	0.52
C8:0	0.59	0.47	0.61	0.44	0.59	0.45	0.03	0.77	0.93	<0.01	0.62	0.64
C10:0	1.24	1.07	1.20	0.93	1.12	0.97	0.08	0.18	0.79	0.01	0.82	0.51
C12:0	2.28	1.86	2.15	1.70	2.05	1.72	0.14	0.19	0.78	<0.01	0.89	0.70
C14:0	11.4	10.6	10.8	9.7	10.1	9.5	0.66	0.09	0.47	0.13	0.98	0.71
C14:1 c9	0.48	0.60	0.36	0.50	0.34	0.46	0.05	0.01	0.54	<0.01	0.92	0.80
C16:0	35.2	40.9	34.0	40.7	33.7	40.4	1.20	0.41	0.81	<0.01	0.66	0.98
C16:1 c9	1.62	2.15	1.45	1.95	1.38	1.76	0.07	<0.01	0.07	<0.01	0.43	0.43
C18:0	9.70	7.15	11.14	8.14	10.63	8.57	0.47	<0.01	0.94	<0.01	0.98	0.33
C18:1 t4	0.02	0.01	0.01	0.01	0.02	0.02	0.002	0.02	<0.01	<0.01	0.71	0.50
C18:1 t5	0.01	0.01	0.01	0.01	0.02	0.01	0.001	0.01	<0.01	<0.01	0.87	0.92
C18:1 t6-8	0.22	0.15	0.21	0.15	0.25	0.19	0.01	0.12	<0.01	<0.01	0.91	0.84
C18:1 t9	0.18	0.13	0.19	0.14	0.21	0.15	0.01	0.01	0.07	<0.01	0.43	0.84
C18:1 t10	0.21	0.16	0.21	0.19	0.28	0.30	0.03	0.02	0.01	0.51	0.40	0.58
C18:1 t11	1.08	0.59	1.04	0.57	1.33	0.77	0.06	0.07	<0.01	<0.01	0.83	0.40
C18:1 t12	0.26	0.18	0.25	0.19	0.33	0.25	0.01	<0.01	<0.01	<0.01	0.65	0.47
C18:1 c9	21.7	22.7	22.0	23.3	22.3	21.9	1.32	0.89	0.70	0.55	0.78	0.56
C18:1 c11	0.99	0.89	0.97	0.89	1.04	0.87	0.06	1.00	0.68	0.02	0.84	0.49
C18:2 n-6	2.16	2.34	2.33	2.28	3.20	3.50	0.10	<0.01	<0.01	0.08	0.77	0.10
C18:3 n-6	0.03	0.03	0.03	0.04	0.03	0.03	0.003	0.68	0.29	0.01	0.65	0.90
C18:3 n-3	0.44	0.31	0.46	0.32	0.45	0.35	0.02	0.23	0.41	<0.01	0.80	0.31
CLA c9 t11	0.25	0.15	0.22	0.13	0.33	0.20	0.02	0.12	<0.01	<0.01	0.61	0.19
CLA t10 c12	0.000	0.000	0.000	0.000	0.001	0.002	0.001	0.26	0.06	0.75	0.82	0.71
C20:2 n-6	0.03	0.02	0.03	0.02	0.04	0.03	0.002	0.00	<0.01	<0.01	0.37	0.69
C20:3 n-9	0.03	0.01	0.03	0.01	0.03	0.01	0.002	0.09	0.05	<0.01	0.62	0.96
C22:0	0.09	0.06	0.09	0.06	0.09	0.06	0.005	0.88	0.82	<0.01	0.38	0.98
C20:3 n-6	0.29	0.21	0.28	0.27	0.33	0.24	0.02	0.04	0.65	<0.01	0.31	0.02
C20:3 n-3	0.01	0.00	0.01	0.01	0.01	0.01	0.001	0.71	0.27	0.01	0.10	0.53
C20:4 n-6	0.49	0.37	0.54	0.43	0.61	0.39	0.02	<0.01	0.42	<0.01	0.23	0.03
C20:5 n-3	0.11	0.05	0.13	0.07	0.11	0.05	0.01	0.16	<0.01	<0.01	0.59	0.99

Table 3-4. Continued.

Measure	Dam diets ¹						SEM	P - values ²				
	Control		SFA		EFA			FAT	FA	Parity (P)	FAT x P	FA x P
	Parity ³											
	Null	Parous	Null	Parous	Null	Parous						
C24:0	0.06	0.04	0.06	0.04	0.07	0.04	0.003	0.99	0.35	<0.01	0.18	0.93
C22:4 n-6	0.09	0.07	0.10	0.08	0.13	0.08	0.01	<0.01	0.01	<0.01	0.09	0.01
C22:5 n-3	0.35	0.13	0.38	0.16	0.41	0.14	0.02	0.04	0.79	<0.01	0.26	0.10
C22:6 n-3	0.05	0.01	0.06	0.01	0.06	0.004	0.003	0.64	0.52	<0.01	0.83	0.52
Unknown FA	0.35	0.33	0.35	0.31	0.35	0.32	0.01	0.53	0.84	0.03	0.94	0.84
Other FA	4.77	3.59	4.79	3.62	4.73	3.57	0.13	0.99	0.68	<0.01	0.95	0.99
Total <C16	21.1	18.6	20.5	17.4	19.3	17.1	1.0	0.12	0.45	<0.01	0.96	0.66
Total C16	36.8	43.1	35.5	42.6	35.1	42.2	1.2	0.29	0.73	<0.01	0.70	0.98
Total >C16	41.7	38.0	43.7	39.7	45.3	40.5	1.8	0.13	0.54	0.01	0.82	0.83
Σ SFA	65.8	66.1	65.6	65.8	63.6	65.8	1.5	0.55	0.49	0.46	0.74	0.52
Σ MUFA cis	26.0	27.5	26.0	27.7	26.4	26.1	1.4	0.87	0.69	0.41	0.76	0.51
Σ PUFA cis	4.07	3.56	4.35	3.69	5.40	4.83	0.14	<0.01	<0.01	<0.01	0.68	0.77
Total CLA	0.30	0.18	0.26	0.15	0.41	0.25	0.02	0.05	<0.01	<0.01	0.67	0.21
Total BCFA	1.37	1.00	1.37	0.98	1.33	0.93	0.07	0.59	0.57	<0.01	0.78	0.98
Σ < C18:1 <i>trans</i>	0.10	0.08	0.11	0.08	0.11	0.08	0.01	0.31	0.32	<0.01	0.99	0.72
Σ C18:1 <i>trans</i>	1.97	1.23	1.91	1.24	2.44	1.68	0.09	0.01	<0.01	<0.01	0.85	0.68
Σ n-3	0.96	0.51	1.02	0.56	1.03	0.55	0.04	0.08	0.98	<0.01	0.78	0.69
Σ n-6	3.08	3.04	3.30	3.12	4.34	4.27	0.12	<0.01	<0.01	0.36	0.68	0.65
n-6 : n-3	3.24	6.14	3.26	5.88	4.25	7.92	0.36	0.04	<0.01	<0.01	0.69	0.17

¹ Control = no fat supplemented; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² Null = nulliparous.

³ P values for orthogonal contrasts and interactions. FAT = (SFA + EFA) vs. Control, FA = EFA vs. SFA, P = parity.

Table 3-5. Passive immunity related parameters in calves born from Holstein cattle fed diets supplemented with no fat (control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before calculated calving date.

Measure	Dam Diet ¹						SEM	P values ³								
	Control		SFA		EFA			FAT	FA	P	FAT x P	FA x P	G	FAT x G	FA x G	
	Parity ²															
	Null	Parous	Null	Parous	Null	Parous										
N° calves	8	17	11	16	9	17										
Birth																
BW ⁴ , kg	37.2	39.8	37.8	43.7	35.5	43.8	1.32	0.13	0.40	<0.01	0.06	0.38	0.02	0.69	0.06	
STP ⁵ , g/dL	4.83	4.82	4.78	4.62	4.79	4.80	0.11	0.44	0.39	0.57	0.75	0.42	0.85	0.19	0.57	
IgG intake ⁶ , g	410	383	344	487	336	431	37.0	0.94	0.42	0.04	0.04	0.54	-	-	-	
ST IgG ⁷ , g/dL	0.02	0.02	0.03	0.02	0.01	0.02	0.01	0.77	0.34	0.95	0.66	0.44	0.29	0.09	0.37	
24 h after birth																
STP, g/dL	6.35	6.16	6.21	6.58	6.33	6.23	0.21	0.67	0.59	0.90	0.39	0.25	0.75	0.11	0.71	
ST IgG, g/dL	2.40	2.21	2.69	2.97	2.51	2.36	0.22	0.09	0.07	0.90	0.52	0.32	0.92	0.03	0.89	
ST IgG, % of STP	37.5	35.2	42.3	44.6	39.1	37.3	2.57	0.05	0.05	0.79	0.59	0.42	0.82	0.03	0.94	
Anti-OVA IgG, OD	1.03	1.04	1.16	1.10	0.91	0.89	0.08	0.80	0.01	0.76	0.71	0.85	0.47	0.74	0.99	
AEA ⁸ , %	23.7	23.0	30.5	28.6	27.3	25.1	2.27	0.03	0.14	0.41	0.73	0.95	0.02	0.20	0.33	

¹ Control = no fat supplemented; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² Null = nulliparous.

³ P-values for orthogonal contrasts and interactions. FAT = (SFA + EFA) vs. Control, FA = EFA vs. SFA, P = parity, G = gender. Three way interactions were not significant.

⁴ Parity by gender, *P* = 0.07.

⁵ Serum total protein.

⁶ Gender not included in the model.

⁷ Serum total IgG.

⁸ Apparent efficiency of IgG absorption, % = [IgG concentration in serum at 24 h of life × (0.099 × BW at birth)] ÷ IgG intake] × 100.

Table 3-6. Concentrations of insulin and insulin-like growth factor I in serum of calves born from Holstein cattle fed diets supplemented with no fat (control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before calculated calving date.

Measure	Dam diet ¹						SEM	P values ³								
	Control		SFA		EFA			FAT	FA	P	FAT x P		G	FAT x G		FA x G
	Null	Parous	Null	Parous	Null	Parous					P	P		FA x P	FAT x G	
Parity ²																
N° calves	8	17	11	16	9	17										
Birth																
Insulin, ng/mL	1.31	0.70	1.24	1.30	0.99	0.73	0.24	0.72	0.12	0.11	0.20	0.45	0.13	0.25	0.24	
IGF-I, ng/mL	97.3	87.8	100.5	89.3	81.4	102.0	10.7	0.96	0.74	0.99	0.46	0.12	0.33	0.04	0.90	
24 h after birth																
Insulin, ng/mL	1.27	1.77	1.73	1.82	1.47	1.84	0.43	0.63	0.79	0.33	0.67	0.70	0.10	0.87	0.58	
IGF-I, ng/mL	71.4	84.2	72.7	77.7	57.4	70.4	8.08	0.26	0.19	0.12	0.88	0.55	0.02	0.09	0.87	

¹ Control = no fat supplemented; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² Null = nulliparous.

³ P values for orthogonal contrasts and interactions. FAT = (SFA + EFA) vs. Control, FA = EFA vs. SFA, P = parity, G = gender. Three way interactions were not significant.

Table 3-7. Correlation coefficients among several variables¹ in calves born from Holstein cattle fed diets supplemented with no fat (control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before calculated calving date. First row within each measure corresponds to r values and second row corresponds to P values.

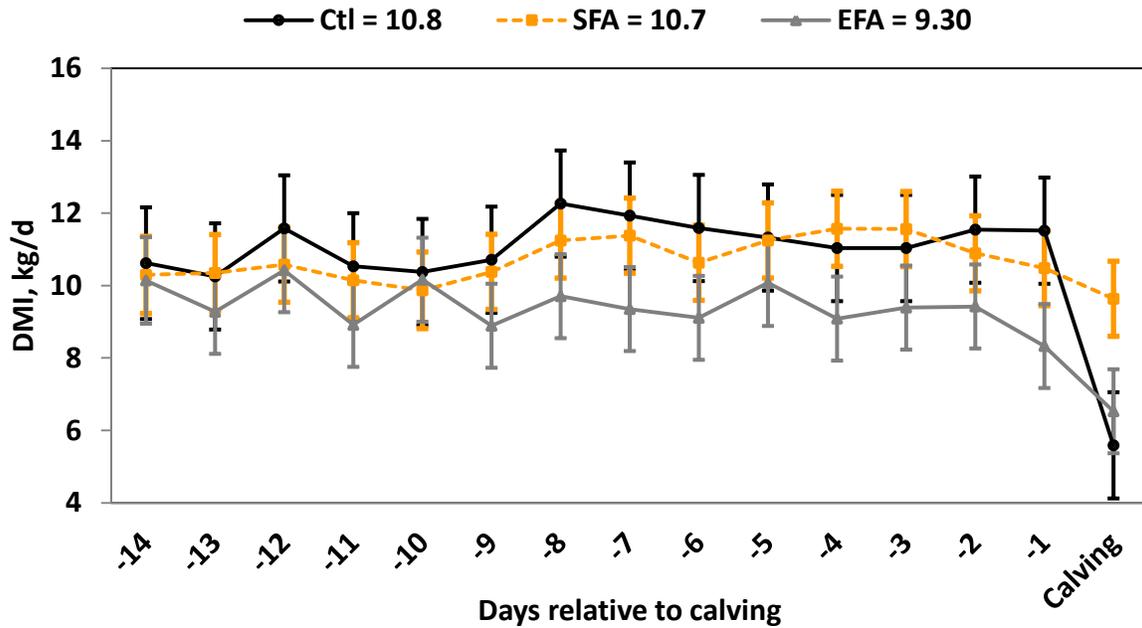
	PP BWC	PP DMI	Colost . IgG	BW birth	IgG 0 h	IgG 24 h	TSP 0 h	TSP 24 h	TSP diff	AEA	IGF-I 0 h	IGF-I 24 h	Insulin 0 h	Insulin 24 h	IGF-I diff	Insulin diff
Gest. Length	0.22	0.31	0.11	0.59	0.00	-0.11	-0.20	-0.09	0.01	0.16	0.10	-0.05	0.20	-0.02	-0.17	-0.17
PP BW change	0.07	0.01	0.38	<0.01	0.97	0.30	0.07	0.43	0.93	0.17	0.33	0.65	0.05	0.86	0.11	0.10
PP		0.58	-0.01	0.03	0.18	-0.06	-0.17	-0.20	-0.12	0.00	0.15	0.17	0.28	0.31	-0.02	0.13
DMI		<0.01	0.96	0.83	0.15	0.64	0.18	0.10	0.33	0.97	0.24	0.18	0.02	0.01	0.88	0.30
Colost. IgG			0.09	0.21	0.28	-0.09	-0.24	-0.18	-0.08	-0.07	0.07	-0.03	0.12	0.22	-0.10	0.14
BW birth			0.51	0.10	0.03	0.47	0.06	0.14	0.54	0.58	0.60	0.80	0.34	0.07	0.44	0.26
IgG 0 h				0.10	0.02	0.54	-0.10	0.50	0.54	-0.39	0.03	0.19	0.11	0.07	0.14	0.04
IgG 24 h				0.42	0.89	<0.01	0.42	<0.01	<0.01	<0.01	0.80	0.12	0.38	0.57	0.26	0.77
TSP 0 h					0.06	-0.13	-0.14	-0.11	-0.02	0.27	0.27	0.13	0.24	-0.13	-0.21	-0.25
TSP 24 h					0.59	0.24	0.18	0.31	0.82	0.02	0.01	0.22	0.03	0.22	0.05	0.02
TSP diff							0.08	0.02	-0.02	-0.05	-0.01	0.02	0.12	0.08	0.04	-0.07
AEA							0.47	0.86	0.84	0.68	0.90	0.83	0.27	0.47	0.72	0.53
IGF-I 0 h							0.11	0.81	0.76	0.42	-0.02	-0.01	-0.07	0.18	0.02	0.17
IGF-I 24 h							0.32	<0.01	<0.01	<0.01	0.85	0.92	0.51	0.10	0.85	0.12
Insulin 0 h									-0.25	0.15	-0.02	0.17	-0.04	0.25	0.18	0.21
Insulin 24 h									0.02	0.20	0.85	0.12	0.73	0.02	0.09	0.06
IGF-I diff									0.87	0.24	-0.10	-0.05	-0.10	0.19	0.08	0.20
Insulin diff									<0.01	0.03	0.35	0.66	0.36	0.07	0.46	0.07
IGF-I 0 h									0.16	-0.09	-0.13	-0.08	0.05	-0.01	0.10	
IGF-I 24 h									0.17	0.40	0.23	0.46	0.64	0.89	0.38	
Insulin 0 h										0.16	-0.05	0.12	0.23	-0.25	0.14	
Insulin 24 h										0.16	0.66	0.31	0.04	0.03	0.25	
IGF-I diff											0.59	0.27	0.04	-0.65	-0.12	
Insulin diff											<0.01	0.01	0.69	<0.01	0.26	
IGF-I 0 h													0.19	0.23	0.06	
IGF-I 24 h													0.07	0.03	0.56	

Table 3-7. Continued.

	PP BWC	PP DMI	Colost . IgG	BW birth	IgG 0 h	IgG 24 h	TSP 0 h	TSP 24 h	TSP diff	AEA	IGF-I 0 h	IGF-I 24 h	Insulin 0 h	Insulin 24 h	IGF-I diff	Insulin diff
Insulin 0 h														0.12	-0.13	-0.54
Insulin 24 h														0.25	0.24	<0.01
IGF-I diff															0.15	0.77
															0.16	<0.01
																0.20
																0.06

¹ Gest. Length= gestation length; PP BW change= Body weight change during the last 60 d of gestation; PP DMI= prepartum dry matter intake; Colost. IgG= Concentration of IgG in colostrum; BW birth= body weight at birth; 0 h = corresponding variable measured in serum of calves before colostrum feeding; 24 = corresponding variable measured in serum of calves after 24 – 30 h of colostrum feeding; diff= difference of measures after and before colostrum feeding; TSP= total serum protein; AEA= apparent efficiency of IgG absorption.

A



B

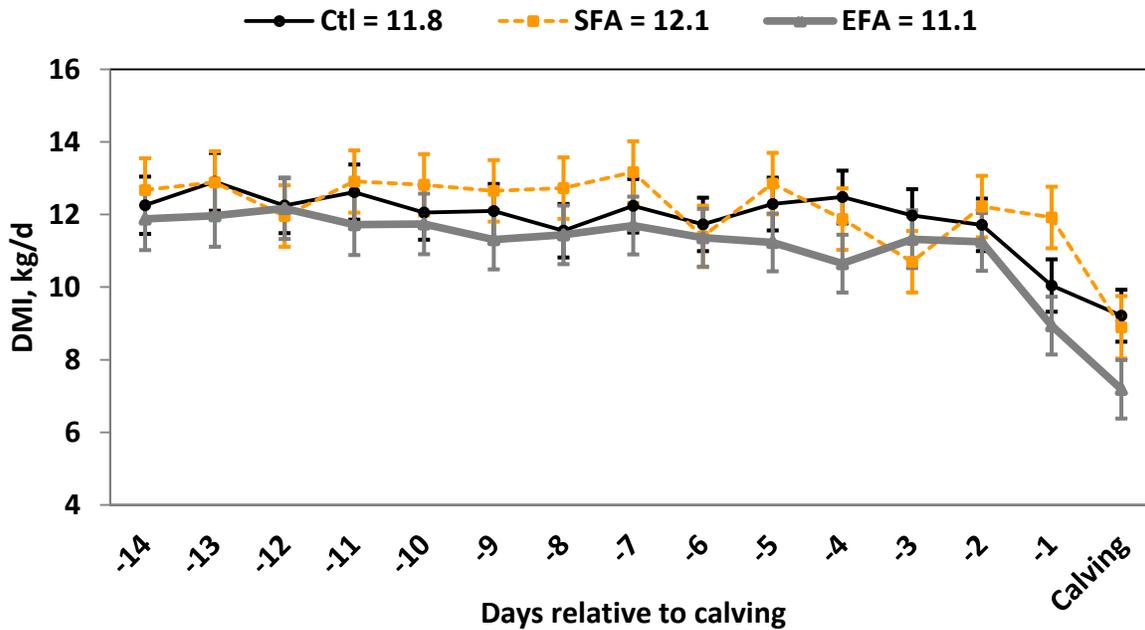


Figure 3-1. Dry matter intake of Holstein cattle supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. A) Nulliparous heifers. B) Parous cows. Effect of parity, $P = 0.03$. Effect of days relative to calving, $P < 0.01$.

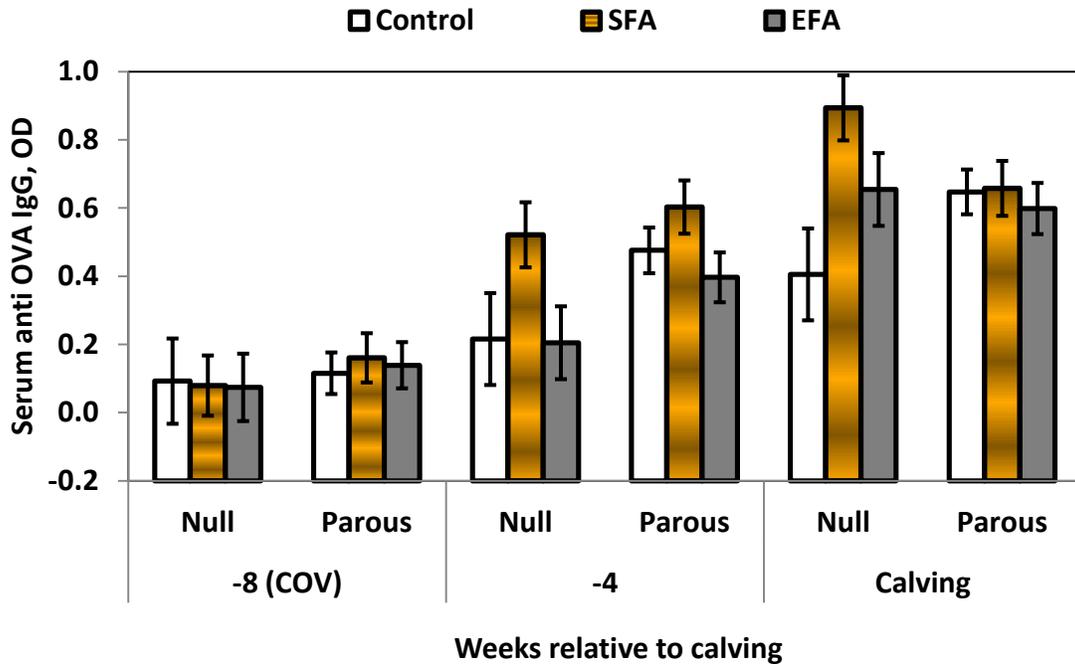


Figure 3-2. Bovine anti-OVA IgG concentration in serum of Holstein cattle supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. A) Nulliparous heifers (Null). B) Parous cows. Cows were injected with 1 μ g of ovalbumin at weeks 8 and 4 relative to calving. Effect of day was $P < 0.01$. Effect of feeding SFA vs. EFA was $P = 0.01$. Effect of interaction of parity by day was $P = 0.03$.

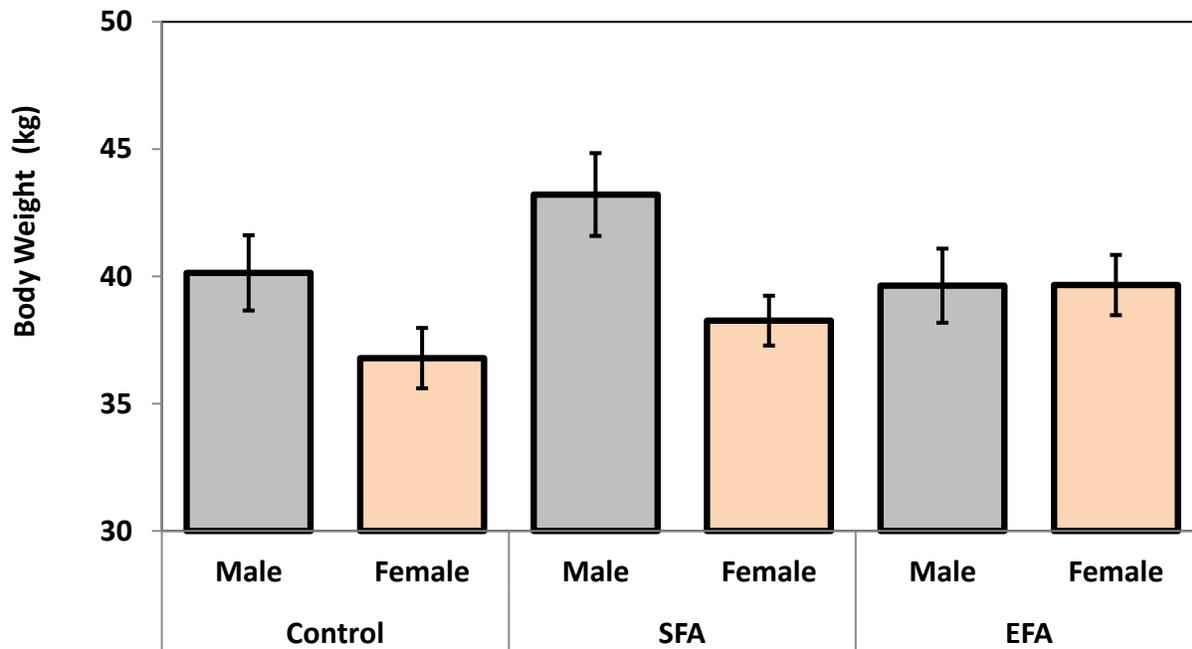
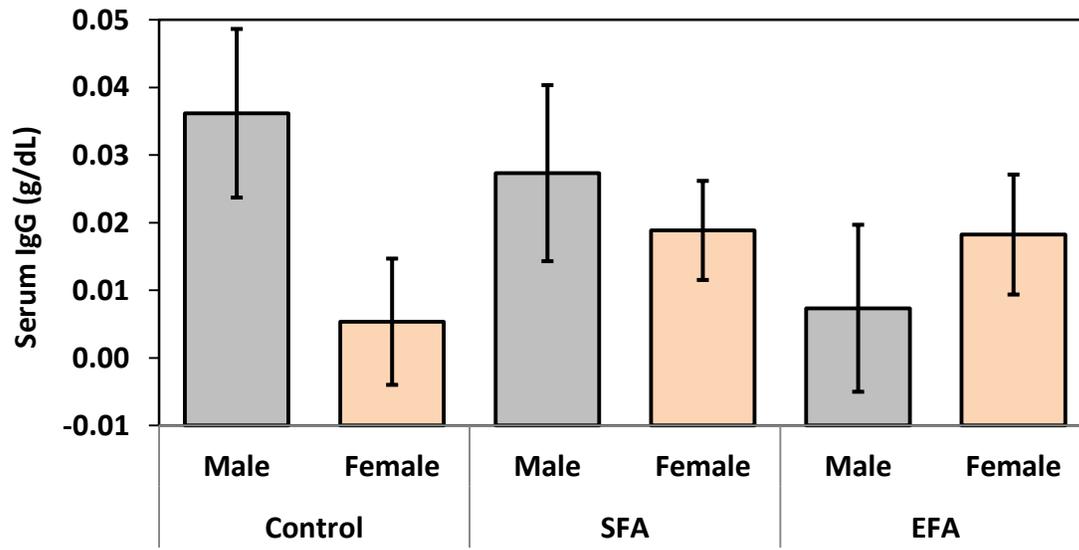


Figure 3-3. Body weight at birth of calves born from Holstein cattle supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Gender, $P = 0.02$, interaction fatty acid by gender, $P = 0.06$.

A



B

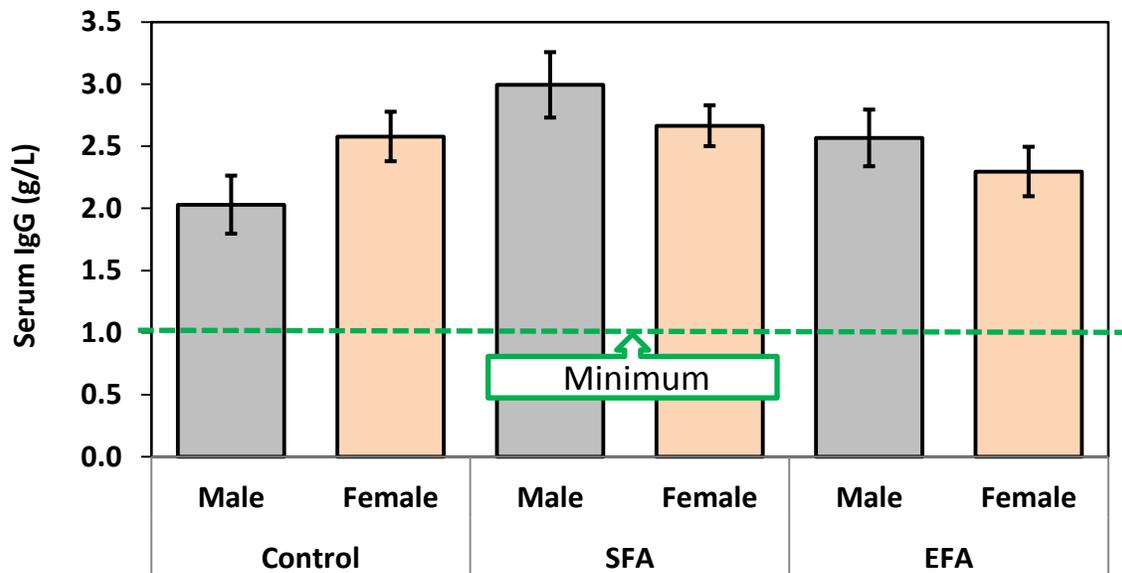
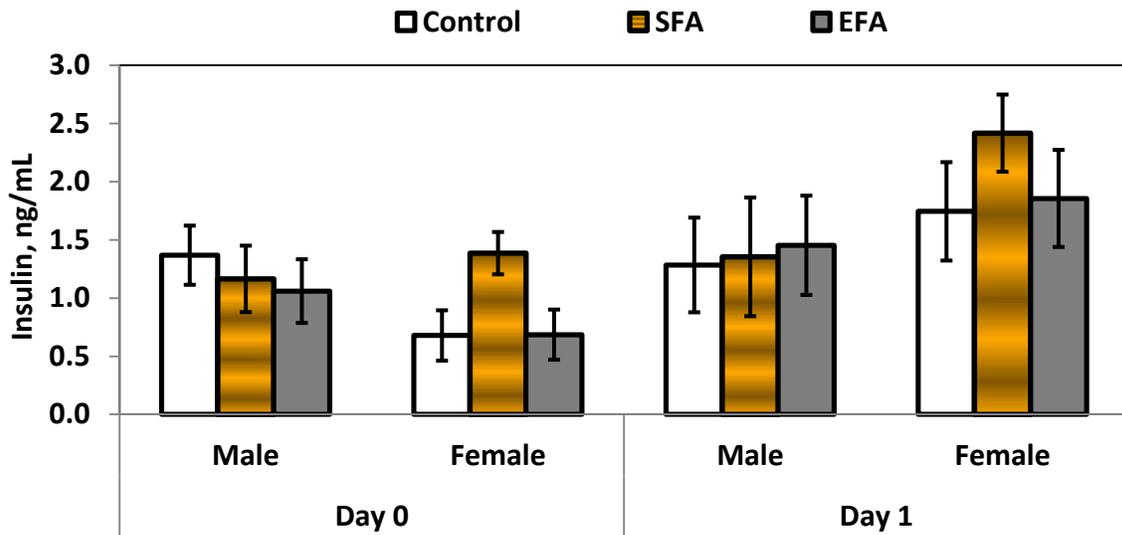


Figure 3-4. Concentrations of total IgG in serum of calves born from Holstein cattle supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. A) Before colostrum feeding, effect of fat (SFA + EFA) by gender, $P = 0.09$. B) After 24 to 30 h of colostrum feeding, effect of feeding SFA vs. EFA, $P = 0.07$ and effect of fat (SFA + EFA) by gender, $P = 0.03$.

A



B

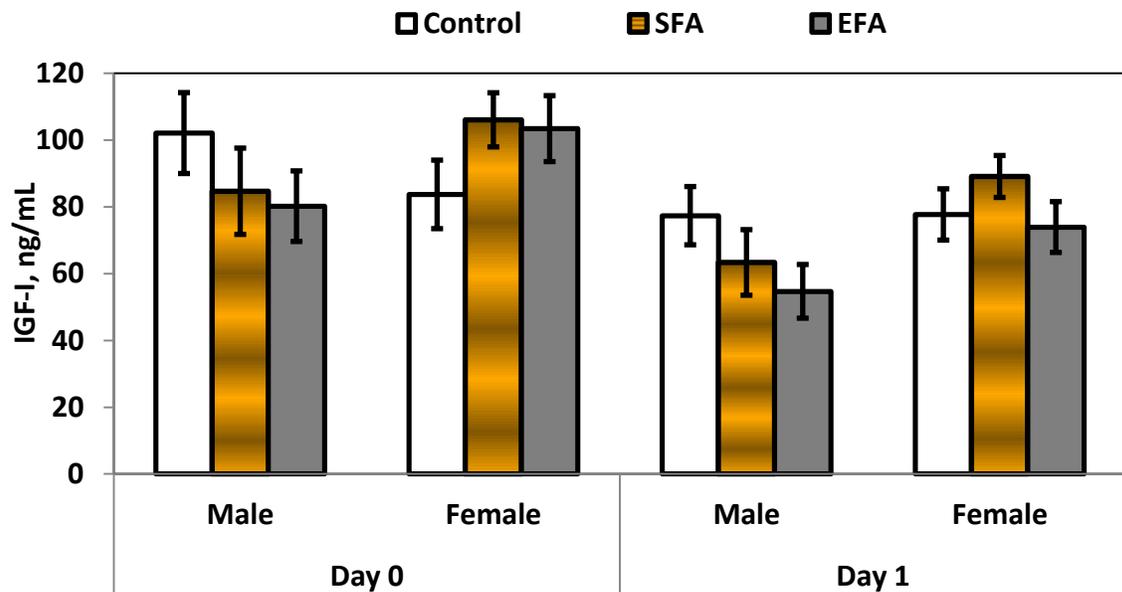


Figure 3-5. Serum concentrations of hormones of calves born from Holstein cattle supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. A) Insulin concentration, Effect of gender at day 1, $P = 0.10$. B) IGF-I concentration, Effect of fat by gender at day 0, $P = 0.04$, at day 1, $P = 0.09$, effect of age, $P = 0.02$.

CHAPTER 4
EFFECT OF SUPPLEMENTING ESSENTIAL FATTY ACID TO PREGNANT
HOLSTEIN COWS AND THEIR PREWEANED CALVES ON CALF PERFORMANCE,
IMMUNE RESPONSE AND HEALTH

Background

Doubling the birth weight at weaning and minimize the incidence of diseases is the primary goals of dairy herd management. Dairy farmers have to deal with critical circumstances and health challenges once the calf is born (Beam et al. 2009, Donovan et al., 1998). Therefore, to prevent high incidence of calf diseases and to avoid jeopardizing the profitability of the herd, effective care should be taken not only during the preweaning period but also during the gestation period, particularly during the last trimester of gestation, during which time the fetus has its greatest development.

Early studies in human subjects have reported a direct effect of the nutritional status of pregnant women during late pregnancy on fetal growth and birth weight. Kramer (1987) reviewed 895 publications related to potential causes of intrauterine growth retardation in human subjects and reported that poor gestational nutrition was a common cause of lighter birth weight. More recent studies in ruminants found contradictory effects of undernutrition during late gestation on birth weight (Osgerby et al., 2002; Dwyer et al. 2003). Hess (2003) evaluated 18 studies that supplemented fat to late gestation beef cows and concluded that fat supplementation did not affect birth weight. Funston et al. (2010) reviewed the effects of maternal nutrition on future performance of beef cows, whereas Singh et al. (2010) reviewed the factors accounting for phenotypic variation in milk production by dairy cows. Both authors concluded that a substantial proportion of the unexplained phenotypical variations were due to epigenetic regulation (change in gene expression without modifying DNA sequence) as a

consequence of maternal nutrition during fetal life or nutrition during the first year of life. Recently Soberon et al. (2012) reported an increase of 850 kg of milk in the first lactation per 1 kg increase of ADG during the preweaning period. They concluded that increased growth rate before weaning resulted in some form of epigenetic programming with a positive effect on milk yield.

Few studies have evaluated the effect of supplementing diets with different FA sources during late gestation on overall calf performance. The few available studies were done in beef cattle and resulted in no effect of supplemental fat on birth and weaning weight (Bottger et al., 2002; Encinias et al., 2001, 2004). Beef calves suckling cows supplemented with LA affected metabolic profile and antibody production but growth was not affected (Lake et al., 2005; 2006a, 2006b, 2006c). A limited number of studies have evaluated the supplementation of increased intakes of LA to preweaned dairy calves. The laboratory of J.K. Jenkins in Ontario, Canada was among the first ones to evaluate the replacement of milk fat with other sources of less expensive fat such as vegetable oils. These studies (Jenkins et al., 1985, 1986; Jenkins and Kramer, 1986) are the foundation to better understand the effects of feeding vegetable and animal oils, aiming to increase the intake of EFA on calf growth, diarrhea incidence, and FA profile of important tissues involved in lipid metabolism such as liver, heart and plasma.

Some work was recently published to evaluate the effect of omega-3 (n-3) FA from animal or vegetable origin (Ballou and DePeters., 2008; Hill et al., 2011). However, there is no study evaluating the inclusion of LA in MR to modify activity of different markers of immune responses in newborn calves. The hypothesis of the current study

was that supplementing prepartum and preweaning diets with LA would improve overall performance of calves. In addition, it was hypothesized that calves born to dams not supplemented with LA would have a greater response to LA feeding in MR than calves born to dams fed diets supplemented with LA. The objective was to evaluate the effect of supplementing diets with fat enriched with LA during late gestation and feeding LA-enriched MR during the first two months of life on calf growth, health, and immune responses.

Materials and Methods

Prepartum Management

The experiment was conducted at the University of Florida's dairy farm (Hague, FL) from October 2008 to June 2009. All procedures for animal handling and care were approved by the University of Florida's Animal Research Committee. Pregnant nulliparous (n = 35) and previously parous (n = 61) Holstein cattle were sorted according to calving date, parity, body weight (BW), and body condition score (BCS) and assigned to one of the three treatments at 8 wk before their expected calving date. Prepartum treatments: supplementation (Control), 1.7% of dietary dry matter (DM) of mostly free saturated FA (SFA, "Energy Booster 100", Milk specialties, Dundee, IL), and 2.0% of dietary DM as Ca salts of FA enriched with essential FA (EFA, "Megalac R", Church and Dwight, Princeton, NJ) as well as cattle general management were the same as those indicated in chapter 3.

Calves Dietary Treatments, Feeding Management and Analyses

All procedures regarding calving management at birth and colostrum feeding were done according details presented in Chapter 3. Calves were blocked by gender (n = 56 females and 40 males) and dam diet and randomly assigned to receive a MR containing

low (LLA, 0.56% LA, DM basis) or high concentrations of LA (HLA, 1.78% LA, DM basis) for 60 d starting at birth. Milk replacer (Tables 4-1 and 4-2, Land O'Lakes, Webster City, IA) was fed at 0600 and 1230 h daily at a constant rate of 0.149 g of LA/kg of BW^{0.75} for the LLA treatment group and 0.487 g of LA/kg of BW^{0.75} for the HLA treatment group, respectively. Milk replacer was fed exclusively the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}. Warm water (~38 to 42°C) was added to the powdered MR at the time of each feeding in order to prepare an 11% DM MR. The amount of fat intake per kg of BW^{0.75} remained constant throughout the experiment. Calves were weighed weekly and amounts of MR fed were adjusted weekly based upon BW. Refusal of MR was recorded daily. Coconut oil was the sole fat source in the LLA MR whereas a mixture of CCO and porcine lard were the fat sources in the HLA MR. The LA intake from the LLA MR was below the minimum recommend for laboratory rats (NRC, 1995) for optimum growth performance. For comparison, typical on-farm practice for calves weighing 40 kg to be fed 4 L of milk daily (10% of BW) containing 3.5% triglycerides of which 3.13% is LA. Intake of LA would be ~3.8 g of LA daily. Calves weighing 40 kg in the current study consumed 2.5 and 7.8 g of LA daily when fed LLA and HLA MR, respectively.

A single grain mix (1.17% LA, DM basis) was offered in ad libitum amounts from 31 to 60 d of age (Tables 4-1 and 4-2). Barley and peanut meal were chosen to formulate the grain mix because they contain the lowest concentration of LA among traditional grain and protein meal supplements, respectively. Peanut meal contained 2 ppb of aflatoxin (Quamta Lab. Selma, TX). Amounts of grain mix offered and refused were measured daily. Clean water was available at all times. Powdered MR and grain

mix were sampled weekly and composited monthly. Monthly composites were analyzed (Dairy One, Ithaca, NY) for minerals (Ca, P, Mg, K, Na, I, Zn, Cu, Mn, Mo, Co, and S) and CP. Additional analyses for the grain mix were ether extract, ADF, and NDF.

Housing, Body Weight and Immunizations

During the 60 d of the experimental period, calves were housed outside in individual wire hutches (1 m × 1.5 m) bedded with sand. Body weights for measures of growth were taken at birth, before colostrum feeding, and at 30 and 60 d before the morning feeding. At birth, calves were administered intranasal TVS-2 (Pfizer Co., New York, NY) to prevent infectious bovine rhinotracheitis (IBR) and parainfluenza 3 (PI3) and oral calf guard (Pfizer Co. New York, NY) to control infection for rotavirus and corona virus.

At 3 wk of age Bovishield Gold-5 (Pfizer Co., New York, NY) was administered by s.c. injection for prevention of IBR, bovine virus diarrhea [types I and II], PI3, and bovine respiratory syncytial virus. The same dose of Bovishield Gold-5 was repeated at wk 5 plus an injection of Ultrabac-7 (Pfizer Co., New York, NY) to protect calves from diseases caused by Clostridium. A dose of Ultrabac-7 was repeated at wk 7 including an injection of Pinkeye Shield XT4 (Novartis, Inc., Larchwood, IA). Starting at 6 wk of age, a 5-day oral treatment with Corid (Merial Limited, Duluth, GA) to treat and prevent coccidiosis was followed by 5 d with an antihelmintic (Safeguard; Merck & Co., Inc., Whitehouse Station, NJ). Calves experiencing diarrhea were given electrolytes (Gener-Lyte, Bio-Vet Inc., Blue Mound, WI), bismuth subsalicylate (Bismusol; First Priority, Inc., Elgin, IL), and sulfadimethoxine (Albon boluses, Pfizer Co., New York, NY) for 5 d. If the diarrheic condition reoccurred in a given calf, the same treatment was re-administered.

Calves Scoring for Health Assessment and Incidence of Health Disorders

Attitude and fecal scores were recorded daily according to the scoring system of Magalhães et al. (2008). Attitude [1) responsive, 2) non-active, 3) depressed, or 4) moribund] and fecal consistency scores [1) feces of firm consistency, no diarrhea, 2) feces of moderate consistency, soft, no diarrhea, 3) runny feces, mild diarrhea, or 4) watery feces, diarrhea] were recorded for each calf after the first MR feeding between 0800 to 1000 h. Incidence of health disorders were recorded daily for each individual calf. Rectal temperature of calves displaying signs of any disease was measured. Fever was diagnosed by rectal temperature $\geq 39.5^{\circ}\text{C}$. Diarrhea was diagnosed by presence of watery feces (fecal score > 2). One calf was diagnosed with chronic pneumonia starting at 38 d of age, consequently only its measures before 30 d of age were considered for all statistical analyses.

Hormone and Metabolite Analyses

Before colostrum was fed, a jugular blood sample was collected from each calf and again within 24 to 30 h after colostrum feeding. Blood samples were collected into a clot-activated tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), and serum was separated at room temperature. Tubes were centrifuged for 15 min at $2095 \times g$ (Allegra X-15R centrifuge, Beckman Coulter, Inc). Blood was collected from the jugular vein twice a week for the first 30 d of age and once a week thereafter, into clot-activated and K_2EDTA tubes for serum and plasma collection, respectively. Plasma and serum were separated by centrifugation and then stored at -20°C for later analyses.

Plasma metabolites such as glucose, plasma urea N (PUN), and cholesterol were analyzed 2 times weekly the first 30 d and 1 time per week from 31 to 60 d of age. Concentrations of nonesterified FA (NEFA) and β -hydroxybutyric acid (BHBA) were

measured once a week from 1 to 60 d of age. Serum samples at 0 and 1 d of age and plasma samples at 14, 28, 42 and 56 d of age were used to analyze insulin and insulin like growth factor I (IGF-I).

A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and PUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965). Samples were run in singlet, including in each run a control sample which was run in duplicate. Inter-assay variations were 2.6 and 4.4% for PUN and glucose, respectively. Plasma concentrations of NEFA were determined using a commercial kit (NEFA-C kit; Wako Diagnostics, Inc., Richmond, VA) with a method modified by Johnson (1993). Plasma concentrations of BHBA also were determined using a commercial kit (Wako Autokit 3-HB; Wako Diagnostics, Inc., Richmond, VA). Samples were run in duplicate for NEFA (intra-assay variation of 2.2%), whereas samples for BHBA were run in singlet, including a control sample which was run in duplicate. Intra- and inter-assay variations for BHBA were 3.5 and 5.9% respectively. Total cholesterol concentrations (Cholesterol E kit, Wako Diagnostics Inc., Richmond, VA) were analyzed in serum at 0 h and in plasma twice a week the first 30 d of life and once a week thereafter. Each sample was analyzed in triplicate and one sample was removed if the coefficient of variation was greater than 5%. Intra- and inter-assay variations were 2.5 and 4.8%, respectively.

Concentrations of IGF-I were analyzed following the manufacturer's protocol (Active non extraction IGF-I ELISA, Diagnostic Systems Laboratory, Inc., Webster TX) with some modifications in sample pre-treatment similar to those indicated in chapter 3.

The intra-plate variation for IGF-I of control samples was 2.4%, whereas the inter-plate variation was 3.2%. Insulin concentrations were analyzed using a double antibody radioimmunoassay (Badinga et al., 1991). Intra- and inter-assay variations were 7.3 and 14.6%, respectively.

The FA extraction and methylation procedures were the same for feed and plasma samples. It was performed by the 2 step methylation procedure according to Kramer et al. (1997) with some modifications. Briefly, feed ingredients (500 mg) and freeze-dried plasma samples (1.5 ml of fresh plasma of calves at 0 d before colostrum feeding, and at 30 and 60 d of age) were weighed or transferred respectively to a screw capped (TeflonTM lined caps) culture tubes. One mL of internal standard (C19:0, 1mg/mL of benzene) was added in order to calculate total FA concentration. Lipid was extracted by adding 2 mL of sodium methoxide (Acros, New Jersey, USA), vortexing, and incubating in a 50°C water bath for 10 min. After cooling for 7 min, 3 mL of 5% methanolic HCl (Fisher Scientific, Hampton, NH, USA) was added and the tubes were vortexed. The tubes were incubated in an 80°C water bath for 10 min, removed from water bath, and allowed to cool for 10 min. One mL of hexane and 6.5 mL of 6% K₂CO₃ were added. The tubes were vortexed and centrifuged at 1455 x g for 10 min. The upper layer was carefully transferred into crimp-top vials and stored at -20°C for further analysis.

Fatty acid methyl esters were determined using a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with auto-sampler (Varian CP-8400), flame ionization detector, and a Varian capillary column (CP-SIL 88 FS, 100 m x 0.25 mm x 0.2 µm). The carrier gas was He, the split ratio was 10:1, and the injector and detector temperatures were maintained at 250°C, respectively. One µl of sample

was injected via the auto-sampler into the column. The oven temperature was set initially at 120°C for 1 min, increased by 5°C/min up to 190°C, held at 190°C for 30 min, increased by 2°C/min up to 220°C, and held at 220°C for 15 min. The peak was identified and calculated based on the retention time and peak area of known standards.

Markers of Immunity Analyses

Blood for hematologic analysis and for markers of immunity in fresh blood, were collected from puncture of the jugular vein into heparinized vacutainer tubes at 2, 7, 14, 21, 30, 40, and 60 ± 1 d of age. Samples were kept at ambient temperature with constant inversion. A Bayer Advia 120 cell counter (Fisher Diagnostic, Middletown, VA) was used to quantify the population of blood cells. Analysis was performed within 2 h of collection.

Phagocytic activity of blood neutrophils was evaluated the same days as blood cells population was quantified. Whole blood samples were collected in duplicate for quantification of blood cells. Samples were kept under constant rotation on a Clay Adams nutator (BD, San Jose, CA) until the neutrophil concentration was obtained from the laboratory. Activation of phagocytic cells was measured using pHrodo™ E.coli BioParticles® Conjugate for phagocytosis (Molecular Probes™, Invitrogen™). Briefly, a sample of the heparinized blood (100 µL) with a neutrophil concentration fewer than 5 x 10³ cell/ µL was incubated with 40 µL of reconstituted pHrodo™ E.coli BioParticles® Conjugate. For samples with greater concentrations of neutrophils, proportional amounts of reconstituted product were added. Samples were incubated for 2 h at 37°C with continuous rotation (Clay Adams nutator; BD, San Jose, CA). A control sample for each animal was included, following the same process as described above but without

using Conjugate *E. coli*. After incubation, phagocytosis initiated by the presence of *E. coli* was stopped by placing the samples on crushed ice. Samples were lysed for red blood cells using 2.5 mL of lysing buffer (44.94 g of NH_4Cl , 5.0 g of KHCO_3 , and 0.185 g of K_2EDTA in 10 L of double distilled water). Tubes were vortexed and left at room temperature for 15 min followed by a 5 min centrifugation at 931 x *g* (Allegra X-15R centrifuge, Beckman Coulter, Inc). The supernatant was removed and the pellet was broken apart by gently shaking. To each tube 2.5 mL of FACS buffer (2% of fetal bovine serum, 0.1% of sodium azide in PBS) was added and immediately centrifuged for 5 min at 931 x *g*. Tubes were then placed on crushed ice and transported to the University of Florida Flow Cytometry Core Lab. FACSFlow sheath fluid (200 μL , BD Biosciences, San Jose, CA) was added to each tube. For each sample the optical features of 50,000 neutrophils were acquired using a Facsort flow cytometer equipped with a 488-nm argon ion laser for excitation at 15 mW (BD Biosciences, San Jose, CA) and CellQuest software (Becton Dickinson, San Jose, CA). Forward (roughly proportional to the diameter of the cell) and side (proportional to membrane irregularity) scatters were used for preliminary identification of neutrophil cells on dot plots (Jain et al., 1991). Density cytograms were generated by linear amplification of the signals in the forward and side scatters. Percentage fluorescence of positive events was correlated with the proportion of neutrophils able to phagocytize *E. coli*, whereas geometric mean fluorescence intensity (MFI) was interpreted as mean number of bacteria ingested per neutrophil.

Expression of adhesion molecules on neutrophil surface was performed according to Silvestre et al. (2011) with some modifications. Briefly, monoclonal mouse anti-bovine L-selectin (CD62L, IgG1 isotype, Serotec, Raleigh, NC) and a mouse anti-canine β 2-

integrin (CD18, IgG1 isotype, Serotec, Raleigh, NC) that cross-reacts with bovine CD18 were used. Additionally, an isotype mouse control antibody (IgG1 isotype, Serotec) was used to correct for non-specific binding of CD62L and CD18 antibodies to the cells. Blood from each sample (3 mL) was placed in a 50 mL polypropylene tube and lysis buffer (44.94 g of NH_4Cl , 5.0 g of KHCO_3 , and 0.185 g of K_2EDTA in 10 L of double distilled water) was added up to a final volume of 50 mL, left at room temperature for 15 min, and then centrifuged for 10 min at $931 \times g$. Supernatant was decanted and pellet re-suspended in 15 mL of lysis buffer and left for 10 min at room temperature, then centrifuged for 10 min at $931 \times g$. Supernatant was decanted and reconstituted with 15 mL of FACS buffer (2% of fetal bovine serum, 0.1% of sodium azide in PBS) and centrifuged for 10 min at $931 \times g$. Supernatant was decanted and the pellet cells were re-suspended in 1 mL of FACS buffer and kept on crushed ice until staining. The cell suspension (100 μL) was divided into four separate 5 mL polystyrene tubes for immunostaining of a negative control with and without any antibody and for each antibody. Working dilution antibodies (10 μL of 1:10 dilution of CD62L, CD18, and control antibody in FACS buffer) were added to each individual tube and incubated at room temperature for 25 min. FACS buffer was added into each tube (2.5 mL) and centrifuged for 5 min at $233 \times g$. Supernatants were decanted and each tube received 5 μL of antimouse IgG (polyclonal IgG isotype, Serotec) and then incubated for another 25 min. Cells were washed with FACS buffer (2.5 mL) and centrifuged for 5 min at $233 \times g$. Supernatants were decanted and 0.4 mL of the FACS fixative solution (2% of fetal bovine serum and 0.1% of sodium azide in 0.5% formalin) was added to each tube to re-suspend the cell pellet. Flow cytometer settings were similar to that for neutrophil

phagocytic activity. Percentage of neutrophil cells positive for CD62L and CD18 were obtained based upon gated cells. Also, the geometric MFI of the labeling kit, an indicator of the number of receptors on the surface of each neutrophil cell, was obtained in the histogram for the gated cell populations.

Blood was collected from the jugular vein twice a wk the first 30 d of age and once a week thereafter into clot-activated and K₂EDTA tubes. Before obtaining the plasma from each sample, hematocrit concentration was measured using heparinized micro-hematocrit capillary tubes (Fisherbrand, Thermo Fisher Scientific Inc.) centrifuged (Microspin 24 tube micro hematocrit centrifuge, Vulcon Technologies, Grandview, Mo) for 3 min and read in a micro hematocrit tube reader (Model CR, Damon/IEC, Needham Heights, MA). Plasma and serum were separated by centrifugation for 15 min at 2095 x *g* (Allegra X-15R centrifuge, Beckman Coulter, Inc) and then stored at - 20°C for later analyses. Serum before storing was analyzed for serum total protein (STP) using an automatic temperature compensated hand refractometer. Concentrations of haptoglobin (Hp) and acid soluble protein (ASP) were measured in all collected samples.

Calves were injected subcutaneously (s.c.) with 0.5 mg of OVA (Sigma Aldrich, Saint Louis, MO) diluted in Quil A adjuvant solution (0.5 mg of Quil A in 1 mL of PBS, Accurate Chemical & Scientific Corp., Westbury, NY) at 2, 20, and 40 d of age. Concentrations of bovine anti-OVA IgG were measured in serum on the same days of injection and at 60 d of age. Serum concentrations of bovine anti-OVA IgG were measured by enzyme linked immunosorbent assay (ELISA) as described by Mallard et al. (1997) and detailed in chapter 3. Intra- and inter-assay coefficients of variation were 9.2 and 9.7%, respectively.

Concentrations of plasma Hp were determined by measuring the differences of H_2O_2 with Hp-hemoglobin (Hb) as described previously (Makimura and Suzuki, 1982). Concentrations of Hp are reported as arbitrary units (optical density x 100) resulting from the absorption reading at 450 nm. Intra- and inter-assay coefficients of variation were 6.0 and 10.9%, respectively. Concentrations of ASP were determined according to Nakajima et al. (1982) with some modifications. Plasma samples (50 μL) were incubated with PCA solution (1 mL, 6 M perchloric acid, Fisher Scientific, Hampton, NH, USA). The intra- and inter-assay coefficients of variations were 2.6 and 5.9%, respectively.

Isolation of peripheral blood mononuclear cells (PBMC) was done at 15 ± 2 and 30 ± 1 d of age according to Caldari (2009) with some modifications. Briefly, 5 tubes of blood (10 mL each) were collected from each calf from the jugular vein into heparinized tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Blood samples were transported to the laboratory at ambient temperature and the isolation was initiated within 3 h of blood collection. Tubes were centrifuged for 15 min at $931 \times g$ at room temperature (Allegra X-15R centrifuge, Beckman Coulter, Inc). The buffy coat, containing most of the white blood cells, was transferred using sterile transfer pipettes to a 13 mL tube (Sarstedt Inc., Newton, NC) containing 2 mL of medium 199 (M-199, Sigma- Aldrich, Saint Louis, MO). The buffy coat and M-199 medium were mixed by pipetting up and down several times. This cell suspension was transferred slowly on top of 2 mL of Fico/Lite LymphoH (Atlanta Biologicals, Lawrenceville, GA). The cell suspension/Fico/Lite LymphoH solution was centrifuged for 30 min at $524 \times g$ at room temperature. Mononuclear cells were collected from the Fico/Lite interface and

transferred to pre-labeled 13 mL culture tubes containing 2 mL of red blood cell lysing buffer (Sigma- Aldrich, Saint Louis, MO). Exactly 20 sec after transferring, the solution was neutralized with 8 mL of 1X DPBS (Sigma-Aldrich, Saint Louis, MO). The solution was centrifuged at 524 x *g* for 15 min at room temperature. The supernatant was removed by aspiration with a sterile glass pipette attached to a vacuum pump and the pellet containing the PBMC was resuspended in 4 mL of M-199 media by pipetting up and down 10 times with a sterile transfer pipette. The supernatant was resuspended in modified M-199 (M-199 media supplemented with 5% horse serum, 500 U/mL of penicillin, 0.2 mg/mL of streptomycin, 2 mM of glutamine, 10⁻⁵ M β-mercaptoethanol; all reagents from Sigma-Aldrich, Saint Louis, MO).

The PBMC were counted using the Trypan blue dye (Sigma-Aldrich, Saint Louis, MO) by exclusion method. The cell suspension was adjusted to 2 x 10⁶ cells/mL. Cell suspension in a total volume of 2 mL was plated in triplicate with modified M-199 media and stimulated or not stimulated with 10 µg/mL of concanavalin A (Sigma-Aldrich, Saint Louis, MO) on a 6-well plate (Corning Inc., Corning, NY). Plates were incubated for 48 h at 37°C at 5% CO₂. After incubation, plates were centrifuged for 10 min at 524 x *g* and the supernatant was stored at -80°C for analysis of cytokine production. Quantification of IFN-γ concentration was performed using the bovine IFN-γ DuoSet ELISA development kit (R&D systems, Minneapolis, MN). Stimulated and non-stimulated samples were run in triplicate and the most variable replicate was not considered. The intra-assay coefficient of variation was 9.2%.

Statistical Analyses

Dam diets (n = 3) and MR (n = 2) were arranged in a 3 x 2 factorial randomized block design. On a weekly basis, a cohort of Holstein cows at 8 wk before the expected

calving date was blocked by parity (nulliparous and parous) and BCS. Within each block, cattle were assigned randomly to one of the three dietary treatments. Calves after birth were blocked by dam diet and gender and randomly assigned to one of the two MR. A total of 40 male and 56 female calves were enrolled.

Repeated measurement analysis was conducted on nearly all variables using the PROC MIXED procedure of SAS (Release 9.2) according to the following model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + CI_{(ijk)} + W_m + (\alpha W)_{im} + (\beta W)_{jm} + (\alpha\beta W)_{ijm} + (\gamma W)_{km} + (\alpha\gamma W)_{ikm} + (\beta\gamma W)_{jkm} + (\alpha\beta\gamma W)_{ijkm} + \epsilon_{ijklm}$$

Where Y_{ijklm} is the observation, μ is overall mean, α_i is the fixed effect of dam diet (control, SFA, and EFA), β_j is the fixed effect of MR (LLA and HLA), $(\alpha\beta)_{ij}$ is the interaction of dam diet and MR, γ_k is the fixed effect of gender (male and female), $(\alpha\gamma)_{ik}$ is the interaction of dam diet and gender, $(\beta\gamma)_{jk}$ is the interaction of MR and gender, $(\alpha\beta\gamma)_{ijk}$ is the interaction of dam diet, MR, and gender, $CI_{(ijk)}$ is the random effect of calf within dam diet, MR, and gender ($l = 1, 2, \dots, n$), W_m is the fixed effect of age ($m =$ days or weeks of age), $(\alpha W)_{im}$ is the interaction of dam diet and age, $(\beta W)_{jm}$ is the interaction of MR and age, $(\alpha\beta W)_{ijm}$ is the interaction of dam diet, MR, and age, $(\gamma W)_{km}$ is the interaction of gender and age, $(\alpha\gamma W)_{ikm}$ is the interaction of dam diet, gender, and age, $(\beta\gamma W)_{jkm}$ is the interaction of MR, gender, and age, $(\alpha\beta\gamma W)_{ijkm}$ is the interaction of dam diet, MR, gender, and age; and ϵ_{ijklm} is the residual error. For nonrepeated measures, the same model was used after removing the age effect and their interactions.

All variables were tested for normality of residuals using the Shapiro-Wilk test (SAS version 9.2, SAS Inst. Inc., Cary, NC). Non-normally distributed data were transformed as suggested using the guided data analysis of SAS and back transformed

using the LINK and ILINK function of GLIMMIX respectively. Data were tested to determine the structure of best fit, namely compound symmetry, compound symmetry heterogeneous, autoregressive-1, and autoregressive-1 heterogeneous as indicated by a Schwartz Bayesian information criteria value closest to zero (Littell et al., 1996). If repeated measures were taken on unequally spaced intervals, the sp(pow) covariance structure was used. Different temporal responses to treatments were further examined using the SLICE option of the MIXED or GLIMMIX procedure.

The following orthogonal contrasts were performed [1) dam diet of no fat vs. fat (SFA + EFA), 2) dam diet of SFA vs. EFA, 3) HLA vs. LLA MR, 4) interaction of contrasts 1 and 3, and 5) interaction of contrasts 2 and 3]. If any 3 or 4-way interaction including the effect of time were not significant ($P > 0.25$), the interactions were dropped from the model and the model was rerun (Bancroft, 1968). Differences discussed in the text were significant at $P \leq 0.05$ and tended to be significant at $0.05 < P \leq 0.10$.

Results

Plasma Fatty Acid Concentration and Profile

Mean plasma concentrations of total FA at birth ranged from 1.14 to 1.34 mg/mL (Table 4-3). Regardless of the diet fed prepartum, palmitic acid and OA made up ~60% (~30% each) of the total FA in plasma of calves at birth followed by stearic acid at approximately 13.5%, palmitoleic at 5.2%, AA at 4.7%, and LA at 3.7%.

Docosahexaenoic acid was the n-3 FA with the greatest concentration with a mean of approximately 0.7%. Total FA concentration in plasma was not affected by parity (1.23 vs. 1.31% for calves born from nulliparous heifers and parous cows, respectively, $P = 0.23$). However, calves from nulliparous heifers had lower concentrations ($P < 0.01$) of n-6 FA, namely LA (2.9 vs. 4.5% of total FA) and AA (4.0 vs. 5.4% of total FA) but

greater concentrations ($P < 0.01$) of n-3 FA, namely EPA (0.39 vs. 0.08% of total FA) and DHA (0.87 vs. 0.51% of total FA). Although all cattle consumed the same basic TMR the last 8 wk before calving, these specific FA differences may have been because nulliparous heifers consumed more fresh pasture than parous cows in previous months as fresh grass usually contains more n-3 FA than stored forages. In summary, calves born from nulliparous heifers had lower concentrations of total n-6 FA (8.7 vs. 12.8, $P < 0.01$) but greater concentrations of total n-3 FA (1.82 vs. 1.13, $P < 0.01$).

Compared to cattle fed control diet, feeding fat prepartum did not appreciably change the total FA concentration or the profile of FA in plasma of the calves at birth (Table 4-3). Total proportions of SFA, MUFA, and PUFA were not affected by dam diets. Plasmatic concentrations of total FA at birth tended to be greater for calves born from dams fed EFA instead of SFA (1.33 vs. 1.21 mg/mL, $P = 0.09$). Cattle supplemented with EFA prepartum gave birth to calves having or tending to have greater proportions ($P = 0.03$) of LA (4.4 vs. 3.3%) and total n-6 FA (11.8 vs. 10.3%; $P = 0.06$) in plasma compared to calves born from cattle fed SFA. The effect of fat type was the opposite for some n-3 FA. Cattle supplemented with EFA prepartum gave birth to calves with lower plasmatic proportions of total n-3 FA (1.30 vs. 1.67%; $P < 0.02$), specifically EPA (0.19 vs. 0.29%; $P = 0.03$) and DHA (0.60 vs. 0.80%; $P < 0.01$) compared to calves born from cattle fed SFA. Although calves born from cattle fed EFA tended to have more circulating FA ($P = 0.09$), the increase was only 10%, hence when correcting the proportions of FA by this increased total FA, calves born from cattle fed EFA still had lower circulating amounts of DHA ($P = 0.05$) but circulating amounts of EPA ($P = 0.52$) were not different. Plasma concentrations of some FA found in greater

concentrations in SFA (C16:0, C18:0, and C18:1) instead of in EFA supplement were not increased in calf plasma by feeding SFA prepartum.

Mean daily intake of LA during the first 30 d, when MR was the only feed, was 2.6 and 8.6 g/d by calves fed LLA and HLA MR, respectively, whereas for the second 30 d of life, intake of LA from MR and grain mix was 9.4 and 16.4 g/d for calves fed LLA and HLA MR, respectively. Intake of ALA was minimal since the LLA MR did not contain ALA and the HLA MR only contained ALA at 0.15% of DM. Average intake of ALA during the first 30 d was 0 and 0.5 g/d by calves fed LLA and HLA MR, respectively and 0.5 and 1.3 g/d for calves fed LLA and HLA MR for the second 30 d, respectively.

The FA profile of plasma changed dramatically from birth (Table 4-3) to that when calves were 30 to 60 d old (Table 4-4). The main changes were in proportions of C16:0, C18:1cis, LA, and ALA. Mean concentrations at birth and at the 30 to 60 d of age period were approximately 30 and 16% for C16:0, 29 and 11% for C18:1cis, 4 and 44% for LA, and 0.06 and 0.70% for ALA. Fat concentration in plasma increased from 1.27 at birth to 2.02 mg/100 mL of plasma for older calves, an increase of ~60%.

The feeding of fat or different FA during the prepartum period had no or little effect on the FA profile of plasma of calves at 30 to 60 d of age (Table 4-4). Proportion of total saturated FA in plasma of calves born from cattle fed SFA was greater ($P = 0.01$) than in those born from EFA-fed cattle, however only proportions of C12:0 (0.74 vs. 0.53%) and C14:0 (3.7 vs. 3.4%) were increased ($P \leq 0.05$). The LA and DHA were in greater and lower concentrations in newborn calves born from cattle fed EFA instead of SFA, respectively and the same pattern tended to be evident ($P \leq 0.10$) in plasma of calves at 30 to 60 d of age. Interactions of dam diet and MR were not detected for any FA except

2 minor FA, C12:0 and C14:1 and some EFA derivatives. Calves fed HLA MR instead of LLA MR and born from cattle fed control diets tended to have a decreased proportion of plasma AA (3.21 vs. 2.82%) and DHA (0.26 vs. 0.20%) whereas proportions of AA (3.15 vs. 3.10%) and DHA (0.22 vs. 0.22%) in plasma of calves fed LLA and HLA MR respectively, and born from dams fed fat, did not differ (FAT by MR interaction, $P \leq 0.10$). Likewise, calves fed HLA MR and born from cattle supplemented with EFA had a greater proportion of plasma DPA (0.36 vs. 0.28%) whereas DPA proportions in plasma of calves were not affected (0.30 vs. 0.30%) when fed HLA and born from SFA-supplemented cattle (FA by MR interaction, $P = 0.01$).

As expected, the main factor affecting plasma FA atd 30 to 60 was the type of MR fed. Plasma concentrations of LA and ALA at birth did not differ in calves assigned to receive LLA or HLA MR treatments. By replacing a portion of the CCO in the LLA MR with porcine lard in the HLA MR, the proportions of MCFA were decreased ($P < 0.01$, Table 4-4) in plasma, namely C12:0 from 0.82 to 0.48% and C14:0 from 4.8 to 2.3%. Likewise, feeding HLA MR decreased proportion ($P < 0.01$) of C18:1 ω 9, from 11.3 to 10.1%. Fatty acids found in greater concentrations in porcine lard compared to CCO were increased in plasma of calves fed porcine lard, namely C16:1 (1.1 vs. 1.4%, $P < 0.01$), LA (40.9 vs. 46.3%, $P < 0.01$), and ALA (0.68 vs. 0.81%, $P < 0.01$). Calves fed HLA had an reduced proportion of intermediate FA perhaps reflecting attenuation of the enzymatic elongation and desaturation processes of LA, namely GLA(0.19 vs. 0.35, $P < 0.01$), C20:3 (0.95 vs. 1.35%, $P < 0.01$), and AA (3.0 vs. 3.2%, $P = 0.05$) but not of C22:4 (0.23 vs. 0.24, $P = 0.84$). Responses of the n-3 FA to feeding HLA MR were not consistent. Plasma proportions of EPA were decreased (0.12 vs. 0.07%, $P < 0.01$), of

DPA were increased (0.29 vs. 0.33%, $P < 0.01$), and of DHA were unchanged (0.23 vs. 0.22%, $P = 0.40$). Despite similar intakes of MR, total FA concentration in plasma was about 8% less in calves fed HLA vs. LLA MR (1.94 vs. 2.09 mg/100mL of plasma, $P = 0.01$).

The dietary FA profile changed when grain feeding started at 31 d of age and this resulted in a change in the FA profile of the plasma of calves at 30 compared to 60 d of age (Figure 4-1). Plasma proportions of C16:0, LA, AA, and DHA decreased ($P < 0.01$) whereas plasma proportions of C14:0, C18:0, and ALA increased ($P \leq 0.04$) in calves at 30 compared to 60 d of age. Interaction of age and MR were not significant ($P > 0.05$) for any FA except LA was reduced to a greater extent due to age when LLA was fed (42.1 vs. 39.7% of total FA) instead of HLA MR (46.6 vs. 46.0% of total FA, MR by age interaction, $P = 0.08$).

Measures of Growth and Feed Efficiency

Body weight of calves at birth did not differ due to dam diet and averaged 40.2, 41.5, and 41.0 kg for calves born from dams fed Control, SFA, and EFA diets respectively (Table 4-5). Male calves enrolled in the HLA MR group were heavier than that of male calves enrolled in the LLA MR group (45.3 vs. 42.0 kg), whereas mean female birth weights did not differ (37.6 vs. 38.6 kg, MR by gender interaction, $P = 0.04$, data not shown). Serum concentrations of IgG measured 24 to 30 h after feeding colostrum were not affected by dam diet or MR. All calves but one had ≥ 1 g of total IgG per 100 mL of serum which indicates an appropriate passive transfer (Tyler et al., 1996; Weaver et al., 2000). The calf that failed to meet an appropriate passive transfer (0.65 g of IgG/dL) was born from a SFA cow and assigned to the HLA MR.

Calves fed the HLA MR had consistently greater ADG than calves fed the LLA MR (an increase of 18, 9 and 15% for the first 30 d ($P = 0.02$), the second 30 d ($P = 0.05$), and the whole 60-d period ($P < 0.01$), respectively, Table 4-5) for both female and male calves. Total intake of grain mix (mean of 11.7 kg of DM across genders) during the last 30 d of the study was not affected by type of MR fed. However intake of MR was greater ($P = 0.03$) for calves fed the HLA MR during the 31 to 60-d period because the heavier calves in the HLA group would have been offered more MR per the design of the feeding regimen. Nevertheless total DMI (kg or kg as a % of BW) did not differ between MR groups over the 60-d study. This improved gain without changing DMI over the 60 d resulted in better efficiency ($P = 0.01$) of BW gain from feed intake during the 60-d study for calves fed the HLA MR (0.63 vs. 0.59). Therefore the improved ADG and FE was due to the superiority of the HLA MR formulation rather than to greater intake of the grain mix. The effect of the HLA MR was independent of the type of diet fed to the dams of the calves (dam diet by MR interaction, $P > 0.10$). However the type of fat supplement prepartum did influence calf performance. Calves of both genders born from cattle fed SFA prepartum gained more BW over the 60-d period ($P = 0.04$) compared with calves born from cattle fed EFA prepartum (30.0 vs. 27.4 kg). This greater gain was due to a tendency ($P = 0.07$) for calves to consume more DM (48.8 vs. 45.6 kg) mainly as a result of a tendency for greater intake of grain mix during the last 30 d of the study (13.1 vs. 10.9 kg of DM, $P = 0.06$). However FE of calves was not improved by feeding SFA prepartum.

Metabolic and Hormonal Profile

Concentrations of plasma glucose were greatest at 2 d of age, exceeding 100 mg/dL, but decreased to between 85 and 95 mg/dL for the remainder of the study

(effect of age, $P < 0.01$, Figure 4-2). Although mean concentrations of plasma glucose were not affected by dam diet, mean plasmatic concentration of glucose tended to be greater at 2 d of life but lower at 19 and 30 d of life for calves born from cattle fed fat compared to calves born from control cows (dam diet by age interaction, $P = 0.07$, Figure 4-2). Mean glucose concentration in plasma was 3.1 percentage units greater (92.7 vs. 89.9 mg/100 dL, $P = 0.03$) in calves fed HLA than in calves fed LLA (Table 4-6). This was true throughout the 60-d study as the MR by age interaction was not significant. Plasma concentrations of PUN were greater the first 30 d and began decreasing upon initiation of grain intake (effect of age, $P < 0.01$, Figure 4-3). Mean concentration of PUN was greater ($P = 0.05$) in calves born from dams fed fat (8.27 vs. 7.61 mg/dL) than dams fed control diets. Mean plasma concentrations of PUN tended to be lower ($P = 0.06$) for calves fed HLA 7.75 vs. 8.35 mg/dL and this held true throughout the study (Figure 4-3).

Plasma concentrations of BHBA peaked during the second week of life, gradually decreased until 30 d of age, then gradually increased once grain intake began (effect of age, $P < 0.01$, Table 4-6, Figure 4-4 A). Mean concentration of BHBA in plasma of calves born from cattle fed fat tended to be greater than that for calves born from control dams (1.21 vs. 0.94 mg/dL, $P = 0.06$). Calves fed LLA MR had greater mean concentrations of plasma BHBA than those fed HLA MR (1.36 vs. 0.87 mg/dL, $P < 0.01$). Plasma concentrations of NEFA were greatest in the first wk of life (approximately 312 $\mu\text{Eq/L}$), gradually decreasing for 3 wk before plateauing at less than half of initial values of approximately 150 $\mu\text{Eq/L}$ (Figure 4-4 B, effect of age, $P < 0.01$). Neither prepartum nor preweaning diets affected concentrations of plasma NEFA.

Plasma concentrations of total cholesterol rose from approximately 30 mg/dL at birth to ≥ 120 mg/dL by 60 d of age (effect of age, $P < 0.01$, Figure 4-5). Both the type of dam diet and MR affected plasma cholesterol. Calves fed HLA MR, regardless of the diet fed to their dams, had lower plasma concentrations of total cholesterol starting at approximately d 19 compared to those fed LLA MR (MR by age interaction, $P = 0.01$, Figure 4-5). In addition, the dam diet tended to influence the effect of the MR. Plasma cholesterol concentrations of calves born from control dams were not affected by MR (87.9 vs. 85.3 mg/dL) but concentrations tended to be greater when calves born from dams fed fat were fed LLA vs. HLA MR (96.1 vs. 82.1 mg/dL, FAT by MR interaction, $P = 0.08$).

Plasma concentrations of insulin were low at birth as expected, but doubled once feeding commenced (Figure 4-6 A, B). Concentrations were relatively steady until grain intake began (after wk 4) after which concentrations increased as a mean of all diets (effect of age, $P < 0.01$). Neither dam diet nor MR affected mean concentration of plasma insulin although feeding HLA MR resulted in a greater numerical mean concentration of plasma insulin compared to feeding LLA MR (1.44 vs. 1.28 ng/mL, $P = 0.14$, Table 4-6). For IGF-1, plasma concentrations were greatest at birth, decreased to $< \text{half}$ 2 wk later, then rising until reaching concentrations by 8 wk, similar to those recorded at birth (effect of age, $P < 0.01$, Figure 4-7 A, B). In a similar pattern to that for insulin, calves fed HLA tended to have greater mean concentrations of plasma IGF-1 compared to those fed LLA (59.7 vs. 53.2, $P = 0.08$). Concentrations of STP were not affected by prepartum or preweaned diets, but greater concentrations were seen the first wk of life (Figure 4-8).

Incidence of Diarrhea and Poor Attitude

Calf attitude was generally responsive throughout the 60-d study with a mean of 1.04 (Table 4-7). Likewise fecal consistency across the study also was quite acceptable with a mean of 1.18. Severity (greater mean score) of poor attitude was greater during the first 2 wk of age whereas severity of diarrhea increased at 2 wk of age (age, $P < 0.01$, Figures 4-9 and 4-10). Neither main effects of prepartum diet nor type of MR had any effect on scores. However, the mean score for attitude tended to be greater in calves fed HLA vs. LLA MR if they were born from control cattle (1.06 vs. 1.03) but were not changed if the dam was fed either fat source prepartum (1.04 vs. 1.03, FAT by MR interaction, $P = 0.06$). This pattern also was true for mean fecal score. The mean score for feces was greater in calves fed HLA vs. LLA MR if they were born from control cattle (1.22 vs. 1.12) but were not changed if the dam was fed either fat source prepartum (1.21 vs. 1.17, FAT by MR interaction, $P = 0.03$). The treatment effects on the percentage of days with poor attitude and diarrhea followed the same pattern. During the first 30 d, feeding HLA rather than LLA MR to calves born from dams not fed fat increased the percentage of days with poor attitude (12.3 vs. 5.3%) whereas no effect of MR was detected on attitude if fat was fed to calves born from dams fed fat prepartum (5.8 vs. 8.0%, FAT by MR interaction, $P = 0.01$). This was a 2-d difference in poor attitude during the first 30 d of life. This same interaction was detected ($P = 0.02$) for attitude when the first 60 d were evaluated. Feeding a HLA MR proved beneficial if dams were fed fat prepartum. During the first 30 d of life, percentage of days with diarrhea were reduced if calves born from dams fed fat were fed the HLA MR (9.2 vs. 15.4%) whereas diarrhea days were increased by feeding HLA MR to calves born from

dams not fed fat (5.3 vs. 12.3%, FAT by MR interaction, $P < 0.01$). This same interaction was detected ($P < 0.01$) for attitude when the first 60 d were evaluated.

Blood Cell Population

Concentrations of total red (mean of $8.4 \times 10^3/\mu\text{L}$) and white (mean of $8.65 \times 10^3/\mu\text{L}$) blood cells were not affected by diets but increased with age ($P < 0.01$, Table 4-8, Figures 4-11 A and B). Similarly, blood concentrations of neutrophils (mean of $3090/\mu\text{L}$), monocytes (mean of $380/\mu\text{L}$), and basophils (mean of $110/\mu\text{L}$) were not affected by diets but by age ($P < 0.01$, Figure 4-12 A, 4-13 A and 4-15 respectively). Concentrations of blood neutrophils were greater the first wk of life and decreased to the lowest starting at 2 wk of life which matches with the period of greatest health challenges. Lymphocyte concentrations were greater in calves fed HLA vs. LLA MR (4.61 vs. $4.20 \times 10^3/\mu\text{L}$, $P = 0.04$) and increased with age ($P < 0.01$, Figure 4-12 B) with the greatest increase occurring between birth and 2 wk of age. Blood concentrations of eosinophils of calves fed HLA MR tended to be greater at 7 and 14 d of age compared to those fed LLA MR (MR by age interaction, $P = 0.07$, Figure 4-13 B). This decrease in eosinophil concentration at d 7 of life of calves fed LLA MR occurred primarily in calves born from cattle fed the control or SFA diets prepartum (dam diet by MR by age interaction, $P = 0.01$, Figures 4-14 A and B). Platelet concentrations in calves increased 2 to 3 fold from birth to the second week of age and then gradually decreased (effect of age, $P < 0.01$, Figure 4-16 A). Feeding EFA prepartum resulted in calves having lower platelet concentrations at 7 d of age ($P = 0.06$) but greater concentrations at 60 d of age ($P = 0.05$) compared with other diets (dam diet by age interaction, $P = 0.03$, Figure 4-16). Mean platelet concentration was greater for calves fed LLA vs. HLA MR (801 vs. $715 \times 10^3/\mu\text{L}$, $P = 0.03$, Figure 16 B).

Proportion of individual classes of white blood cells (%) followed the same pattern as their concentration per μL of blood (Table 4-8) with two exceptions. Proportion of lymphocytes was not affected by the MR fed but that of monocytes was greater for calves fed HLA vs. LLA MR and born from cattle fed the control diet (4.51 vs. 4.09%) whereas the opposite was true for those born from cattle fed fat prepartum (3.87 vs. 4.32%, FAT by MR interaction, $P = 0.05$). Calves fed HLA MR tended to have greater hematocrit than those fed LLA MR (35.9 vs. 34.4%, $P = 0.08$). Concentrations increased after birth but started falling after 9 d of age until d 42 when they increased again (Figure 4-17).

Expression of Adhesion Molecules and Phagocytic Activity of Neutrophils

Proportion of neutrophils expressing CD18 and CD62L was not affected by dam or calf diets and means were 94.4 and 98.2% across diets, respectively. Likewise the MFI of CD18, an indicator of mean number of CD18 expressed per neutrophil, was not affected by diets. However MFI of CD62L tended to be greater in calves born from dams fed the control diet than calves born from dams fed fat-supplemented diets (382 vs. 338, $P = 0.10$, Table 4-9, Figure 4-18). Mean fluorescence intensity, an indicator of the number of *E. coli* phagocytized per neutrophil, was greater for calves born from dams fed EFA compared to those born from dams fed SFA (121 vs. 113, $P = 0.04$, Figure 4-19 A) however calves born from dams fed SFA tended to have greater concentrations of phagocytic neutrophils (3.40 vs. $2.89 \times 10^3/\mu\text{L}$ of blood, $P = 0.08$, Figure 4-19 B) in blood. Phagocytic activity of blood neutrophils tended to be greater for calves fed HLA vs. LLA MR (96.3 vs. 95.6%), with the difference observed after 7 d of age (Figure 4-20).

Concentration of Acute Phase Proteins

Plasma concentration of ASP was greatest right after calving (~230 mg/L) and decreased gradually until plateauing at ~60 mg/L around 30 d of age (effect of age, $P < 0.01$, Figure 4-21). Feeding HLA rather than LLA MR reduced ASP concentrations to a greater extent in calves born from control dams (94.1 vs. 72.3 mg/L) compared to the response in calves of dams fed fat prepartum (90.0 vs. 82.0 mg/L, FAT by MR interaction, $P = 0.04$, Table 4-10). Concentration of ASP was lower in calves fed HLA (78.8 vs. 91.4 mg/L, $P < 0.01$) but the difference tended to be accentuated after 12 d of age (Figure 4-21, MR by age, $P = 0.09$). Calves born from dams fed fat tended to have increased plasma concentrations of haptoglobin (1.04 vs. 0.95, $P = 0.06$) and the concentrations increased after 2 d of age reaching a peak at 9 d of age (Figure 4-22 A).

Humoral and Cell Mediated Immune Responses

Injection of OVA into calves at 2 and 20 d of age did not have any effect on the concentration of anti-OVA IgG in serum, whereas the increase was minimal after the 3rd injection at 40 d of age (Figure 4-22A). Production of bovine anti-OVA IgG was greater in calves born from dams fed SFA than in calves born from dams fed EFA between 2 and 20 d of age (dam diet by age interaction, $P < 0.01$, Table 4-10, Figure 4-22 B).

Production of IFN- γ by PBMC stimulated with concanavalin-A is presented as the difference in concentrations of stimulated minus nonstimulated cells. In general, values of IFN- γ produced were low and close to the sensitivity value of the commercial kit used. At 15 d of age, calves born from cows fed SFA tended to have greater differential production of IFN- γ than calves born from dams fed EFA (44.1 vs. 23.3 pg/mL, $P = 0.08$, Table 4-10). At 30 d of age, stimulated PBMC from calves fed the HLA MR had a

greater differential production of IFN- γ than calves fed the LLA MR (48.1 vs. 25.6 pg/mL, $P = 0.05$).

Discussion

Prepartum Supplementation of Fatty Acids Affects FA Profile and Immunity Measures of Calves

Calves have a high demand for EFA derivatives such as DHA for central nervous system development. However the epitheliochorial placenta of cows is less permeable to free FA, partially limiting their uptake (Moallem and Zachut, 2012). In sheep (Campbell et al., 1994) and humans (Koletzko et al., 2007) a preferential materno-fetal transfer of DHA across the placenta has been demonstrated, which is aided by the presence of placental FA transport proteins. Mean plasma concentrations of total FA at birth were similar to those reported by Jenkins et al. (1988) for 3-d old calves but greater than that of Noble et al. (1975) for newborn calves. The FA profiles of calf plasma were quite similar to those reported by Moallem and Zachut (2012) for newborn calves from cows fed 240 g/d of saturated fat, 300 g of linseed oil, or 300 g of FO prepartum. In this study, only proportions of ALA and DHA differed due to diet.

Dams supplemented with EFA had an expected daily intake of 116 g of LA compared to 57 and 62 g/d for dams fed no supplemental fat or SFA, respectively. Intake of ALA was influenced minimally by the type of fat supplemented. As previously reported for newborn lambs (Noble et al., 1978; Soares, 1986), calves born from cows fed EFA (rich in LA) had increased concentrations of LA in plasma but AA concentration was unaffected by type of diet. However FA such as GLA and C20:3 n-6, which are precursors of AA in the elongation-desaturation steps, were greater in calves born from dams fed EFA, in agreement with the findings of Soares (1986) studying lambs born

from LA-supplemented ewes. The increased proportions of these intermediate FA might indicate that the enzymatic activity of FA desaturases and elongases that are the same for both n-6 and n-3 groups of FA were preferentially metabolizing LA over ALA in dams supplemented with fat enriched in LA, although final end products of AA and C22:4 were not increased significantly.

Interestingly, supplementing SFA prepartum increased the proportions of EPA and DHA in plasma of newborn calves. This result is opposite to that of Elmes et al. (2004) who reported that increased intake of LA in pregnant ewes not only increased the proportion of LA, GLA, C20:3 n-6, and AA but also of DPA and DHA but not of ALA. These authors concluded that the overall activity of desaturases and elongases were very active in ewes fed more LA, so that the synthesis of longer chain FA were enhanced in both n-6 and n-3 FA groups. Burdge and Calder (2005) reviewed 23 studies supplementing ALA and concluded that greater supplementation of ALA prioritized the synthesis of its derivate LCFA, similarly greater supplementation of LA should increase the synthesis of its derivatives. However Moallem and Zachut (2012) did not find increased proportions of ALA derivatives (EPA and DHA) when feeding prepartum cows linseed oil as compared to cows supplemented with saturated FA. These results indicate that the enzymatic processes of desaturation/elongation were either not activated by the increased supply of ALA or that the extra supply of ALA was metabolized.

On the other hand, the greater synthesis of n-6 derivatives (GLA and C20:3) in plasma of calves from dams fed LA in the current study might have depressed the elongation and desaturation of ALA, hence calves born from cattle fed EFA had lower

proportions of those ALA-derived FA (EPA and DHA). The current results are in agreement with most studies using humans where supplementation of high amounts of LA reduced the synthesis of long chain n-3 FA, thus favoring the elongation of n-6 FA because of competition for $\Delta 6$ desaturase, the first limiting enzyme in this process. Chan et al. (1993) reported increased concentrations of EPA in the plasma phospholipid fraction of men fed low LA whereas Liou et al. (2007), feeding healthy men a diet rich in LA, reported greater concentrations of LA but lower concentrations of EPA in the plasma phospholipid fraction.

Another important finding is the parity effect on proportion of EFA and their derivatives. Calves born from nulliparous heifers had increased plasma concentrations of n-3 FA such as EPA, DPA, and DHA but decreased LA and AA. Although the plasma of dams was not analyzed for FA, the FA profile of colostrum was analyzed. Nulliparous heifers produced colostrum with greater concentrations of ALA, AA, EPA, DPA, and DHA whereas LA was greater in colostrum of parous cows (Chapter 3). A previous study in humans reported a negative relationship of parity with DHA concentrations in blood of mothers and their neonates (Al et al., 1997) but another study did not detect negative effect of increased parity on dam n-3 FA in the offspring (Van Gool et al., 2004). It is not known why mature cows might have a preferential synthesis of FA derivate from LA instead of those derived from ALA, which could increase the risk of deficiency of critical FA for brain development in offspring. However, nulliparous animals may have mobilized fat with greater proportions of PUFA and possibly transferred this to their calves because unluckily parous cows, they were raised in sod-base pens, with some access to pasture.

Several studies have reported that undernutrition during pregnancy can decrease birth weight in humans (Naeye et al., 1973; Kramer, 1987) and sheep (Osgerby et al., 2002; Dwyer et al., 2003). Yet, the fetal metabolic environment can have long-term metabolic effects on the offspring without necessarily affecting birth weight (Pettitt et al., 1987; Ferezou-Viala et al., 2007). However supplementation of different lipid sources to nutritionally adequate diets for pregnant beef cows have not affected calf birth weight (Hess, 2003; Banta et al., 2006; Banta et al., 2011) when isocaloric and isonitrogenous diets were fed.

Dams fed SFA ate more DM than dams fed EFA (Greco et al., 2010) and calves born from dams fed SFA were numerically 0.5 kg heavier than calves born from dams fed EFA. Whether these positively related responses of DMI and birth weight (Osgerby et al., 2002; Dwyer et al., 2003) were the drivers promoting increased grain intake (75 g/d average) during 31 to 60 d of age by calves born from cows fed SFA is unclear. This greater intake of grain helped contribute to calves gaining 2.7 kg more between birth and weaning than calves born from dams fed EFA. This increased intake of grain would not necessarily cause a change in plasma concentrations of energy and protein metabolites. Feeding a grain mix along with MR to dairy calves did not change plasma concentrations of glucose, insulin, BHBA, or IGF-1 compared to calves fed MR alone (Laarman et al., 2012). Similarly calves born from dams fed SFA and EFA did not differ in plasma concentrations of glucose, PUN, IGF-I, insulin, and BHBA.

Calves born from dams fed the control diet had lower concentrations of PUN than those fed fat. Elevation of circulating PUN could result from supply of more protein than the calf could utilize. Bascom et al. (2007) fed calves with MR of 29 or 20% CP, and

found reduced concentrations of PUN in calves fed the 20% CP MR. However other studies feeding increased concentrations of CP in MR did not affect PUN concentrations (Daniels et al. 2008). Metabolism of dietary nutrients basically occurs after feeding, hence it would be improbable that dietary CP in prepartum diets could directly affect PUN concentrations of their offspring in early life than is the MR fed, even more considering that all three prepartum diets were isonitrogenous. However, it might be that a low-fat diet prepartum modified the ability of calves to use energy and protein to meet their needs, although plasma concentrations of glucose were not affected by prepartum diets.

In addition to aiding the clotting process, platelets, have been reported to be involved in recruitment of leukocytes to sites of vascular injury and inflammation and release of pro- and antiinflammatory factors, all mostly associated with incidence of atherosclerosis, sepsis, or hepatitis (Smyth et al, 2009). Lam et al. (2011) reported that platelets enhanced transendothelial migration of neutrophils. It is important to indicate that regardless of the diet, plasma concentrations of platelets increased dramatically during the first 2 wk of life which is in agreement with Knowles et al. (2000) and Brun-Hansen et al. (2006) and support the hypothesis that platelets have a clear role enhancing neutrophil migration to injured tissues in calves undergoing an outbreak of diarrhea.

Activity of immune cells, more than their concentration per se, could be influenced by the FA composition of their membrane. This may affect cell signaling, production of eicosanoids, and fluidity to modify activity of receptors or their expression by regulating activity of target genes (Jump, 2002; Yaqoob and Calder, 2007; Calder, 2012). The

importance of CD62L and CD18 expression on the neutrophil surface is due to their role in the processes of rolling and tethering neutrophils on the endothelium to enhance its migration to the injured tissue (Simon et al., 2000; Ley et al. 2007). These receptors are said to be constitutively expressed, therefore they should not be influenced by diet as happened in the current study and other using calves (Pang et al. 2009; Corrigan et al., 2009). However, current findings contrast to that of Novak et al. (2012a) who reported a lower proportion of monocytes expressing CD62L in diarrheic calves and to Silvestre et al. (2011) who reported an increase in percentage of neutrophils positive to CD62L and CD18 in transition dairy cows fed Ca salts of SAO.

Even though CD62L and CD18 are constitutively expressed, they could be down regulated or enhanced according to the animal's physiological status and dietary management. After calving, a lower number of CD62L expressed per neutrophil was associated with neutrophilia in cows, which might indicate the inability of neutrophils to migrate to the infection zone, hence increasing the risk of infections (Weber et al. 2001). A similar association was reported in abruptly weaned calves 2 d postweaning when compared with preweaned calves (Lynch et al., 2010). In this study, circulating neutrophils from calves born from dams fed fat tended to have a decreased number of CD62L receptors (MFI) compared to calves born from cows not fed fat. Weber et al. (2001) and Lynch et al. (2010) indicated that adhesion molecule expression can be inversely related to neutrophil concentration because the greater the adhesion intensity, the greater the movement of neutrophils out of circulation. If this relationship holds true in the current study, calves born from fat-supplemented dams would have experienced a reduced movement of neutrophils from the blood stream.

Neutrophil function is incomplete if the neutrophils that are able to migrate to the infection zone are not able to phagocytose pathogens. Consequently an enhanced ability to phagocytose would potentially result in reduced incidence of diseases. However, such effects have been equivocal in studies using calves to evaluate the effect of different stressors on phagocytic activity of blood neutrophils (Pang et al., 2009; Hulbert et al., 2011). Circulating neutrophils in calves born from dams fed EFA phagocytized more bacteria per neutrophil compared to those of calves born from dams fed SFA. Although the neutrophils in EFA calves were more efficient, number of circulating neutrophils with ability to phagocytose tended to be lower, likely resulting in similar number of bacteria phagocytized by neutrophils in calves from dams fed the 2 fat sources.

Actual concentrations of Hp increased before the attitude and fecal scores reached their highest point, which agree with studies reporting the validity of Hp as a predictor of the inflammatory process (Ganheim et al., 2007; Cray et al. 2009). Haptoglobin is absent or present in very low concentrations in healthy animals but under subclinical inflammatory disorders, its concentration increases (Ganheim et al., 2007; Cray et al., 2009). When calves had respiratory and digestive tract infections, plasma concentrations of Hp were increased compared to healthy calves (Deignan et al., 2000; Heegaard et al., 2000; da Silva et al., 2011). In the current study calves born from dams fed SFA or EFA had greater plasma concentrations of Hp compared to calves born from cows fed the control diet, which was due to a greater rise in concentration at the time Hp peaked in all calves (~9 d of age). This agrees with the finding of Bueno and coworkers (2010) who reported that mice supplemented with lard (rich in long chain SFA) instead of SO increased the expression of genes coding for Hp in white adipose

tissue. Authors hypothesized that lard could induce a proinflammatory condition, increasing the production of proinflammatory cytokines which are inducers of acute phase protein production. In the current study, the time when Hp reached its highest concentration was between 5 and 9 d of age which coincided with the period of initiation of diarrheic events in calves.

Interferon- γ is a cytokine with a variety of roles such as enhancement of antigen presentation, cell cycle growth and apoptosis, leukocyte trafficking, and B cell depletion (Arens et al., 2001; Chen and Liu, 2009). On the other hand, Ig, specifically IgG, directly could kill or neutralize pathogens or indirectly serve as a cell-surface receptor for antigens permitting cell signaling and activation through its presentation by professional antigen presenting cells (Schroeder and Cavacini, 2010; Rath et al, 2012). Stimulating the PBMC from 15-d old calves born from dams fed SFA resulted in increased production of IFN- γ and a consistently greater plasma concentration of anti-OVA IgG from 2 to 20 d of age when compared to calves born from dams fed EFA. Anti-OVA IgG concentrations found in calves were primarily those derived from passive transfer with colostrum because all dams were vaccinated with OVA prepartum.

Newborn calves have a biased preferential T helper-2 (Th2) response, which is responsible for a strong antibody production and a reduced Th1 response (Chase et al., 2008). The Th1 type cytokines play a key role initiating early resistance to pathogens and induction of cell-mediated immunity (Marodi, 2002). In the current study, greater production of IFN- γ in calves born from dams fed SFA might indicate a switching from a Th2- to a Th1-mediated immune response. This preferential Th pattern might be aided by the greater anti-OVA IgG response. Total IgG concentrations in serum were also

greater (Chapter 3) in calves born from dams fed SFA after 24 to 30 h of colostrum feeding. Other immune cells in colostrum were not measured but it is possible that colostrum from dams fed SFA in addition to having greater total IgG might have had a greater number of CD4+, CD8+, and $\gamma\delta$ T cells, because the latter two cell types can produce IFN- γ (Hagiwara et al., 2008).

In summary, prepartum supplementation of EFA changed the FA status of calves as evidenced by changes in their FA profile. Feeding fat prepartum did not have a negative influence on health and immunity with the exception that plasma concentrations of haptoglobin were greater at 5 and 9 d after birth suggesting that inflammation was increased in these calves whereas lower expression of CD62L indicated a reduced proinflammatory response. Specific source of FA differentially affected some markers of immune response such as concentrations of anti-OVA IgG, and production of IFN- γ . Calves born from dams fed SFA gained more BW overall and this may have been due to greater intake of grain than calves born from EFA-supplemented dams. However this increased intake did not affect the concentration of energetic metabolites and anabolic hormones

Feeding Milk Replacer Enriched with Linoleic Acid Improved Growth, Feed Efficiency, and Immune Responses

Calves are born as preruminants with a preference for milk intake which delays the initiation of ruminal development or makes it very limited until grain intake increases. Consequently, metabolism of nutrients in the rumen, including fat, is very limited. Limited microbial activity in the underdeveloped rumen prevents or limits hydrolysis and biohydrogenation of dietary FA; thus the FA profile of plasma of preweaned calves is expected to reflect the diet.

Newborn calves, assigned to either MR, at birth had similar plasma concentrations of LA, ALA and all their FA derivatives as well as total plasmatic concentrations of these FA. Plasma concentrations of LA increased markedly at 30 and 60 d of life from that of birth (~11.5 fold increase). This change occurred gradually starting right after the first day of life (~ 2.5 fold increase from that at birth). Concentrations of LA became relatively stable around 3 wk of age (Noble et al., 1975). Two potential mechanisms occurring in placenta might account for the decreased plasma concentrations of LA in newborn calves, namely increased desaturation activity in the placenta and selective uptake of FA by placental FA-binding proteins (Moallem and Zachut, 2012). Gradual increase of LA postpartum might be a combination of a release from the regulatory effects of the placenta in transferring FA and an enhancement by the increased dietary intake of fat from colostrum, milk, and grain.

Concentration of total FA in plasma was less in calves fed the HLA MR which may have resulted from a greater digestibility of the FA in porcine lard compared to CCO. Murley et al. (1949) reported that plasma fat concentration was reduced in calves consuming a more vs. a less digestible SO. The effect of feeding MR of different FA profiles had a profound impact on the FA profile of calf plasma. Feeding a MR containing a highly saturated FA fat source (CCO containing a high concentration of medium chain FA, LLA) resulted in elevated plasma concentrations of C10:0, C12:0, and C14:0. These results are in agreement with previous studies supplementing short and medium chain FA in humans (Hill et al., 1990) and calves (Jenkins and Kramer, 1986). Reveneau et al. (2012) found increased proportions of medium chain FA in omasal digesta of CCO-supplemented cows, resulting in milk with greater proportions of

medium chain FA. Swift et al. (1990) reported that human enterocytes can incorporate medium chain FA as substrates for triglyceride synthesis when diets contain high proportions of those FA.

Other studies also have documented transfer of dietary medium chain FA into milk fat of dairy cows supplemented with CCO, even though efficiency of transfer decreased with increased intakes of CCO (Vyas et al., 2012; Hollmann and Beede, 2012). Likewise, calves fed a MR containing a combination of CCO and a highly unsaturated FA fat source (porcine lard containing mainly C18:1 and LA, HLA) had increased plasma concentrations of LA and ALA. These results were similar to the findings of Wrenn et al. (1973), and Jenkins and Kramer (1986, 1990) when diets rich in LA or ALA were fed to calves during the preweaning period.

Plasma of calves fed HLA MR at 30 to 60 d of age had decreased proportions of LA derivatives (GLA, C20:3, and AA) compared to those fed LLA MR from 30 to 60 d of age. However, newborn calves born from dams fed EFA as compared to those fed SFA had similar proportions of all identified LA derivatives. The lack of effect in LA derivatives when feeding EFA prepartum, particularly in AA, also contrasts with several previous studies which reported an increased concentration of AA due to enhanced synthesis from its parent FA, LA (lambs, Soares, 1986; rats, Lands et al., 1990; and pigs, Novak et al., 2008) and also was recently reviewed by Gibson et al. (2011). It is important to point out that the liver of calves fed HLA MR tended to have greater proportions of AA (Chapter 5).

Jenkins et al. (1985) reported a greater proportion of LA but decreased AA in plasma of calves fed CO compared to those fed CCO or beef tallow as the fat sources

in MR. Later Jenkins and Kramer (1990) found that increased intake of LA from CO increased LA but reduced AA concentration in plasma compared to calves fed tallow plus CCO which agrees with our current findings. Authors suggested a reduced activity of $\Delta 6$ desaturase in calves fed CO that not only reduced synthesis of AA but also that of EPA yet concentrations of DPA and DHA did not change. In the current study calves fed HLA MR had increased plasma concentrations of DPA. It is not clear why, in early life, calves fed HLA MR may not have enhanced desaturase/elongase activity favoring elongation of LA as that of pregnant dams fed greater amounts of LA. A potential reason might be that neonatal calves have a preferential synthesis of DHA to cope with the needs of brain tissue, enhancing DHA synthesis regardless of the balance of LA and ALA. This hypothesis is supported by the similar plasma concentrations of DHA in calves fed any of the MR and by the increased proportions of EPA and DPA, precursors of DHA synthesis, found in calves fed LLA and HLA MR, respectively.

Improved ADG and FE in calves fed HLA MR were consistent during the periods of feeding MR alone (1 to 30 d), MR plus grain mix (31 to 60 d), and total experimental days (1 to 60 d). Efficiency of gain was improved because calves fed HLA MR had greater ADG and no difference in MR intake and total DMI during the first and second 30 d, respectively. Studies regarding the feeding of increased amounts of LA on calf performance are limited. Beef calves born from cows supplemented with safflower seed rich in LA during the prepartum and lactation periods resulted in calves having similar BW at birth and at weaning, even though diets rich in LA were more energetically dense (Encinias et al., 2001, 2004; Bottger et al., 2002;). When Lake and coworkers (2005, 2006a,b,c) fed isocaloric and isonitrogenous diets enriched in LA to lactating beef cows,

BW at weaning was not affected. Intake of LA from the control diets and intake of LA from dry feed that calves were consuming may have been sufficient so that intake of LA above those values did not improve calf performance.

The first studies using dairy calves that totally replaced milk fat with vegetable oils rich in LA resulted in poor ADG and FE. However the major reason for poor diet utilization (greater incidence of diarrhea and poor gain) was the inferior quality of high LA vegetable oil (crude expeller SO) plus poor preparation of CO (large size of oil droplets) (Jacobson et al., 1949; Murley et al., 1949; Jenkins et al., 1985, 1986). However in current commercial practice, no MR is composed of 100% vegetable oils derived from long chain PUFA (≥ 18 C). A recent study by Lewis et al. (2008) supplementing a basal MR to orphaned lambs with daily intakes of 1 g of SAO or FO did not produce any change in ADG or FE. Authors did not report whether lambs had diarrhea. Jenkins et al. (1985) reported that ADG and FE by calves were not different when CCO (3.2% of FA as LA) or tallow (3.8% of FA as LA) were the sources of dietary fat. Hence both fat sources supported the same performance in calves even though mean carbon length of FA differed and LA supply was similar. The replacement of tallow by lard rich in LA in the current study could have an enhanced improvement of ADG and FE in calves fed CCO due to increased intake of LA.

Pattern of metabolites in plasma of calves are in agreement with the difference in growth performance between calves fed LLA and HLA MR. Regardless of age, calves fed HLA MR had increased plasma concentrations of glucose, which was accompanied by a tendency for increased plasma concentrations of IGF-I and only a numerical increase in plasma insulin ($P = 0.14$). Plasma concentrations of PUN and BHBA were

maintained lower in calves fed HLA MR, as well as concentrations of cholesterol starting to differentiate after 16 d of age. Calves in our current study were fed MR at 0600 h and blood collection occurred within 1 to 2 h after this first feeding, hence concentrations reported here are postprandial in response to the type of MR fed.

Fresh colostrum has been identified as a good source of endocrine factors and hormones such as insulin and IGF-I (Georgiev, 2008b, Blum and Baumrucker, 2008). Those compounds can exert effects on the gastrointestinal tract (GIT). The IGF-I gets into the GIT in an active form and survives the digestion process, having an important role in the growth and development of the GIT and on the functional maturation of the calf and its adaptation to the new external environment after birth (Georgiev, 2008a; Flaga et al., 2011). Prepartum diets did not affect plasma concentrations of these two hormones after colostrum feeding (Chapter 3). However calves randomly assigned to LLA MR had lower concentrations of insulin than calves assigned to the HLA MR before colostrum feeding but, once colostrum was fed, concentrations of insulin did not differ between calves fed the two MR. However, calves fed HLA MR tended to have greater plasma concentrations of IGF-I.

Preweaned calves fed an increased amount of nutrients had an enhanced ADG and FE, with increased plasma concentrations of insulin, glucose, and IGF-I as potent anabolic metabolites or hormones, with an additional reduction in PUN concentrations (Smith et al., 2002). This pattern of growth and metabolites are in complete agreement with our current findings. Similarly, Quigley et al. (2006) reported increased concentrations of glucose and IGF-I when calves were fed increased amounts of MR which was reflected in a greater ADG and improved FE. However Quigley and

coworkers (2006) observed an increase in PUN concentrations when calves had greater intake of MR which contrasts with Smith et al. (2002) and our current finding.

Etherton and Bauman (1998) reviewed the main functions of growth hormone (GH), with the enhanced synthesis of IGF-I in liver being one of the most important. Smith et al. (2002) administered external GH to calves and reported increased plasma concentrations of IGF-I as a response. Authors concluded that the GH:IGF-I axis was functional in preweaned calves. Under this assumption, it can be hypothesized that other functions of GH might be happening also in calves with greater circulation concentrations of IGF-I, one of which would be the reduction of amino acid oxidation associated with greater efficiency of protein accretion. This should reduce the need for amino acid catabolism and, as a consequence, a reduction in PUN concentrations. Another role of GH is the reduction in clearance and oxidation of circulating glucose, whereas in liver, GH should increase the output of glucose and might reduce the ability of insulin to inhibit gluconeogenesis. This should result in increased plasma concentrations of glucose as was reported by Smith et al. (2002) and in our current study. Recently, Piantoni et al. (2012) demonstrated the importance of the milk-feeding period in mammary development (functionality and growth). Preweaned heifers that consumed more nutrients from birth to 65 d of age (a MR of 28% CP and 28% fat vs. a MR of 20% CP and 20% fat) had a dramatic change in expression of genes (genes associated with cell morphology, cell to cell signaling, and immune responses) in mammary parenchyma and fat pad tissues. In an earlier study (Daniels et al., 2008) using the same feeding treatments, authors reported that heifers fed increased amounts of nutrients (28% CP and 28% fat) had a numerically greater plasma concentration of

IGF-I. Piantoni et al., (2012) speculated that this increased concentration of IGF-I might have been associated with some of the gene expression responses observed in their 2012 study.

Cholesterol and BHBA are synthesized primarily in liver of preruminant calves, as products of lipid metabolism. Both metabolites were in greater concentrations in plasma of calves born from dams fed LLA MR; these greater plasmatic concentrations were accompanied by an increased accumulation of lipids in the liver of these calves (Chapter 5). Considering that the production of BHBA by the ruminal epithelium due to minimal microbial activity is low, the increased plasma BHBA was likely due to incomplete oxidation of FA in the liver. Sato (1994) fed medium chain FA (C8 and C10) to neonatal calves causing a marked hyperketonemia within a few hours after feeding due to preferential transport of these FA through the portal vein and greater availability for FA oxidation and synthesis of ketogenic products. This same biological process likely was happening in calves fed more medium chain FA coming from CCO in the current study. Incomplete oxidation of medium chain FA by the liver was likely responsible for elevated plasma concentrations of BHBA of calves fed more CCO. Polyunsaturated FA have been reported to reduce circulating concentrations of cholesterol whereas medium chain FA such (C12:0, C14:0, and C16:0) have been identified as the most potent inducers of cholesterolemia (Fernandez and West, 2005). Cholesterolemic effect of CCO has been documented by Jenkins et al. (1985), Berr et al. (1993), and Chechi and Chema (2006) in rats. Fernandez and West (2005) stated that upregulation of low density lipoprotein receptors and increased activity of

cytochrome P450 7A are the most potential mechanisms by which n-6 FA reduces the concentrations of circulating cholesterol.

Increased concentration of hematocrit commonly is caused by calf dehydration due to diarrhea whereas a reduced concentration is associated with anemic conditions (Moonsie-Shageer and Mowat, 1993). Calves fed HLA MR tended to have a greater mean concentration of hematocrit as well as hemoglobin (data not shown). However it is unlikely that those calves were under-hydrated since type of MR did not affect the incidence of diarrhea. Lower concentrations of hemoglobin in calves fed LLA MR might indicate an increased risk of anemia. However hemoglobin concentrations were within the normal range for all preweaned calves (Brun-Hansen et al., 2006).

The blood lymphocyte population is primarily composed by $\gamma\delta$ T cells, CD4+, and CD8+ T cells, B cells, and natural killer cells; however their proportions change with calf age. Kampen et al. (2006) reported that the proportion of $\gamma\delta$ T cells in plasma of newborn calves is between 20 to 25% and they decreased with age. This proportional decrease is not due to a change in the number of cells but due to an increase in absolute number of CD4+ and B cells, with the latter being remarkably lower in younger calves and reaching adult proportions at 11 to 12 wk of age. Calves fed the HLA MR had an increased concentration of circulating lymphocytes. Therefore it could be assumed that these calves also had increased concentrations of CD4+, which are the precursors of T-helper cells that have a regulatory function in the interaction of innate and adaptive immunity, as well as increased B cells, components of the humoral adaptive immunity responsible for antibody production. All of these cells are potential aiding factors for an improved immune response of calves fed HLA MR.

Platelet concentrations in plasma of calves were within normal ranges reported for preweaned calves (Knowles et al., 2000). Platelet concentration increased by the second week in all calves whereas it was lower throughout the study in calves fed HLA MR. Platelets have been reported to enhance neutrophil migration by facilitating the endothelial membrane extravasation (Lam et al., 2011). However in vitro analysis of blood neutrophil expression of CD18 and CD62L was not affected by the type of MR fed indicating that platelet concentrations were not sufficiently depressed to affect the neutrophil-adhesion molecule relationship in calves fed HLA MR. On the other hand, results might indicate an antiinflammatory effect of LA considering studies that have related increased concentrations of platelets with development of various inflammatory diseases (Smyth et al., 2009).

One of the goals towards “maturity” of the neonatal calves’ immunity is the early switch from a preferential Th2 response to a Th1 response. The pattern of cytokine production is used to verify the predominant type of Th response. An increased concentration of IFN- γ with a constant or a decreased production of IL-4 is indicative of Th1 predominance (Chase et al., 2008). The in vitro stimulation of PBMC with concanavalin A produced greater concentrations of IFN- γ at 30 d of age of calves fed HLA vs. LLA MR. Foote et al. (2007) reported that calves at a high growth rate (1.16 kg/d) due to greater DM intake compared with calves at a low growth rate (0.11 kg/d) had similar production of IFN- γ by stimulated PBMC. Hence we could hypothesize that the increased intake of LA rather than the improved growth was responsible for the better IFN- γ response by calves fed HLA MR, although a study using human cells reported that LA inhibited production of IFN- γ by PBMC (Karsten et al., 1994). Fritsche

et al. (1997) reported no change in IFN- γ production by stimulated PBMC when they were cultured with lard, SO, or FO. However in vivo concentration of murine IFN- γ during listeriosis infection was elevated when FO was fed.

Acid soluble protein has dual inflammatory and immuno-modulatory properties. One of the mechanisms by which ASP can exert its antiinflammatory effect is by inhibiting platelet aggregation, hence platelet recruitment (Hochepped et al., 2003). In order to downregulate platelet aggregation and recruitment, a really high physiological concentration is needed (Costello et al., 1979). Based on this requirement, we hypothesize that the slight increased concentrations of ASP in calves fed LLA MR in comparison with calves fed HLA MR did not prevent platelet aggregation and migration that could lead to a potential negative effect of thrombosis and risk of exacerbated inflammatory responses of calves in this group. Moreover incidence of diseases was not different between groups of calves.

In summary, feeding of MR enriched with LA changed the plasma FA profile of calves. Transfer of dietary FA to calf plasma was verified through increased proportions of LA and ALA in calves fed HLA MR while maintaining similar concentrations of DHA regardless of the MR fed. Calves fed HLA MR improved BW gain and feed efficiency. This enhanced performance was accompanied by increased concentrations of energy metabolites and anabolic hormones. Feeding HLA MR appeared to improve immune response by increasing the number of circulating lymphocytes and possibly by enhancing the switch from a Th2 to a Th1 response by the increased production of IFN- γ .

Prepartum Supplementation of Fatty Acids Affects Calf Responses to a Linoleic Acid-Enriched Milk Replacer

No clear modification of the effect of MR on the FA profile of plasma of calves at 30 to 60 d of age by prepartum diets was detected. In studies evaluating the synthesis of DHA from ALA-deficient rats, the enzymes involved in this synthetic process were found to be upregulated in the liver but not in the brain, with enhanced activity under ALA deprivation (Rapoport et al., 2007; Igarashi et al., 2007). Therefore tissues can differ in their ability to synthesize longer chain FA from EFA. In the current study, the efficiency of conversion of LA to AA and C20:3 and conversion of ALA to EPA and DHA were better when LA and ALA were in shorter supply. This is borne out by the fact that calves fed HLA had greater plasma concentrations of LA but lower concentrations of AA and C20:3 compared to calves fed LLA. Likewise calves fed HLA had greater plasma concentrations of ALA but lower concentrations of EPA and similar concentrations of DHA compared to calves fed LLA.

Prepartum diets did not affect the growth and performance of calves fed a specific MR (no interaction of dam diet by MR). However, it has been stated that prepartum diets can induce a fetal programming event affecting the future performance of calves without affecting birth weight (Hess, 2003; Banta et al., 2006, 2011; Pettitt et al, 1987; Ferezou-Viala et al., 2007). In addition, the preweaning period is another critical period where programming of future events might occur (Fowden et al., 2006). Recently Soberon et al. (2012) reported a potential epigenetic programming of lifetime productivity (milk yield) due to an improved growth rate during the preweaning period.

Severity of diarrhea was not affected by the single effect of prepartum diet or MR but it was affected by their interaction. This interaction is actually expected if calves

are fed colostrum harvested from their respective dams and receive by passive transfer a pool of antibodies to be used to fight against potential pathogen invasion during the first week of life until the calf's own immune system is able to reach maturity and produce their own memory cells against invaders (Weaver et al., 2000; Heinrichs and Elizondo-Salazar, 2009). In our current study, calves born from dams fed fat and supplemented with HLA instead of LLA MR had the fewer number of days of diarrhea. This improved response of calves born from dams fed fat could be due to the fact that calves born from dams fed fat had a trend for greater serum total IgG after colostrum feeding (Chapter 3).

In summary feeding fat prepartum may modify the ability of tissues to synthesize essential FA derivatives due to differential proportion of LA and ALA they had when they are born. No apparent effect of prepartum diets to modify performance of calves fed LA in MR was observed. Calves fed a MR enriched in LA and born from dams fed fat experienced fewer days of diarrhea and poor attitude.

Summary

Strategic feeding of EFA, both during the nonlactating pregnant period and in early life, can change the FA status of calves as evidenced by changes in plasma FA. These changes affected calf metabolism, health, and performance. Supplementing LA and ALA to dams increased plasma concentrations of LA but decreased those of EPA and DHA of neonatal calves. In addition, feeding a MR enriched in LA and ALA increased plasma concentrations of LA and ALA but decreased that of EPA but not DHA. Synthesis of DHA in the growing calf overcame the inhibiting effect of EFA in utero. Feeding fat prepartum did not have a negative influence on calf performance or health with the exception that plasma concentrations of haptoglobin tended to be greater at 5

and 9 d after birth suggesting that inflammation was increased but a tendency for lower expression of CD62L may suggest that the inflammatory process was not excessive in these calves. Increased intake of LA from approximately 6.2 to 13.2 g/d on average over the 60-d period by partially replacing CCO with porcine lard in the MR increased BW gain by 3 kg over a 60-d period. Because feed intake was not changed, conversion of feed to gain was improved by 8%. This enhanced performance was accompanied by increased plasma concentrations of glucose and IGF-I and lower plasma concentrations of urea N and cholesterol, which corroborate the enhanced anabolic process that these calves were undergoing during the preweaning period. Feeding more LA in the MR also influenced health and immunity as evidenced by greater hematocrit and blood lymphocyte concentrations, lower plasma concentrations of ASP, greater proportion of phagocytosis by blood neutrophils, and greater synthesis of IFN- γ by PBMC. Feeding a HLA MR may have improved the switch from a Th2 to a Th1 response based upon the increased in vitro production of IFN- γ , which might enhance cell-mediated immunity in these calves. Supplementing SFA prepartum resulted in calves consuming more DM (primarily grain) and gaining 2.6 kg more BW by 60 d of age compared to calves born from dams supplemented with EFA during the nonlactating period. These same calves also demonstrated improvements in immunity as evidenced by a greater concentration of anti-OVA IgG and greater synthesis of IFN- γ by PBMC at 15 d of life. When these calves were fed MR enriched in LA, they had lower fecal and better attitude scores at 2 wk of age.

Table 4-1. Ingredient and chemical composition of milk replacers (MR) and grain mix.

Ingredients, % of DM	Milk replacer ¹		Grain mix
	LLA	HLA	
Coconut base MR ²	100	---	---
Porcine lard base MR ³	---	100	---
Barley, ground	---	---	51.7
Peanut meal	---	---	16.5
Beet pulp shreds	---	---	24.5
Sugarcane molasses	---	---	5.3
Mineral mix ⁴	---	---	2.0
Nutrient composition (DM basis)			
Lactose, %	34.1	34.2	---
Protein, %	29.0	28.7	18.7
Fat, %	19.4	19.8	4.2
NDF, %	-	-	23.0
C, %	0.8	0.8	0.5
P, %	0.8	0.8	0.5
Mg, %	0.1	0.1	0.4
K, %	2.4	2.4	0.9
Na, %	1.2	1.2	0.2
S, %	0.4	0.4	0.2
Fe, mg/kg	96.7	110.3	440.0
Zn, mg/kg	40.3	41.3	55.5
Cu, mg/kg	8.3	6.8	14.5
Mn, mg/kg	49.0	47.8	46.5
Mo, mg/kg	1.4	1.3	2.8
Co, mg/kg	0.5	0.6	---

¹ Milk replacers were classified as low linoleic acid (LLA) or high linoleic acid (HLA).

² Prepared by Land O'lakes®. Contains pre-homogenized coconut oil (30.5%), milk derivate products (68.6%), Neo-Terramycin® 100/50 (0.1%) and vitamin and mineral mixes (0.8%). Each kg contains 0.90% Ca, 0.87% P, 0.1 mg of Co, 10.1 mg of Cu, 1.0 mg of I, 100 mg of Fe, 45,374 IU of vitamin A, 11,345 IU of vitamin D and 220 IU of vitamin E.

³ Prepared by Land O'lakes®. Contains pre-homogenized coconut oil (13.3%) and porcine lard (19.6%), milk derivate products (66.3%), Neo-Terramycin® 100/50 (0.1%) and vitamin and mineral mixes (0.7%). Each kg contains 0.90% Ca, 0.87% P, 0.1 mg of Co, 10.1 mg of Cu, 1.0 mg of I, 100 mg of Fe, 45,374 IU of vitamin A, 11,345 IU of vitamin D and 220 IU of vitamin E.

⁴ Each kg contains 8.8% Ca, 4.2% P, 11.4% Mg, 12.4% Cl, 0.49% K, 8.1% Na, 0.36% S, 58 mg of Co, 263 mg of Cu, 26 mg of I, 1933 mg of Fe, 923 mg of Mn, 8.46 mg of Se, 1109 mg of Zn, 259,000 IU of vitamin A, 70,000 IU of vitamin D, and 2,400 IU of vitamin E.

Table 4-2. Fatty acid (FA) profile of milk replacers and grain mix.

FA	Milk replacer ¹		Grain mix
	LLA	HLA	
	% of identified FA		
C8:0	8.5	6.1	ND ²
C10:0	6.1	4.5	0.0
C12:0	42.5	29.9	0.1
C14:0	15.9	11.9	0.2
C16:0	10.6	14.6	13.2
C16:1	0.3	0.7	0.1
C18:0	4.4	6.7	2.0
C18:1	8.9	15.7	47.1
C18:2	2.9	9.0	28.2
C18:3 α	ND	0.8	2.1
C20:1	ND	ND	1.9
C22:0	ND	ND	2.3
C24:0	ND	ND	1.7
Others FA	ND	ND	1.1

¹ Milk replacers are classified as low linoleic acid (LLA) or high linoleic acid (HLA).

² ND = Not detected.

Table 4-3. Mean concentration of total plasma fatty acids (FA, mg/mL of plasma) and individual and group of FA expressed as % of total FA (g of FA/100 g of total FA) before colostrum feeding in calves born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

FA	Dam Diet ¹						SEM	P values				
	Control		SFA		EFA			FAT	FA	Parity	FAT by P	FA by P
	Null	Parous	Null	Parous	Null	Parous						
FA mg/mL plasma	1.23	1.33	1.14	1.28	1.34	1.33	0.07	0.91	0.09	0.21	0.81	0.33
C12:0	0.11	0.10	0.00	0.04	0.00	0.00	0.06	0.06	0.72	0.82	0.78	0.72
C14:0	1.83	2.12	1.52	1.58	1.99	1.58	0.23	0.15	0.30	0.92	0.27	0.31
C14:1 c9	0.56	0.44	0.50	0.40	0.50	0.32	0.07	0.26	0.54	0.02	0.93	0.54
C15:0	0.07	0.12	0.13	0.21	0.17	0.17	0.05	0.12	0.97	0.30	0.87	0.44
C16:0	29.6	30.4	30.0	29.3	30.5	29.9	0.54	0.80	0.30	0.66	0.12	0.95
C16:1 c9	5.26	4.82	5.46	5.21	4.89	5.21	0.42	0.69	0.50	0.72	0.53	0.50
C17:0	0.76	0.96	0.99	0.67	0.74	0.60	0.21	0.57	0.43	0.62	0.26	0.66
C17:1 c9	0.86	0.67	0.98	0.67	0.86	0.57	0.09	0.96	0.20	<0.01	0.47	0.86
C18:0	13.7	13.1	13.4	13.6	13.5	13.6	0.37	0.60	0.90	0.72	0.26	0.86
C18:1 c9	31.5	28.0	30.4	29.1	30.6	26.7	0.84	0.47	0.19	<0.01	0.56	0.12
C18:2 n-6	2.25	4.43	2.68	3.97	3.71	5.06	0.49	0.25	0.03	<0.01	0.34	0.95
C18:3 n-6	0.09	0.32	0.09	0.25	0.21	0.41	0.04	0.43	<0.01	<0.01	0.50	0.57
C18:3 n-3	0.00	0.11	0.02	0.03	0.11	0.04	0.04	0.85	0.16	0.53	0.03	0.26
C20:2	0.06	0.01	0.03	0.01	0.00	0.00	0.02	0.12	0.15	0.07	0.19	0.47
C20:3 n-6	1.31	2.03	1.80	2.08	1.63	2.91	0.20	0.01	0.09	<0.01	0.86	0.01
C20:4 n-6	4.13	5.23	4.27	5.32	3.67	5.77	0.29	0.78	0.80	<0.01	0.37	0.07
C20:5 n-3	0.37	0.07	0.44	0.14	0.34	0.03	0.05	0.73	0.03	<0.01	0.91	0.97
C22:4 n-6	0.02	0.12	0.00	0.15	0.00	0.25	0.03	0.34	0.16	<0.01	0.12	0.16
C22:5 n-3	0.50	0.45	0.62	0.50	0.44	0.47	0.07	0.62	0.11	0.36	0.92	0.23
C22:6 n-3	0.88	0.51	0.99	0.61	0.75	0.44	0.07	1.00	<0.01	<0.01	0.77	0.55

Table 4-3. Continued.

	Dam Diet ¹						SEM	P - values				
	Control		SFA		EFA			FAT	FA	Parity	FAT by P	FA by P
	Parity (P)											
	Prim	Mult	Prim	Mult	Prim	Mult						
Unknowns	6.18	5.97	5.66	6.21	5.37	6.02	0.41	0.48	0.55	0.33	0.27	0.90
Σ SFA	46.1	46.8	46.1	45.4	47.0	45.8	0.72	0.56	0.37	0.53	0.19	0.76
Σ MUFA	38.1	33.9	37.3	35.4	36.8	32.8	1.00	0.61	0.13	<0.01	0.50	0.29
Σ PUFA	9.6	13.3	10.9	13.0	10.8	15.4	0.87	0.16	0.19	<0.01	0.83	0.16
Σ n-6	7.9	12.1	8.9	11.8	9.22	14.4	0.78	0.13	0.06	<0.01	0.86	0.15
Σ n-3	1.76	1.14	2.06	1.27	1.63	0.99	0.15	0.78	0.02	<0.01	0.72	0.61

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² Null = nulliparous

³ P values for orthogonal contrasts and interactions. FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA.

Table 4-4. Mean concentration of total plasma fatty acids (FA, mg/mL of plasma) and individual and group of FA expressed as % of total FA (g of FA/100 g of total FA) of calves fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. All interactions with gender did not differ unless footnoted.

FA	Dam Diet ¹						SEM	P values ³								
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR	A	DD x A	MR x A	DD x MR x A
	LLA	HLA	LLA	HLA	LLA	HLA										
FA mg/mL plasma	2.04	1.95	2.12	1.95	2.12	1.93	0.07	0.62	0.88	0.02	0.48	0.90	0.91	0.36	0.05	0.02
C10:0	0.03	0.00	0.06	0.01	0.02	0.03	0.02	0.36	0.32	0.06	0.61	0.06	0.34	0.61	0.96	0.41
C12:0	0.91	0.44	0.89	0.59	0.65	0.42	0.06	0.55	<0.01	< 0.01	0.07	0.63	0.89	0.41	0.53	0.78
C14:0	4.73	2.22	4.89	2.58	4.70	2.17	0.16	0.42	0.05	< 0.01	0.60	0.21	0.04	0.63	0.46	0.73
C14:1 c9	0.18	0.24	0.25	0.22	0.24	0.20	0.02	0.43	0.62	0.81	0.04	0.90	<0.01	0.73	0.59	0.15
C15:0	0.41	0.48	0.45	0.43	0.46	0.45	0.05	0.96	0.75	0.71	0.35	0.90	0.03	0.96	0.77	0.97
C16:0	16.0	16.6	16.2	16.7	16.4	16.2	0.27	0.73	0.63	0.21	0.41	0.17	<0.01	0.11	0.74	0.80
C16:1 c9	1.21	1.34	1.11	1.40	1.09	1.35	0.06	0.46	0.58	< 0.01	0.18	0.82	0.68	0.39	0.22	0.74
C17:0	0.37	0.36	0.34	0.37	0.34	0.37	0.03	0.68	0.92	0.47	0.34	0.97	<0.01	0.67	0.20	0.46
C17:1 c9	0.07	0.09	0.07	0.07	0.07	0.10	0.02	0.38	0.54	0.02	0.25	0.27	0.04	0.14	0.64	0.62
C18:0	13.8	13.3	13.9	13.9	13.7	13.2	0.25	0.47	0.11	0.10	0.71	0.38	<0.01	0.47	0.18	0.51
C18:1 c9	10.9	10.0	11.6	10.5	11.3	9.9	0.42	0.31	0.27	< 0.01	0.77	0.71	0.87	0.41	0.11	0.88
C18:2 n-6	41.6	46.8	39.9	45.1	41.1	47.0	0.89	0.23	0.09	< 0.01	0.79	0.75	0.00	0.42	0.08	0.52
C18:3 n-6	0.34	0.22	0.37	0.19	0.32	0.17	0.03	0.45	0.20	< 0.01	0.43	0.52	<0.01	0.90	0.30	0.24
C18:3 ⁵ n-3	0.70	0.86	0.65	0.77	0.68	0.81	0.05	0.23	0.50	< 0.01	0.74	0.84	<0.01	0.53	0.55	0.78
C20:2	0.19	0.25	0.21	0.25	0.21	0.29	0.03	0.41	0.28	0.01	0.97	0.46	<0.01	0.30	0.13	0.57
C20:3 ⁶ n-6	1.34	0.90	1.38	0.98	1.32	0.98	0.06	0.39	0.59	< 0.01	0.48	0.68	<0.01	0.41	0.09	0.22
C20:4 n-6	3.21	2.82	3.14	3.03	3.17	3.17	0.11	0.23	0.45	0.05	0.07	0.58	<0.01	0.76	0.52	0.60
C20:5 n-3	0.12	0.08	0.13	0.07	0.12	0.07	0.02	0.88	0.74	< 0.01	0.74	0.83	0.11	0.49	0.55	0.96
C22:4 n-6	0.22	0.23	0.24	0.22	0.25	0.25	0.03	0.41	0.33	0.84	0.81	0.74	0.05	0.57	0.36	0.79
C22:5 n-3	0.29	0.33	0.30	0.30	0.28	0.36	0.02	0.78	0.24	<0.01	0.76	0.01	<0.01	0.97	0.66	0.55
C22:6 n-3	0.26	0.20	0.25	0.23	0.19	0.22	0.02	0.85	0.10	0.43	0.10	0.20	<0.01	0.05	0.61	0.09

Table 4-4. Continued.

	Dam Diet ¹						SEM	P values ³								
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR	A	DD x A	MR x A	DD x MR x A
	LLA	HLA	LLA	HLA	LLA	HLA										
Unknowns	3.54	3.03	3.53	2.91	3.43	2.97	0.27	0.75	0.94	0.02	0.95	0.77	<0.01	0.81	<0.01	0.17
Σ SFA	36.3	33.4	36.8	34.6	36.4	32.9	0.44	0.42	0.01	< 0.01	0.94	0.14	0.19	0.70	0.30	0.71
Σ MUFA	11.9	10.8	13.1	11.3	12.5	10.8	0.41	0.11	0.20	< 0.01	0.30	0.95	0.89	0.28	0.17	0.91
Σ PUFA	48.3	52.7	46.5	51.2	47.7	53.3	0.88	0.28	0.07	< 0.01	0.60	0.57	<0.01	0.58	0.05	0.60
Σ n-6	47.0	51.2	45.2	49.8	46.4	51.8	0.88	0.32	0.07	< 0.01	0.61	0.63	<0.01	0.49	0.05	0.57
Σ n-3	1.37	1.47	1.33	1.37	1.26	1.47	0.06	0.26	0.80	0.02	0.86	0.17	0.04	0.25	0.77	0.65

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ P values for orthogonal contrasts and interactions: FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, MR = milk replacer, A = age, DD = dam diet. Three way interactions were removed from the model if $P > 0.25$.

⁴ FA by gender, $P = 0.02$.

⁵ FAT by gender, $P = 0.01$; FAT by MR by gender, $P = 0.05$.

⁶ FA by gender, $P = 0.03$.

Table 4-5. Dry matter intake (DMI), body weight (BW) gain and feed efficiency (FE) of Holstein calves fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

Measure	Dam Diet ¹						SEM	P values ³							
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR	G	DD x G	MR x G
	Milk replacer (MR) ²														
	LLA	HLA	LLA	HLA	LLA	HLA									
Birth to 30d															
Birth weight, kg	38.7	41.6	40.6	42.4	41.7	40.3	1.31	0.32	0.71	0.30	0.23	0.23	<0.01	0.71	0.04
MR intake, kg of DM	14.7	15.6	15.3	16.0	15.3	15.2	0.42	0.43	0.35	0.15	0.41	0.35	<0.01	0.62	0.33
MR intake, % of BW	1.15	1.12	1.14	1.13	1.11	1.14	0.02	0.75	0.70	0.73	0.22	0.20	<0.01	0.86	0.34
BW gain, kg	7.59	9.42	8.16	10.2	8.07	8.39	0.72	0.75	0.19	0.02	0.61	0.24	<0.01	0.28	0.66
ADG, Kg/d	0.25	0.31	0.27	0.34	0.27	0.28	0.02	0.76	0.19	0.02	0.60	0.23	<0.01	0.28	0.68
FE, (kg BW gain/kg MR intake)	0.51	0.60	0.53	0.63	0.52	0.56	0.05	0.99	0.34	0.05	0.87	0.54	0.19	0.42	0.81
31d to weaning															
MR intake, Kg of DM	18.8	20.1	19.5	20.6	19.4	19.6	0.47	0.50	0.28	0.03	0.42	0.30	<0.01	0.50	0.22
Grain mix intake, Kg of DM	10.4	11.9	13.8	12.5	11.3	10.6	1.14	0.36	0.06	0.81	0.22	0.79	0.41	0.99	0.52
Total DMI, kg of DM	29.3	32.0	33.3	33.1	30.7	30.2	1.38	0.32	0.05	0.58	0.19	0.90	0.01	0.92	0.91
Total DMI, % of BW	1.75	1.74	1.87	1.75	1.75	1.70	0.05	0.61	0.10	0.19	0.37	0.50	0.07	0.76	0.15
BW gain, Kg	19.0	20.3	20.2	21.4	17.8	20.5	1.07	0.71	0.13	0.05	0.76	0.50	<0.01	0.98	0.81
ADG, Kg/d	0.63	0.68	0.68	0.71	0.59	0.68	0.03	0.69	0.11	0.05	0.81	0.44	<0.01	0.97	0.81
FE, (kg BW gain/kg total DMI)	0.65	0.64	0.62	0.64	0.58	0.68	0.03	0.66	0.93	0.09	0.13	0.19	0.40	0.81	0.96
Birth to weaning															
Final BW, Kg	65.3	71.7	69.0	74.1	67.6	69.3	1.97	0.36	0.12	0.01	0.40	0.39	<0.01	0.55	0.84
Total DMI, Kg	44.0	47.7	48.6	49.0	46.0	45.3	1.66	0.32	0.07	0.39	0.18	0.73	<0.01	0.85	0.90
Total DMI, % of BW	1.41	1.40	1.47	1.41	1.40	1.38	0.02	0.63	0.04	0.16	0.38	0.43	0.01	0.80	0.03
BW gain, Kg	26.6	29.6	28.4	31.6	25.9	28.9	1.27	0.55	0.04	<0.01	0.95	0.92	<0.01	0.78	0.91
ADG, Kg/d	0.44	0.49	0.47	0.53	0.43	0.48	0.02	0.49	0.04	<0.01	0.94	0.92	<0.01	0.77	0.87
FE, (kg BW gain/kg total DMI)	0.60	0.62	0.59	0.64	0.57	0.64	0.03	0.93	0.63	0.01	0.23	0.61	0.10	0.95	0.88

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ *P* values for orthogonal contrasts and interactions: FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, DD = dam diet, MR = milk replacer, G = gender. Three way interactions were not significant.

Table 4-6. Plasma concentrations of metabolites and hormones in Holstein calves fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. All interactions with gender did not differ unless footnoted.

Measure	Dam Diet ¹						SEM	P values ³								
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR	A	DD x A	MR x A	DD x MR x A
	LLA	HLA	LLA	HLA	LLA	HLA										
Glucose, mg/dL	91.4	92.8	88.8	93.2	89.5	92.0	1.52	0.35	0.90	0.03	0.44	0.53	<0.01	0.07	0.99	0.35
PUN, mg/dL	7.59	7.63	8.95	7.88	8.52	7.74	0.38	0.05	0.48	0.06	0.16	0.74	<0.01	0.75	0.97	0.99
BHBA, mg/dL	1.08	0.80	1.52	0.88	1.49	0.94	0.17	0.06	0.93	<0.01	0.27	0.80	<0.01	0.40	0.11	0.98
NEFA, μ Eq/L	180	170	171	165	169	166	7.4	0.25	0.98	0.28	0.67	0.82	<0.01	0.19	0.43	0.60
Total cholesterol, mg/dL	87.9	85.3	92.7	79.7	99.6	84.6	3.67	0.45	0.12	<0.01	0.08	0.83	<0.01	0.41	0.01	0.41
Insulin ⁵ , ng/mL	1.21	1.46	1.30	1.45	1.33	1.41	0.13	0.69	0.98	0.14	0.52	0.78	<0.01	0.52	0.42	0.10
IGF-I, g/mL	57.0	63.7	50.7	62.1	52.0	53.2	4.33	0.12	0.40	0.08	0.99	0.25	<0.01	0.21	0.83	0.03
STP ⁶ , g/dL	5.75	5.88	5.76	5.87	5.80	5.75	0.08	0.76	0.66	0.37	0.47	0.35	<0.01	0.73	0.13	0.35

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ P values for orthogonal contrasts and interactions: FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, MR = milk replacer, A = age, DD = dam diet. Three way interactions were not significant.

⁴ Gender, $P < 0.01$, FAT by gender, $P = 0.03$.

⁵ MR by gender, $P = 0.05$.

⁶ Serum total protein. Gender, $P = 0.02$, FA by MR by gender, $P = 0.01$

Table 4-7. Attitude and fecal scores and percentage of days with poor attitude and diarrhea in Holstein calves fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. All interactions with gender did not differ unless footnoted.

Measure	Dam Diet ¹						SEM	P values ³								
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR	A	DD x A	MR x A	DD x MR x A
	LLA	HLA	LLA	HLA	LLA	HLA										
Health score ⁴																
Attitude	1.03	1.06	1.03	1.02	1.05	1.03	0.01	0.29	0.32	0.89	0.06	0.85	<0.01	0.74	0.85	0.30
Fecal	1.12	1.22	1.24	1.14	1.19	1.20	0.04	0.48	0.96	0.85	0.03	0.14	<0.01	0.96	0.92	0.77
Percentage of days with ⁵																
Poor attitude, 30 d	5.3	12.3	7.4	4.5	8.7	7.1	2.0	0.28	0.33	0.63	0.01	0.74	-	-	-	-
Poor attitude, 60 d	3.3	6.4	4.2	2.0	5.0	3.7	1.1	0.24	0.27	0.89	0.02	0.67	-	-	-	-
Diarrhea ⁶ , 30 d	8.9	17.7	15.6	6.6	15.3	11.9	2.2	0.63	0.26	0.51	<0.01	0.21	-	-	-	-
Diarrhea, 60 d	4.5	8.9	8.3	3.5	7.5	6.2	1.1	0.76	0.37	0.54	<0.01	0.11	-	-	-	-

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ P values for orthogonal contrasts and interactions. FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, MR = milk replacer, A = age, DD = dam diet. Three way interactions were not significant.

⁴ Scoring criteria for attitude was the following: 1 = responsive, 2 = non-active, 3 = depressed, or 4 = moribund. Scoring criteria for feces was the following: 1 = feces of firm consistency, no diarrhea; 2 = feces of moderate consistency, soft, no diarrhea; 3 = Runny feces, mild diarrhea; or 4 = watery feces, diarrhea.

⁵ Percentage of days with poor attitude (if score > 1) and diarrhea (if score > 2).

⁶ FA by gender, P = 0.04.

Table 4-8. Mean concentration of blood cell number and white blood cells percentages in Holstein calves fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. All interactions with gender did not differ unless footnoted.

Measure	Dam Diet ¹						SEM	P values ³									
	Control		SFA		EFA			FAT	FA	MR	FAT x MR		FA x MR	A	DD x A	MR x A	DD x MR x A
	LLA	HLA	LLA	HLA	LLA	HLA											
Blood cells number																	
Total red ⁴ , 10 ⁶ /μL	8.40	8.61	8.24	8.33	8.25	8.58	0.24	0.46	0.58	0.30	0.99	0.63	<0.01	0.24	0.75	0.35	
Total white ⁵ , 10 ³ /μL	8.46	8.75	8.60	9.35	8.17	8.58	0.49	0.88	0.23	0.23	0.73	0.77	<0.01	0.31	0.95	0.30	
Neutrophils, 10 ³ /μL	3.08	3.06	3.06	3.58	2.90	2.87	0.26	0.93	0.11	0.51	0.58	0.33	<0.01	0.17	0.91	0.31	
Lymphocytes, 10 ³ /μL	4.26	4.57	4.29	4.57	4.05	4.68	0.24	0.93	0.75	0.04	0.72	0.46	<0.01	0.76	0.96	0.81	
Monocytes, 10 ³ /μL	0.37	0.39	0.38	0.38	0.38	0.36	0.38	0.82	0.79	0.96	0.61	0.85	<0.01	0.45	0.42	0.41	
Eosinophils ⁴ , 10 ³ /μL	0.11	0.12	0.11	0.12	0.11	0.12	0.01	0.96	0.78	0.30	0.90	0.92	<0.01	0.60	0.07	0.01	
Basophils, 10 ³ /μL	0.11	0.11	0.11	0.12	0.10	0.11	0.01	0.95	0.44	0.37	0.54	0.62	<0.01	0.73	0.59	0.71	
Platelets, 10 ³ /μL	781	710	833	698	789	738	46.1	0.65	0.99	0.03	0.79	0.37	<0.01	0.03	0.41	0.95	
White Blood cells, %																	
Neutrophils	39.0	36.8	38.5	40.5	38.5	35.5	1.57	0.78	0.11	0.39	0.54	0.12	<0.01	0.38	0.93	0.40	
Lymphocytes ⁴	52.7	54.5	53.1	51.7	52.6	56.4	1.58	0.94	0.19	0.29	0.81	0.11	<0.01	0.45	0.91	0.34	
Monocytes ⁶	4.09	4.51	4.22	3.88	4.43	3.87	0.26	0.36	0.70	0.45	0.05	0.68	<0.01	0.62	0.65	0.61	
Eosinophils ⁷	1.32	1.36	1.29	1.27	1.38	1.45	0.10	0.96	0.19	0.74	0.87	0.67	<0.01	0.14	0.21	<0.01	
Basophils	1.30	1.26	1.28	1.25	1.23	1.33	0.06	0.89	0.77	0.88	0.48	0.27	<0.01	0.64	0.20	0.54	
Hematocrit ⁸ , %	34.9	35.8	33.8	35.5	34.6	36.3	0.97	0.72	0.39	0.08	0.63	0.97	<0.01	0.45	0.46	0.98	

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ P values for orthogonal contrasts and interactions: FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, MR = milk replacer, A = age, DD = dam diet. Three and four way interactions with gender were removed from the model if P > 0.25.

⁴ Gender, P ≤ 0.03.

⁵ FAT by gender, P = 0.05.

⁶ FAT by gender, P = 0.05.

⁷ Gender, P = 0.04, FA by MR by gender, P = 0.02.

⁸ Gender, P = 0.04.

Table 4-9. Expression of adhesion molecules (CD18 and CD62L) on surface of blood neutrophils and phagocytic activity of blood neutrophils as in Holstein calves fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. All interactions with gender did not differ unless footnoted.

Measure	Dam Diet ¹						SEM	P values ³										
	Control		SFA		EFA			FAT	FA	MR	FAT x MR		FA x MR		A	DD x A	MR x A	DD x MR x A
	LLA	HLA	LLA	HLA	LLA	HLA					MR	MR	MR	MR				
CD18 Expression																		
CD18+, %	94.7	94.3	93.7	94.3	94.9	94.7	0.70	0.87	0.27	0.98	0.66	0.56	0.06	0.36	0.22	0.18		
MFI	52.4	50.2	47.3	47.4	50.5	50.8	5.20	0.61	0.52	0.89	0.79	0.98	0.27	0.65	0.60	0.54		
CD62L Expression																		
CD62L+ ⁴ , %	98.2	98.3	97.8	98.2	98.2	98.3	0.20	0.49	0.23	0.23	0.50	0.50	0.23	0.12	0.36	0.22		
MFI	376	389	329	301	357	364	31.2	0.10	0.13	0.85	0.65	0.56	<0.01	0.79	0.57	0.25		
Phagocytic activity																		
Phagocytosis, %	95.7	96.3	95.9	96.5	95.2	96.1	0.49	0.90	0.27	0.09	0.84	0.85	<0.01	0.19	0.87	0.28		
MFI	118	120	111	114	120	121	4.3	0.41	0.04	0.57	0.98	0.82	<0.01	0.65	0.46	0.95		
Phagocytic neutrophils ⁵ , 10 ³ /μL	3.19	3.26	3.24	3.88	3.06	2.99	0.29	0.84	0.07	0.41	0.71	0.27	<0.01	0.18	0.93	0.28		

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ P values for orthogonal contrasts and interactions: FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, MR = milk replacer, A = age, DD = dam diet. Three and four way interactions were not significant.

⁴ Gender, P=0.04, FA by gender, P= 0.01, MR by gender, P= 0.01.

⁵ Gender, P=0.05.

Table 4-10. Mean concentration of serum total protein, acute phase proteins, serum anti OVA-IgG and interferon gamma produced by peripheral blood mononuclear cells stimulated with concanavalin A in Holstein calves fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. All interactions with gender did not differ unless footnoted.

Measure	Dam Diet ¹						SEM	P values ³									
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR	A	DD x A	MR x A	DD x MR x A	
	Milk replacer ²																
	LLA	HLA	LLA	HLA	LLA	HLA											
ASP ⁴ , mg/L	94.1	72.3	90.0	75.7	90.0	88.3	4.01	0.34	0.11	<0.01	0.04	0.11	<0.01	0.90	0.09	0.59	
Haptoglobin, OD x 100	0.94	0.96	1.04	1.02	1.02	1.05	0.03	0.06	0.89	0.88	0.78	0.65	<0.01	0.85	0.80	1.00	
Anti OVA-IgG, OD	0.87	0.86	0.87	0.94	0.82	0.84	0.04	0.99	0.07	0.51	0.39	0.55	<0.01	<0.01	0.38	0.99	
IFN-γ-15d, pg/mL	22.3	21.8	38.9	49.3	22.7	23.9	11.4	0.23	0.08	0.69	0.75	0.69	-	-	-	-	
IFN-γ-30d, pg/mL	19.9	48.7	35.5	61.5	21.5	34.2	13.67	0.74	0.14	0.05	0.69	0.63	-	-	-	-	

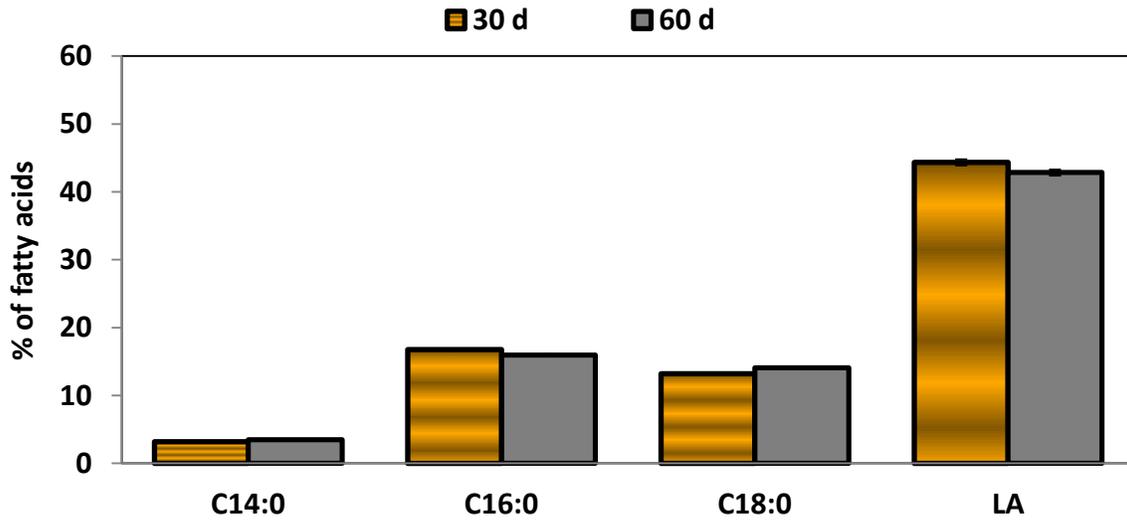
¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30 d of life to provide 6.72 g of fat/kg of BW^{0.75}.

³ P values for orthogonal contrasts and interactions: FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, MR = milk replacer, A = age, D = dam diet. Three and four way interactions were not significant.

⁴ Acid soluble protein. FA by Gender, P = 0.04.

A



B

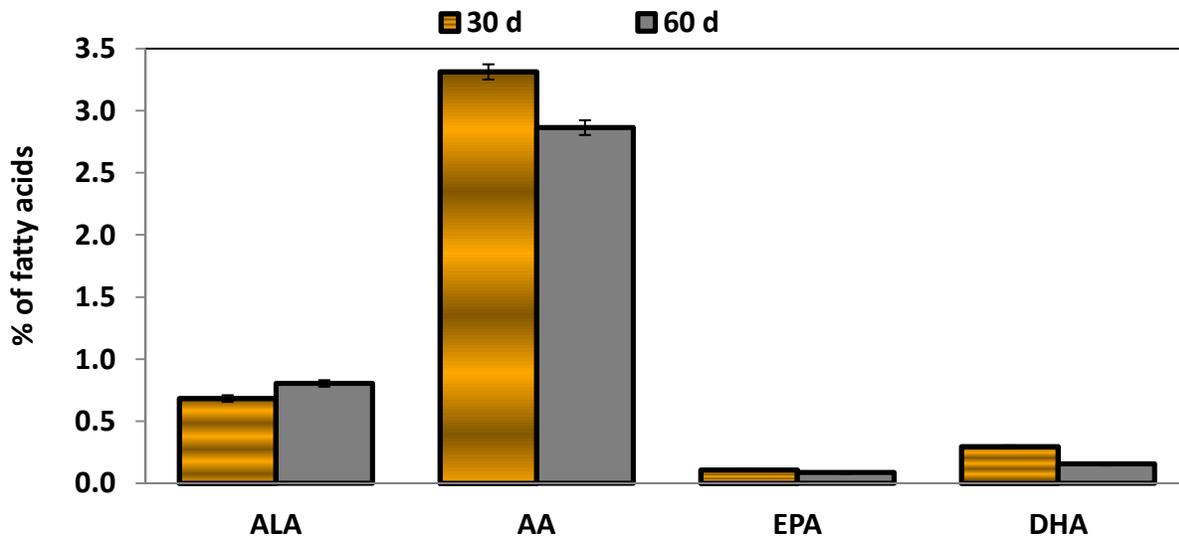


Figure 4-1. Plasma fatty acid concentrations in Holstein calves from 30 to 60 days of age. A) Concentrations of 14:0, C16:0, C18:0 and linoleic acid (LA). B) Concentrations of α -linolenic acid (ALA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Calves were fed milk replacers containing low or high linoleic acid and were born from dams fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of age for all fatty acids (FA) was $P < 0.01$ except for C14:0 ($P = 0.04$) and EPA ($P = 0.11$).

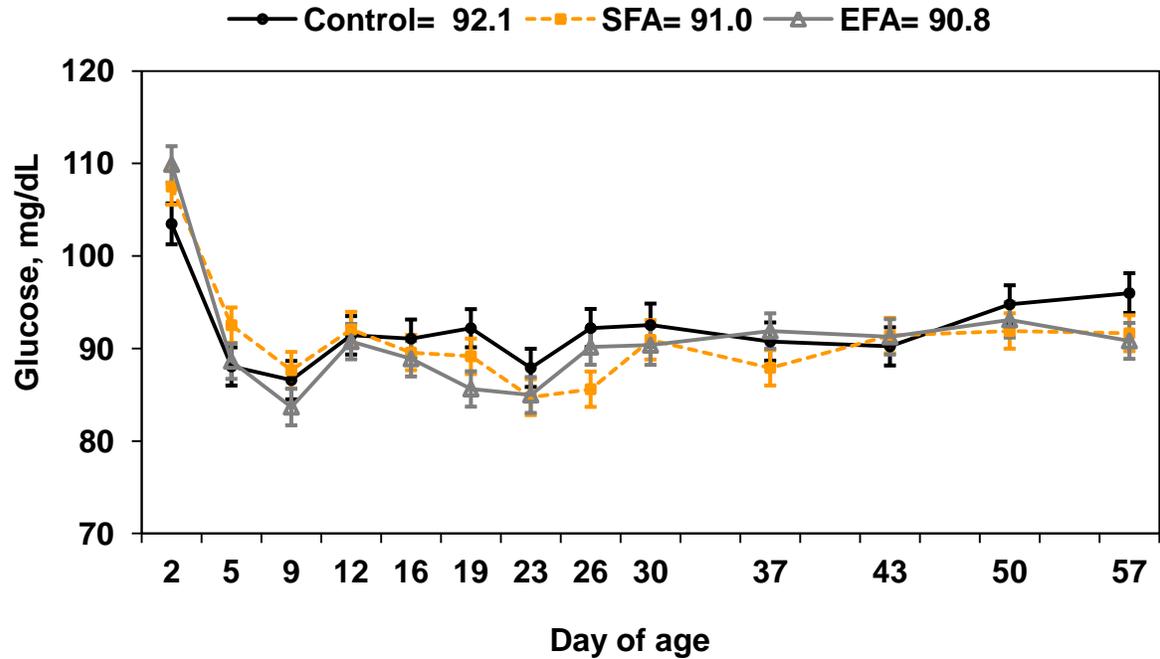


Figure 4-2. Plasmatic concentrations of glucose in Holstein calves fed milk replacer containing low or high linoleic acid from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of dam diet by age, $P = 0.07$ [slice effect, $P = 0.08$ (day 2), $P = 0.05$ (day 19) and $P = 0.06$ (day 26)].

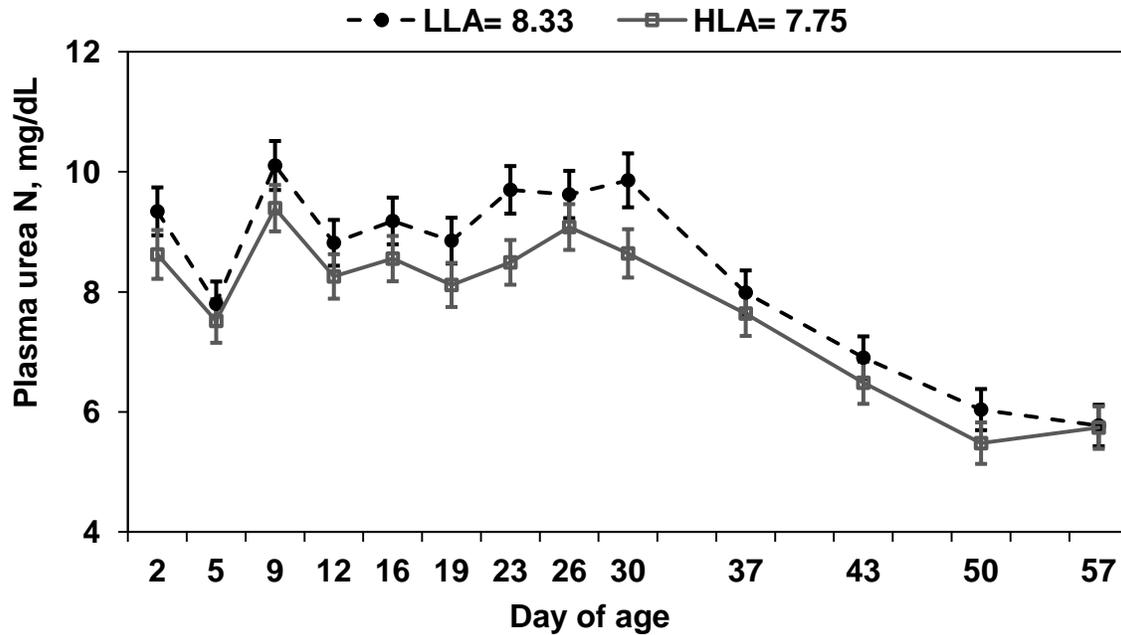
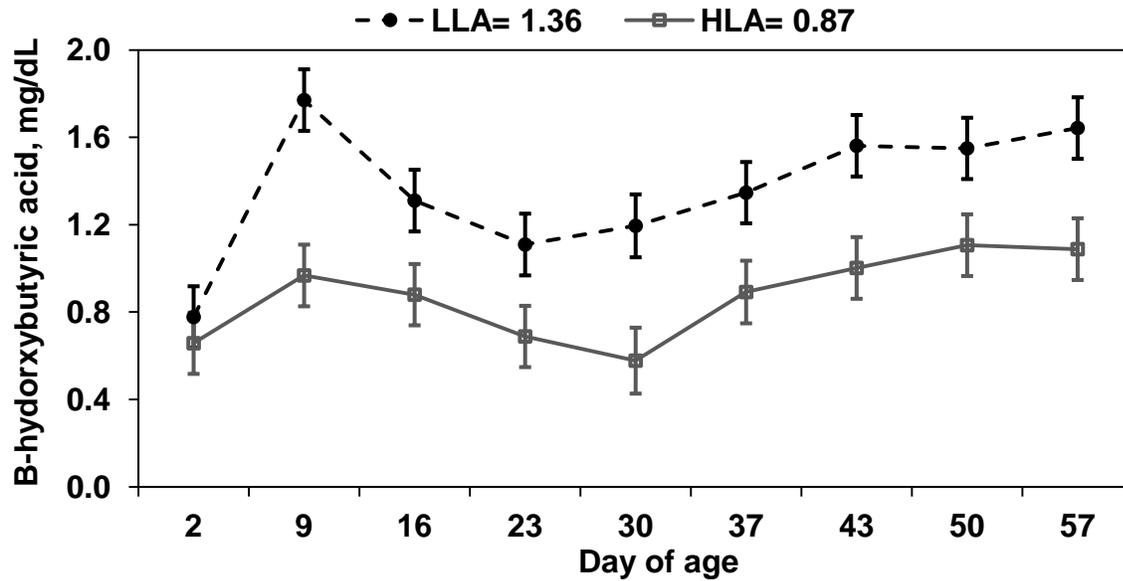


Figure 4-3. Plasmatic concentrations of urea N in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of milk replacer, $P = 0.03$ and effect of age, $P < 0.01$.

A



B

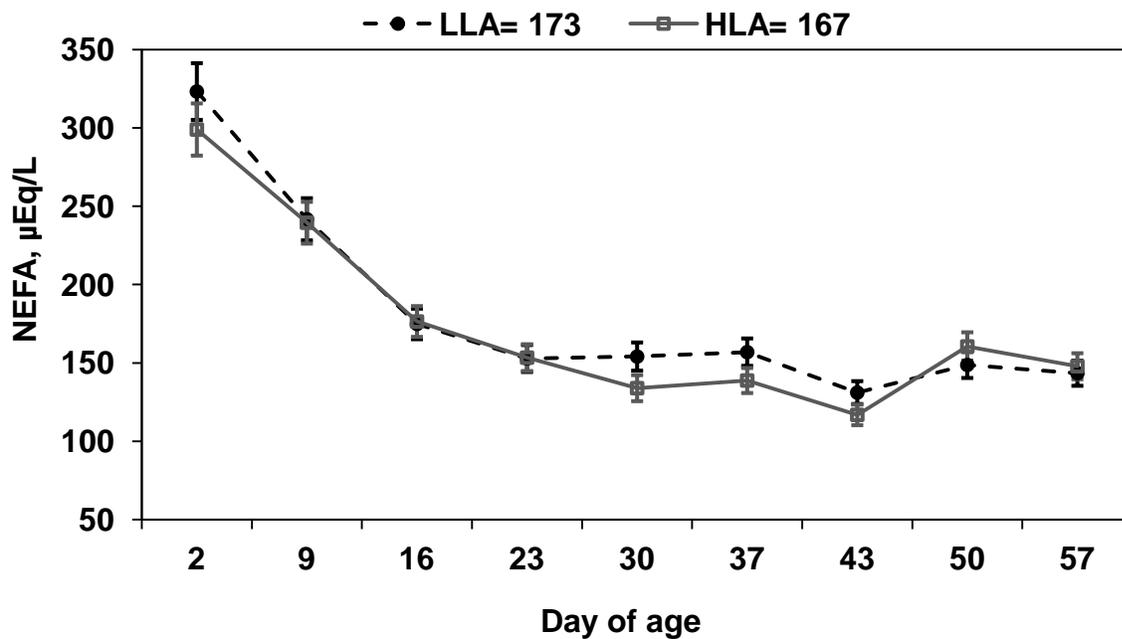


Figure 4-4. Plasmatic concentrations of β -hydroxybutyric acid (BHBA), and nonesterified fatty acids (NEFA) in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. A) Effects of milk replacer, $P < 0.01$ and age, $P < 0.01$. B) Effect of age, $P < 0.01$.

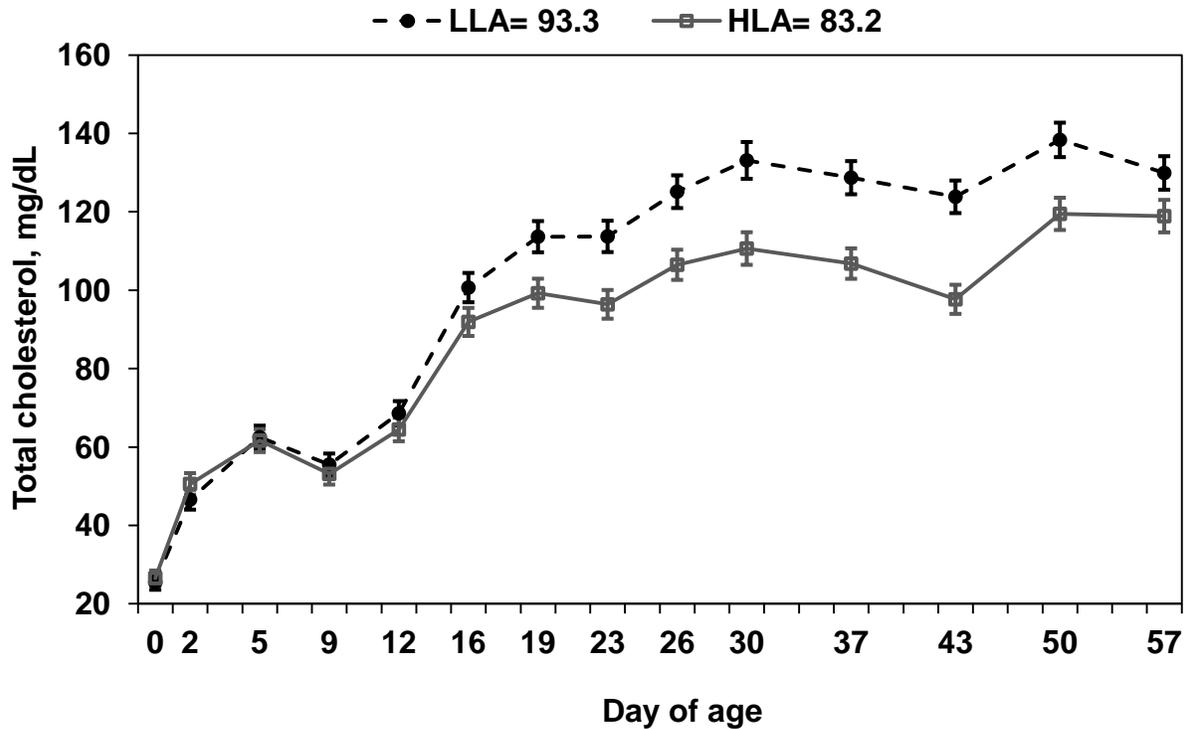
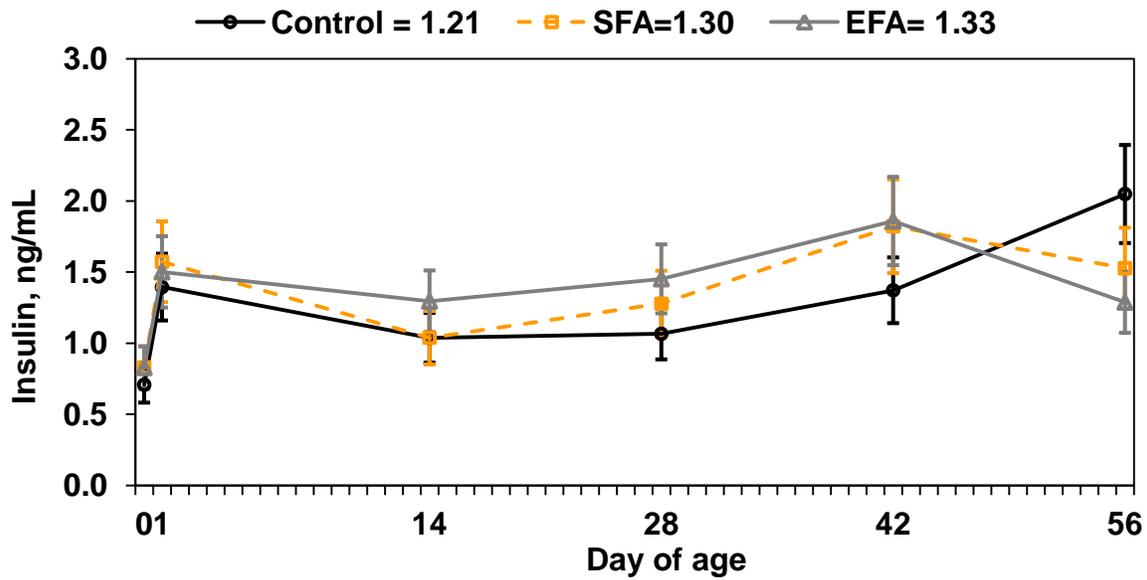


Figure 4-5. Plasmatic concentrations of total cholesterol in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of milk replacer by age, $P = 0.01$ [slice effect, $P = 0.09$ (day 16), $P \leq 0.01$ (from 19 to 49 d), $P = 0.06$ (day 57)].

A



B

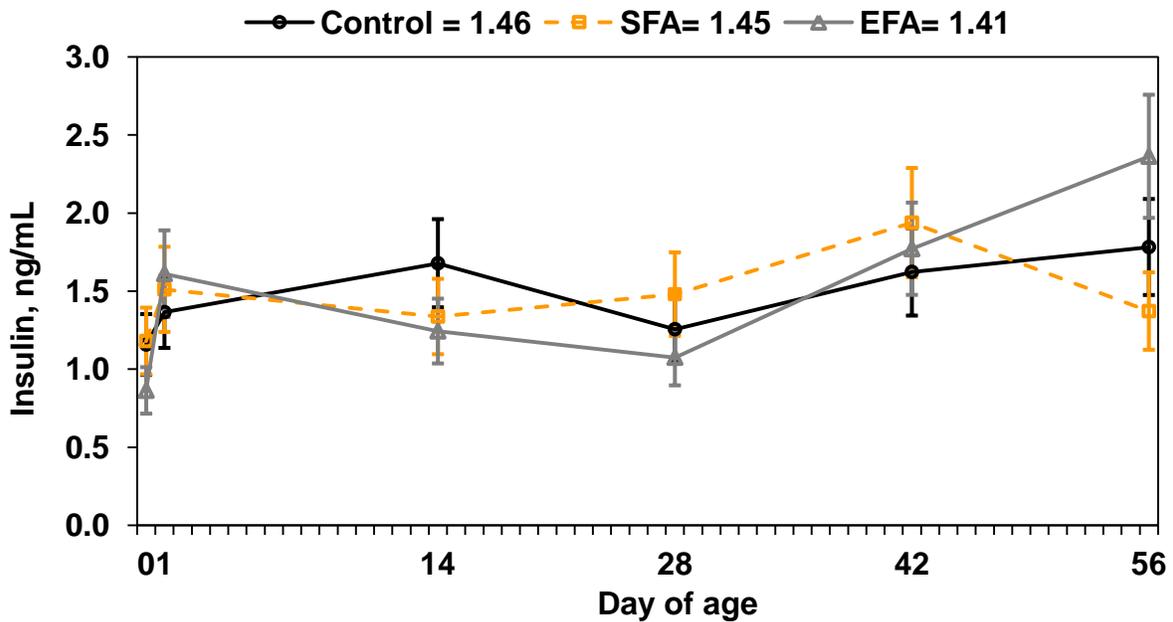
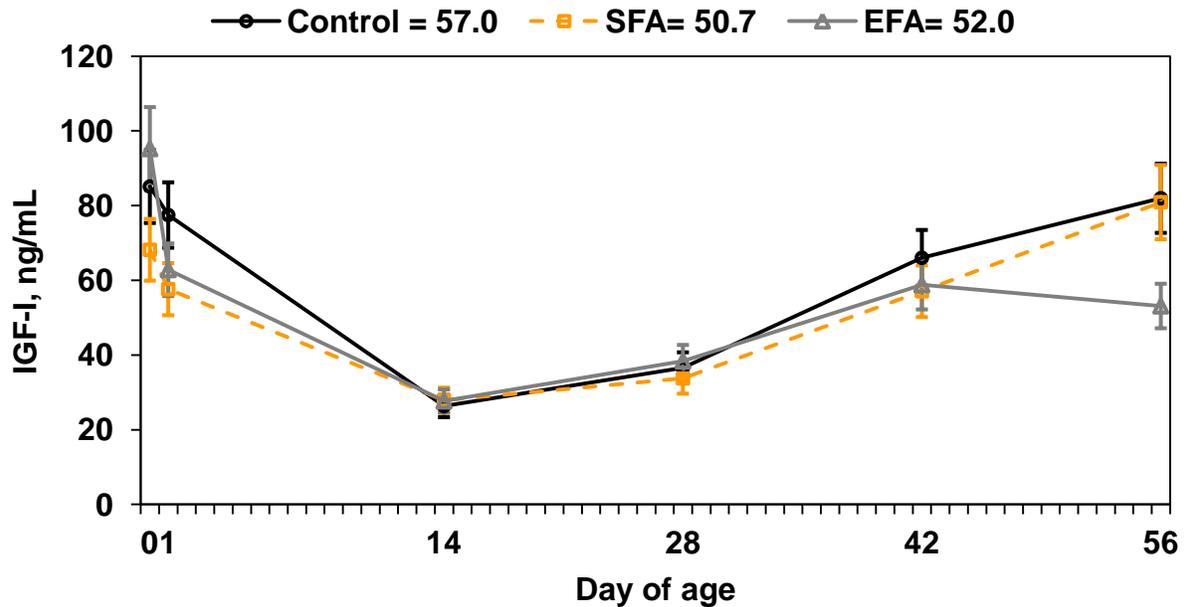


Figure 4-6. Plasmatic concentrations of insulin in Holstein calves from 0 to 60 days of age. A) Calves were fed milk replacer containing low linoleic acid. B) Calves were fed milk replacer containing low linoleic acid. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Interaction dam diet by milk replacer by age, $P = 0.10$ (slice effect at day 56, $P = 0.07$).

A



B

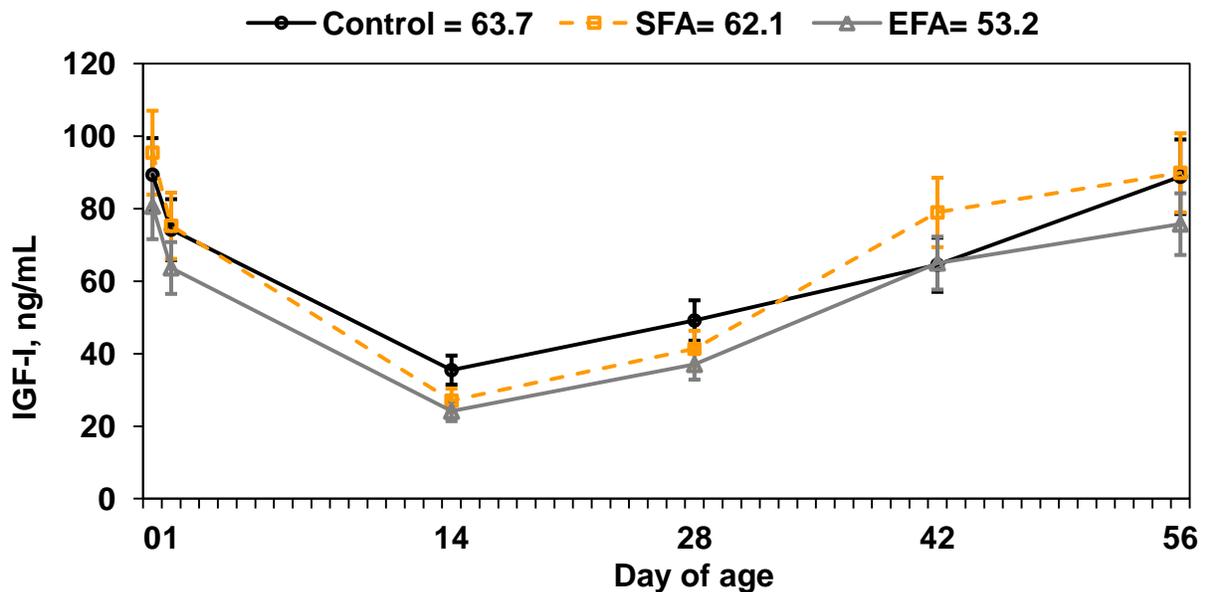


Figure 4-7. Plasmatic concentrations of insulin like growth factor I (IGF-I) in Holstein calves from 0 to 60 days of age. A) Calves were fed milk replacer containing low linoleic acid. B) Calves were fed milk replacer containing low linoleic acid. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Interaction dam diet by milk replacer by age, $P = 0.03$ (slice effect at day 56, $P = 0.01$).

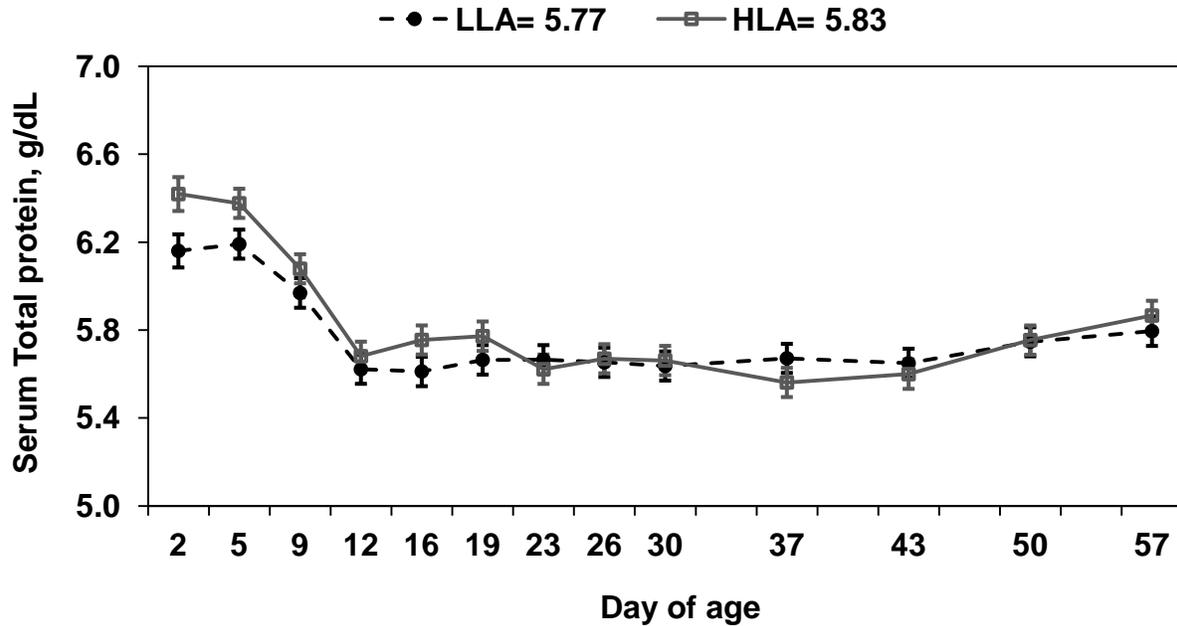
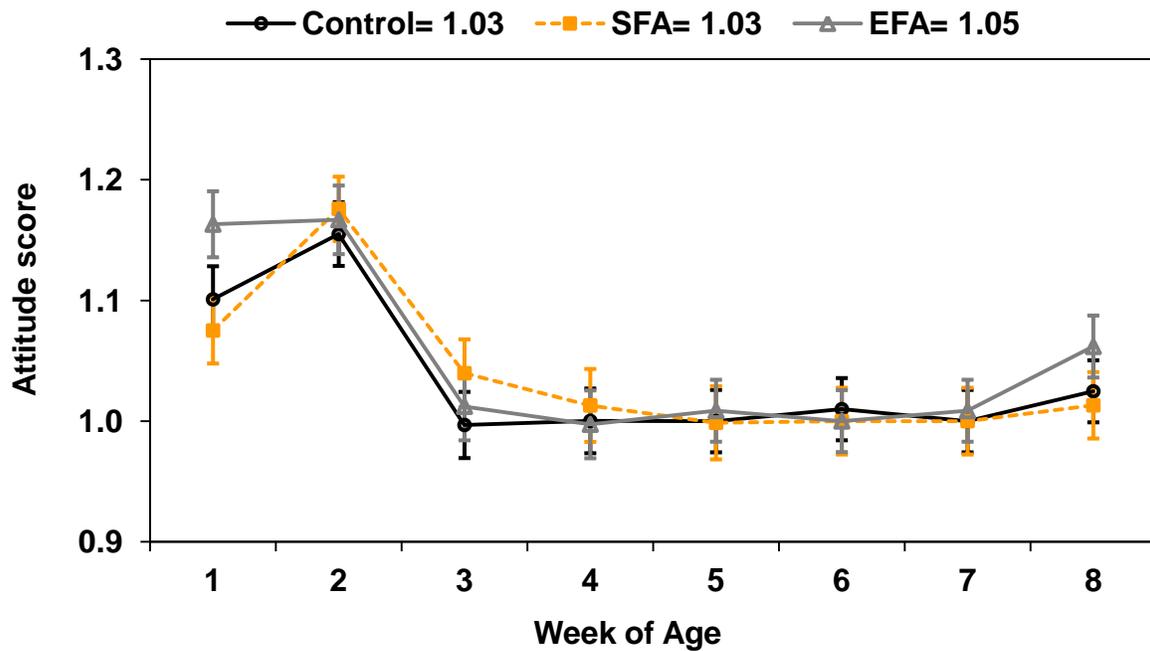


Figure 4-8. Serum total protein concentrations in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of age, $P < 0.01$; effect of milk replacer by age, $P = 0.13$ (slice effect, $P = 0.02$ at day 2).

A



B

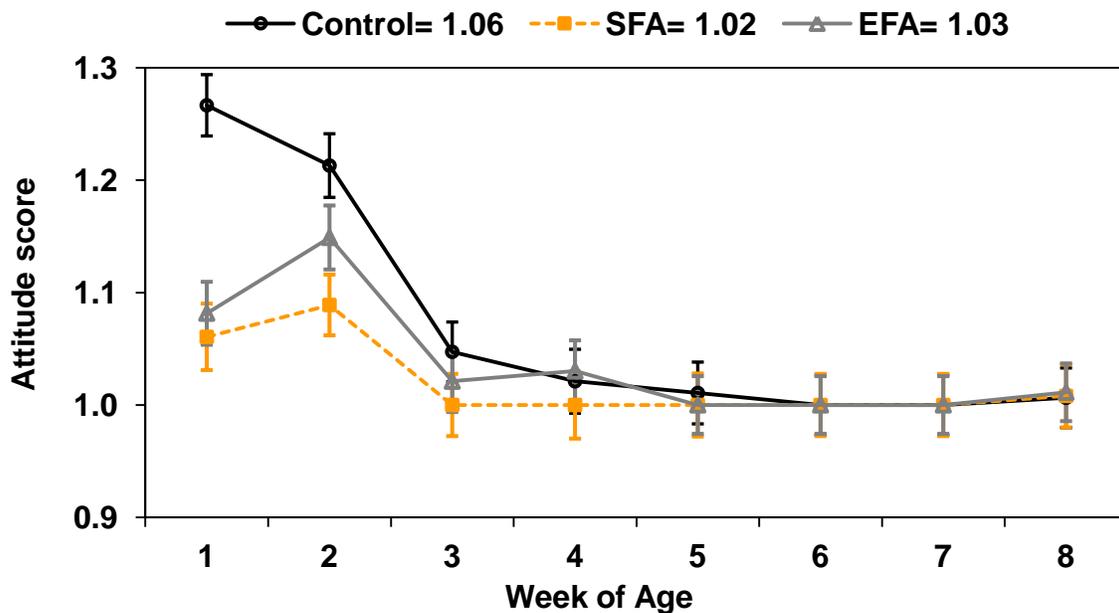
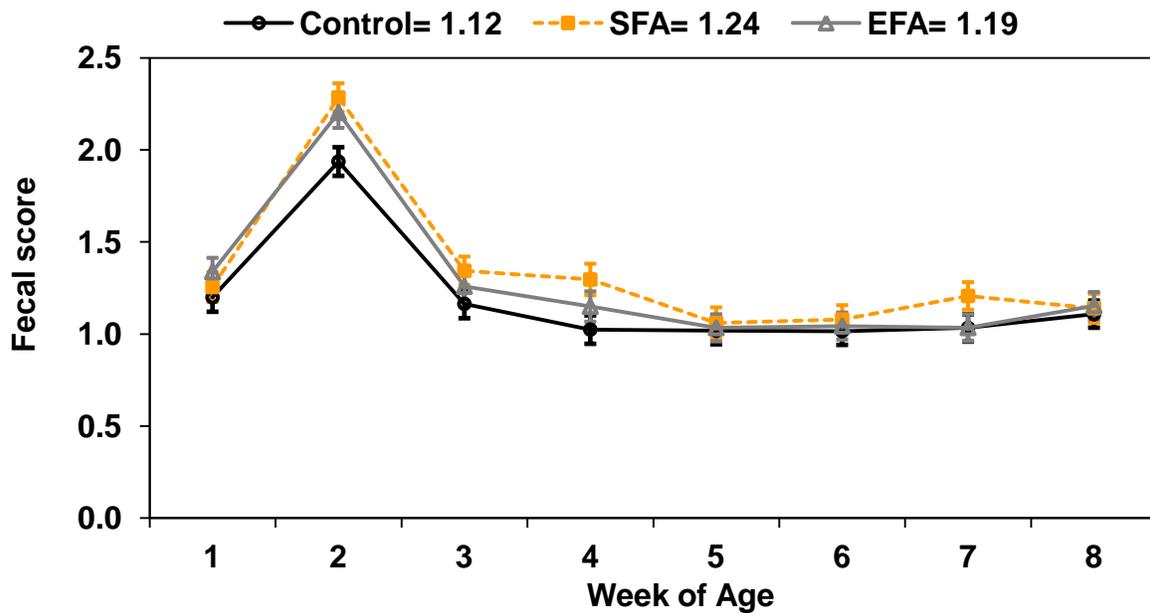


Figure 4-9. Attitude score of Holstein calves from 0 to 60 days of age. A) Calves were fed milk replacer containing low linoleic acid. B) Calves were fed milk replacer containing low linoleic acid. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Interaction of fat by milk replacer was $P = 0.06$ and of age was $P < 0.01$.

A



B

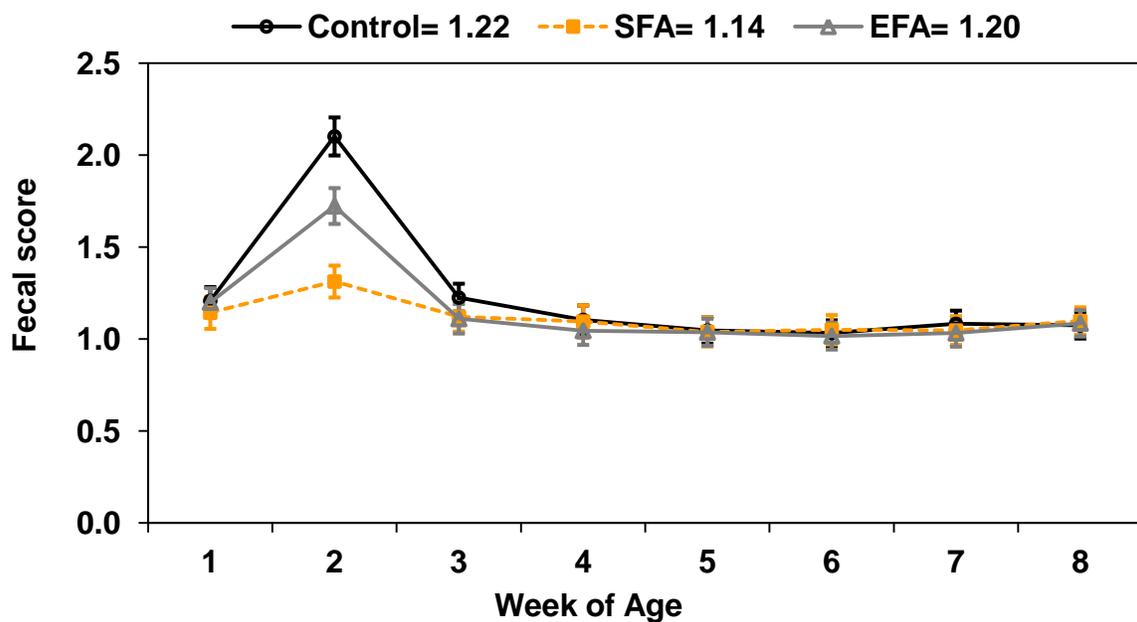
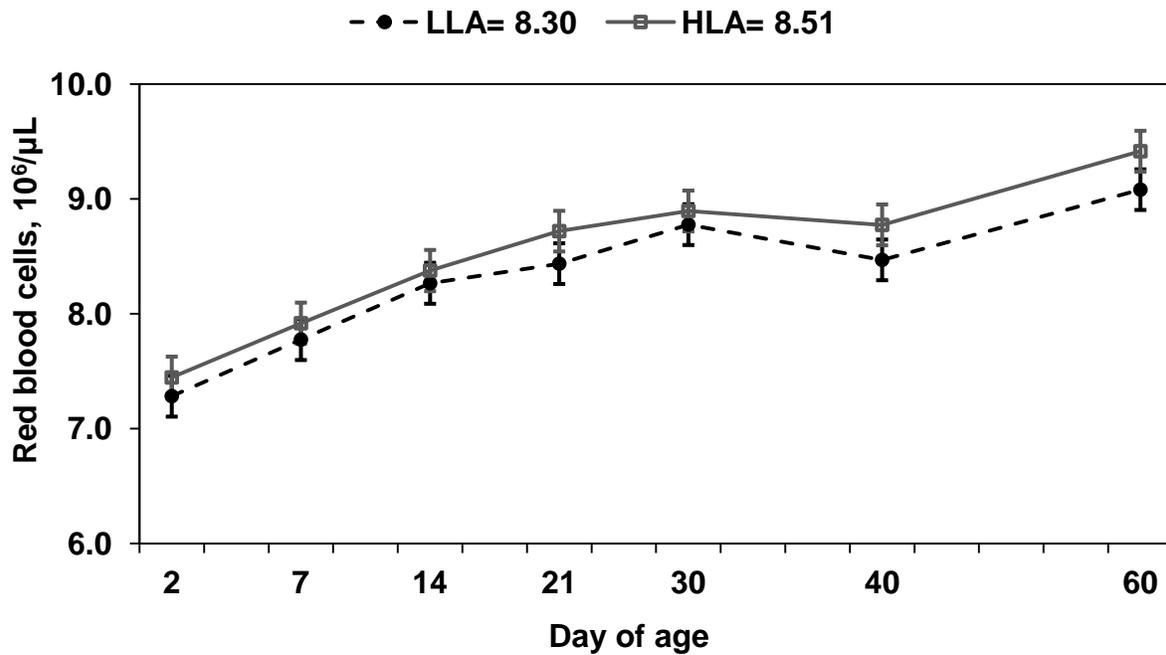


Figure 4-10. Fecal score of Holstein calves from 0 to 60 days of age. A) Calves were fed milk replacer containing low linoleic acid. B) Calves were fed milk replacer containing high linoleic acid. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Interaction of fat by milk replacer, $P = 0.01$ and of age, $P < 0.01$.

A



B

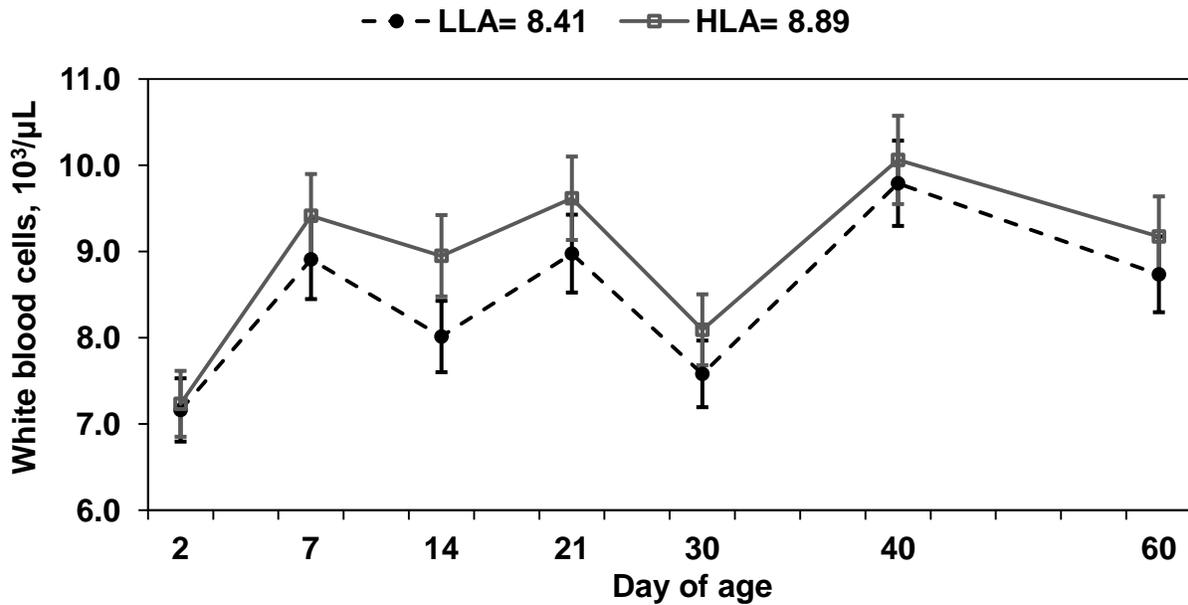
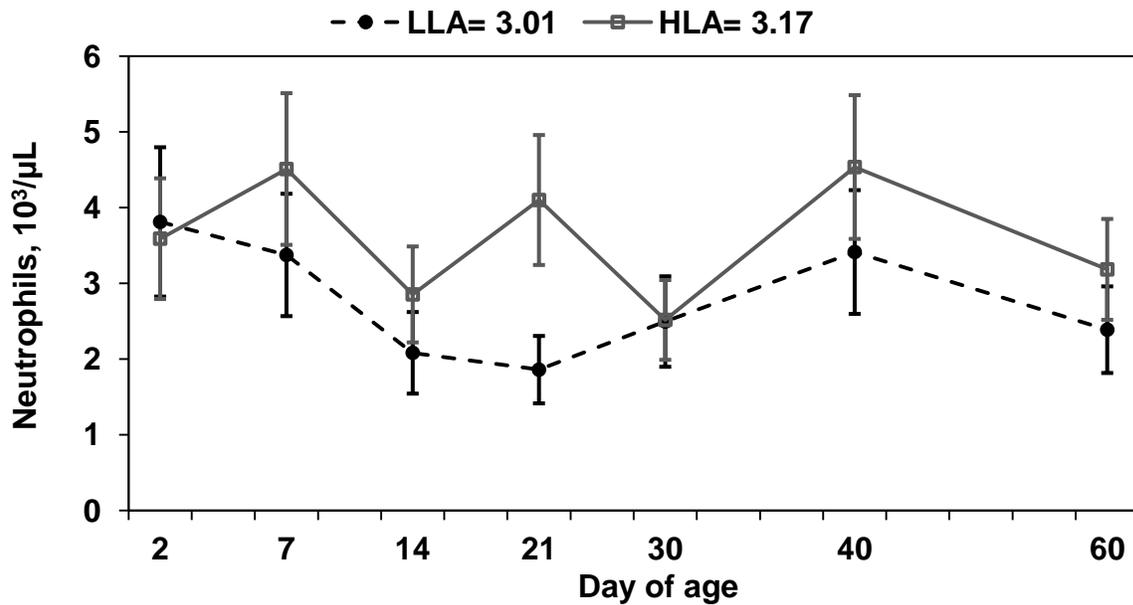


Figure 4-11. Blood concentrations of red and white blood cells in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of age in red and white blood cells, $P < 0.01$.

A



B

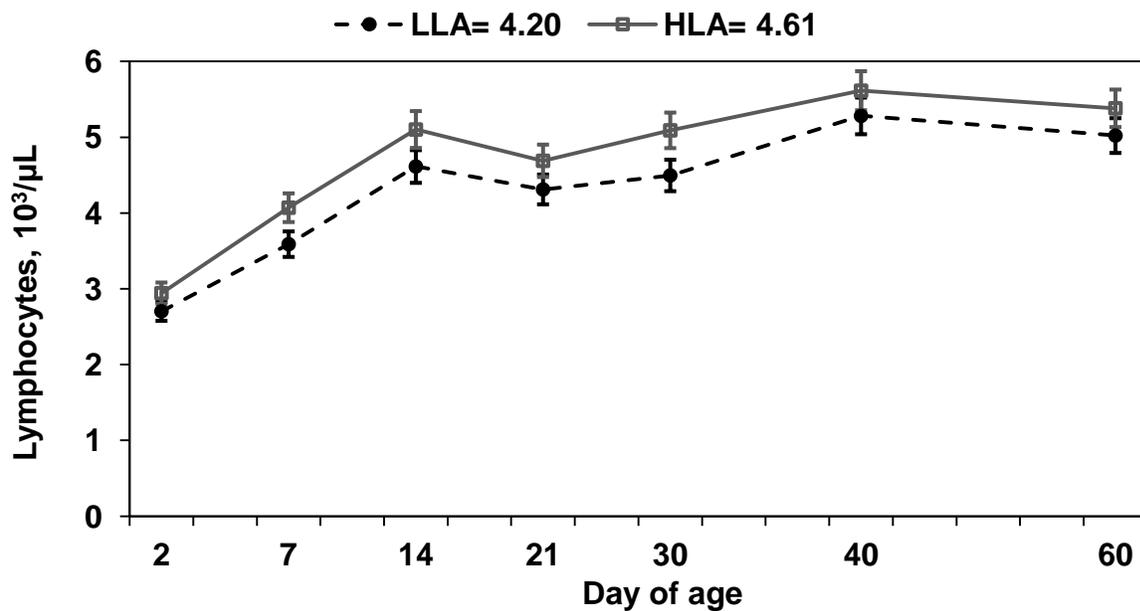
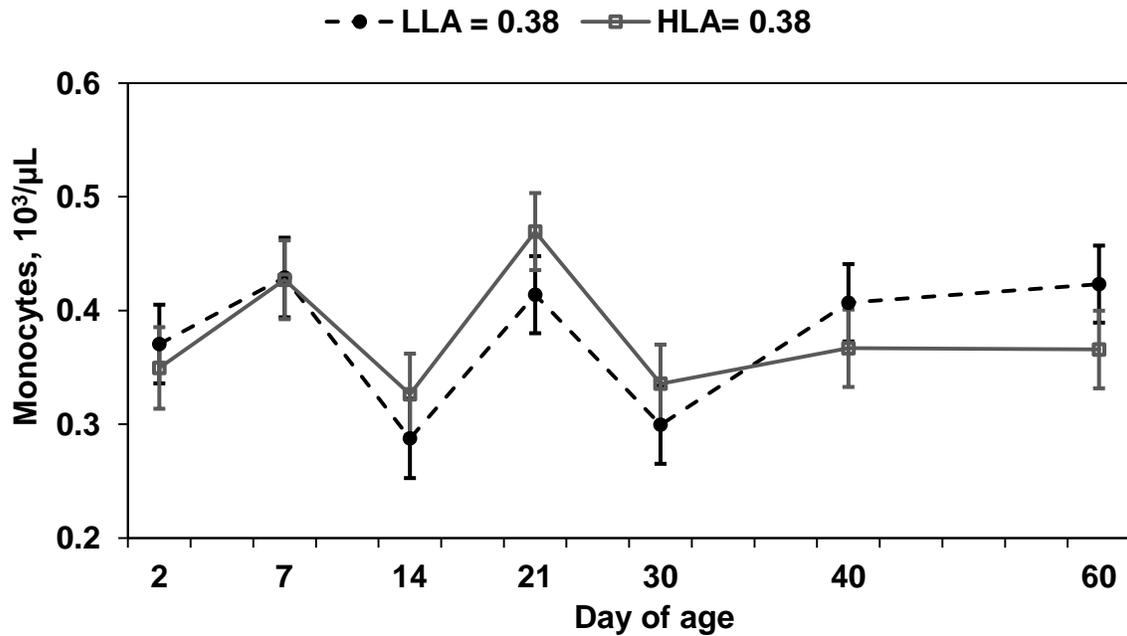


Figure 4-12. Blood concentrations of neutrophils and lymphocytes in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. A) Effect of age, $P < 0.01$. B) Effect of milk replacer, $P = 0.04$ and age, $P < 0.01$.

A



B

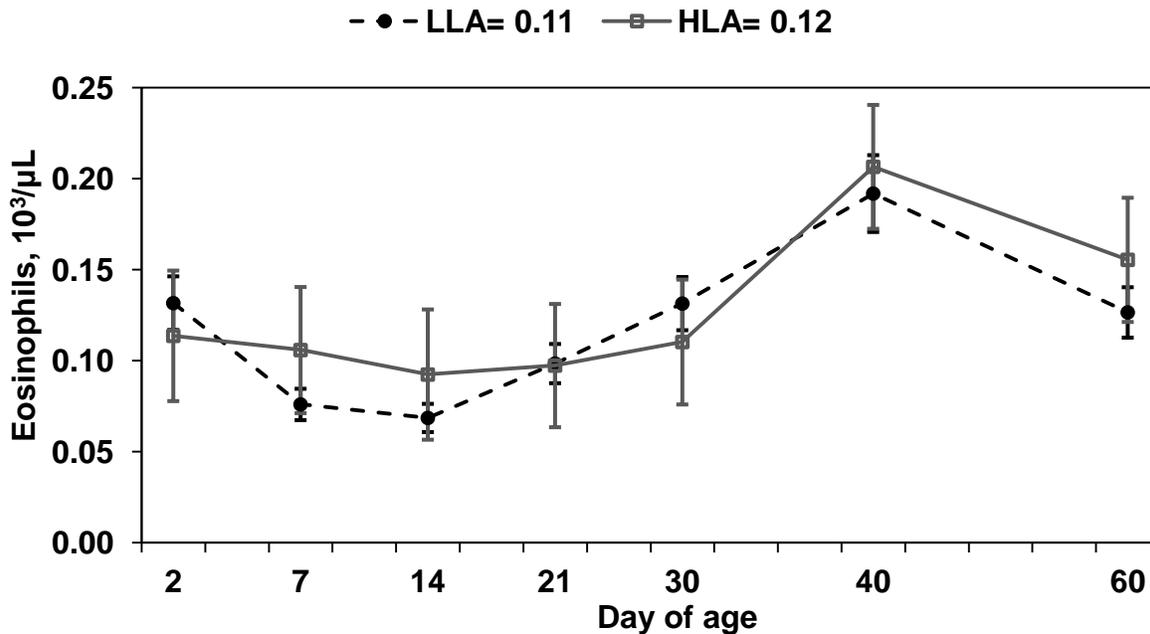
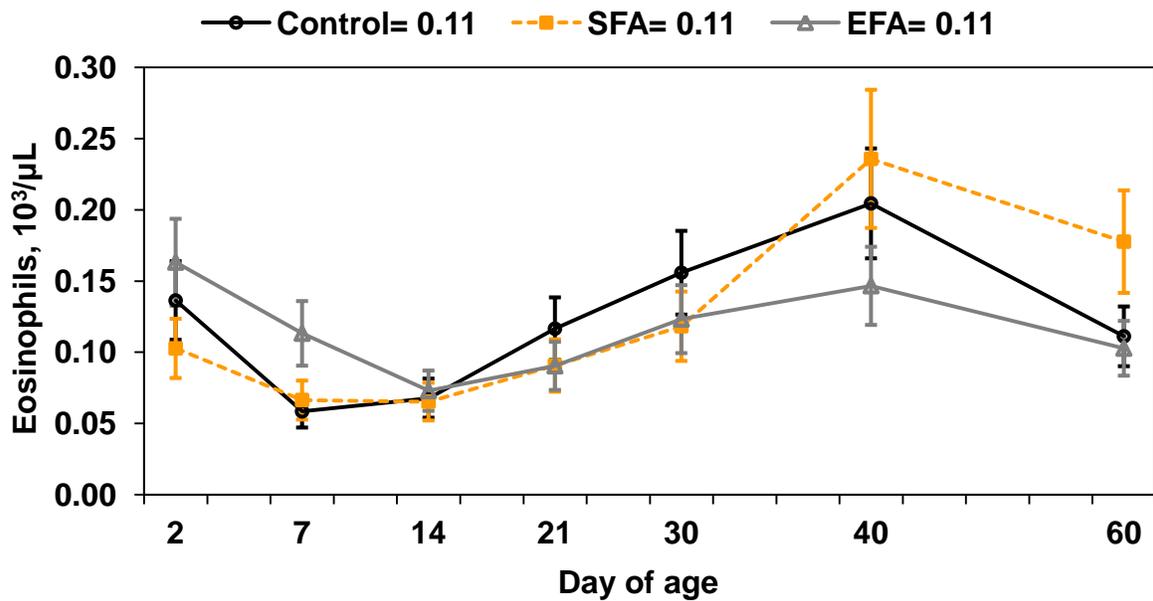


Figure 4-13. Blood concentrations of monocytes and eosinophils in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. A) Effect of age, $P < 0.01$. B) Effect of milk replacer by age, $P = 0.01$ (slice effect at day 7, $P = 0.04$ and at day 14, $P = 0.06$).

A



B

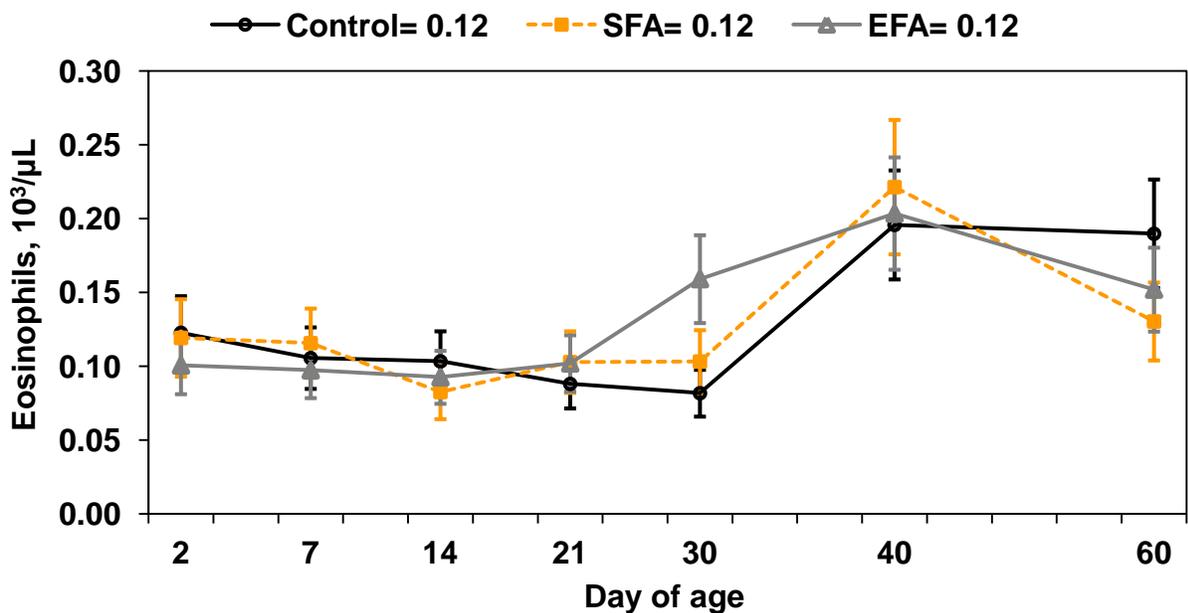


Figure 4-14. Blood concentrations of eosinophils in Holstein calves from 0 to 60 days of age. A) Calves were fed milk replacer containing low linoleic acid. B). Calves were fed milk replacer containing high linoleic acid. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain mix was offered starting at 31 d of life. Interaction of dam diet by milk replacer by age, $P < 0.01$ (slice effect at day 8, $P = 0.05$).

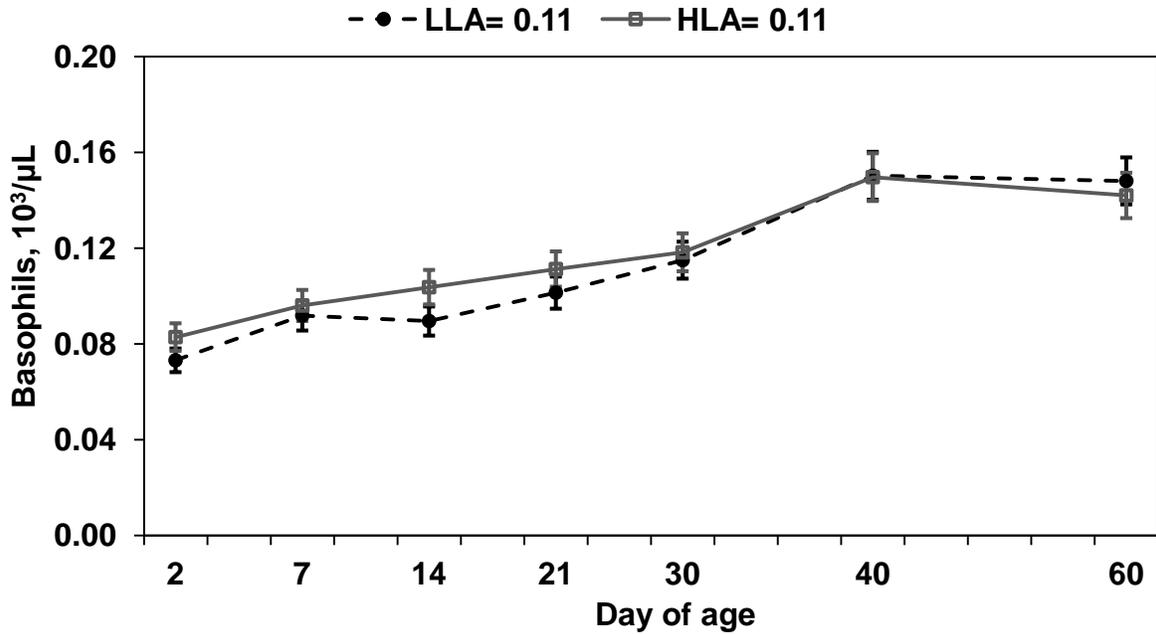
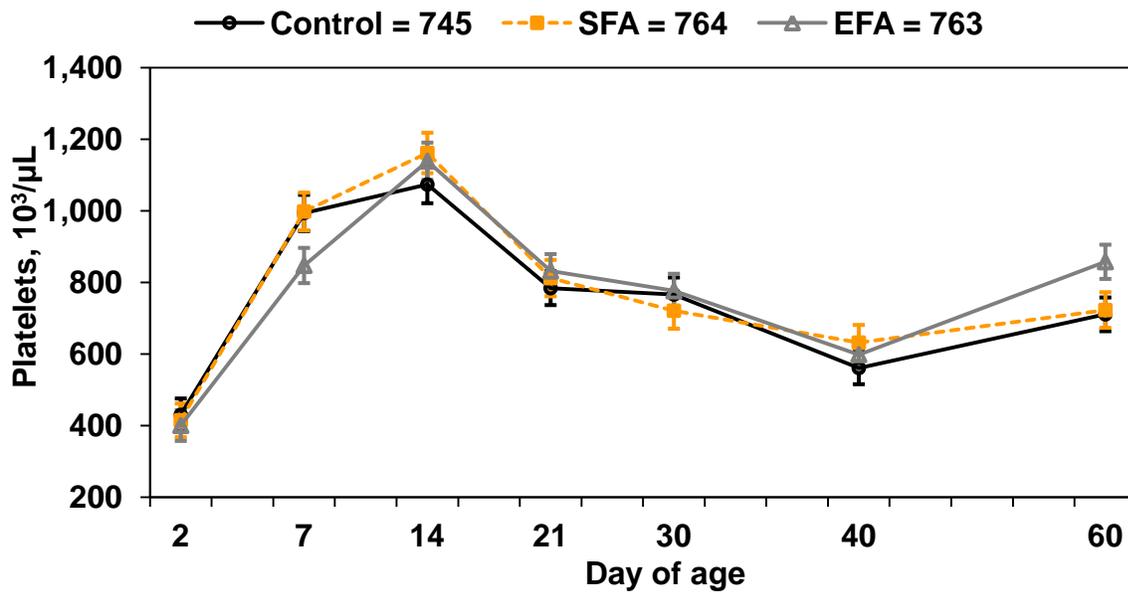


Figure 4-15. Blood concentrations of basophils in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of age, $P < 0.01$.

A



B

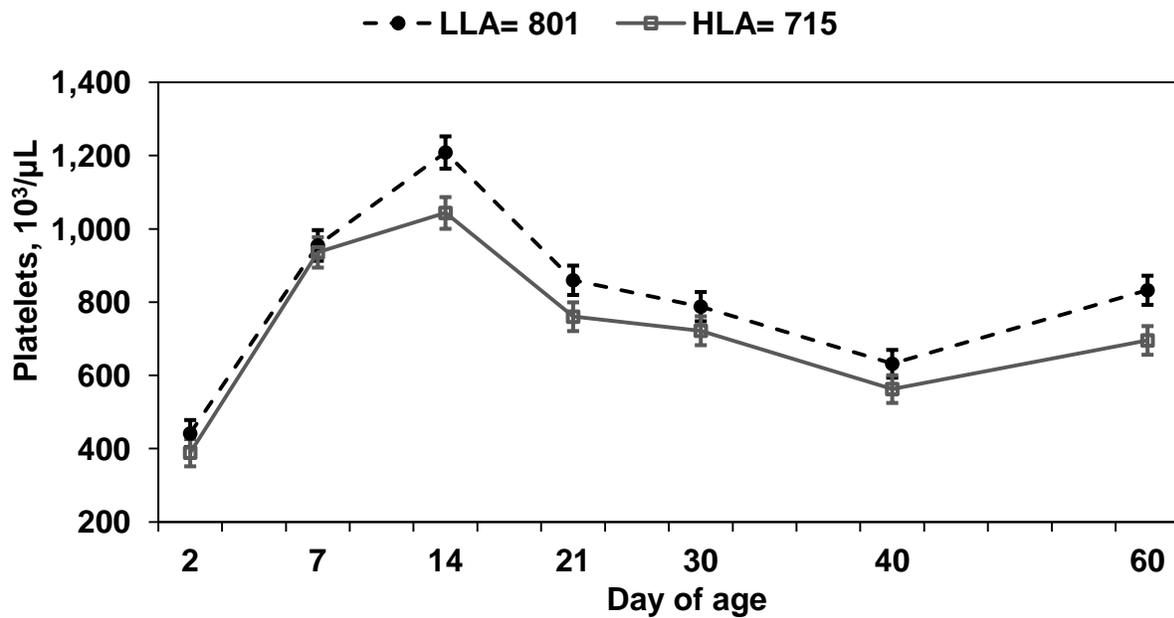


Figure 4-16. Blood concentrations of platelets in Holstein calves fed milk replacer containing low or high linoleic acid from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. A) Effect of dam diet by age interaction, $P = 0.03$ (slice effect, $P = 0.06$ (day 7) and $P = 0.05$ (day 60). B) Effect of age, $P < 0.01$, effect of milk replacer, $P = 0.03$.

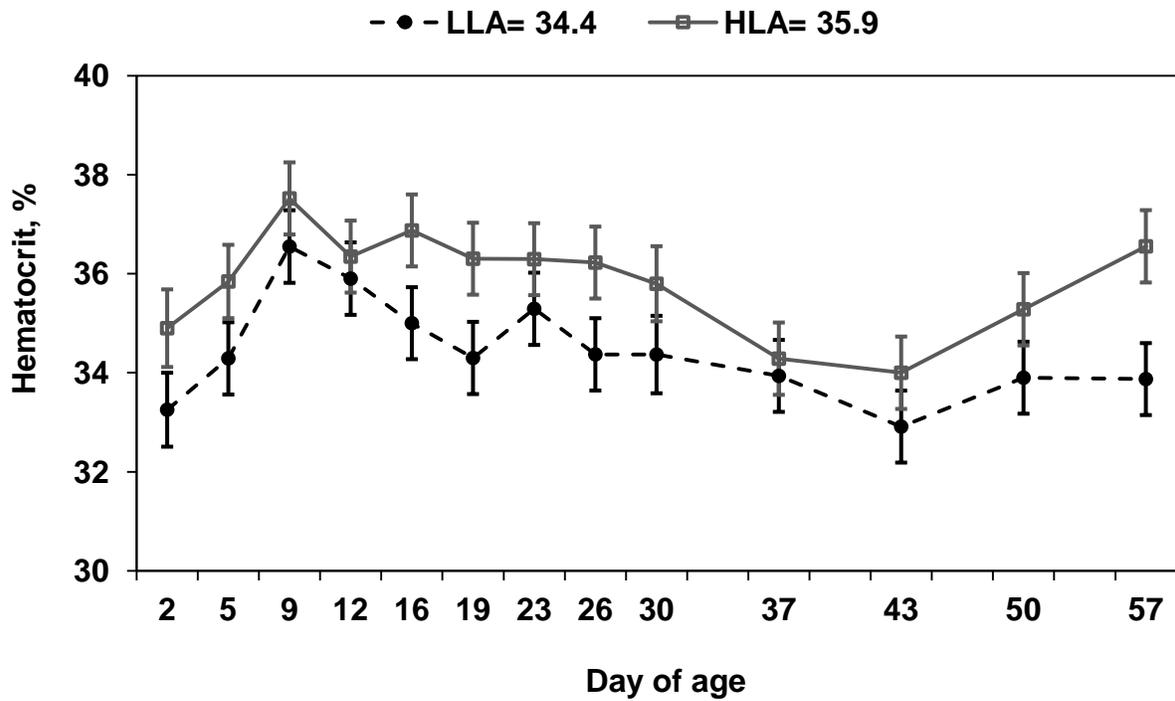


Figure 4-17. Hematocrit concentrations in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of age, $P < 0.01$; effect of milk replacer on hematocrit, $P = 0.08$.

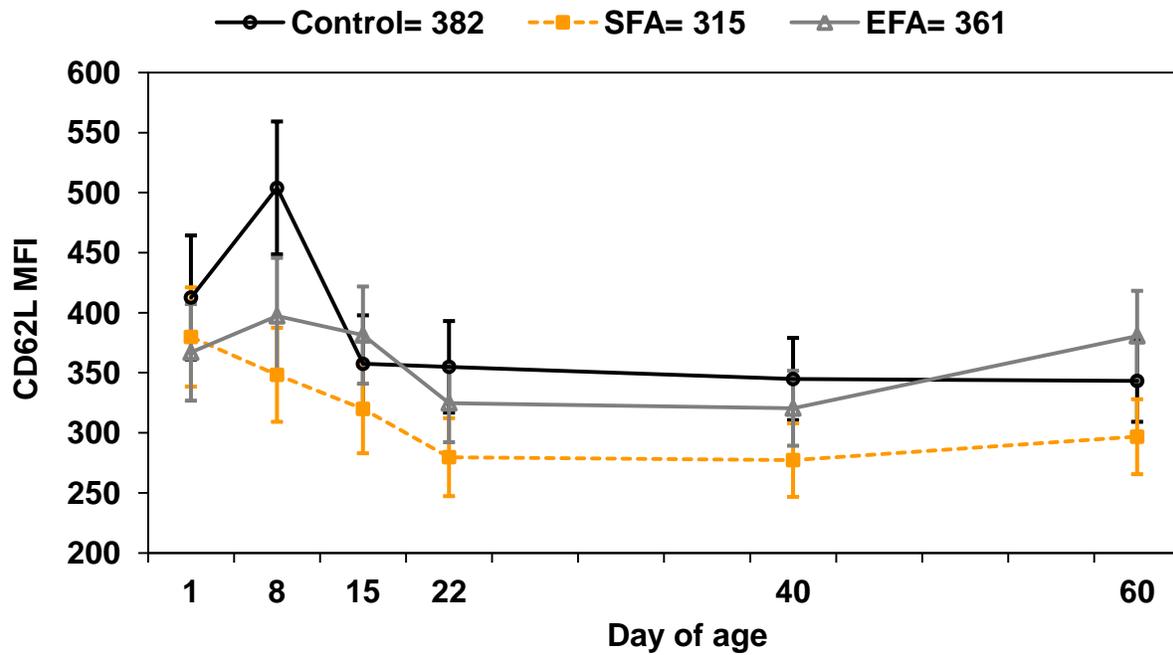
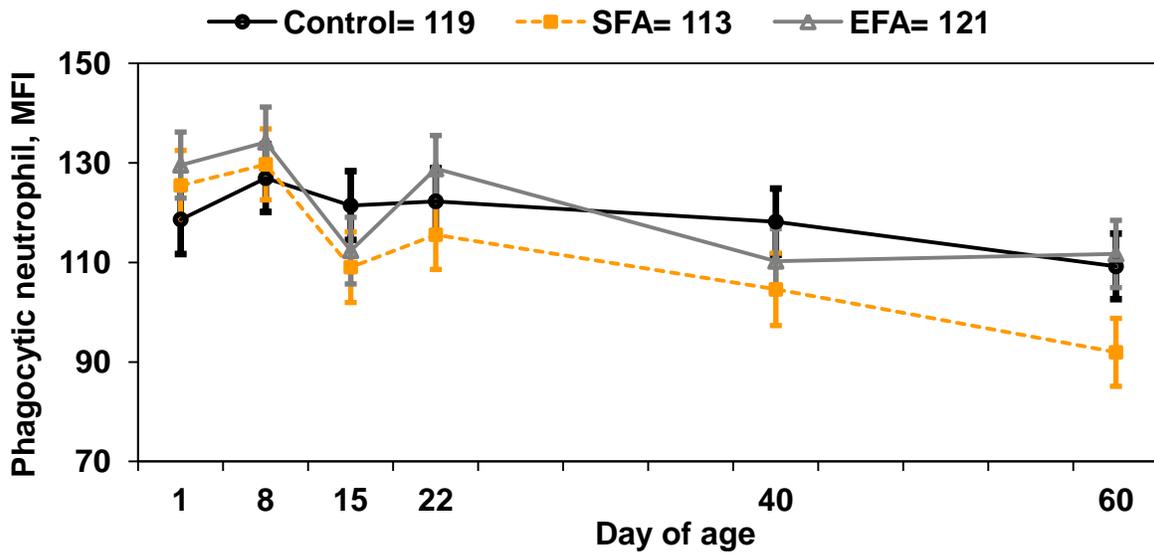


Figure 4-18. Mean fluorescence intensity (MFI) of neutrophils positive to CD62L in blood of Holstein calves fed milk replacer containing low or high linoleic acid from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Interaction SFA vs. EFA, $P = 0.10$.

A



B

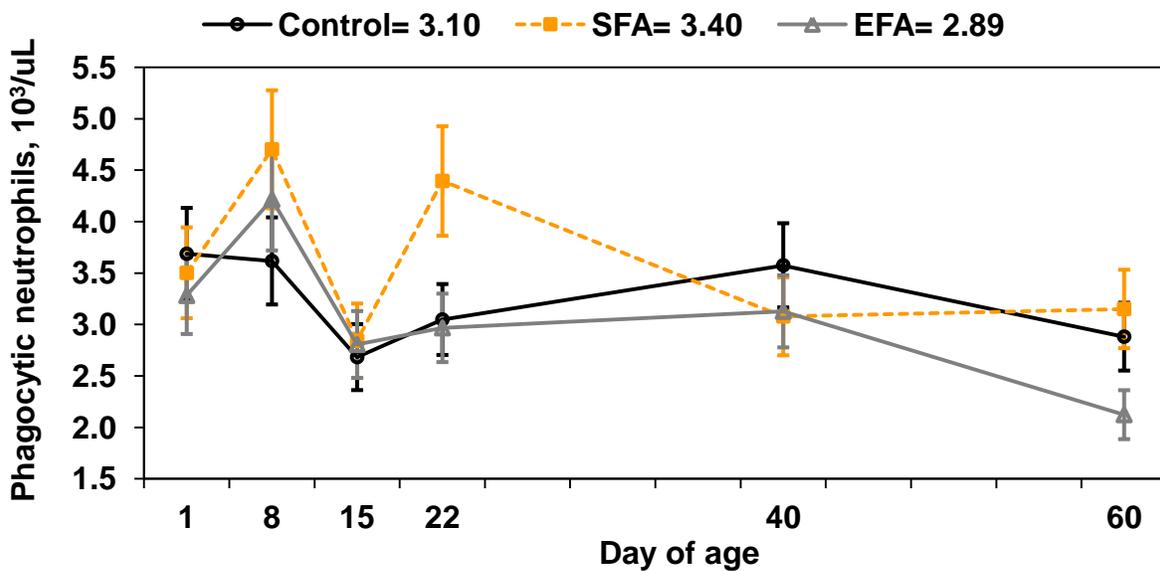


Figure 4-19. Mean Fluorescence intensity (MFI) and concentration of phagocytic blood neutrophils (B) in Holstein calves fed milk replacer containing low or high linoleic acid from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. A) Interaction SFA vs. EFA, $P = 0.04$. B) Interaction SFA vs. EFA, $P = 0.08$.

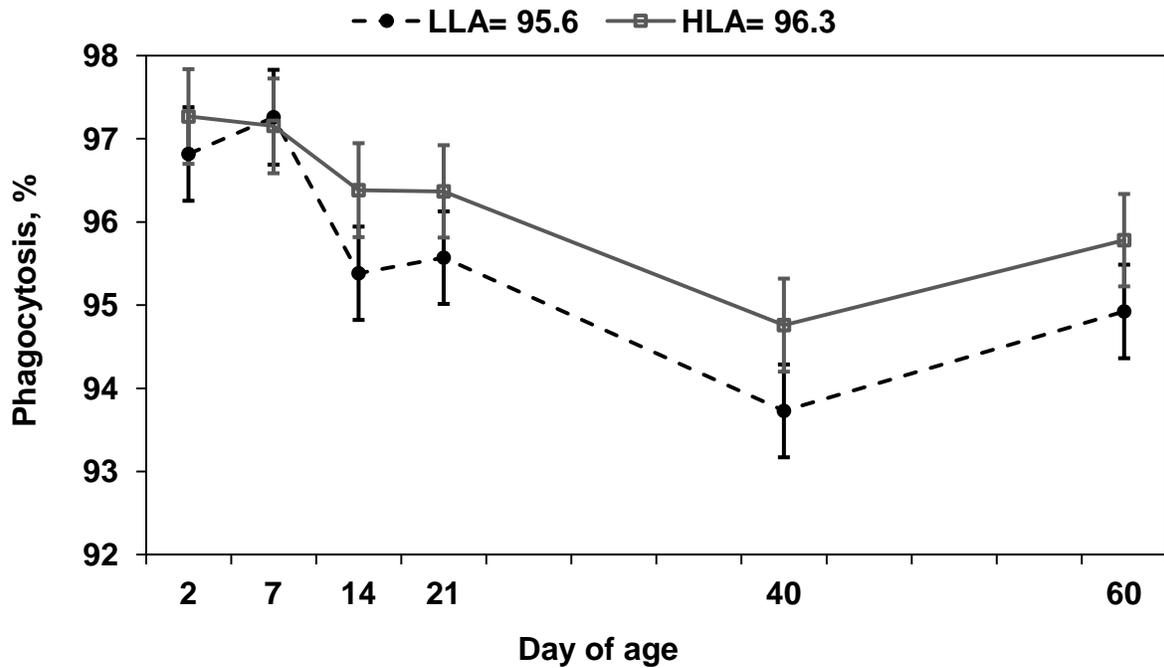


Figure 4-20. Percentage of blood neutrophils undergoing phagocytosis in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of milk replacer, $P = 0.09$; effect of age, $P < 0.01$.

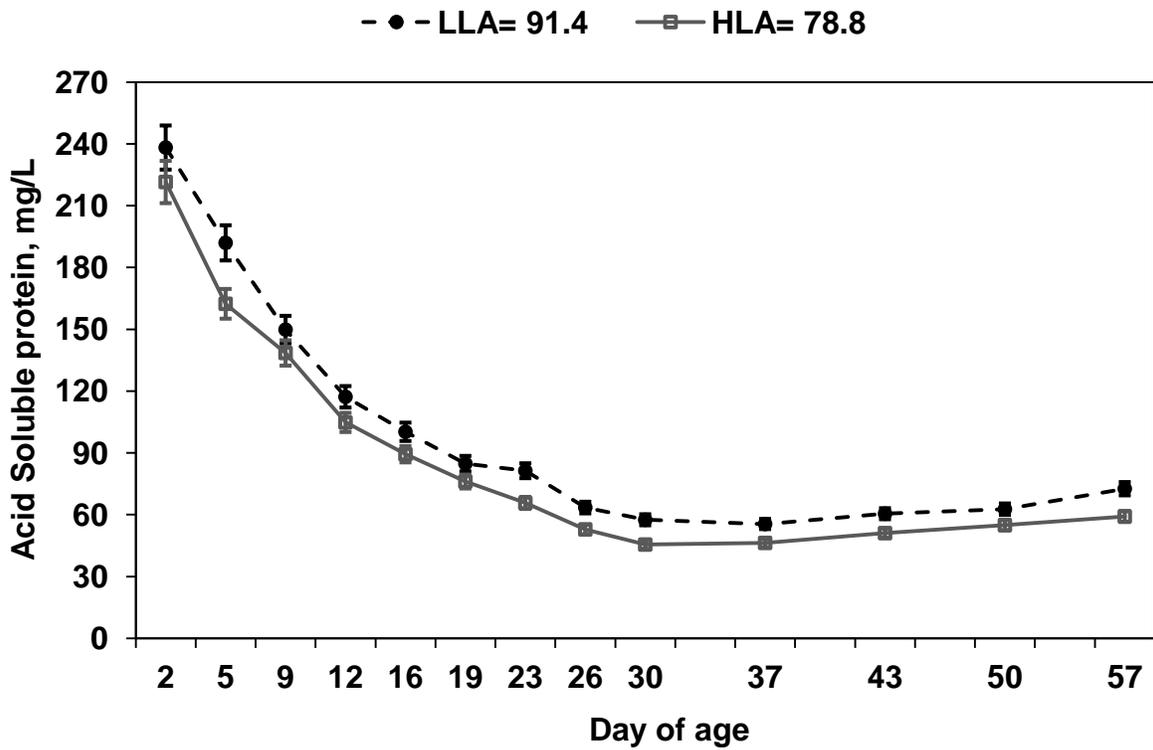
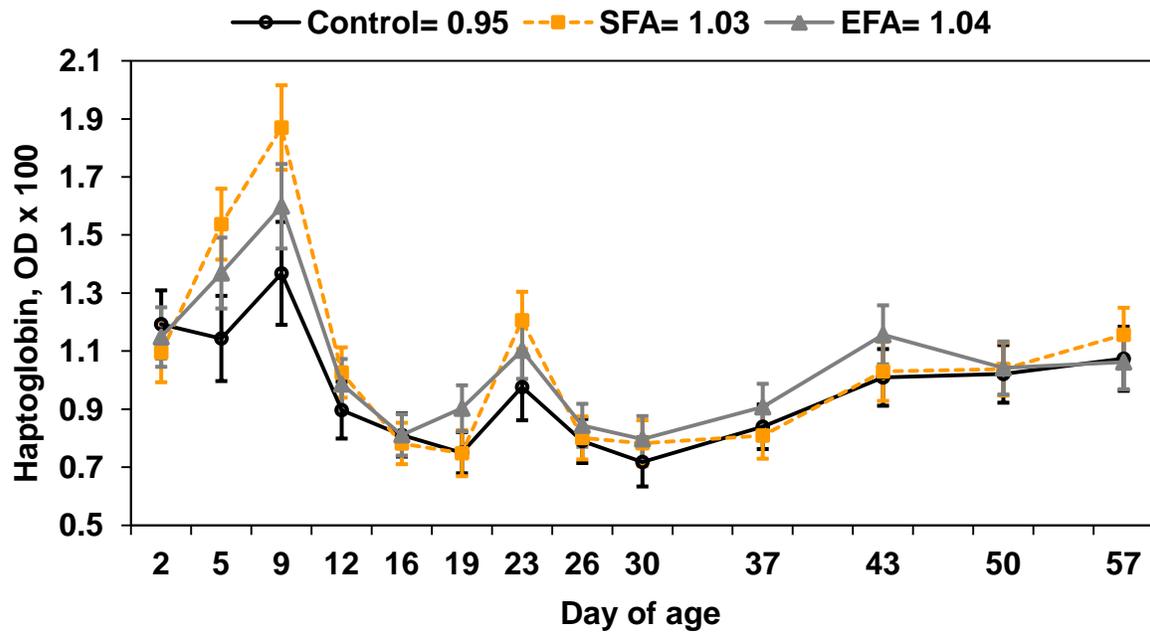


Figure 4-21. Plasmatic concentration of acid soluble protein in Holstein calves fed milk replacer containing low (LLA) or high linoleic acid (HLA) from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. Effect of milk replacer and age, $P < 0.01$. Effect of milk replacer by age interaction, $P = 0.09$ (slice effect starting at 16 d of age ≤ 0.10).

A



B

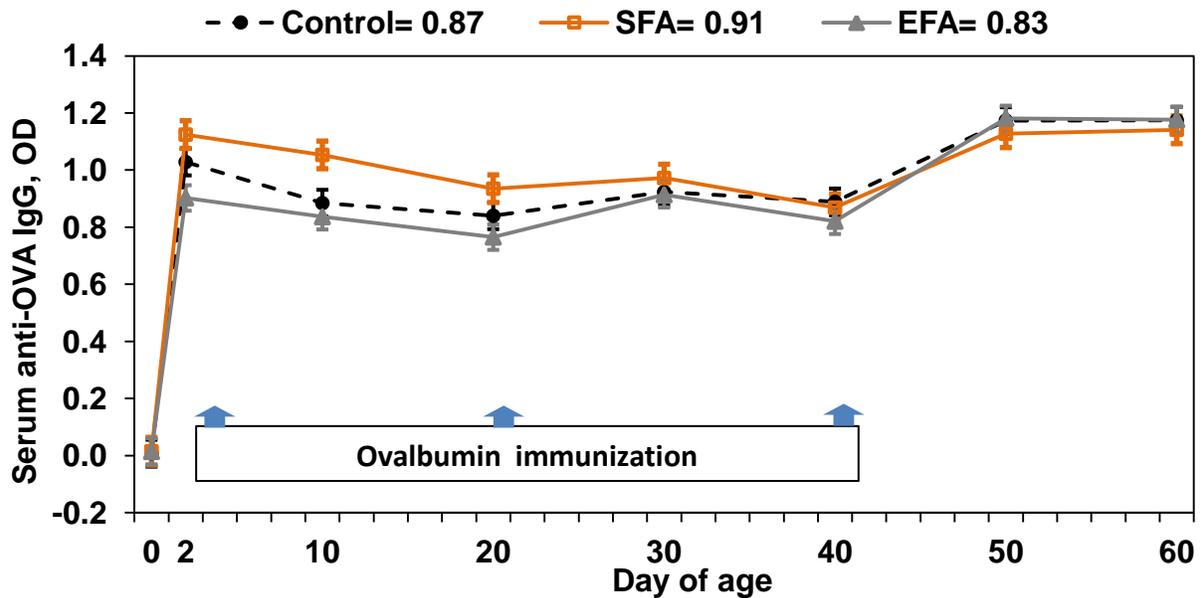


Figure 4-22. Plasmatic concentration of haptoglobin and serum anti-OVA IgG in Holstein calves fed milk replacer containing low or high linoleic acid from 0 to 60 days of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Grain was offered starting at 31 d of life. A) Effect of fat, $P = 0.06$) and of age, $P < 0.01$. B) Effect of dam diet by age interaction, $P < 0.01$ (slice effect at days 2, 10, and 20, $P < 0.04$).

CHAPTER 5
EFFECT OF SUPPLEMENTAL ESSENTIAL FATTY ACIDS TO PREGNANT
HOLSTEIN COWS AND THEIR PREWEANED CALVES ON CALF HEPATIC FATTY
ACID PROFILE AND GENE EXPRESSION

Background

Raising good quality replacement heifers, able to calf between 22 to 24 months and reaching at least 80% of adult weight is critical to gradually improve overall herd performance. Raising heifers is the most challenge area of management on dairy farms. After birth, dairy calves are removed immediately from their dams and transferred to a different unit to initiate the preweaning period which can take a few weeks to approximately 8 wk. The preweaning period which starts with an adequate passive transfer of immunity is considered one of the most critical periods affecting future performance (Beam et al., 2009; Furman-Fratczac, 2011). In addition, optimized feeding management of heifers during the preweaning period has a positive impact on future milk production (Soberon et al., 2012). A relative new concept adopted from human studies labeled “fetal programming” indicates that future milking performance of calves could be affected by the type of diet fed to their dams during late gestation (Fowden et al., 2006; Gicquel et al, 2008).

During the preweaning period, newborn calves have to cope with different environmental challenges such as adaptation to an external uterine life, pathogens, and different nutritional value of feeds. An appropriate management of all these factors should result with in calves able to overcome health problems, increase feed intake, and maintain a rapid growth rate. Feeding high-energy diets for rapid growth during the preweaning period has reduced both the age to reach the target breeding weight and costs

associated with raising of replacement heifers (Radcliff et al., 2000; Raeth-Knight et al., 2009).

The rapid growth rate of calves during the preweaning period implies a need for their bodies to have an efficient utilization of nutrients. The liver plays a key role in nutrient utilization due to its strategic position in the circulatory system; hence a profound understanding of its mechanism of nutrient utilization is needed. Pioneer studies (Jenkins et al., 1985; Jenkins et al., 1986; Jenkins and Kramer, 1986) supplemented the MR of newborn calves with different sources of fat and reported that concentration of essential EFA in liver and plasma reflected the composition of FA in the MR. Hence selective supplementation of FA would be expected to modify FA profile of different tissues and by that means its functionality. Early studies (Mashek et al. 2002; Mashek and Grummer, 2003, 2004) cultured preruminant calf hepatocytes with different FA to evaluate oxidative and gluconeogenic activity. Authors reported that different SFA, MUFA or PUFA did not affect gluconeogenesis as they did in liver of ruminant calves whereas LA, CLA c9 t11 and CLA t10 c12 did not affect propionic acid metabolism to produce glucose. However, regardless the type of FA, the formation of both glucose and glycogen were decreased when FA concentrations increased from 0.1 to 1.0 mM. Limited information has been generated regarding the role dietary EFA might have in modifying the expression of genes in liver of preweaned calves.

However, no study had evaluated the effect of supplementing EFA prepartum and continued supplementation of EFA during early life of the calf on liver metabolism through global gene expression analysis. The hypothesis was that feeding increased amounts of LA during late gestation and the preweaning period would modify the FA

profile of calf's liver and differentially impact the expression of hepatic genes. An additional hypothesis was that early strategic feeding would have a long-term effect on heifers' future milking performance. Therefore the objective was to evaluate the supplementation of EFA to prepartum cows during the last two months of pregnancy and during the preweaning period on hepatic FA profile and global gene expression in liver of 30 d of age with MR as only feed. An additional objective was to evaluate productive and reproductive responses of heifers at their first lactation.

Materials and Methods

Prepartum Management

The experiment was conducted at the University of Florida's dairy farm (Hague, FL) from October 2008 to June 2009. All procedures for animal handling and care were approved by the University of Florida's Animal Research Committee. Pregnant nulliparous (n = 35) and previously parous (n = 61) Holstein cattle were sorted according to calving date, parity, body weight (BW), and body condition score (BCS) and assigned to one of the three treatments at 8 wk before their expected calving date. Prepartum treatments: supplementation (Control), 1.7% of dietary dry matter (DM) of mostly free saturated FA (SFA, "Energy Booster 100", Milk specialties, Dundee, IL), and 2.0% of dietary DM as Ca salts of FA enriched with essential FA (EFA, "Megalac R", Church and Dwight, Princeton, NJ) as well as cattle general management were the same as those indicated in chapter 3.

Calves Dietary Treatments, Feeding Management and Analyses

All procedures regarding calving management at birth and colostrum feeding were done according details presented in Chapter 3. Calves were blocked by gender (n = 56 females and 40 males) and dam diet and randomly assigned to receive a MR containing

low (LLA, 0.56% LA, DM basis) or high concentrations of LA (HLA, 1.78% LA, DM basis) for 60 d starting at birth. Milk replacers and grain mix fed in this study were similar to that used in study reported in chapter 4. Similarly, all feeding management of calves was performed as indicated for calves in chapter 4. Procedures for housing, weighting and immunization of newborn calves were performed according to that indicated in chapter 4.

Liver Biopsy

Liver biopsies were performed at 30 ± 2 d using a percutaneous liver biopsy needle (Aries Surgical, Davis, CA). Briefly, an ultrasound imaging on the right flank was used to determine the optimal intercostal liver biopsy location. The area that was previously shaved and disinfected was anesthetized with 10 mL of 2% lidocaine HCl (Pfizer Inc., New York, NY). A 1-cm stab incision was made through the skin, after a thorough re-sterilization of the target zone. The biopsy instrument was inserted through the incision crossing the muscle layer reaching the liver and a liver sample (approximately 500 mg) was obtained. The open skin was closed with a surgical disposable sterile skin stapler (Oasis Inc., IL). Biopsied calves were subcutaneously injected with 1 mL of antibiotic at the base of the ear (Excede®, Pfizer Inc., New York, NY), and their post-surgical behavior was monitored for the following 12 h. The liver sample was rinsed immediately with sterile saline, sample was split into 2 vials and snap-frozen in liquid N, and stored at -80°C until analyzed for liver FA profile and mRNA abundance.

Calves Liver Fatty Acid Profile

Liver samples (~250 mg) were freeze dried for 48 h (Labconco Kansas City, MO) and delivered to Michigan State University for analysis of FA profile. Briefly, total FA

from freeze-dried liver samples were extracted using the standard procedure of Bligh and Dyer (1959) and then extracted FA were methylated by the 2-step procedure of Nuerberg et al. (2007) with some modifications. The FA methyl esters were quantified using a GC-2110 Plus gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector (1:100 split ratio) and a flame ionization detector using a CP-Sil 88 WCOT fused silica column (100 m × 0.25-mm i.d. × 0.2-µm film thickness; Varian Inc., Lake Forest, CA).

Total RNA isolation

Total cellular RNA was isolated from liver tissue (n = 18) using Qiazol reagent (Qiagen, Valencia, CA) and purified (RNA MIDI isolation kit, Qiagen, Valencia, CA) according to the manufacturer's recommendation. Briefly, frozen liver (200 mg) samples were immersed in 3 mL of Qiazol (quiagen, Valencia, CA) just prior to their homogenization in a conventional Rotor-Stator homogenizer. Homogenated solution in each tube was left for 5 min at room temperature and then 0.6 mL of chloroform was added to each tube and maintained at room temperature for 3 min. Tubes were centrifuged at 5000 × g at 4°C for 10 min. After the upper phase containing RNA (1.5 mL) was transferred from each tube to a tube containing 1.5 mL of ethanol (70%) and mixed immediately to suspend the precipitates, the mixed solution was added to the RNeasy midi spin column. Column tubes with the RNA suspension were centrifuged at 5000 × g at 23°C for 5 min. The flow through was discarded, and 2 mL of RW1 buffer was added to the column and centrifuged at 5000 × g at 23°C for 5 min. After the flow through was discarded, 160 µL of DNase working solution (12.5% of DNase stock solution in RDD buffer) was added carefully on the membrane of the column to ensure complete DNA digestion. A series of three additional washing steps with corresponding

buffers followed by centrifugation were performed, before a final 1.5 mL of RNase-free water was added to collect the RNA after centrifugation. Integrity and concentration of the RNA was then analyzed using a micro-volume spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA). Purified RNA was aliquoted and then stored at -80°C.

Affymetrix Array Hybridization, washing, staining and scanning

Isolated RNA samples were delivered to the Interdisciplinary Center for Biotechnology Research (ICBR) of the University of Florida. Briefly, amplification and biotin labeling were performed with an initial 200 ng of RNA by using MessageAmp III (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's guidelines. Samples were then tested in the Bioanalyzer for quality determination (all samples had an RNA integrity number > 7.5) and subsequently submitted for fragmentation and hybridization following Affymetrix's protocol. (Affymetrix GeneChip Bovine Genome Array, Affymetrix Inc., Santa Clara, CA). Arrays were washed on a fluidics station 450 (Affymetrix, Inc., Santa Clara, CA) with the hybridization wash and stain kit from Affymetrix. Fluorescent signals were measured with the Affymetrix GeneChip scanner 3000 7G.

Affymetrix Data Analysis

The Affymetrix GeneChip Bovine Genome array contains 24,027 probe sets corresponding to approximately 23,000 transcripts including assemblies from ~19,000 UniGene clusters. The Affymetrix CEL files, obtained after the fluorescence signal measure of each Affymetrix chip, were loaded into an AffyBatch object using R/Bioconductor environment (Gentleman et al., 2004).

Data normalization and background correction were performed using guanine-cytosine Robust Multichip Average (gcRMA) function as described by Wu et al. (2004). All Affymetrix control probes (AFFX prefix) were excluded as non informative probes using the informative/ non-informative (I/NI) calls from the enhanced-FARMS algorithm (Talloe et al., 2007). Differential gene expression was analyzed using linear models for microarray (LIMMA) as described by Smyth (2005). Treatments were arranged in a 3 x 2 factorial design that included the evaluation of 5 contrasts, as detailed in the Statistical design section. Enrichment analysis of DEG was evaluated using the functional annotation clustering method within the Data Base for Annotation, Visualization and Integrated Discovery (DAVID, Huang et al., 2009) bioinformatics resource. The enriched DEG were grouped according to their biological process (BP) and molecular function (MF) terms based on the gene ontology (GO, Ashburner et al., 2000) and Kiotto Encyclopedia of Genes and Genomens (KEGG, Kanehisa and Goto, 2000) pathways.

Statistical Analysis

Dam diets (n = 3) and MR diets (n = 2) were arranged in a 3 x 2 factorial randomized block design. On a weekly basis, a cohort of Holstein cows at 8 wk before the expected calving date was blocked by parity (nulliparous and parous) and BCS. Within each block, cattle were assigned randomly to one of the three dietary treatments. Calves after birth were blocked by dam diet and gender and randomly assigned to one of the two MR. A total of 40 male and 56 female calves were enrolled.

Liver FA profile and all productive and reproductive measures were analyzed using the MIXED procedure of SAS (Release 9.2) according to the following model: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$. Where Y_{ijk} is the observation, μ is overall mean, α_i is the fixed

effect of dam diet (control, SFA, and EFA); β_j is the fixed effect of MR (LLA and HLA), $(\alpha\beta)_{ij}$ is the interaction of dam diet by MR; and ϵ_{ijk} is the residual error.

The following orthogonal contrasts were performed for all variables [1) FAT: dam diet of fat (SFA + EFA) vs. no fat (control), 2) FA: dam diet EFA vs. SFA, 3) MR: milk replacer HLA vs. LLA, 4) FAT by MR: interaction of contrasts 1 and 3, 5) FA by MR: interaction of contrasts 2 and 3]. For FA profile and all productive and reproductive measures, a P-value ≤ 0.05 was considered significant and a trend was considered when P values were > 0.05 but ≤ 0.10 .

Analysis with the LIMMA package (Smyth, 2005) was used for Identification of DEG after using the method of Benjamini and Hochberg (BH) to adjust for multiple tests and control the false discovery rate (FDR) up to 5%, a cut-off for adjusted P-value ≤ 0.05 and a fold change (FC) ≥ 1.4 . For the effects of comparing DEG in pre-determined contrasts of experimental groups, the appropriate reference group was defined for each comparison per contrast as follows:

Arrangement of treatments

Treatment	Dam Diet	Milk replacer	Number of samples
1	Control	LLA	3
2	Control	HLA	3
3	SFA	LLA	3
4	SFA	HLA	3
5	EFA	LLA	3
6	EFA	HLA	3

1. Contrast FAT: Dam diet (SFA + EFA)/2 \div Control (reference).
2. Contrast FA: Dam diet EFA \div SFA (reference).
3. Contrast MR: Milk replacer HLA \div LLA (reference)

4. Interaction FAT by MR: $[(\text{SFA-HLA} + \text{EFA-HLA})/2 : \text{Control-HLA (reference)}] \div [(\text{SFA-LLA} + \text{EFA-LLA})/2 : \text{Control-LLA (reference)}]$
5. Interaction FA by MR: $[\text{EFA-HLA} : \text{SFA-HLA (reference)}] \div [\text{EFA-LLA} : \text{SFA-LLA (reference)}]$

Binary data were all analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). The models included the effects of dam diet and milk replacer. Adjusted odds ratio and the 95% confidence interval (CI) were calculated. Differences discussed in the text were significant at $P \leq 0.05$ and tended to be significant at $0.05 < P \leq 0.10$, unless another probability is indicated. The modified fisher's exact probability test was used to identify statistically over represented GO terms and KEGG pathways within the DAVID annotation tool.

Results

Liver Fatty Acid Content and Profile

Mean FA concentration on liver of male calves was not affected by dam diet. but by the type of MR fed. Calves fed the HLA MR had a lower mean concentration of total FA in liver (7.56 vs. 8.47% of total DM, Table 5-1). Mean proportions of SFA, MUFA, and PUFA across treatments were 42.6, 15.4, and 39.3% respectively. These groups of FA were affected only by the type of MR fed to calves. Calves fed HLA MR had a lower proportion (of total FA) of SFA (40.0 vs. 45.1%) and MUFA (14.3 vs.16.4%) but greater proportions of PUFA (43.6 vs. 35.56%, Table 5-1).

As expected, most of the individual FA in liver of calves also were affected by the MR fed. Calves fed LLA MR, whose only source of fat was CCO, had increased proportions ($P < 0.01$, Figure 5-1) of C12:0, C14:0, and C16:0, with the greatest proportional difference detected for C14:0 (5.22 vs. 1.30%). Regarding MUFA, OA

represented 75% of total MUFA in liver of calves fed LLA MR, followed by C16:1 which occurred in minor proportions of total MUFA but also occurred in greater proportions in liver of males fed LLA vs. HLA MR (0.48 vs. 39%, $P = 0.02$). Of the six identified n-6 FA, four were increased or tended to be increased in liver of calves fed HLA MR, namely LA (22.1 vs. 15.9% of total FA, $P < 0.01$), C20:2 (1.01 vs. 0.54% of total FA, $P < 0.01$), AA (10.78 vs. 10.17% of total FA, $P = 0.09$), and C22:4 (1.27 vs. 1.13% of total FA, $P = 0.03$, $P = 0.03$) whereas proportions of GLA (0.03 vs. 0.07% of total FA, $P < 0.01$) and C20:3 (2.70 vs. 3.36% of total FA, $P = 0.01$) were decreased in liver of calves fed HLA MR. Proportions of LA and AA accounted for ~76% of total n-6 FA in calves fed HLA MR, hence proportions of total n-6 FA were greater in calves fed HLA as compared to those fed LLA MR (37.6 vs. 31.1% of total FA, $P < 0.01$, Table 5-1, Figure 5-2 A). Four n-3 FA were identified in the liver of calves. Of these ALA (0.99 vs. 0.70% of total FA, $P < 0.01$) and DPA (2.06 vs. 1.57% of total FA, $P < 0.01$) were greater in liver of calves fed HLA MR whereas EPA was greater in liver of calves fed LLA MR (0.24 vs. 0.19% of total FA, $P < 0.01$) and proportions of DHA did not differ due to the type of MR fed. Because ALA and DPA accounted for 60% of total n-3 FA in liver of calves fed HLA MR, the total proportion of n-3 FA was greater in this group of calves compared to calves fed LLA MR (5.08 vs. 4.17% of total FA, $P < 0.01$, Table 5-1, Figure 5-2 B). However the effect of MR on the proportions of n-3 FA of liver was influenced by the type of fat fed to their dams. If the dam was fed SFA, the effect of MR on the shorter chain n-3 FA (ALA and EPA) was magnified; that is, the increase in ALA proportions due to the feeding of HLA MR was greater if SFA (1.03 vs. 0.65%) rather than EFA (0.91 vs. 0.71%) was fed prepartum (FA by MR interaction, $P = 0.04$, Table 5-1). Similarly EPA proportions were

increased in liver fat of calves by HLA MR only if SFA (0.30 vs. 0.18%) and not EFA (0.22 vs. 0.22%) was fed to their dams prepartum (FA by MR interaction, $P = 0.04$, Table 5-1). Lastly, only HLA and not LLA MR increased DHA proportions in liver fat of calves if their dams were fed EFA (1.90 vs. 1.34%) rather than SFA (1.84 vs. 2.05%) prepartum (FA by MR interaction, $P = 0.03$, Table 5-1).

Feeding fat to prepartum cows produced some minor effects on liver FA profiles of their calves such as greater proportions of AA (10.73 vs. 9.97% of total FA, $P = 0.05$) and DPA (1.87 vs. 1.70% of total FA, $P = 0.02$) but lower proportions of ALA (0.82 vs. 0.89% of total FA, $P = 0.05$) compared to calves from dams not supplemented with fat.

Differential Expression of Genes in Liver

A total of 58 transcripts were upregulated according to the criteria of false discovery rate ≤ 0.05 and fold change ≥ 1.4 (Figure 5-3) in liver of calves born from dams fat (EFA + SFA) compared to that of calves born from dams not fed fat, but only 41 transcripts were either annotated or identified with the bovine DAVID annotation tool. Feeding a specific type of fat during the prepartum period resulted in the upregulation of 75 transcripts (Figure 5-3) in liver of calves born from dams fed EFA compared to those fed SFA. From these 75 transcripts, only 63 were recognized by bovine-David. Those 2 contrasts of dam diet effects shared a total of 7 transcripts (Figure 5-3) that were differentially expressed in the same manner and 2 of them were not annotated. Regarding the effect of feeding a HLA MR, 53 transcripts were upregulated when HLA rather than LLA MR was fed (Figure 5-3). From those transcripts only 42 were recognized by bovine-DAVID.

A total of 208 transcripts were upregulated differentially in liver from calves fed HLA instead of LLA MR in a manner that differed between dams fed or not fed fat

prepartum (interaction of FAT by MR, Figure 5-3). Of the 208 transcripts, 167 were read by bovine-DAVID. A specific comparison between type of fats fed prepartum indicated that a total of 132 transcripts were upregulated in liver of calves born from dams fed EFA instead of SFA in a manner that differed due to the type of MR fed (interaction of FA by MR, Figure 5-3). Of these 132 differentially expressed transcripts, 107 were read by bovine-DAVID. Among both interactive contrasts, 13 of the differentially expressed transcripts were commonly upregulated. It is strikingly clear that distinct differences in gene expression are detected, and the differences are much more pronounced when looking at the interactive effects between prepartum supplementation and type of MR fed preweaning.

In contrast, feeding fat prepartum downregulated 51 transcripts in liver of calves, with 39 of these transcripts being read by DAVID (contrast FAT, Figure 5-4). Liver of calves born from EFA-fed dams had 56 downregulated transcripts compared to calves born from SFA-fed dams (contrast FA, Figure 5-4). From these 56 transcripts, 51 were read by DAVID. These two dam diet-contrasts had 5 common downregulated genes with 4 of them read by DAVID. If calves were fed HLA instead of LLA MR, 31 transcripts were downregulated with 19 being read by DAVID. A total of 187 genes were differentially downregulated in liver of calves if they were fed HLA instead of LLA while born from fat-fed dams (interaction FAT by MR, Figure 5-4). From these 197 transcripts, 132 were read by DAVID. When comparing the effect of feeding a specific profile of FA prepartum, liver of calves born from dams fed EFA instead of SFA had a differential downregulated response when fed HLA instead of LLA MR. These calves had 182

downregulated transcripts with 134 being read by DAVID. Among both interactive contrasts, 17 of the differentially expressed transcripts were commonly downregulated.

Enriched Gene Ontology Terms

The groups of DEG within GO terms were identified using the DAVID analysis of functional annotation clusters with medium stringency. The enrichment score (ES) of each cluster represents the $-\log_{10}$ value of the geometric mean of all adjusted Fisher P values for each GO within a cluster. Hence the greater the ES the smaller is the P value. Authors of DAVID annotation tool recommend giving more attention to clusters with $ES \geq 1.3$ and adjusted Fisher P values for GO terms ≤ 0.10 . However they also recommend evaluating ES with lower values in terms of the expected biological meaning according to the experimental condition (Huang et al., 2009). Therefore, after analyzing all clusters and GO (only BP and MF) terms within each cluster for all five contrasts evaluated in the current study, it was decided to present only clusters with $ES \geq 1.00$ ($P \leq 0.10$) and within cluster, only GO terms with an adjusted fisher P value of ≤ 0.10 . A single exception was done for a cluster with an $ES = 0.97$ (contrast FA by MR) due to its significant biological meaning.

From the 41 upregulated and recognized genes for the effect of feeding fat prepartum, the analysis with bovine-DAVID resulted in 3 enriched clusters. Yet not one of these clusters or GO terms fit within the selected enrichment criteria ($ES \geq 1$, adjusted Fisher P value ≤ 0.10). On the other hand, the enrichment analysis of upregulated genes within the contrast of FA resulted in a total of 9 enriched clusters but only 1 cluster including 2 BP met the criteria of selection (Table 5-2). The enriched biological processes were 1) negative regulation of metabolic and transcription processes which included 4 genes and 2) negative regulation of transcription, which

resulted in the enrichment of 3 genes. Feeding HLA MR instead of LLA MR resulted in the enrichment of 4 clusters but only 3 of them met the enrichment criteria (Table 5-2). The first enriched cluster included the MF calcium ion binding with 7 enriched genes; the second included 2 MF, namely actin binding and motor activity and the BP striated muscle tissue development; the last cluster included 2 MF, namely cation binding and calcium ion binding, and included 1 BP, namely, proteolysis involved in cellular protein catabolic process.

The interaction contrasts of dam diet and MR resulted in a greater number of DEG and hence in a greater number of enriched clusters. For the interaction FAT by MR, only 7 clusters were selected from a total of 27 clusters enriched with upregulated genes (Table 5-3). The top enriched cluster included the highest number of DEG involved in electron carrier activity, oxidation reduction, and iron ion binding. The other clusters had at most 2 GO terms involved in processes such as binding, transport, and metabolic processes. The other combined effect of feeding a specific type of fat and HLA MR (interaction of FA by MR) resulted in the enrichment of 22 clusters with only 3 meeting the criteria assumed in this current study (Table 5-4). The enriched clusters included different BP terms involved in catabolic processes to generate energy intermediates, phospholipid biosynthetic process, organophosphate metabolic processes, and protein complex assembly.

The analysis of downregulated DEG resulted in a few enriched clusters affected by dam diets or MR but a greater number of enriched clusters affected by the interaction of dam diet and MR. This pattern was similar to that observed with the upregulated DEG. The two contrasts involving dam diets (FAT and FA) resulted in a total of 6 enriched

clusters within each contrast, but using the enrichment criteria set for this study, only 2 clusters were selected for the contrast of FAT which included actin binding, striated muscle tissue development, and motor activity (Table 5-5). Only 1 cluster was selected for the contrast of FA which included the genes involved in catabolic processes. Calves fed HLA instead of LLA MR had 3 enriched clusters with their downregulated DEG but only 1 cluster met the criteria of enrichment. The GO included iron ion binding and oxidation reduction.

Similar to the upregulated DEG enrichment analysis, prepartum diets influenced the effect of HLA feeding on the enrichment of clusters for downregulated DEG in the liver of calves. Feeding HLA MR to calves born from dams fed fat instead of control diets resulted in the enrichment of 19 clusters but only 4 clusters met the criteria of enrichment (Table 5-6). The main enriched GO terms in this interaction group were different binding activities, striated muscle development, and heart morphogenesis. Calves fed HLA MR and born from dams fed EFA instead of SFA resulted in the enrichment of 25 clusters with downregulated DEG but only 3 clusters met the criteria used in the current study (Table 5-7). The top enriched cluster included GO terms involved in proteolysis, peptidase activity, and thiolesterase mediated by ubiquitin. Other clusters included genes involved in different signaling pathways and different immune activities.

Enriched KEGG Pathways

Enriched pathways within each contrast of evaluation were identified with DAVID using cut-off criteria to contain at least 3 genes in a given pathway and have an adjusted Fisher P-value ≤ 0.10 . Under these cut-off settings, the upregulated DEG in dam diet contrasts (FAT and FA) did not enrich any KEGG pathway. However, feeding

MR enriched 4 pathways (Table 5-8). Two up regulated pathways shared the same genes that encode for sarcomeric proteins (Tajsharghi, 2008) and were related to cardiomyopathy. The upregulation of the PPAR pathway included the upregulation of the gene coding for PPAR α receptor and 2 of its target genes (OLR1 and ANGPTL4; Table 5-8). The upregulation of this PPAR pathway is an indicator of enhanced lipid transport (OLR1) and adipocyte differentiation (ANGPTL4, also labeled as PGAR) in calves fed HLA MR (Figure 5-5 so designated by diamond symbol). The last upregulated KEGG pathway in liver of calves fed HLA MR was the tight junction pathway which included 3 genes (MYL2, MYH7, and ACTN2) which encode for two sarcomeric proteins, actin and myosin, that might be related to handling of cardiomyopathy disorders (Tajsharghi, 2008).

The enriched KEGG pathways of liver of calves fed MR was influenced greatly by the prepartum diet fed to their dams. Calves fed HLA instead of LLA MR and born from dams fed fat instead of the control diet (interaction of FAT by MR) experienced an upregulation of 8 KEGG pathways (Table 5-8). One of the upregulated pathways was the PPAR signaling pathway. The gene coding for PPAR- α receptor per se was not upregulated but 6 PPAR α target genes were upregulated (Table 5-8 and Figure 5-5, so designated by star symbol). In addition to the PPAR pathway, well known for its regulatory process in lipid oxidation, other catabolic KEGG pathways, involved in metabolism of lipids, carbohydrates, and drugs also were upregulated (Table 5-8). In contrast, when calves were fed HLA MR instead of LLA MR and were born from dams fed EFA- instead of SFA (interaction of FA by MR), 4 KEGG pathways were enriched (Table 5-8). The enriched pathways are involved in catabolic processes and generation

of intermediate products to generate energy, with a marked upregulation of the oxidative phosphorylation pathway. Interestingly, adipocytokine pathway was upregulated that included 3 genes involved in regulation of insulin sensitivity (ADIPOR2, STAT3, and ACSL5 labeled as FACS so designated by star symbol, Figure 5-6).

Neither the type of fat fed prepartum nor the type of MR fed preweaning downregulated any KEGG pathways. Four KEGG pathways were downregulated due to feeding of FAT prepartum (Table 5-9). Of these 4 pathways, 3 pathways were downregulated mainly due to HLA rather than to LLA MR (FAT by MR interaction). For 2 of the pathways, the 3 genes affected were identical, namely MYL2, TNNC1, and TPM2 for hydrotrophic cardiomyopathy and dilated cardiomyopathy. For the tight junction pathway involved in maintaining the impermeable integrity of all cell membranes, the main effect of FAT feeding influenced 3 genes (MYL2, MYH7, and ACTN2) whereas the interaction of FAT by MR influenced these same 3 genes plus MYH1 and CASK (Table 5-9). Genes MYL2, MYH1, and MYH7 help code for the myosin protein and ACTN2 codes for α -actinin protein as illustrated in Figure 5-7 (so designated with arrow symbol). The CASK (calcium/calmodulin-dependent serine protein kinase) protein functions as a scaffolding protein. In addition, calves born from dams fed fat instead of control diet had 3 downregulated genes (ICAM1, MYL2, and ACTN2) within the leukocyte transendothelial migration KEGG pathway (Table 5-9 and Figure 5-8 so designated with star symbol, MYL2 is shown as MLC). Lastly liver from calves fed HLA MR and born from dams fed fat had 5 genes (SOCS1, UBA7, PML, HERC4, and BIRC3) downregulated from the ubiquitin mediated proteolysis KEGG pathway (Table 5-9). This same KEGG pathway (ubiquitin mediated proteolysis) was influenced in liver

of calves fed HLA and born from dams fed EFA but 3 different (CUL3, KLHL9, and ITCH) and 1 common (BIRC3) genes were downregulated (Table 5-9). The other KEGG pathway affected in the liver of calves fed HLA and born from dams fed EFA was the pyrimidine metabolism pathway involving 4 genes (UPP2, ENTPD4, EPYD, and NME7).

Heifers Productive and Reproductive Performance

Performance of experimental heifers was evaluated until the end of their first lactation. A total of 56 heifers participated in the experiment, however only 33 heifers were included in the data set because 23 heifers were culled before finishing at least 150 d in their first lactation. The effect of MR and its interaction with dam diet had minimal impact on all productive and reproductive variables measured (Table 5-10). In contrast, prepartum feeding of fat had major influences on future outcomes. Age at first insemination did not differ due to dam diet (mean of 13.1 mo). However heifers born from dams fed fat during the last 8 wk prior to calving had a greater number of inseminations at first conception (2.6 vs. 1.7, $P = 0.04$, Table 5-10). In agreement with a greater number of inseminations it was an older age at first calving (24.2 vs. 22.9 mo, $P = 0.02$, Table 5-10) in heifers born from dams fed fat.

Because heifers born from fat-fed dams were older at first calving, they also were heavier (548 vs. 512 kg, $P = 0.04$, Table 5-10) and had greater BCS (3.3 vs. 3.1, $P = 0.04$, Table 5-10) than heifers born from control-fed dams. The length of lactation did not differ with diets (296 vs. 302 days, $P = 0.56$). Heifers from fat-fed dams tended to have a greater BCS (3.53 vs. 3.43, $P = 0.08$, Table 5-10) at the end of lactation.

Days in milk at peak of lactation tended to be earlier for heifers born from fat-fed dams (80.5 vs. 96.3, $P = 0.08$, Table 5-10). Heifers born from dams fed fat prepartum

produced more mature equivalent milk during their first lactation (12,004 vs. 10,605 kg, $P = 0.02$, Table 5-10). Concentrations of fat and protein in milk did not differ due to prepartum diets but lactose concentration tended to be greater for heifers born from dams fed fat (4.81 vs. 4.78, $P = 0.08$, Table 5-10). The type of FA fed prepartum did not affect any of the variables except BCS at dry off. Heifers fed the LLA MR and born from dams fed EFA prepartum tended to have more body condition than those fed the HLA MR (3.8 vs. 3.5) whereas the type of MR fed did not affect BCS at dry off if dams were fed SFA prepartum (3.4 vs. 3.4,), $P = 0.07$, Table 5-10).

Culling incidence was evaluated as total incidence and additional, the most frequent reasons for culling. No effect of any diet was observed on the incidence of culling (Table 5-11). Mean culling rate was 27.8% (5/18), 50% (11/22), and 43.8% (7/16) for heifers born from dams fed control, SFA, or EFA diets, respectively. Regarding MR diets, heifers fed the LLA MR had a culling rate of 46.4% (13/28) whereas that of heifers fed HLA MR was 35.7% (10/28) but the difference was not significant. The most common reasons for culling were reproductive problems ($n = 5$), poor growth ($n = 8$), and mastitis and low production ($n = 5$). Neither prepartum dam diet nor preweaning calf diet affected the incidence of a particular reason of culling.

Discussion

Regulation of Hepatic Total and Individual Fatty Acid Concentration

Fatty liver is a critical condition that can lead to impairment of liver function. The negative effects and etiology of this condition have been well documented in humans (Reddy and Rao, 2006; Cave et al., 2007; Semple et al., 2009; Thomson and Knolle, 2010) and in dairy cows (Bobe et al., 2004). Fat concentration of liver in preweaned dairy calves was increased by feeding CCO in the MR by Jenkins and Kramer (1986).

Upon in vitro incubation of liver tissue from calves fed CCO or tallow in MR, Gruffat-Mouty et al. (2001) reported that the liver from CCO-fed calves had reduced concentration of Apo-protein B and reduced in vitro secretion of VLDL.

In the current study, calves fed LLA MR had 12% greater proportions of FA in liver compared to calves fed HLA MR but it is unlikely that excessive steatosis occurred. Jenkins and Kramer (1986) documented an increase of 48% in proportion of FA in fresh liver when feeding a CCO-based MR compared to a MR containing 95% CCO and 5% corn oil (% of fat), however, calves fed CCO-MR had a better performance, which might indicate that the liver was not affected by this increase in fat. In vitro studies using liver of calves fed CCO reported a reduction in FA oxidation which suggests an increase in esterification in liver (Graulet et al., 2000). Coconut oil is composed by MCFA which leave the enterocyte and directly arrives to the liver by portal vein, greater and faster availability of these MCFA are partially oxidized and elongated to synthesize longer chain FA and TG that due cannot leave the liver at same rate as they are synthesized ending up accumulating (Sato, 1994). Hence calves fed LLA-MR rich in CCO, might follow same mechanism.

Studies performed by Jenkins and Kramer (1986, 1990) and Leplaix-Chalat et al. (1996) suggested that the amount of fat provided to calves and more important the type of dietary FA can impact the accumulation of fat in liver. This agrees with the results of the current study. As stated in previous studies, CCO, a fat rich in medium chain FA, has been associated with a steatotic condition, whereas LA, and other PUFA, are potent inducers of lipid oxidation in liver (Clarke et al., 1977; Sampath and Ntambi, 2005). The primary mechanism by which PUFA enhance fat oxidation is by the activation of PPAR-

α , an upregulator of key genes involved in lipid oxidation (Forman et al., 1997; Hostetler et al., 2005). In the current study LA and AA (as well as all PUFA) were present in greater proportions in liver fat of calves fed HLA MR. This increased proportion of natural ligands of PPAR- α might account for the upregulation of PPARG gene when calves were fed HLA MR.

Up to the time of liver biopsy (30 ± 1 d of age), calves were fed only MR. As a result, microbial activity in the rumen was not fully active which likely limited hydrolysis and biohydrogenation of dietary FA. Consequently, the FA profile of the liver reflected the FA profile of the type of MR fed. Concentrations of C12:0 and C14:0 were greater in liver of calves fed LLA MR. Even though C12:0 was dramatically greater in CCO compared to porcine lard (42.5 vs. 29.9%), the differences in liver proportions of C12:0 were small but significant (1.23 vs. 0.29%). This because much of the C12:0 would have been readily oxidized for energy by the liver, leaving little to accumulate in hepatic tissue. Concentration of C16:0 was greater in liver of calves fed LLA vs. HLA (16.5 vs. 13.9%) despite being in greater concentration in the HLA MR (14.6 vs. 10.6%). Palmitic acid is the longest chain FA in CCO and would be the predominant FA absorbed in the lymphatic system as part of the chylomicron matrix from the LLA MR. As the liver takes up these C16:0 dominated lipoproteins, they would be synthesized into triglycerides and stored by hepatic tissues and likely be found in greater concentrations compared to calves storing the longer chain C18 FA from the HLA MR. As expected concentration of LA was increased in liver of calves fed more LA. However the other 18-carbon FA in liver tissue did not follow exactly the MR pattern. Concentrations of C18:0 in liver matched those in MR but that of C18:1 did not. The HLA MR contained 76% more

C18:1 than the LLA MR, but concentrations of C18:1 in liver were lower in calves fed HLA MR (10.1 vs. 11.3%). This may have occurred if biohydrogenation of some C18:1 by ruminal microorganism took place, although it is more likely that ruminal activity was minimal due to feeding of MR alone. However, some of the ingested MR would have ended up in the rumen rather than the abomasum, providing a substrate for the population of anaerobic microbes established there. The conversion of some C18:1 to C18:0 in calves fed HLA MR which have more C18:1 may have caused the decreased concentration of C18:1 in hepatic tissues.

Regarding the n-6 FA derivatives, the desaturases/elongase enzymes were operational, since the proportion of AA, C20:2, and 22:4 increased in liver of calves fed HLA MR. Only 1 n-3 derivative (DPA) was increased in liver of calves fed HLA despite the fact that the parent FA (ALA) was greater from HLA feeding. This indicates that these same desaturase/elongase enzymes were less active in PUFA metabolism. Interestingly some studies have documented a preferential use of the desaturase / elongase enzymes by a parent FA when it is in greater proportion and conversely limiting the synthesis of longer chain FA from the parent FA found in lower concentrations (Chan et al., 1993; Goyens et al., 2006; Liou et al., 2007).

Feeding of High Linoleic Acid in Milk Replacer Up regulated PPAR α and its Target Genes

Based upon DEG analysis, The HLA MR fed to calves greatly influenced the PPAR signaling pathway thus potentially impacting FA oxidation at the tissue level and delivering net energy for cell functions. Upregulation of PPARA should be expected to enhance some hepatic catabolic processes such as lipid oxidation and gluconeogenesis (Rakhshandehroo et al., 2010). However response was not clear-cut. Upregulation of

OLR1 and ANGPTL4 genes, accompanying the upregulation of the PPARA in calves fed HLA MR have an opposite effect to reduce the clearance of lipid from liver tissue. Expression of OLR1, the receptor responsible for binding to oxidized low density lipoprotein–cholesterol (ox-LDL) in order to prevent its elimination from the liver, is constitutively in low concentrations, but its activation can be induced under pathological conditions such as diabetes mellitus, hypertension, myocardial ischemia, and atherosclerosis (Mehta et al., 2006). In addition, OLR1 also can be induced by elevated amounts of ox-LDL and reactive oxygen species (Khaidakov et al., 2011). Based on the metabolic profile of calves fed HLA, there was no evidence that calves were undergoing any of the above pathological conditions. However the gene expression of the antioxidant enzyme, SOD2, was downregulated in calves fed HLA MR (fold change of 1.40, $P < 0.01$, Appendix 4). Because SOD is a member of the reactive oxygen species family, reactive oxygen species were not likely responsible for OLR1 inducement. Moreover, pathways related to catabolic processes that generate reactive oxygen species (i.e., mitochondrial respiration, peroxisomal FA β -oxidation, microsomal cytochrome P450 metabolism) were not enriched by any KEGG pathway or GO term in liver of calves fed HLA MR. It is well documented that activation of PPAR- α will enhance oxidative processes by upregulating the expression of several target genes, among them the CYP4 family (Rakhshandehroo et al., 2009). In addition, enhanced oxidative processes have been associated with increased production of reactive oxygen species. These oxygen species are known to increase tissue damage (West, 2000; Sun et al., 2002). However, the oxidative process was apparently reduced in calves fed HLA MR

since 3 genes in addition to SOD2 (CYP4A22, CYP2C19, and HAAO) involved in oxidative / reduction processes were downregulated (Appendix 4).

The other gene upregulated in the PPAR signaling pathway was ANGPTL4. It is directly upregulated by PPAR- α through the PPAR α response element present in the ANGPTL4 gene (Zhu et al., 2012). A role of PPAR- α is to help clear TG from plasma by upregulating the activity of different lipoprotein lipases. The gene, ANGPTL4, has an inhibitory effect on lipoprotein lipase (Duval et al., 2007). As expression of ANGPTL4 was upregulated by feeding of more LA, plasma concentrations of TG should have increased. However, total FA in plasma of calves fed HLA MR was lower than that of calves fed the LLA MR (Chapter 4). These 3 identified upregulated genes in the PPAR signaling pathway seem to be exerting pro- and anti-lipolytic effects. The option to exert a pro or an anti-lipolytic effect may allow the calf to better adapt to the immediate energy circumstances. The activation of PPAR- α is required for normal adaptive responses to starvation (Inagaki et al., 2007). However, calves fed HLA MR were under normal feeding conditions and undergoing increased anabolic processes, verified by the greater BW gain and plasmatic IGF-I concentrations (Chapter 4). Therefore, although increased availability of PUFA in calves fed HLA MR might increased the activity of PPARA gene, but the not urged need to synthesis energy intermediate products as well as glucose might prevented further activations of other catabolic enzymes by PPARA .

Feeding Fat Prepartum and High Linoleic Acid in Milk Replacer Upregulated PPAR α Target Genes

In the previous section, the effect of HLA on expression of genes of PPAR signaling pathway has both anti- and pro-lipolytic effects. In this section, all upregulated genes associated with PPAR had a clear pro-lipolytic function. Expression of CYP4A

genes is sensitive to PPAR- α ligand activation (Harmon et al., 2006). Calves born from dams fed any source of fat and supplemented with HLA MR had 6 upregulated genes (CYP4A11, CYP4A22, CYP27A1, APOA5, ACADL, and ACAA1) within the PPARA signaling pathway. However expression of the specific PPARA gene did not change. All of the 6 upregulated genes have well-defined functions regarding lipid transport, cholesterol synthesis, and lipid oxidation. The CYP4A11 and CYP4A22 genes act through enhancing microsomal ω -oxidation and mitochondrial β -oxidation (Savas et al., 2003). Synthesis of bile acids from cholesterol is a catabolic process to eliminate excess cholesterol and CYP27A1 has a clear role in this process (Chen and Chiang, 2003). Clearance of TG from circulation is aided by the activity of APOA5 which has high affinity for lipids. Metabolic studies using mice documented that APOA5 can lower plasma TG by reducing the hepatic VLDL-TG production rate and by enhancing the lipolytic conversion of TG-rich lipoproteins (Pennacchio and Rubin, 2003). Finally ACADL and ACCA1 are two enzymes that play key roles in mitochondrial β -oxidation and peroxisomal β -oxidation, respectively (Rakhshandehroo et al., 2010).

A significant number of additional pathways were upregulated in liver of calves fed HLA instead of LLA and born from dams fed fat instead of control diets (FAT by MR). The pathways included FA metabolism, glycerolipid metabolism, arachidonic acid metabolism, and drug metabolism pathways (Table 5-12). The upregulation of these pathways are indicative that these groups of calves were certainly undergoing a degradation of lipids through microsomal ω -hydroxylation and mitochondrial and peroxisomal β -oxidation and, by these means, might be generating energy intermediate products such as NADPH. Electron carrier activity, oxidation/reduction, transmembrane

transport, NAD/NADPH binding, and coenzyme metabolic processes include genes that are related closely with the processing and further metabolism of lipid catabolic products originated by the upregulated activity of the aforementioned genes.

Genes associated with the PPAR signaling pathway were upregulated due to supplementing of fat during prepartum. Furthermore, the stimulatory effect of HLA MR occurred in calves born from dams fed either SFA or EFA prepartum (i.e., no FA by MR interaction was detected, Appendix 1). Certain nutritional conditions occurring during the fetal period or early life have a more marked effect on fetal programming occurrence (Fowden et al., 2006; Gicquel et al, 2008). Any of these prepartum and preweaning diets could have a fetal programming effect, which generates a “metabolic plasticity” in the later life of offspring. The potential effects of dam diet on fetal programming could be modified by the preweaning diet offered.

Feeding of Essential Fatty Acids Prepartum and High Linoleic Acid in Milk Replacer Enhanced Catabolic Processes and ATP Generation

Neither feeding EFA nor HLA MR alone as main effects influenced catabolic processes and ATP generation. Although the interaction of FA and MR was essentially not significant for FA profile of liver (Table 5-5), these diets influenced gene expression in the liver. Feeding HLA MR to calves modified the effect of EFA-fed prepartum (interaction FA by MR). It is possible that provision of greater amounts of LA and ALA during the fetal period, through prepartum feeding, might modify the fetus's ability to deal with continued feeding of greater amounts of LA once they are born. The upregulated pathways in the contrast of FA by MR support this hypothesis. Although the PPARA pathway, which has a big role in FA oxidation, was not upregulated, other pathways that are indicative of oxidation of nutrients were upregulated such as

glycolysis and most importantly, oxidative phosphorylation, the end point pathway to generate high energy compounds.

Glycolysis and alcohol catabolic processes were two upregulated BP within the top enriched cluster in the interaction response of FA by MR (Table 5-8). Feeding HLA MR to calves born from dams fed EFA promote the upregulation of genes within a cluster as compared to calves fed LLA MR and born from dams fed SFA. These 2 BP shared the same set of genes, namely ALDOA, TPI1, ENO1, OGDH, and MDH2. The first three genes code for enzymes within the glycolytic pathway whereas OGDH synthesizes succinyl CoA from α -ketoglutarate within the Krebs cycle and MDH2 exports oxaloacetate from mitochondria through conversion to malate in a reversible reaction (Hartsock and Nelson, 2008). Another enriched BP within the top enriched cluster was the “generation of precursor metabolites and energy,” which included the 5 genes listed before plus UQCR1, COX10, ATP6V1E1, ATP5B, and NDUFS2. These genes likewise are listed for the upregulated oxidative phosphorylation pathway. All of these latter 5 genes are enzymes involved in four of the five complexes of the oxidative respiratory chain responsible for the intermediate products of oxidation (NADPH, FADPH) to be converted to ATP (Osellame et al., 2012).

The enhanced catabolic processes in this group of calves (interaction of FA by MR) are indicative of greater glucose availability to be used as a source of energy. Indeed calves fed HLA MR had greater plasma concentrations of glucose and IGF-I (Chapter 4). This greater availability of glucose in liver is derived from the diet, specifically lactose, which was the only source of glucose to these calves at the time the liver biopsy was performed. In fact, the glycolysis pathway in these calves was

upregulated with the greater expression of GALM in calves fed HLA MR and born from dams fed EFA (FA by MR interaction, Table 5-12), the gene that encodes for the first enzyme of four needed to get glucose 1-P from β -D galactose. Specifically, the GALM gene mutarotates the β - α - configuration to form α -D galactose, which follows a series of conversion steps to UDP-glucose (Thoden et al., 2004). In addition, because several key genes of the oxidative respiratory chain were upregulated, several intermediate energy products would have been diverted to mitochondria for ATP synthesis. Oxidation of lipids through β -oxidation is another important contributor to intermediate energy products. It is speculative that this mechanism was also active in this group of calves. Although no individual gene involved in the β -oxidation process was found upregulated, there was a linear fold change increase of 1.94 (false discovery rate = 0.14) in expression of PPAR α relative to calves born from cows fed EFA but fed LLA MR.

Synthesis of phospholipids, as well as “organophosphate metabolic processes,” were upregulated via 2 BP enriched in liver of calves fed HLA instead of LLA MR and born from dams fed EFA instead of SFA diets (interaction of FA by MR). Both of these BP had the common enriched genes CDIPT, LPCAT3, and ALG12 and the organophosphate metabolic process also had TPI1 enriched. Phospholipids are not just structural components of the cell membrane but are critical to such functions as second messenger molecules, membrane receptors for the recruitment of specific proteins, chaperones to aid in protein folding, and modulators of protein function (McMaster and Jackson, 2004). Thus an upregulation of phospholipid synthesis is an indirect indicator of modified functionality of liver cells.

Regulation of Carbohydrate Metabolism

One of the main roles of activated PPAR- α is to upregulate genes that increase synthesis of glucose during fasting conditions (Rakhshandehroo et al., 2009). Based on the DEG upregulated genes detected in the current study, neither prepartum diets nor MR nor the interaction between them upregulated expression of gluconeogenic genes (Appendices 1, 2, 3, 4, and 5). Increased need for gluconeogenesis due to fasting conditions in calves of the current study was not expected to occur under the feeding regimen used for calves in this study. When mice were fasted for 12, 24, 48, or 72 h, genes that code for enzymes aiding in the production of energy in the early fasting period were upregulated, but gluconeogenesis per se was not initiated until after prolonged fasting (Sokolovic et al., 2008). Calves in the current study were experiencing constant growth and appropriate feeding conditions thus there would have been little to no utilization of aminoacids for potential synthesis of glucose via gluconeogenesis. However, since the main source of carbohydrate in calves' diet was lactose, a disaccharide composed by 1 mole of glucose and 1 mole of galactose, an enhancement in the mechanism of galactose isomerization was logical and perhaps needed. Liver genes of calves within the interaction groups of FAT by MR and FA by MR experienced an upregulatory effect on expression of several genes involved in galactose metabolism to conversion into glucose (Tables 5-4 and 5-8). It is possible that calves fed HLA and born from dams fed SFA or EFA were having a more efficient conversion of galactose into glucose. However, the overall better response of calves fed HLA MR in regard to ADG and feed efficiency was not affected by the supplemental fat when compared to control diets (no FAT by MR interaction; Chapter 4).

Regulation of Protein Turnover

Degradation of proteins occurs through the ubiquitin-proteasome pathway and involves the following two successive steps: 1) tagging of the substrate by covalent attachment of multiple ubiquitin molecules and 2) degradation of the tagged protein by the 26S proteasome complex with release of free and reusable ubiquitin (Glickman and Ciechanover, 2002). The roles of protein ubiquitination include intra-cellular controls over a wide range of biological processes including: protein degradation, DNA repair, endocytosis, autophagy, transcription, immunity, and inflammation (Husnjak and Dikic, 2012). Thus, a tight regulation of ubiquitination processes will ensure appropriate balance between degradation and maintenance of activity of many active proteins within cells.

Regulation of ubiquitin-mediated protein degradation can happen at any point of the three enzymatic reactions occurring in the cascade via updown regulation of any of the several enzymes of the cascade (Gao and Karin, 2005). Calves fed HLA instead of LLA MR and born either from dams fed fat (interaction FAT by MR, genes: SOCS1, UBA7, PML, HERC4, and BIRC3) or from dams fed EFA (interaction FA by MR, genes: CUL3, KLHL9, ITCH, and BIRC3) had a different set of downregulated genes coding for enzymes involved in the activation of ubiquitin-mediated proteolysis in one of the three enzymatic reactions (Glickman and Ciechanover, 2002). Massive degradation of skeletal muscle proteins could upregulate the activity of the ubiquitin-proteasome pathway. Calves in this study did not undergo prolonged fasting periods (2 feedings per day). In addition, instead of degradation of muscle they were under muscle accretion conditions (Chapter 4). Calves in the current study had greater BW gain and plasmatic IGF-I concentrations at least in calves fed HLA MR ($P < 0.08$, Table 4-6) regardless of

the diet fed prepartum. Hence it should be expected that ubiquitination of proteins, by upregulation of its coding genes, should not be of high activity. An interesting gene, USP2, was found to be up- and downregulated by the interactions FAT by MR and FA by MR, respectively (Appendices 5-5 and 5-6). The differential regulation of this gene is primarily due to the greater upregulation (greater mean expression value) in liver of calves fed HLA and born from dams fed SFA diets (Appendix 1).

The USP2, is another proteolytic enzyme that has been found over-expressed in human prostate cancer and has been associated to increase the half-life of FASN, an enzyme associated with the malignancy of aggressive prostate cancer (Renatus et al., 2006). Metzigg et al. (2011) documented that downregulation of USP2 inhibited TNF- α / NFkB signaling, hence reducing the risk of inflammation. The current finding that calves fed HLA MR and born from dams fed EFA instead of SFA were able to downregulate the expression of USP2 might mean that these calves had an improved ability to cope with inflammatory processes. Generally LA is considered a proinflammatory FA compared with ALA or other n-3 FA (Calder, 2006; Whelan, 2008; Weaver et al., 2009). However the current finding indicates that when compared to SFA supplementation, supplementation of LA during the prepartum and preweaning periods could prevent excessive inflammatory processes.

Regulation of Inflammation and other immune processes

Ubiquitination of proteins can modify the activity of immune cells or immune metabolites as it clearly alters gene expression of USP2, potentially leading to the downregulation of the TNF- α / NFkB pathway, which is a critical pathway enhancing inflammatory conditions (Harhaj and Dixit, 2012). Although n-6 FA are mostly considered proinflammatory FA, some studies have reported that n-6 FA also can have

ant inflammatory activities (Fritsche, 2008; Bjermo et al., 2012). The principal mechanism by which the inflammatory response is implemented is through activation of NFkB transcription factor, the key mediator of the inflammatory response (Weaver et al., 2009) Other mechanisms may include cessation of neutrophil recruitment by reduction of migration and increased apoptosis of neutrophils and other leukocytes (Lawrence et al., 2002).

Expression of 3 genes involved in “leukocyte transendothelial migration” in liver from calves born from cows fed dams fed fat (contrast FAT), regardless of the subsequent MR fed, were downregulated (Table 5-9). One gene was ICAM1, a gene that directly regulates leukocyte migration, as it is an intracellular adhesion molecule critical to moving leukocytes from the circulation and allowing transmigration into the infected tissue for subsequent phagocytic activity (Lawson and Wolf, 2009). On the other hand, MYL2 and ACTN2 are involved in structural support of the leukocyte, by formation of cytoskeleton, regulation of leukocyte movement allowing the leukocyte to move forward and finally enhancing migration (Sanchez-Madrid and Del Pozo, 1999). Leukocyte migration to infected or damaged tissues is a necessary process to aid in the healing of cellular damage from pathogens. Liver has a high demand for leukocytes to migrate into hepatocytes and help fight potential microbial infections and tissue trauma. However an excessive migration of leukocytes to hepatocytes could be detrimental and increase hepatocyte damage leading to chronic liver injury (Jaeschke, 2006). Although liver was examined at only one point in time (30 d of age), it cannot be ruled out that a ability of leukocytes to migrate into the hepatocytes was reduced over a long period of time and could negatively impact the stability of the hepatic tissue.

The apparent mechanism of the downregulated response of leukocyte migration in calves born from dams fed fat (contrast FAT) is not clear. It would be expected that only calves born from dams fed EFA, regardless of the MR fed (contrast FA), would have a better and more effective resolution of inflammation, leading to a downregulation of inflammatory mechanisms, than calves born from dams fed SFA. This assumption is based on the mechanism that PUFA (preferentially n-3 followed by n-6 FA) can induce the inactivation of the TNF- α /NF κ B pathway (Calder, 2012), a mechanism that was apparently downregulated in calves fed HLA but only when born from EFA-fed and not SFA-fed dams (interaction FA by MR) as presented in a previous section. However, a recent study (Bjermo et al, 2012) fed obese subjects with supplements rich in SFA or PUFA reported no differential expression of inflammatory and oxidative stress genes, which might indicate that both sources of FA had similar regulatory effect on expression of genes within the inflammatory process.

Based on the down regulation of UPS2 as evidenced in the FA by MR interaction, increased activation of the TNF- α /NF κ B pathway is a potential indicator of reduced or controlled inflammatory processes. Another mechanism could be the down regulation of leukocyte transendothelial migration; however this mechanism was not directly influenced by the interaction of FA by MR but only affected in calves born from dams fed fat regardless of the MR fed (contrast FAT). However, in addition to UPS2 which was exclusively downregulated by the interaction FA by MR, 3 additional DEG were downregulated (Appendix 6) but not enriched in any GO term or KEGG pathway. This might indicate that calves of this interaction were able to better resolve inflammation. The genes were BCL10, CASP3, and ITCH. The BCL10 gene encodes the B-cell

lymphoma 10. An over-expressed BCL10 induces a constitutive activation of the NFkB / JNK resulting in the over-activation, differentiation, and proliferation of specific T and B cells (Thome, 2004). The CASP3 gene, when upregulated is a potent inducer of apoptosis of immune cells such as lymphocytes. An upregulated CASP3 could be detrimental to lymphocyte function during sepsis conditions and result in death (Hotchkiss et al., 2000). An over-expressed ITCH gene was reported to inhibit TNF- α -mediated NFkB mice cells (Shembade et al., 2008). The listed functions of these 3 genes appear to be antagonistic; the BCL10 (+) and ITCH (-) both have roles in activation of NFkB but in different directions; whereas the CASP3 by being downregulated prevented the excessive apoptosis of leukocytes that could prevent them from performing under inflammatory processes. Under the circumstances of the current study and considering that LA is well known to have proinflammatory effects, these genes acting in different ways to resolve inflammatory processes confirm our aforementioned hypothesis of a potential greater ability of calves fed HLA and born from dams fed EFA (interaction FA by MR) to resolve inflammation.

Feeding of Essential Fatty Acids Prepartum and High Linoleic Acid in Milk Replacer Improved Insulin Sensitivity

Adipocytokines are soluble factors namely cytokines which are produced by the adipose tissue. The most common adipocytokines are adiponectin, leptin, resistin, and visfatin, all of which have important roles in regulating insulin resistance (Tilg and Moschen, 2006). Adiponectin prevents insulin resistance, acting intracellularly, by binding to its receptor, ADIPOR2, which is the most abundant receptor of adiponectin in liver tissue (Kadowaki and Yamauchi, 2005). Although adiponectin per se was not upregulated, the expression of its receptor, ADIPOR2 was certainly upregulated in

calves fed HLA MR and born from dams fed EFA (interaction FA by MR, Table 5-8). Consequently, it can be postulated that calves of this interaction were less likely to develop insulin resistance. The mechanisms by which adiponectin performs an insulin-sensitizing action have been discovered recently. One mechanism is by activating AMPK, thus downregulating the expression of gluconeogenic genes (Kadowaki et al., 2006). In fact, calves in this group have the KEGG glycolysis pathway upregulated with five genes (ALDOA, TPI1, GALM, PGM1, and ENO1, Table 5-8) as well as five genes in the BP of glycolysis (3 shared with the KEGG pathway - ALDOA, TPI1, and ENO1 and 2 different genes – OGDH and MDH2, Table 5-4). Unfortunately some key genes regulating glycolysis (phosphofructokinase and piruvate kinase) or gluconeogenesis (phosphoenolpyruvate carboxykinase and glucose 6- phosphatase) were not up- or downregulated respectively (Appendix 6), which could have provided a clearer picture about the prevalence of glycolysis or gluconeogenesis. Another postulated mechanism of adiponectin sensitizing insulin is via increased β -oxidation and energy consumption, in part via PPAR- α activation, leading to a decreased triglyceride content in liver (Kadowaki et al., 2006). However, regarding fat content in liver, similar concentrations of total FA were found in calves fed MR if they were born from dams fed SFA or EFA (interaction FA by MR), which might indicate that the most probably mechanism of insulin sensitization was though reduction of gluconeogenesis rather than change in the proportion of FA in liver by enhancing their oxidation.

Another upregulated gene in this adipocytokine signaling pathway was STAT3 (Table 5-8). This gene can inhibit SREBP-1c promoter activity. By inhibiting the expression of SREBP-I, the synthesis of FA may be reduced thus preventing steatosis

and dyslipidemia, hence reducing the risk of insulin resistance (Ueki et al., 2004). In addition to the upregulation of STAT3, SOCS6 was downregulated by the interaction of FA by MR (Appendix 5-6), although it was not enriched in any GO term or KEGG pathway. The SOCS6 gene has been reported to reduce the active form of STAT3 protein (Hwang et al., 2007). Therefore the down regulation of SOCS6 gene could be associated with the increased expression of STAT-3 which would support the reduced risk of insulin resistance in calves born from cows fed EFA and supplemented with HLA MR.

Fat and Fatty Acid Supplementation and its Risk and Prevention of Cardiomyopathic Diseases

The sarcomere is the fundamental unit of cardiac and skeletal muscle contraction. Recent studies have identified mutations in genes coding for these proteins as the main drivers of different cardiomyopathic disorders (Tajsharghi, 2008). The four identified upregulated genes in calves fed HLA MR coding for sarcomeric proteins related to hypertonic cardiomyopathy and dilated cardiomyopathy (Table 5-8) indicate a potential accumulation of those proteins which have been indicated as one of the reasons for incidence of myopathy (Fielitz et al., 2007). Mutation of sarcomeric genes are one of the most common etiologies for cardiomyopathic diseases (Probst et al., 2011), and this mutation is commonly accompanied by an over expression of the upregulated genes found in liver of calves fed HLA MR. However, the microarray analysis does not indicate whether a gene has mutated. Additional work would be required to verify gene mutation. Bovine dilated cardiomyopathy is a terminal myocardial disease with common age at onset between 2-4 years (Owczarek-Lipska et al., 2011). Heifers in this study (n = 56) were followed throughout their first 45 mo of life. Only 1 death was reported due to

endocarditis and that was for a calf not in the group of upregulated genes for cardiomyopathies.

Feeding HLA instead of LA MR (contrast MR) also upregulated genes from the tight junction pathway (Table 5-8) as well as genes from BP and MF related to actin and calcium binding, as well as striated muscle tissue development (Table 5-2). Cardiac and skeletal muscle contractions are regulated by calcium dependent interactions with the thick and thin filaments of tropomyosin and troponin of sarcomeric proteins. Thus, when intracellular calcium concentrations increase, it binds to troponin C resulting in regulation of muscle contraction (Lee et al., 2010). Upregulation of calcium binding might be a result of a change in its sensitivity to troponin C. Karibe et al. (2001) reported that a mutation of tropomyosin modified the affinity to calcium. The tight junction pathway is responsible for regulating the paracellular movement of Ca, ions and solutes between cells (Hartsock and Nelson, 2008). Some genes of the tight junction pathway coding for sarcomeric proteins also were upregulated. The upregulation of this pathway could potentially increase the risk of heart disease, but as stated early, heart problems were not reported in heifers fed HLA MR throughout their first 45 months of age. Perhaps the increased gene expression in the liver of tight junction responses associated with feeding of HLA MR contributed to a greater cardiac function if also expressed in the heart (not determined in the present study). This may be associated with increased milk production in the first lactation due to increased cardiac output and blood flow to mammary gland

In the previous section, the upregulation of sarcomeric genes due to feeding HLA MR was discussed. Interestingly, these same genes were downregulated in livers of

calves born from dams fed fat but only if the calves were fed HLA. as well as have down regulated pathways related to this condition such as tight junction, including BP and MF such as actin and calcium binding. The reason for this interaction is unclear. It can be hypothesized that a potential fetal programming may have occurred in this group of calves born from cows fed fat prepartum, which may pre-condition the calves in this group to respond differentially to either high or low levels of LA in the preweaned diet. Feeding increased amounts of fat, primarily saturated fat, has been reported to induce cardiomyopathies in obese mice (Fang et al., 2008). However, feeding PUFA, primarily n-3 FA, reduced the risk of cardiomyopathies in mice (Takahashi et al., 2005). A recent study reported that feeding FO to sheep induced cardiac dysfunction after infusion of doxorubicin, as displayed by a greater level of ventricular dilatation compared with placebo sheep (Carbone et al., 2012). The aforementioned studies have led to different conclusions regarding the influence of fat on risk of cardiac problems. However the most common postulation is that PUFA have a protective effect, hence it is not clear why calves fed HLA instead of LLA MR (contrast MR) had a potential increased risk of cardiac problems thorough upregulation of some genes involved in these pathogenesis. Current results warrant further investigation of understanding potential interactions of prenatal dam diets with neonatal diets of the newborn on subsequent development and metabolic/endocrine regulation of productivity and health traits.

Prepartum Fat Feeding Influenced Future Adult Performance

Late gestation and preweaning periods have been identified as two of the most critical periods during which nutritional management could have long term effects in future offspring performance (Fowden et al., 2006). Studies conducted using humans have documented a detrimental effect on birth weight and health of offspring born from

undernourished women (Barker, 1997; Pettitt et al., 1987). In the current study calves fed the HLA MR during the preweaning period had a marked improved performance with a greater body weight gain and feed efficiency (Chapter 4). However, increased intake of LA via MR had no effect on all post-pubertal measures of production and reproduction, with the exception of a trend for greater concentration of milk lactose when calves were fed HLA MR. There was a numerical difference of 552 kg of mature equivalent milk for heifers fed HLA MR. Soberon et al. (2012) recently reported that every 1 kg increase in ADG by heifers during the preweaning period resulted in an additional 850 kg of mature equivalent milk during the first lactation. In the current study, heifers born from dams fed SFA had better ADG during the preweaning period than heifers born from dams fed EFA (Chapter 4). This advantage in BW gain due to prepartum fat type did not translate into better lactation performance. In fact, heifers from dams fed EFA had numerically greater (517 kg) mature equivalent milk. The most significant change in milk yield was observed as result of supplementing fat prepartum, regardless of the type of FA provided (even though supplementing fat prepartum did not influence the performance of heifers during the preweaning period, Chapter 4). Heifers fed fat prepartum resulted in the most dramatic increase in milk yield at first lactation, producing ~13% more milk than heifers born from dams not supplemented with fat. Because these same heifers conceived later, they were ~45 d older and 36 kg heavier at calving. Since heavier heifers can consume more feed DM, milk yield may have been increased partly due to greater feed intake based on body size but it is unlikely that an additional 1400 kg of milk (4.6 kg/d in a 305 d lactation period) would be

produced by heifers that have a 36 kg BW advantage. Moreover, the mature equivalent milk is corrected by age and BW at calving.

Most of the first studies documented that additional intake of nutrients during the prepubertal period had a negative impact on future milk production (Foldager and Sejrsen, 1987). More recent studies have documented increased intake of nutrients during the preweaning period improved future milk production [Shamay et al., 2005 (n = 40, 5.1% increase in milk); Moallem et al., 2010 (n = 46, 10.3% increase in milk); Soberon et al., 2012 (1244 kg);]. However, in the current study, heifers did not have an increased intake of nutrients as heifers from all other studies, but ADG was improved during the preweaning period due to feeding of HLA MR. Feeding of fat to dams during prepartum, increased milk production of their calves during their subsequent lactation as first calf heifers. Although not significant, heifers fed HLA MR during the first 60 d of life had a non-significant 4.9% greater milk production as first calf heifers.

The increased number of inseminations to initiate a first pregnancy and the greater milk production during first lactation due to fat feeding of the dam in late gestation on subsequent heifer performance suggests some alteration in neonatal programming that influences subsequent heifer performance. Certainly future studies should focus on the potential long-lasting effect of prepartum dam diets and postnatal calf diets on programming subsequent heifer performance reproductive and lactational performances.

Summary

Supplementing greater amounts of LA and ALA during the prepartum and preweaning periods modified the response of liver to different metabolic processes. This differential profile of liver FA might have modified the activity of the liver regarding

expression of hepatic genes. Similarity of liver dietary FA profile, depended more on the MR. Greater effect of MR were verified by the increased proportions of C12:0 and C14:0 in calves fed a MR formulated with CCO, whereas calves supplemented with porcine had liver with greater proportions of LA and three of its derivative FA. The analysis of representative genes within a cluster or metabolic pathway resulted d in fewer enriched genes due to prepartum diets and MR as compared to the amount of enriched genes obtained by the interaction of prepartum diets and MR. The DEG identified in all preplanned interaction were related to processes such as lipid and carbohydrate metabolism, protein metabolism, and inflammatory processes.

Polyunsaturated FA, such as LA, are potent ligands of PPAR- α and by this mechanism FA can exert their function in different metabolic processes. Calves fed MR containing porcine lard, regardless of prepartum diets, enhanced the expression of PPARA gene and two PPAR- α target genes with pro-lipolytic effects. However greater lipolytic effect of prepartum diets was observed in liver of calves fed MR containing porcine lard and born from dams fed fat instead control diet. Calves of this group had 6 upregulated genes, targeted for activation by the PPAR- α . In addition same calves had upregulated other group of genes involved in FA metabolism, glycerolipid metabolism and AA metabolism. The upregulation of genes in all aforementioned pathways might indicate that these calves were undergoing a preferential degradation of lipids. Interestingly, the key enzymes in the gluconeogenic pathway were neither upregulated by prepartum diets nor by MR or their combined effect.

The different profile of FA provided prepartum also affected the expression of genes in liver of calves fed a particular MR. Calves fed porcine lard and born from dams

fed EFA instead of SFA had upregulated genes involved in glycolysis and oxidative phosphorylation. Although increased oxidative phosphorylation could negatively impact the liver by excessive generation of free radicals. Calves in this group had more down regulated genes involved in inflammatory response as compared to the other four preplanned contrasts. This effect could have a positive impact limiting exaggerated inflammatory response that could negatively impact liver function. However, a potential attenuated inflammatory response, which could negatively impact calf survival, could not be ruled out.

A long term effect of preweaning diets on performance of heifers at first lactation, regardless its considerable impact in liver gene expression at 30 d of calf age was not apparent. However the effect of prepartum diets appeared to impact more dramatically the future performance of heifers. Heifers born from dams supplemented with fat had ~13% greater milk production at first lactation compared to those born from dams not supplemented with fat. Other studies have reported positive impact of improved ADG during the preweaning on future milk production. In the current study a numerical increase of 5.3% in milk production was observed for calves fed MR containing porcine lard instead of CCO.

Findings in this study reveal a strong effect of prepartum diet during the fetal period to modify the response of calves to strategic supplementation of FA during the preweaning period. However, the greater long term effect of prepartum diets versus preweaning diet, might indicate that the most critical period of programming effect of diets occurs during the late gestation rather than the preweaning period. Future research should focus on detailing the mechanisms by which strategic lipid

supplementation actually modifies the production and activity of proteins encoded for the DEG. Moreover, more efforts should be attained to evaluate different nutritional strategies during the late gestation period that would positively impact the future performance of dairy cattle.

Table 5-1. Mean concentration of liver fatty acid (FA, g of FA/100g of total FA) of Holstein male calves fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Calves were born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), and essential fatty acid (EFA)

Fatty acid	Dam Diet ¹						SEM	P values ³				
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR
	Milk replacer (MR) ²											
	LLA	HLA	LLA	HLA	LLA	HLA						
Total FA, % DM	8.49	6.76	8.6	7.85	8.32	8.06	0.44	0.13	0.94	0.02	0.12	0.59
C12:0	1.25	0.36	1.25	0.25	1.21	0.27	0.20	0.74	0.94	<0.01	0.81	0.88
C14:0	5.29	1.52	5.08	1.15	5.28	1.24	0.71	0.72	0.84	<0.01	0.86	0.94
C16:0	16.76	14.05	16.36	14.09	16.31	13.56	0.57	0.51	0.63	<0.01	0.8	0.68
C16:1 c9	0.50	0.43	0.48	0.39	0.45	0.36	0.04	0.26	0.53	0.02	0.84	0.98
C17:0	0.38	0.39	0.40	0.43	0.46	0.41	0.53	0.62	0.40	0.05	0.76	0.23
C18:0	20.48	22.69	21.84	23.82	21.68	24.10	0.94	0.13	0.95	0.01	1.00	0.82
C18:1 t6-8	0.02	0.01	0.05	0.02	0.02	0.02	0.01	0.39	0.26	0.24	0.74	0.26
C18:1 t9	0.07	0.07	0.10	0.07	0.06	0.06	0.01	0.96	0.10	0.47	0.60	0.21
C18:1 t10	0.13	0.08	0.15	0.11	0.14	0.18	0.06	0.42	0.68	0.73	0.67	0.55
C18:1 t11	0.19	0.16	0.21	0.20	0.22	0.24	0.04	0.19	0.55	0.85	0.69	0.68
C18:1 t12	0.09	0.09	0.10	0.10	0.10	0.13	0.01	0.08	0.25	0.31	0.30	0.08
C18:1 c9	12.37	10.94	12.33	10.00	11.47	9.98	0.60	0.17	0.48	<0.01	0.64	0.50
C18:1 c11	2.62	2.67	2.57	2.61	2.38	2.57	0.09	0.17	0.21	0.21	0.67	0.40
C18:2 n-6	15.87	23.00	15.20	22.04	16.57	21.27	0.74	0.30	0.70	<0.01	0.30	0.17
C18:3 n-6	0.07	0.03	0.08	0.01	0.05	0.04	0.05	0.62	0.97	<0.01	0.77	0.05
C18:3 n-3	0.74	1.04	0.65	1.03	0.71	0.91	0.04	0.05	0.41	<0.01	0.87	0.04
CLA 9c,t11	0.04	0.02	0.02	0.02	0.04	0.03	0.01	0.45	0.13	0.15	0.71	0.74
C20:2 n-6	0.56	1.05	0.50	1.00	0.56	0.99	0.04	0.23	0.58	<0.01	0.76	0.50
C20:3 n-6	3.13	2.53	3.78	2.48	3.17	3.09	0.28	0.22	1.00	0.01	0.84	0.04
C20:4 n-6	9.94	10.02	10.09	10.84	10.48	11.49	0.43	0.05	0.24	0.09	0.28	0.76
C20:5 n-3	0.21	0.16	0.30	0.18	0.22	0.22	0.02	<0.01	0.28	<0.01	0.65	<0.01
C22:4 n-6	1.20	1.24	0.96	1.28	1.23	1.30	0.08	0.68	0.07	0.03	0.27	0.12
C22:5 n-3	1.47	1.92	1.59	2.09	1.64	2.16	0.08	0.02	0.52	<0.01	0.64	0.90
C22:6 n-3	1.51	1.61	2.05	1.84	1.34	1.90	0.16	0.13	0.06	0.27	0.77	0.03
Σ Others	3.93	2.73	2.52	3.90	3.70	2.32	0.29	0.38	0.50	<0.01	0.72	0.99

Table 5-1. Continued

Fatty acid	Dam Diet ¹						SEM	P values ³				
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR
	Milk replacer (MR) ²											
	LLA	HLA	LLA	HLA	LLA	HLA						
Σ Unknowns	2.82	1.94	2.13	1.66	2.24	1.84	0.46	0.30	0.75	0.47	0.25	0.36
Σ SFA	44.64	39.68	45.38	40.32	45.37	40.00	0.66	0.29	0.80	<0.01	0.82	0.82
Σ MUFA <i>cis</i>	16.96	15.12	16.66	14.00	15.57	13.88	0.79	0.14	0.46	<0.01	0.81	0.55
Σ PUFA <i>cis</i>	34.94	42.75	35.53	42.92	36.13	43.50	1.11	0.48	0.61	<0.01	0.82	1.00
Σ CLA ⁴	0.04	0.02	0.02	0.02	0.04	0.03	0.01	0.45	0.13	0.15	0.71	0.74
Σ > C18:1 <i>trans</i>	0.09	0.07	0.09	0.11	0.10	0.07	0.01	0.28	0.37	0.12	0.98	0.83
Σ C18:1 <i>trans</i>	0.52	0.43	0.65	0.53	0.57	0.68	0.13	0.24	0.80	0.77	0.72	0.41
Σ n-3	3.97	4.8	4.61	5.21	3.93	5.24	0.23	0.07	0.17	<0.01	0.75	0.14
Σ n-6	30.76	37.88	30.62	37.64	32.05	38.19	1.03	0.73	0.36	<0.01	0.76	0.68

¹ Control = no fat supplemented; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA= 0.175 g LA/BW^{0.75}, HLA=.562 g LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30d of life to provide 6.72 g fat / kg BW^{0.75}.

³ P-values for orthogonal contrasts and interactions; FAT: contrast of dam diet (SFA+EFA) vs. control,; FA: contrast of dam EFA vs. SFA; MR= milk replacer

⁴ Concentration of CLA t10, c12 were 0 for all treatments.

Table 5-2. Functional annotation clusters for main effects of upregulated enriched GO terms in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Cluster ² #	GO ³	Term	Fold E ⁴ .	Count ⁵	P-value ⁶
Contrast FA ⁷					
1 (ES = 1.44)	BP_GO:0045934	negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	6.8	4	0.019
	BP_GO:0016481	negative regulation of transcription	5.5	3	0.098
Contrast MR ⁸					
1 (ES = 2.81)	MF_GO:0005509	calcium ion binding	4.5	7	0.003
	MF_GO:0003779	actin binding	10.0	4	0.006
2 (ES = 2.39)	BP_GO:0014706	striated muscle tissue development	21.3	3	0.008
	MF_GO:0003774	motor activity	9.4	3	0.037
	MF_GO:0043169	cation binding	2.1	15	0.002
	MF_GO:0005509	calcium ion binding	4.5	7	0.003
	BP_GO:0051603	proteolysis involved in cellular protein catabolic process	5.6	3	0.090

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Clustering of GO terms using the DAVID functional annotation clustering option. Table presents the most relevant GO terms within each cluster. Each cluster shows the group enrichment score (ES= -log 10 scale) that represents the geometric mean of all P-values of each annotation term in the group.

³ Gene ontology codes prefix BP = biological process and MF= molecular function. The GO terms cellular component and the detail of each GO terms is present in supplemental material.

⁴ Fold enrichment of each GO term within a cluster.

⁵ The gene members which belong to an annotation term. Some genes can be repeated in different GO terms within each cluster.

⁶ Fisher exact P-value.

⁷ Main effect of FA: Effect of feeding EFA prepartum with SFA diet as reference (Contrast FA).

⁸ Main effect of MR: Effect of feeding HLA milk replacer to newborn calves with LLA milk replacer as reference (Contrast MR).

Table 5-3. Functional annotation clusters for the interaction fat by milk replacer of upregulated enriched GO terms in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Cluster ² #	GO ³	Term	Fold E ⁴	Count ⁵	P-value ⁶
Interaction FAT by MR ⁷					
1 (ES = 2.88)	MF_GO:0009055	electron carrier activity	8.6	13	0.000
	BP_GO:0055114	oxidation reduction	3.7	22	0.000
	MF_GO:0005506	iron ion binding	4.5	11	0.000
2 (ES = 2.78)	BP_GO:0055085	transmembrane transport	2.0	9	0.078
3 (ES = 2.54)	MF_GO:0051287	NAD or NADH binding	11.8	4	0.004
4 (ES 1.84)	BP_GO:0006732	coenzyme metabolic process	5.5	6	0.004
	BP_GO:0019362	pyridine nucleotide metabolic process	13.6	3	0.020
5 (ES = 1.78)	BP_GO:0042364	water-soluble vitamin biosynthetic process	15.2	3	0.016
6 (ES = 1.68)	BP_GO:0006869	lipid transport	5.6	5	0.012
7 (ES = 1.49)	BP_GO:0005996	monosaccharide metabolic process	4.2	6	0.014

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Clustering of GO terms using the DAVID functional annotation clustering option. Table presents the most relevant GO terms within each cluster. Each cluster shows the group enrichment score (ES= -log 10 scale) that represents the geometric mean of all P-values of each annotation term in the group.

³ Gene ontology codes prefix BP = biological process and MF= molecular function. The GO terms cellular component and the detail of each GO terms is present in supplemental material.

⁴ Fold enrichment of each GO term within a cluster.

⁵ The gene members which belong to an annotation term. Some genes can be repeated in different GO terms within each cluster.

⁶ Fisher exact P-value.

⁷ Interaction fat by milk replacer: $\left[\frac{(\text{SFA-HLA} + \text{EFA-HLA})/2}{\text{Control-HLA}}\right] \div \left[\frac{(\text{SFA-LLA} + \text{EFA-LLA})/2}{\text{Control-LLA}}\right]$

Table 5-4. Functional annotation clusters for the interaction fatty acid by milk replacer of upregulated enriched GO terms in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Cluster ² #	GO ³	Term	Fold E ⁴	Count ⁵	P-value ⁶
Interaction FA by MR ⁷					
1 (ES = 2.87)	BP_GO:0006091	generation of precursor metabolites and energy	6.1	10	0.000
	BP_GO:0006096	glycolysis	17.0	5	0.000
	BP_GO:0046164	alcohol catabolic process	11.1	5	0.001
2 (ES = 1.22)	BP_GO:0008654	phospholipid biosynthetic process	9.0	3	0.042
	BP_GO:0019637	organophosphate metabolic process	4.9	4	0.047
3 (ES = 1.21)	BP_GO:0006461	protein complex assembly	4.3	6	0.012

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Clustering of GO terms using the DAVID functional annotation clustering option. Table presents the most relevant GO terms within each cluster. Each cluster shows the group enrichment score (ES= -log 10 scale) that represents the geometric mean of all P-values of each annotation term in the group.

³ Gene ontology codes prefix BP = biological process and MF= molecular function. The GO terms cellular component and the detail of each GO terms is present in supplemental material.

⁴ Fold enrichment of each GO term within a cluster.

⁵ The gene members which belong to an annotation term. Some genes can be repeated in different GO terms within each cluster.

⁶ Fisher exact P-value.

⁷ Interaction fatty acid by milk replacer: [EFA-HLA : SFA-HLA (reference)] ÷ [EFA- LLA : SFA-LLA (reference)]

Table 5-5. Functional annotation clusters for main effects of downregulated enriched GO terms in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Cluster ² #	GO ³	Term	Fold E ⁴	Count ⁵	P-value ⁶
Contrast FAT ⁷					
1 (ES = 2.44)	MF_GO:0003779	actin binding	14.2	4	0.002
	BP_GO:0014706	striated muscle tissue development	20.3	3	0.008
	MF_GO:0003774	motor activity	13.4	3	0.019
2 (ES = 2.29)	MF_GO:0005509	calcium ion binding	4.6	5	0.017
Contrast FA ⁸					
1 (ES = 1.19)	BP_GO:0051603	proteolysis involved in cellular protein catabolic process	5.9	3	0.081
	BP_GO:0044257	cellular protein catabolic process	5.9	3	0.082
Contrast MR ⁹					
1 (ES = 1.08)	MF_GO:0005506	iron ion binding	11.9	3	0.020
	BP_GO:0055114	oxidation reduction	5.3	4	0.029

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Clustering of GO terms using the DAVID functional annotation clustering option. Table presents the most relevant GO terms within each cluster. Each cluster shows the group enrichment score (ES= -log 10 scale) that represents the geometric mean of all P-values of each annotation term in the group.

³ Gene ontology codes prefix BP = biological process and MF= molecular function. The GO terms cellular component and the detail of each GO terms is present in supplemental material.

⁴ Fold enrichment of each GO term within a cluster.

⁵ The gene members which belong to an annotation term. Some genes can be repeated in different GO terms within each cluster.

⁶ Fisher exact P-value.

⁷ Main effect of FAT: Effect of feeding fat prepartum (SFA + EFA)/2 with control diet as reference (contrast FAT).

⁸ Main effect of FA: Effect of feeding EFA prepartum with SFA diet as reference (contrast FA).

⁹ Main effect of MR: Effect of feeding HLA milk replacer to newborn calves with LLA milk replacer as reference (contrast MR).

Table 5-6. Functional annotation clusters for the interaction fat by milk replacer of downregulated enriched GO terms in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Cluster ² #	GO ³	Term	Fold E ⁴ .	Count ⁵	P-value ⁶
Interaction FAT by MR ⁷					
1 (ES = 2.62)	MF_GO:0003779	actin binding	5.4	5	0.013
	BP_GO:0014706	striated muscle tissue development	8.4	3	0.047
2 (ES = 1.75)	MF_GO:0030554	adenyl nucleotide binding	1.9	14	0.022
	MF_GO:0005524	ATP binding	1.9	13	0.032
3 (ES = 1.66)	BP_GO:0003007	heart morphogenesis	18.9	3	0.010
4 (ES = 1.41)	MF_GO:0005509	calcium ion binding	1.4	5	0.463

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Clustering of GO terms using the DAVID functional annotation clustering option. Table presents the most relevant GO terms within each cluster. Each cluster shows the group enrichment score (ES= -log 10 scale) that represents the geometric mean of all P-values of each annotation term in the group.

³ Gene ontology codes prefix BP = biological process and MF= molecular function. The GO terms cellular component and the detail of each GO terms is present in supplemental material.

⁴ Fold enrichment of each GO term within a cluster.

⁵ The gene members which belong to an annotation term. Some genes can be repeated in different GO terms within each cluster.

⁶ Fisher exact P-value.

⁷ Interaction fat by milk replacer: $\left[\frac{(\text{SFA-HLA} + \text{EFA-HLA})/2}{\text{Control-HLA (reference)}}\right] \div \left[\frac{(\text{SFA-LLA} + \text{EFA-LLA})/2}{\text{Control-LLA (reference)}}\right]$

Table 5-7. Functional annotation clusters for the interaction fatty acid by milk replacer of downregulated enriched GO terms in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Cluster ² #	GO ³	Term	Fold E ⁴	Count ⁵	P-value ⁶
Interaction FA by MR ⁷					
1 (ES = 1.97)	BP_GO:0051603	proteolysis involved in cellular protein catabolic process	4.4	8	0.002
	BP_GO:0006511	ubiquitin-dependent protein catabolic process	7.1	5	0.005
	MF_GO:0070011	peptidase activity, acting on L-amino acid peptides	3.1	9	0.008
	MF_GO:0004221	ubiquitin thiolesterase activity	10.0	3	0.035
2 (ES = 1.01)	BP_GO:0007179	transforming growth factor beta receptor signaling pathway	19.6	3	0.010
	BP_GO:0007178	transmembrane receptor protein serine/threonine kinase signaling pathway	9.8	3	0.036
3 (ES = 0.97)	BP_GO:0050863	regulation of T cell activation	6.5	3	0.075
	BP_GO:0051249	regulation of lymphocyte activation	5.4	3	0.103

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Clustering of GO terms using the DAVID functional annotation clustering option. Table presents the most relevant GO terms within each cluster. Each cluster shows the group enrichment score (ES= -log 10 scale) that represents the geometric mean of all P-values of each annotation term in the group.

³ Gene ontology codes prefix BP = biological process and MF= molecular function. The GO terms cellular component and the detail of each GO terms is present in supplemental material.

⁴ Fold enrichment of each GO term within a cluster.

⁵ The gene members which belong to an annotation term. Some genes can be repeated in different GO terms within each cluster.

⁶ Fisher exact P-value.

⁷ Interaction fatty acid by milk replacer: [EFA-HLA : SFA-HLA (reference)] ÷ [EFA- LLA : SFA-LLA (reference)]

Table 5-8. Functional annotation chart for enriched upregulated KEGG pathways for main factors and interactions in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Entry ID ²	Pathway	Fold E ³	Count ⁴	P-Value ⁵	Genes ⁶
Contrast MR ⁷					
bta05410	Hypertrophic cardiomyopathy	15.2	4	0.002	DES, MYL2, TNNC1, TPM2
bta05414	Dilated cardiomyopathy	14.3	4	0.002	DES, MYL2, TNNC1, TPM2
bta03320	PPAR signaling pathway	12.3	3	0.022	PPARA, OLR1, ANGPTL4
bta04530	Tight junction	6.7	3	0.065	MYL2, MYH7, ACTN2
Interaction FAT by MR ⁸					
bta00071	Fatty acid metabolism	9.4	5	0.002	CYP4A11, CYP4A22, ACADL, DCI, ACAA1
bta03320	PPAR signaling pathway	6.4	6	0.002	CYP4A11, CYP4A22, CYP27A1, APOA5, ACADL, ACAA1
bta00561	Glycerolipid metabolism	8.7	5	0.002	GLYCTK, AKR1A1, PPAP2A, LIPC, AGPAT2
bta03010	Ribosome	5.1	6	0.006	RPL13, RPL34, RPL8, RPS9, RPS4Y1, RPS8
bta00520	Amino sugar and nucleotide sugar metabolism	6.8	4	0.020	GALK1, PGM1, HEXB, GALT
bta00590	Arachidonic acid metabolism	5.2	4	0.039	CYP4A11, CYP4A22, LTA4H, CYP2E1
bta00052	Galactose metabolism	8.9	3	0.042	GALK1, PGM1, GALT
bta00983	Drug metabolism	6.5	3	0.075	CES2, DPYD, GMPS
Interaction FA by MR ⁹					
bta00010	Glycolysis / Gluconeogenesis	10.7	5	0.001	ALDOA, TPI1, GALM, PGM1, ENO1
bta00190	Oxidative phosphorylation	4.5	5	0.022	UQCRC1, COX10, ATP6V1E1, ATP5B, NDUFS2
3bta00260	Glycine, serine and threonine metabolism	10.7	3	0.030	GCAT, PSAT1, GLDC
bta04920	Adipocytokine signaling pathway	5.6	3	0.096	ADIPOR2, STAT3, ACSL5

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Entry ID for the corresponding Kyoto encyclopedia for gene and genomes (KEGG) pathway.

³ Fold enrichment for each corresponding pathway.

⁴ The number of gene members for each corresponding pathway.

⁵ Fisher exact P-value.

⁶ List of genes in each corresponding KEGG pathway.

⁷ Main effect of MR, comparing the effect of feeding HLA milk replacer to newborn calves with LLA milk replacer as reference.

⁸ Interaction fat by milk replacer: $\frac{[(SFA-HLA + EFA-HLA)/2 : Control-HLA (reference)]}{[(SFA-LLA + EFA-LLA)/2 : Control-LLA (reference)]}$

⁹ Interaction fatty acid by milk replacer: $\frac{[EFA-HLA : SFA-HLA (reference)]}{[EFA-LLA : SFA-LLA (reference)]}$

Table 5-9. Functional annotation chart for enriched downregulated KEGG pathways for main factors and interactions in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Calves were born from dams fed diets supplemented with no fat, saturated fatty acids, and essential fatty acids¹

Entry ID ²	Pathway	Fold E ³	Count ⁴	P-Value ⁵	Genes ⁶
Contrast FAT ⁷					
bta05410	Hypertrophic cardiomyopathy	12.9	3	0.019	MYL2, TNNC1, TPM2
bta05414	Dilated cardiomyopathy	12.0	3	0.022	MYL2, TNNC1, TPM2
bta04670	Leukocyte transendothelial migration	8.3	3	0.044	ICAM1, MYL2, ACTN2
bta04530	Tight junction	7.6	3	0.052	MYL2, MYH7, ACTN2
Interaction FAT by MR ⁸					
bta04530	Tight junction	5.8	5	0.009	MYH1, MYL2, CASK, MYH7, ACTN2
bta04120	Ubiquitin mediated proteolysis	5.3	5	0.013	SOCS1, UBA7, PML, HERC4, BIRC3
bta05410	Hypertrophic cardiomyopathy (HCM)	5.9	3	0.087	MYL2, TNNC1, TPM2
bta05414	Dilated cardiomyopathy	5.5	3	0.098	MYL2, TNNC1, TPM2
Interaction FA by MR ⁹					
bta00240	Pyrimidine metabolism	5.7	4	0.030	UPP2, ENTPD4, DPYD, NME7
bta04120	Ubiquitin mediated proteolysis	3.9	4	0.078	CUL3, KLHL9, ITCH, BIRC3

¹ Calves were fed milk replacer (MR) containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 30 d of age. Calves were born from cows fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

² Entry ID for the corresponding Kyoto encyclopedia for gene and genomes (KEGG) pathway.

³ Fold enrichment for each corresponding pathway.

⁴ The number of gene members for each corresponding pathway.

⁵ Fisher exact P-value.

⁶ List of genes in each corresponding KEGG pathway.

⁷ Main effect of MR, comparing the effect of feeding HLA milk replacer to newborn calves with LLA milk replacer as reference.

⁸ Interaction fat by milk replacer: $\frac{[(SFA-HLA + EFA-HLA)/2 : Control-HLA (reference)]}{[(SFA-LLA + EFA-LLA)/2 : Control-LLA (reference)]}$

⁹ Interaction fatty acid by milk replacer: $\frac{[EFA-HLA : SFA-HLA (reference)]}{[EFA-LLA : SFA-LLA (reference)]}$

Table 5-10. Productive and reproductive parameter of Holstein heifers fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Heifers were born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

	Dam Diet ¹						SEM	P values ³				
	Control		SFA		EFA			FAT	FA	MR	FAT x MR	FA x MR
	Milk replacer (MR) ²											
	LLA	HLA	LLA	HLA	LLA	HLA						
Age at 1st Insemination, months	13.2	13.1	13.2	12.8	13.2	13.1	0.2	0.69	0.35	0.20	0.66	0.35
N of Inseminations	1.6	1.9	2.4	2.3	2.8	2.8	0.5	0.04	0.39	0.92	0.66	0.99
Age 1st calving, years	1.9	1.9	2.1	2.0	2.0	2.0	0.1	0.02	0.76	0.44	0.43	0.45
BW at calving, kg	515	508	545	545	565	538	19.5	0.04	0.75	0.49	0.85	0.51
BCS at calving	3.1	3.0	3.3	3.3	3.4	3.2	0.1	0.04	0.89	0.64	0.86	0.35
BW at drying, kg	606	635	637	645	715	650	32.4	0.14	0.23	0.72	0.29	0.29
BCS at drying	3.4	3.5	3.4	3.4	3.8	3.5	0.1	0.08	0.02	0.55	0.03	0.07
Length of lactation, d	301	302	302	301	276	304	12.4	0.56	0.38	0.37	0.54	0.25
DIM at peak of lactation, d	107.4	85.3	76.4	89.5	78.0	78.0	10.2	0.08	0.64	0.72	0.11	0.54
Mature equivalent Milk, kg	10,107	11,103	11,542	11,948	12,136	12,389	694	0.02	0.48	0.34	0.57	0.92
Fat, %	3.65	3.64	3.67	3.63	3.63	3.53	0.10	0.71	0.53	0.56	0.75	0.80
Protein, %	3.09	3.05	3.08	3.07	3.05	3.03	0.04	0.69	0.31	0.48	0.68	0.93
Lactose, %	4.78	4.78	4.77	4.85	4.80	4.83	0.02	0.08	0.71	0.07	0.16	0.30

¹ Control = no fat supplement; SFA = Energy Booster 100 (Milk Specialties, Dundee, IL); EFA = Megalac-R (Church & Dwight, Princeton, NJ).

² LLA = 0.175 g of LA/BW^{0.75}, HLA = 0.562 g of LA/BW^{0.75}. Milk replacer (20% fat) was exclusively fed the first 30d of life to provide 6.72 g fat/kg BW^{0.75}.

³ P- values for orthogonal contrasts and interactions. FAT = fat (SFA + EFA) vs. Control, FA = EFA vs. SFA, DD= dam diet, MR = milk replacer.

Table 5-11. Incidence and main causes of culling of Holstein heifers fed milk replacer containing low linoleic acid (LLA) or high linoleic acid (HLA) from 1 to 60 days of age. Heifers were born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

Item	Factor	% (n/n)	AOR ²	95% CI		P
Total culling	Dam Diet					
	Control	27.8 (5/18)	Ref.			
	SFA	50.0 (11/22)	2.6	0.69	10.0	0.28
	EFA	43.8 (7/16)	2.0	0.49	8.6	0.71
	Milk Replacer					
	LLA	46.4 (13/28)	Ref.			
Reproductive Problems	HLA	35.7 (10/28)	0.63	0.21	1.9	0.41
	Dam Diet					
	Control	- (0/18)	Ref.			
	SFA	13.6 (3/22)	-	-	-	-
	EFA	12.5 (2/16)	-	-	-	-
	Milk Replacer					
Poor growth	LLA	10.7 (3/28)	Ref.			
	HLA	7.1 (2/28)	0.63	0.09	4.25	0.63
	Dam Diet					
	Control	11.1 (2/18)	Ref.			
	SFA	13.6 (3/22)	1.27	0.18	8.91	0.53
	EFA	18.8 (3/16)	1.90	0.26	13.7	0.14
Mastitis and Low production	Milk Replacer					
	LLA	21.4 (6/28)	Ref.			
	HLA	7.1 (2/28)	0.28	0.05	1.54	0.14
	Dam Diet					
	Control	5.6 (1/18)	Ref.			
	SFA	9.1 (2/22)	1.72	0.14	21.5	0.92
Others ²	EFA	12.5 (2/16)	2.51	0.20	31.9	0.52
	Milk Replacer					
	LLA	3.6 (1/28)	Ref.			
	HLA	14.3 (4/28)	4.56	0.47	44.0	0.19
	Dam Diet					
	Control	11.1 (2/18)	Ref.			
Others ²	SFA	13.6 (3/22)	1.26	0.19	8.52	0.53
	EFA	3.3 (1/16)	0.53	0.04	6.51	0.51
	Milk Replacer					
	LLA	10.7 (3/28)	Ref.			
	HLA	10.7 (3/28)	1.0	0.18	5.48	1.0

¹ Adjusted odds ratio, Control was reference (Ref.) for treatment dam diets and LLA was reference for milk replacer.

² Includes: dead (2), accidentally ill (2), pneumonia (1), and foreign body (1).

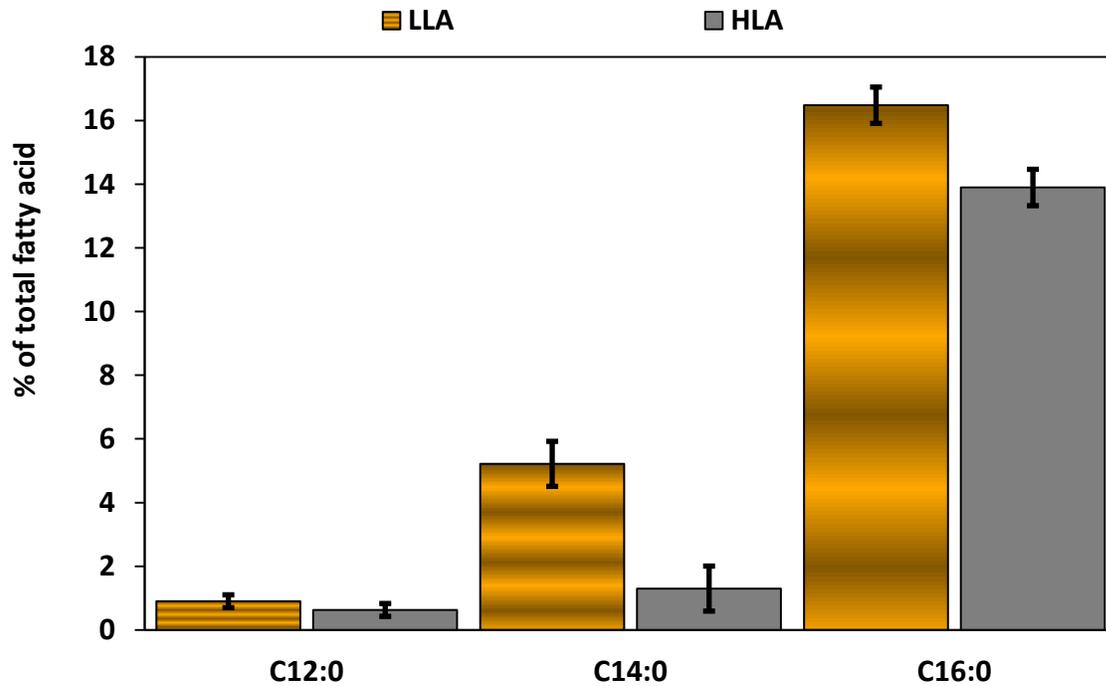
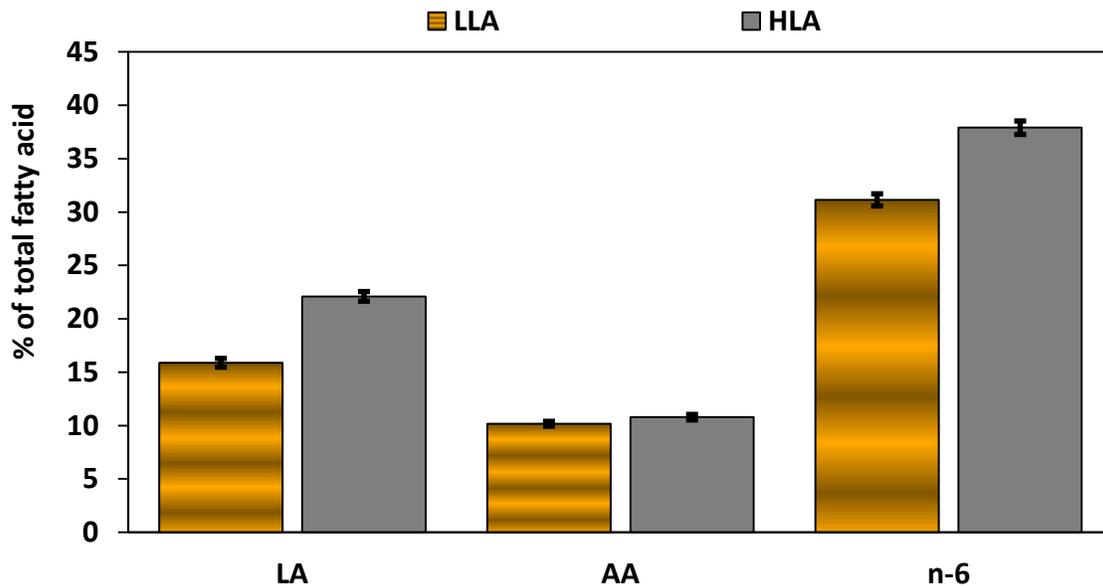


Figure 5-1. Concentrations of C12:0, C14:0 and C16:0 in liver of Holstein calves fed milk replacer containing low (LLA) or high LA (HLA) from 1 to 30 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. For all fatty acids, effect of milk replacer, $P < 0.01$.

A



B

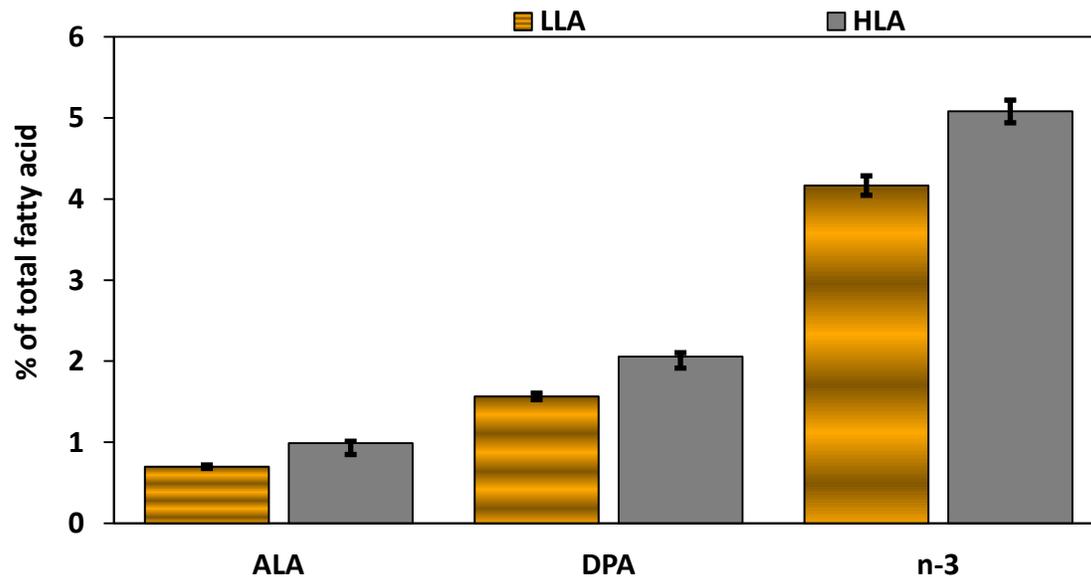


Figure 5-2. Concentrations of omega-3 and 6 fatty acids in liver of Holstein calves fed milk replacer containing low (LLA) or high LA (HLA) from 1 to 30 days of age. Calves were born from cows fed diets supplemented with no fat, saturated fatty acids, or essential fatty acids starting at 8 wk before expected calving date. A) Concentrations of linoleic acid (LA), arachidonic acid (AA) and total n-6 FA; effect of milk replacer on LA and total n-6, $P < 0.01$, on AA, $P = 0.09$. B) Concentrations of α -linolenic acid (ALA), docosapentaenoic acid (DPA) and total n-3 FA; effect of milk replacer, for all fatty acids, $P < 0.01$.

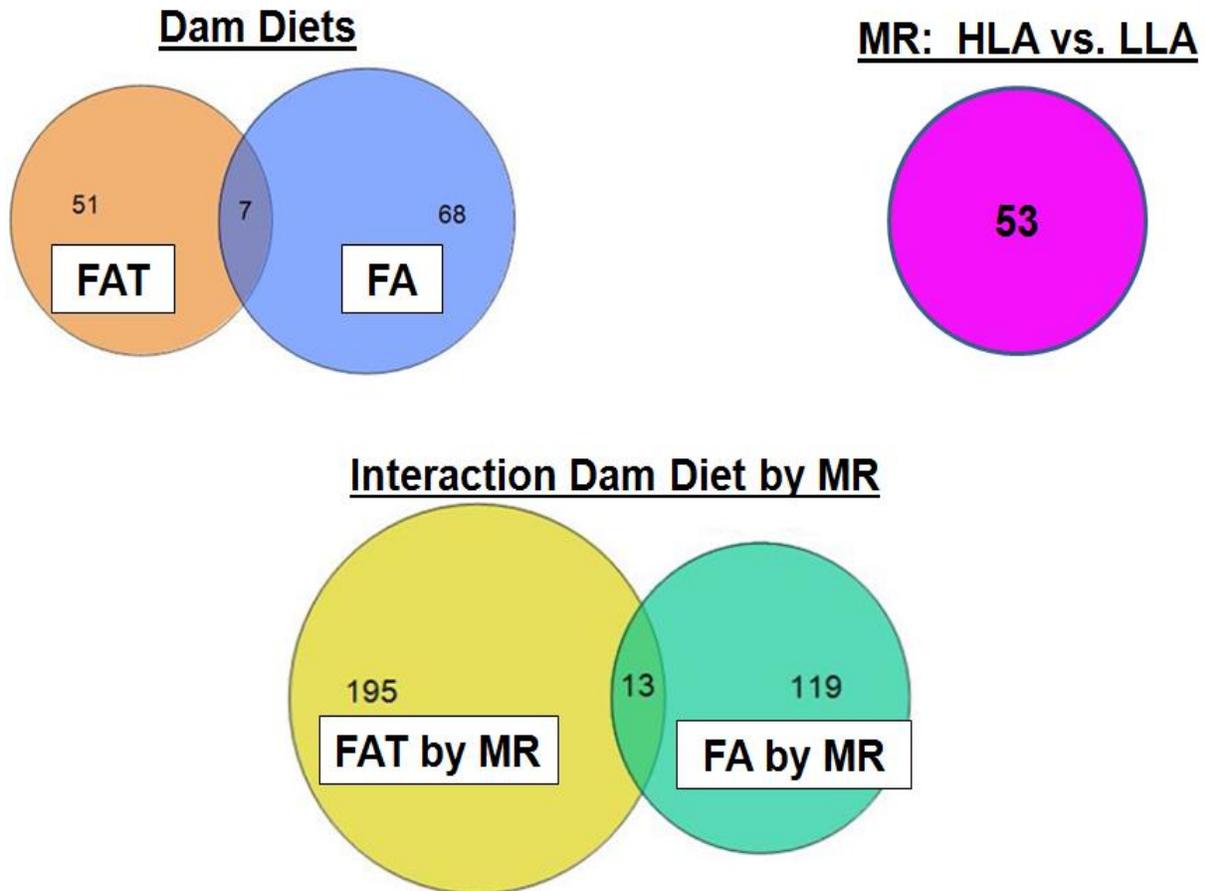


Figure 5-3. Venn diagram of the upregulated differential expressed genes in liver of male calves fed milk replacer (MR) containing low (LLA) or high (HLA) from 1 to 30 days of age. Calves were born from cows fed either control diets (no fat), saturated fatty acids (SFA) or essential fatty acids (EFA) starting at 8 wk of expected calving date. 1) Contrast of FAT: $[(SFA + EFA)/2 \text{ vs. control (reference)}]$. 2) Contrast of FA: $[EFA \text{ vs. SFA (reference)}]$. 3) Contrast of MR: $[HLA \text{ vs. LLA (reference)}]$. 4) Interaction FAT by MR: $[(SFA-HLA + EFA-HLA)/2 : \text{Control-HLA (reference)}] \div [(SFA-LLA + EFA-LLA)/2 : \text{Control-LLA (reference)}]$. 5) Interaction FA by MR: $[EFA-HLA : SFA-HLA \text{ (reference)}] \div [EFA-LLA : SFA-LLA \text{ (reference)}]$.

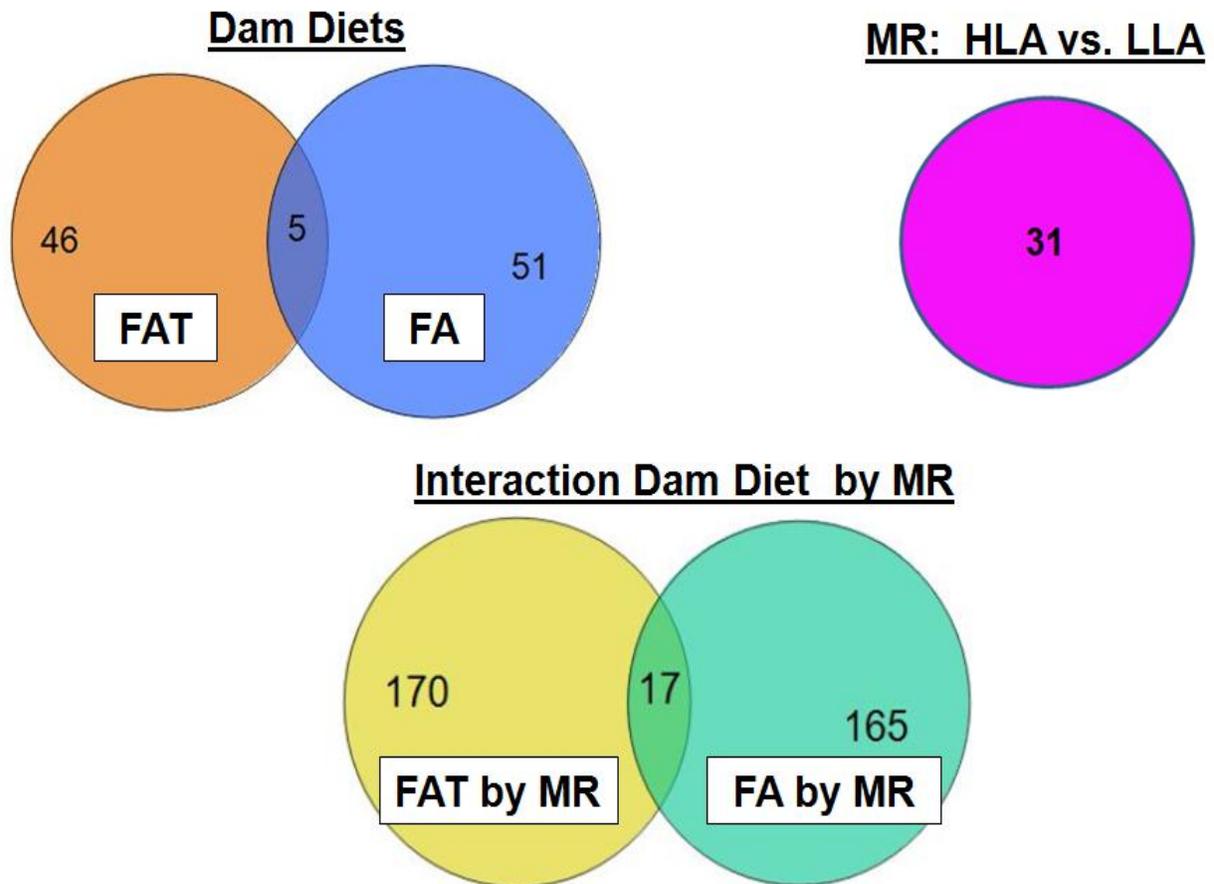


Figure 5-4. Venn diagram of the downregulated differential expressed genes in liver of male calves fed milk replacer (MR) containing low (LLA) or high (HLA) from 1 to 30 days of age. Calves were born from cows fed either control diets (no fat), saturated fatty acids (SFA) or essential fatty acids (EFA) starting at 8 wk e expected calving date. 1) Contrast of FAT: $[(SFA + EFA)/2 \text{ vs. control (reference)}]$. 2) Contrast of FA: $[EFA \text{ vs. SFA (reference)}]$. 3) Contrast of MR: $[HLA \text{ vs. LLA (reference)}]$. 4) Interaction FAT by MR: $[(SFA-HLA + EFA-HLA)/2 : \text{Control-HLA (reference)}] \div [(SFA-LLA + EFA-LLA)/2 : \text{Control-LLA (reference)}]$. 5) Interaction FA by MR: $[EFA-HLA : SFA-HLA \text{ (reference)}] \div [EFA-LLA : SFA-LLA \text{ (reference)}]$.

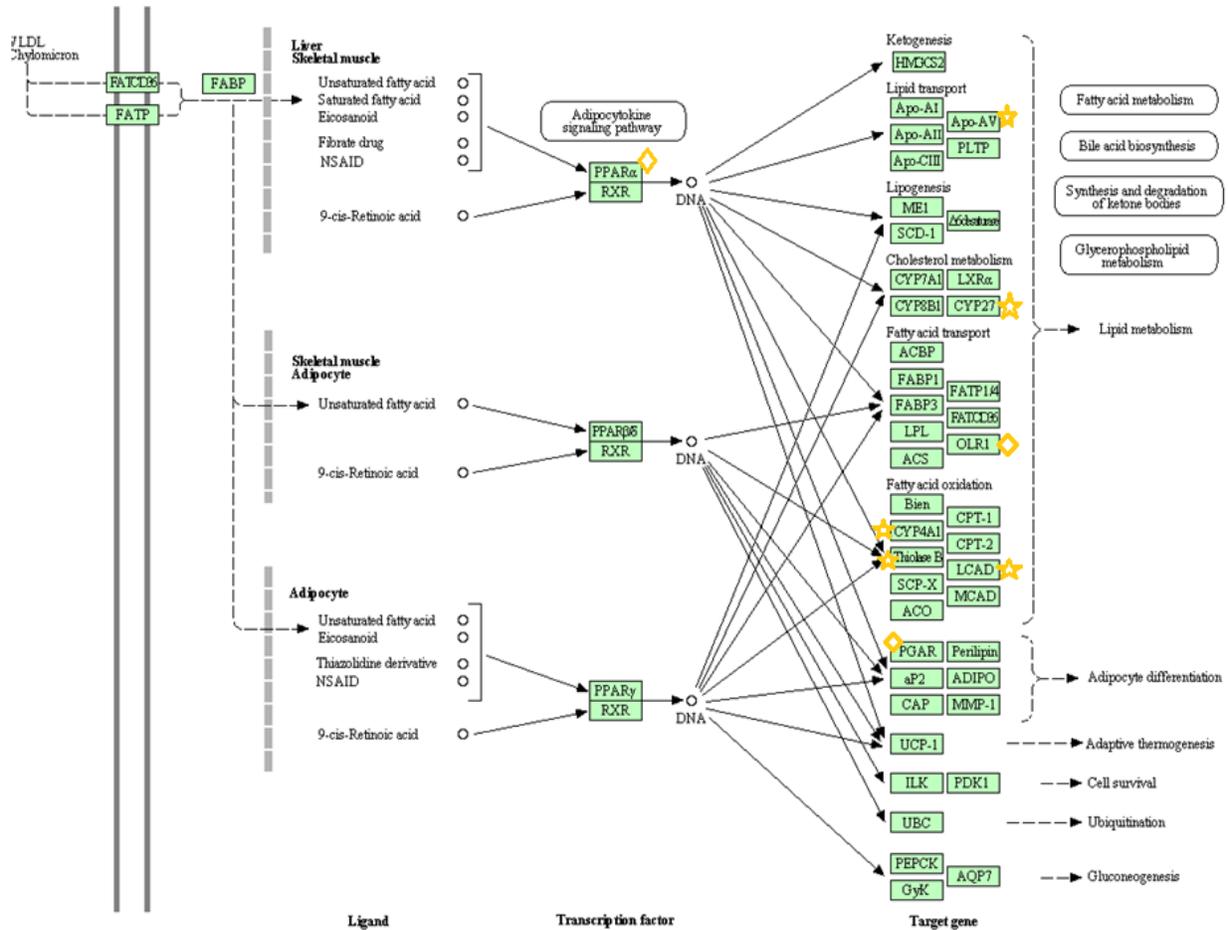


Figure 5-5. Upregulated genes of the PPARA KEGG pathway in calf's liver. Diamond symbol corresponds to upregulated genes by the contrast high linoleic acid milk replacer vs. low linoleic acid milk replacer (reference). Genes are: peroxisome proliferator receptor α (PPARA), oxidized lipoprotein receptor 1 (OLR1) and angiopoietin-like 4 (ANGPTL4 = PGAR). Star symbol corresponds to genes upregulated by the contrast FAT by milk replacer. Genes are: cytochrome P450 subfamily 27A1 (CYP27A1), cytochrome P450 subfamily 4A11 (CYP4A11), cytochrome P450 subfamily 4A22 (CYP4A22), acyl-CoA dehydrogenase, long chain (LCAD), apolipoprotein A5 (APO-A5) and thiolase B.

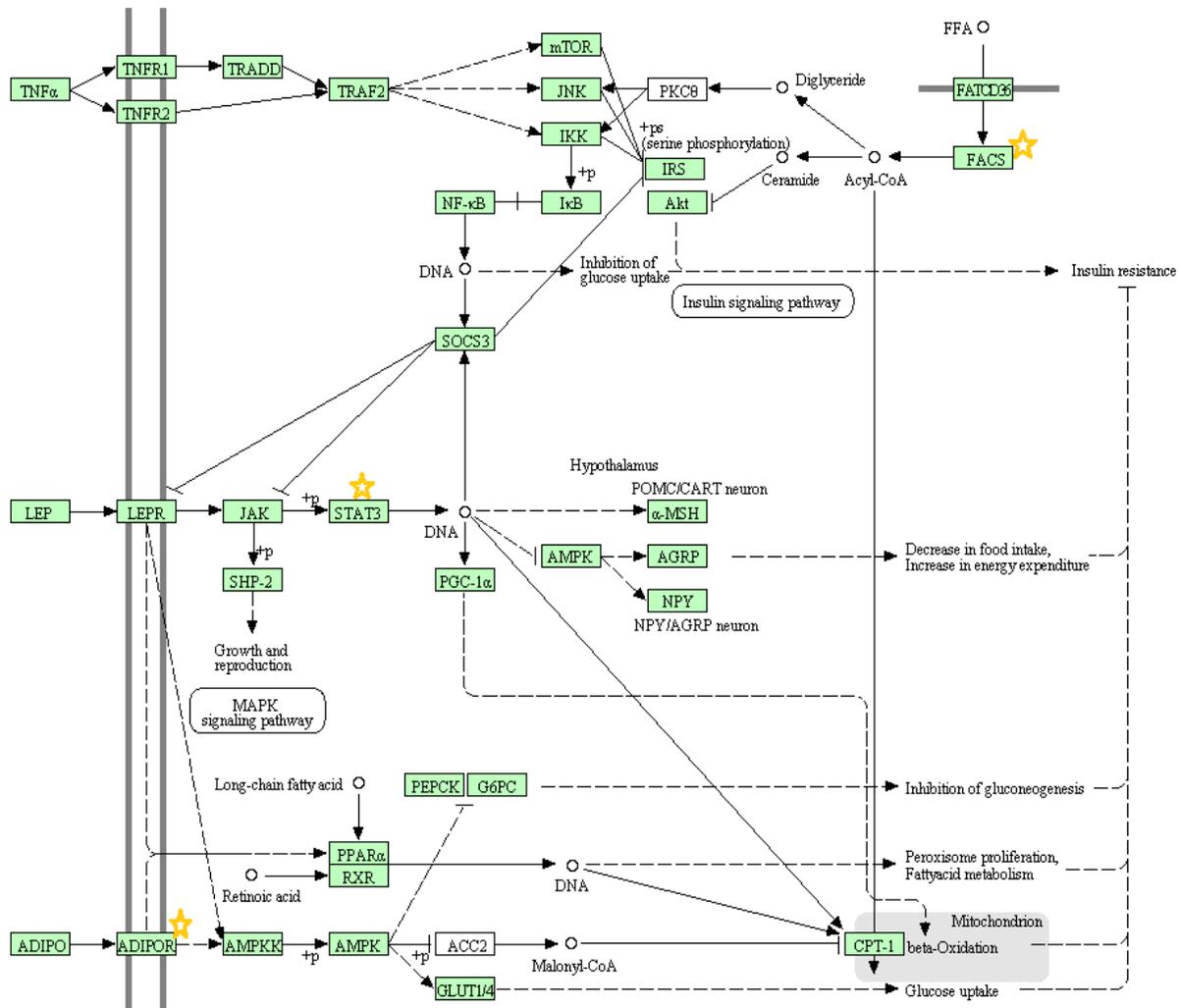


Figure 5-6. Upregulated genes of the adipocytokine KEGG pathway in calf's liver. Star symbol corresponds to upregulated genes by the contrast FA by MR. Genes are: fatty acyl CoA synthetase (FACS), signal transducer and activator of transcription 3 (STAT3), and adiponectin receptor 2 (ADIPOR).

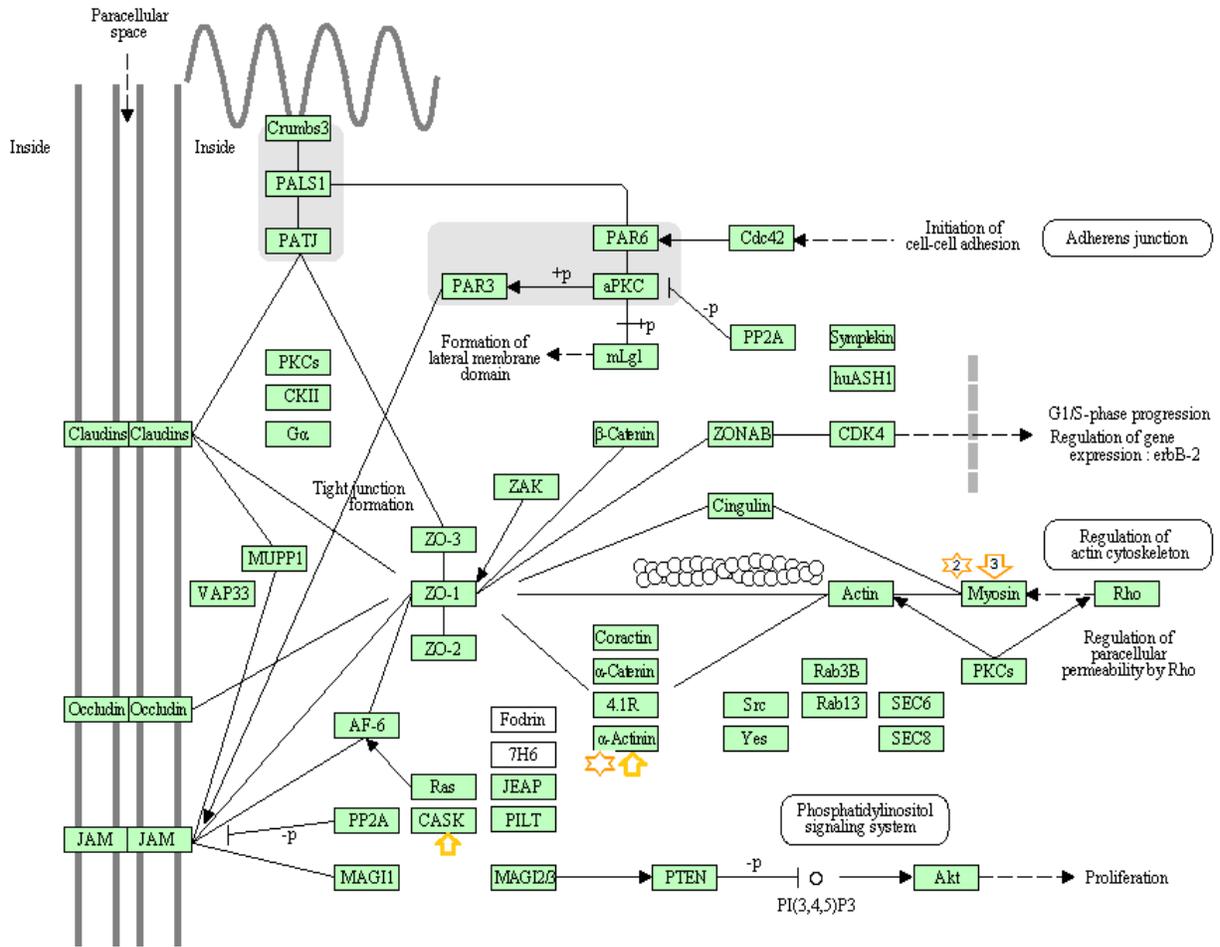


Figure 5-7. Differentially expressed genes within the tight junction KEGG pathway in calf's liver. Star symbol corresponds to downregulated by the contrast of FAT vs. control (reference). Genes are: two myosin subfamilies, myosin regulatory light chain 2 (MYL2) and myosin heavy chain 7 (MYH7), and α -actinin (ACTN2). Arrow symbol corresponds to genes downregulated by the interaction FAT by milk replacer. Genes are: three myosin subfamilies, heavy chain 1 (MYH1), MYL2, and MYH7; calcium/calmodulin- dependent serine protein kinase (CASK), and α -actinin (ACTN2).

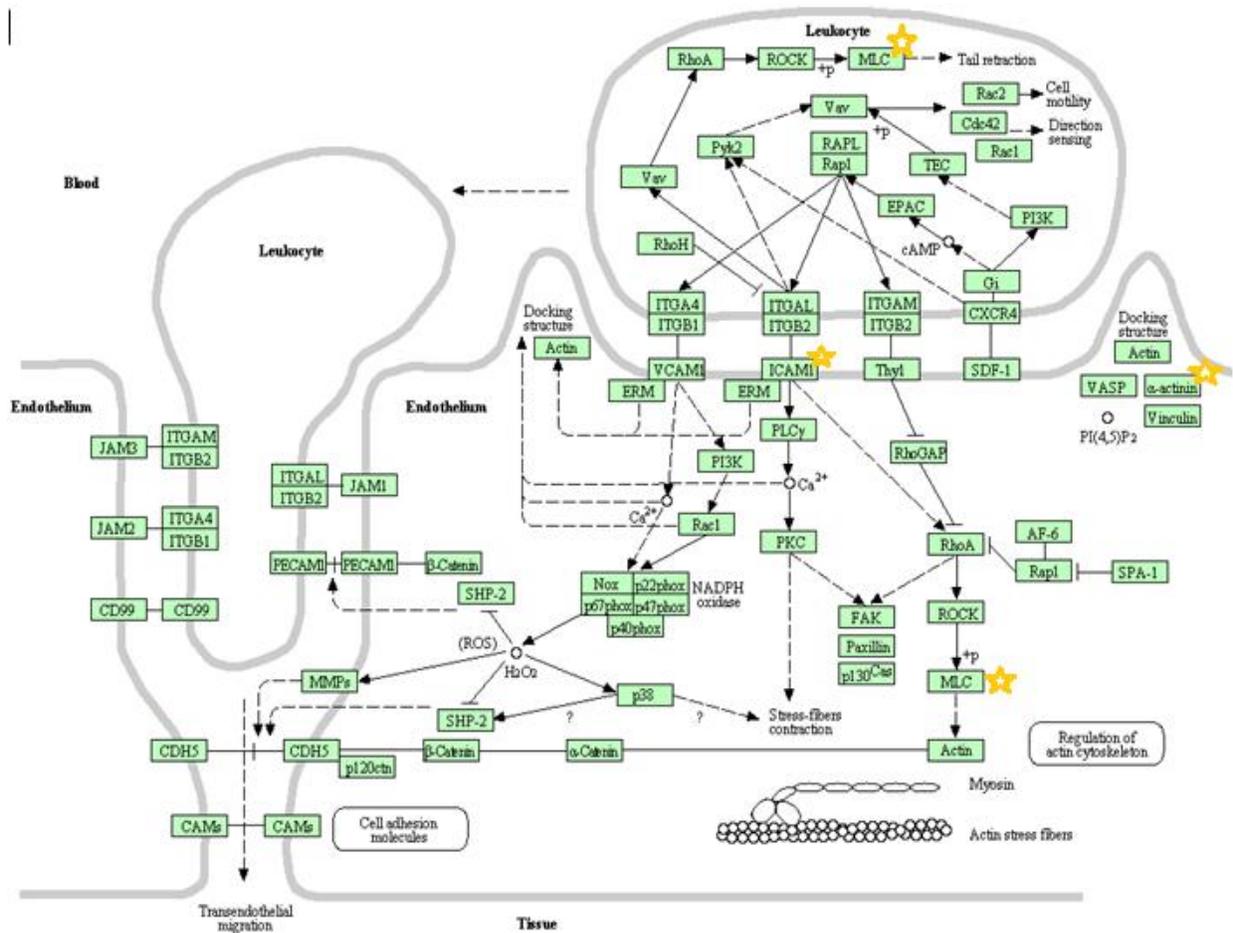


Figure 5-8. Downregulation of genes in the leukocyte transendothelial migration KEGG pathway in calf's liver. Star symbol corresponds to downregulated genes by the interaction FA by milk replacer. Downregulated genes are marked with start and are: intracellular adhesion molecule 1 (ICAM1), myosin heavy light chain 2 (MLC = MYL2), and α-actinin (ACTN2).

CHAPTER 6
EFFECT OF FEEDING MILK REPLACER ENRICHED WITH INCREASING LINOLEIC
ACID ON HOLSTEIN CALF PERFORMANCE,
IMMUNE RESPONSE AND HEALTH

Background

Essentiality of certain long chain fatty acids (FA) was discovered by Burr and Burr (1929, 1930, 1932) in pioneer studies performed with rats fed fat-free diets and supplemented with purified FA or mixtures of them. These authors identified the symptoms of linoleic acid (LA) deficiency, namely poor growth, dermatitis, poor reproduction, and death. A LA requirement was documented later using swine, poultry, and guinea pigs (Hill et al., 1961; Bieri and Prival, 1966; Reid et al., 1964). Authors also concluded that α -linolenic acid (ALA) was able to prevent these signs of deficiency. However, it was not until the late 1970's and early 1980's that essentiality of ALA was determined by identifying the role of their derivatives, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in brain development, as reviewed by Innis (1991).

Although the dietary essentiality of LA and ALA has been clearly demonstrated, specific requirements of LA have only been established for mice and rats. The National Research Council (1995) recommends a daily intake of 1.3 and .055% of LA from the total daily intake of metabolizable energy for laboratory rats. An adjustment for metabolic body weight (BW = 100 g) results in a daily intake of 0.551 and 0.212 g of LA/BW^{0.75}. Some recommendations have been released for humans, but most of these recommendations have focused on groups (e.g. n-3 or n-6) or ratios (e.g. LA:ALA and n-6:n-3) of FA instead of single FA. In a review paper, Palmquist (2009) pointed out the inaccuracy of recommending intake of LA and ALA in terms of their ratio, particularly because this practice leads to reduction of the absolute intake of ALA. Czernichow et al.

(2010) reviewed studies on intake of n-6 FA and risk of cardiac diseases and recommended an intake of n-6 FA above 10% of the total energy intake in order to reduce the risk of cardiac diseases. Ramsden et al. (2010) used the same studies reviewed by Czernichow et al. (2010) in another analytical approach. They concluded that if dietary intake of n-6 are increased without a parallel increase of n-3 intake, a greater risk of cardiac diseases would result. Calder and Deckelbaum (2011) agreed with the analysis of Ramsden et al. (2010) but pointed out that both studies grouped FA without considering their individual effects which could potentially lead to confounding effects.

A limited number of studies have evaluated the supplementation of fat sources enriched with LA to preruminant dairy calves. Dr. Jenkins' research group in Ontario, Canada was among the first to evaluate the replacement of milk fat with sources of less expensive fat such as vegetable oils. Their studies (Jenkins et al., 1985, 1986; Jenkins and Kramer, 1986) are the foundation to evaluate the effects of total or partial replacement of milk fat with vegetable oil in order to enrich the milk replacer (MR) with essential FA (EFA). They evaluated calf responses in terms of growth, diarrhea incidence, and FA profile of most of the important tissues and organs involved in lipid metabolism. Authors concluded that commonly supplemented milk contains enough LA to avoid signs of deficiency, but that the requirement of EFA might be greater under conditions of high stress on preweaned or newly-weaned calves.

To the best of our knowledge no studies have been although

The most recent studies, in preweaned calves, supplementing EFA to evaluate the growth response and the activity of different markers of immune responses have been

conducted supplementing n-3 FA. The hypothesis was that immune responses would be improved by increasing intakes of LA. This improved immune response could positively affect calf productive performance. Therefore the objective was to evaluate the effect of supplementing increasing amounts of LA in a MR to newborn calves during the first 60 d of life on calf growth, health and different markers of immune responses.

Materials and Methods

Enrollment and Management of Pregnant Cows

The experiment was conducted at the University of Florida's dairy farm (Hague, FL) from October 2010 to June 2011. All procedures for animal handling and care were approved by the University of Florida's Institutional Animal Care and Use Committee. A weekly cohort of pregnant nulliparous (n = 39) and previously parous (n = 64) Holstein cattle were enrolled in the study starting at 8 wk before expected calving day.

Experimental cattle were fed once daily (0800 h) with a single diet prepared as a totally mixed ration formulated to have low concentrations of total and essential FA (Table 6-1). Offered feed was adjusted daily to achieve 5 to 10% orts. Orts were collected and weighed daily. A bermudagrass silage sample was collected once a week and dried for 1 h using a Koster® (Koster Crop Tester, Inc., Strongsville, OH) for determination of dry matter (DM). Proportions of forages and concentrates in the diet were adjusted weekly based on the weekly DM values in order to maintain the formulated forage to concentrate ratio (55.3:44.7). Weekly samples of silage, hay, and concentrate were ground to pass through a 1-mm screen using a Wiley Mill (Arthur H. Thomas, Company, Philadelphia, PA). Samples were composited monthly, pooled in a single sample and analyzed (Dairyland Laboratories, Inc., Arcadia, WI) for crude protein

(CP), ash-free acid detergent fiber, ether extract, ash, and individual minerals (Ca, P, Mg, K, S, Na, Cl, Mn, Zn, Cu, Fe, and Mo).

Calving Management at Birth and Colostrum Feeding

Calves were born from January 4th, 2011 through April 5th, 2011. Pregnant cows gave birth to calves in a sod-based pen. All cows were monitored for signs of parturition initiation every 30 min between 0530 to 1530 h and then every 2 hours between 1530 and 0530 hours. Ease of calving was scored according to Sewallem et al. (2008) as unassisted (1), easy pull (2), hard pull (3), and surgery (4). Within 2 h of birth calves were weighed, ear-tagged, and the umbilical cord was disinfected with 10% Betadine solution (Purdue Frederick Co., Norwalk, CT).

Parturient cows were milked within 6 h of calving and colostrum was harvested. Concentration of total immunoglobulin G (IgG) in colostrum was measured using a colostrometer. Colostrum of good quality (> 50g/L of IgG) was frozen (-20°C) in 4-L amounts. Immediately after weighing, calves were given 4 L of thawed and warmed colostrum having a minimum IgG concentration of 55 g/L using an esophageal feeder. Calves were housed temporarily in individual hutches (1 x 1 m) equipped with a heat lamp and moved to individual wire hutches (1 x 1.5 m) on sand bedding when they were between 2 to 16 h of age.

Appropriate Passive Immune Transfer Identification

Blood samples were collected via jugular venipuncture before colostrum feeding, and again within 24 to 30 h after feeding colostrum. Calf blood samples were collected in a clot-activated tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and serum was separated at room temperature. Tubes were centrifuged for 15 min at 2095 x *g* (Allegra X-15R centrifuge, Beckman Coulter, Inc). Serum total protein (STP)

concentrations were determined using an automatic temperature-compensated hand refractometer (Reichert Jung; Cambridge Instruments Inc. Buffalo, NY).

Sera and colostrum total IgG concentrations were measured using a single radial immunodiffusion method (Triple J Farms, Bellingham, WA) following the manufacturer's protocol with some modifications. Briefly, sera and colostrum samples were diluted with sterile saline (0.9% NaCl) at a ratio of 7:10 and 1:15 respectively. Diluted samples (5 μ L) were applied to serial radial immunodiffusion plates containing agarose gel with anti-bovine IgG. Plates were left undisturbed for 27 h at room temperature and resulting ring diameters were measured with a monocular comparator (VMRD, Inc., Pullman WA). A standard curve was plotted with reference sera supplied by the manufacturer (1.96, 14.02, and 27.48 g/L of IgG). Concentrations of IgG in diluted samples were read from the standard curve and the corresponding correction factor, due to dilution, was applied afterwards. Samples were run in singlet, but a control sample, included in each plate, was run in duplicate resulting in a 3.6% intra-assay variation.

Calves were considered as having an appropriate passive transfer (APT) if they had a serum total IgG \geq 1 g/dL after 24 to 30 h of colostrum feeding (Tyler et al., 1996; Weaver et al., 2000). Alternatively, STP was another measure to evaluate APT by considering a minimum plasma concentration of STP \geq 5.0 g/dL after 24 to 30 h of colostrum feeding (Donovan et al., 1998, Calloway et al., 2002). The apparent efficiency of IgG absorption (AEA, %) was calculated according to (Quigley et al., 1998) assuming that serum was 9.9% of calf body weight (BW) using the following equation: (IgG concentration in serum at 24 to 30 h of colostrum feeding (g/L) \times [0.099 \times BW (kg) at birth] \div IgG intake (g) \times 100%.

Dietary Treatments, Feeding Management and Analyses

Calves were blocked by parity of the dam and gender (females = 60, males = 43) and assigned randomly to receive one of four MR from 0 to 60 d of life. Treatments of differing LA concentrations were prepared by mixing preplanned ratios of hydrogenated coconut oil (CCO; (Welch, Holme & Clark Co., Inc, Newark, NJ)) and soybean oil (SO; Winn Dixie Co.). The treatment (T) ratios of CCO and SO were the following: T1 = 100:0, T2 = 96.0:4.0, T3 = 87.9:12.1, and T4 = 71.8:28.2 and the FA profile is described in Table 6-2.

Reconstitution of MR was done consistently throughout the experiment. Briefly, amounts of each fat source and emulsifier (3% of the oils, GRINDSTED® MONO-DI HV 52 K-A, Danisco, USA Inc.) needed to feed the number of calves assigned to each treatment were calculated. Fats were kept in a walk-in cooler (4°C). Every day at 0530 h, the needed amounts of each fat source and emulsifier per treatment were weighed (Ohaus®, TAJ4001 series, 0.1 g resolution). The required amount of CCO was melted to just reach the liquid form using a conventional microwave oven followed by the addition of the required amounts of SO. Oils were warmed to 70 to 80°C which is the required temperature for proper dissolution of the emulsifier. Immediately, the blend of fats and emulsifier were transferred into insulated containers and transferred to the calf area.

The corresponding amounts of powdered MR (9.5% fat DM basis, Land O'Lakes Animal Milk Products Co., Shoreview, MN) and warm water (40 to 43°C) were weighed and (11% DM solution) mixed for 5 to 10 min using an electric drill with a wire whisk attachment (12.5 cm diameter). Then the blend of oils and emulsifier were added and mixed again with the electric drill. Surface oil droplets were not observed. Immediately

upon mixing, individual calves were offered amounts (L) of MR to achieve LA intakes of 0.144 (T1), 0.206 (T2), 0.333 (T3), or 0.586 (T4) g of LA per kg of $BW^{0.75}$. Targeted intakes of LA formulated in the current study were selected with reference to the recommended intake of LA in rats (NRC, 1995) and from results of our previous study (Chapter 4) in which 2 rates of LA (0.487 vs. 0.149 g/kg $WB^{0.75}$) were provided with the MR. Laboratory rats have a LA requirement of 0.5 and 1.3% of the metabolizable energy for females and males, respectively (NRC for Laboratory Animals (1995)). The LA requirement of rats expressed in relation to $BW^{0.75}$ was calculated for a 100 g BW growing rat consuming 16.4 g/d (Kennedy and Mitra, 1963) of a 4 kcal of ME/g of DM diet. Gross energy value of LA and its digestibility was considered to be 9 kcal/g and 96.7% (NRC, 1995). Using the previous specifications the LA requirement of male and female rats were 0.551 and 0.212 g/kg of $BW^{0.75}$, respectively. The LA intake rates formulated for the current study diets were below and above those calculated for rats on a metabolic BW basis and within the range of LA rates used in Chapter 4. An attempt was made to feed the minimum rate feasible using feedstuffs commonly available to the dairy industry.

The temperature of the liquid MR placed in front of calves was always between 35 to 38°C. At each feeding, each calf was monitored to ensure that the MR was consumed within 10 min of offer. Those calves not willing to drink quickly were fed using a nipple bottle preferentially or an esophageal feeder alternatively. Temperature of MR was verified and warmed in a hot water bath if needed for these calves.

Calves were fed MR exclusively during the first 30 d of life and supplemented with a single grain mix of low concentration of LA starting at 31 d of age (Table 6-3). Amounts

of MR offered were increased weekly according to BW measured weekly throughout the 60 d of the experimental period whereas grain mix was offered in ad libitum amounts. Clean water was available in ad libitum amounts at all times. Powdered MR and grain mix were sampled weekly. Weekly samples were composited monthly and then composited in a single sample. Samples were analyzed (Dairyland Laboratories, INC., Arcadia, WI) for CP, ash-free acid detergent fiber (only for grain mix), ether extract (grain mix), mojonier fat (MR), ash, and individual minerals (Ca, P, Na, Cl, Mg, K, S, Mn, Zn, Cu, Fe, and Mo).

Milk replacer was fed at a constant rate per kg of $BW^{0.75}$, and adjusted weekly based on a new BW; however, calves that lost BW in a 7-d period were offered the same amount of MR as that offered the previous week. If calves did not consume all of their morning MR within a few minutes of offer, the remaining MR was given using an esophageal feeder whereas the afternoon feeding was replaced with electrolytes if calves were not willing to drink voluntarily.

Body Weight and Immunizations

Calf BW was measured at birth before colostrum intake. This measure was used to assign the amount of MR each calf was offered until the next weekly BW measure. Weekly BW measures were done every Monday at 1700 h (about 4 h after the second MR feeding) and the new intakes were adjusted starting on every Wednesday of the same week. Body weight and wither and hip heights (as measures of growth) also were recorded at 0, 30 and 60 d of age. The 30 and 60 d BW were measured before the morning milk feeding (0530 h). All immunization protocols were done according details in chapter 4.

Calf Scoring for Health Assessment and Incidence of Health Disorders

Calves were scored daily using the calf health scoring system from the University of Wisconsin (<http://www.vetmed.wisc.edu/dms/fapm/fapmtools/calves.htm>). Attitude, fecal consistency, nasal discharge, ocular discharge, and cough were scored daily after the first feeding of MR (0830 to 0930 h) using a 0 to 3 scale. For attitude, calves were categorized as 0 when alert and responsive, 1 when non-active, 2 when depressed, and 3 when moribund. Fecal consistency was scored as 0 when firm, 1 when soft or of moderate consistency, 2 when runny or mild diarrhea, and 3 when watery and profuse diarrhea. For nasal score, 0 was normal serous discharge, 1 was when a small amount of unilateral cloudy discharge was present, 2 was when bilateral cloudy or excessive mucus discharge was present, and 3 was when copious bilateral mucopurulent discharge was present. Ocular discharge was scored as 0 when normal, 1 when a small amount of ocular discharge was present, 2 when moderate amount of bilateral discharge was present, and 3 when heavy ocular discharge was present. Cough was scored after pressing the trachea as 0 when absent, 1 when a single cough was induced, 2 when repeated cough or occasional spontaneous cough was induced, and 3 when repeated spontaneous cough was detected. Weekly averages of all scores were generated per calf for statistical analysis. Calves with fecal score > 1 were considered to have diarrhea and severe diarrhea when score = 3, whereas calves with score > 0 for other occurrences were considered as being abnormal for that measure.

Incidence of health disorders were recorded daily for individual calves. Rectal temperature was measured daily during the first 14 d of age, and on days when the calf displayed clinical signs of disease such as diarrhea, bloat, coughing, increased respiratory frequency, depression, or lack of appetite. Calves with rectal temperature \geq

39.5°C were categorized as febrile. Day when disease was first diagnosed was recorded and duration of each illness event was determined. Number of episodes of fever, diarrhea, and pneumonia were determined. To distinguish between different episodes, an interval of 4, 4, and 10 d between diagnoses of fever, diarrhea and pneumonia, respectively, had to elapse to characterize a new event. Calves with digestive and respiratory problems were treated by farm personnel according to protocols established by the herd veterinarian.

Hormone and Productive Metabolite Analyses

Before colostrum was fed, a jugular blood sample was collected from each calf and again after 24 to 30 h of feeding colostrum into clot-activated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Serum was separated at room temperature and tubes were centrifuged for 15 min at 2095 x g (Allegra X-15R centrifuge, Beckman Coulter, Inc). Weekly samplings of blood into clot-activated and K₂EDTA tubes were centrifuged for 15 min at 2095 x g for harvesting of serum and plasma, respectively. Before storing of serum, STP was measured using an automatic temperature-compensated hand refractometer (Reichert Jung; Cambridge Instruments Inc. Buffalo, NY). Plasma samples for all productive metabolites were analyzed once a week at approximate ages of 1, 8, 15, 22, 29, 36, 43, 50, and 57 ± 1 d whereas analyses of hormones were done on sera sample from d 0 and in plasma samples at 1, 15, 29, 43, and 57 ± 1 d of age.

A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and PUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965). A total of twelve runs (each balanced for treatment and gender)

were performed. Each run included a common control sample which was run in duplicate with a final intra- and interassay variations of 1.0 and 1.3% and 3.0 and 4.2% for glucose and PUN, respectively.

Plasma concentrations of β -hydroxybutyric acid (BHBA) were determined using a commercial kit (Wako Autokit 3-HB; Wako Diagnostics, Inc., Richmond, VA). Unknown samples were run in singlet including a control sample which was run in duplicate. A total of twelve plates, balanced for same number of calves per treatment, were run. Intra- and inter-plate variations were 5.6 and 7.8%, respectively. Total cholesterol concentrations were determined using a commercial kit (Cholesterol E kit, Wako Diagnostics Inc., Richmond, VA). Each sample was analyzed in duplicate, including a common control sample in each of the 24 plates. Intra- and inter-assay variations were 3.2 and 6.8%, respectively.

Plasma concentrations of insulin-like-growth factor-I (IGF-I) were analyzed following the manufacturer's protocol (Quantine Elisa, Human IGF-I Immunoassay, R&D Systems Inc.) with some modifications in sample preparation. Briefly, serum and plasma samples were run in singlet. The pre-treatment of samples, to release the IGF-I from their binding proteins, was done with half of the indicated volumes for sample pre-treatment reagents to maintain the final suggested dilution of samples (1:100); control sample was included in duplicated wells per plate. The intra-plate variation for control sample was 3.6%, whereas the inter-plate variation was 8.1%. Insulin concentrations were analyzed by a double antibody radioimmunoassay (Badinga et al., 1991). Intra- and interassay variations were 7.3 and 14.6%, respectively.

Markers of Immunity Analyses

Blood was collected from puncture of the jugular vein into heparinized vacutainer tubes at 7, 14, 28, and 42 ± 2 d of age. Samples were transported at ambient temperature with constant gentle inversion. Quantification of individual cells and cell populations were performed using a ProCyt Dx hematology analyzer (IDEXX Laboratories, Inc., Westbrook, ME). Tubes were kept at room temperature with gentle inversion and analyzed within 2 h of collection.

Neutrophil phagocytosis and oxidative burst were measured on blood of calves at 7, 14, 28, and 42 d of age using a dual color flow cytometry assay using methodology modified from Smits et al. (1997). Whole blood samples were collected in replicate for this analysis and for quantification of cell populations. Tubes were kept at room temperature with gentle inversion and assayed immediately after the hematologic results were done. Briefly, whole blood (100 μ L) was transferred into each of 3 polystyrene round-bottom tubes (12 x 75 mm) and 10 μ L of 50 μ M dihydrorhodamine 123 (DHR, Sigma-Aldrich, Saint Louis, MO) was added to all tubes. Tubes were vortexed slowly and incubated at 37°C for 10 min with constant rotation using a nutator (BD, San Jose, CA). A 10 μ L solution of 20 μ g/L of phorbol myristate acetate (PMA, Sigma-Aldrich) was added into tube number 2 (positive control for oxidative burst). A pathogenic *E. coli* bacterial suspension (10^6 CFU/mL) isolated from a case of bovine mastitis and labeled with propidium iodide (Sigma-Aldrich) was added to tube number 3 to establish a 40:1 ratio of bacteria to neutrophil, using the concentration of neutrophils in blood provided by the hematologic results. Tubes were slowly vortexed and incubated at 37°C for 30 min with constant rotation. After incubation, tubes were placed immediately on crushed ice to stop neutrophil activity. Tubes were processed into a Q-

Prep Epics immunology workstation (Coulter Corp., Miami, FL) on a 35-sec cycle using three lysing reagents, followed by the addition of 500 uL of cold distilled water to complete the hemolysis and 10 µL of 0.4% trypan blue to quench extracellular oxidized DHR. Tubes were vortexed slowly and kept on crushed ice until flow cytometry analysis within 2 h of fixation at the University of Florida Flow Cytometry Core Lab. For each sample the optical features of 10,000 neutrophils were acquired using a Facsort flow cytometer equipped with a 488-nm argon ion laser for excitation at 15 mW (BD Biosciences, San Jose, CA) and CellQuest software (Becton Dickinson, San Jose, CA). Forward (roughly proportional to the diameter of the cell) and side (proportional to membrane irregularity) scatters were used for preliminary identification of neutrophil cells on dot plots (Jain et al., 1991). Density cytograms were generated by linear amplification of the signals in the forward and side scatters. Parameters analyzed included the percentage of neutrophils that phagocytized bacteria and the percentage of neutrophils with a phagocytosis-induced oxidative burst. Also, mean fluorescence intensity (MFI) of green (DHR oxidation) and red (PI-labeled bacteria) wave lengths were used as an estimation of the total gated neutrophil mean oxidative burst intensity (interpreted as the mean number of reactive oxygen species produced per neutrophil) and mean phagocytic activity (indicator of mean number of bacteria engulfed per neutrophil), respectively.

Before harvesting of plasma from the blood collected in K₂EDTA tubes, concentrations of hematocrit were determined by centrifuging (Microspin 24 tube micro hematocrit centrifuge, Vulcon Technologies, Grandview, Mo) heparinized micro-hematocrit capillary tubes (Fisherbrand, Thermo Fisher Scientific Inc.) for 3 min and

read in a micro hematocrit tube reader (Model CR, Damon/IEC, Needham Heights, MA). Concentrations of STP and acute phase proteins were determined on weekly plasma samples at approximate ages of 1, 8, 15, 22, 29, 36, 43, 50, and 57 ± 1 d whereas determination of IgG against ovalbumin (OVA) were done in sera sample at 1, 22, 43, and 57 ± 1 d of age. Blood was collected into heparinized tubes at 7, 14, 28, and 42 ± 2 d of age for in vitro analysis of neutrophil activity whereas proliferation of lymphocytes and production of cytokines were done at 14, 28, and 42 ± 2 d of age. These analyses were performed within 2 h of blood harvest.

Calves were injected s.c. with 0.5 mg of OVA (Sigma Aldrich, Saint Louis, MO) diluted in Quil A adjuvant solution (0.5 mg of Quil A in 1 mL of phosphate buffered saline (PBS), Accurate Chemical & Scientific Corp., Westbury, NY) using sterile procedures at 1, 22, and 43 d of age. Serum concentrations of bovine anti-OVA IgG were measured on the same days of injection and at 57 d of age by the method described by Mallard et al. (1997) and detailed in chapter 3. Intra- and interassay coefficients of variation based on the positive control were 3.6 and 3.8%, respectively.

Concentrations of plasma haptoglobin (Hp) were determined by measuring the differences of H_2O_2 activity with haptoglobin-hemoglobin (Hb) as described previously (Mikamura and Suzuki, 1982). Concentration of Hp is reported as arbitrary units (optical density x 100). Intra- and interassay coefficients of variation were 5.6 and 6.8%, respectively. Plasma concentrations of ASP were determined according to Nakajima et al. (1982) with some modifications. Plasma samples (50 μ L) were incubated with PCA solution (1 mL, 6 M perchloric acid, Fisher Scientific, Hampton, NH, USA). (The intra- and interassay coefficients of variations were 4.6 and 8.6%, respectively.

In order to determine whether blood lymphocytes from experimental calves contained detectable amounts of cytokines in a preliminary study, whole blood was stimulated for cell proliferation with phytohaemagglutinin (PHA, L1668; Sigma-Aldrich) + lipopolysaccharide (LPS, E. coli 0111:B4; Sigma-Aldrich) at a dose of 0.2 + 1 µg/mL. This dose was selected from three doses tested (PHA + LPS: 0.2 + 1 µg/L; 1 + 5 µg/mL; 5 + 25 mg/mL) based upon earlier work using human whole blood cell proliferation (De Groote et al., 1992). Stimulated and non-stimulated blood samples from four preweaned calves at the University of Florida dairy herd were analyzed (Aushon Biosystems, Billerica, MA) for tumor necrosis factor α (TNF- α), interferon γ (IFN- γ), interleukin-2 (IL-2), and IL-4. The non-stimulated samples had very low concentrations of all cytokines (TNF- α at < 5, IFN- γ at < 13, IL-2 at < 12, and IL-4 at < 40 pg/mL), whereas stimulated samples had greatly increased concentrations of all cytokines with the lowest stimulation dose of mitogens selected for use with all samples collected for the experimental calves in the current study.

The analysis of whole blood lymphocyte proliferation was performed following the protocol of Hulbert et al. (2011) with some modifications. Briefly, whole blood was diluted at 1:5 with RPMI 1640 (Invitrogen) containing 1% antibiotics (Gibco Antibiotic-Antimycotic, Invitrogen). Whole blood was stimulated with a combination of 0.2 µg/mL of PHA + 1 µg/mL of LPS. Stimulated and non-stimulated samples were incubated in sterile 24-well cell culture plates (2mL wells) for 48 h in a humidified 5% CO₂ chamber. The cell culture plates were centrifuged for 12 min at 1455 x *g* (Allegra X-15R Centrifuge, Beckman Coulter, Inc). The supernatant fraction from 3 wells was pooled

and aliquoted into 200 μ L microtubes and stored at -80°C until analyzed for bovine TNF- α and IFN- γ .

Quantification of TNF- α and IFN- γ concentration was performed only on stimulated supernatant samples, based on the preliminary results from the validation test in which the concentrations of cytokines of non-stimulated cells were very low. Bovine TNF- α and IFN- γ Vet SetsTM Elisa Development Kit (Kingfisher Biotech, Inc.) were used according to manufacturer's procedure. Stimulated samples were analyzed in duplicate including a pool of stimulated samples as a control. Standards were diluted in RPMI with 4% BSA and 1% antibiotics; stimulated samples were not diluted. The intra- and interassay coefficients of variation were 2.0 and 11.4% and 8.4 and 13.2% for TNF- α and IFN- γ , respectively. The sensitivity of the assay was 78 and 125 pg/mL for TNF- α and IFN- γ , respectively.

Cell-mediated hypersensitivity to epidermal injection of PHA (L1668; Sigma-Aldrich) was done in calves at 29 and 59 ± 2 d of age. The treated shoulder was shaved and sterilized with 78% alcohol. The injected area was identified by circling it with a marker. The epidermal injection of PHA (200 μ g of PHA dissolved in 100 μ L of sterile isotonic saline solution) was made in the middle of the created circle using insulinic syringes. The skin fold thickness was measured before injection at 6, 24, and 48 h after injection using a digital caliper (Mitutoyo, Kawasaki, Kanagawa, Japan). Delayed type Hypersensitivity (DTH) response to PHA injection was determined by the increase in the diameter of the skin fold thickness related to the diameter before injection as a proportion (%) of increase with respect to the baseline (diameter before injection).

Statistical Analysis

The experiment was of a completely randomized design. Calves were stratified by gender and randomly assigned to one of the four MR on the day of birth. Nearly all dependent variables were measured repeatedly and analyzed using the PROC GLIMMIX procedure of SAS (Release 9.2) using the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + Cl_{(ij)} + W_l + (\alpha W)_{il} + (\beta W)_{jl} + (\alpha\beta W)_{ijl} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the observation, μ is overall mean, α_i is the fixed effect of MR (T1, T2, T3, and T4); β_j is the fixed effect of gender (male and female); $(\alpha\beta)_{ij}$ is the interaction of MR and gender; $Cl_{(ij)}$ is the random effect of calf nested within MR and gender ($l = 1, 2, \dots, n$); W_l is the fixed effect of age ($l = d$ or wk of age); $(\alpha W)_{il}$ is the interaction of MR and age; $(\beta W)_{jl}$ is the interaction of gender and age; $(\alpha\beta W)_{ijl}$ is the interaction of MR, gender, and age, and ε_{ijkl} is the residual error.

Repeated measures data were tested to determine the structure of best fit, namely compound symmetry, compound symmetry heterogeneous, autoregressive-1, and autoregressive-1 heterogeneous as indicated by a Schwartz Bayesian information criteria value closest to zero (Littell et al., 1996). If repeated measures were taken on unequally spaced intervals, the sp(pow) covariance structure was used. For non repeated measures, the same model was used after removing the age effect and their interactions. All variables were tested for normality of residuals using the Shapiro-Wilk test of SAS version 9.2 (SAS Inst. Inc., Cary, NC). Non-normally distributed data were transformed as suggested using the guided data analysis of SAS and back transformed using the link and ilink function of PROC GLIMMIX procedure. Different temporal responses to treatments were further examined using the SLICE option of the GLIMMIX procedure.

Coefficients for testing of orthogonal contrasts when using unequally spaced quantitative treatments were generated using PROC IML of SAS. Orthogonal contrasts performed were the following: 1) linear effect of treatment, 2) quadratic effect of treatment, 3) cubic effect of treatment, 4) gender effect, 5) interaction of contrasts 1 and 4, 6) interaction of contrasts 2 and 4, and 7) interaction of contrasts 3 and 4. If a 3-way interaction of time with the main effects of treatment and gender or interaction of age with gender had a $P > 0.25$ (Bancroft, 1968), the interactions were dropped from the model and the model was rerun.

Binary data were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). The models included the effects of treatment and gender of calf. Adjusted odds ratio and the 95% confidence interval (CI) were calculated. Birth weight and height deviations within each gender were covariates for analysis of BW gain and growth, respectively. First day measure of plasma metabolites was used as covariate for the same metabolites. Finally, serum total IgG concentration at 1 d of life was used as a covariate for health measures. Differences discussed in the text were significant at $P \leq 0.05$ and tended to be significant at $0.05 < P \leq 0.10$ unless another probability is indicated.

Results

A total of 103 calves were enrolled in the study ($n = 43$ males and 60 females), born from nulliparous ($n = 39$) and parous ($n = 64$) Holstein animals fed a low fat and low EFA diet during the last 8 wk of expected calving date. Six male and 2 female calves were removed from the study at an average of 13 d of age due to death from causes other than the treatments or unwillingness to drink the MR. A total of 95 calves completed the study, however 7 calves were removed (T1: 1, T2: 2, T3: 3, and T4: 1)

from the data set because they lost BW during the first 30 d of life regardless of severity of disease. Treatment effects on calf performance were not affected by including or excluding these 7 calves from the data set. Distribution of genders to treatments was as follows: T1: 7 males and 14 females, T2: 9 males and 13 females, T3: 9 males and 13 females, and T4: 9 males and 14 females.

Birth weight of calves assigned to treatments did not differ and averaged 38.9, 41.1, 39.3, and 40.2 kg for calves assigned to T1, T2, T3, and T4 respectively (Table 6-4), however males were heavier than females (42.0 vs. 37.7 kg, $P < 0.01$). The IgG concentration of colostrum fed to male calves decreased linearly as LA intake increased (95, 97, 77, and 79 g/L for T1, T2, T3, and T4, respectively) whereas IgG concentration of colostrum fed to female calves was unchanged across LA treatments (82, 72, 76, and 87 g/L for T1, T2, T3, and T4, respectively, gender by linear LA interaction, $P = 0.05$). Because target intake of colostrum was 4 L of colostrum, intake of IgG from colostrum followed the same pattern, namely (379, 382, 308, and 317 g for males and 330, 286, 304, and 346 g for females for T 1, 2, 3, and 4, respectively, gender by linear LA interaction, $P = 0.07$). Serum concentration of total IgG after consumption of colostrum did not differ among treatments or genders (mean of 2.14 g/dL). The AEA of colostral IgG was unchanged by LA intake in female calves (26.1, 26.3, 22.5, and 24.4% for T1, T2, T3, and T4, respectively), whereas males assigned to T1 had the lowest AEA (21.8, 26.3, 28.3, and 27.4% for T1, T2, T3, and T4, respectively, gender by quadratic LA interaction, $P = 0.04$). Two calves assigned to T2 and one calf assigned to T3 failed to attain APT after colostrum feeding (serum total IgG < 1 g/dL) which was corroborated by

their low (< 5 g/dL) STP concentration. Concentrations of serum total protein after colostrum consumption did not differ among treatments or genders (mean of 5.8 g/dL).

Measures of Growth and Feed Efficiency

Male calves assigned to T2 tended to consume more MR DM than males on other treatments due to less MR refusal whereas female calves consumed similar amounts of MR (gender by LA cubic interaction, $P = 0.09$, Table 6-5). As a result of greater intake of MR by male calves fed T2, BW gain ($P = 0.02$, Figure 6-1A), ADG ($P = 0.02$), and FE ($P = 0.04$) had quadratic patterns whereas female calves tended to linearly increase in, BW gain ($P = 0.07$), ADG ($P = 0.07$), and FE ($P = 0.10$) with increasing intake of LA (gender by linear LA interaction, Figures 6-1A and B). During the period when MR and grain were offered together (31 to 60 d of age), no effect of LA intake on BW gain or FE was detected. This lack of LA treatment effect held true for the total 60-d period.

During the first 30 d of life, wither height (cm) and wither growth rate (cm/d) of females tended to increase linearly with increasing intake of LA whereas wither height and growth rate of males did not differ among LA treatments (gender by linear LA interaction, $P = 0.09$, Table 6-6). During these same 30 d, hip height (cm) and hip growth rate of both genders tended to increase as intake of LA increased from 0.144 to 0.333 g/kg of BW^{0.75} before decreasing for calves fed T4 (quadratic effect, $P = 0.09$). For the following 30 d of life, this same quadratic pattern was detected for height of both withers ($P = 0.05$) and hips ($P = 0.04$) as well as growth rate of both withers ($P = 0.04$) and hips ($P = 0.04$, Table 6-6, Figure 6-2).

Metabolic and Hormonal Profile in Plasma

Concentrations of plasma glucose were greatest at the first d of age, exceeding 100 mg/dL, and decreased to between 80 and 90 mg/dL for 5 wk, then rose during the

last 3 wk (effect of age, $P < 0.01$, Figure 6-3A). Increased feeding of LA from T1 to T3 to female calves resulted in decreasing plasma concentrations of glucose before rebounding in female calves fed T4 (89.2, 87.6, 84.9, and 90.3 mg/dL) whereas that of male calves did not differ according to LA treatment (90.1, 87.8, 90.2, and 86.6 mg/dL, gender by quadratic LA interaction, $P = 0.02$, Table 6-7). Concentrations of PUN gradually increased during the first 30 d of age peaking between 10 and 11 mg/dL and then gradually decreased once grain intake commenced (effect of age, $P < 0.01$, Figure 6-3B). Mean concentrations of PUN of female calves tended to follow a quadratic response to LA feeding being lowest when fed T2 and greatest when fed T3 (8.0, 7.6, 8.5, and 7.9 mg/dL) whereas PUN concentrations of male calves were steady for T1, T2, and T3 until increasing when T4 was consumed (7.7, 7.7, 7.3, and 8.5 mg/dL, gender by quadratic LA interaction, $P = 0.07$, Table 6-7).

Plasma concentrations of BHBA were low the first 30 d of life (below 0.7 mg/dL) before gradually increasing when grain mix intake commenced (effect of age, $P < 0.01$, Figure 6-4A). Mean concentrations of plasma BHBA tended to decrease as intake of LA increased (T1 = 0.88, T2 = 0.80, T3 = 0.76, T4 = 0.76 mg/dL, linear effect of LA treatment, $P = 0.06$, Table 6-7) for both genders. Plasma concentrations of cholesterol increased with age starting with values around 40 mg/dL during the first 8 d of age and increasing gradually until grain was offered after which concentrations held steady (90 to 110 mg/dL, effect of age, $P < 0.01$, Figure 6-4B) till the study ended. Mean concentrations of plasma cholesterol increased quadratically as intake of LA increased (T1 = 77.3, T2 = 82.5, T3 = 89.2, T4 = 86.9 mg/dL, quadratic effect of LA treatment, $P = 0.04$, Table 6-7). Male calves, regardless of treatment, had greater mean concentrations

of BHBA (0.84 vs. 0.75 mg/dL, $P = 0.04$) and total cholesterol (87.3 vs. 80.7 mg/dL, $P = 0.03$, Table 6-7).

Mean concentrations of the anabolic hormones insulin and IGF-I did not differ due to LA treatment. Concentrations of plasma insulin were low at birth, increasing one d after feeding of colostrum, decreasing at 2 wk of age, and then increasing steadily thereafter (effect of age, $P < 0.01$, Figure 6-5A). On the other hand, IGF-I had its greatest concentration at birth, decreasing dramatically until d 15 of age when, similar to insulin, concentrations steadily increased thereafter (effect of age, $P < 0.01$, Figure 6-5B). Compared to female calves, male calves had greater mean concentrations of insulin (2.7 vs. 2.0 ng/mL, $P < 0.01$) and IGF-I (42.0 vs. 39.0 ng/mL, $P = 0.06$). Mean of STP concentrations were about 5.8 g/dL the first wk of life after colostrum feeding but decreased at ~15 d to 5.5 to 5.6 g/dL throughout the remainder of the study (age effect, $P < 0.01$, Figure 6-6). Treatment with LA did not affect STP concentration.

Incidence of Diarrhea and Other Diseases

Calves were generally responsive and without signs of diseases except for diarrhea. Mean scores for attitude and ocular discharge were 0.15 and 0.01 and were not affected by LA treatments (Table 6-8). Severity (greater mean attitude score) of poor attitude and diarrhea increased at the second wk of age (effect of age, $P < 0.01$, Figures 7A, B). Severity of diarrhea (lower mean fecal score) tended to decrease as intake of LA increased (0.70, 0.66, 0.66, and 0.60, linear effect of LA treatment, $P = 0.07$, Table 6-8). In addition, the number of days of age to first evidence of diarrhea (score ≥ 2) tended to increase linearly as the intake of LA increased (7.0, 7.3, 7.5, and 7.6 d, linear effect of LA treatment, $P = 0.10$, Table 6-8). Mean score of nasal discharge tended to be greater in calves fed T3 and lowest when calves were fed T1 or T2 (quadratic effect of LA

treatment, $P = 0.10$). Rectal temperature was lowest at the first day of age (mean of 38.3°C) and gradually increased until peaking around d 8 (39.0°C , effect of age, $P = 0.01$, Figure 6-8). Males had lower or tended to have lower mean scores for attitude (0.13 vs. 0.18, $P = 0.02$), fecal consistency (0.62 vs. 0.69, $P = 0.08$), and nasal discharge (0.03 vs. 0.06, $P = 0.09$), as well as lower mean rectal temperature during the first 14 d of age (38.9 vs. 38.8°C , $P = 0.02$).

When abnormal scores or days with fever were calculated as percentage of days of life (Table 6-8), no effect of treatment was detected except for percentage of days with nasal discharge (Table 6-8) which peaked for calves on T3 ($P = 0.04$). Treatment did not affect the risk of pneumonia (18.1% incidence), navel infection (4.5% incidence), bloody diarrhea (43.1% incidence), or fever (62.1% incidence). Gender also was not a risk factor for disease with the exception of fever. Female calves had a 2.9 fold increase ($P = 0.03$) in risk of developing fever compared to male calves apart from dietary treatment (Table 6-9).

Blood Cell Populations

Concentrations of red blood cells increased with age the first 30 d of life and then decreased at 42 d (effect of age, $P < 0.01$, Figure 6-9A). Mean concentration of red blood cells tended to decrease as intake of LA increased starting at T2 (T1 = 8.16, T2 = 8.71, T3 = 8.32, and T4 = $7.90 \times 10^3/\mu\text{L}$, linear effect of LA treatment, $P = 0.10$, Figure 6-9B). However hematocrit measures were not affected by LA treatments but by age in a similar pattern as to red blood cell concentrations (effect of age, $P < 0.01$, Figure 6-9B).

Concentrations of total white blood cells were greatest at 7 d (mean of $11.5 \times 10^3/\mu\text{L}$), falling to $< 9 \times 10^3/\mu\text{L}$ at 14 d of age before gradually increasing thereafter

(effect of age, $P < 0.01$, Figure 6-10). Mean concentration of total white blood cells increased in males consuming increasing amounts of LA between T1 and T3 before decreasing in males fed T4 (8.7, 9.2, 10.0, and $8.9 \times 10^3/\mu\text{L}$) whereas female calves demonstrated the opposite effect (10.4, 10.1, 9.4, and $11.0 \times 10^3/\mu\text{L}$, gender by quadratic LA interaction, $P = 0.04$, Table 6-10). These changes in white blood cells were primarily due to changes in neutrophils as treatment effects on neutrophils mimicked that effect on white blood cells (gender by quadratic LA interaction, $P = 0.04$, Table, 6-10). Concentrations of neutrophils accounted for about 42% of the total population of white blood cells (Table 6-10). Therefore as expected the pattern due to age mimicked that pattern for total white blood cell concentrations. Neutrophil concentrations were greatest at 7 d of age (effect of age, $P < 0.01$, Figure 6-11A).

Lymphocytes were ~50% of total white blood cells (Table 6-10) and their mean concentrations were not affected by treatment or gender. Calves at 7 d of age had lower concentrations of lymphocytes and concentrations increased gradually with age of the calf (effect of age, $P < 0.01$, Figure 6-11B). Similarly mean concentrations of blood monocytes (mean of $483/\mu\text{L}$), eosinophils (mean of $57/\mu\text{L}$) and platelets (mean of $497 \times 10^3/\mu\text{L}$) were not affected by LA treatment but by age (effect of age, $P < 0.01$, Table 6-10, Figures 6-12A, 6-12 B and 6-13B, respectively).

Mean concentration of blood basophils decreased by about 50% with increasing age (effect of age, $P < 0.01$, Table 6-10, Figure 6-13A). Males fed T3 or T4 had greater mean concentrations of basophils than males fed T2 (32, 26, 54, and $43/\mu\text{L}$) whereas LA treatment did not have an effect on basophils of female calves (34, 44, 32, and

43/ μ L, gender by cubic LA interaction, $P = 0.03$ Table 6-10). A similar response was detected for the proportion of basophils in total white blood cells ($P = 0.02$, Table 6-10).

Neutrophil Phagocytosis and Oxidative Burst

Proportion of blood neutrophils undergoing phagocytosis did not change with age (effect of age, $P = 0.12$, Figure 6-14A) but throughout the study, mean proportion of phagocytic neutrophils tended to be greater in calves fed T2 or T3, with proportions in calves fed T1 or T4 not differing from each other (T1 = 62.1, T2 = 66.6, T3 = 64.2, and T4 = 62.8 %, cubic effect of treatment, $P = 0.09$, Table 6-11). Proportion of neutrophils producing oxidative radicals did not differ due to treatment or age. Mean fluorescence intensity for phagocytic activity and production of oxidative radicals was not affected by treatment but by age, with greater proportions at 7 d of age (effect of age, $P < 0.01$, Figure 6-14B).

Concentration of Acute Phase Proteins

Age had a big impact ($P < 0.01$) on concentrations of both acute phase proteins evaluated. Plasma concentrations of ASP were greater the first wk of age, decreasing gradually to a nadir from 29 d of age (effect of age, $P < 0.01$, Figure 6-15A). Changes in plasma concentrations due to LA treatments were detected at different ages (age by treatment interaction, $P < 0.01$; d 9, 16, and 23, $P \leq 0.01$; d 30, 37, and 57, $P \leq 0.08$, Figure 6-15A), the differences among treatments were minimal.

Plasma concentrations of Hp followed the same pattern as that for fecal and attitude scores. Haptoglobin concentrations reached the highest values at 8 d of life, with calves fed T1 having the greatest concentration, and falling to nadir values from 15 d till the experiment ended (treatment by age interaction, $P = 0.02$, Figure 6-15B).

Females had greater mean concentrations of plasma ASP throughout the study (91.3

vs. 83.8 mg/L, $P = 0.02$, Table 6-12) and similarly, mean Hp concentrations tended to be greater in females compared to males (0.87 vs. 0.78 OD x 100, effect of gender, $P = 0.06$, Table 6-12).

Humoral and Cell Mediated Immune Responses

Plasma concentrations of anti-OVA IgG at 1 d of age were high which was unexpected considering that dams of the current study were not injected with OVA; however, they might have retained some circulating antibodies from injection of OVA in previous trials at the University of Florida's dairy research unit. Because of these high values prior to OVA injection, concentration of anti-OVA IgG at day 1 were used as a covariate for each calf. Calves, regardless of LA treatment, were not responsive to the first and second OVA injection but were responsive to the third injection (d 1 = 0.16, d 22 = 0.14, d 43 = 0.13, and d 57 = 0.27, effect of age, $P = 0.01$, Figure 6-16A, B). Males fed T2 and T3 were responsive to the second and third injections of OVA, hence had the greater mean anti-OVA IgG concentration throughout the study whereas females had similar responses throughout the study regardless of LA treatment (gender by quadratic LA interaction, $P = 0.04$).

Lymphocyte proliferation in whole blood after 48 h of stimulation with PHA and LPS differed due to calf age. Proliferation of stimulated lymphocytes characterized as an increase above proliferation of nonstimulated cells (stimulation index) was similar at 14 and 28 d of age but was greater at 42 d of age (effect of age, $P < 0.01$, Figure 6-17A). Stimulated blood lymphocytes proliferated 23 to 36 times greater than that of nonstimulated blood lymphocytes collected from calves at 14 and 28 d of age. Proliferation was greater at 42 d of age, ranging between a 28 and 48 fold increase. Proliferation of stimulated lymphocytes from calves fed T1 or T4 did not change much

from 14 to 28 to 42 d but proliferation was dramatically changed at 42 d compared to earlier time points when blood lymphocytes were stimulated from calves fed T2 or T3 (Figure 6-17A).

Lymphocytes from calves fed T2 demonstrated greater proliferation at all 3 measuring days of age as reflected in stimulation index means of 26.3, 39.5, 28.9, and 28.2 for T1, T2, T3, and T4, respectively (cubic effect of LA, $P = 0.01$, Table 6-12). If response is measured as number of lymphocytes proliferated per number of lymphocytes present in 1 μL of whole blood, proliferation increased with age, with the greatest increase occurring between 28 and 42 d (effect of age, $P < 0.01$, Figure 6-17B). Again, when averaged across days, lymphocytes from calves fed T2 proliferated to a greater degree than calves fed other LA treatments (3.0, 4.7, 3.5, and 3.8, cubic effect of LA, $P = 0.01$, Table 6-12) and this was most apparent at d 42 (Figure 6-17B). Mean lymphocyte proliferation was lower when T1 was compared to the other treatments as a group (3.31 vs. 4.33 counts per minute, $P = 0.04$).

Concentration of TNF- α in supernatant of whole blood stimulated with LPS and PHA decreased at 28 d of age (14 d = 416, 28 d = 294, and 42 d = 415 pg/mL, effect of age, $P < 0.01$, Figure 6-18A). Although LA treatments did not have an effect on mean concentrations of TNF- α , concentrations of TNF- α were greater numerically at 42 d of age of calves fed T3. Mean concentrations of IFN- γ were similar at 14 and 28 d but increased at 42 d of age (14 d = 227, 28 d = 268, and 42 d = 373 pg/mL, effect of age, $P < 0.01$, Figure 6-18B). Mean concentrations of IFN- γ produced by stimulated whole blood cells tended to be greater in male calves fed T3 (T1 = 260, T2 = 357, T3 = 411, and T4 = 209 pg/mL, Figure 6-19A) whereas females fed T2 had the greater IFN- γ

production compared to females fed the other LA diets (T1 = 256, T2 = 327, T3 = 227, and T4 = 265 pg/mL, gender by quadratic effect of LA interaction, $P = 0.09$, , Figure 6-19B, Table 6-12).

Not all calves were responsive to an intradermal injection of PHA, hence delayed type hypersensitivity (DTH) to PHA injection was evaluated using only the responsive calves (calves having an increase in skin fold thickness after PHA injection on any of the 3 measuring times post injection). The adjusted risk ratio analysis at 30 and 60 d of age indicated that neither of treatments 2, 3, or 4 differed from T1 (reference, $P > 0.40$) and averaged 91% (73/80) and 78% (68/78) at 30 and 60 d of age, respectively.

At 30 d of age, response at each hour of measurement decreased with h post injection ($P < 0.01$) with means of 15.2, 11.7, and 9.5% for 6, 24, and 48 h, respectively (Figure 6-20A). Mean skin fold thickness increased linearly with increasing intake of LA (7.7, 11.0, 14.4, and 15.6% for T1, T2, T3, and T4, respectively, linear effect of treatment, $P = 0.03$, Table 6-13). However this pattern differed when gender was considered. Extent of response of female calves peaked when fed T3 and T4 whereas that of male calves peaked when fed T2, T3, and T4 (gender by LA diet interaction, $P < 0.01$, Table 6-13). When PHA was injected at 60 d of age, skin fold change likewise decreased ($P < 0.01$) with hours after injection (10.8, 5.2, and 5.5%, for 6, 24, and 48 h, respectively, Figure 6-20B). However at 60 d of age, calves fed T3 tended to have the smallest mean skin fold change (8.2, 9.0, 5.8, and 10.0% for T1, T2, T3, and T4, respectively, quadratic effect of LA treatment, $P = 0.09$, Table 6-13).

Discussion

Serum total IgG or STP concentrations are used as estimators of APT. The use of STP concentration after colostrum feeding is preferred by commercial farms because it

is a cheaper and faster tool to estimate APT than serum IgG. In the current study those calves identified as failing to achieve APT using the minimum serum concentrations of IgG also were so identified by failing to achieve the minimum STP concentration. A positive correlation of serum IgG and STP at 24 h of colostrum was detected in this study ($r = 0.74$, $P < 0.01$, data not shown), which agrees with results of others (Colloway et al., 2002; Campbell et al., 2007).

Dairy calves reared by commercial farms usually are fed milk or MR at fixed amounts per calf. Some farms use a step-down method which consist in gradually reducing the liquid feed offered in order to encourage intake of grain mix generally after the first 4 wk of age, with grain mix offered free choice starting the first day of life. In the current study the MR (29.7% CP, 18.7% fat) was fed in increasing amounts weekly as a proportion of each calfs' $BW^{0.75}$ during the whole preweaning period and intake of grain mix was delayed until 31 d of age. Accordingly, it was expectable that calves would not perform similarly to commercial calves.

Consequently, calf performance in the first 30 d of life was poor with ADG averaging 111 g/d and FE at 175 g of gain/kg of DMI. This first 30-d period was the only period in which LA intake affected gain, namely, males fed T2 having a better ADG (176 vs. 93 g/d) and FE (268 vs. 146 g of gain/kg of DMI) than males fed the other LA diets (gender by cubic LA diet interaction). This positive response of male calves fed T2 was not replicated, even numerically, in the second 30 d of life. Greenberg et al. (1950) and Pudelnkewicz et al. (1968) concluded that male rats have a greater requirement for LA than female rats when using BW gain, skin lesions, and accumulation of tetraene FA as measures of response to LA supplementation. The National Research Council (1995)

recommends a minimum intake of LA in rat diets (0.5% of ME as energy from LA for females and 1.3% of ME as energy from LA for males) based on results from previous studies (Greenberg et al., 1950; Pudelkewicz et al., 1968). In the current study it is difficult to explain why T2 stimulated BW gain and feeding whereas T3 and T4 had similar gains to calves fed T1. Slightly more MR was consumed by male calves fed T2 but the 17 to 23 g/d increase in MR intake would not account for nearly doubling the BW gain for this treatment group.

Body weight gain and FE in the first 30 d by female calves tended to increase linearly with increasing LA intake (2.6, 3.1, 3.3, and 3.4 kg for T1, T2, T3, and T4, respectively) as did FE (0.15, 0.17, 0.18, and 0.19 g of gain/kg of DMI). In a previous study (Chapter 4) in which 2 intakes of LA (0.149 or 0.487 g/kg of $BW^{0.75}$) in MR were tested, calves fed the greater amount of LA, regardless of gender, had better ADG and FE. Hence, a LA feeding rate of 0.149 g/kg of MBW was deficient. The current lower feeding rate of 0.144 g of LA per kg of $BW^{0.75}$ is below that recommended for growing female rats of (0.212 g of LA per kg of $BW^{0.75}$) by 33%. It may be that the LA requirement for female Holstein calves is at least 0.206 g of LA per kg of $BW^{0.75}$ (T2) which equals 3.0 g of LA/d for a 35-kg calf. If a 20% fat MR is fed at 454 g of DM daily, LA concentration is 0.66% of DM or 3.3% of fat. A 100% tallow-based MR containing 3.8% LA would supply 3.5 g of LA per day and meet the proposed LA requirement. However if the LA requirement is closer to that supplied by T3, (0.333 g of LA per kg of MBW), a 20% fat MR fed at 454 g of DM/d to a 35-kg calf would need to supply 4.8 g of LA/d. This would require the MR to contain 1% LA (DM basis) or 5.3% LA (fat basis)

and the fat source would need to be a mix of approximately 85% tallow and 15% porcine lard (13.9% LA).

All calves in the current study suffered from diarrhea starting at a mean of 7 d of age in calves fed T1, with the onset tending to be linearly delayed slightly with increasing intake of LA. Episodes of disease in preweaned calves are the main drivers of reduced performance. Morrison et al. (2009) fed 900 g/d of MR DM (27% CP, 17% fat) to Holstein female calves and reported an ADG of 320 g during the first 28 d of life but calves also were fed free choice a commercial grain mix. Authors did not report incidence of diseases in these calves. On the other hand, Jenkins et al. (1985) fed a 24% CP, 20% fat MR (DM basis) as the only feed fed the first 4 wk of age to male calves using tallow, CCO, or corn oil (CO) as sources of MR fat. Intake of DM from MR averaged 800 g/d. Calves fed CO had the poorest ADG (392 g/d) which was associated with “severe scours” whereas calves fed tallow or CCO had ADG of 533 and 519 g, respectively. In a later study in which only MR was fed from 3 to 31 d of life, Jenkins et al. (1986) fed male calves a MR (24% CP, 20% fat, DM basis) with tallow, canola oil, or reclaimed restaurant cooking fat as fat sources. Mean DMI of MR was 823 g/d and mean ADG was 570 g and diarrhea was not detected, when half the tallow was replaced with CO, severe scours was observed and ADG decreased to 310 g. Jenkins and Kramer (1986) fed MR containing one of 4 sources of fat, namely 100% CCO, 95% CCO + 5% CO, 92.5% CCO + 7.5% canola oil, and 100% tallow to male calves for 42 d without grain mix. Mean DMI was 979 g/d and ADG of 660 g. Mean intake of LA was 1, 27, 16, and 51 g/d. Authors reported that “there were no problems with diarrhea” and “none showed any of the EFA deficiency signs that occur in nonruminants.”.

Certainly performance of calves in the current study was inferior to that of calves in aforementioned studies but major differences exist between these studies. Calves in the 4 aforementioned studies consumed much more DM (875 vs. 618 g) and were housed in a warm, insulated building vs. outside during the winter season. Differences in diarrhea severity may have occurred in the previous studies but this was not tested. Mean fecal scores of calves in the current study peaked at 2 (mild diarrhea) during wk 2 of life whereas previous authors often indicated that diarrhea was not a problem in their studies. Nevertheless, ADG of calves in the current study in the first 30 d was 90 g apart from male calves fed T2 whereas ADG of all calves in chapter 4 managed in a similar fashion was 288 g. Fat density of MR used in chapter 4 was a bit greater (19.6 vs. 18.7%) but intake of DM from MR was actually greater in the current study (618 vs. 512 g/d). It may have been that the fat in the MR in the current study was not emulsified properly leading to reduced digestibility of MR fat even though a proven emulsifier was used at the correct amount and mixing was extensive. If fat digestibility was reduced in the current study, it was not reflected by greater incidence of diarrhea. Incidence and severity of diarrhea were similar between the two studies.

Jenkins (1988) repeated a previous study from 1985 using CCO or CO as sources of fat. Even though the exact same diets were fed, calves fed CO had appreciably less diarrhea than in their previous study. The only difference between those studies was the fat dispersion method, low pressure dispersion in first study and homogenization in the second study, with the latter producing smaller sized fat globules (< 1 μm vs. 10 to 20 μm). In the current study a commercial emulsifier was used and the solution was vigorously stirred using an electric drill. The size of fat globules was not measured.

However globule size in this study might be lower than 10 μm based on the findings of Jenkins et al. (1985) and Jenkins (1988) who reported that fat globules greater than 10 μm resulted in increased incidence of diarrhea when feeding CO. In contrast in the current study inclusion of SO decreased the severity of diarrhea. Therefore, the size of fat globule in the MR of the current study should not be a big risk factor for poor fat digestibility considering that the normal size of fat globules in raw milk ranges from 0.15 to 15 μm (Michalski et al., 2006).

In the current study, SO replaced up to 24% of CCO, however, mean fecal score was actually reduced linearly as intake of LA increased. The main cause of diarrhea in calves of the current study was likely of environmental (infection) rather than nutritional (size of fat globule) origin, because another study conducted at the same location (Perdomo, 2011) also reported a 100% incidence of diarrhea by experimental calves fed pasteurized milk. In that study, ADG for the first 28 d of age was 350 g but these calves were fed 1 kg of milk DM of high nutrient density (28.5% CP, 26.8% fat, DM basis) and were offered a commercial grain mix in ad libitum amounts. Calves of the current study were fed 618 g of MR DM (29.7% CP, 18.7% fat, DM basis) as the only feed for the first 30 d.

Body weight gain of female calves between 31 and 60 d of age (630 g/d) was somewhat typical of that of commercial dairy farms. Soberon et al. (2012), aiming to evaluate the effect of ADG during the preweaned period on future milk production, evaluated heifer growth on 2 farms. The Cornell University farm with a population of 1244 heifers reported an ADG of 820 g/d (range of 100 to 1580 g/d), whereas that from a commercial farm was 660 g/d (range of 320 to 1270 g/d) for 623 heifers. Heifers at

both farms were fed commercial MR (28% CP and 15 to 20% fat) at a rate of ~900 g/d (DM basis) and were offered a commercial grain mix in ad libitum amounts. No effect of treatment was detected for ADG or FE for the second 30 d of life and the whole 60-d period. In contrast, hip and wither growth for the overall preweaning period was better for calves fed T2 and T3. Deficiency of LA led to impaired growth of rats as reported by (Burr and Burr, 1929, 1930).

Plasma concentrations of metabolites and hormones in the current study were estimated in the postprandial period because calves always were bled within 1 to 2 h after their morning feeding. Also important to remember is that the gross nutritional composition of the MR in terms of concentrations of protein, fat, lactose, minerals, and vitamins was the same for all LA treatments. Differences in plasma concentrations of anabolic metabolites and hormones normally are seen when groups of calves experience different growth rates. Smith et al. (2002) fed preweaned calves with increased amount of nutrients resulting in enhanced ADG and FE with parallel increased concentrations of insulin, glucose, and IGF-I but a reduction in PUN concentrations. Similarly, Quigley et al. (2006) reported increased concentrations of glucose and IGF-I when calves were fed increased amounts of MR which was reflected in a greater BW gain and FE. Better ADG and FE of calves fed greater intake of LA in chapter 4, also resulted in increased plasma concentrations of glucose and IGF-I but reduced PUN, and even though calves fed low or high amounts of LA were fed diets of similar nutrient density. In the current study a lack of effect of LA treatment in ADG and FE was accompanied by a lack of difference in all aforementioned metabolites and hormones.

Linoleic acid is known to have a potent effect activating the peroxisome proliferator activator receptor- α (PPAR- α) in liver and hence enhancing β -oxidation and regulating cholesterol synthesis in many species (Forman et al., 1997; Li and Chiang, 2009). Lower plasma concentrations of BHBA, an intermediate product of β -oxidation, with increasing intake of LA might indicate that the amount of LA evaluated in the present study did promote complete β -oxidation, leading to a complete oxidation of FA. More likely, calves with lower intakes of LA (thus greater intake of CCO) had greater intakes of medium chain FA (C10 and C12) which resulted in calves with greater concentrations of BHBA. Results from the current study are in agreement with the findings from Sato (1994) who fed medium chain FA (C8 and C10) to neonatal calves and caused a marked hyperketonemia a few hours after feeding. It was thought that this was due to preferential transport of these FA through the portal vein and greater availability for oxidation and synthesis of ketogenic products. Likewise, in the previous study (Chapter 4), calves fed greater amounts of CCO and lower amounts of porcine lard had increased plasma concentrations of BHBA.

Medium chain SFA such as C12:0, C14:0, and C16:0 have been identified as the most potent inducers of cholesterolemia in laboratory animals (Fernandez and West, 2005). In a previous study (Chapter 4), calves fed a MR with a greater proportion of CCO had greater plasma concentrations of cholesterol. In contrast, feeding polyunsaturated FA (PUFA) to rats resulted in reduced concentrations of circulating plasmatic cholesterol compared to rats fed CCO (Berr et al., 1993; Chechi and Chema, 2006). Authors agreed that increased concentrations of cholesterol in plasma were related to greater concentrations of LDL -cholesterol and vice versa. A review article by

Fernandez and West (2005) proposed different mechanisms by which n-6 FA might lower plasma cholesterol. First n-6 FA may upregulate LDL receptors and secondly, they may increase the activity of cytochrome P450 7A, hence increasing the synthesis of bile acid as a means to remove cholesterol from circulation. Strong evidence exists for diets rich in saturated FA to induce an increase in plasmatic cholesterol, with diets rich in n-6 FA doing the opposite. Findings in this current study contradict the general acceptance of n-6 FA as reducers of cholesterol in plasma. At this point, we cannot offer a potential reason why may this have occurred.

Plasma concentration of red blood cells was measured only at 4 d of age during the experimental period, and calves fed T2 had the greatest mean concentration at the time. However, hematocrit concentration was measured once a week and the values did not differ due to LA treatment. Increased concentration of red blood cells usually is related to calf dehydration, often caused by increased incidence or severity of diarrhea whereas a reduced concentration of red blood cells is associated with anemic conditions (Moonsie-Shageer and Mowat, 1993). Severity of diarrhea (using mean fecal scores) decreased linearly with intake of LA, hence greater red blood cells in calves in T2 could not be due to dehydration, otherwise calves in T1 should have had greater concentrations of red blood cells or hematocrit. Mean values of hematocrit were within normal ranges for preweaned calves (Brun-Hansen et al., 2006).

If these calves were experiencing nutritional stress based upon low BW gain the first 30 d of life, increased feeding of LA may not have been able to optimize gain but may have been able to influence immune responses. Concentrations of white blood cells were greater at 7 d of age falling thereafter and a similar pattern was observed for

blood neutrophil concentrations. In current study we did not analyze the expression of receptors on neutrophil surfaces. It is known that although neutrophil receptors (CD18, CD62L) are expressed constitutively, their expression could be downregulated by immunosuppression. Therefore, the number of receptors expressed per neutrophil could be reduced, this was found in cows after parturition and in calves abruptly weaned (Weber et al. 2001; Lynch et al., 2010). Fewer receptors expressed per neutrophil was associated with neutrophilia, possibly indicating the inability of neutrophils to migrate to the infection zone, hence increasing the risk of infections (Weber et al. 2001). If it is assumed that the lower mean concentration of neutrophils detected in female calves fed T2 or T3 was due to increased migration from the blood to sites of inflammation, it would indicate that these calves were better able to mount an attack against infection; however a decreased production of neutrophils in bone marrow could not be ruled out. Regardless of gender, a greater proportion of blood neutrophils from calves fed T2 or T3 performed phagocytosis and produced oxidative radicals, which indicates a more efficient activity of neutrophils in these calves. If these neutrophils were in lower concentrations due to increased migration to the sites of inflammation and had improved immune activity, calves would be more efficient to respond to inflammatory processes to resist pathogen invasions. Studies evaluating the effect of different stressors on neutrophil phagocytic activity of calves have reported variable results (Pang et al., 2009; Hulbert et al., 2011).

Concentrations of Hp are absent in healthy calves but elevated under subclinical inflammatory disorders (Ganheim et al., 2007; Cray et al. 2009). Experimental models of respiratory and digestive tract infection in calves reported increased plasma

concentrations of Hp in sick calves as compared to healthy ones (Deignan et al. 2000; Heegaard et al., 2000; da Silva et al. 2011). Concentrations of Hp peaked at 8 d of age when episodes of diarrhea were greatest. Calves fed T1 had greater plasma concentration of Hp at this point time. However fecal score at this time did not differ among LA treatments so calves fed T1 had a greater immune reaction to inflammation of the small intestine suggesting the feeding more LA reduced the inflammatory response as compared to the feeding of saturated FA. Acid soluble protein is identified as having dual inflammatory and immunomodulatory properties. One of the mechanisms by which ASP can exert its antiinflammatory effect is by inhibiting the proliferation of blood lymphocytes after mitogen stimulation (Hochepped et al., 2003). At d 15, calves fed T3 had lower concentrations of ASP which matched with the lower in vitro proliferation of lymphocytes for T3 calves collected at d 14.

Selective proliferation of T cells after 48-h in vitro stimulation with LPS + PHA was greater in calves fed T2 and this held true at every time of measure whereas calves fed T3 responded well only at 42 d of age. Some human studies however failed to detect an effect of LA on cell proliferation but this was due likely to the short duration of the studies and or to minimal or no change in the profile of FA in blood cells which may have prevented LA from having an opportunity to exert an effect on cell proliferation (Kelley et al., 1989, 1992; Yaqoob et al., 2000). In contrast, Thanasak et al. (2005) cultured bovine PBMC with 2 doses (125 or 250 μ M) of LA or ALA and reported that the higher concentration of LA inhibited proliferative response of PBMC to mitogens. Later Gorjao et al. (2007) evaluated the proliferative response of human lymphocytes to IL-2 stimulation and reported that lower concentrations of LA stimulated proliferation of

lymphocytes preventing apoptosis and necrosis (< 75 uM) but that greater concentrations of LA reduced the proliferation of lymphocytes with respect to the control media. Based upon the results of Gorjao et al. (2007), it can be hypothesized that none of the current treatments had toxic effects so as to induce apoptosis and necrosis of lymphocytes which would have prevented their proliferation because all LA treatments stimulated lymphocytes equal to or than that of T1.

Another reason why LA intakes greater than that of T2 would not have toxic effects on immune cells that could prevent their proliferation was that the production of IFN- γ was increased by stimulated cells especially from males fed T2 and T3 and from females fed T2, whereas T4 and T3 and T4 from and males and females, respectively did not differ from that of calves fed T1. These results contrast with those of Wallace et al. (2001) who fed mice diets, of low fat or high fat supplemented with CCO (2.3% of LA), safflower oil (SAO, 61% of LA) or FO (9% of LA). The FA profile of the phospholipids in spleen lymphocytes reflected the dietary FA but IFN- γ production was decreased when mice were fed SAO or FO. The current study, however agrees with the previous study (Chapter 4) where stimulated PBMC of calves fed greater amounts of LA (between the amount offered with T3 and T4) produced more IFN- γ . One of the goals towards “maturity” of the neonatal calf’s immunity is the early switch from a preferential T helper-2 (Th2) response towards a Th1 response. The pattern of cytokine production is used to verify the predominant type of Th response. An increased concentration of IFN- γ with constant or decreased production of IL-4 is indicative of Th1 predominance (Chase et al., 2008). Greater mean production of IFN- γ by males fed T2 and T3 or

females fed T2 might indicate an improved ability of these calves to switch to the Th1 response.

Interferon- γ is a cytokine with a variety of roles such as enhancement of antigen presentation, cell cycle growth and apoptosis, leukocyte trafficking, and B cell depletion (Arens et al., 2001; Chen and Liu, 2009). Hence, it should be expected that as long as IFN- γ increases, activity of B cells to produce Ig will be reduced. Unexpectedly, production of anti-OVA IgG was greater by male calves when fed T2 or T3, which also matched with an increased production of IFN- γ and TNF- γ (the latter just numerically). Foote et al. (2007) fed increased amounts of nutrients to preweaned calves and reported better growth but production of TNF- γ by stimulated blood cells and production of anti-OVA IgG were not affected by treatment. The finding of Foote et al. (2007) could be interpreted as that the activation or inactivation of a cell type response (T cells producing IFN- γ) would induce the opposite humoral response (greater production of IgG). Hence, it can be concluded that male calves fed T2 and T3 had an overall better function of T and B cells, whereas female calves only had improved T cell function.

Delayed type hypersensitivity tests the ability of mononuclear immune cells to infiltrate and/or accumulate into regions of antigen deposition. It is strictly a cell-mediated response and not an antibody-mediated response (Berhagen et al. 1996). The DTH skin test produces a characteristic response which includes induration, swelling, and monocytic infiltration into the site of the lesion within 24 to 72 h (Black, 1999). Use of antigen rather than mitogens is the best approach to evaluate DTH responses; however, the use of antigens has been reported to cross-react with *Mycobacterium tuberculosis* leading to false positives (Hernandez et al., 2005). On the other hand,

mitogens such as PHA, without being a strong inducer of DTH, has been demonstrated to induce a moderate response after an intradermal injection in calves (Stanton et al., 2000; Ballou and DePeters, 2008) and in cows (Hernandez et al., 2005; Caroprese et al., 2009).

Previous research suggests that increasing LA intake would increase the proliferation of lymphocytes up to a point beyond which further increases in LA would suppress lymphocyte proliferation. However this was not documented in the current study. Ballou and DePeters (2008) hypothesized that a positive correlation exists between in vitro lymphocyte proliferation and a DTH response of cells to explain their results. However Hernandez et al. (2005) reported that the main cells infiltrating into the skin of cows challenged with PHA were eosinophils, macrophages, and neutrophils but not lymphocytes, whereas in the skin of sheep intradermally challenged with avidin, the major infiltrating cells were CD4+, CD8+, $\gamma\delta$ T-cells, neutrophils, macrophages, and CD45R⁺ B-cells (Lofthouse et al., 1995). The current findings at 30 d of age indicate that skin thickness responded linearly to PHA injection with increasing intake of LA (Table 6-13) but without a concomitant increase in lymphocyte proliferation (Figure 6-17). Therefore current results may support the work of Hernandez et al. (2005).

Measures of DTH at both 30- and 60-d measures were affected by time, with the greater response at 6h after PHA injection at both measurement times. This differential response due to time after injection is in agreement to Staton et al. (2000) and Hernandez et al. (2005) who reported that the largest responses to a PHA challenge were seen at 8 and 6 h post injection respectively. Hernandez et al. (2005) concluded that PHA is not a viable alternative to determine true DTH. Responses to PHA injection

in current study were variable; this might be due to other factors that could influence the response of calves, with the exception of gender which did not affect the results in this study. Moreover the lack of ability of PHA to maintain a true DTH (greater skin fold changes at 24 or 48 h) in the current study might indicate that other alternative mitogens or true antigens should be used to test DTH response in dairy calves.

Summary

Intake of LA was progressively adjusted by partially replacing hydrogenated CCO with SO in MR. Male calves fed LA at 0.206 g/kg of BW^{0.75} had better ADG and FE during the first 30 d of age and this was accompanied by a tendency for greater intake of MR. However ADG returned to baseline when male calves were fed LA at the greater rates of 0.333 and 0.586 g/kg of BW^{0.75}. Female calves tended to improve ADG and FE with increasing intake of LA in the first 30 d of life. However these responses to increasing LA intake after initiation of grain feeding at 31 d of life. On the other hand, wither and hip growth was greater by calves consuming LA at or exceeding 0.206 g/kg of BW^{0.75} during the 60-d study. These changes in gain and growth were not accompanied by increases in circulation concentrations of glucose, insulin or IGF-1.

Circulating concentrations of white blood cells, neutrophils, and monocytes were generally greater in female compared to male calves but gender effects on white blood cells and neutrophils were modified by LA intake. Similarly, some of the measured immune markers differed with gender and intake of LA. Males fed LA at 0.206 and/or 0.333 g/kg of BW^{0.75} had increased production of anti-OVA IgG, production of IFN- γ by stimulated blood cells, and DTH in response to an intradermal injection of PHA at 30 d. Female calves fed LA at 0.206 g/kg of BW^{0.75} had increased production of IFN- γ by stimulated blood cells and DTH in response to an intradermal injection of PHA at 30 d

were increased in females fed 0.333 or 0.586g/kg of $BW^{0.75}$. Regardless the gender calves fed 0.206 and/or 0.333 g/kg of $BW^{0.75}$ had greater phagocytosis activity by blood neutrophils, proliferation of stimulated whole blood cells, and DTH in response to an intradermal injection of PHA at 60 d.

Diarrhea affected all calves. Mean score of feces and age at first outbreak of diarrhea decreased and increased linearly respectively with increasing intake of LA. Plasma concentrations of haptoglobin were lower in calves fed LA at or > 0.206 g/kg of $BW^{0.75}$ at 8 d of age when diarrhea was most evident. Risk of diseases (pneumonia, naval infection, bloody diarrhea, or fever) was not reduced by increased feeding of LA.

Feeding T2 or T3 diets to preweaned Holstein calves increased responses for most of the markers of immunity evaluated in this study and improved wither and hip growth and feces and attitude scores. Hence under the conditions of the present study, intakes of LA of between 0.206 and 0.333 g/kg of $BW^{0.75}$ promoted better productive performance possibly by improving the immune status of calves. Future research should seek to clarify the mechanisms by which increased intake of LA might differentially modify the response of healthy and unhealthy female and male calves.

Table 6-1. Ingredient and chemical composition of diet fed to nonlactating, pregnant Holstein animals.

Prepartum diet	
Ingredient, % of DM	
Bermudagrass silage	46.50
Corn silage	8.80
Citrus pulp	31.70
Soybean meal	9.20
Mineral mix ¹	3.80
Nutrients, DM basis	
Crude protein, %	13.80
NE _L ² , Mcal/kg	1.46
NDF, %	39.85
Ether extract, %	3.20
Ash, %	8.12
Ca, %	1.26
P, %	0.34
Mg, %	0.40
K, %	1.52
S, %	0.34
Na, %	0.18
Cl, %	0.87
Mn, mg/kg	64.00
Zn, mg/kg	59.00
Cu, mg/kg	20.00
Fe, mg/kg	212.00
Mo, mg/kg	0.62
DCAD, mg/100 g	20.07

¹ Contains (DM basis) 34.5% corn meal, 5.0% dicalcium phosphate, 16.0% calcium carbonate, 10% calcium sulfate, 5% magnesium oxide, 10% magnesium sulfate, 4% sodium chloride, 1.7% Zinpro 4-plex (Zinpro, Minneapolis, MN), 0.4% Rumensin 80 (Elanco Animal Health, IN), 0.35% Sel-Plex 2000 (Alltech Biotechnology, Nicholasville, KY), 0.002% Ca iodate, and a vitamin premix. Each kg contains 24.5% CP, 9.8% Ca, 1.5% P, 4.2% Mg, 3.2% S, 1.7% Na, 10.7% Cl, 475 mg of Zn, 160 mg of Cu, 456 mg of Mn, 7.4 mg of Se, 37.4 mg of Co, 13.2 mg of I, 118,000 IU of vitamin A, 27,500 IU of vitamin D, 2,600 IU of vitamin E, and 770 mg of monensin.

² Calculated from the estimation of energetic values of individual ingredients using the NRC software (2001) and considering intake at 3X of maintenance.

Table 6-2. Fatty acid (FA) profile of sources if fat, emulsifier and basal milk replacer

FA	Coconut oil ¹	Soybean oil ²	Emulsifier ³	Milk replacer ⁴
C6:0	0.6	ND ⁵	ND	0.3
C8:0	7.8	ND	ND	2.0
C10:0	6.2	ND	ND	4.2
C12:0	50.0	ND	ND	30.6
C14:0	18.1	0.1	0.1	14.9
C16:0	8.4	11.4	11.1	17.6
C16:1	ND	0.1	ND	0.7
C18:0	8.7	4.0	87.9	7.6
C18:1	0.1	20.5	0.1	15.2
C18:2	0.0	55.3	0.1	5.9
C18:3 α	0.0	8.1	0.0	0.1
C20:0	0.1	0.3	0.5	ND
Other FA	0.0	0.2	0.2	0.9

¹ Welch, Holme & Clark Co., Inc, Newark, NJ

² Winn Dixie Co..

³ Grindsted® mono-di HV 52, Gillco Ingredients, San Marcos, CA.

⁴ Prepared by Land O'lakes®. Contains whey products, dried skimmed milk, dried milk protein, coconut oil, vitamins, and minerals. Each kg contains 1.03% Ca, 0.80% P, 0.11 mg of Co, 10.9 mg of Cu, 1.1 mg of I, 110 mg of Fe, 49,560 IU of vitamin A, 12,400 IU of vitamin D, and 241 IU of vitamin E.

⁵ Non detected

Table 6-3. Ingredient and chemical composition of milk replacers and grain mix fed to preweaned Holstein calves.

	Milk replacer	Grain mix
Ingredients, % of DM		
31:7 milk replacer ¹	89.80	-
Oil combination ²	9.68	-
Emulsifier ³	0.48	-
Steam rolled barley	-	51.7
Soybean meal	-	16.5
Beet pulp shreds	-	24.5
Sugarcane molasses	-	5.3
Mineral mix ⁴	-	2.0
Nutrients, DM basis		
Lactose, %	39.70	-
Crude protein, %	29.70	18.30
Ether extract, %	18.70	2.10
Ash, %	6.08	5.42
Ca, %	0.77	0.57
P, %	0.72	0.45
Mg, %	0.13	0.35
K, %	2.12	0.92
S, %	0.39	0.26
Na, %	0.76	0.16
Cl, %	1.27	0.32
Mn, mg/kg	49.50	55.00
Zn, mg/kg	53.00	57.00
Cu, mg/kg	11.70	16.00
Fe, mg/kg	132.00	362.00
Mo, mg/kg	0.60	1.40

¹ Prepared by Land O'lakes®. Contains whey products, dried skimmed milk, dried milk protein, coconut oil, vitamins, and minerals. Each kg contains 1.03% Ca, 0.80% P, 0.11 mg of Co, 10.9 mg of Cu, 1.1 mg of I, 110 mg of Fe, 49,560 IU of vitamin A, 12,400 IU of vitamin D, and 241 IU of vitamin E.

² Contains proportions of coconut oil:soybean oil according to treatments (T), T1 = 100:0, T2 = 95.99:4.01, T3 = 87.93:12.07, and T4 = 71.77:28.23.

³ Grindsted® mono-di HV 52, Gillco Ingredients, San Marcos, CA.

⁴ Each kg of DM contains 8.8% Ca, 4.2% P, 11.4% Mg, 12.4% Cl, 0.49% K, 8.1% Na, 0.36% S, 58 mg of Co, 263 mg of Cu, 26 mg of I, 1933 mg of Fe, 923 mg of Mn, 8.46 mg of Se, 1109 mg of Zn, 259,000 IU of vitamin A, 70,000 IU of vitamin D, and 2,400 IU of vitamin E.

Table 6-4. Passive immunity-related measures of newborn male (M) and female (F) Holstein calves assigned to treatments with increasing amounts of linoleic acid (LA)

Measure	Treatments ¹								SEM	Contrasts ² , P values						
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G	Cb*G
	M	F	M	F	M	F	M	F								
Total calves	7	14	9	13	9	13	9	14								
Colostrum fed																
Quantity, L	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	0.02	0.73	0.88	0.63	0.61	0.29	0.71	0.24
Total IgG, g/L	94.6	82.4	96.5	71.5	77.0	76.1	79.1	87.4	7.49	0.55	0.14	0.89	0.17	0.05	0.97	0.22
Total IgG intake, g	379	330	382	286	308	304	317	346	30.3	0.52	0.15	0.94	0.17	0.07	0.94	0.25
Birth																
Body weight, kg	41.1	36.8	43.4	38.8	40.3	38.2	43.2	37.2	1.57	0.76	1.00	0.13	<0.01	0.61	0.32	0.57
STP, g/dL	4.87	4.55	4.86	4.58	4.83	4.59	4.64	4.83	0.11	0.86	0.90	0.88	0.04	0.01	0.48	0.87
24 h after birth																
Serum total IgG, g/dL ³	1.99	2.30	2.26	2.01	2.06	1.91	2.24	2.34	0.21	0.46	0.24	0.67	1.00	0.97	0.26	0.36
AEA ⁴ , %	21.8	26.1	27.1	26.3	28.3	22.5	27.4	24.4	2.00	0.61	0.60	0.24	0.36	0.12	0.04	0.87
STP, g/dL	5.90	5.75	6.10	5.72	5.91	5.59	5.72	5.96	0.19	0.87	0.68	0.48	0.26	0.16	0.28	0.73
STP increase, g/dL	1.03	1.22	1.24	1.14	1.08	1.00	1.08	1.13	0.18	0.76	0.69	0.53	0.91	0.94	0.47	0.62

¹ Targeted intakes of linoleic acid from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² P values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

³ Four out of 88 calves had an IgG concentration < 10 g/dL, two fed treatment 2 and one fed treatment 3.

⁴ % AEA = [IgG concentration in serum at 24 h of life × (0.099 × body weight at birth)] / IgG intake × 100%.

Table 6-5. Dry matter intake (DMI), body weight (BW) gain, and feed efficiency (FE, kg of BW gain/kg of DMI) of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA)

Measure	Treatments ¹								SEM	Contrasts ² , P values						
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G	Cb*G
	M	F	M	F	M	F	M	F								
Birth to 30 d																
Birth weight ³ , kg	41.1	36.8	43.4	38.8	40.3	38.2	43.2	37.2	1.57	0.76	1.00	0.13	<0.01	0.61	0.32	0.57
BW gain, kg	2.75	2.62	5.26	3.09	2.80	3.30	2.70	3.44	0.54	0.66	0.44	0.01	0.49	0.07	0.98	0.02
ADG, kg/d	0.09	0.09	0.18	0.10	0.09	0.11	0.09	0.11	0.02	0.65	0.47	0.01	0.41	0.07	1.00	0.02
MR intake, kg DM	19.2	17.6	19.7	17.8	19.0	18.0	19.2	17.9	0.17	0.96	0.54	0.03	<0.01	0.17	0.27	0.09
LA intake, g	95	87	143	129	212	208	393	358	5.85	<0.01	0.76	0.21	<0.01	0.02	0.16	0.35
FE	0.14	0.15	0.27	0.17	0.15	0.18	0.15	0.19	0.03	0.84	0.44	0.01	0.93	0.10	0.93	0.04
31 to 60 d																
Weight at 30 d, kg	44.9	40.3	47.4	40.8	44.9	41.0	44.8	41.1	0.54	0.66	0.44	0.01	<0.01	0.07	0.98	0.02
BW gain, kg	23.5	19.2	21.3	18.9	23.7	19.1	24.1	19.3	1.39	0.51	0.84	0.34	<0.01	0.58	0.95	0.44
ADG, kg/d	0.78	0.64	0.71	0.63	0.79	0.64	0.80	0.64	0.05	0.51	0.83	0.34	<0.01	0.59	0.95	0.44
MR intake, kg DM	22.9	20.9	23.6	21.0	23.1	21.1	22.9	21.1	0.22	0.88	0.24	0.17	<0.01	0.17	0.67	0.13
Grain intake, kg DM	16.3	13.7	13.4	13.3	16.1	13.5	15.6	13.3	1.66	0.92	0.88	0.30	0.11	0.82	0.95	0.40
FE	0.58	0.56	0.57	0.55	0.60	0.55	0.63	0.56	0.02	0.14	0.86	0.54	<0.01	0.18	0.96	0.70
Birth to weaning																
BW at 60 d, kg	68.3	59.5	68.7	59.7	68.6	60.1	68.9	60.4	1.35	0.61	0.91	0.92	<0.01	0.88	0.95	0.90
Total BW gain, kg	26.2	21.9	26.6	22.0	26.5	22.4	26.8	22.7	1.35	0.61	0.92	0.92	<0.01	0.88	0.95	0.90
ADG, kg/d	0.44	0.36	0.44	0.37	0.44	0.37	0.45	0.38	0.02	0.64	0.94	0.88	<0.01	0.89	0.95	0.90
MR intake, kg DM	42.1	38.5	43.3	38.8	42.1	39.1	42.0	39.0	0.34	0.89	0.28	0.05	<0.01	0.12	0.77	0.07
Total DMI, kg	58.4	52.2	56.7	52.1	58.2	52.6	57.7	52.4	1.76	0.94	0.95	0.55	<0.01	0.93	0.90	0.66
FE	0.44	0.42	0.47	0.42	0.45	0.43	0.47	0.43	0.01	0.23	0.86	0.30	<0.01	0.93	0.99	0.45

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² P values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

³ Birth weight deviations from the mean birth weight within each gender were covariates for analysis of BW gains. Hence birth weight added to any later variable of gain will not give the expected body weight.

Table 6-6. Wither and hip height and growth of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA)

Measure	Treatments ¹								SEM	Contrasts ² , <i>P</i> values						
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G	Cb*G
	M	F	M	F	M	F	M	F								
Height, cm																
Day 0 withers	76.9	74.2	76.7	74.6	75.9	73.3	76.9	73.7	0.98	0.67	0.35	0.54	<0.01	0.66	0.83	0.74
Day 0 hip	81.1	78.5	81.2	79.5	80.3	78.1	81.5	78.2	1.03	0.83	0.56	0.37	<0.01	0.56	0.67	0.73
Day 30 withers	79.1	76.5	79.3	76.3	79.6	77.9	78.7	77.6	0.54	0.41	0.12	0.35	<0.01	0.09	0.83	0.45
Day 30 hip	83.2	81.6	84.3	80.9	84.3	82.5	83.4	82.2	0.53	0.44	0.09	0.58	<0.01	0.22	0.55	0.08
Day 60 withers	84.5	81.2	84.9	82.7	85.1	83.4	84.7	82.4	0.66	0.54	0.05	0.58	<0.01	0.56	0.27	0.69
Day 60 hip	89.8	85.9	90.2	87.7	90.3	88.1	89.3	87.1	0.69	0.97	0.04	0.48	<0.01	0.32	0.38	0.55
Growth, cm/d																
Wither, 1 st 30 d	0.08	0.09	0.09	0.08	0.10	0.13	0.07	0.12	0.02	0.38	0.13	0.39	0.13	0.09	0.85	0.41
Hip, 1 st 30 d	0.07	0.10	0.11	0.08	0.11	0.13	0.08	0.12	0.02	0.48	0.09	0.62	0.18	0.21	0.52	0.07
Wither, 2 nd 30 d	0.18	0.15	0.19	0.21	0.18	0.18	0.20	0.16	0.02	0.96	0.43	0.11	0.45	0.32	0.29	0.25
Hip, 2 nd 30 d	0.22	0.14	0.20	0.23	0.20	0.19	0.20	0.16	0.02	0.46	0.35	0.18	0.08	0.92	0.10	0.02
Wither, all period	0.13	0.12	0.14	0.15	0.14	0.16	0.13	0.14	0.01	0.55	0.04	0.63	0.71	0.48	0.29	0.61
Hip, all period	0.15	0.12	0.15	0.15	0.15	0.16	0.14	0.14	0.01	0.90	0.04	0.44	0.57	0.34	0.38	0.56

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² *P* values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

Table 6-7. Plasma concentrations of glucose, plasma urea nitrogen (PUN), B-hydroxybutyrate (BHBA), total cholesterol, insulin, and insulin like growth factor I (IGF-I) of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA). All interactions with age did not differ unless footnoted

Measure	Treatments ¹								SEM	Contrasts ² , <i>P</i> values						Age	
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G		Cb*G
	M	F	M	F	M	F	M	F									
Glucose, mg/dL	90.1	89.2	87.8	87.6	90.2	84.9	86.6	90.3	1.46	0.70	0.18	0.46	0.53	0.12	0.02	0.23	<0.01
PUN, mg/dL	7.69	8.02	7.74	7.64	7.32	8.49	8.47	7.86	0.35	0.24	0.70	0.62	0.41	0.27	0.07	0.15	<0.01
BHBA, mg/dL	0.92	0.84	0.81	0.78	0.81	0.71	0.82	0.69	0.06	0.06	0.17	0.58	0.04	0.35	0.96	0.64	<0.01
Total cholesterol, mg/dL	78.6	76.0	83.8	81.2	92.3	86.0	94.4	79.4	4.08	0.03	0.04	0.98	0.03	0.09	0.81	0.90	<0.01
Insulin, ng/mL	2.66	2.20	2.66	1.72	2.48	2.03	2.92	2.04	0.29	0.63	0.40	0.44	<0.01	0.75	0.89	0.26	<0.01
IGF-I ³ , g/mL	39.5	38.4	45.1	38.6	41.1	39.5	42.3	39.4	2.22	0.68	0.66	0.25	0.06	0.93	0.94	0.21	<0.01
STP ⁴ , g/dL	5.59	5.60	5.52	5.74	5.57	5.67	5.56	5.66	0.07	0.93	0.75	0.67	0.03	0.90	0.54	0.15	<0.01

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² *P* values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

³ Gender by age *P* = 0.10.

⁴ Serum total protein, gender by age, *P* < 0.01.

Table 6-8. Health scores and percentage of days with poor attitude, fever, diarrhea and nasal discharge of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA). All interactions with age did not differ unless footnoted

Measure	Treatments ¹								SEM	Contrasts ² , <i>P</i> values							Age
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G	Cb*G	
	M	F	M	F	M	F	M	F									
Scores ³																	
Feces	0.66	0.73	0.64	0.68	0.59	0.73	0.60	0.60	0.05	0.07	0.92	0.60	0.08	0.53	0.32	0.41	<0.01
Attitude	0.14	0.19	0.11	0.18	0.12	0.20	0.13	0.15	0.03	0.53	0.78	0.53	0.02	0.51	0.57	0.92	<0.01
Nasal discharge ⁴	0.02	0.03	0.02	0.06	0.04	0.09	0.04	0.05	0.02	0.44	0.10	0.83	0.09	0.77	0.30	0.64	0.01
Ocular discharge	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.86	0.81	0.89	0.49	0.58	0.82	0.46	0.06
Rectal temp, °C	38.8	38.9	38.8	38.9	38.8	38.9	38.8	38.9	0.05	0.36	0.93	0.22	0.02	0.99	0.56	0.68	<0.01
Days to diarrhea	7.21	6.82	7.18	7.49	7.68	7.38	7.75	7.52	0.35	0.10	0.39	0.72	0.54	0.85	0.86	0.30	-
Days with ⁵ , %																	
Poor attitude	12.9	16.0	9.6	15.1	12.0	16.4	10.9	13.3	2.54	0.53	0.81	0.36	0.04	0.72	0.69	0.71	-
Nasal discharge	2.05	3.02	1.54	5.37	4.99	8.25	3.19	4.34	1.85	0.51	0.04	0.54	0.08	0.79	0.46	0.60	-
Ocular discharge	1.44	1.29	0.55	2.19	1.30	2.07	1.48	1.41	0.90	0.91	0.74	0.86	0.39	0.71	0.51	0.41	-
Cough	0.01	0.35	0.18	0.27	0.00	0.91	0.37	0.46	0.30	0.38	0.52	0.78	0.10	0.84	0.25	0.30	-
Fever, first 14 d	4.86	8.54	5.69	5.12	3.93	6.61	4.06	7.05	2.30	0.74	0.61	0.75	0.18	0.81	0.78	0.36	-
Diarrhea	14.7	18.6	15.7	15.8	14.2	18.3	15.1	13.7	2.00	0.28	0.81	0.61	0.25	0.30	0.59	0.24	-
Severe diarrhea	3.92	5.10	5.64	4.54	3.69	5.49	4.31	3.33	0.97	0.29	0.61	0.57	0.75	0.49	0.48	0.12	-

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² *P* values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

³ Nasal score scale: 0 = normal serous discharge, 1 = small amount of unilateral cloudy discharge, 2 = bilateral cloudy or excessive mucus discharge, and 3 = copious bilateral mucopurulent discharge.

Attitude score scale: 0 = responsive, 1 = non-active, 2 = depressed, and 3 = moribund.

Feces score scale: 0 = firm feces, no diarrhea; 1 = soft feces, no diarrhea, 2 = mild diarrhea and 3 = watery diarrhea, severe diarrhea.

Ocular score scale: 0 = normal, 1 = small amount of ocular discharge, 2 = moderate amount of bilateral discharge, 3 = heavy ocular discharge.

Cough score scale: 0 = none, 1= induced single cough, 2 =: induced repeated cough or occasional spontaneous cough, 3 = repeated spontaneous cough.

⁴ Treatment by age, *P* = 0.08

⁵ Percentage of days with health issue over a total of a 60-d period unless another time period is indicated. Poor attitude, nasal, and ocular discharges if score scale > 0. Fever if temperature ≥ 39.44 C (103°F). Diarrhea if score scale > 1 and severe diarrhea if score scale = 3.

Table 6-9. Incidence of diseases in preweaned Holstein calves fed increasing amounts of linoleic acid (LA)

Item	Treatment ¹	%(n/n)	AOR ²	95% CI		P
Pneumonia	T1	14.3 (3/21)	Ref.	-	-	-
	T2	18.2 (4/22)	1.42	0.20	7.36	0.95
	T3	18.2 (4/22)	1.42	0.27	7.36	0.95
	T4	21.7 (5/23)	1.75	0.36	8.59	0.59
	Male	11.8 (4/34)	Ref.	-	-	-
	Female	22.2 (12/54)	2.19	0.64	7.49	0.21
Navel infection	T1	4.8 (1/21)	Ref.	-	-	-
	T2	4.5 (1/22)	0.83	0.05	14.90	0.96
	T3	0.0 (0/22)	-	-	-	0.96
	T4	8.7 (2/23)	1.77	0.14	22.10	0.95
	Male	8.8 (3/34)	Ref.	-	-	-
	Female	1.9 (1/54)	0.18	0.02	1.88	0.15
Bloody diarrhea ³	T1	47.6 (10/21)	Ref.	-	-	-
	T2	40.9 (9/22)	0.69	0.21	2.24	0.80
	T3	31.8 (7/22)	0.46	0.14	1.51	0.21
	T4	52.2 (12/23)	1.18	0.37	3.79	0.31
	Male	50.0 (17/34)	Ref.	-	-	-
	Female	38.9 (21/54)	0.60	0.26	1.40	0.29
Fever	T1	68.2 (14/21)	Ref.	-	-	-
	T2	58.3 (14/22)	0.94	0.26	3.42	0.99
	T3	72.0 (16/22)	1.47	0.38	5.52	0.29
	T4	50.0 (12/23)	0.56	0.16	1.97	0.18
	Male	51.4 (17/34)	Ref.	-	-	-
	Female	69.0 (39/54)	2.86	1.07	6.70	0.03

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² Adjusted odds ratio, T1 was reference (Ref.) for treatment diets and male was reference for gender.

³ Diarrhea occurred in all calves.

Table 6-10. Mean concentration of blood cell number and white blood cells percentages in preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA). All interactions with age did not differ unless footnoted.

Measure	Treatments ¹								SEM	Contrasts ² , P values						Age	
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G		Cb*G
	M	F	M	F	M	F	M	F									
Blood cells																	
Total Red, 10 ⁶ /uL	8.28	8.03	8.85	8.56	8.41	8.22	8.22	7.57	0.32	0.10	0.24	0.11	0.13	0.50	0.69	0.85	<0.01
Total white, 10 ³ /uL	8.7	10.4	9.2	10.1	10.0	9.4	8.9	11.0	0.62	0.64	0.91	0.59	<0.01	0.99	0.04	0.67	<0.01
Neutrophils, 10 ³ /uL	3.26	4.14	3.35	3.89	3.61	3.43	3.27	4.45	0.31	0.57	0.43	0.94	0.01	0.55	0.04	0.71	<0.01
Lymphocytes, 10 ³ /uL	4.11	4.74	4.67	4.71	4.63	4.73	4.60	4.47	0.28	0.95	0.36	0.55	0.41	0.28	0.60	0.45	<0.01
Monocytes, 10 ³ /uL	0.42	0.61	0.44	0.49	0.41	0.57	0.33	0.65	0.09	0.84	0.83	0.51	<0.01	0.25	0.60	0.56	<0.01
Eosinophils ³ , 10 ³ /uL	48.9	75.2	56.7	50.6	59.2	64.6	43.4	54.0	8.21	0.18	0.54	0.21	0.13	0.81	0.24	0.12	<0.01
Basophils, #/uL	31.8	34.5	26.1	43.9	54.5	32.5	43.1	43.3	7.04	0.12	0.36	0.68	0.95	0.42	0.10	0.03	<0.01
Platelets, 10 ³ /uL	488	521	527	515	561	415	518	447	49.8	0.48	0.84	0.56	0.15	0.27	0.13	0.66	<0.01
White cells, %																	
Neutrophils	41.6	43.3	39.7	42.7	41.4	39.4	40.2	45.7	2.05	0.64	0.22	0.90	0.17	0.41	0.17	0.36	<0.01
Lymphocytes	51.2	48.1	53.1	49.6	51.6	52.0	53.6	45.3	1.93	0.70	0.18	0.71	0.01	0.18	0.12	0.46	<0.01
Monocytes	6.21	7.14	5.43	6.24	5.22	7.18	4.98	7.19	0.99	0.73	0.72	0.49	0.04	0.42	0.80	0.79	<0.01
Eosinophils ⁴	0.59	0.69	0.61	0.51	0.65	0.69	0.50	0.50	0.09	0.19	0.31	0.18	0.86	0.86	0.79	0.25	<0.01
Basophils	0.35	0.32	0.26	0.46	0.54	0.35	0.51	0.37	0.07	0.11	0.35	0.78	0.44	0.09	0.51	0.02	<0.01
Hematocrit, %	33.1	32.3	32.8	32.9	32.3	32.2	32.1	32.1	0.65	0.23	0.80	0.62	0.62	0.74	0.69	0.65	<0.01

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² P values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

³ Gender by age, P = 0.05. ⁴ Gender by age, P <0.01

Table 6-11. Phagocytosis, oxidative burst, and mean fluorescence intensity (MFI) of neutrophils in peripheral blood of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA)

Measure	Treatments ¹								SEM	Contrasts ² , P values						Age	
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G		Cb*G
	M	F	M	F	M	F	M	F									
Phagocytosis, % of neutrophils	60.2	64.0	67.4	65.8	64.3	64.1	62.9	62.7	2.28	0.58	0.27	0.09	0.81	0.64	0.50	0.38	0.14
Phagocytosis, MFI	21.2	22.8	23.2	24.6	21.2	24.5	23.0	21.4	2.10	0.78	0.62	0.42	0.45	0.41	0.45	0.71	<0.01
Oxidative burst % of neutrophils	48.9	53.9	55.8	55.9	53.4	53.8	50.5	50.4	2.52	0.24	0.18	0.15	0.46	0.46	0.55	0.53	0.95
Oxidative burst, MFI	32.2	33.1	35.5	35.5	31.5	36.8	36.9	34.6	2.90	0.45	0.88	0.36	0.63	0.62	0.29	0.52	<0.01

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² P values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

Table 6-12. Mean concentration of plasma acute phase proteins, serum anti OVA-IgG, cytokines produced by whole blood cells stimulated with LPS + PHA, and proliferation of whole blood cells by thymidine incorporation in preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic acid (LA). All interactions with age did not differ unless footnoted

Measure	Treatments ¹								SEM	L	Q	Contrasts ² , P values					
	T1		T2		T3		T4					Cb	G	L*G	Q*G	Cb*G	Age
	M	F	M	F	M	F	M	F									
ASP ³ , mg/L	88.7	96.1	81.8	98.1	85.0	85.0	93.4	94.8	4.70	0.07	0.83	0.58	0.05	0.25	0.70	0.15	<0.01
Haptoglobin ⁴ , OD x 100	0.82	0.86	0.75	0.84	0.77	0.90	0.77	0.88	0.10	0.06	0.49	0.94	0.91	0.64	0.62	0.84	<0.01
Anti OVA-IgG, OD	0.10	0.17	0.22	0.18	0.24	0.18	0.13	0.22	0.04	0.71	0.33	0.58	0.08	0.47	0.04	0.47	<0.01
TNF- α , pg/mL	311	362	424	321	457	390	345	389	64.2	0.68	0.94	0.72	0.20	0.66	0.26	0.40	<0.01
IFN- γ , pg/mL	260	256	357	327	411	227	209	265	66.4	0.39	0.38	0.38	0.19	0.62	0.09	0.57	<0.01
Whole blood cell proliferation																	
Control ⁵	0.50	0.52	0.56	0.56	0.55	0.58	0.59	0.65	0.08	0.71	0.65	0.23	0.81	0.75	0.93	0.87	<0.01
Stimulated ⁵	12.8	14.7	23.5	20.4	14.0	18.8	16.2	19.0	2.83	0.33	0.01	0.67	0.44	0.63	0.71	0.22	<0.01
Difference ⁵	12.1	14.1	22.8	19.8	13.3	17.9	15.5	18.0	2.78	0.33	0.01	0.71	0.44	0.65	0.72	0.22	<0.01
Stimulation index ⁶	25.5	28.4	41.7	36.5	25.3	32.7	27.6	29.4	4.47	0.47	0.01	0.47	0.51	0.80	0.61	0.22	<0.01
Stimulated per Lymphocyte ⁷	2.87	3.13	4.98	4.36	3.00	4.04	3.56	4.11	0.59	0.37	0.01	0.61	0.50	0.57	0.58	0.26	<0.01

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² P values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

³ Treatment by age, P < 0.01

⁴ Treatment by age, P = 0.02.

⁵ Proliferation is expressed as KCPM (1000 counts per minute of thymidine incorporation).

⁶ CPM of stimulated cells divided by CPM of nonstimulated cells.

⁷ CPM of stimulated cells divided by the number of lymphocytes in whole blood.

Table 6-13. Skin fold change measured after 6, 24, and 48 h of intradermal injection of 150 ug of phytohaemagglutinin in preweaned male (M) and female (F) Holstein calves. All interactions with age did not differ unless footnoted

Measure	Treatments ¹								SEM	Contrasts ² , P values						Hour after injection	
	T1		T2		T3		T4			L	Q	Cb	G	L*G	Q*G		Cb*G
	M	F	M	F	M	F	M	F									
Total calves	7	14	9	13	9	13	9	14									
Responsive calves																	
At 30 d, n =	7	13	7	9	8	12	5	11									
At 30 d	6.5	8.8	16.3	5.7	11.8	17.0	15.6	15.6	3.22	0.03	0.21	0.85	0.73	0.60	0.68	0.01	<0.01
At 60 d, n =	5	11	6	10	7	11	8	11									
At 60 d	6.6	7.0	8.5	7.4	3.5	5.8	11.7	6.6	1.92	0.33	0.09	0.18	0.54	0.17	0.25	0.42	<0.01

¹ Targeted intakes of LA from milk replacer was as follows: T1 = 0.144, T2 = 0.206, T3 = 0.333, T4 = 0.586 g of LA/kg of BW^{0.75}. Milk replacer was the only feed during the first 30 d of life.

² P values for orthogonal contrasts of treatments, gender, and treatment by gender interactions. L= linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, G = gender.

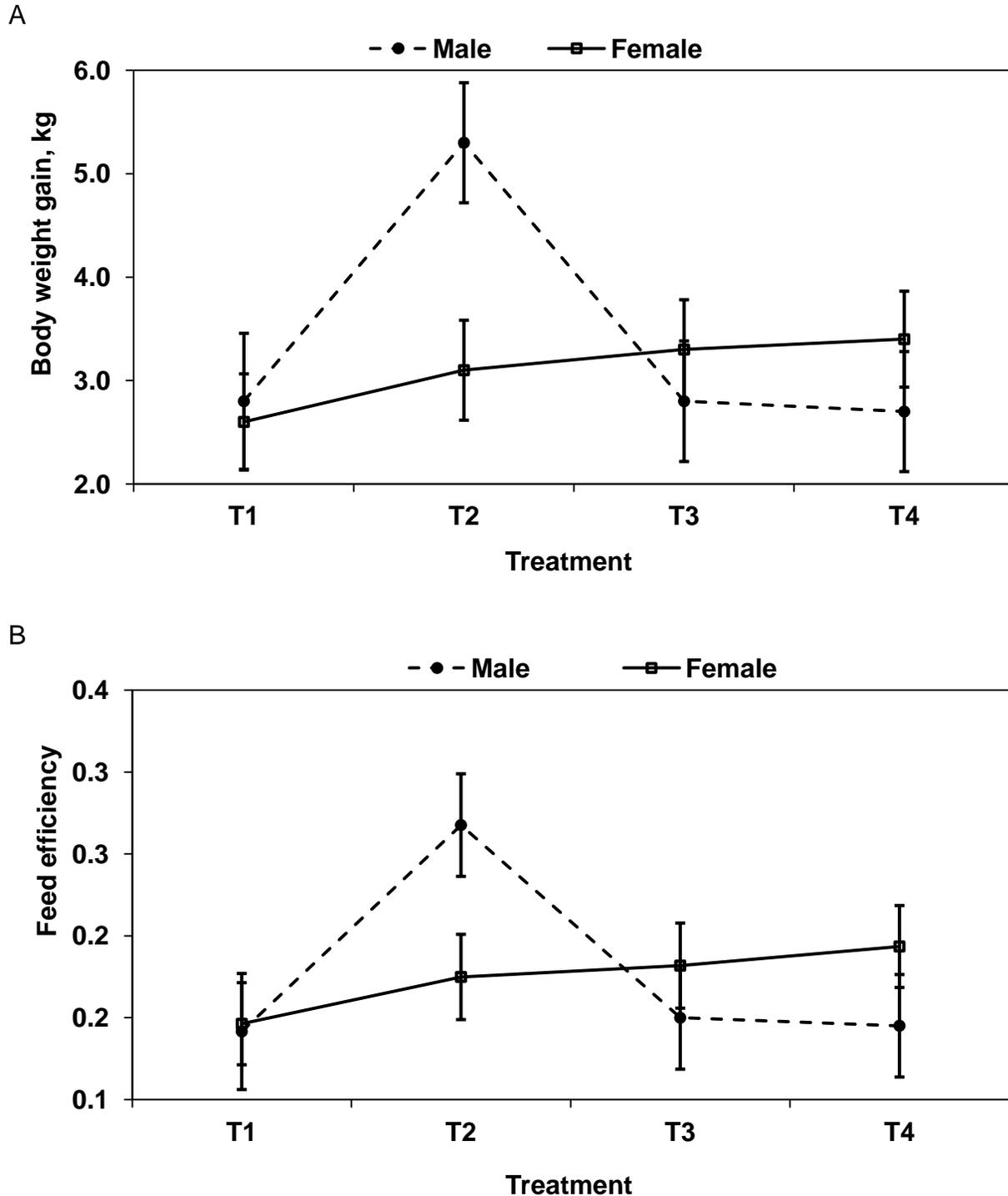


Figure 6-1. Body weight gain and feed efficiency (gain : intake) during the first 30 d of life of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Cubic effect of treatment by gender, $P = 0.02$, linear effect of treatment by gender, $P = 0.07$. B) Cubic effect of treatment by gender, $P = 0.04$, linear effect of treatment by gender, $P = 0.10$.

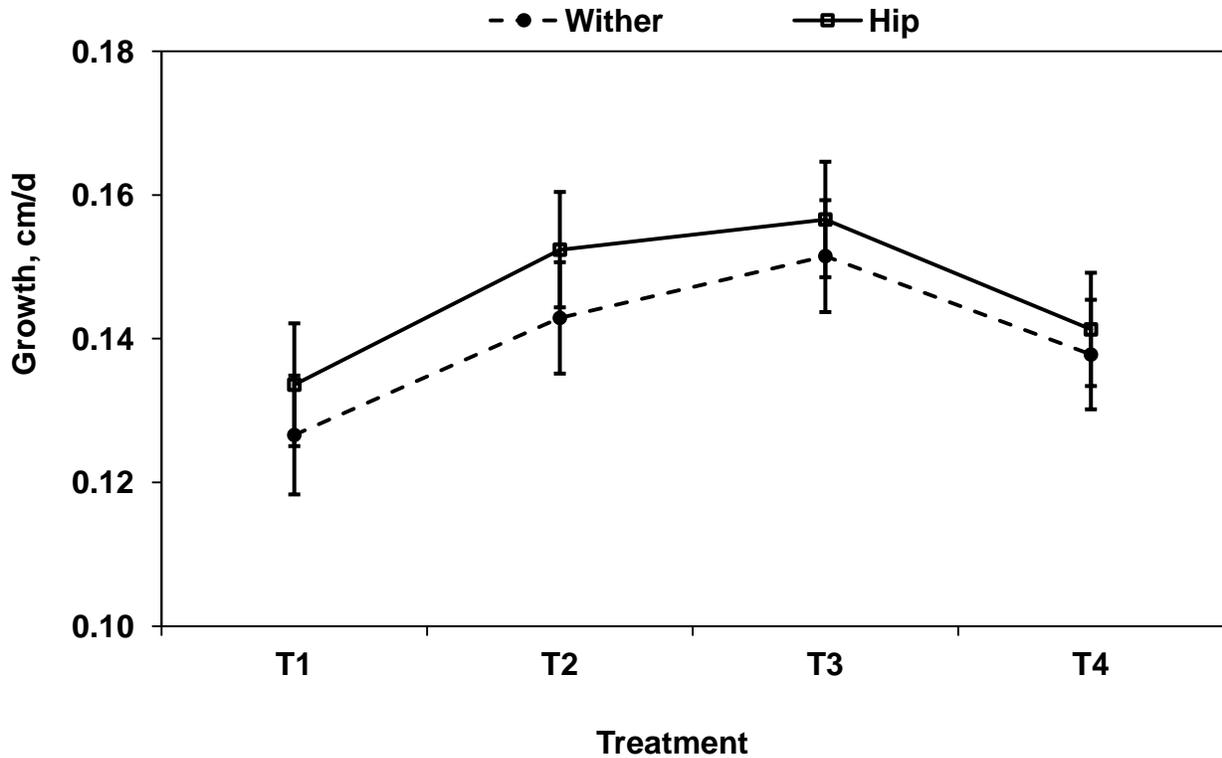


Figure 6-2. Averages daily wither and hip growth during first 60 d of life of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). Quadratic effect of treatment in wither growth, $P = 0.04$. Quadratic effect of treatment in hip growth, $P = 0.04$.

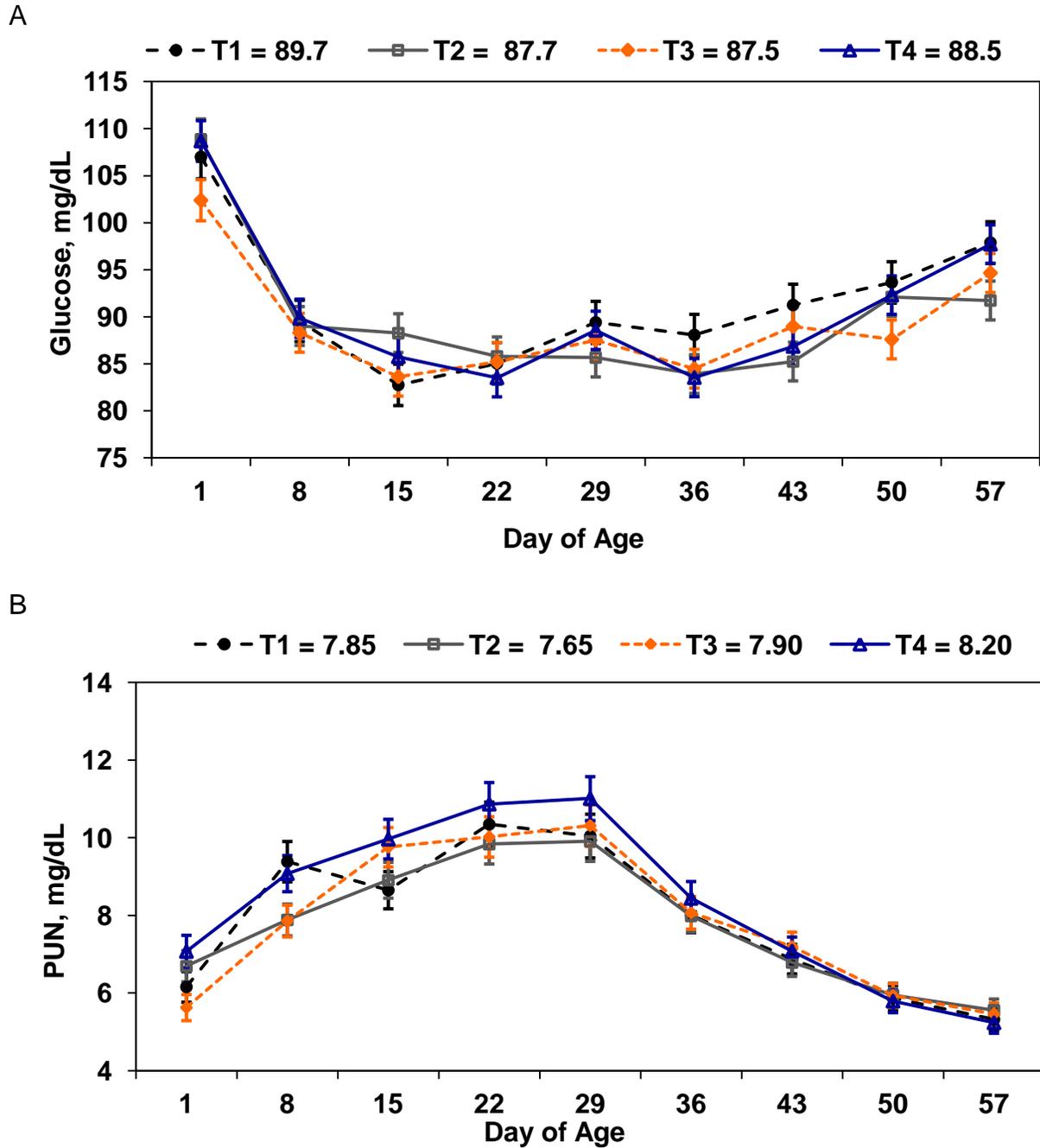
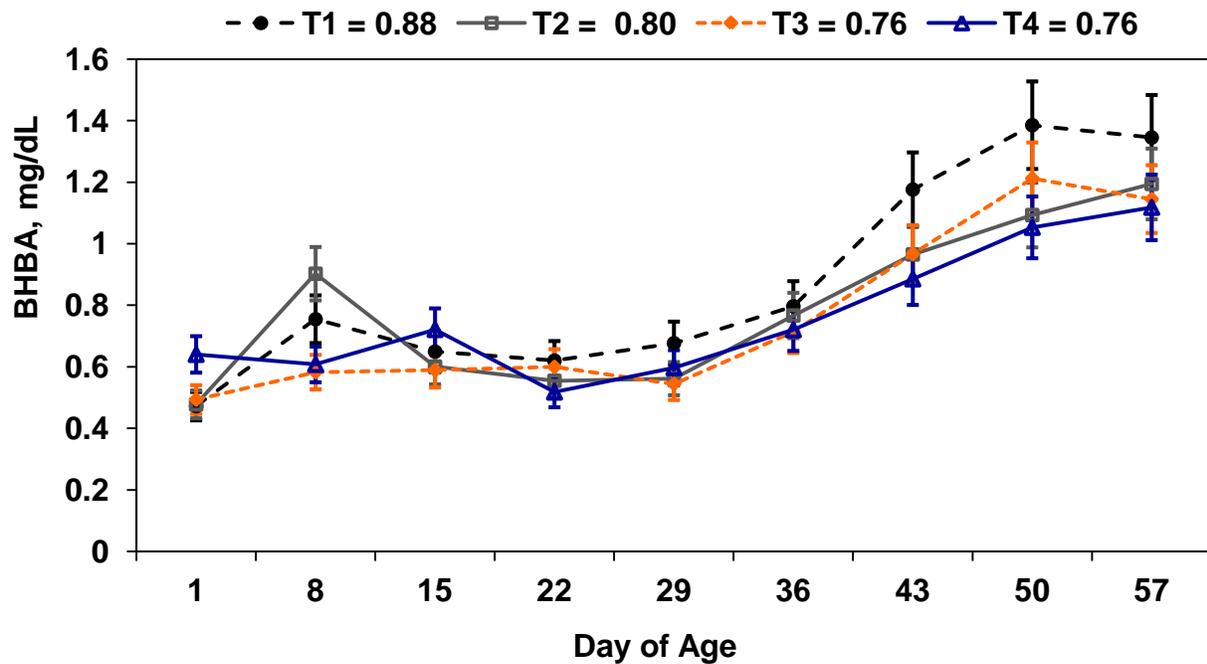


Figure 6-3. Plasma concentrations of glucose and urea N (PUN) of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Quadratic effect of treatment by gender, $P = 0.02$ and effect of age, $P < 0.01$. B) Quadratic effect of treatment by gender, $P = 0.07$ and effect of age, $P < 0.01$.

A



B

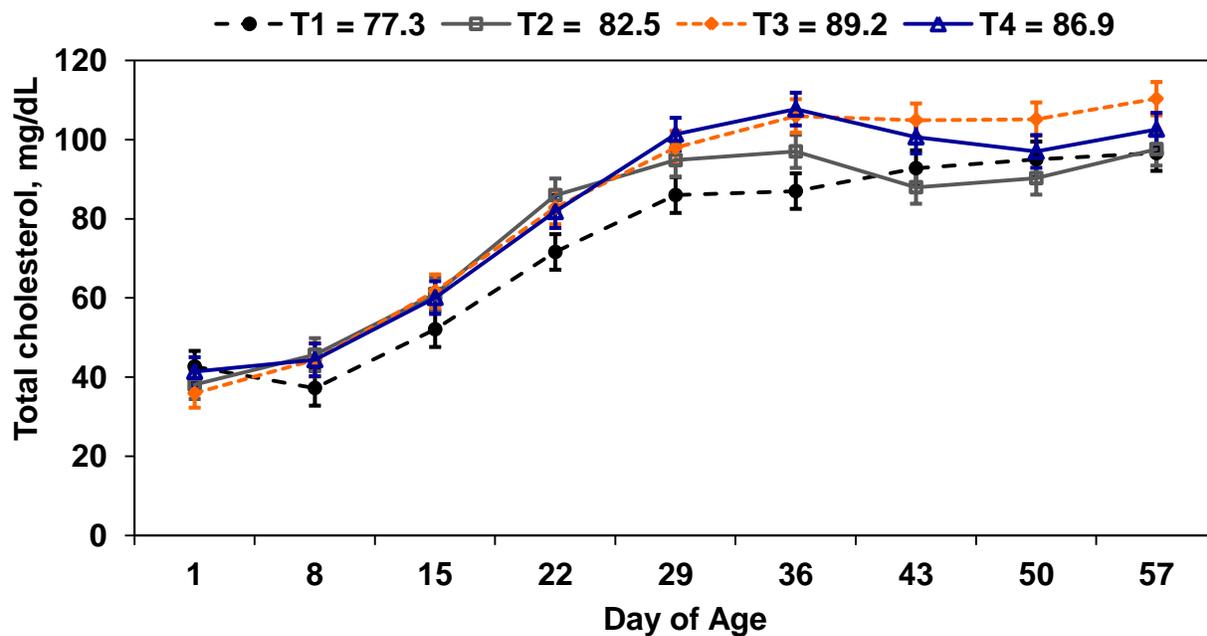
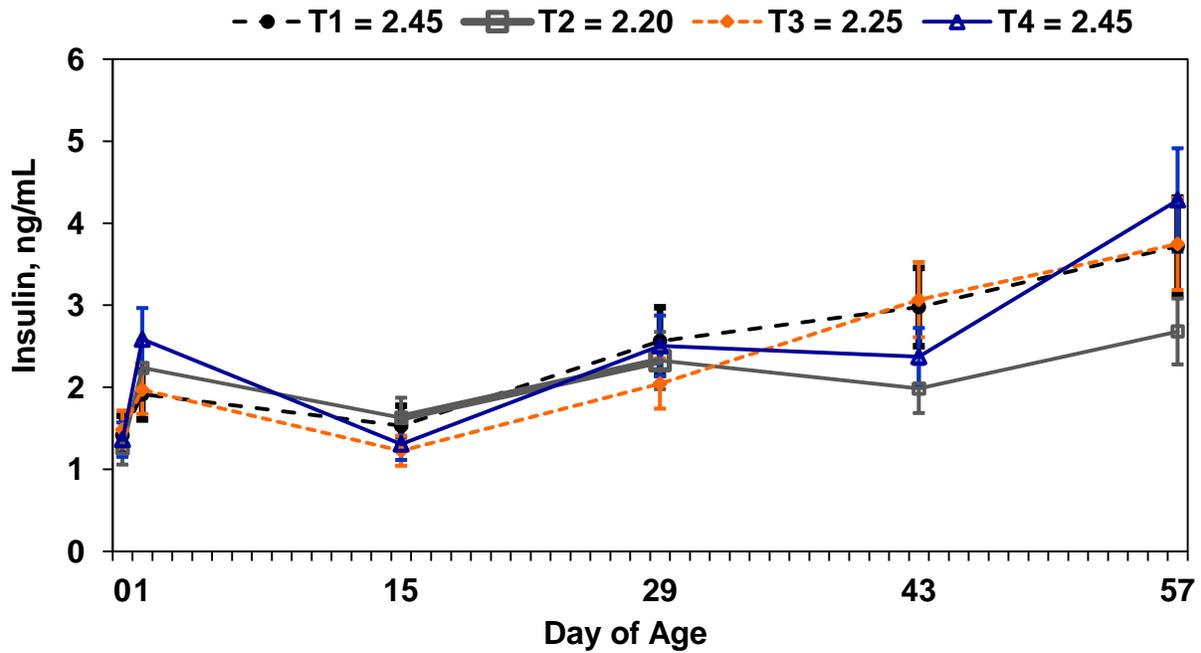


Figure 6-4. Plasma concentrations of BHBA and total cholesterol in preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Linear effect of treatment, $P = 0.06$ and effect of age, $P < 0.01$. B) Linear effect of treatment, $P = 0.03$ and effect of age, $P < 0.01$.

A



B

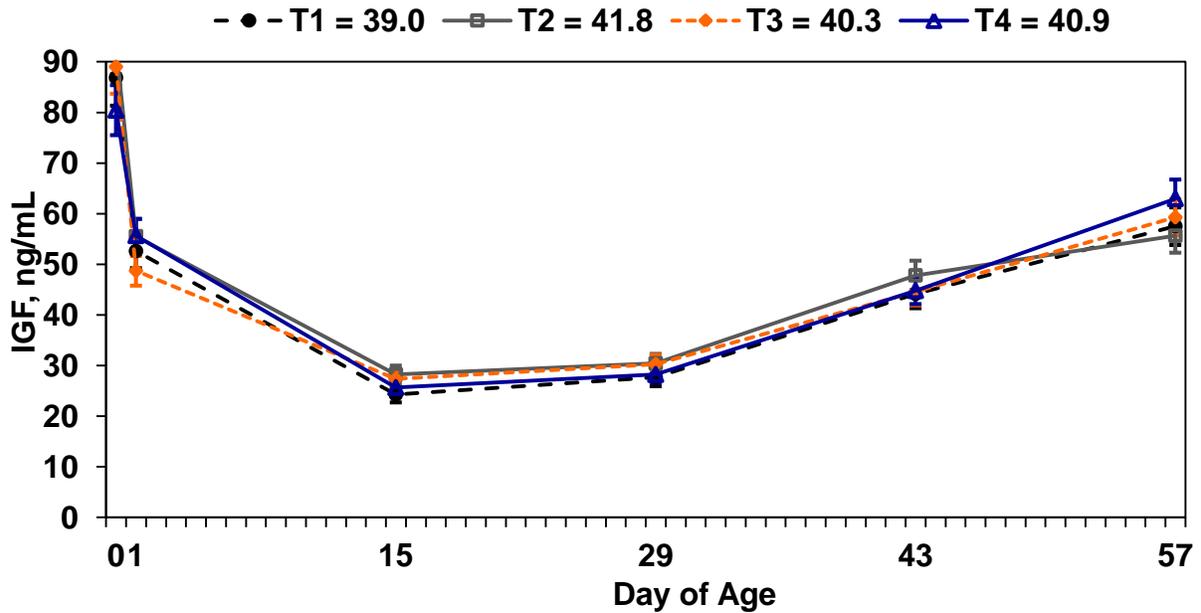


Figure 6-5. Plasma concentrations of insulin and IGF-I in preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Effect of age, $P < 0.01$. B) Effect of age, $P < 0.01$.

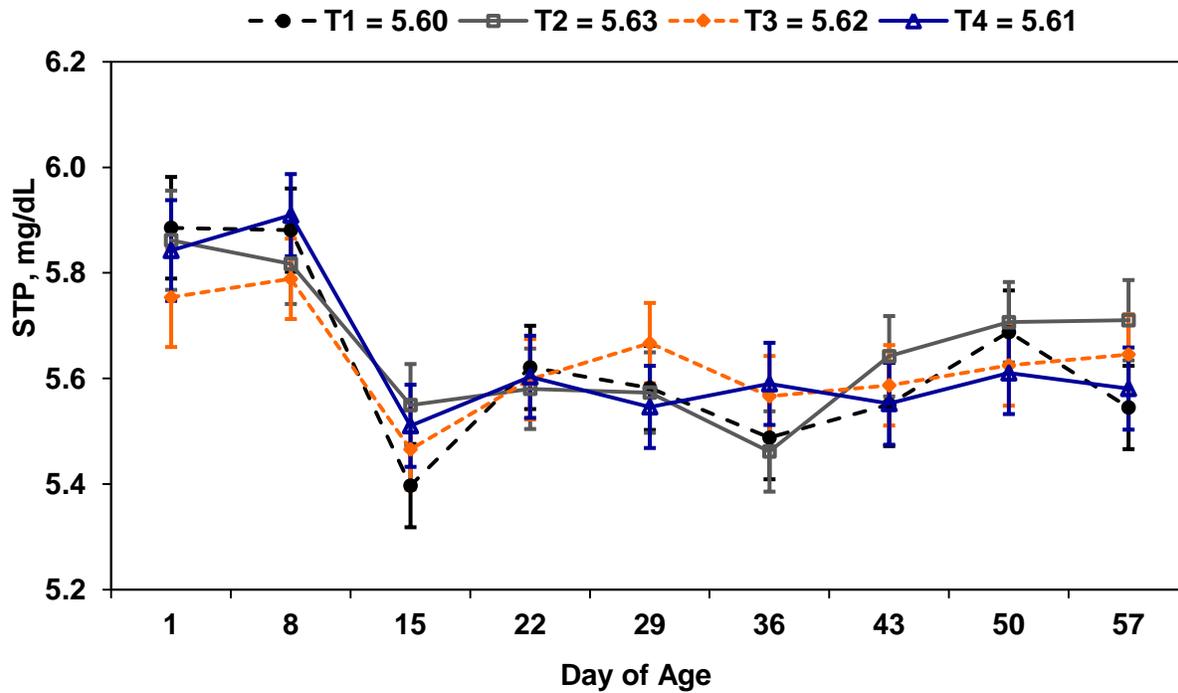
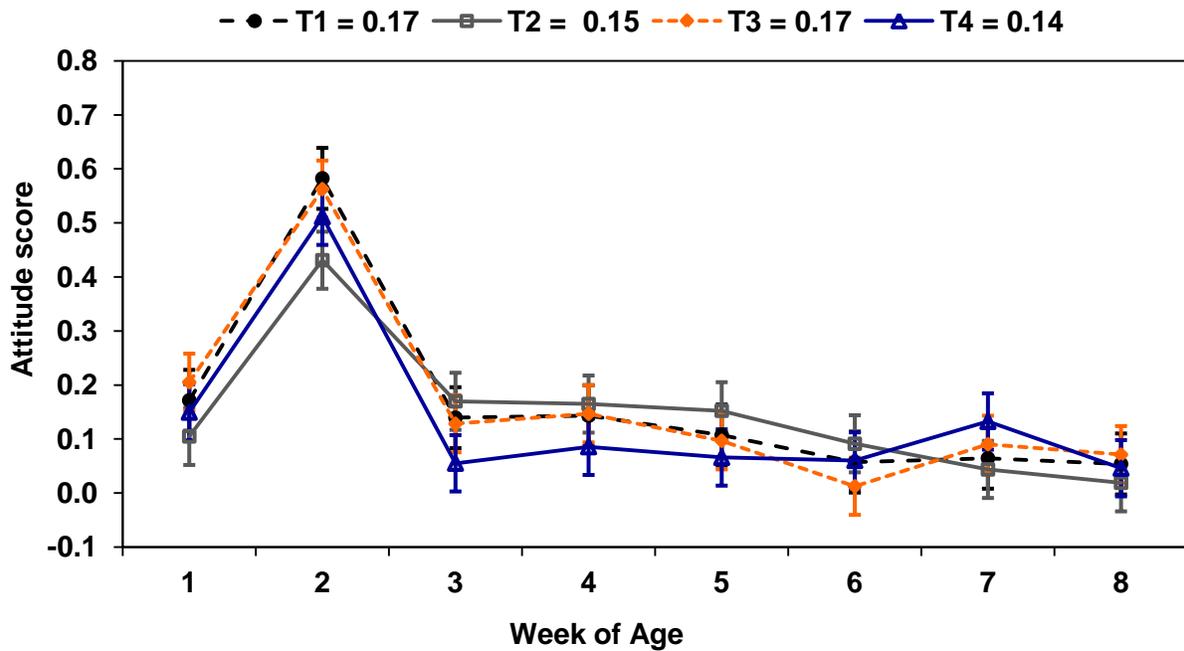


Figure 6-6. Total serum protein (STP) in preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). Effect of age, $P < 0.01$.

A



B

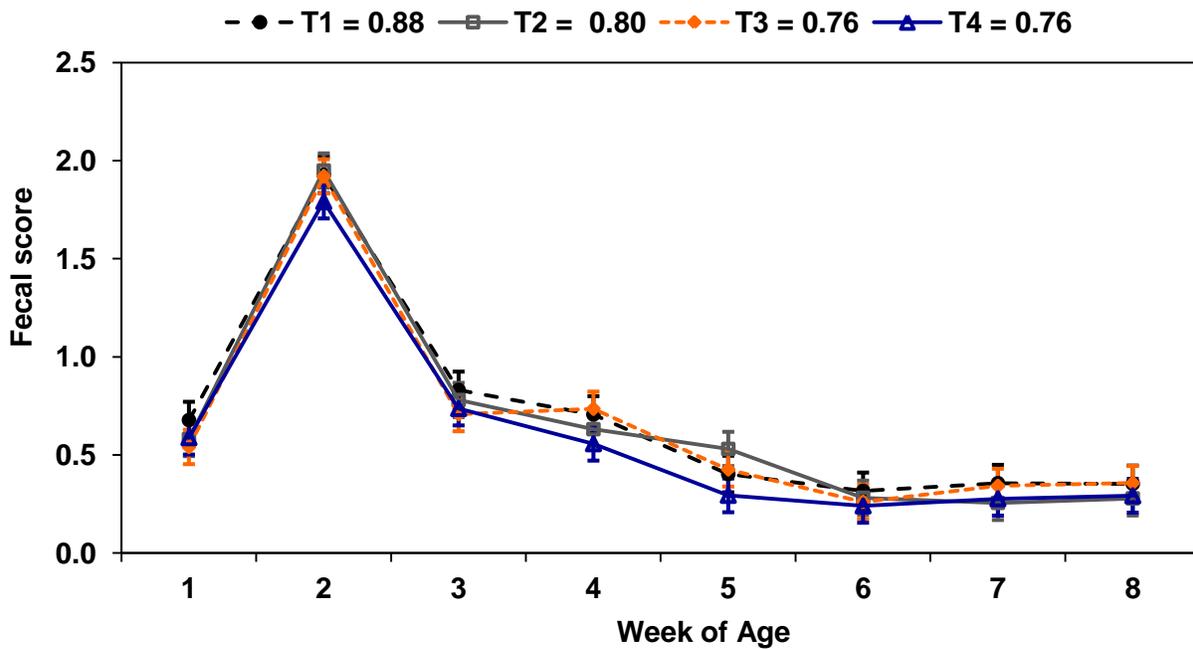


Figure 6-7. Attitude and fecal average weekly scores of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Effect of age, $P < 0.01$. B) Linear effect of treatment, $P = 0.05$, and effect of age, $P < 0.01$.

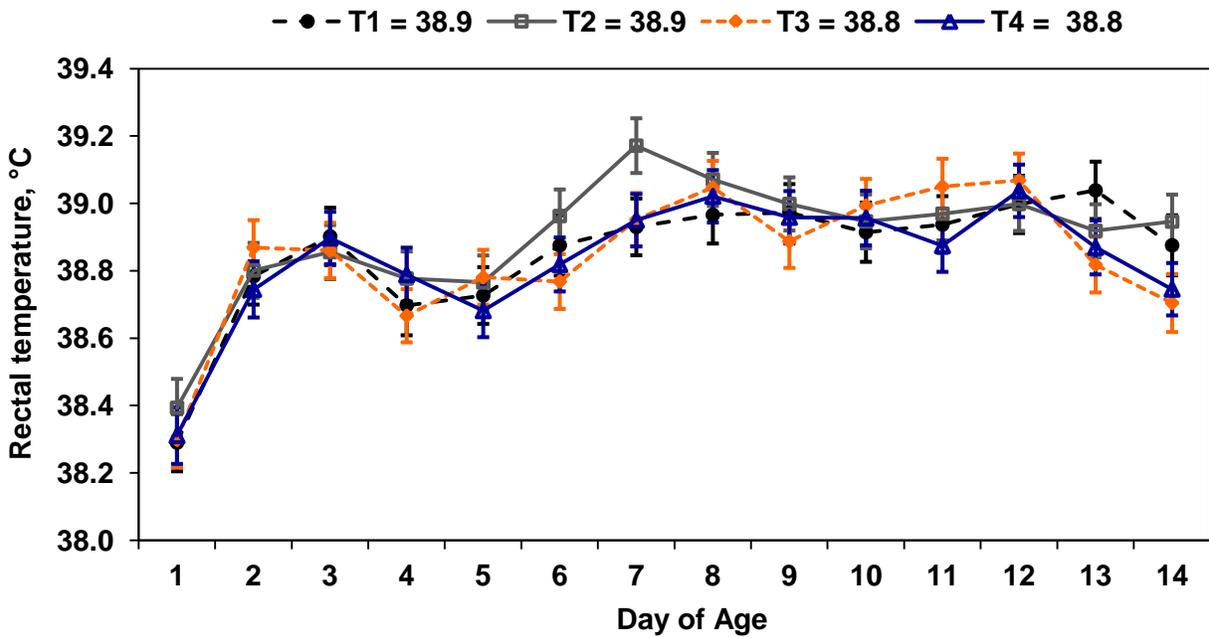


Figure 6-8. Rectal temperature first 14 days of life of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). Effect of age, $P < 0.01$.

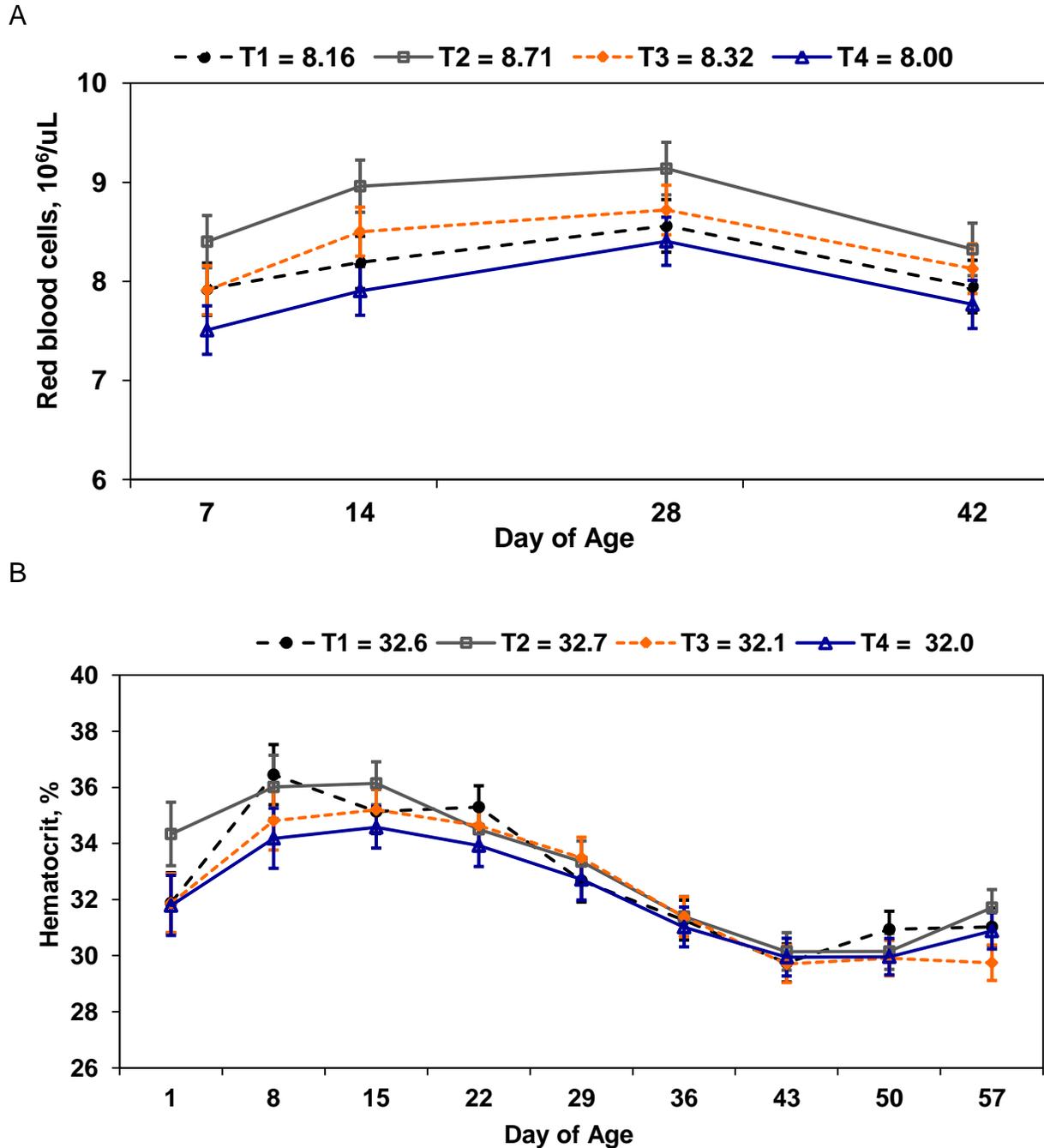


Figure 6-9. Red blood cells and hematocrit concentration in Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Linear effect of treatment, $P = 0.10$ and effect of age, $P < 0.01$. B) Effect of age, $P < 0.01$.

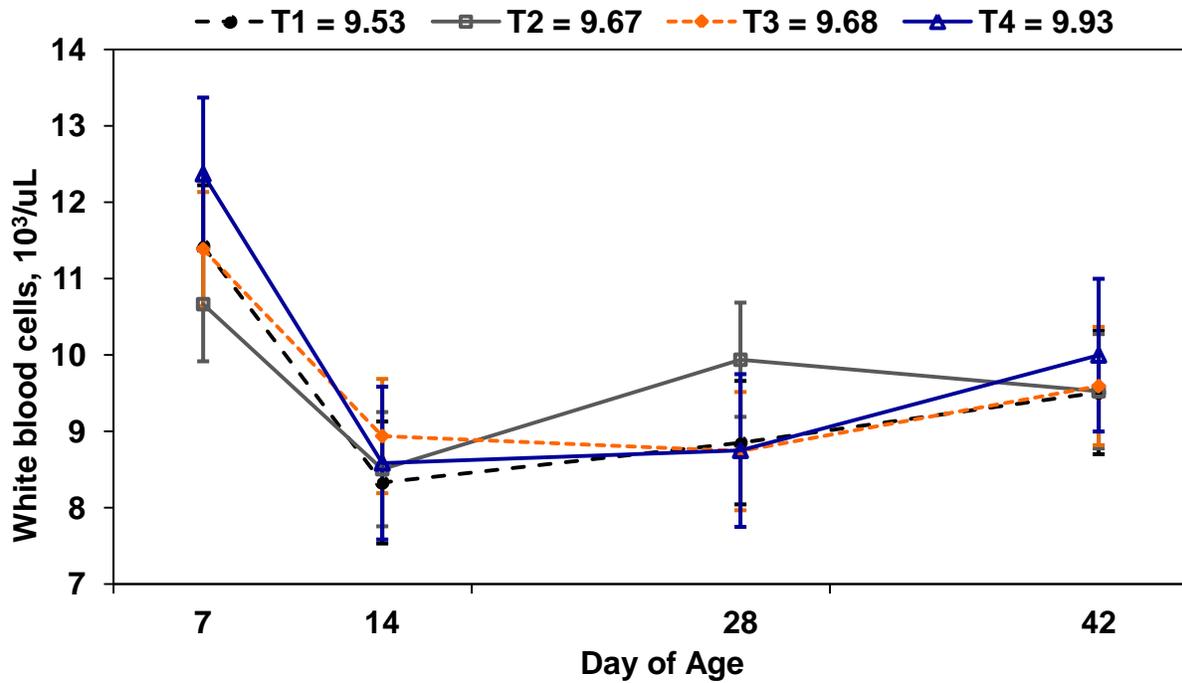


Figure 6-10. Concentrations of white blood cells of Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). Effect of age, $P < 0.01$.

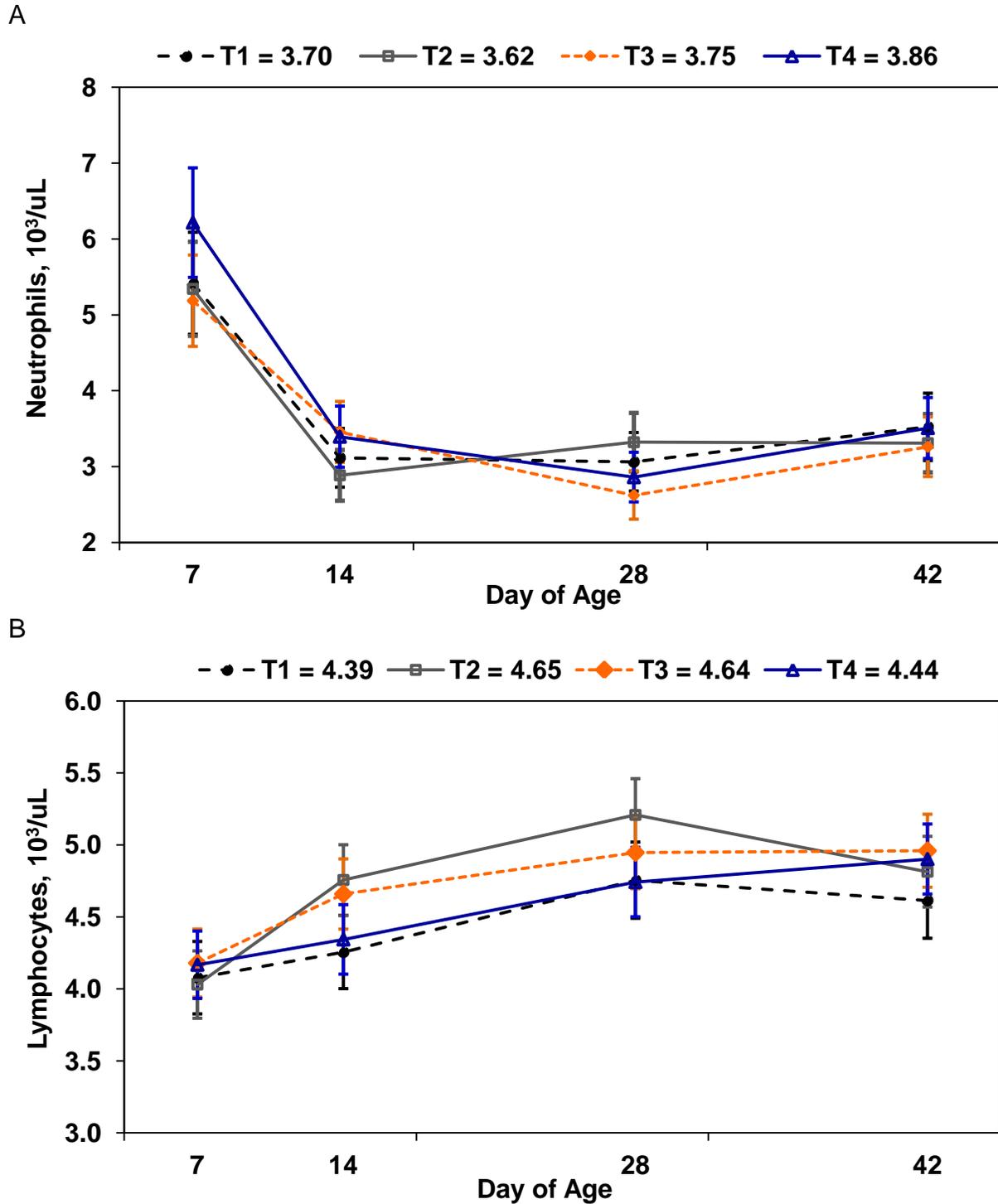


Figure 6-11. Concentrations of neutrophil and lymphocyte in blood of Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Effect of age, $P < 0.01$. B) Effect of age, $P < 0.01$.

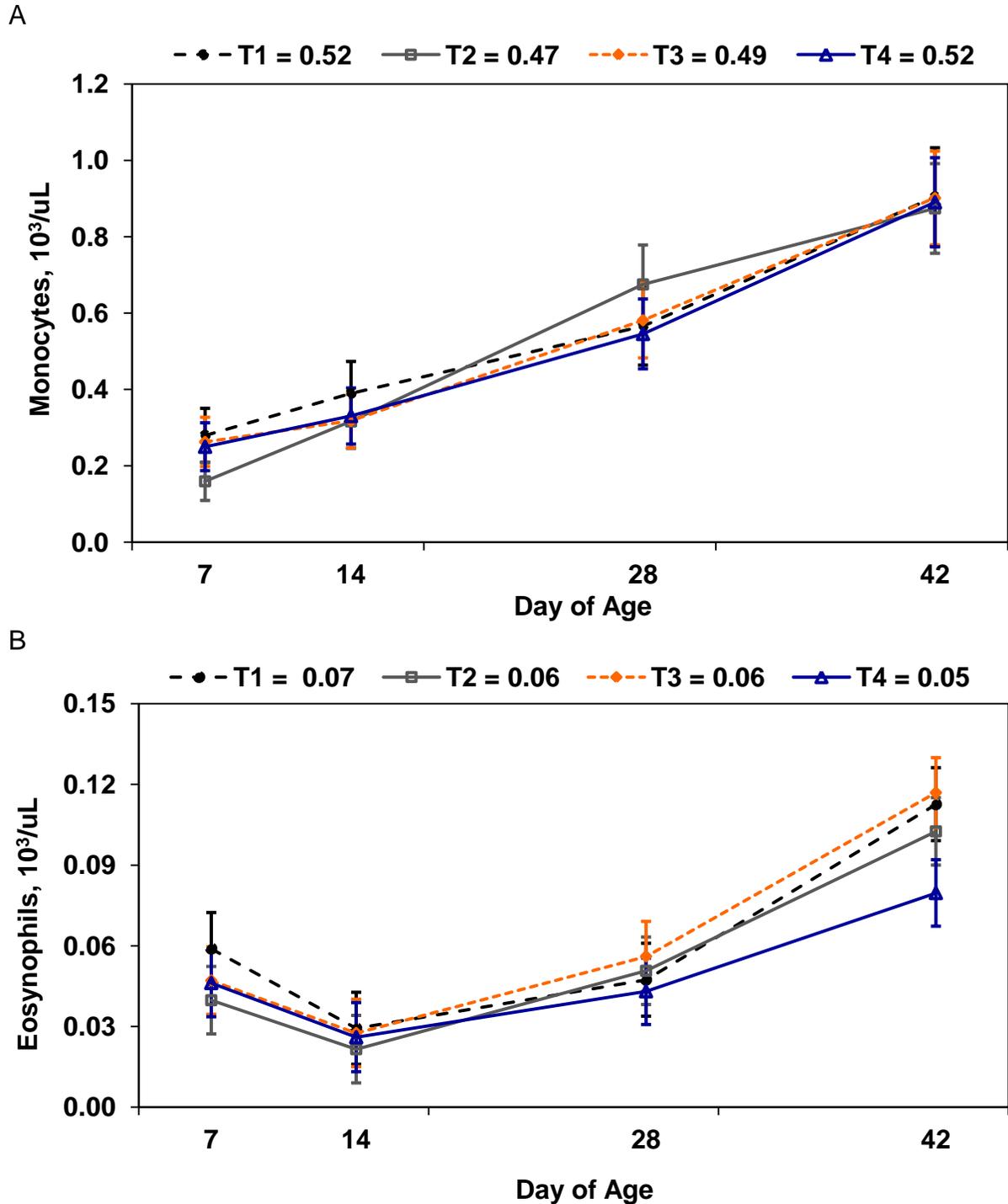


Figure 6-12. Concentrations of monocytes and eosinophils in blood of Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Effect of age, $P < 0.01$. B) Effect of age, $P < 0.01$.

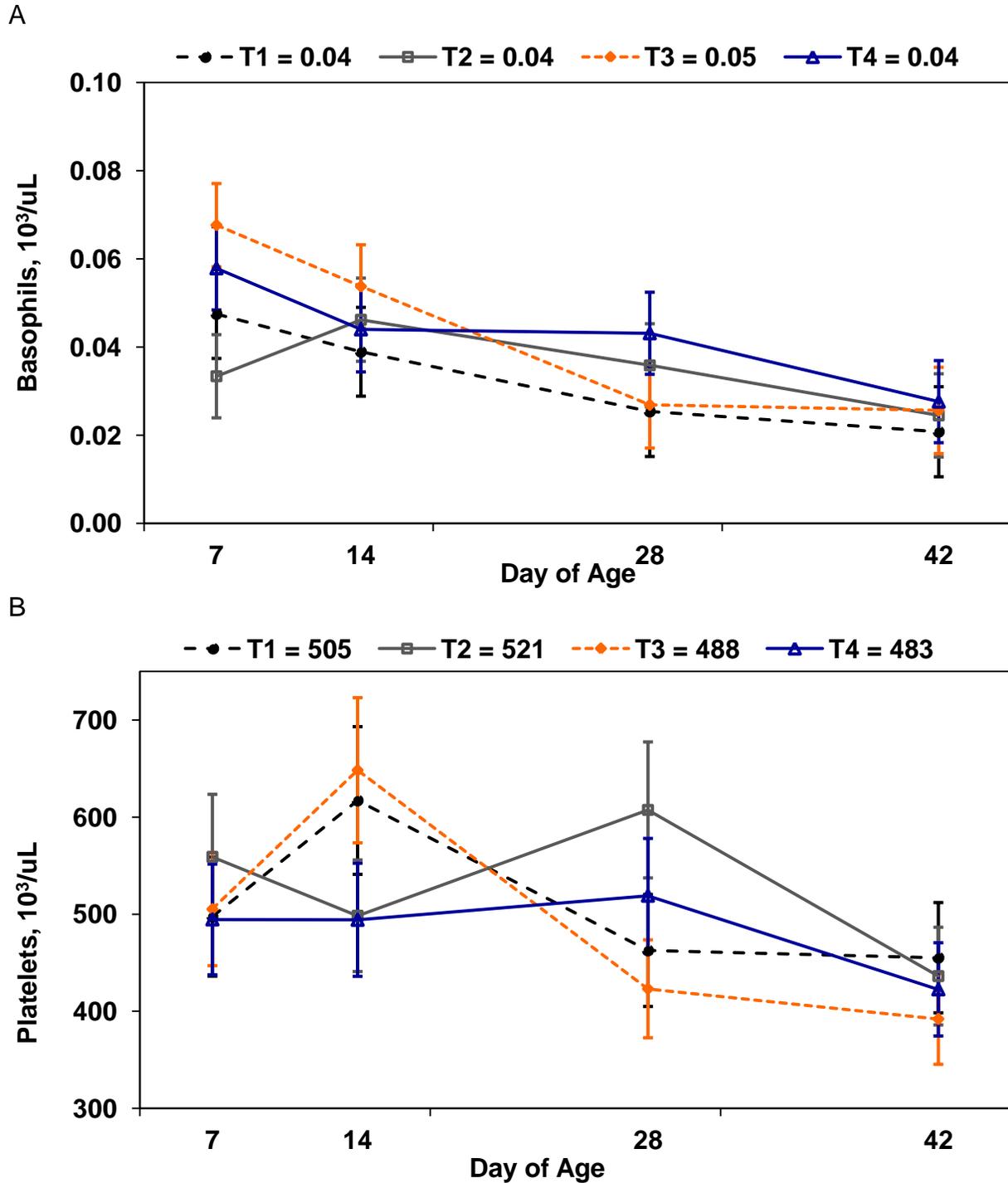
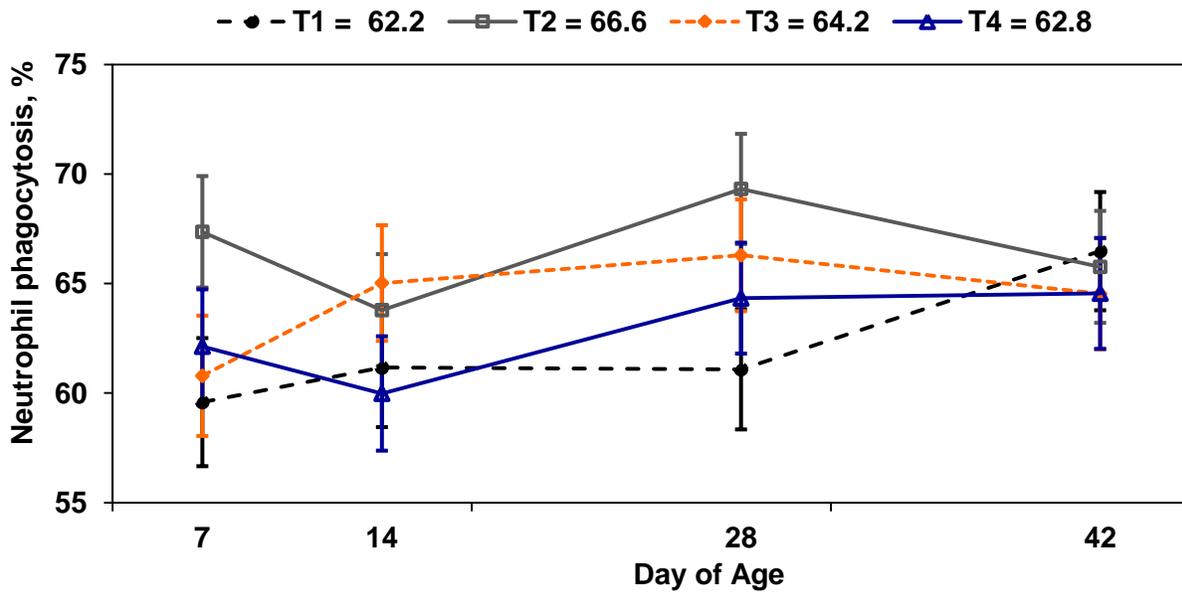


Figure 6-13. Concentrations of basophils and platelets in blood of Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Effect of age, $P < 0.01$. B) Effect of age, $P < 0.01$.

A



B

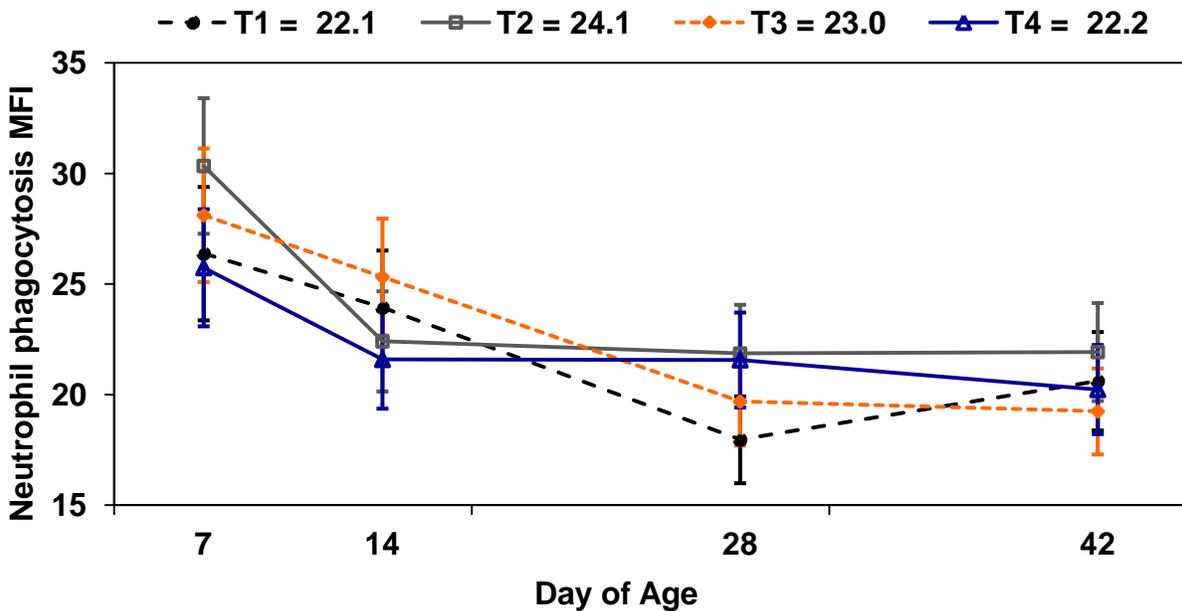
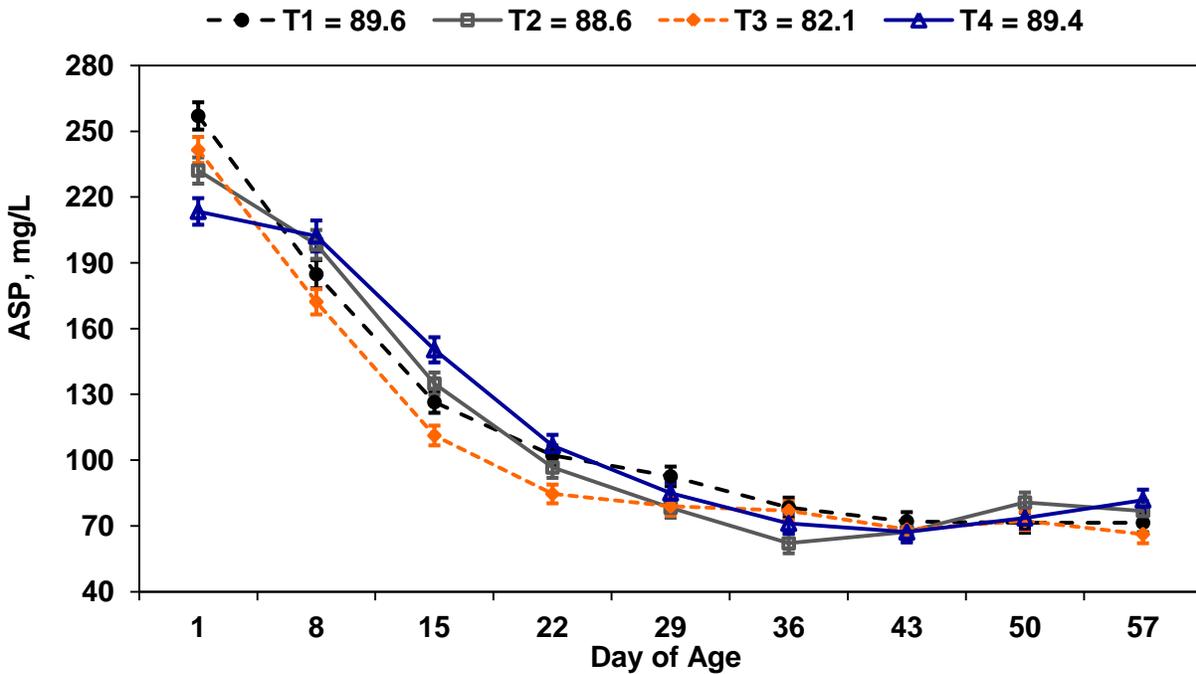


Figure 6-14. Neutrophil phagocytosis and mean fluorescence intensity (MFI) of neutrophils in blood of Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Cubic effect of treatment, $P = 0.09$. B) Effect of age, $P < 0.01$.

A



B

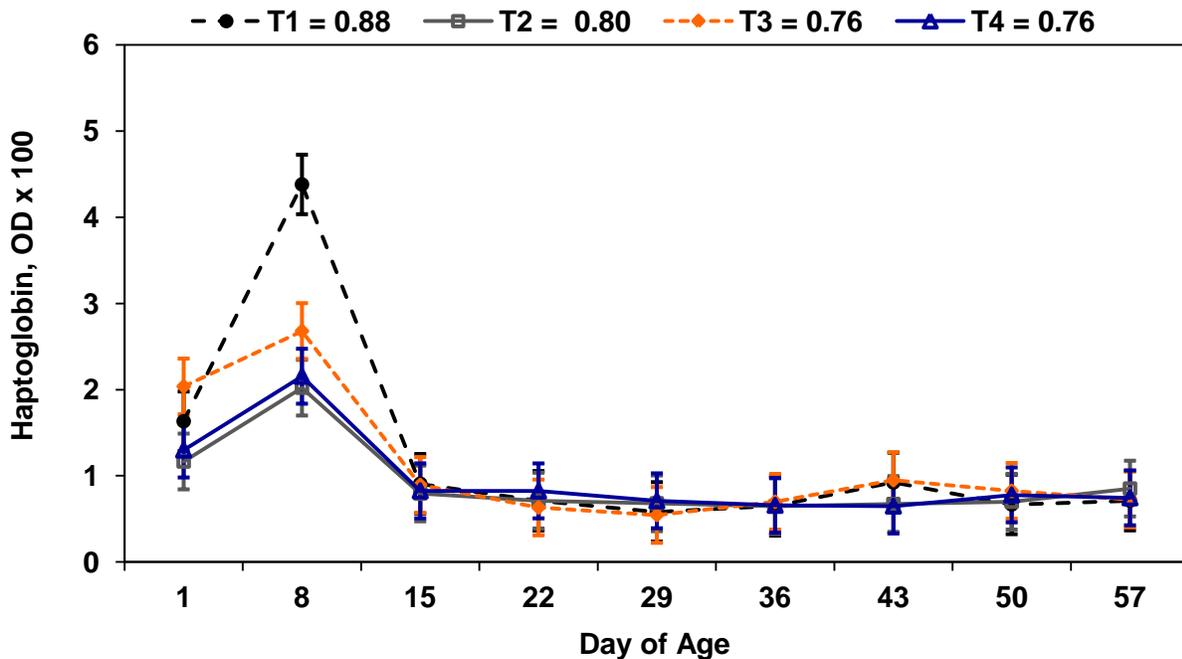
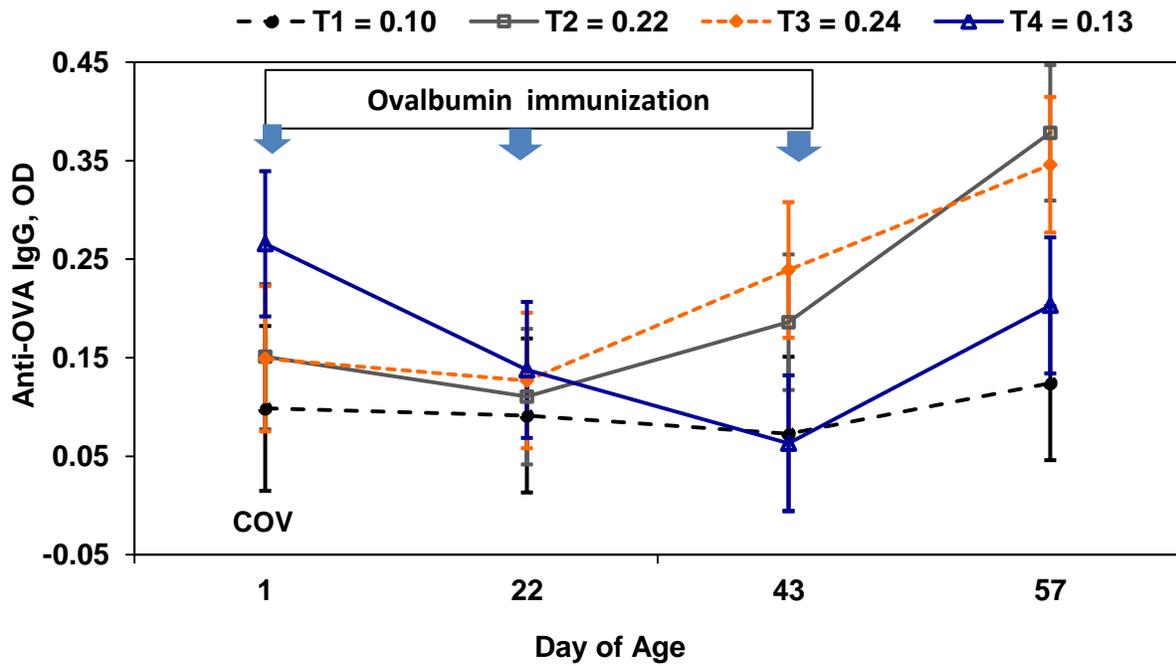


Figure 6-15. Concentrations of acid soluble protein (ASP) and Haptoglobin in plasma of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). A) Effect of treatment by age, $P < 0.01$ [slice effect at 8, 15, and 22 days ($P \leq 0.01$) at 29, 36, and 57 days ($P \leq 0.08$)] B) Effect of treatment by age, $P = 0.02$ [slice effect at days 8 and 43 ($P < 0.05$)].

A



B

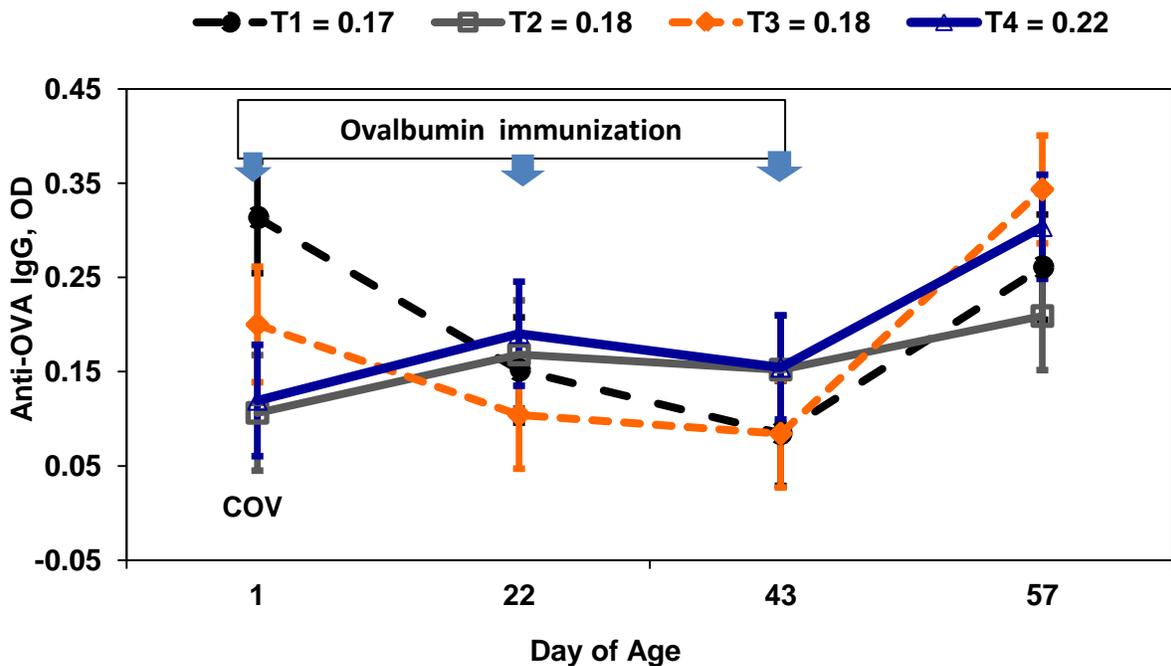


Figure 6-16. Serum Anti-OVA IgG concentrations in preweaned Holstein calves fed increased intake of linoleic acid. A) Males. B) Females. Day 1 was used as covariate. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1 = 0.144 g LA/WB^{0.75}, T2 = 0.206 g LA/WB^{0.75}, T3 = 0.333 g LA/WB^{0.75}, T4 = 0.586 g LA/WB^{0.75}). Quadratic effect of treatment by gender, $P = 0.04$, effect of age, $P < 0.01$.

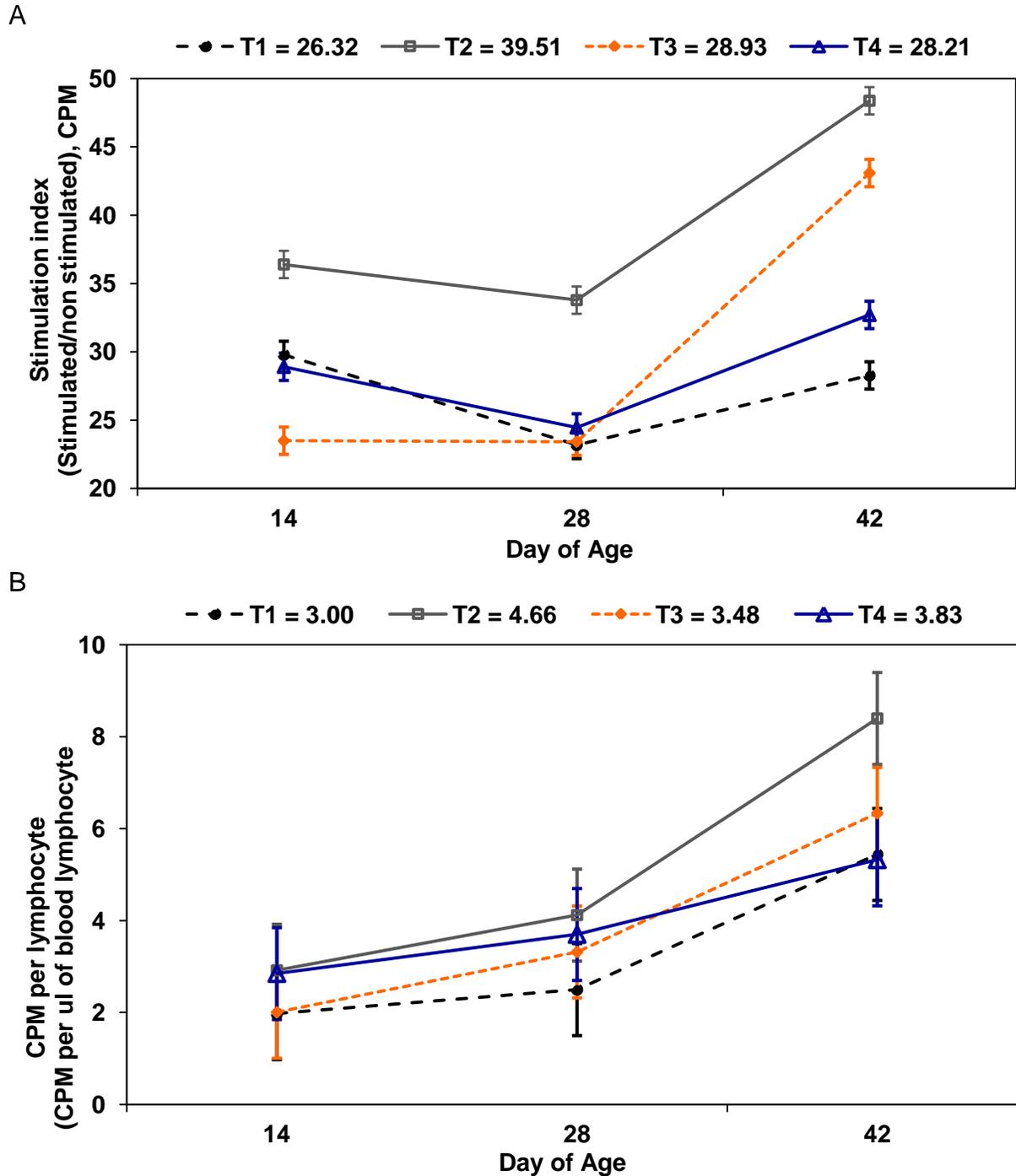


Figure 6-17. Lymphocyte proliferation in Holstein calves fed increased intake of linoleic acid. A) Lymphocyte proliferation measured as counts per minute (CPM) of thymidine incorporation respect to non stimulated cells. B) Lymphocyte proliferation measured as respect to number of blood lymphocytes. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). For both variables, cubic effect of treatment, $P = 0.01$ and effect of age, $P < 0.01$.

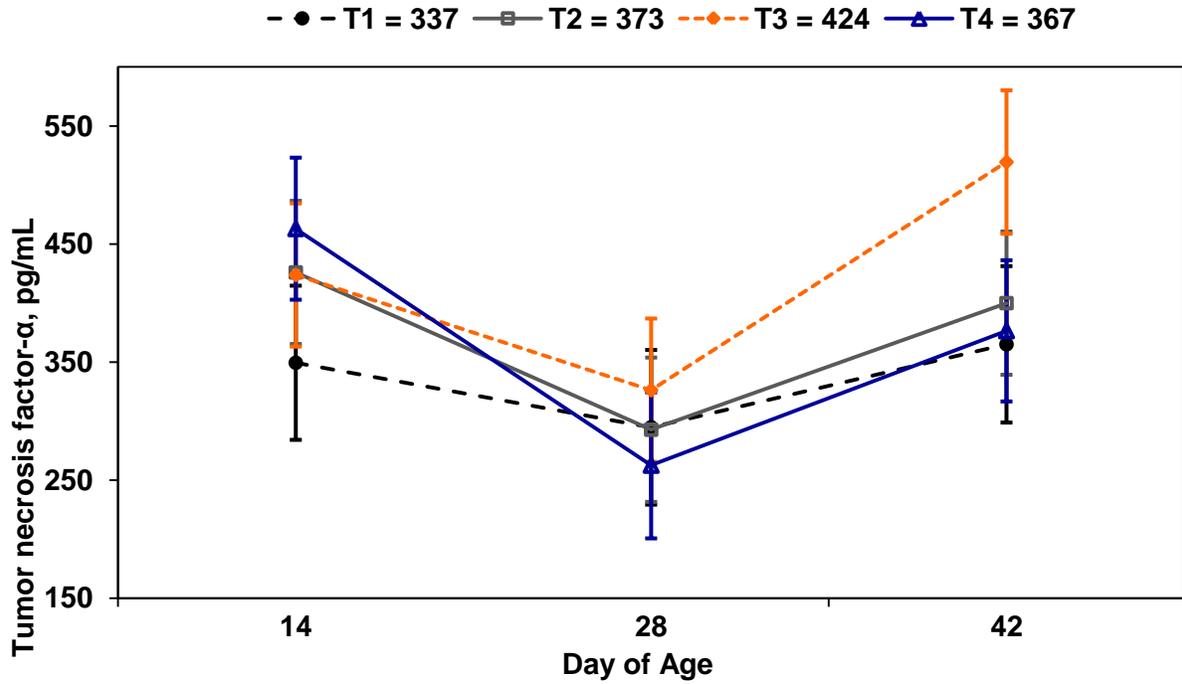


Figure 6-18. Tumor necrosis factor - α produced by stimulated whole blood cells of preweaned Holstein calves fed increased intake of linoleic acid. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). Effect of age, P< 0.01

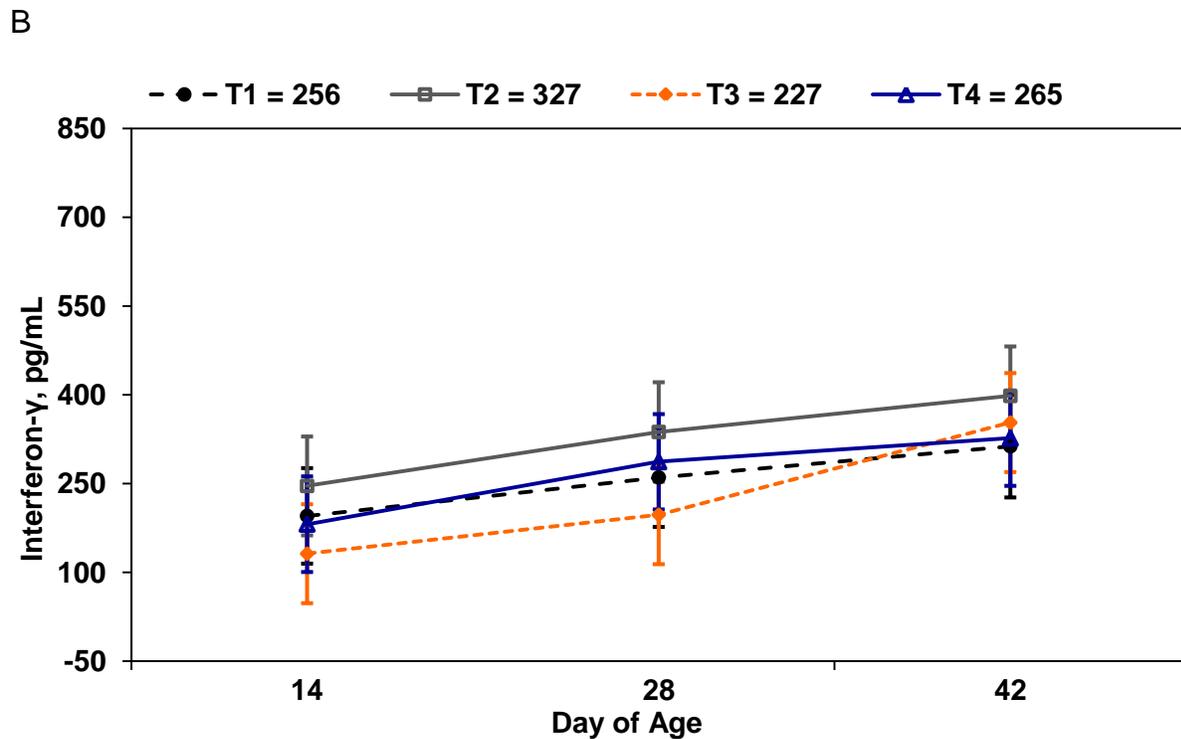
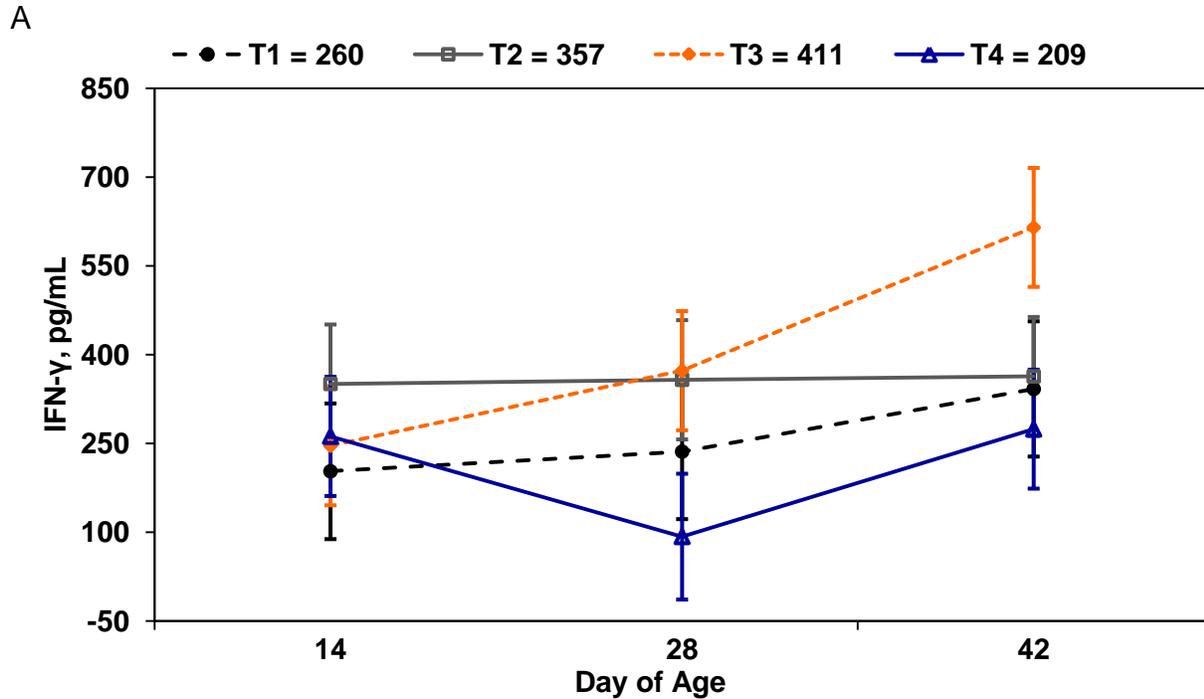
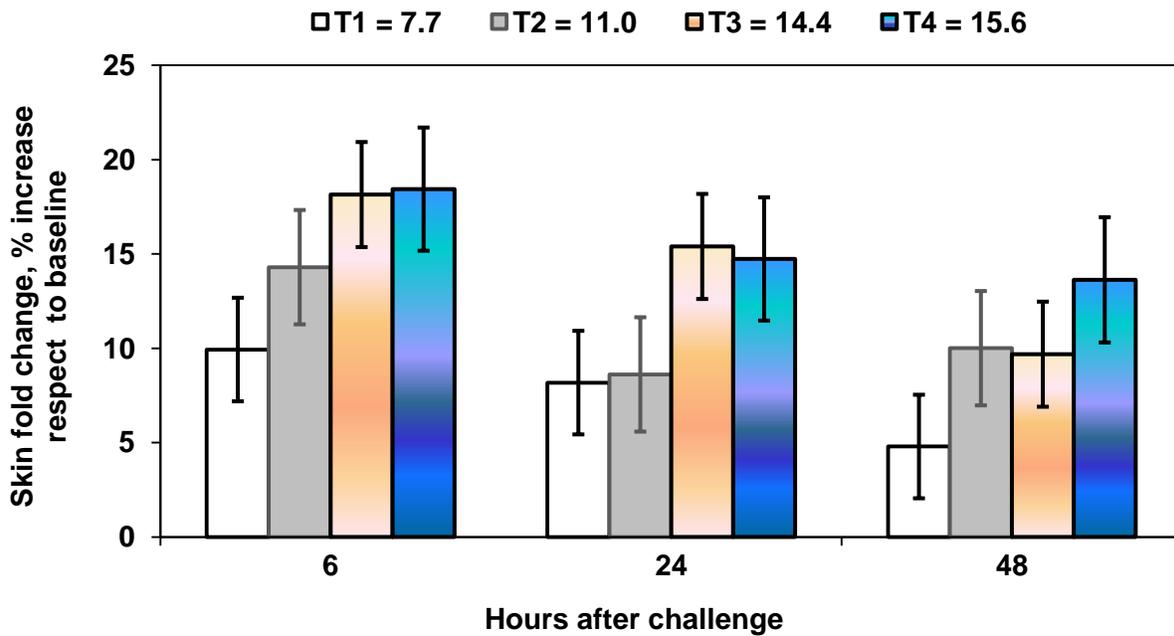


Figure 6-19. Interferon - γ produced by stimulated whole blood cells of preweaned Holstein calves fed increased intake of linoleic acid. A) Males. B) Females. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1= 0.144 g LA/WB^{0.75}, T2= 0.206 g LA/WB^{0.75}, T3= 0.333 g LA/WB^{0.75}, T4= 0.586 g LA/WB^{0.75}). Quadratic effect of treatment by gender, $P = 0.09$, effect of age, $P = 0.01$.

A



B

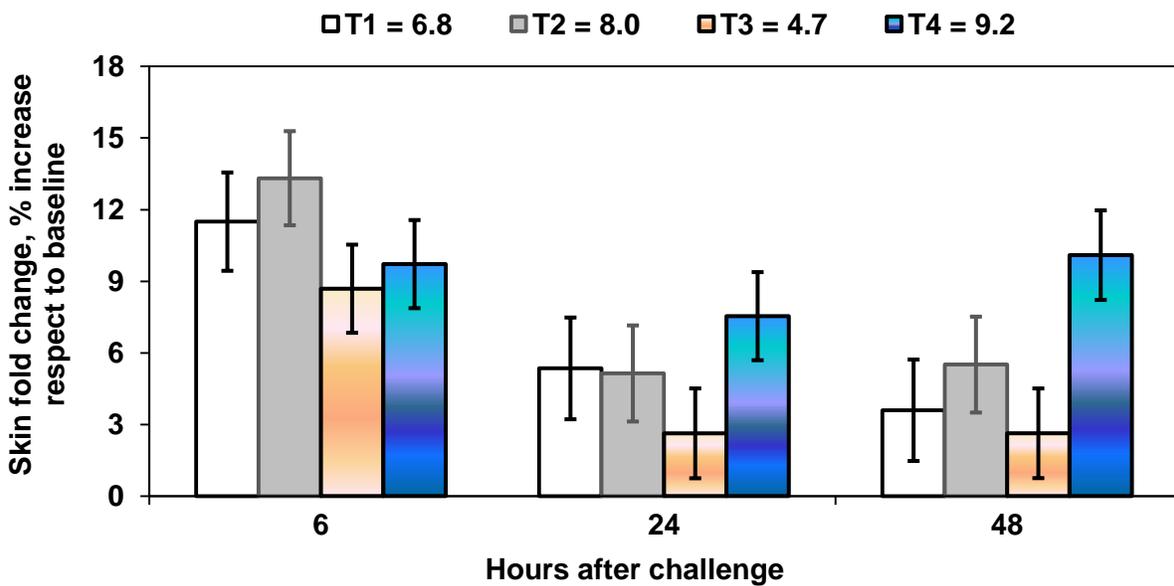


Figure 6-20. Skin fold change after phytohaemagglutinin injection as percentage of the baseline measure in responsive Holstein calves fed increased intake of linoleic acid. A) Measured at 30 days of age. B) Measured at 60 days of age. Calves were assigned to one of four treatments with increased intake of linoleic acid (T1 = 0.144 g LA/WB^{0.75}, T2 = 0.206 g LA/WB^{0.75}, T3 = 0.333 g LA/WB^{0.75}, T4 = 0.586 g LA/WB^{0.75}). A) Linear effect of treatment, $P = 0.03$, effect of h post challenge, $P < 0.01$. B) Quadratic effect of treatment, $P = 0.09$, effect of h post challenge, $P < 0.01$.

CHAPTER 7 GENERAL DISCUSSION AND CONCLUSIONS

The experiments presented here were conducted with two objectives. The first general objective was to evaluate the effect of supplementing essential fatty acids (FA) to prepartum Holstein cattle and to their newborn calves during the first 60 days of life on calf growth and development. The transfer of FA fed prepartum to colostrum was influenced by the dietary FA profile and by its metabolism in the rumen of the pregnant cattle. Colostrum of nulliparous heifers had greater proportions of ALA, AA, EPA, DPA, and DHA whereas LA was greater in colostrum of fat from parous cows. The major individual CLA detected in the current study was CLA c9, t11, whereas CLA t10, c12 was detected only in cows fed EFA but in limited concentrations. Increased proportions of LA and its n-6 derivatives indicate that elongase / desaturase activities in the mammary gland were taking place. However, increased proportions of total and individual CLA, as well as total C18:1 *trans* FA in colostrum of dams fed EFA, indicate that the Ca salt of EFA were not completely effective in preventing the processes of biohydrogenation by ruminal microbes.

Intake of IgG did not differ due to dietary treatments but serum concentrations of total IgG (2.83 vs. 2.44 g/dL) and anti-OVA IgG (1.13 vs. 0.90 OD) after colostrum feeding were greater in calves born from cattle supplemented with SFA vs. EFA. Feeding of fat prepartum improved AEA across parities from 23.3 to 27.9% regardless of type of fat supplemented. It is possible that cattle fed fat gave birth to calves that had a more efficient mechanism to transfer IgG into circulation, possibly by modifying the activity of FcRn receptors in the intestinal tract due to the likely differential composition of FA in the cell membrane.

The second study involved the strategic feeding of EFA, both during the nonlactating pregnant period and in early life. The FA status of newborn calves was affected by the type of fat supplemented prepartum. Calves born from cows fed EFA had increased concentrations of LA in plasma but AA concentration was unaffected by type of diet; however GLA and C20:3 n-6, which are precursors of AA in the elongation-desaturation steps, were greater in plasma of calves born from dams fed EFA. The increased proportions of these intermediate FA might indicate that the enzymatic activity of FA desaturases and elongases that are shared by both n-6 and n-3 groups of FA was preferentially metabolizing LA over ALA in dams supplemented with fat enriched in LA, although final end products of AA and C22:4 were not increased significantly. Interestingly, supplementing SFA prepartum increased the proportions of EPA and DHA in plasma of newborn calves. Another important finding is the parity effect on proportion of EFA and their derivatives. Calves born from nulliparous heifers had increased plasma concentrations of n-3 FA such as EPA, DPA, and DHA but decreased LA and AA. Although the plasma of dams was not analyzed for FA, the FA profile of colostrum was analyzed. This result is in agreement with the FA profile reported for colostrum of nulliparous heifers that had concentrations of ALA, AA, EPA, DPA, and DHA whereas LA was greater in colostrum of parous cows (Chapter 3).

Calves born from dams fed SFA, although not statistically different, were 0.5 kg heavier than calves born from dams fed EFA. In addition dams fed SFA ate more DM than dams fed EFA (Greco et al., 2010). Calves of SFA-fed dams had greater grain mix intake during 31 to 60 d of age. This greater intake resulted in a better ADG. Whether a direct relation between dam and calf performance exists is not clear. The increased

intake of grain did not change the plasma concentrations of energy and protein metabolites as reported by (Laarman et al., 2012) because calves born from SFA- or EFA-fed dams did not differ in plasma concentrations of glucose or PUN. Moreover, calves born from dams fed SFA demonstrated improvements in immunity as evidenced by a greater concentration of anti-OVA IgG and greater synthesis of IFN- γ by PBMC at 15 d of life. When these calves were fed MR enriched in LA, they had lower fecal and better attitude scores at 2 wk of age.

Plasma concentrations of LA in newborn calves increased markedly at 30 and 60 d of life from that at birth (~11.5 fold increase). The concentration of fat in plasma was less in calves fed the HLA MR which may result from a greater digestibility of the FA in porcine lard compared to CCO (Murley et al., 1949). Feeding a MR containing a highly saturated FA fat source (CCO) resulted in elevated plasma concentrations of C10:0, C12:0, and C14:0, as reported by others (Jenkins and Kramer, 1986). Likewise, calves fed a MR containing a combination of CCO and a highly unsaturated FA fat source (porcine lard) had increased plasma concentrations of LA and ALA, similar to the findings of Wrenn et al. (1973) and Jenkins and Kramer (1986, 1990). Calves fed HLA MR (0.487 g of LA/kg of BW^{0.75}) had an improved ADG and FE during throughout the 60-d preweaning period. Increased ADG was not accompanied by greater DMI. However, this better growth was accompanied by increased concentrations of anabolic metabolites and hormones, which agrees with studies reporting increased concentrations of plasma anabolic metabolites and hormones in faster-growing calves (Smith et al., 2002; Quigley et al., 2006). On the other hand, plasma concentrations of cholesterol and BHBA were in lower concentrations in plasma of calves fed HLA MR.

One of the “healthy properties” of PUFA, including LA, is to reduced circulating concentrations of total lipids and cholesterol by regulating their metabolism at the liver by enhancing lipid oxidation and reducing lipid accumulation and export. Thus reduced circulating concentrations of cholesterol, BHBA and total lipid in plasma might indicate a better efficiency of nutrient utilization. Moreover, feeding HLA MR appeared to improve immune responses by increasing the number of circulating lymphocytes and possibly by enhancing the switch from a Th2 to a Th1 response by the increased production of IFN- γ observed under in vitro stimulation of PBMC.

The combined effect of feeding fat prepartum and a LA-enriched MR during the preweaning period appeared to modify the ability of tissues to synthesize essential FA derivatives due to differential proportion of LA and ALA calves had when they were born. No apparent effect of prepartum diets to modify performance of calves fed LA in MR was observed. However calves fed a MR enriched in LA and born from dams fed fat experienced fewer days of diarrhea and poor attitude. This interaction effect might be mediated by the passive transfer of IgG which tended to be in greater concentrations in calves born from dams fed fat as compared to those born from control dams.

In Chapter 5, strategic feeding of FA during the prepartum and preweaning periods modified the response of liver to different metabolic processes. This differential profile of liver FA might have modified the activity of liver regarding expression of hepatic genes. Ability of liver to mimic dietary FA profile, was markedly affected by the MR fed rather than by prepartum diets or its combined effect with MR. Greater effect of MR were verified by the increased proportions of C12:0 and C14:0 in calves fed a MR formulated with CCO, whereas when CCO was partially replaced by porcine lard, the liver

contained greater proportion of LA and three of its derivative FA. Concentrations of total FA was greater in calves fed LLA MR, which was expected based on the results in Chapter(4 where calves fed LLA MR had increased concentrations of circulating BHBA, cholesterol and total FA. First calves fed LLA MR had also greater proportions of total FA in liver as compared to calves fed HLA MR. Liver of calves fed HLA MR had upregulated the expression of PPARA gene, which is a potent inducer of lipid oxidation and utilization in liver. However an interesting interaction was observed in calves fed HLA MR when they were born from dams fed FAT instead of control diet. A greater number of genes (n = 6) coding for enzymes involved in lipid utilization might indicate that the prepartum feeding of fat increased the effect of MR per se. In addition calves in this interaction also had upregulated another group of genes involved in FA metabolism, glycerolipid metabolism and AA metabolism. The upregulation of genes in all aforementioned pathways might indicate that these calves were certainly undergoing a preferential degradation of lipids.

Feeding a specific profile of FA in the late gestation period also modified the response of calves fed a MR enriched in LA. Liver of calves fed porcine lard and born from dams fed EFA instead of SFA had upregulated genes involved in glycolysis and oxidative phosphorylation. The increased oxidative phosphorylation could have a negative impact on tissue stability if excessive amount of free radicals are produced. On the other hand, calves in this group had more downregulated genes involved in regulation of inflammatory responses. This effect could have a positive impact limiting exaggerated inflammatory responses that could negatively impact liver function. However, a potential

attenuated inflammatory response that could negatively impact calf survival could not be ruled out.

First lactation milk yield by heifers born and used in this study was not influenced by the MR fed. However feeding fat during late gestation instead of a control no fat-diet resulted in 13% greater milk production at first lactation (12,004 vs. 10,605 kg). Other studies have reported positive impacts of improved ADG during preweaning on future milk production but in the current study only a numerical increase of 5.3% in milk yield was observed for calves having a faster growth rate due to consumption of a MR containing porcine lard instead of CCO. Findings in this study reveal a strong effect of prepartum diets during the fetal period to modify the response of calves to strategic supplementation of FA during the preweaning period. However, long term effects of prepartum diets, regardless of the preweaning diet, suggests that the more critical period of programming through nutrition occurred during late gestation. Future research should focus on detailing the mechanisms by which designated expressed genes (DEG) due to strategic lipid supplementation modify the production and activity of the proteins encoded by the DEG. Moreover, more efforts should be made to evaluate nutritionally strategies that would positively impact fetus and newborn calves so as to improve their future performance.

The last study aimed to determine the requirement of LA in preweaned Holstein calves. Four dietary intakes of LA were formulated (0.144, 0.206, 0.333 and 0.586 g of LA/kg of BW^{0.75}) based on the recommendation of LA for laboratory rats. Calves in this study were exclusively fed MR during the first 30 d of life. However, body weight gain in this study was poor, ADG averaged 111 g/d and FE at 180 g of gain/g of DMI. The first

30 d of life was the only period in which LA intake affected BW gain. Male calves fed 0.206 g of LA/kg of BW^{0.75} had the greater ADG whereas females linearly increased the ADG as intake of LA increased. Studies performed in rats reported that female rats have about one third of the male requirement for LA (0.5% vs. 1.3% of ME;(Greenberg et al., 1950; Pudelnkewicz et al., 1968). The current results, based on the performance obtained in the first 30 d, seem to oppose of the findings observed in rats. All calves in this study suffered from diarrhea starting at a mean of 7 d of age in calves fed T1, with the onset tending to be linearly delayed slightly with increasing intake of LA. Episodes of disease in preweaned calves are the main drivers of reduced performance. Early studies replaced milk fat with vegetable oils such as coconut oil, corn oil, and tallow. Feeding CO resulted in calves with greater episodes of diarrhea and hence resulted in more attenuated BW gain (Jenkins et al., 1985). However in the current study, increasing intake of LA linearly reduced the severity of diarrhea. Body weight of female calves between 31 to 60 d of age, was similar to that obtained by commercial farms but did not differ with increased intake of LA. As expected due to poor BW the first 30 d of life and recovered BW the second 30 d of life, but without effect of treatments, plasma concentrations of metabolites, glucose, PUN, and hormones, insulin, and IGF-I did not differ along the 60-d period. However, concentrations of BHBA increased linearly with intake of LA whereas concentrations of total plasma cholesterol surprisingly increased linearly with intake of LA. In a previous study, presented in chapter 4, calves fed increased intake of LA had reduced concentrations of total cholesterol in plasma. Feeding PUFA have been well documented to reduce circulating levels of cholesterol

and triglycerides, hence it is not clear why in the current study this mechanism did not work.

If these calves were experiencing nutritional stress based upon low BW gain the first 30 d of life, increased feeding of LA may not have been able to optimize gain but may have been able to influence immune responses. Population of blood cells were not influenced by increased intake of LA but it changed along calf age, in a pattern expected for their age. However, regardless of the treatment, concentrations of neutrophils and haptoglobin peaked around d 7 to 8 which was the period in which episodes of diarrhea began. Proliferation of T cells after 48-h of in vitro stimulation with LPS + PHA was greater in calves fed 0.206 g of LA/kg of BW^{0.75}) and this held true at 14, 28 and 42 d of age whereas calves fed 0.333 g of LA/kg of BW^{0.75} responded well only at 42 d of age. Linoleic acid is commonly identified as having proinflammatory activities. However an antiinflammatory activity, by reducing the proliferation of lymphocytes has been reported when increased concentrations of LA were added to media containing PBMC (Thanasak et al., 2005; Gorjao et al., 2007). Based on the proliferative responses of calves fed 0.333 or 0.586 g of LA/kg of BW^{0.75} as compared to 0.144 g of LA/kg of BW^{0.75}, although the rate of proliferation was minimal, it can be inferred that the different intakes of LA provided in the current study would not have toxic effects on lymphocytes that could prevent its proliferation. The production of IFN- γ by stimulated lymphocytes in whole blood was increased in calves fed 0.333 and 0.586 g of LA/kg of BW^{0.75}. This result also corroborates the postulation that the intakes of LA were not preventing lymphocytes to proliferate. Moreover, the delayed type hypersensitivity analysis indicated that 60-d old calves fed 0.586 g of LA/kg of BW^{0.75}

had the greatest skin response to an intradermal injection of PHA. Overall, feeding diets of 0.333 or 0.586 g of LA/kg of BW^{0.75} to preweaned Holstein calves increased responses for most of the markers of immunity evaluated in this study and improved wither and hip growth and severity of feces and attitude scores, hence a minimum intake of LA by dairy calves should be at least 0.206 g/kg of BW^{0.75}.

Strategic feeding of LA during the first 60 d (0.487 g/kg of BW^{0.75}) of life improved overall performance in a first study; however intakes at and above 0.206 g/kg of BW^{0.75} improved the response of calves in a second study. Future research should clarify the mechanisms by which targeted intake of LA might differentially modify the response of healthy and unhealthy calves.

APPENDIX A
LIST OF DIFFERENTIALLY EXPRESSED GENES

List of differential expressed genes in liver of Holstein male calves fed milk replacer containing low or high linoleic acid from 1 to 30 days of age. Males were born from dams fed diets supplemented with no fat (CTL), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Genes are ranked for alphabetical order according gene symbol

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.6156.1.S1_at	3290025600	apoptosis related protein 3	1187	642	958	974	899	1074
Bt.9298.1.S1_at	AARSD1	alanyl-tRNA synthetase domain containing 1	71.51	58.26	96.24	63.98	51.55	87.66
Bt.20249.1.S1_a_at	ABCD3	ATP-binding cassette, sub-family D (ALD), member 3	1480	1044	1380	1383	1445	1445
Bt.10387.1.S1_at	ABCF1	ATP-binding cassette, sub-family F (GCN20), member 1	181	262	234	165	114	233
Bt.20453.1.S1_at	ABHD14A	abhydrolase domain containing 14A	116	66.12	91.61	100	71.98	95.25
Bt.2858.1.S1_at	ABHD6	abhydrolase domain containing 6	44.06	46.67	69.33	38.77	29.14	44.11
Bt.5188.1.S1_at	ABTB1	ankyrin repeat and BTB (POZ) domain containing 1	133	147	129	155	169	76.64
Bt.2050.1.A1_at	ACAA1	acetyl-CoA acyltransferase 1	7042	4021	6667	7640	5981	7710
Bt.27073.1.S1_at	ACADL	acyl-CoA dehydrogenase, long chain	802	493	731	980	985	1050
Bt.28278.1.S1_at	ACE2	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	867	247	1177	1326	859	1016
Bt.21101.1.A1_at	ACMSD	aminocarboxymuconate semialdehyde decarboxylase	355	153	426	128	454	293
Bt.6177.1.S1_at	ACOT8	acyl-CoA thioesterase 8	146	40.99	103	83.20	74.73	141
Bt.5193.1.S1_at	ACP5	acid phosphatase 5, tartrate resistant	354	192	225	267	210	316
Bt.5193.2.S1_a_at	ACP5	acid phosphatase 5, tartrate resistant	2197	1213	1426	1685	1385	1999
Bt.15886.1.S1_at	ACSL5	acyl-CoA synthetase long-chain family member 5	7508	8409	9820	8272	4538	9079
Bt.4604.1.S1_a_at	ACSM1	acyl-CoA synthetase medium-chain family member 1	6824	7425	7465	6766	4089	7292
Bt.19544.1.A1_at	ACSM2A	acyl-CoA synthetase medium-chain family member 2A	4049	3041	5609	4253	5104	4881
Bt.8435.1.S1_at	ACTA1	actin, alpha 1, skeletal muscle	4.76	402	4.94	4.75	4.93	4.94
Bt.20557.1.S1_at	ACTN2	actinin, alpha 2	5.09	41.28	4.76	4.89	5.17	5.01
Bt.12030.2.S1_at	ACTN4	actinin, alpha 4	79.59	83.85	98.96	79.15	58.56	97.26
Bt.19723.1.A1_at	ACTR10	actin-related protein 10 homolog (S. cerevisiae)	2390	2135	1489	2163	2827	2019
Bt.26992.1.A1_at	ADAM10	ADAM metallopeptidase domain 10	938	1017	641	917	1055	798
Bt.805.1.S1_at	ADIPOR2	adiponectin receptor 2	410	223	372	357	202	520
Bt.22590.1.S1_at	AGPAT2	1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	108	47.38	71.25	88.94	35.16	103
Bt.22170.1.S1_a_at	AGPAT5	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon)	394	303	350	404	428	479

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.2048.1.S1_at	AGPS	Alkylglycerone phosphate synthase	195	190	142	204	216	196
Bt.6813.1.A1_at	AKAP5	A kinase (PRKA) anchor protein 5	61.18	54.75	111	74.24	124	83.74
Bt.4449.1.S1_at	AKR1A1	aldo-keto reductase family 1, member A1 (aldehyde reductase)	1176	490	1050	913	834	1062
Bt.11078.2.S1_at	AKR7A2	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	69.41	45.06	68.41	61.05	33.40	75.85
Bt.24662.1.S1_at	AKT1S1	AKT1 substrate 1 (proline-rich)	70.89	64.02	64.05	53.06	37.41	67.76
Bt.3248.1.S1_at	ALDH4A1	aldehyde dehydrogenase 4 family, member A1	284	217	350	300	164	321
Bt.16137.1.S1_at	ALDH9A1	aldehyde dehydrogenase 9 family, member A1	387	288	700	508	346	688
Bt.22533.1.S1_at	ALDOA	aldolase A, fructose-bisphosphate	751	928	804	641	501	1120
Bt.20207.1.A1_at	ALG12	asparagine-linked glycosylation 12, alpha-1,6-mannosyltransferase homolog (S. cerevisiae)	40.21	37.37	35.21	28.79	23.52	32.68
Bt.18435.3.A1_at	ANGEL1	angel homolog 1 (Drosophila)	156	103	81.28	153	125	127
Bt.24203.1.S1_at	ANGPTL3	angiopoietin-like 3	3778	3232	3045	3139	4713	2926
Bt.4816.1.S1_at	ANGPTL4	angiopoietin-like 4	58.16	83.01	62.49	70.46	60.56	135
Bt.9069.1.S1_at	ANKRD10	ankyrin repeat domain 10	414	499	456	575	493	412
Bt.22626.1.A1_at	ANKRD12	ankyrin repeat domain 12	132	232	160	166	198	159
Bt.28798.1.A1_at	ANKRD22	Ankyrin repeat domain 22	6.61	10.77	5.99	5.63	7.92	5.74
Bt.21981.3.S1_at	ANTXR1	anthrax toxin receptor 1	109	199	164	134	195	183
Bt.12745.1.A1_at	ANTXR2	anthrax toxin receptor 2	66.95	72.80	79.83	89.73	97.62	149
Bt.27322.1.S1_at	AP1AR	adaptor-related protein complex 1 associated regulatory protein	160	150	138	188	302	157
Bt.8775.1.S1_at	AP1B1	adaptor-related protein complex 1, beta 1 subunit	426	452	520	430	338	452
Bt.2056.1.S1_at	APEH	N-acylaminoacyl-peptide hydrolase	474	318	501	519	412	484
Bt.26604.1.S1_at	APLNR	apelin receptor	335	170	214	325	159	245
Bt.22694.1.A1_at	APOA5	apolipoprotein A-V	3686	1726	3750	3676	2751	3798
Bt.17961.1.S1_at	APOC4	apolipoprotein C-IV	6157	4066	5987	5874	4642	6016
Bt.9735.1.S1_at	APOM	apolipoprotein M	1541	586	1348	884	1157	1132
Bt.9735.2.A1_at	APOM	apolipoprotein M	2499	1100	2211	1653	1850	2086
Bt.19980.2.S1_at	ApoN	ovarian and testicular apolipoprotein N	1313	764	1257	1305	978	1320
Bt.28934.1.S1_at	AREG	amphiregulin	6.04	7.72	7.62	5.70	7.77	61.27
Bt.14075.1.S1_at	ARHGAP5	Rho GTPase activating protein 5	223	176	209	242	381	222
Bt.20329.2.S1_at	ARL4D	ADP-ribosylation factor-like 4D	221	149	221	247	132	290
Bt.17432.1.S1_at	ARL5B	ADP-ribosylation factor-like 5B	277	348	281	312	394	277
Bt.8078.1.S1_at	ARPC4	actin related protein 2/3 complex, subunit 4, 20kDa	61.30	60.51	78.09	53.70	33.96	54.87
Bt.16276.1.A1_at	ARSK	arylsulfatase family, member K	402	234	300	478	688	447
Bt.18330.2.S1_at	ASGR2	asialoglycoprotein receptor 2	580	322	582	543	479	685
Bt.18037.2.A1_at	ASPDH	aspartate dehydrogenase domain containing	125	45.29	96.25	65.81	72.86	78.18
Bt.24211.1.A1_at	ASPN	asporin	1700	2582	1779	1689	2802	1850
Bt.8053.1.S1_at	ATAD1	ATPase family, AAA domain containing 1	1136	1913	1202	1480	1236	1154
Bt.20514.1.S1_at	ATG2B	similar to ATG2 autophagy related 2 homolog B	226	258	361	344	333	351

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.20206.1.A1_at	ATP11B	ATPase, class VI, type 11B	502	394	377	626	629	501
Bt.1059.3.S1_a_at	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	429	543	628	455	267	553
Bt.4431.1.S1_a_at	ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	4577	4319	4634	3881	3743	4903
Bt.1753.1.S1_at	ATP6V1E1	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1	828	875	855	683	601	850
Bt.25471.1.S1_at	ATXN3	ataxin 3	12.07	21.97	13.02	18.84	33.42	14.13
Bt.25471.2.A1_at	ATXN3	ataxin 3	56.98	70.51	55.05	75.07	117	64.05
Bt.14059.1.A1_at	AUH	AU RNA binding protein/enoyl-CoA hydratase	2639	2349	2087	2764	3512	2901
Bt.4898.1.S1_at	BASP1	brain abundant, membrane attached signal protein 1	689	932	787	806	928	699
Bt.22524.2.A1_at	BBS5	Bardet-Biedl syndrome 5	190	186	117	203	198	181
Bt.5412.1.S1_at	BCKDHB	branched chain keto acid dehydrogenase E1, beta polypeptide	2811	1938	2420	2569	2871	2533
Bt.11445.1.A1_at	BCL10	B-cell CLL/lymphoma 10	518	430	290	516	659	437
Bt.11043.1.S1_a_at	BCL2L12	BCL2-like 12 (proline rich)	4.51	6.45	4.63	4.63	4.63	4.51
Bt.9391.2.S1_at	BIRC3	baculoviral IAP repeat-containing 3	170	241	191	242	282	181
Bt.2824.1.S1_at	BLOC1S1	biogenesis of lysosomal organelles complex-1, subunit 1	1098	661	853	977	829	931
Bt.29823.1.S1_x_at	BOLA	MHC class I heavy chain	15.02	148	15.35	16.98	23.70	27.24
Bt.29823.1.S1_at	BOLA	MHC class I heavy chain	14.45	111	20.85	15.79	26.79	20.57
Bt.8121.1.S1_x_at	BOLA	MHC class I heavy chain	4138	2983	3645	2467	1999	3497
Bt.4762.1.S1_at	BOLA-NC1	non-classical MHC class I antigen	49.15	60.96	46.95	29.48	29.65	29.37
Bt.1048.1.S1_at	BORA	aurora borealis	48.39	72.93	52.36	47.06	71.65	74.67
Bt.21099.1.A1_at	BRMS1L	breast cancer metastasis-suppressor 1-like	101	112	101	114	180	116
Bt.26364.1.A1_at	BTBD8	BTB (POZ) domain containing 8	16.46	19.91	11.39	16.05	46.71	15.96
Bt.19064.1.A1_at	BTD	biotinidase	354	195	256	265	205	283
Bt.22510.1.S1_at	C11H2ORF7	chromosome 2 open reading frame 7 ortholog	477	290	317	365	236	465
Bt.8903.1.S1_at	C14H8ORF70	chromosome 8 open reading frame 70 ortholog	280	201	175	181	301	221
Bt.9310.1.S1_at	C16orf5	chromosome 16 open reading frame 5	54.91	38.46	38.30	49.40	37.32	45.14
Bt.26522.2.S1_at	C1H3ORF34	chromosome 3 open reading frame 34 ortholog	47.12	37.52	46.77	34.56	62.48	53.69
Bt.19274.1.A1_at	C1QTNF7	C1q and tumor necrosis factor related protein 7	4.65	4.60	4.65	4.65	11.76	4.68
Bt.2481.2.S1_at	C23H6ORF105	Chromosome 6 open reading frame 105 ortholog	1172	596	769	1187	888	1072
Bt.3865.3.S1_a_at	C25H16orf14	chromosome 16 open reading frame 14 ortholog	396	207	326	403	188	354
Bt.20997.1.S1_at	C2H1orf144	chromosome 1 open reading frame 144 ortholog	57.10	44.93	83.49	50.31	27.07	59.60
Bt.19664.1.A1_at	C3H1ORF210	chromosome 1 open reading frame 210 ortholog	168	79.30	77.95	121	73.45	139
Bt.4507.1.S1_at	C4A	complement component 4A	8023	15537	10673	8089	6089	8548
Bt.16789.1.A1_at	C5H12orf11	chromosome 12 open reading frame 11 ortholog	76.27	75.02	79.71	85.26	141	76.27
Bt.25752.1.A1_at	C7H5orf24	chromosome 5 open reading frame 24 ortholog	53.96	36.64	72.85	69.09	66.32	54.91

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.5164.1.S1_at	CA14	carbonic anhydrase XIV	82.25	24.90	41.91	89.65	22.29	46.59
Bt.23960.1.S1_at	CA5B	carbonic anhydrase VB, mitochondrial	44.81	40.52	34.29	57.14	66.45	43.96
Bt.16382.1.A1_at	CALCRL	calcitonin receptor-like	235	215	169	219	396	289
Bt.26832.1.S1_at	CANT1	calcium activated nucleotidase 1	72.90	38.14	32.06	82.11	22.15	61.95
Bt.6686.1.S1_at	CASK	calcium/calmodulin-dependent serine protein kinase (MAGUK family)	123	160	134	109	198	132
Bt.10084.1.S1_at	CASP3	caspase 3, apoptosis-related cysteine peptidase	233	205	180	210	287	174
Bt.13989.1.A1_at	CAV2	caveolin 2	161	150	117	156	232	155
Bt.15971.1.S1_at	CCAR1	cell division cycle and apoptosis regulator 1	402	571	451	459	658	413
Bt.4405.1.S1_s_at	CCDC104	coiled-coil domain containing 104	241	246	230	241	339	234
Bt.18220.1.A1_at	CCDC112	coiled-coil domain containing 112	26.11	29.07	23.00	35.79	63.76	26.54
Bt.29506.1.S1_at	CCDC82	coiled-coil domain containing 82	65.79	66.53	54.94	79.09	117	52.48
Bt.26562.2.S1_at	CCDC86	coiled-coil domain containing 86	4.75	7.60	4.89	4.84	4.72	4.79
Bt.9974.1.S1_at	CCL3	chemokine (C-C motif) ligand 3	83.61	127	80.02	216	169	82.50
Bt.9974.1.S1_a_at	CCL3	chemokine (C-C motif) ligand 3	20.20	22.73	17.36	25.69	24.05	12.33
Bt.154.1.S1_at	CCL8	chemokine (C-C motif) ligand 8	9.56	35.58	11.23	20.96	9.82	11.16
Bt.23572.1.S1_at	CCNDBP1	cyclin D-type binding-protein 1	908	569	528	713	471	829
Bt.20977.3.S1_at	CCPG1	cell cycle progression 1	97.36	95.49	87.88	95.34	180	92.65
Bt.22069.1.A1_at	CCPG1	Cell cycle progression 1	278	266	221	248	370	220
Bt.5415.1.S1_at	CCS	copper chaperone for superoxide dismutase	522	299	441	445	138	161
Bt.5096.1.S1_at	CCT3	chaperonin containing TCP1, subunit 3 (gamma)	485	454	691	381	313	555
Bt.16580.1.S1_at	CD2AP	CD2-associated protein	16.88	30.42	23.17	48.32	48.85	28.77
Bt.13864.1.A1_at	CDC26	cell division cycle 26 homolog (S. cerevisiae)	689	445	587	655	553	565
Bt.1667.1.S1_at	CDC34	cell division cycle 34 homolog (S. cerevisiae)	846	498	644	771	434	709
Bt.20490.1.S1_at	CDC42EP4	CDC42 effector protein (Rho GTPase binding) 4	1397	1417	1406	2103	828	1261
Bt.23366.1.S1_at	CDIPT	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	254	222	299	201	159	286
Bt.2.1.S1_at	CDK1	cyclin-dependent kinase 1	19.63	20.54	20.86	17.60	47.67	22.80
Bt.27042.1.S1_at	CENPC1	centromere protein C 1	54.53	79.14	53.38	65.42	109	53.18
Bt.14213.1.A1_at	CES2	carboxylesterase 2 (intestine, liver)	2528	1830	2596	2389	2099	3155
Bt.4336.1.S1_at	CFD	complement factor D (adipsin)	1905	792	1613	2077	1539	2023
Bt.13556.1.S1_at	CFH	complement factor H	1346	1065	1317	1358	2035	704
Bt.17612.2.S1_at	CFHR4	complement factor H-related 4	4578	6584	4003	2920	4835	4204
Bt.24506.2.A1_at	CHIC2	cysteine-rich hydrophobic domain 2	25.22	24.93	18.72	24.76	34.92	25.85
Bt.11411.1.S1_at	CIAPIN1	cytokine induced apoptosis inhibitor 1	228	250	254	151	119	199
Bt.13381.1.S1_at	CIDEC	cell death-inducing DFFA-like effector c	5.03	4.65	4.85	4.73	4.88	9.09
Bt.10007.1.A1_at	CKAP2	cytoskeleton associated protein 2	73.40	75.56	54.64	56.17	144	78.75
Bt.12980.3.S1_a_at	CL43	collectin-43	10800	9177	13456	9711	4595	9921
Bt.11279.1.A1_at	CLCN4	chloride channel 4	94.58	57.27	101	83.43	60.42	129
Bt.27474.1.S1_at	CLEC4F	C-type lectin domain family 4, member F	23.63	130	32.44	147	17.24	60.25

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.21113.1.S1_at	CNDP2	CNDP dipeptidase 2 (metallopeptidase M20 family)	1399	1447	1738	1273	955	1606
Bt.11256.1.S1_at	CNOT1	CCR4-NOT transcription complex, subunit 1	1032	1321	1503	1095	682	1081
Bt.19218.2.S1_at	CNOT6	CCR4-NOT transcription complex, subunit 6	368	341	326	404	509	365
Bt.8617.1.S1_at	CNRIP1	cannabinoid receptor interacting protein 1	178	110	118	132	115	151
Bt.26828.1.S1_at	CNTLN	centlein, centrosomal protein	84.17	94.10	56.66	108	182	66.68
Bt.21467.1.S1_at	COG4	component of oligomeric golgi complex 4	141	171	197	114	125	163
Bt.4141.1.S1_at	COPE	coatamer protein complex, subunit epsilon	406	283	386	331	247	414
Bt.1332.1.S1_a_at	COX10	COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast)	90.90	93.65	131	87.06	65.56	85.72
Bt.395.1.S1_at	COX8B	cytochrome c oxidase subunit VIII-H (heart/muscle)	4.55	8.58	4.54	4.55	4.54	4.55
Bt.22479.1.S1_at	CPEB4	cytoplasmic polyadenylation element binding protein 4	13.95	14.04	14.73	15.06	28.78	16.89
Bt.25663.1.A1_at	CPNE8	copine VIII	107	109	138	176	196	199
Bt.24779.2.S1_at	CREM	cAMP responsive element modulator	5.34	5.08	10.63	5.17	12.38	6.84
Bt.1927.1.S1_at	CRISPLD2 /// TIMM13	cysteine-rich secretory protein LCCL domain containing 2 /// translocase of inner mitochondrial membrane 13 homolog (yeast)	69.73	156	109	95.87	96.37	99.39
Bt.23143.2.S1_at	CSDE1	cold shock domain containing E1, RNA-binding	2118	1503	1814	2085	2360	2163
Bt.22563.1.A1_s_at	CSDE1	cold shock domain containing E1, RNA-binding	1228	889	1062	1257	1456	1173
Bt.6646.1.S1_at	CTDSP1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	60.17	45.44	48.46	61.50	23.83	52.55
Bt.5240.1.S1_at	CTGF	connective tissue growth factor	57.00	136	113	95.23	78.91	82.21
Bt.4150.1.S1_at	CTNBL1	catenin, beta like 1	618	390	572	647	431	619
Bt.4902.1.S1_at	CTSZ	cathepsin Z	3142	2751	3730	2462	2973	3733
Bt.18003.1.S1_at	CUL3	cullin 3	9.34	9.10	9.64	15.51	23.48	11.33
Bt.23998.1.A1_a_at	CUX2	cut-like homeobox 2	100	176	69.49	160	173	94.93
Bt.21216.1.S1_at	CXorf56	chromosome X open reading frame 56 ortholog	336	350	416	313	245	396
Bt.10609.2.A1_at	CYP20A1	cytochrome P450, family 20, subfamily A, polypeptide 1	623	392	360	604	552	605
Bt.9699.1.S1_at	CYP26A1	cytochrome P450, family 26, subfamily A, polypeptide 1	3801	2270	1496	4297	754	3765
Bt.16001.1.S1_at	CYP27A1	cytochrome P450, family 27, subfamily A, polypeptide 1	3443	1869	2926	2793	2367	2772
Bt.12255.1.A1_at	CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	24.47	19.90	34.35	24.68	38.41	20.55
Bt.23912.1.A1_a_at	CYP2E1	cytochrome P450, family 2, subfamily E, polypeptide 1	1650	1346	884	2057	753	1838
Bt.14369.1.A1_at	CYP39A1	cytochrome P450, family 39, subfamily A, polypeptide 1	110	131	126	87.39	185	168
Bt.4126.1.A1_at	CYP4A11	cytochrome P450, family 4, subfamily A, polypeptide 11	6763	5881	6267	7116	5348	7182

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.27036.1.S1_at	CYP4F2	cytochrome P450, family 4, subfamily F, polypeptide 2	3251	1464	1955	3247	1338	2597
Bt.13530.1.S1_at	DCI	dodecenoyl-CoA isomerase	3449	2063	3493	3439	2770	3376
Bt.23178.1.S2_at	DCN	decorin	4023	4244	3077	4081	5309	4139
Bt.18792.1.S1_at	DCTN6	Dynactin 6	27.36	28.49	25.51	27.88	104	29.94
Bt.12508.1.S1_at	DCTPP1	dCTP pyrophosphatase 1	70.20	57.73	78.96	46.72	42.61	79.22
Bt.22199.1.S1_at	DDIT4L	DNA-damage-inducible transcript 4-like	5.68	14.18	5.90	5.41	5.84	5.93
Bt.9047.1.S1_at	DDT	D-dopachrome tautomerase	5288	3157	4816	4503	4453	4461
Bt.8323.1.S1_at	DDX21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	668	921	668	657	682	664
Bt.6334.1.A1_at	DEGS1	degenerative spermatocyte homolog 1, lipid desaturase (Drosophila)	1237	1083	950	1269	921	1180
Bt.6141.1.S1_at	DES	desmin	14.09	20.47	14.61	16.01	10.63	19.86
Bt.16832.1.A1_at	DHDPSL	dihydrodipicolinate synthase-like, mitochondrial	438	214	482	498	362	395
Bt.13376.1.S1_at	DHRS1	dehydrogenase/reductase (SDR family) member 1	488	264	710	348	351	542
Bt.8915.1.A1_at	DHTKD1	dehydrogenase E1 and transketolase domain containing 1	67.28	125	80.98	112	115	170
Bt.2506.1.S1_at	DKK3	dickkopf homolog 3 (Xenopus laevis)	48.86	63.94	43.82	39.24	64.12	92.21
Bt.27889.1.S1_at	DLD	Dihydroliipoamide dehydrogenase	49.94	52.11	50.58	49.55	125	53.07
Bt.9632.2.S1_at	DMBT1	deleted in malignant brain tumors 1	3594	6452	5969	3592	3012	4917
Bt.27589.1.A1_at	DNAH12L /// LOC781795	dynein, axonemal, heavy chain 12-like /// similar to ciliary dynein heavy chain 7	23.39	34.57	27.74	23.09	29.16	26.19
Bt.6341.1.S1_at	DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1	82.57	79.23	70.50	83.54	80.89	44.67
Bt.6020.1.S1_at	DNAJC11	DnaJ (Hsp40) homolog, subfamily C, member 11	149	140	201	129	90.56	125
Bt.211.1.S1_at	DNAJC3	DnaJ (Hsp40) homolog, subfamily C, member 3	1323	897	1347	965	1925	1134
Bt.869.1.S1_at	DPM1	dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit	1234	1688	1423	1277	1621	1166
Bt.2110.1.S1_at	DPP3	dipeptidyl-peptidase 3	601	649	853	516	368	665
Bt.2424.1.S1_at	DPYD	dihydropyrimidine dehydrogenase	5585	3430	4807	5765	7575	5005
Bt.15705.1.S2_at	DSTN	destrin (actin depolymerizing factor)	406	336	334	462	385	449
Bt.15705.1.S1_at	DSTN	destrin (actin depolymerizing factor)	1945	1417	1067	1517	1707	1774
Bt.28523.1.S1_at	DTX3L	deltex 3-like (Drosophila)	1322	5711	1991	2159	1390	1156
Bt.13768.1.S1_at	DYNLT3	dynein, light chain, Tctex-type 3	642	739	662	773	1117	693
Bt.27286.2.S1_at	ECD	ecdysoneless homolog (Drosophila)	50.97	50.46	65.06	40.71	50.16	80.81
Bt.20265.1.A1_at	ECD	ecdysoneless homolog (Drosophila)	692	653	688	519	571	797
Bt.7963.1.S1_at	EHD1	EH-domain containing 1	264	207	236	229	112	181
Bt.11769.2.S1_at	EID3	EP300 interacting inhibitor of differentiation 3	13.70	12.08	16.66	12.16	22.94	11.22
Bt.18928.1.A1_at	EIF4E3	eukaryotic translation initiation factor 4E family member 3	216	228	119	206	315	224

Appendix A. Continued

Affimetrix ID	Gene	Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
				CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.19745.1.S1_at	ELL2		elongation factor, RNA polymerase II, 2	414	282	309	346	633	440
Bt.1983.1.S1_at	EMR1		egf-like module containing, mucin-like, hormone receptor-like 1	302	316	364	247	164	394
Bt.3857.1.S1_at	ENDOG		endonuclease G	456	245	334	390	259	422
Bt.22783.1.S1_at	ENO1		enolase 1, (alpha)	2064	1860	2691	1498	1069	2132
Bt.22169.1.S1_at	ENO3		enolase 3 (beta, muscle)	11.31	40.81	10.10	9.72	9.36	10.10
Bt.16000.1.S1_at	ENTPD4		ectonucleoside triphosphate diphosphohydrolase 4	343	314	297	542	336	304
Bt.22737.1.S1_at	ERBB2IP		erb2 interacting protein	1658	2095	1899	1736	2630	1800
Bt.18026.1.A1_at	ERBB2IP		erb2 interacting protein	20.04	20.82	20.05	27.46	30.22	21.38
Bt.28586.1.S1_at	ERMP1		endoplasmic reticulum metalloproteinase 1	169	144	202	143	165	183
Bt.17415.3.A1_at	ERRFI1		ERBB receptor feedback inhibitor 1	6.11	5.90	21.72	5.90	6.16	6.10
Bt.23905.1.A1_at	ERRFI1		ERBB receptor feedback inhibitor 1	3683	2726	6077	3458	4158	2775
Bt.24361.1.S1_at	ESF1		ESF1, nucleolar pre-rRNA processing protein, homolog (S. cerevisiae)	35.95	55.32	39.31	38.44	66.56	33.94
Bt.5350.1.S1_at	ETFA		electron-transfer-flavoprotein, alpha polypeptide	2268	1831	1770	2511	2021	2394
Bt.4555.1.S1_at	ETFB		electron-transfer-flavoprotein, beta polypeptide	455	302	447	421	308	513
Bt.1817.1.S1_at	ETV1		ets variant 1	21.96	24.37	24.70	15.85	55.16	20.89
Bt.4758.1.S1_at	FABP3		fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)	7.46	12.17	8.33	6.44	8.13	8.13
Bt.22869.1.S2_at	FABP5		fatty acid binding protein 5 (psoriasis-associated)	10.26	22.25	11.81	10.39	32.80	19.19
Bt.7023.1.S1_at	FAHD2A		fumarylacetoacetate hydrolase domain containing 2A	545	383	583	752	564	751
Bt.26318.1.S1_a_at	FAIM		Fas apoptotic inhibitory molecule	16.67	19.90	18.29	26.78	55.85	16.67
Bt.28623.1.S1_at	FAT1		FAT tumor suppressor homolog 1 (Drosophila)	364	751	503	523	302	556
Bt.6449.1.S1_at	FBLN5		fibulin 5	90.70	155	85.46	109	74.70	115
Bt.20361.2.A1_at	FBXL20		F-box and leucine-rich repeat protein 20	130	85.78	138	72.46	109	203
Bt.24950.1.S1_at	FBXL5		F-box and leucine-rich repeat protein 5	1619	1148	1329	1391	1161	1521
Bt.24205.1.A1_at	FGB		fibrinogen beta chain	3765	2393	1656	2819	2867	2386
Bt.22730.1.S1_at	FGFR1OP2		FGFR1 oncogene partner 2	41.21	54.92	46.90	65.21	63.91	51.63
Bt.2587.2.S1_a_at	FH		fumarate hydratase	293	235	364	366	277	545
Bt.19999.1.A1_at	FICD		FIC domain containing	159	35.33	60.98	91.15	114	160
Bt.2899.1.S2_at	FOS		FBJ murine osteosarcoma viral oncogene homolog	75.66	111	75.64	244	119	82.66
Bt.21181.1.S1_at	FOXK2		forkhead box K2	81.32	116	89.57	89.57	60.02	62.61
Bt.10777.1.S1_at	FOXP1		forkhead box P1	45.11	47.25	52.62	65.01	89.05	88.03
Bt.6180.1.S1_at	FRG1		FSHD region gene 1	333	354	300	352	617	336
Bt.121.1.S1_at	FRZB		frizzled-related protein	24.42	29.78	38.54	29.23	30.14	75.30
Bt.18415.1.A1_at	FTSJD1		FtsJ methyltransferase domain containing 1	409	928	186	455	403	342
Bt.15854.1.A1_at	FUBP1		far upstream element (FUSE) binding protein 1	626	892	754	585	826	560
Bt.2190.1.S1_at	FUBP3		far upstream element (FUSE) binding protein 3	628	669	453	680	784	571

Appendix A. Continued

Affimatrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.2169.1.S1_at	FUCA1	fucosidase, alpha-L-1, tissue	1146	825	883	916	885	1034
Bt.26635.2.S1_at	FZD1	frizzled homolog 1 (Drosophila)	102	103	74.98	67.34	144	125
Bt.5197.1.S1_at	G3BP1	GTPase activating protein (SH3 domain) binding protein 1	873	1267	925	790	1200	918
Bt.20252.2.S1_a_at	GALK1	galactokinase 1	163	83.08	128	168	87.18	150
Bt.2580.1.S1_at	GALM	galactose mutarotase (aldose 1-epimerase)	832	454	921	571	711	902
Bt.21464.2.S1_a_at	GALT	galactose-1-phosphate uridylyltransferase	264	123	188	275	154	276
Bt.21464.3.S1_a_at	GALT	galactose-1-phosphate uridylyltransferase	227	107	160	210	116	202
Bt.21464.1.S1_at	GALT	galactose-1-phosphate uridylyltransferase	859	562	625	957	480	821
Bt.28744.1.S1_at	GBP4	guanylate binding protein 4	123	737	363	210	215	127
Bt.16350.2.A1_s_at	GBP5	guanylate binding protein 5	5.23	7.62	4.94	5.37	5.57	5.11
Bt.14207.1.S1_at	GCAT	glycine C-acetyltransferase	340	229	633	327	285	332
Bt.20267.1.S1_at	GCLM	glutamate-cysteine ligase, modifier subunit	184	165	157	129	180	244
Bt.25088.1.A1_at	GCSH	Glycine cleavage system protein H (aminomethyl carrier)	29.44	18.55	27.03	28.52	34.62	27.87
Bt.21798.1.S1_at	GIMAP6	GTPase, IMAP family member 6	31.19	235	34.98	93.29	35.67	150
Bt.13777.2.S1_at	GIMAP7	GTPase, IMAP family member 7	14.19	34.78	54.54	30.10	72.62	32.93
Bt.13777.1.S1_at	GIMAP7	GTPase, IMAP family member 7	221	322	357	251	406	243
Bt.26769.1.S1_at	GIMAP8	GTPase, IMAP family member 8	4.59	4.86	4.86	4.86	4.58	94.75
Bt.12579.1.A1_at	GK5	glycerol kinase 5	1700	914	2422	2730	2291	2281
Bt.13486.1.A1_at	GLDC	glycine dehydrogenase (decarboxylating)	2512	2731	3104	1967	1424	2129
Bt.24597.1.S1_at	GLG1	golgi apparatus protein 1	31.31	53.49	42.15	33.24	13.26	45.51
Bt.11167.1.S1_at	GLRX5	glutaredoxin 5	978	485	581	369	365	588
Bt.12240.1.A1_at	GLYATL3	glycine-N-acyltransferase-like 3	1853	1021	1242	1171	1199	1328
Bt.13942.1.S1_at	GLYCTK	glycerate kinase	439	203	351	454	260	477
Bt.22350.1.A1_at	GMCL1	germ cell-less homolog 1 (Drosophila)	507	482	416	490	867	483
Bt.9140.1.S1_at	GMNN	geminin, DNA replication inhibitor	206	182	132	180	305	211
Bt.25097.1.S1_at	GMPS	guanine monophosphate synthetase	359	244	319	321	346	406
Bt.18321.1.A1_at	GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	302	283	139	163	181	155
Bt.20919.2.A1_at	GNMT	glycine N-methyltransferase	99.05	25.32	77.59	64.64	45.20	71.71
Bt.29268.1.S1_at	GOLT1A	golgi transport 1 homolog A (S. cerevisiae)	526	343	396	502	333	499
Bt.11178.1.S1_at	GPC3	glypican 3	19510	11064	19030	14169	15175	16879
Bt.14464.1.A1_at	GPHN	gephyrin	176	261	239	198	280	184
Bt.22676.1.A1_at	GPN3	GPN-loop GTPase 3	436	498	357	444	619	414
Bt.7575.1.A1_at	GPT2	glutamic pyruvate transaminase (alanine aminotransferase) 2	212	134	312	237	440	218
Bt.5170.1.S1_at	GRHPR	glyoxylate reductase/hydroxypyruvate reductase	1365	686	1267	1416	904	1465
Bt.7413.1.S1_at	GRN	granulin	266	295	300	227	188	271
Bt.27623.2.S1_a_at	GRTP1	growth hormone regulated TBC protein 1	324	164	226	331	187	283

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.3201.1.S1_at	GRWD1	glutamate-rich WD repeat containing 1	72.42	79.82	74.21	73.93	35.30	54.35
Bt.227.3.A1_x_at	GSTA1	glutathione S-transferase A1	10042	10167	11208	7377	7898	10169
Bt.227.2.A1_at	GSTA1	glutathione S-transferase A1	15853	15223	16920	13053	8989	13963
Bt.28076.1.A1_at	GSTO1	glutathione S-transferase omega 1	1683	1051	1551	1472	1321	1797
Bt.13641.1.S1_at	GSTZ1	glutathione transferase zeta 1	6300	5074	7227	5706	4463	6512
Bt.20241.1.S1_at	HAAO /// LOC786774	3-hydroxyanthranilate 3,4-dioxygenase	785	331	708	439	494	514
Bt.7237.2.S1_a_at	HADHA	hydroxyacyl-CoA dehydrogenase	420	326	608	327	254	568
Bt.15687.1.S1_at	HERC4	hect domain and RLD 4	1158	1491	1615	1184	1778	1193
Bt.27463.1.A1_at	HERC6	hect domain and RLD 6	4.57	14.16	4.60	4.62	5.47	4.55
Bt.22498.2.S1_at	HES4	Hairy and enhancer of split 4 (Drosophila)	10.53	27.49	9.87	37.05	11.62	11.31
Bt.2183.1.A1_at	HEXB	hexosaminidase B (beta polypeptide)	1182	754	817	1200	852	1101
Bt.19899.1.A1_at	HGD	homogentisate 1,2-dioxygenase	15581	12993	18375	12777	12975	15842
Bt.6171.1.A1_at	HIBADH	3-hydroxyisobutyrate dehydrogenase	3357	2026	3695	2779	3696	3699
Bt.1738.1.S1_at	HIBCH	3-hydroxyisobutyryl-CoA hydrolase	521	483	469	496	734	472
Bt.19519.1.S1_at	HLTF	Helicase-like transcription factor	1229	1110	1067	1238	2034	1272
Bt.6397.2.S1_at	HMGB2	high-mobility group box 2	1392	1372	769	1016	1949	1159
Bt.3928.1.S1_at	HNRNPAB	heterogeneous nuclear ribonucleoprotein A/B	1627	1945	1668	1838	1033	1452
Bt.21801.2.S1_at	HNRNPL	heterogeneous nuclear ribonucleoprotein L	200	277	268	221	209	217
Bt.19922.1.S1_at	HPD	4-hydroxyphenylpyruvate dioxygenase	2248	1521	2019	1082	1161	2144
Bt.22672.1.A1_at	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	785	384	434	924	1351	863
Bt.20399.1.S1_at	HSD17B13	hydroxysteroid (17-beta) dehydrogenase 13	788	646	531	452	790	1340
Bt.23179.1.S1_at	HSP90AA1	heat shock 90kD protein 1, alpha	1688	1448	3975	1519	1973	2218
Bt.19575.1.S1_at	HSPA14	heat shock 70kDa protein 14	445	454	450	580	517	385
Bt.19575.2.S1_at	HSPA14	heat shock 70kDa protein 14	46.40	39.89	40.53	65.45	65.99	44.45
Bt.5372.1.S1_at	ICAM1	intercellular adhesion molecule 1	202	253	153	165	125	179
Bt.1730.1.A1_at	ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	1379	1839	777	2417	546	635
Bt.2415.1.S1_at	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	2031	1638	1323	1585	1270	1439
Bt.13324.4.S1_at	IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	6527	3234	6119	5994	5185	7139
Bt.13324.1.S1_a_at	IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	734	346	652	625	614	915
Bt.27759.2.S1_at	IDO1	indoleamine 2,3-dioxygenase 1	5.28	12.35	6.39	6.18	7.37	6.02
Bt.22021.1.S1_at	IFI16	interferon, gamma-inducible protein 16	257	905	426	458	581	240
Bt.17223.1.S1_at	IFI35	interferon-induced protein 35	169	266	201	247	92.98	158
Bt.20785.2.S1_at	IFI44	interferon-induced protein 44	301	1913	536	1363	377	207
Bt.20785.1.A1_at	IFI44	interferon-induced protein 44	474	2854	815	1825	569	305
Bt.19620.1.A1_at	IFI44	interferon-induced protein 44	459	2885	709	1263	529	315

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.8436.1.S1_at	IFI6	interferon, alpha-inducible protein 6	524	5793	4070	1704	771	589
Bt.24098.1.A1_at	IFIH1	interferon induced with helicase C domain 1	96.55	505	98.70	128	163	114
Bt.14054.1.A1_at	IFRD1	interferon-related developmental regulator 1	354	587	393	374	444	352
Bt.14054.2.S1_at	IFRD1	interferon-related developmental regulator 1	24.46	60.30	45.61	38.18	44.91	37.13
Bt.8829.1.S1_a_at	IFT122	Intraflagellar transport 122 homolog (Chlamydomonas)	149	120	113	146	165	229
Bt.11379.1.S1_at	IFT52	intraflagellar transport 52 homolog (Chlamydomonas)	35.01	75.79	50.26	53.39	45.49	47.17
Bt.190.1.A1_at	IGFBP1	insulin-like growth factor binding protein 1	43.74	18.58	26.62	20.71	116	105
Bt.3843.1.S1_at	IGJ	immunoglobulin J chain	767	540	584	735	1092	896
Bt.22116.1.A1_at	IL18BP	interleukin 18 binding protein	10.64	33.21	14.48	10.46	10.06	8.56
Bt.12760.1.S1_at	INHBA	inhibin, beta A	41.73	371	252	146	400	189
Bt.24767.1.S1_at	INTS3	integrator complex subunit 3	176	225	209	198	314	193
Bt.5768.1.S1_at	IRF7	interferon regulatory factor 7	91.67	330	94.29	190	59.20	71.28
Bt.11259.1.S1_at	ISG12(A)	putative ISG12(a) protein	1811	13433	8824	6225	1113	1745
Bt.9779.1.S1_at	ISG12(B)	similar to TLH29 protein precursor	6.28	59.92	7.26	7.25	7.00	5.92
Bt.12304.1.S1_at	ISG15	ISG15 ubiquitin-like modifier	1388	14191	2938	8522	785	485
Bt.3212.1.S1_at	ISOC2	isochorismatase domain containing 2	1397	873	1275	1408	1021	1530
Bt.8905.1.S1_at	ITCH	itchy E3 ubiquitin protein ligase homolog (mouse)	120	149	123	141	174	98.79
Bt.5536.1.S1_at	ITGB5	integrin, beta 5	785	623	692	923	559	846
Bt.21565.1.S1_at	IWS1	IWS1 homolog (S. cerevisiae)	305	433	340	287	378	315
Bt.29879.1.S1_at	KAT2B	K(lysine) acetyltransferase 2B	69.81	48.30	55.08	94.07	133	73.94
Bt.6972.1.S1_at	KBTBD10	kelch repeat and BTB (POZ) domain containing 10	4.89	11.65	4.74	4.74	5.80	4.89
Bt.16187.1.A1_at	KBTBD6	kelch repeat and BTB (POZ) domain containing 6	169	174	137	136	389	279
Bt.15691.1.S1_at	KCNK5	potassium channel, subfamily K, member 5	67.82	112	182	141	92.77	152
Bt.9170.1.A1_at	KIAA1147	KIAA1147	456	228	289	454	281	378
Bt.9527.2.S1_at	KLF10	Kruppel-like factor 10	11.69	11.70	11.21	16.94	28.77	14.76
Bt.11751.1.A1_at	KLHL23	kelch-like 23	73.58	61.33	42.76	67.03	112	62.99
Bt.3191.1.A1_at	KLHL24	kelch-like 24 (Drosophila)	602	386	541	354	808	1091
Bt.19212.1.S1_at	KLHL9	kelch-like 9 (Drosophila)	942	863	723	905	1058	864
Bt.16496.1.A1_at	KNTC1	kinetochore associated 1	199	167	230	324	191	256
Bt.12663.1.S1_at	KRT19	keratin 19	5.19	9.77	8.67	5.00	5.22	6.01
Bt.26150.1.A1_at	L2HGDH	L-2-hydroxyglutarate dehydrogenase	195	175	145	153	313	206
Bt.14129.1.S1_at	LACTB2	lactamase, beta 2	1245	1108	954	1141	1548	1128
Bt.27891.1.S1_at	LARS2	leucyl-tRNA synthetase 2, mitochondrial	59.33	103	124	66.65	95.88	84.29
Bt.19614.1.A1_at	LIPC	lipase, hepatic	3438	2059	3810	3490	3957	3843
Bt.4643.1.S1_at	LMAN2	lectin, mannose-binding 2	2328	1912	2616	2040	1793	2463
Bt.20934.1.S1_at	LOC100137763	hypothetical protein LOC100137763	145	121	46.45	95.35	177	89.72
Bt.8724.1.S1_at	LOC100299281	---	788	402	751	902	515	995
Bt.5692.1.S1_at	LOC100425208	---	141	124	132	218	215	137
Bt.24749.1.S1_at	LOC100430496	---	522	393	370	727	753	461
Bt.24001.1.A1_at	LOC100433242	---	3253	1408	1757	2740	1309	2494
Bt.28945.1.A1_at	LOC100440461	---	186	174	152	210	242	169
Bt.29398.1.S1_at	LOC100582155	---	722	384	576	613	824	656

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.16058.2.S1_at	LOC100583040	---	30.49	16.08	42.24	29.81	91.77	46.50
Bt.8421.2.S1_at	LOC100623159	---	2695	1769	2344	3395	2863	3117
Bt.17814.1.A1_at	LOC100736585	---	799	730	922	1081	518	912
Bt.26804.1.S1_at	LOC100847122	---	190	278	274	164	299	206
Bt.18114.1.A1_at	LOC100851000	---	96.56	43.59	40.79	36.06	55.50	41.03
Bt.18577.2.A1_at	LOC472962	---	346	400	272	386	583	327
Bt.6556.1.S1_at	LOC504773	regakine 1	2726	1984	1969	1527	850	2097
Bt.4937.1.S1_at	LOC505941	similar to KIAA1398 protein	2285	5253	3167	2494	1521	2436
Bt.15796.1.S1_at	LOC508226	similar to CDC42-binding protein kinase beta	53.46	71.60	62.98	51.13	29.51	44.06
Bt.25111.1.A1_at	LOC508347	Similar to interferon-induced protein 44-like	299	2041	410	755	314	253
Bt.12586.1.A1_at	LOC508439	similar to CG2943 CG2943-PA	371	417	480	312	341	379
Bt.643.1.S1_at	LOC508666	Similar to MPIF-1	3295	2008	2772	3001	3207	6186
Bt.21461.1.S1_at	LOC509034	similar to Feline leukemia virus subgroup C receptor-related protein 2 (Calcium-chelate transporter) (CCT)	20.22	11.18	5.79	6.58	5.79	5.79
Bt.26538.1.S1_at	LOC509420	similar to chromosome 9 open reading frame 61	19.93	17.08	60.64	18.18	32.62	27.17
Bt.23696.1.A1_at	LOC509457	WD repeat domain 73-like	4.56	370	4.56	4.56	4.56	369
Bt.18323.1.A1_at	LOC509506	similar to Cytochrome P450, family 4, subfamily F, polypeptide 2	203	89.68	152	178	122	164
Bt.18440.2.S1_at	LOC510382	similar to guanylate binding protein 4	5.19	6.92	6.25	6.93	25.65	5.48
Bt.18440.3.A1_at	LOC510382	similar to guanylate binding protein 4	18.94	15.41	10.99	28.88	113	17.86
Bt.2049.1.S1_at	LOC510634	hypothetical LOC510634	1101	448	717	665	492	839
Bt.27118.1.A1_at	LOC510651	hypothetical LOC510651	799	1515	852	803	1263	839
Bt.3300.1.S1_at	LOC511523	similar to SLC2A4 regulator	447	249	369	487	289	371
Bt.18316.1.A1_at	LOC513587	Similar to UPF0474 protein C5orf41	137	89.08	83.41	91.93	231	118
Bt.12704.1.S1_at	LOC514801	similar to retina copper-containing monoamine oxidase	11.96	37.45	20.07	17.94	16.23	12.61
Bt.10371.1.S1_at	LOC516241	similar to cysteine sulfinate decarboxylase	107	68.04	49.00	84.64	76.19	79.90
Bt.8736.1.S1_at	LOC520588	similar to chromosome 1 open reading frame 9	763	898	886	865	1234	891
Bt.28626.2.S1_at	LOC521363	similar to GC-rich sequence DNA-binding factor (GCF) (Transcription factor 9) (TCF-9)	8.44	10.65	11.44	9.93	16.15	9.95
Bt.13184.1.S1_at	LOC523126	similar to ATP-binding cassette, sub-family C, member 4	12.71	111	55.67	152	9.61	22.34
Bt.22421.1.A1_at	LOC530325	similar to signal peptide peptidase-like 2A	1717	1873	1028	1825	2482	1465
Bt.26568.2.S1_a_at	LOC531049	similar to Putative eukaryotic translation initiation factor 3 subunit (eIF-3)	131	150	215	116	127	154
Bt.12665.1.A1_at	LOC531600	similar to AAT1-alpha	49.38	69.10	60.44	57.80	69.34	52.35
Bt.1785.1.A1_at	LOC532189	similar to carboxypeptidase D	245	172	244	309	347	305
Bt.19937.1.S1_at	LOC532189	similar to carboxypeptidase D	1221	938	1102	1274	1189	1349
Bt.27966.1.S1_at	LOC532789	similar to PAWR	57.99	36.56	38.17	47.53	69.63	57.01
Bt.21869.1.S1_at	LOC537017	similar to CMP-N-acetylneuraminic acid hydroxylase	334	431	301	534	580	446

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.24881.1.S1_at	LOC539690	similar to Complement component C1q receptor precursor (Complement component 1 q subcomponent receptor 1) (C1qR) (C1qRp) (C1qR(p)) (C1q/MBL/SPA receptor) (Matrix-remodeling-associated protein 4) (CD93 antigen) (CDw93)	329	168	232	297	204	308
Bt.2859.1.A1_at	LOC540253	hypothetical LOC540253	210	159	141	221	393	185
Bt.27403.1.S1_at	LOC540987	similar to Uncharacterized protein C5orf5 (GAP-like protein N61)	251	344	297	371	424	288
Bt.20758.1.S1_at	LOC541014	hypothetical protein LOC541014	233	211	172	249	257	189
Bt.6162.1.S1_at	LOC613560	similar to putative c-Myc-responsive	69.40	32.13	60.19	69.92	51.05	72.51
Bt.28139.1.S1_at	LOC614107	similar to Hexokinase-2 (Hexokinase type II) (HK II)	12.77	36.13	13.06	11.70	15.62	11.11
Bt.11772.2.S1_at	LOC614339	hypothetical protein LOC614339	73.28	43.28	59.86	64.83	151	88.54
Bt.10797.2.S1_a_at	LOC615093	hypothetical protein LOC615093	1229	746	965	1116	1205	1203
Bt.2965.1.A1_at	LOC618434	hypothetical LOC618434	1631	891	1280	1652	1016	1406
Bt.16672.1.A1_at	LOC698727	---	22.50	17.91	11.17	25.88	51.93	13.31
Bt.1978.3.S1_at	LOC780933	cationic trypsin	80.98	15.67	41.51	43.49	38.59	64.96
Bt.5466.2.S1_a_at	LOC783142	ribosomal protein S4, Y-linked 1 /// ribosomal protein S4, Y-linked 2 /// similar to ribosomal protein S4 /// hypothetical protein LOC783463	9895	7390	8909	9955	8466	9879
Bt.2999.1.A1_at	LOC783843	similar to seven transmembrane helix receptor	135	148	90.95	116	180	123
Bt.22065.1.S1_at	LOC783920	similar to mCG1046517	5.10	11.69	5.03	4.59	4.85	5.03
Bt.15530.1.S1_at	LOC784762	similar to 60S ribosomal protein L12 /// ribosomal protein L12	3946	3157	3318	3740	2995	4049
Bt.6899.1.S1_at	LOC784769	similar to MGC127725 protein	529	511	448	624	632	498
Bt.17352.1.A1_at	LOC785119	similar to programmed cell death 10	179	189	128	227	193	181
Bt.23566.2.S1_at	LOC785936	Hypothetical protein LOC785936	18.98	23.60	17.74	14.68	63.34	30.42
Bt.28764.1.A1_at	LOC787057	similar to zinc finger protein 415	39.28	57.52	46.44	36.75	88.81	56.30
Bt.18080.2.S1_at	LOC787094	similar to tescalcin	5.47	8.94	13.00	5.95	6.13	5.99
Bt.11233.1.S1_at	LOC787143 /// TOP2B	similar to DNA topoisomerase II, beta isozyme /// topoisomerase (DNA) II beta 180kDa	1453	1312	1494	1748	1903	1516
Bt.19994.1.S1_at	LOC789597	similar to PDZ domain-containing guanine nucleotide exchange factor PDZ-GEF2	351	385	368	450	621	392
Bt.9655.2.S1_at	LOC790332	similar to enterocytin	117	73.85	21.73	17.89	20.37	110
Bt.27204.1.S1_at	LPCAT3	lysophosphatidylcholine acyltransferase 3	150	89.18	168	67.33	38.15	95.31
Bt.8135.1.S1_at	LRAT	lecithin retinol acyltransferase (phosphatidylcholine--retinol O-acyltransferase)	103	90.47	62.69	85.81	192	104

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.6143.1.S1_at	LTA4H	leukotriene A4 hydrolase	661	417	491	579	429	584
Bt.13257.2.A1_at	LTV1	LTV1 homolog (<i>S. cerevisiae</i>)	146	249	216	174	208	128
Bt.22150.1.A1_at	LZTFL1	leucine zipper transcription factor-like 1	277	316	262	285	449	276
Bt.21336.1.S1_a_at	MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)	128	131	118	184	76.89	102
Bt.24258.2.S1_at	MAN1A1	mannosidase, alpha, class 1A, member 1	566	506	435	659	869	815
Bt.6774.2.S1_at	MAP1LC3B	microtubule-associated protein 1 light chain 3 beta	564	360	448	583	438	579
Bt.25957.1.S1_at	MAVS	mitochondrial antiviral signaling protein	71.93	83.44	61.87	45.41	31.74	61.43
Bt.20529.1.A1_at	MBLAC1	metallo-beta-lactamase domain containing 1	316	194	291	281	261	310
Bt.21433.1.S1_at	MCM6	minichromosome maintenance complex component 6	252	260	190	230	379	292
Bt.7915.1.S1_at	MDH2	malate dehydrogenase 2, NAD (mitochondrial)	4161	3775	5167	3727	3564	4191
Bt.13251.1.S1_at	MFNG	MFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	156	111	94.87	120	117	125
Bt.7327.2.S1_a_at	MGC133692	hypothetical LOC506714	6266	6082	4934	6281	8276	5715
Bt.17517.1.S1_at	MGC134574	hypothetical LOC505226	527	469	321	457	622	468
Bt.18540.1.A1_at	MGC165715	Hypothetical LOC530484	427	302	403	362	710	533
Bt.9774.1.S1_a_at	MGC165862	hypothetical LOC614805	265	222	146	314	455	315
Bt.3678.1.S1_at	MKI67IP	MKI67 (FHA domain) interacting nucleolar phosphoprotein	417	492	341	509	426	385
Bt.12370.1.S1_at	MLF2	myeloid leukemia factor 2	395	405	500	265	185	375
Bt.24793.1.S1_at	MN1	meningioma (disrupted in balanced translocation) 1	5.27	9.94	5.01	5.27	5.03	10.45
Bt.15685.1.A1_at	MOSC2	MOCO sulphurase C-terminal domain containing 2	11838	10364	9867	11363	13442	9858
Bt.17219.1.A1_at	MPDU1	mannose-P-dolichol utilization defect 1	402	457	467	436	282	469
Bt.27187.1.S1_at	MPHOSPH10	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)	197	286	219	261	395	158
Bt.11135.1.S1_at	MPV17	MpV17 mitochondrial inner membrane protein	726	511	643	602	549	726
Bt.4985.1.S1_at	MRPL23	mitochondrial ribosomal protein L23	7129	4393	6496	6411	4345	5934
Bt.4985.1.S1_a_at	MRPL23	mitochondrial ribosomal protein L23	4430	2490	4062	3992	2836	3656
Bt.26953.1.A1_at	MRPL36	mitochondrial ribosomal protein L36	138	92.73	119	124	99.84	121
Bt.3811.1.S1_at	MRPS18B	mitochondrial ribosomal protein S18B	263	226	343	221	228	299
Bt.20270.1.S1_at	MSL1	male-specific lethal 1 homolog (<i>Drosophila</i>)	285	486	356	333	322	350
Bt.4503.1.S2_at	MTCH2	mitochondrial carrier homolog 2 (<i>C. elegans</i>)	2956	2025	2709	2545	2336	2883
Bt.26410.1.A1_at	MTERF	mitochondrial transcription termination factor	159	184	120	211	223	174
Bt.18045.1.S1_at	MTPAP	mitochondrial poly(A) polymerase	130	183	195	149	172	143
Bt.8143.1.S1_at	MX2	myxovirus (influenza virus) resistance 2 (mouse)	5.36	32.14	5.83	6.29	5.29	4.96
Bt.8090.2.S1_at	MYBBP1A	MYB binding protein (P160) 1a	65.02	101	88.07	68.53	32.47	54.73

Appendix A. Continued

Affimatrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.10310.1.S1_at	MYBPC1	myosin binding protein C, slow type	4.76	10.74	4.71	4.71	4.66	4.76
Bt.12300.1.S1_at	MYH1	myosin, heavy chain 1, skeletal muscle, adult	4.51	7.02	4.51	4.67	4.67	4.67
Bt.12300.2.S1_at	MYH2	myosin, heavy chain 2, skeletal muscle, adult	4.52	579	4.52	4.52	4.52	4.55
Bt.6620.1.S1_at	MYH7	myosin, heavy chain 7, cardiac muscle, beta	4.56	30.59	4.60	4.63	4.56	4.63
Bt.4922.1.S1_at	MYL1	myosin, light chain 1, alkali; skeletal, fast	4.52	377	4.52	4.53	4.53	4.53
Bt.1905.1.S1_at	MYL2	myosin, light chain 2, regulatory, cardiac, slow	4.53	109	4.52	4.52	4.53	4.72
Bt.11199.1.S1_at	MYOZ1	myozenin 1	5.38	10.03	5.30	5.30	5.37	5.38
Bt.5399.1.S2_at	NADK	NAD kinase	1501	1374	2014	1591	979	1448
Bt.5399.1.S1_at	NADK	NAD kinase	86.34	86.01	103	85.83	54.21	95.69
Bt.3999.1.S1_at	NAGA	N-acetylgalactosaminidase, alpha-	281	198	220	217	210	245
Bt.5542.2.S1_at	NAP1L1	nucleosome assembly protein 1-like 1	2074	1580	1453	2265	2607	2009
Bt.26892.1.S1_at	NBN	nibrin	963	1330	1005	1035	1325	818
Bt.2905.1.S1_at	NDRG2	NDRG family member 2	1507	1397	2268	2027	1756	2422
Bt.4475.1.S1_at	NDUFS2	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	2585	1794	2887	2602	1933	2691
Bt.653.1.S1_at	NEK6	NIMA (never in mitosis gene a)-related kinase 6	3586	4248	4811	3629	2713	4099
Bt.17428.1.A1_at	NHLRC3	NHL repeat containing 3	390	230	304	311	308	513
Bt.3023.1.S1_at	NIT1	nitrilase 1	318	218	365	229	219	343
Bt.9705.1.S1_at	NKTR	natural killer-tumor recognition sequence	355	481	465	504	467	342
Bt.6993.2.A1_a_at	NME7	non-metastatic cells 7, protein expressed in (nucleoside-diphosphate kinase)	360	311	214	328	406	324
Bt.12285.3.S1_a_at	NMI	N-myc (and STAT) interactor	792	1711	767	864	868	786
Bt.5129.1.S1_a_at	NNAT	neuronatin	80.39	19.67	7.77	34.99	32.49	23.36
Bt.5129.2.A1_at	NNAT	neuronatin	259	65.21	27.99	123	105	75.81
Bt.7381.1.S1_at	NPLOC4	nuclear protein localization 4 homolog (S. cerevisiae)	134	133	148	142	97.72	109
Bt.3599.1.S1_at	NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	5466	5845	4659	5714	6540	5290
Bt.6316.1.S1_at	NR2F6	nuclear receptor subfamily 2, group F, member 6	1185	1073	1104	1418	683	963
Bt.20373.1.S1_at	NRP1	neuropilin 1	941	918	590	830	1096	948
Bt.20932.1.S1_at	NSA2	NSA2 ribosome biogenesis homolog (S. cerevisiae)	1236	1250	806	1387	1323	1031
Bt.1946.1.S1_at	NSFL1C	NSFL1 (p97) cofactor (p47)	168	162	190	144	111	160
Bt.20677.1.S1_at	NSL1	NSL1, MIND kinetochore complex component, homolog (S. cerevisiae)	64.52	53.98	36.70	51.89	92.81	53.78
Bt.17805.2.A1_at	NUDT12	nudix (nucleoside diphosphate linked moiety X)-type motif 12	90.41	83.95	82.08	79.85	174	124
Bt.26961.1.S1_at	NUDT14	nudix (nucleoside diphosphate linked moiety X)-type motif 14	83.89	43.42	71.23	82.65	63.31	109
Bt.17124.1.A1_s_at	NUDT14	nudix (nucleoside diphosphate linked moiety X)-type motif 14	255	157	265	324	219	366
Bt.20891.1.S1_at	OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	1105	4606	2338	2372	579	653

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.27143.1.A1_at	ODF2L	Outer dense fiber of sperm tails 2-like	133	164	141	131	232	131
Bt.12910.1.S1_at	OGDH	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	74.36	72.23	107	63.77	46.93	79.52
Bt.367.1.S1_at	OLR1	oxidized low density lipoprotein (lectin-like) receptor 1	13.35	34.27	12.06	52.91	16.24	11.74
Bt.17777.1.S1_at	OPTN	optineurin	663	1436	900	726	881	707
Bt.17777.3.S1_at	OPTN	optineurin	97.72	217	156	121	167	119
Bt.17777.2.S1_at	OPTN	optineurin	331	691	531	461	586	400
Bt.13189.1.A1_at	ORC4L	Origin recognition complex, subunit 4-like (yeast)	153	202	165	170	209	165
Bt.28245.1.S1_at	OSTBETA	organic solute transporter beta	1072	1112	983	1581	481	894
Bt.15997.1.S1_at	P2RX4	purinergic receptor P2X, ligand-gated ion channel, 4	311	169	258	324	192	383
Bt.5360.1.S1_a_at	PAPOLA	poly(A) polymerase alpha	315	411	478	331	478	331
Bt.6521.1.A1_at	PARD6B	par-6 partitioning defective 6 homolog beta (C. elegans)	57.79	39.38	65.58	78.98	67.40	57.62
Bt.18116.1.S1_at	PARP12	poly (ADP-ribose) polymerase family, member 12	6.73	13.78	11.87	9.25	7.93	8.28
Bt.18116.2.A1_at	PARP12	poly (ADP-ribose) polymerase family, member 12	12.12	28.53	15.18	15.08	11.67	10.93
Bt.23171.2.S1_at	PCBD1	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha	6186	4720	8324	5700	4442	6127
Bt.4718.1.S1_at	PCTP	phosphatidylcholine transfer protein	3920	2300	2844	3065	2012	3886
Bt.3736.1.A1_at	PDE4DIP	phosphodiesterase 4D interacting protein (myomegalin)	7.75	7.75	11.94	6.94	7.38	8.22
Bt.444.1.S1_at	PDE6C	phosphodiesterase 6C, cGMP-specific, cone, alpha prime	496	206	137	568	661	321
Bt.6460.1.S1_at	PDIA6	protein disulfide isomerase family A, member 6	5001	4358	5410	3275	3920	4674
Bt.11475.1.A1_at	PDLIM5	PDZ and LIM domain 5	5.81	10.02	6.31	5.69	6.52	6.47
Bt.5916.1.S1_at	PGCP	plasma glutamate carboxypeptidase	345	374	358	490	530	361
Bt.20281.2.S1_a_at	PGM1	phosphoglucomutase 1	707	527	644	615	508	686
Bt.20281.3.S1_a_at	PGM1	phosphoglucomutase 1	197	172	219	183	142	260
Bt.12820.1.S1_at	PGRMC1	progesterone receptor membrane component 1	5596	3171	6074	5967	6873	6656
Bt.15306.1.A1_at	PHF3	PHD finger protein 3	1091	992	1198	1394	1528	1099
Bt.23955.1.A1_at	PHOSPHO2	phosphatase, orphan 2	823	536	655	681	902	783
Bt.12864.1.S1_at	PHPT1	phosphohistidine phosphatase 1	723	433	502	677	391	675
Bt.21680.2.S1_at	PIR	pirin (iron-binding nuclear protein)	76.17	49.18	57.37	68.62	41.11	68.97
Bt.29432.1.A1_at	PKHD1	similar to polycystic kidney and hepatic disease 1 (autosomal recessive)	27.26	51.65	51.16	19.39	29.92	30.47
Bt.13534.1.S1_at	PLA2G16	phospholipase A2, group XVI	823	583	632	654	492	735
Bt.15713.2.S1_at	PLEK	pleckstrin	6.03	8.92	4.63	10.98	4.66	12.77
Bt.22283.1.S1_at	PLEKHA2	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 2	351	486	351	328	389	388
Bt.29194.1.S1_at	PLIN4	similar to plasma membrane associated protein, S3-12	15.77	17.04	41.29	14.80	15.47	18.45

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.28162.1.S1_at	PLN	phospholamban	111	114	97.93	86.08	222	155
Bt.15906.1.S1_at	PLS3	plastin 3	1561	1430	1396	1312	2198	1828
Bt.12638.1.S1_at	PML	promyelocytic leukemia	26.73	57.34	34.26	28.75	24.86	25.20
Bt.23599.1.S1_at	PON2	paraoxonase 2	1170	793	916	1077	1131	1205
Bt.6626.1.S1_at	PPAP2A	phosphatidic acid phosphatase type 2A	793	545	652	606	578	665
Bt.12803.1.S1_at	PPARA	peroxisome proliferator-activated receptor alpha	77.00	98.10	64.16	80.09	44.00	89.23
Bt.9791.1.S1_at	PPIF	peptidylprolyl isomerase F	1633	1666	1109	960	1014	1161
Bt.19839.1.A1_at	Ppig	peptidylprolyl isomerase G (cyclophilin G)	44.07	46.14	41.98	46.82	65.41	42.38
Bt.18634.1.A1_at	PPM1K	protein phosphatase, Mg2+/Mn2+ dependent, 1K	684	456	586	465	989	714
Bt.5319.1.S1_at	PRDX6	peroxiredoxin 6	1346	993	1390	1646	1293	1704
Bt.20145.1.S1_at	PRELID1	PRELI domain containing 1	2029	1635	2245	1643	1345	2010
Bt.21189.1.S1_at	PRKD2	protein kinase D2	192	237	238	206	116	170
Bt.6225.2.A1_at	PRKD3	protein kinase D3	411	611	425	461	713	465
Bt.4404.1.A1_at	PRSS2	protease, serine, 2 (trypsin 2)	4.87	4.87	4.51	135	4.87	4.51
Bt.13588.2.S1_at	PSAT1	phosphoserine aminotransferase 1	33.41	15.87	49.01	22.87	21.42	45.88
Bt.13588.3.A1_at	PSAT1	phosphoserine aminotransferase 1	108	40.63	127	76.79	50.96	166
Bt.9048.2.S1_a_at	PSENE1	presenilin enhancer 2 homolog (C. elegans)	584	377	508	488	420	509
Bt.12290.1.S1_at	PSIP1	PC4 and SFRS1 interacting protein 1	997	988	916	1192	1956	938
Bt.20110.1.S1_at	PSMF1	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)	299	672	384	315	190	250
Bt.3715.1.S1_at	PSMG4	proteasome (prosome, macropain) assembly chaperone 4	1074	589	851	1012	495	988
Bt.1645.1.S1_at	PTGDS	prostaglandin D2 synthase 21kDa (brain)	80.17	128	85.09	134	48.32	89.95
Bt.20261.1.S1_at	PTPN3	protein tyrosine phosphatase, non-receptor type 3	30.06	48.18	53.85	59.45	61.03	47.50
Bt.24848.1.A1_at	PTPRD	protein tyrosine phosphatase, receptor type, D	57.63	54.58	103	94.80	174	79.25
Bt.21708.1.S1_at	RAB4A	RAB4A, member RAS oncogene family	391	253	357	402	337	418
Bt.26308.2.A1_at	RAD18	RAD18 homolog (S. cerevisiae)	7.65	7.65	7.15	8.22	14.93	6.70
Bt.8997.1.S1_at	RANGAP1	Ran GTPase activating protein 1	104	412	161	192	61.07	84.54
Bt.8730.1.S1_at	RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	860	950	1232	769	684	797
Bt.22323.1.A1_a_at	RASSF5	Ras association (RalGDS/AF-6) domain family member 5	350	315	454	392	253	327
Bt.22683.1.S1_at	RBM10	RNA binding motif protein 10	189	312	244	188	164	155
Bt.17614.1.S1_at	RBM25	RNA binding motif protein 25	52.73	102	87.44	60.50	107	61.42
Bt.27964.1.A1_at	RCL1	RNA terminal phosphate cyclase-like 1	3344	1769	2865	2794	1978	2998
Bt.20711.1.S1_at	RDH16	retinol dehydrogenase 16 (all-trans)	9295	7254	9157	6543	6134	7357
Bt.13743.1.A1_at	RFK	riboflavin kinase	1134	1151	629	1124	1540	1135
Bt.20477.1.S1_at	RFTN1	raftlin, lipid raft linker 1	52.21	20.49	23.74	53.05	27.33	28.75
Bt.6802.1.S1_at	RGS5	regulator of G-protein signaling 5	197	303	74.40	128	428	267

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.28182.1.A1_at	RGS5	regulator of G-protein signaling 5	31.93	26.82	10.13	22.32	69.12	55.91
Bt.27940.1.A1_at	RHBG	Rh family, B glycoprotein (gene/pseudogene)	70.50	37.81	100	35.93	36.70	73.26
Bt.24892.1.A1_at	RIT1	Ras-like without CAAX 1	427	422	273	366	598	375
Bt.6822.1.S1_at	RNF150	similar to RING finger protein 150	14.66	22.33	19.97	15.64	18.31	16.33
Bt.10686.1.S1_at	RNF170	ring finger protein 170	664	425	649	763	1048	742
Bt.920.1.S1_at	RNF181	ring finger protein 181	78.12	93.55	81.91	94.58	76.12	180
Bt.28207.1.S1_at	RNF19A	ring finger protein 19A	401	442	386	531	740	419
Bt.15692.1.A1_at	RNF19B	ring finger protein 19B	61.63	88.13	51.45	103	44.99	65.76
Bt.6645.1.S1_at	RNPC3	RNA-binding region (RNP1, RRM) containing 3	339	290	343	393	530	278
Bt.28914.1.A1_at	RP2	retinitis pigmentosa 2 (X-linked recessive)	153	129	103	87.51	101	91.49
Bt.23317.1.S1_at	RPL13	ribosomal protein L13	5393	2809	4534	5850	4506	5606
Bt.23548.1.S1_at	RPL34	ribosomal protein L34	5877	4149	5191	5944	5568	6202
Bt.2822.1.S1_at	RPL8	ribosomal protein L8	4798	3580	4545	4708	2963	4711
Bt.21268.1.S2_at	RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	365	257	355	441	516	355
Bt.1034.1.S1_at	RPS8	ribosomal protein S8	18003	14263	15641	17448	14874	18099
Bt.4711.1.S1_at	RPS9	ribosomal protein S9	2640	1838	2488	2499	1803	2637
Bt.5334.1.S1_at	RPSA	ribosomal protein SA	5533	4764	5548	4573	3737	5316
Bt.22064.2.S1_at	RSRC2	arginine/serine-rich coiled-coil 2	1061	1467	1277	1358	1681	973
Bt.196.1.S1_at	S100A13	8kDa amlexanox-binding protein	1032	354	425	821	465	875
Bt.17537.1.A1_at	SAA4	serum amyloid A4, constitutive	1260	1042	1537	632	757	1174
Bt.1552.1.S1_at	SARS	seryl-tRNA synthetase	514	638	559	430	342	545
Bt.26302.1.A1_at	SCML1	Sex comb on midleg-like 1 (Drosophila)	23.15	16.89	21.50	17.62	62.56	28.09
Bt.11055.1.S1_at	SDPR	serum deprivation response	2274	2271	1801	2025	3705	2150
Bt.22483.1.S1_at	SEC31B	SEC31 homolog B (S. cerevisiae)	148	176	123	182	148	130
Bt.27099.1.A1_at	SEC62	SEC62 homolog (S. cerevisiae)	936	857	711	910	1325	736
Bt.28577.1.S1_at	SENP6	SUMO1/sentrin specific peptidase 6	1468	1873	1454	1449	2146	1388
Bt.17451.2.A1_at	SESTD1	SEC14 and spectrin domains 1; similar to SEC14 domain and spectrin repeat-containing protein 1 (Huntingtin-interacting protein-like protein) (Protein Solo)	6.16	6.20	5.45	21.74	5.35	17.17
Bt.16234.2.S1_at	SFRS18	splicing factor, arginine/serine-rich 18	52.98	73.69	115	92.25	154	71.87
Bt.16448.2.A1_at	SFRS2IP	splicing factor, arginine/serine-rich 2, interacting protein	112	169	117	105	164	128
Bt.26408.1.A1_at	SFRS2IP	splicing factor, arginine/serine-rich 2, interacting protein	1031	1201	1301	1296	1915	1076
Bt.8206.1.S1_at	SFRS7	splicing factor, arginine/serine-rich 7, 35kDa	1328	2015	1730	1974	1598	1395
Bt.633.2.S1_a_at	SFXN1	sideroflexin 1	285	295	1085	644	675	689
Bt.633.1.S1_at	SFXN1	sideroflexin 1	429	434	1416	975	1004	824
Bt.27320.1.A1_at	SGOL2	shugoshin-like 2 (S. pombe)	99.46	108	104	106	206	85.05
Bt.5582.1.S1_at	SH3BGR	similar to SH3 domain-binding glutamic acid-rich protein (SH3BGR protein)	27.54	35.45	25.91	28.81	45.04	41.16
Bt.5220.1.S1_at	SHBG	sex hormone-binding globulin	527	217	511	428	412	619

Appendix A. Continued

Affimatrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.7116.1.A1_at	SIAE	sialic acid acetyltransferase	230	133	190	176	162	235
Bt.6038.1.S1_at	SIGLEC1	sialic acid binding Ig-like lectin 1, sialoadhesin	382	554	412	622	255	595
Bt.714.1.S1_at	SIGMAR1	sigma non-opioid intracellular receptor 1	89.59	60.87	74.67	72.40	63.46	89.73
Bt.23169.1.S1_at	SIRPA	signal-regulatory protein alpha	277	402	340	204	175	303
Bt.16250.2.S1_at	SLC10A1	solute carrier family 10 (sodium/bile acid cotransporter family), member 1	393	208	390	566	368	433
Bt.24007.1.A1_at	SLC15A2	solute carrier family 15 (H ⁺ /peptide transporter), member 2	298	209	493	192	305	343
Bt.1207.1.S1_at	SLC16A13	solute carrier family 16, member 13 (monocarboxylic acid transporter 13)	714	372	754	518	446	811
Bt.27443.1.S1_at	SLC22A18	solute carrier family 22, member 18	136	75.40	134	106	113	134
Bt.3358.1.S1_at	SLC25A1	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1	594	448	561	601	298	548
Bt.20520.1.S1_at	SLC25A10	solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10	979	565	748	830	627	776
Bt.11770.1.S1_at	SLC25A20	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	479	368	451	453	267	577
Bt.4880.1.S1_at	SLC25A3	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	2977	2929	3104	2532	1797	2926
Bt.22577.2.S1_at	SLC25A33	solute carrier family 25, member 33	16.08	6.33	7.77	8.19	5.98	16.18
Bt.13332.1.S1_at	SLC25A46	solute carrier family 25, member 46	913	1075	964	1271	1184	938
Bt.5083.1.S1_at	SLC27A4	solute carrier family 27 (fatty acid transporter), member 4	112	104	116	63.46	27.03	108
Bt.28697.1.S1_at	SLC31A1	solute carrier family 31 (copper transporters), member 1	6677	4474	6746	6669	6767	6431
Bt.8169.1.S1_at	SLC39A6	solute carrier family 39 (zinc transporter), member 6	303	339	256	290	476	289
Bt.3195.1.S1_at	SLC7A9	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 9	101	27.02	75.47	55.28	82.00	70.47
Bt.15872.1.S1_at	SLU7	SLU7 splicing factor homolog (<i>S. cerevisiae</i>)	386	323	223	288	859	440
Bt.27590.1.A1_at	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	89.68	164	143	93.75	79.51	87.00
Bt.22976.1.S1_at	SMC4	structural maintenance of chromosomes 4	32.40	32.69	33.34	39.61	86.58	35.92
Bt.13336.1.A1_at	SMC4	structural maintenance of chromosomes 4	339	396	269	368	648	315
Bt.8491.1.S1_at	SMOC2	SPARC related modular calcium binding 2	59.17	71.69	131	131	130	132
Bt.835.1.A1_at	SNTB1	syntrophin, beta 1 (dystrophin-associated protein A1, 59kDa, basic component 1)	166	139	128	190	218	151

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.27468.1.A1_at	SOAT2	sterol O-acyltransferase 2	96.08	18.70	36.52	82.79	56.25	57.81
Bt.1736.1.A1_at	SOCS1	suppressor of cytokine signaling 1	8.40	12.11	8.04	8.06	8.03	8.04
Bt.19339.3.A1_at	SOCS6	similar to suppressor of cytokine signaling 6	302	327	183	308	405	250
Bt.2501.1.S1_at	SOD2	superoxide dismutase 2, mitochondrial	611	360	597	532	754	519
Bt.24317.1.A1_at	SOX6	SRY (sex determining region Y)-box 6	95.50	106	130	80.38	138	103
Bt.27830.1.A1_at	SP140	SP140 nuclear body protein	364	842	604	698	397	375
Bt.6289.1.S1_at	SPTLC1	serine palmitoyltransferase, long chain base subunit 1	1392	1319	1090	1409	1825	1250
Bt.11687.1.S1_a_at	SRL	sarcalumenin	4.52	16.90	4.52	4.52	4.52	4.52
Bt.13705.1.S1_at	SSR2	signal sequence receptor, beta (translocon-associated protein beta)	1983	1472	1924	1464	1077	1787
Bt.15037.1.S1_at	ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	556	646	537	512	248	445
Bt.11739.1.S1_a_at	STAP2	signal transducing adaptor family member 2	1039	698	909	845	718	858
Bt.1920.2.S1_at	STARD10	StAR-related lipid transfer (START) domain containing 10	1280	734	826	1065	499	1076
Bt.24492.1.S1_at	STAT2	signal transducer and activator of transcription 2, 113kDa	448	896	515	419	383	417
Bt.15334.2.A1_at	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	115	127	219	95.90	61.99	107
Bt.13278.1.S1_at	STEAP3	STEAP family member 3	408	351	407	441	270	437
Bt.28617.1.S1_at	STOM	Stomatin	1378	704	1083	1378	932	1513
Bt.27430.1.S1_at	STRADB	STE20-related kinase adaptor beta	237	136	150	209	197	196
Bt.7161.1.S1_at	STRBP	spermatid perinuclear RNA binding protein	285	194	275	240	230	263
Bt.3206.1.A1_at	SUSD2	sushi domain containing 2	30.32	16.84	60.93	19.11	28.90	22.03
Bt.24249.1.S1_at	SUV420H1	suppressor of variegation 4-20 homolog 1 (Drosophila)	53.00	56.11	69.72	85.08	111	57.87
Bt.8054.1.S1_at	SYAP1	synapse associated protein 1, SAP47 homolog (Drosophila)	275	326	286	274	474	285
Bt.16614.1.A1_s_at	SYNCRIP	Synaptotagmin binding, cytoplasmic RNA interacting protein	229	349	227	346	282	186
Bt.20416.1.S1_at	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	145	334	191	202	171	140
Bt.4079.2.S1_a_at	TARDBP	TAR DNA binding protein	281	311	407	311	312	250
Bt.1987.1.S1_at	TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3	1278	990	1298	967	891	1234
Bt.21764.1.S1_at	TBC1D15	TBC1 domain family, member 15	217	199	148	210	404	285
Bt.21021.1.S1_at	TBC1D7	TBC1 domain family, member 7	188	96.57	182	133	118	196
Bt.20229.1.S1_at	TBRG4	transforming growth factor beta regulator 4	114	144	137	115	87.27	107
Bt.4053.1.S1_at	TBXA2R	thromboxane A2 receptor	145	92.85	96.98	130	107	189
Bt.3026.1.A1_at	TCEA3	transcription elongation factor A (SII), 3	81.41	32.84	45.07	90.09	38.01	56.55
Bt.5635.1.S1_at	TCEAL1	transcription elongation factor A (SII)-like 1	428	497	421	704	747	530
Bt.20091.1.S1_at	TCF20	transcription factor 20 (AR1)	64.62	95.86	95.10	66.96	112	79.39

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.25103.1.S1_at	TDRD7	tudor domain containing 7	321	584	375	359	299	299
Bt.13834.1.S1_at	TFRC	transferrin receptor (p90, CD71)	3439	2637	4924	3246	4572	4708
Bt.6275.1.S1_at	TGFBR1	transforming growth factor, beta receptor 1	476	419	288	543	618	402
Bt.4619.1.S1_at	TH1L	TH1-like (Drosophila)	514	376	559	538	417	519
Bt.23605.2.S1_at	THRA	thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian)	198	176	394	161	178	209
Bt.10880.1.S1_at	TIMM50	translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae)	138	108	113	117	70.76	135
Bt.22554.1.A1_at	TK2	thymidine kinase 2, mitochondrial	116	171	175	171	122	116
Bt.22413.1.A1_at	TLE4	Transducin-like enhancer of split 4 (E(sp1) homolog, Drosophila)	87.59	122	128	97.08	110	96.48
Bt.13981.1.S1_at	TM2D2	TM2 domain containing 2	847	692	685	1150	1196	673
Bt.20586.1.S1_a_at	TM4SF5	transmembrane 4 L six family member 5	4265	2497	4507	3863	3462	3931
Bt.9567.1.S1_at	TM7SF2	transmembrane 7 superfamily member 2	414	179	340	375	273	404
Bt.2416.1.S2_at	TMBIM6	transmembrane BAX inhibitor motif containing 6	9072	6141	9467	9925	8946	9457
Bt.11176.2.S1_at	TMEM14A	transmembrane protein 14A	364	232	243	248	321	365
Bt.8039.1.S1_at	TMEM170A	transmembrane protein 170A	947	682	679	891	1093	645
Bt.26998.1.A1_s_at	TNNC1	troponin C type 1 (slow)	4.57	94.99	4.57	4.57	4.57	4.57
Bt.6012.1.S1_at	TNNC1	troponin C type 1 (slow)	4.51	33.57	4.51	4.67	4.54	4.54
Bt.9992.1.S1_at	TNNC2	troponin C type 2 (fast)	6.58	101	6.77	6.55	6.52	6.55
Bt.12957.1.A1_at	TNRC6B	trinucleotide repeat containing 6B	751	970	938	885	502	736
Bt.21839.1.A1_at	TOP1	Topoisomerase (DNA) I	1397	2081	1534	1591	1630	1255
Bt.19057.1.S1_at	TOR1A	torsin family 1, member A (torsin A)	97.29	32.48	44.88	41.19	47.25	83.48
Bt.842.1.A1_at	TOR1AIP1	torsin A interacting protein 1	547	588	461	678	746	536
Bt.3487.1.S1_at	TPI1	triosephosphate isomerase 1	1744	1287	1822	1263	908	1956
Bt.12477.2.S1_at	TPM2	tropomyosin 2 (beta)	4.53	62.96	4.55	4.55	4.58	5.08
Bt.12477.1.S1_a_at	TPM2	tropomyosin 2 (beta)	124	228	82.25	110	89.41	219
Bt.17628.1.A1_at	TRAK2	trafficking protein, kinesin binding 2	59.75	7.97	7.81	62.23	9.44	65.33
Bt.8235.1.S1_at	TRAPPC5	trafficking protein particle complex 5	187	121	165	167	137	173
Bt.22980.1.S1_at	TRIM21	tripartite motif-containing 21	8.81	27.90	8.63	9.83	7.69	9.30
Bt.27071.1.S1_at	TRIM38	tripartite motif-containing 38	258	460	322	269	248	261
Bt.1529.2.A1_at	TSG118	protein C16orf88 homolog	137	191	119	208	103	189
Bt.5444.1.S1_at	TSPAN3	tetraspanin 3	2360	1511	1841	2254	1796	2353
Bt.16052.2.A1_at	TSPYL1	TSPY-like 1	1125	1269	697	1330	1133	1114
Bt.460.1.S1_at	TST	thiosulfate sulfurtransferase (rhodanese)	3375	2078	2946	3039	2207	2978
Bt.20848.1.A1_at	TTC36	tetratricopeptide repeat domain 36	2636	938	1992	2670	1714	2921
Bt.21767.1.S1_at	TTN	titin	5.15	87.46	5.02	5.02	5.13	5.10
Bt.21767.1.S1_a_at	TTN	titin	7.33	325	8.82	10.04	10.30	10.02
Bt.5183.1.S1_at	TUBA4A	tubulin, alpha 4a	373	463	553	380	216	493
Bt.27119.1.A1_at	TUBE1	tubulin, epsilon 1	268	281	219	241	442	280
Bt.2294.1.S1_a_at	UBA7	ubiquitin-like modifier activating enzyme 7	73.08	482	132	124	72.93	76.83
Bt.19006.2.A1_at	UPB1	Ureidopropionase, beta	167	128	309	232	301	243

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.17653.1.A1_at	UPP2	uridine phosphorylase 2	1701	3367	969	2002	4761	2368
Bt.23164.1.S1_at	UQCRC1	UQCRC1 protein	545	497	604	482	335	592
Bt.24095.1.A1_at	USP1	Ubiquitin specific peptidase 1	568	742	559	605	921	582
Bt.21721.1.A1_at	USP2	ubiquitin specific peptidase 2	4.74	6.27	4.52	17.26	4.74	4.75
Bt.14124.2.S1_at	USP33	ubiquitin specific peptidase 33	118	142	104	161	242	120
Bt.17717.1.A1_at	USPL1	ubiquitin specific peptidase like 1	204	327	346	260	278	228
Bt.20427.2.S1_at	UTP6	UTP6, small subunit (SSU) processome component, homolog (yeast)	56.55	236	234	136	131	133
Bt.25537.1.A1_at	UXS1	UDP-glucuronate decarboxylase 1	42.73	71.63	54.73	53.55	97.25	39.91
Bt.3549.1.A1_at	VAMP4	vesicle-associated membrane protein 4	178	176	123	173	273	144
Bt.24281.1.S1_at	VAPA	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa	976	796	844	1106	1047	1114
Bt.11270.2.S1_at	VARS	valyl-tRNA synthetase	41.86	61.05	59.62	41.51	23.90	54.54
Bt.282.1.S1_at	VDAC1P5	voltage-dependent anion channel 1 pseudogene 5	1278	1118	1117	1207	711	1303
Bt.28243.1.S1_a_at	VNN1	vanin 1	2470	891	1744	2361	2126	2547
Bt.2170.1.A1_at	VPS33A	vacuolar protein sorting 33 homolog A (<i>S. cerevisiae</i>)	210	150	173	216	139	237
Bt.29587.1.S1_at	WAC	WW domain containing adaptor with coiled-coil	113	115	135	164	205	127
Bt.20322.3.S1_a_at	WDR18	WD repeat domain 18	75.31	51.90	104	49.18	51.90	70.11
Bt.5196.1.S1_at	WDR55	WD repeat domain 55	643	676	754	611	497	702
Bt.28187.1.S1_at	WEE1	WEE1 homolog (<i>S. pombe</i>)	120	126	79.14	195	125	130
Bt.26825.1.A1_at	XRN2	5'-3' exoribonuclease 2	392	649	541	814	463	400
Bt.11237.1.S1_at	YTHDC1	YTH domain containing 1	926	1284	1115	1134	1189	915
Bt.27876.1.A1_at	ZCCHC10	zinc finger, CCHC domain containing 10	17.59	28.98	13.44	17.77	17.15	17.26
Bt.12141.2.S1_a_at	ZCCHC6	zinc finger, CCHC domain containing 6	310	621	375	316	403	294
Bt.23941.1.A1_at	ZFP161	zinc finger protein 161 homolog (mouse)	240	306	283	214	325	278
Bt.3863.1.S1_at	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)	140	307	165	320	119	233
Bt.13489.1.S1_at	ZMIZ1	zinc finger, MIZ-type containing 1	95.10	186	109	126	124	148
Bt.12664.2.S1_at	ZMYM5	Zinc finger, MYM-type 5	96.90	82.83	101	141	212	90.81
Bt.17848.2.S1_at	ZMYND8	zinc finger, MYND-type containing 8	54.37	79.99	99.68	55.69	64.59	68.53
Bt.18023.1.S1_at	ZNF322	zinc finger protein 322	271	237	285	335	399	264
Bt.10631.1.A1_at	ZNF547	zinc finger protein 547	105	114	160	149	104	103
Bt.18479.1.A1_at	ZNF608	zinc finger protein 608	142	211	174	121	123	178
Bt.1602.1.S1_at	ZNF613	zinc finger protein 613	93.39	92.13	184	139	130	141
Bt.2186.1.S1_at	ZNFX1	zinc finger, NFX1-type containing 1	243	1480	338	469	170	254
Bt.17229.1.A1_at	ZNFX1	zinc finger, NFX1-type containing 1	4.62	7.64	4.63	5.27	4.63	4.62
Bt.7208.1.S1_at	ZP2	zona pellucida glycoprotein 2 (sperm receptor)	46.64	15.48	204	24.62	51.46	22.87
Bt.29175.1.A1_at	ZUFSP	zinc finger with UFM1-specific peptidase domain	285	379	301	374	382	272
Bt.26650.1.S1_at	---	---	16.66	9.37	17.83	17.81	43.39	22.64
Bt.841.1.S1_at	---	Transcribed locus	10.73	11.66	12.51	16.97	26.97	12.63
Bt.16425.1.A1_at	---	Transcribed locus	11.51	14.89	15.42	27.25	20.33	17.15

Appendix A. Continued

Affimetrix ID	Gene Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
			CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.13815.1.S1_at	---	Transcribed locus	103	59.15	103	123	154	107
Bt.17034.1.A1_at	---	---	4.73	4.65	4.73	18.47	4.52	4.86
Bt.24524.2.A1_at	---	---	86.29	57.66	137	139	115	88.23
Bt.22188.1.S1_at	---	---	457	421	945	678	769	482
Bt.17364.1.A1_at	---	---	1029	428	829	2525	3215	1162
Bt.19906.1.A1_at	---	---	18.89	8.97	14.98	29.04	39.94	18.61
Bt.16509.1.A1_at	---	Transcribed locus	144	118	219	413	302	207
Bt.16058.1.A1_at	---	---	44.46	32.09	91.39	38.12	135	69.51
Bt.19120.1.A1_at	---	---	47.81	40.36	48.95	42.94	93.84	83.78
Bt.6636.1.S1_at	---	Transcribed locus	18.13	59.09	7.92	8.46	8.64	8.11
Bt.19792.1.A1_at	---	---	36.89	95.64	36.34	35.04	39.15	28.17
Bt.28164.2.S1_at	---	---	85.22	197	62.81	152	59.84	64.27
Bt.23735.1.A1_s_at	---	---	5.02	22.02	10.90	4.87	10.31	11.26
Bt.15807.1.S1_at	---	---	152	140	117	148	232	181
Bt.9785.1.S1_at	---	---	74.59	60.19	67.60	60.70	114	84.10
Bt.24316.1.A1_at	---	---	457	297	374	285	654	712
Bt.20501.1.S1_at	---	---	7.25	6.90	7.51	6.97	10.81	27.43
Bt.19232.1.A1_at	---	---	26.24	22.81	7.01	14.00	39.85	14.87
Bt.29581.1.A1_at	---	---	4.77	5.29	15.84	4.97	5.56	4.93
Bt.22543.1.S1_at	---	---	113	118	139	129	73.19	101
Bt.26926.1.S1_at	---	---	57.61	61.11	134	63.84	57.91	59.95
Bt.18206.1.A1_at	---	---	289	160	460	359	188	229
Bt.18861.1.A1_at	---	---	303	143	278	449	245	224
Bt.6575.1.A1_at	---	Transcribed locus, strongly similar to NP_663476.1 [Mus musculus]	114	127	218	264	155	86.81
Bt.5771.1.S1_at	---	Transcribed locus	221	141	266	190	133	186
Bt.10130.1.S1_at	---	Transcribed locus	28.58	54.53	51.04	37.94	17.10	26.57
Bt.13308.1.S1_at	---	Transcribed locus	67.89	70.75	98.90	112	65.27	78.63
Bt.28739.1.S1_at	---	---	686	956	399	1353	237	893
Bt.6890.1.S1_at	---	Transcribed locus	3302	4919	2963	3959	2688	4242
Bt.28238.1.A1_at	---	---	2814	1669	3155	1789	4600	2916
Bt.17263.1.S1_at	---	---	514	402	567	285	544	428
Bt.22076.1.A1_at	---	---	279	169	250	178	296	229
Bt.20592.1.S1_at	---	---	65.36	31.50	66.84	36.22	66.60	49.91
Bt.13429.2.S1_at	---	Transcribed locus	68.33	28.75	79.35	41.39	73.70	55.60
Bt.15706.1.A1_at	---	---	49.78	47.99	52.09	44.80	104	38.28
Bt.26416.1.A1_at	---	---	117	71.20	90.14	107	321	77.13
Bt.3555.1.S1_at	---	Transcribed locus	71.43	47.21	52.21	74.13	46.12	60.80
Bt.23902.1.A1_at	---	---	2879	2151	2262	2150	1734	2314
Bt.19118.1.A1_at	---	---	552	360	319	470	460	634
Bt.22063.2.S1_at	---	---	1688	985	1266	1718	1710	1680
Bt.12381.1.A1_at	---	Transcribed locus, moderately similar to NP_001026004.1 [Gallus gallus]	62.56	40.36	59.38	60.62	63.22	66.31
Bt.12360.1.S1_at	---	---	334	240	234	303	301	353
Bt.1252.1.S1_at	---	Transcribed locus	413	285	395	504	348	449
Bt.20404.1.S1_at	---	---	374	237	240	358	294	286
Bt.23706.1.A1_at	---	---	125	82.85	92.11	113	118	123
Bt.19284.1.A1_at	---	---	185	377	292	201	222	222
Bt.11918.1.A1_at	---	---	130	203	314	83.90	128	169
Bt.8920.1.S1_at	---	Transcribed locus	285	402	391	362	544	370
Bt.29960.1.S1_at	---	Transcribed locus	181	228	234	127	212	124
Bt.9098.1.A1_at	---	Transcribed locus	4.76	7.68	4.96	5.09	4.71	4.95
Bt.18873.1.A1_at	---	---	77.18	364	133	121	63.16	77.74
Bt.29924.1.S1_at	---	Transcribed locus	1034	1645	1210	1261	1085	1102
Bt.10692.1.S1_at	---	CDNA clone IMAGE:8398549	243	365	318	300	343	248
Bt.16739.1.A1_at	---	Transcribed locus	1312	2385	6793	1848	2457	2075

Appendix A. Continued

Affimetrix ID	Gene	Symbol	Gene Title	Treatment (Dam diet-Milk replacer)					
				CTL-LLA	CTL-HLA	SFA-LLA	SFA-HLA	EFA-LLA	EFA-HLA
Bt.7576.1.S1_at	---		Transcribed locus	23.44	31.94	23.56	24.86	27.80	23.24
Bt.26415.1.A1_at	---		---	129	160	182	115	157	156
Bt.25832.1.S1_at	---		---	36.53	46.31	42.28	41.34	80.74	32.84
Bt.26232.2.A1_at	---		---	28.82	62.09	31.86	31.65	28.36	29.81
Bt.19339.1.S1_at	---		---	30.19	47.60	38.81	55.66	53.51	39.07
Bt.11791.2.S1_at	---		Transcribed locus	485	537	655	484	601	495
Bt.25196.1.A1_at	---		---	100	167	120	153	147	151
Bt.2465.1.S1_at	---		---	44.52	95.88	58.49	47.80	58.80	57.64
Bt.24940.1.A1_at	---		---	53.13	335	177	158	277	177
Bt.19107.1.S1_at	---		---	291	631	339	373	330	280
Bt.7349.1.S1_at	---		Transcribed locus	18.05	29.73	18.69	21.48	34.43	19.29
Bt.12854.1.S1_at	---		Transcribed locus	149	213	188	157	265	138
Bt.22335.1.S1_a_at	---		---	741	1057	920	925	844	848
Bt.23306.1.S1_at	---		---	448	699	526	556	638	596
Bt.25084.1.S1_at	---		---	329	433	421	350	676	327
Bt.17073.1.S1_at	---		---	122	142	120	80.25	70.20	163
Bt.16525.1.A1_at	---		Transcribed locus	356	436	502	320	232	430
Bt.18914.1.S1_at	---		---	311	385	345	273	274	381
Bt.10361.1.S1_at	---		---	6.52	5.82	9.34	6.27	6.02	6.10
Bt.18847.1.A1_at	---		---	14.55	36.38	28.42	17.59	9.14	25.33
Bt.13633.1.A1_at	---		---	144	262	235	113	99.59	191
Bt.29324.1.S1_at	---		---	48.08	51.61	44.40	55.15	95.49	35.23
Bt.21952.1.A1_at	---		---	127	136	113	156	183	127
Bt.2962.1.S1_at	---		---	108	121	94.43	150	342	111
Bt.15299.1.A1_at	---		---	6.60	6.12	6.28	6.91	11.56	6.82
Bt.21957.1.S1_at	---		---	103	109	120	162	210	112
Bt.29107.1.S1_at	---		---	177	182	136	226	239	188
Bt.22044.1.S1_at	---		---	173	180	107	182	305	155
Bt.17883.2.A1_at	---		---	18.85	15.85	11.20	19.37	34.95	15.28
Bt.28101.1.S1_at	---		---	118	102	118	108	180	105
Bt.22656.2.S1_at	---		---	334	399	278	406	540	284
Bt.23900.1.A1_at	---		---	259	280	221	270	396	260
Bt.17846.1.A1_at	---		---	41.86	31.49	18.69	34.76	48.49	25.32
Bt.812.1.S1_at	---		Transcribed locus	215	235	131	204	248	185
Bt.14283.1.A1_at	---		Transcribed locus	123	116	109	118	232	116
Bt.8039.2.S1_a_at	---		---	347	305	209	338	432	281
Bt.23992.1.A1_at	---		---	148	331	109	319	203	124
Bt.25190.1.A1_at	---		---	1023	1155	1092	1180	2108	1056
Bt.20666.1.S1_at	---		---	96.51	111	84.56	122	182	88.08
Bt.16828.1.A1_at	---		Transcribed locus	16.69	22.27	15.72	22.40	19.73	15.17
Bt.2765.1.S1_at	---		---	222	244	199	267	334	226

APPENDIX B
DIFFERENTIALY EXPRESSED GENES FOR THE CONTRAST OF FAT

List of differential expressed genes in liver of Holstein males at 30 d of age. Effect of feeding fat prepartum (contrast FAT, control = reference). Calves born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Genes are ranked by adjusted P value in descendant order.

Affimetrix ID	Gene symbol	Fold change	Ave. Exp. (SFA + EFA)	Ave. Exp. Control	Adjusted P value	Regulation
Bt.26650.1.S1_at	---	1.89	23.63	12.49	1.84E-07	UP
Bt.26769.1.S1_at	GIMAP8	2.13	10.06	4.72	8.40E-07	UP
Bt.7575.1.A1_at	GPT2	1.72	290	168	3.68E-06	UP
Bt.841.1.S1_at	---	1.47	16.40	11.18	3.79E-06	UP
Bt.4404.1.A1_at	PRSS2	2.21	10.76	4.87	1.01E-05	UP
Bt.16425.1.A1_at	---	1.49	19.56	13.09	4.77E-05	UP
Bt.1785.1.A1_at	LOC532189	1.46	299	205	1.57E-04	UP
Bt.6813.1.A1_at	AKAP5	1.66	96.26	57.88	1.94E-04	UP
Bt.6521.1.A1_at	PARD6B	1.40	66.97	47.71	3.61E-04	UP
Bt.13815.1.S1_at	---	1.54	120	77.94	4.01E-04	UP
Bt.24779.2.S1_at	CREM	1.59	8.26	5.20	4.01E-04	UP
Bt.17034.1.A1_at	---	1.41	6.62	4.69	5.32E-04	UP
Bt.18003.1.S1_at	CUL3	1.53	14.12	9.22	5.57E-04	UP
Bt.19614.1.A1_at	LIPC	1.42	3771	2661	6.01E-04	UP
Bt.19006.2.A1_at	UPB1	1.84	269	147	6.01E-04	UP
Bt.17451.2.A1_at	SESTD1	1.65	10.22	6.18	1.01E-03	UP
Bt.24249.1.S1_at	SUV420H1	1.44	78.58	54.53	2.11E-03	UP
Bt.25663.1.A1_at	CPNE8	1.62	176	108	2.22E-03	UP
Bt.26538.1.S1_at	LOC509420	1.70	31.44	18.45	2.51E-03	UP
Bt.10686.1.S1_at	RNF170	1.48	788	532	2.56E-03	UP
Bt.10777.1.S1_at	FOXP1	1.56	71.96	46.17	2.94E-03	UP
Bt.24524.2.A1_at	---	1.67	118	70.54	3.65E-03	UP
Bt.15691.1.S1_at	KCNK5	1.58	138	87.19	5.11E-03	UP
Bt.22188.1.S1_at	---	1.59	698	439	5.60E-03	UP
Bt.17364.1.A1_at	---	2.52	1672	664	8.42E-03	UP
Bt.24848.1.A1_at	PTPRD	1.92	108	56.08	1.35E-02	UP
Bt.16234.2.S1_at	SFRS18	1.67	104	62.48	1.36E-02	UP
Bt.19906.1.A1_at	---	1.83	23.85	13.02	1.65E-02	UP
Bt.12820.1.S1_at	PGRMC1	1.51	6381	4213	1.73E-02	UP
Bt.16509.1.A1_at	---	2.11	274	130	1.99E-02	UP
Bt.16137.1.S1_at	ALDH9A1	1.62	540	334	2.19E-02	UP
Bt.27073.1.S1_at	ACADL	1.48	928	629	2.84E-02	UP
Bt.29879.1.S1_at	KAT2B	1.45	84.48	58.07	2.92E-02	UP
Bt.28278.1.S1_at	ACE2	2.34	1080	463	3.00E-02	UP
Bt.2587.2.S1_a_at	FH	1.44	377	262	3.06E-02	UP
Bt.16580.1.S1_at	CD2AP	1.56	35.42	22.66	3.06E-02	UP
Bt.633.2.S1_a_at	SFXN1	2.60	755	290	3.06E-02	UP
Bt.12745.1.A1_at	ANTXR2	1.45	101	69.81	3.10E-02	UP
Bt.633.1.S1_at	SFXN1	2.40	1034	431	3.24E-02	UP
Bt.26302.1.A1_at	SCML1	1.44	28.57	19.77	3.30E-02	UP
Bt.8491.1.S1_at	SMOC2	2.01	131	65.13	3.30E-02	UP
Bt.16058.1.A1_at	---	2.00	75.65	37.77	3.48E-02	UP
Bt.13834.1.S1_at	TFRC	1.43	4307	3012	3.57E-02	UP
Bt.25752.1.A1_at	C7H5orf24	1.47	65.43	44.47	3.74E-02	UP

Appendix B. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. (SFA + EFA)	Ave. Exp. Control	Adjusted P value	Regulation
Bt.16250.2.S1_at	SLC10A1	1.52	433	286	3.86E-02	UP
Bt.1602.1.S1_at	ZNF613	1.59	147	92.76	3.87E-02	UP
Bt.16058.2.S1_at	LOC100583040	2.17	48.14	22.14	4.04E-02	UP
Bt.20514.1.S1_at	ATG2B	1.44	347	241	4.06E-02	UP
Bt.190.1.A1_at	IGFBP1	1.79	50.90	28.51	4.20E-02	UP
Bt.19544.1.A1_at	ACSM2A	1.41	4937	3509	4.25E-02	UP
Bt.20261.1.S1_at	PTPN3	1.45	55.20	38.05	4.28E-02	UP
Bt.7023.1.S1_at	FAHD2A	1.44	657	457	4.37E-02	UP
Bt.12579.1.A1_at	GK5	1.94	2425	1247	4.37E-02	UP
Bt.2905.1.S1_at	NDRG2	1.45	2103	1451	4.40E-02	UP
Bt.18440.2.S1_at	LOC510382	1.47	8.83	5.99	4.64E-02	UP
Bt.16276.1.A1_at	ARSK	1.49	458	307	4.68E-02	UP
Bt.19120.1.A1_at	---	1.45	63.76	43.93	4.71E-02	UP
Bt.13777.2.S1_at	GIMAP7	2.00	44.52	22.21	4.94E-02	UP
Bt.12300.2.S1_at	MYH2	11.29	4.53	51.17	4.61E-15	DOWN
Bt.4922.1.S1_at	MYL1	9.11	4.53	41.25	5.52E-15	DOWN
Bt.8435.1.S1_at	ACTA1	8.94	4.89	43.70	5.55E-13	DOWN
Bt.26998.1.A1_s_at	TNNC1	4.56	4.57	20.83	5.71E-13	DOWN
Bt.1905.1.S1_at	MYL2	4.87	4.57	22.25	5.71E-13	DOWN
Bt.9992.1.S1_at	TNNC2	3.91	6.60	25.78	3.20E-12	DOWN
Bt.21767.1.S1_at	TTN	4.19	5.07	21.22	3.20E-12	DOWN
Bt.12477.2.S1_at	TPM2	3.61	4.68	16.90	3.36E-12	DOWN
Bt.23696.1.A1_at	LOC509457	3.00	13.69	41.08	3.90E-11	DOWN
Bt.6012.1.S1_at	TNNC1	2.69	4.57	12.31	8.24E-11	DOWN
Bt.6620.1.S1_at	MYH7	2.56	4.60	11.81	2.48E-10	DOWN
Bt.20557.1.S1_at	ACTN2	2.92	4.96	14.49	5.56E-09	DOWN
Bt.11687.1.S1_a_at	SRL	1.93	4.52	8.74	1.01E-08	DOWN
Bt.8143.1.S1_at	MX2	2.36	5.57	13.12	1.29E-07	DOWN
Bt.22169.1.S1_at	ENO3	2.19	9.82	21.49	2.44E-07	DOWN
Bt.27463.1.A1_at	HERC6	1.68	4.80	8.04	5.03E-07	DOWN
Bt.4126.2.S1_at	CYP4A22	1.77	34.05	60.28	5.03E-07	DOWN
Bt.10310.1.S1_at	MYBPC1	1.52	4.71	7.15	5.20E-06	DOWN
Bt.9655.2.S1_at	LOC790332	3.04	30.57	92.93	5.25E-06	DOWN
Bt.22199.1.S1_at	DDIT4L	1.56	5.77	8.97	5.43E-06	DOWN
Bt.9779.1.S1_at	ISG12(B)	2.84	6.83	19.41	1.56E-05	DOWN
Bt.6636.1.S1_at	---	3.95	8.28	32.73	1.75E-05	DOWN
Bt.22065.1.S1_at	LOC783920	1.59	4.87	7.72	2.09E-05	DOWN
Bt.21461.1.S1_at	LOC509034	2.51	5.98	15.03	4.04E-05	DOWN
Bt.6972.1.S1_at	KBTBD10	1.50	5.03	7.55	4.53E-05	DOWN
Bt.21767.1.S1_a_at	TTN	4.99	9.78	48.78	4.77E-05	DOWN
Bt.4937.1.S1_at	LOC505941	1.49	2326	3464	1.73E-03	DOWN
Bt.4762.1.S1_at	BOLA-NC1	1.65	33.13	54.74	2.65E-03	DOWN
Bt.12285.3.S1_a_at	NMI	1.42	820	1164	3.07E-03	DOWN
Bt.22980.1.S1_at	TRIM21	1.78	8.83	15.68	3.07E-03	DOWN
Bt.154.1.S1_at	CCL8	1.46	12.67	18.44	4.66E-03	DOWN
Bt.24492.1.S1_at	STAT2	1.47	431	634	4.74E-03	DOWN
Bt.28914.1.A1_at	RP2	1.47	95.71	141	9.70E-03	DOWN
Bt.19792.1.A1_at	---	1.73	34.42	59.40	1.05E-02	DOWN
Bt.25957.1.S1_at	MAVS	1.60	48.38	77.47	1.35E-02	DOWN
Bt.22116.1.A1_at	IL18BP	1.76	10.69	18.80	1.93E-02	DOWN
Bt.18114.1.A1_at	LOC100851000	1.52	42.78	64.88	1.96E-02	DOWN
Bt.18415.1.A1_at	FTSJD1	1.87	329	616	2.04E-02	DOWN
Bt.15037.1.S1_at	ST3GAL1	1.43	418	599	2.10E-02	DOWN

Appendix B. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. (SFA + EFA)	Ave. Exp. Control	Adjusted P value	Regulation
Bt.5372.1.S1_at	ICAM1	1.47	154	226	2.38E-02	DOWN
Bt.20110.1.S1_at	PSMF1	1.63	275	448	2.41E-02	DOWN
Bt.8090.2.S1_at	MYBBP1A	1.41	57.23	80.95	2.60E-02	DOWN
Bt.6556.1.S1_at	LOC504773	1.53	1522	2326	3.06E-02	DOWN
Bt.18321.1.A1_at	GNB4	1.84	159	293	3.24E-02	DOWN
Bt.2186.1.S1_at	ZNFX1	2.09	288	600	3.24E-02	DOWN
Bt.8997.1.S1_at	RANGAP1	1.84	112	207	3.42E-02	DOWN
Bt.9791.1.S1_at	PPIF	1.56	1058	1649	4.06E-02	DOWN
Bt.29823.1.S1_x_at	BOLA	2.33	20.25	47.12	4.28E-02	DOWN
Bt.5768.1.S1_at	IRF7	1.87	93.23	174	4.37E-02	DOWN
Bt.5129.1.S1_a_at	NNAT	1.87	21.31	39.77	4.96E-02	DOWN
Bt.28164.2.S1_at	---	1.66	77.82	129	4.97E-02	DOWN

APPENDIX C
DIFFERENTIALY EXPRESSED GENES FOR THE CONTRAST OF FATTY ACIDS

List of differential expressed genes in liver of Holstein male at 30 d of age. Effect of feeding essential fatty acids prepartum (Contrast FA, reference = saturated fatty acid diet). Calves were born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date. Genes are ranked by adjusted P value in descendant order.

Affimetrix ID	Gene symbol	Fold change	Av. Exp. EFA	Ave. Exp. SFA	Adjusted P value	Regulation
Bt.26150.1.A1_at	L2HGDH	1.70	254	149	7.34E-05	UP
Bt.18634.1.A1_at	PPM1K	1.61	840	522	2.97E-04	UP
Bt.20977.3.S1_at	CCPG1	1.41	129	91.53	0.0034	UP
Bt.24793.1.S1_at	MN1	1.41	7.25	5.14	0.0092	UP
Bt.26522.2.S1_at	C1H3ORF34	1.44	57.92	40.21	0.0016	UP
Bt.9140.1.S1_at	GMNN	1.65	254	154	4.11E-04	UP
Bt.15872.1.S1_at	SLU7	2.43	614	253	8.40E-04	UP
Bt.6397.2.S1_at	HMGB2	1.70	1503	884	1.54E-03	UP
Bt.3191.1.A1_at	KLHL24	2.15	939	437	1.57E-03	UP
Bt.1048.1.S1_at	BORA	1.47	73.14	49.64	0.0384	UP
Bt.24892.1.A1_at	RIT1	1.50	473	316	3.42E-03	UP
Bt.23735.1.A1_s_at	---	1.48	10.78	7.29	0.0296	UP
Bt.27966.1.S1_at	LOC532789	1.48	63.01	42.59	0.0077	UP
Bt.22479.1.S1_at	CPEB4	1.48	22.05	14.89	0.0291	UP
Bt.21764.1.S1_at	TBC1D15	1.93	339	176	3.72E-03	UP
Bt.17653.1.A1_at	UPP2	2.41	3358	1393	6.36E-03	UP
Bt.3843.1.S1_at	IGJ	1.51	989	655	6.36E-03	UP
Bt.10777.1.S1_at	FOXP1	1.51	88.54	58.49	0.0226	UP
Bt.13743.1.A1_at	RFK	1.57	1322	841	6.36E-03	UP
Bt.15807.1.S1_at	---	1.56	205	131	7.33E-03	UP
Bt.9785.1.S1_at	---	1.53	98.01	64.06	0.0052	UP
Bt.24258.2.S1_at	MAN1A1	1.57	841	536	8.27E-03	UP
Bt.11751.1.A1_at	KLHL23	1.57	83.88	53.54	0.0335	UP
Bt.8903.1.S1_at	C14H8ORF70	1.45	258	178	8.50E-03	UP
Bt.20373.1.S1_at	NRP1	1.46	1019	700	8.55E-03	UP
Bt.5582.1.S1_at	SH3BGR	1.58	43.06	27.32	0.0214	UP
Bt.16187.1.A1_at	KBTBD6	2.42	330	136	8.92E-03	UP
Bt.19274.1.A1_at	C1QTNF7	1.59	7.42	4.65	0.0000	UP
Bt.20267.1.S1_at	GCLM	1.47	209	142	1.25E-02	UP
Bt.24316.1.A1_at	---	2.09	682	327	1.25E-02	UP
Bt.18540.1.A1_at	MGC165715	1.61	615	382	1.33E-02	UP
Bt.20677.1.S1_at	NSL1	1.62	70.65	43.64	0.0043	UP
Bt.27889.1.S1_at	DLD	1.62	81.34	50.06	0.0000	UP
Bt.8829.1.S1_a_at	IFT122	1.52	195	128	1.63E-02	UP
Bt.19745.1.S1_at	ELL2	1.61	528	327	1.97E-02	UP
Bt.18928.1.A1_at	EIF4E3	1.70	266	156	2.39E-02	UP
Bt.27119.1.A1_at	TUBE1	1.53	351	230	2.45E-02	UP
Bt.21433.1.S1_at	MCM6	1.59	333	209	2.45E-02	UP
Bt.1817.1.S1_at	ETV1	1.72	33.94	19.79	0.0034	UP
Bt.2.1.S1_at	CDK1	1.72	32.97	19.16	0.0139	UP
Bt.28764.1.A1_at	LOC787057	1.71	70.71	41.31	0.0053	UP
Bt.9774.1.S1_a_at	MGC165862	1.76	378	214	2.45E-02	UP
Bt.26650.1.S1_at	---	1.76	31.34	17.82	0.0000	UP
Bt.19519.1.S1_at	HLTF	1.40	1608	1149	2.74E-02	UP

Appendix C. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. EFA	Ave. Exp. SFA	Adjusted P value	Regulation
Bt.18440.2.S1_at	LOC510382	1.80	11.85	6.58	0.0140	UP
Bt.17805.2.A1_at	NUDT12	1.82	147	80.96	0.0079	UP
Bt.2506.1.S1_at	DKK3	1.85	76.89	41.47	0.0345	UP
Bt.11772.2.S1_at	LOC614339	1.86	116	62.29	0.0437	UP
Bt.18316.1.A1_at	LOC513587	1.89	165	87.57	0.0468	UP
Bt.26635.2.S1_at	FZD1	1.89	134	71.06	0.0413	UP
Bt.10007.1.A1_at	CKAP2	1.92	106	55.40	0.0117	UP
Bt.17517.1.S1_at	MGC134574	1.41	539	383	3.23E-02	UP
Bt.8135.1.S1_at	LRAT	1.93	141	73.34	0.0251	UP
Bt.19120.1.A1_at	---	1.93	88.67	45.85	0.0052	UP
Bt.26364.1.A1_at	BTBD8	2.02	27.30	13.52	0.0001	UP
Bt.28162.1.S1_at	PLN	2.02	186	91.82	0.0090	UP
Bt.14369.1.A1_at	CYP39A1	1.68	176	105	4.04E-02	UP
Bt.18792.1.S1_at	DCTN6	2.10	55.91	26.67	0.0064	UP
Bt.15906.1.S1_at	PLS3	1.48	2005	1353	4.28E-02	UP
Bt.11055.1.S1_at	SDPR	1.48	2822	1910	4.29E-02	UP
Bt.26302.1.A1_at	SCML1	2.15	41.92	19.47	0.0008	UP
Bt.22869.1.S2_at	FABP5	2.26	25.09	11.08	0.0085	UP
Bt.20501.1.S1_at	---	2.38	17.22	7.23	0.0052	UP
Bt.9655.2.S1_at	LOC790332	2.40	47.41	19.72	0.0002	UP
Bt.2999.1.A1_at	LOC783843	1.44	149	103	4.37E-02	UP
Bt.20399.1.S1_at	HSD17B13	2.10	1029	490	4.44E-02	UP
Bt.16382.1.A1_at	CALCRL	1.76	338	192	4.82E-02	UP
Bt.19232.1.A1_at	---	2.46	24.34	9.91	0.0429	UP
Bt.23566.2.S1_at	LOC785936	2.72	43.89	16.13	0.0296	UP
Bt.28934.1.S1_at	AREG	3.31	21.82	6.59	0.0139	UP
Bt.6802.1.S1_at	RGS5	3.46	338	97.59	0.0000	UP
Bt.28182.1.A1_at	RGS5	4.13	62.17	15.04	0.0090	UP
Bt.26769.1.S1_at	GIMAP8	4.29	20.83	4.86	0.0000	UP
Bt.190.1.A1_at	IGFBP1	4.70	110	23.48	0.0001	UP
Bt.23696.1.A1_at	LOC509457	9.00	41.06	4.56	0.0000	UP
Bt.4404.1.A1_at	PRSS2	5.26	4.69	24.68	3.77E-08	DOWN
Bt.17415.3.A1_at	ERRFI1	1.85	6.13	11.32	4.16E-06	DOWN
Bt.17034.1.A1_at	---	1.99	4.69	9.34	1.32E-05	DOWN
Bt.21721.1.A1_at	USP2	1.86	4.74	8.83	7.50E-05	DOWN
Bt.29581.1.A1_at	---	1.69	5.24	8.87	3.37E-04	DOWN
Bt.22543.1.S1_at	---	1.55	86.06	134	4.11E-04	DOWN
Bt.26926.1.S1_at	---	1.57	58.92	92.33	7.02E-04	DOWN
Bt.12957.1.A1_at	TNRC6B	1.50	608	911	1.12E-03	DOWN
Bt.8090.2.S1_at	MYBBP1A	1.84	42.16	77.68	2.29E-03	DOWN
Bt.5399.1.S2_at	NADK	1.50	1191	1790	3.37E-03	DOWN
Bt.5415.1.S1_at	CCS	2.98	149	443	5.16E-03	DOWN
Bt.6020.1.S1_at	DNAJC11	1.52	107	161	6.36E-03	DOWN
Bt.7963.1.S1_at	EHD1	1.63	142	232	7.35E-03	DOWN
Bt.3248.1.S1_at	ALDH4A1	1.41	230	324	8.27E-03	DOWN
Bt.2858.1.S1_at	ABHD6	1.45	35.85	51.84	8.44E-03	DOWN
Bt.21336.1.S1_a_at	MAD2L2	1.66	88.39	147	8.44E-03	DOWN
Bt.26825.1.A1_at	XRN2	1.54	430	663	8.50E-03	DOWN
Bt.11256.1.S1_at	CNOT1	1.49	859	1283	8.55E-03	DOWN
Bt.28245.1.S1_at	OSTBETA	1.90	656	1247	9.41E-03	DOWN
Bt.11259.1.S1_at	ISG12(A)	5.32	1394	7411	9.98E-03	DOWN
Bt.4937.1.S1_at	LOC505941	1.46	1925	2810	1.17E-02	DOWN
Bt.18206.1.A1_at	---	1.96	208	406	1.17E-02	DOWN

Appendix C. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. EFA	Ave. Exp. SFA	Adjusted P value	Regulation
Bt.12304.1.S1_at	ISG15	8.11	617	5004	1.39E-02	DOWN
Bt.12980.3.S1_a_at	CL43	1.69	6752	11431	1.40E-02	DOWN
Bt.8997.1.S1_at	RANGAP1	2.45	71.86	176	1.53E-02	DOWN
Bt.15037.1.S1_at	ST3GAL1	1.58	333	525	1.64E-02	DOWN
Bt.154.1.S1_at	CCL8	1.47	10.46	15.34	1.81E-02	DOWN
Bt.20891.1.S1_at	OAS2	3.83	615	2355	1.83E-02	DOWN
Bt.3358.1.S1_at	SLC25A1	1.44	404	581	1.97E-02	DOWN
Bt.17223.1.S1_at	IFI35	1.84	121	223	2.14E-02	DOWN
Bt.20785.2.S1_at	IFI44	3.06	280	855	2.39E-02	DOWN
Bt.21181.1.S1_at	FO XK2	1.46	61.30	89.57	2.45E-02	DOWN
Bt.18861.1.A1_at	---	1.51	234	353	2.45E-02	DOWN
Bt.3201.1.S1_at	GRWD1	1.69	43.80	74.07	2.45E-02	DOWN
Bt.17814.1.A1_at	LOC100736585	1.45	687	998	2.51E-02	DOWN
Bt.22323.1.A1_a_at	RASSF5	1.47	288	422	2.51E-02	DOWN
Bt.1332.1.S1_a_at	COX10	1.43	74.97	107	2.65E-02	DOWN
Bt.29194.1.S1_at	PLIN4	1.46	16.89	24.72	2.78E-02	DOWN
Bt.6575.1.A1_at	---	2.07	116	240	2.81E-02	DOWN
Bt.27830.1.A1_at	SP140	1.68	386	649	2.91E-02	DOWN
Bt.20490.1.S1_at	CDC42EP4	1.68	1022	1720	2.96E-02	DOWN
Bt.1730.1.A1_at	ID1	2.33	589	1370	2.99E-02	DOWN
Bt.7381.1.S1_at	NPLOC4	1.41	103	145	3.02E-02	DOWN
Bt.22554.1.A1_at	TK2	1.45	119	173	3.34E-02	DOWN
Bt.21189.1.S1_at	PRKD2	1.57	141	222	3.47E-02	DOWN
Bt.5771.1.S1_at	---	1.43	157	224	3.50E-02	DOWN
Bt.20785.1.A1_at	IFI44	2.93	417	1220	3.60E-02	DOWN
Bt.10130.1.S1_at	---	2.06	21.32	44.00	3.95E-02	DOWN
Bt.6316.1.S1_at	NR2F6	1.54	811	1251	4.04E-02	DOWN
Bt.8436.1.S1_at	IFI6	3.91	674	2633	4.11E-02	DOWN
Bt.3928.1.S1_at	HNRNPAB	1.43	1225	1751	4.39E-02	DOWN
Bt.13308.1.S1_at	---	1.47	71.64	105	4.70E-02	DOWN
Bt.10631.1.A1_at	ZNF547	1.49	104	154	4.82E-02	DOWN
Bt.8078.1.S1_at	ARPC4	1.50	43.17	64.75	4.82E-02	DOWN
Bt.13184.1.S1_at	LOC523126	6.27	14.65	91.91	4.82E-02	DOWN
Bt.15796.1.S1_at	LOC508226	1.57	36.06	56.75	4.98E-02	DOWN

APPENDIX D
DIFFERENTIALY EXPRESSED GENES FOR THE CONTRAST OF MILK REPLACER

List of differential expressed genes in liver of Holstein males at 30 d of age. Effect of feeding high linoleic acid (HLA) in milk replacer (contrast MR, reference = low linoleic acid (LLA) milk replacer). Genes are ranked by adjusted P value in descendant order.

Affimetrix ID	Gene symbol	Fold change	Average Exp. HLA	Average Exp. LLA	Adjusted P value	Regulation
Bt.23696.1.A1_at	LOC509457	18.72	85.43	4.56	2.67E-16	UP
Bt.4922.1.S1_at	MYL1	4.37	19.77	4.52	1.37E-13	UP
Bt.12300.2.S1_at	MYH2	5.05	22.84	4.52	1.37E-13	UP
Bt.26998.1.A1_s_at	TNNC1	2.75	12.56	4.57	1.39E-11	UP
Bt.8435.1.S1_at	ACTA1	4.34	21.13	4.87	1.39E-11	UP
Bt.1905.1.S1_at	MYL2	2.93	13.26	4.53	1.61E-11	UP
Bt.12477.2.S1_at	TPM2	2.49	11.33	4.55	9.93E-11	UP
Bt.21767.1.S1_at	TTN	2.56	13.08	5.10	1.11E-10	UP
Bt.9992.1.S1_at	TNNC2	2.46	16.30	6.62	3.36E-10	UP
Bt.21798.1.S1_at	GIMAP6	4.39	149	33.89	1.33E-09	UP
Bt.6012.1.S1_at	TNNC1	1.97	8.93	4.52	2.36E-09	UP
Bt.6620.1.S1_at	MYH7	1.90	8.68	4.57	5.71E-09	UP
Bt.26769.1.S1_at	GIMAP8	2.80	13.08	4.67	1.75E-08	UP
Bt.4404.1.A1_at	PRSS2	3.03	14.37	4.75	1.18E-07	UP
Bt.20557.1.S1_at	ACTN2	2.01	10.04	5.01	1.86E-07	UP
Bt.11687.1.S1_a_at	SRL	1.55	7.02	4.52	2.75E-07	UP
Bt.15713.2.S1_at	PLEK	2.13	10.77	5.07	3.01E-06	UP
Bt.17451.2.A1_at	SESTD1	2.34	13.23	5.65	4.35E-06	UP
Bt.8143.1.S1_at	MX2	1.82	10.01	5.49	5.83E-06	UP
Bt.154.1.S1_at	CCL8	1.99	20.26	10.18	9.25E-06	UP
Bt.21721.1.A1_at	USP2	1.72	8.01	4.67	1.39E-05	UP
Bt.17034.1.A1_at	---	1.60	7.47	4.66	1.82E-05	UP
Bt.22169.1.S1_at	ENO3	1.55	15.89	10.23	2.68E-05	UP
Bt.24793.1.S1_at	MN1	1.60	8.18	5.10	9.43E-05	UP
Bt.21767.1.S1_a_at	TTN	3.66	31.97	8.73	1.42E-04	UP
Bt.9779.1.S1_at	ISG12(B)	2.00	13.70	6.83	3.66E-04	UP
Bt.6449.1.S1_at	FBLN5	1.50	125	83.35	5.08E-04	UP
Bt.28739.1.S1_at	---	2.61	1049	402	1.10E-03	UP
Bt.6038.1.S1_at	SIGLEC1	1.72	590	343	2.23E-03	UP
Bt.1529.2.A1_at	TSG118	1.65	196	119	2.69E-03	UP
Bt.4937.1.S1_at	LOC505941	1.43	3172	2224	3.38E-03	UP
Bt.3863.1.S1_at	ZFP36	2.03	284	140	3.97E-03	UP
Bt.15692.1.A1_at	RNF19B	1.61	84.18	52.25	4.05E-03	UP
Bt.23912.1.A1_a_at	CYP2E1	1.67	1720	1032	5.47E-03	UP
Bt.9699.1.S1_at	CYP26A1	2.05	3324	1624	7.15E-03	UP
Bt.12803.1.S1_at	PPARA	1.48	88.84	60.13	8.34E-03	UP
Bt.22980.1.S1_at	TRIM21	1.63	13.66	8.36	8.34E-03	UP
Bt.27474.1.S1_at	CLEC4F	4.43	105	23.64	8.56E-03	UP
Bt.2186.1.S1_at	ZNFX1	2.33	561	241	1.21E-02	UP
Bt.4816.1.S1_at	ANGPTL4	1.53	92.49	60.38	1.40E-02	UP
Bt.1645.1.S1_at	PTGDS	1.68	116	69.08	1.51E-02	UP
Bt.12477.1.S1_a_at	TPM2	1.82	176	97.08	1.79E-02	UP
Bt.5768.1.S1_at	IRF7	2.06	165	79.99	1.79E-02	UP
Bt.28164.2.S1_at	---	1.82	124	68.42	1.97E-02	UP
Bt.22498.2.S1_at	HES4	2.12	22.58	10.65	1.97E-02	UP
Bt.920.1.S1_at	RNF181	1.49	117	78.68	2.35E-02	UP
Bt.28623.1.S1_at	FAT1	1.58	602	381	2.35E-02	UP

Appendix D. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. HLA	Ave. Exp. LLA	Adjusted P value	Regulation
Bt.6890.1.S1_at	---	1.46	4355	2974	2.47E-02	UP
Bt.8915.1.A1_at	DHTKD1	1.56	134	85.54	2.63E-02	UP
Bt.8997.1.S1_at	RANGAP1	1.87	188	101	2.68E-02	UP
Bt.29823.1.S1_x_at	BOLA	2.32	40.90	17.61	3.16E-02	UP
Bt.6141.1.S1_at	DES	1.44	18.67	12.98	3.38E-02	UP
Bt.367.1.S1_at	OLR1	2.01	27.71	13.78	3.45E-02	UP
Bt.17415.3.A1_at	ERRFI1	1.57	5.97	9.35	8.09E-09	DOWN
Bt.26650.1.S1_at	---	1.51	15.57	23.45	2.06E-08	DOWN
Bt.7575.1.A1_at	GPT2	1.62	190	308	3.91E-08	DOWN
Bt.4126.2.S1_at	CYP4A22	1.44	34.37	49.37	4.11E-08	DOWN
Bt.29581.1.A1_at	---	1.48	5.06	7.49	1.26E-06	DOWN
Bt.24779.2.S1_at	CREM	1.58	5.64	8.89	2.34E-06	DOWN
Bt.26538.1.S1_at	LOC509420	1.67	20.36	34.04	1.28E-05	DOWN
Bt.1817.1.S1_at	ETV1	1.55	20.06	31.04	1.33E-05	DOWN
Bt.20241.1.S1_at	HAAO /// LOC786774	1.54	421	650	1.74E-05	DOWN
Bt.9735.1.S1_at	APOM	1.60	837	1339	3.48E-05	DOWN
Bt.28238.1.A1_at	---	1.67	2057	3444	3.52E-05	DOWN
Bt.211.1.S1_at	DNAJC3	1.52	994	1508	4.99E-05	DOWN
Bt.26302.1.A1_at	SCML1	1.55	20.30	31.46	7.58E-05	DOWN
Bt.17263.1.S1_at	---	1.48	366	541	8.11E-05	DOWN
Bt.2501.1.S1_at	SOD2	1.40	463	650	9.79E-05	DOWN
Bt.3206.1.A1_at	SUSD2	1.96	19.21	37.65	1.07E-04	DOWN
Bt.11769.2.S1_at	EID3	1.47	11.81	17.36	1.16E-04	DOWN
Bt.18114.1.A1_at	LOC100851000	1.50	40.11	60.24	1.47E-04	DOWN
Bt.21101.1.A1_at	ACMSD	2.29	179	410	1.62E-04	DOWN
Bt.22076.1.A1_at	---	1.44	190	274	2.22E-04	DOWN
Bt.20592.1.S1_at	---	1.72	38.47	66.26	2.68E-04	DOWN
Bt.13429.2.S1_at	---	1.82	40.44	73.66	2.83E-04	DOWN
Bt.3195.1.S1_at	SLC7A9	1.81	47.22	85.36	2.99E-04	DOWN
Bt.23905.1.A1_at	ERRFI1	1.53	2969	4532	3.71E-04	DOWN
Bt.15706.1.A1_at	---	1.49	43.50	64.61	3.89E-04	DOWN
Bt.7208.1.S1_at	ZP2	3.83	20.58	78.81	3.96E-04	DOWN
Bt.12255.1.A1_at	CYP2C19	1.47	21.61	31.84	4.84E-04	DOWN
Bt.26416.1.A1_at	---	1.79	83.69	150	6.12E-04	DOWN
Bt.24007.1.A1_at	SLC15A2	1.48	239	355	7.21E-04	DOWN
Bt.16058.1.A1_at	---	1.86	43.97	81.92	7.52E-04	DOWN
Bt.18440.2.S1_at	LOC510382	1.47	6.41	9.40	8.59E-04	DOWN

APPENDIX E
DIFFERENTIALLY EXPRESSED FOR THE INTERACTION FAT BY MILK REPLACER

List of differential expressed genes in liver of Holstein males at 30 d of age. Effect of fat during prepartum and high linoleic acid in milk replacer during (Interaction of contrasts FAT by MR). Calves were fed a high or low linoleic acid milk replacer from 1 – 30 d of age and were born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.4126.2.S1_at	CYP4A22	3.39	1.04	0.31	2.38E-07	UP
Bt.26769.1.S1_at	GIMAP8	4.30	4.42	1.03	1.49E-06	UP
Bt.4404.1.A1_at	PRSS2	5.26	5.07	0.96	6.40E-06	UP
Bt.17451.2.A1_at	SESTD1	3.55	3.12	0.88	1.22E-04	UP
Bt.1978.3.S1_at	LOC780933	6.86	3.39	0.49	2.91E-04	UP
Bt.27964.1.A1_at	RCL1	2.30	1.64	0.71	3.20E-04	UP
Bt.17034.1.A1_at	---	2.08	2.04	0.98	3.21E-04	UP
Bt.6774.2.S1_at	MAP1LC3B	2.05	1.61	0.79	4.03E-04	UP
Bt.3715.1.S1_at	PSMG4	2.81	1.70	0.60	4.10E-04	UP
Bt.28076.1.A1_at	GSTO1	1.82	1.55	0.85	5.50E-04	UP
Bt.23317.1.S1_at	RPL13	2.43	2.04	0.84	6.99E-04	UP
Bt.13530.1.S1_at	DCI	1.83	1.65	0.90	8.58E-04	UP
Bt.4449.1.S1_at	AKR1A1	2.52	2.01	0.80	1.22E-03	UP
Bt.3212.1.S1_at	ISOC2	2.06	1.68	0.82	1.44E-03	UP
Bt.9699.1.S1_at	CYP26A1	6.34	1.77	0.28	1.51E-03	UP
Bt.17628.1.A1_at	TRAK2	55.66	8.00	0.14	1.70E-03	UP
Bt.2965.1.A1_at	LOC618434	2.45	1.71	0.70	1.82E-03	UP
Bt.20477.1.S1_at	RFTN1	3.91	1.91	0.49	1.86E-03	UP
Bt.21464.2.S1_a_at	GALT	3.46	2.23	0.65	1.90E-03	UP
Bt.5129.1.S1_a_at	NNAT	7.35	1.45	0.20	1.92E-03	UP
Bt.29268.1.S1_at	GOLT1A	2.11	1.46	0.69	1.96E-03	UP
Bt.9655.2.S1_at	LOC790332	3.34	0.60	0.18	2.19E-03	UP
Bt.7116.1.A1_at	SIAE	2.00	1.53	0.76	2.37E-03	UP
Bt.805.1.S1_at	ADIPOR2	2.90	1.93	0.67	2.43E-03	UP
Bt.9170.1.A1_at	KIAA1147	2.91	1.82	0.62	2.46E-03	UP
Bt.1667.1.S1_at	CDC34	2.37	1.48	0.62	2.49E-03	UP
Bt.21021.1.S1_at	TBC1D7	2.16	1.67	0.78	2.77E-03	UP
Bt.18861.1.A1_at	---	2.58	2.22	0.86	2.97E-03	UP
Bt.10880.1.S1_at	TIMM50	1.80	1.17	0.65	3.69E-03	UP
Bt.10609.2.A1_at	CYP20A1	2.15	1.54	0.72	3.69E-03	UP
Bt.29398.1.S1_at	LOC100582155	1.73	1.65	0.95	4.24E-03	UP
Bt.27468.1.A1_at	SOAT2	7.84	3.70	0.47	4.24E-03	UP
Bt.13864.1.A1_at	CDC26	1.65	1.37	0.83	4.60E-03	UP
Bt.3857.1.S1_at	ENDOG	2.57	1.66	0.64	4.60E-03	UP
Bt.23912.1.A1_a_at	CYP2E1	2.92	1.44	0.49	4.60E-03	UP
Bt.24881.1.S1_at	LOC539690	2.72	1.80	0.66	4.65E-03	UP
Bt.6334.1.A1_at	DEGS1	1.49	1.13	0.76	4.78E-03	UP
Bt.12240.1.A1_at	GLYATL3	1.85	1.22	0.66	5.56E-03	UP
Bt.24281.1.S1_at	VAPA	1.45	1.39	0.96	5.61E-03	UP
Bt.27443.1.S1_at	SLC22A18	1.75	1.58	0.91	5.89E-03	UP
Bt.12864.1.S1_at	PHPT1	2.55	1.56	0.61	5.89E-03	UP
Bt.2481.2.S1_at	C23H6ORF105	2.69	1.89	0.70	5.89E-03	UP
Bt.3555.1.S1_at	---	2.07	1.42	0.69	6.17E-03	UP

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.6156.1.S1_at	3290025600	2.04	1.59	0.78	6.34E-03	UP
Bt.6162.1.S1_at	LOC613560	2.77	2.22	0.80	6.34E-03	UP
Bt.23902.1.A1_at	---	1.51	1.04	0.69	6.40E-03	UP
Bt.2049.1.S1_at	LOC510634	3.09	1.67	0.54	6.40E-03	UP
Bt.22577.2.S1_at	SLC25A33	4.29	1.82	0.42	6.40E-03	UP
Bt.3358.1.S1_at	SLC25A1	1.86	1.28	0.69	6.42E-03	UP
Bt.4475.1.S1_at	NDUFS2	1.61	1.47	0.91	6.79E-03	UP
Bt.2050.1.A1_at	ACAA1	2.13	1.91	0.90	6.79E-03	UP
Bt.26604.1.S1_at	APLNR	3.01	1.66	0.55	7.03E-03	UP
Bt.5466.2.S1_a_at	RPS4Y1 /// RPS4Y2	1.53	1.34	0.88	7.38E-03	UP
Bt.17961.1.S1_at	APOC4	1.71	1.46	0.86	7.40E-03	UP
Bt.6143.1.S1_at	LTA4H	2.01	1.40	0.69	7.40E-03	UP
Bt.13381.1.S1_at	CIDEC	1.46	1.41	0.97	7.40E-03	UP
Bt.196.1.S1_at	S100A13	5.55	2.39	0.43	7.69E-03	UP
Bt.4053.1.S1_at	TBXA2R	2.41	1.69	0.70	8.15E-03	UP
Bt.2183.1.A1_at	HEXB	2.16	1.52	0.71	8.57E-03	UP
Bt.20329.2.S1_at	ARL4D	2.32	1.80	0.77	9.42E-03	UP
Bt.4336.1.S1_at	CFD	3.13	2.59	0.83	9.42E-03	UP
Bt.4503.1.S2_at	MTCH2	1.57	1.34	0.85	9.92E-03	UP
Bt.22694.1.A1_at	APOA5	2.48	2.16	0.87	9.92E-03	UP
Bt.19064.1.A1_at	BTD	2.17	1.41	0.65	1.02E-02	UP
Bt.15530.1.S1_at	LOC784762 /// RPL12	1.54	1.23	0.80	1.02E-02	UP
Bt.4141.1.S1_at	COPE	1.72	1.31	0.76	1.02E-02	UP
Bt.4985.1.S1_at	MRPL23	1.88	1.40	0.75	1.02E-02	UP
Bt.25097.1.S1_at	GMPS	1.60	1.48	0.93	1.08E-02	UP
Bt.3248.1.S1_at	ALDH4A1	1.70	1.43	0.84	1.08E-02	UP
Bt.13534.1.S1_at	PLA2G16	1.75	1.19	0.68	1.08E-02	UP
Bt.21464.3.S1_a_at	GALT	3.21	1.93	0.60	1.11E-02	UP
Bt.19999.1.A1_at	FICD	6.52	3.42	0.52	1.20E-02	UP
Bt.714.1.S1_at	SIGMAR1	1.72	1.32	0.77	1.21E-02	UP
Bt.18435.3.A1_at	ANGEL1	2.09	1.36	0.65	1.21E-02	UP
Bt.7161.1.S1_at	STRBP	1.47	1.30	0.88	1.24E-02	UP
Bt.19614.1.A1_at	LIPC	1.57	1.78	1.13	1.24E-02	UP
Bt.10797.2.S1_a_at	LOC615093	1.77	1.55	0.88	1.24E-02	UP
Bt.13411.1.S1_at	LRBA	1.45	1.51	1.04	1.29E-02	UP
Bt.20252.2.S1_a_at	GALK1	2.96	1.91	0.65	1.33E-02	UP
Bt.8421.2.S1_at	LOC100623159	1.91	1.84	0.96	1.41E-02	UP
Bt.16001.1.S1_at	CYP27A1	1.95	1.49	0.76	1.44E-02	UP
Bt.19937.1.S1_at	LOC532189	1.49	1.40	0.94	1.48E-02	UP
Bt.16250.2.S1_at	SLC10A1	2.47	2.38	0.96	1.50E-02	UP
Bt.13942.1.S1_at	GLYCTK	3.33	2.29	0.69	1.50E-02	UP
Bt.28697.1.S1_at	SLC31A1	1.45	1.46	1.01	1.51E-02	UP
Bt.20529.1.A1_at	MBLAC1	1.75	1.52	0.87	1.54E-02	UP
Bt.6521.1.A1_at	PARD6B	1.49	1.71	1.15	1.56E-02	UP
Bt.9735.1.S1_at	APOM	2.10	1.71	0.81	1.56E-02	UP
Bt.18323.1.A1_at	LOC509506	2.84	1.91	0.67	1.56E-02	UP
Bt.282.1.S1_at	VDAC1P5	1.61	1.12	0.70	1.59E-02	UP
Bt.1034.1.S1_at	RPS8	1.47	1.25	0.85	1.62E-02	UP
Bt.23548.1.S1_at	RPL34	1.60	1.46	0.91	1.62E-02	UP
Bt.20241.1.S1_at	HAAO /// LOC786774	1.90	1.43	0.75	1.62E-02	UP

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.3865.3.S1_a_at	C25H16orf14	2.92	1.82	0.62	1.62E-02	UP
Bt.11279.1.A1_at	CLCN4	2.20	1.81	0.82	1.62E-02	UP
Bt.19057.1.S1_at	TOR1A	3.81	1.81	0.47	1.64E-02	UP
Bt.1785.1.A1_at	LOC532189	1.50	1.78	1.19	1.66E-02	UP
Bt.2056.1.S1_at	APEH	1.65	1.58	0.96	1.66E-02	UP
Bt.21464.1.S1_at	GALT	2.47	1.58	0.64	1.66E-02	UP
Bt.1207.1.S1_at	SLC16A13	2.15	1.74	0.81	1.70E-02	UP
Bt.11135.1.S1_at	MPV17	1.58	1.29	0.82	1.73E-02	UP
Bt.27966.1.S1_at	LOC532789	1.60	1.42	0.89	1.80E-02	UP
Bt.4150.1.S1_at	CTNNBL1	2.02	1.62	0.80	1.80E-02	UP
Bt.27430.1.S1_at	STRADB	2.06	1.49	0.73	1.80E-02	UP
Bt.22170.1.S1_a_at	AGPAT5	1.48	1.45	0.98	1.81E-02	UP
Bt.2170.1.A1_at	VPS33A	2.05	1.51	0.74	1.86E-02	UP
Bt.4555.1.S1_at	ETFB	1.89	1.54	0.82	1.87E-02	UP
Bt.9567.1.S1_at	TM7SF2	2.96	2.18	0.74	1.90E-02	UP
Bt.5129.2.A1_at	NNAT	7.05	1.48	0.21	1.91E-02	UP
Bt.11178.1.S1_at	GPC3	1.60	1.40	0.87	1.93E-02	UP
Bt.27623.2.S1_a_at	GRTP1	2.95	1.87	0.63	1.99E-02	UP
Bt.11176.2.S1_at	TMEM14A	1.69	1.29	0.77	2.00E-02	UP
Bt.27036.1.S1_at	CYP4F2	3.99	1.98	0.50	2.00E-02	UP
Bt.9047.1.S1_at	DDT	1.62	1.42	0.88	2.06E-02	UP
Bt.20848.1.A1_at	TTC36	4.25	2.98	0.70	2.06E-02	UP
Bt.22590.1.S1_at	AGPAT2	4.37	2.02	0.46	2.06E-02	UP
Bt.19118.1.A1_at	---	2.19	1.52	0.69	2.08E-02	UP
Bt.4619.1.S1_at	TH1L	1.50	1.41	0.94	2.09E-02	UP
Bt.13278.1.S1_at	STEAP3	1.54	1.25	0.81	2.11E-02	UP
Bt.21708.1.S1_at	RAB4A	1.83	1.62	0.89	2.13E-02	UP
Bt.18330.2.S1_at	ASGR2	2.08	1.89	0.91	2.13E-02	UP
Bt.22510.1.S1_at	C11H2ORF7	2.47	1.42	0.57	2.13E-02	UP
Bt.3026.1.A1_at	TCEA3	4.27	2.17	0.51	2.13E-02	UP
Bt.3999.1.S1_at	NAGA	1.52	1.16	0.77	2.19E-02	UP
Bt.19980.2.S1_at	ApoN	2.03	1.72	0.84	2.19E-02	UP
Bt.16832.1.A1_at	DHDPSL	2.18	2.07	0.95	2.30E-02	UP
Bt.643.1.S1_at	LOC508666	2.37	2.15	0.90	2.30E-02	UP
Bt.5319.1.S1_at	PRDX6	1.69	1.69	1.00	2.36E-02	UP
Bt.20453.1.S1_at	ABHD14A	2.12	1.48	0.70	2.36E-02	UP
Bt.13324.4.S1_at	IDH1	2.34	2.02	0.86	2.40E-02	UP
Bt.26961.1.S1_at	NUDT14	2.74	2.19	0.80	2.49E-02	UP
Bt.24950.1.S1_at	FBXL5	1.65	1.27	0.77	2.50E-02	UP
Bt.20919.2.A1_at	GNMT	4.50	2.69	0.60	2.50E-02	UP
Bt.2824.1.S1_at	BLOC1S1	1.88	1.44	0.77	2.53E-02	UP
Bt.5170.1.S1_at	GRHPR	2.68	2.10	0.78	2.54E-02	UP
Bt.22063.2.S1_at	---	1.98	1.73	0.87	2.56E-02	UP
Bt.5193.1.S1_at	ACP5	2.47	1.51	0.61	2.70E-02	UP
Bt.23599.1.S1_at	PON2	1.65	1.44	0.87	2.72E-02	UP
Bt.13815.1.S1_at	---	1.59	1.95	1.23	2.90E-02	UP
Bt.19664.1.A1_at	C3H1ORF210	3.63	1.64	0.45	2.90E-02	UP
Bt.23143.2.S1_at	CSDE1	1.45	1.41	0.98	3.00E-02	UP
Bt.5193.2.S1_a_at	ACP5	2.36	1.51	0.64	3.00E-02	UP
Bt.2415.1.S1_at	ID2	1.44	0.92	0.64	3.03E-02	UP
Bt.16496.1.A1_at	KNTC1	1.64	1.72	1.05	3.03E-02	UP
Bt.15713.2.S1_at	PLEK	1.73	1.33	0.77	3.03E-02	UP
Bt.5412.1.S1_at	BCKDHB	1.40	1.32	0.94	3.03E-02	UP

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.13251.1.S1_at	MFNG	1.64	1.11	0.68	3.07E-02	UP
Bt.4985.1.S1_a_at	MRPL23	2.00	1.53	0.77	3.07E-02	UP
Bt.2424.1.S1_at	DPYD	1.45	1.57	1.08	3.16E-02	UP
Bt.3195.1.S1_at	SLC7A9	2.95	2.31	0.78	3.16E-02	UP
Bt.5220.1.S1_at	SHBG	2.72	2.37	0.87	3.17E-02	UP
Bt.5536.1.S1_at	ITGB5	1.79	1.42	0.79	3.18E-02	UP
Bt.17428.1.A1_at	NHLRC3	2.21	1.74	0.79	3.18E-02	UP
Bt.24205.1.A1_at	FGB	1.87	1.08	0.58	3.20E-02	UP
Bt.8617.1.S1_at	CNRIP1	1.96	1.29	0.66	3.23E-02	UP
Bt.20586.1.S1_a_at	TM4SF5	1.68	1.56	0.93	3.24E-02	UP
Bt.2822.1.S1_at	RPL8	1.72	1.32	0.76	3.29E-02	UP
Bt.8235.1.S1_at	TRAPPC5	1.75	1.41	0.80	3.39E-02	UP
Bt.23572.1.S1_at	CCNDBP1	2.46	1.35	0.55	3.39E-02	UP
Bt.24001.1.A1_at	LOC100433242	3.98	1.86	0.47	3.43E-02	UP
Bt.20249.1.S1_a_at	ABCD3	1.42	1.35	0.95	3.43E-02	UP
Bt.5350.1.S1_at	ETFA	1.61	1.34	0.83	3.43E-02	UP
Bt.25088.1.A1_at	GCSH	1.46	1.52	1.04	3.45E-02	UP
Bt.5164.1.S1_at	CA14	6.99	2.60	0.37	3.51E-02	UP
Bt.4711.1.S1_at	RPS9	1.74	1.40	0.80	3.54E-02	UP
Bt.27073.1.S1_at	ACADL	1.95	2.06	1.06	3.58E-02	UP
Bt.15705.1.S2_at	DSTN	1.53	1.36	0.88	3.64E-02	UP
Bt.2416.1.S2_at	TMBIM6	1.56	1.58	1.01	3.64E-02	UP
Bt.9310.1.S1_at	C16orf5	1.78	1.23	0.69	3.64E-02	UP
Bt.460.1.S1_at	TST	1.92	1.45	0.76	3.76E-02	UP
Bt.20520.1.S1_at	SLC25A10	2.03	1.42	0.70	3.76E-02	UP
Bt.1920.2.S1_at	STARD10	2.90	1.46	0.50	3.76E-02	UP
Bt.12381.1.A1_at	---	1.60	1.57	0.98	3.84E-02	UP
Bt.26832.1.S1_at	CANT1	5.11	1.87	0.37	3.86E-02	UP
Bt.28617.1.S1_at	STOM	2.81	2.05	0.73	3.95E-02	UP
Bt.14213.1.A1_at	CES2	1.63	1.50	0.92	4.01E-02	UP
Bt.12360.1.S1_at	---	1.72	1.36	0.79	4.01E-02	UP
Bt.1252.1.S1_at	---	1.86	1.67	0.90	4.01E-02	UP
Bt.9735.2.A1_at	APOM	2.09	1.69	0.81	4.01E-02	UP
Bt.13324.1.S1_a_at	IDH1	2.54	2.19	0.86	4.01E-02	UP
Bt.10371.1.S1_at	LOC516241	2.12	1.21	0.57	4.03E-02	UP
Bt.28243.1.S1_a_at	VNN1	3.53	2.75	0.78	4.14E-02	UP
Bt.8724.1.S1_at	LOC100299281	2.99	2.36	0.79	4.14E-02	UP
Bt.20404.1.S1_at	---	1.90	1.35	0.71	4.18E-02	UP
Bt.17124.1.A1_s_at	NUDT14	2.32	2.19	0.95	4.35E-02	UP
Bt.20281.2.S1_a_at	PGM1	1.52	1.23	0.81	4.37E-02	UP
Bt.28278.1.S1_at	ACE2	4.06	4.70	1.16	4.40E-02	UP
Bt.4718.1.S1_at	PCTP	2.46	1.50	0.61	4.42E-02	UP
Bt.23955.1.A1_at	PHOSPHO2	1.46	1.36	0.93	4.50E-02	UP
Bt.6177.1.S1_at	ACOT8	4.38	2.64	0.60	4.54E-02	UP
Bt.26953.1.A1_at	MRPL36	1.68	1.32	0.79	4.59E-02	UP
Bt.3300.1.S1_at	LOC511523	2.34	1.71	0.73	4.63E-02	UP
Bt.9048.2.S1_a_at	PSENNEN	1.67	1.32	0.79	4.65E-02	UP
Bt.21721.1.A1_at	USP2	1.48	1.44	0.98	4.65E-02	UP
Bt.18037.2.A1_at	ASPDH	2.36	1.58	0.67	4.65E-02	UP
Bt.15997.1.S1_at	P2RX4	2.92	2.08	0.71	4.65E-02	UP
Bt.6626.1.S1_at	PPAP2A	1.51	1.16	0.77	4.76E-02	UP
Bt.21680.2.S1_at	PIR	2.19	1.40	0.64	4.76E-02	UP
Bt.6171.1.A1_at	HIBADH	1.44	1.58	1.10	4.82E-02	UP

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.11078.2.S1_at	AKR7A2	2.19	1.51	0.69	4.83E-02	UP
Bt.2169.1.S1_at	FUCA1	1.53	1.18	0.77	4.93E-02	UP
Bt.11770.1.S1_at	SLC25A20	1.92	1.39	0.72	4.93E-02	UP
Bt.4126.1.A1_at	CYP4A11	1.42	1.22	0.86	5.00E-02	UP
Bt.11739.1.S1_a_at	STAP2	1.57	1.22	0.78	5.00E-02	UP
Bt.15705.1.S1_at	DSTN	1.67	1.16	0.69	5.00E-02	UP
Bt.23706.1.A1_at	---	1.71	1.42	0.83	5.00E-02	UP
Bt.6646.1.S1_at	CTDSP1	2.22	1.25	0.56	5.00E-02	UP
Bt.12300.2.S1_at	MYH2	127.59	0.01	1.00	4.60E-15	DOWN
Bt.4922.1.S1_at	MYL1	83.33	0.01	1.00	5.47E-15	DOWN
Bt.8435.1.S1_at	ACTA1	85.98	0.01	1.04	4.43E-13	DOWN
Bt.26998.1.A1_s_at	TNNC1	20.79	0.05	1.00	5.73E-13	DOWN
Bt.1905.1.S1_at	MYL2	23.64	0.04	1.00	5.73E-13	DOWN
Bt.12477.2.S1_at	TPM2	13.18	0.08	1.01	3.13E-12	DOWN
Bt.9992.1.S1_at	TNNC2	15.59	0.06	1.01	3.13E-12	DOWN
Bt.21767.1.S1_at	TTN	17.03	0.06	0.99	3.13E-12	DOWN
Bt.23696.1.A1_at	LOC509457	9.01	0.11	1.00	3.88E-11	DOWN
Bt.6012.1.S1_at	TNNC1	7.31	0.14	1.00	7.87E-11	DOWN
Bt.6620.1.S1_at	MYH7	6.64	0.15	1.00	2.32E-10	DOWN
Bt.20557.1.S1_at	ACTN2	8.14	0.12	0.98	7.50E-09	DOWN
Bt.11687.1.S1_a_at	SRL	3.74	0.27	1.00	1.01E-08	DOWN
Bt.27463.1.A1_at	HERC6	3.39	0.32	1.10	7.12E-08	DOWN
Bt.8143.1.S1_at	MX2	5.96	0.17	1.04	7.12E-08	DOWN
Bt.23735.1.A1_s_at	---	6.28	0.34	2.11	2.63E-06	DOWN
Bt.22199.1.S1_at	DDIT4L	2.59	0.40	1.03	2.72E-06	DOWN
Bt.22169.1.S1_at	ENO3	3.54	0.24	0.86	3.04E-06	DOWN
Bt.9779.1.S1_at	ISG12(B)	10.37	0.11	1.13	4.71E-06	DOWN
Bt.10310.1.S1_at	MYBPC1	2.23	0.44	0.98	8.16E-06	DOWN
Bt.6972.1.S1_at	KBTBD10	2.59	0.41	1.07	8.16E-06	DOWN
Bt.21767.1.S1_a_at	TTN	42.06	0.03	1.30	9.64E-06	DOWN
Bt.22065.1.S1_at	LOC783920	2.35	0.41	0.97	5.52E-05	DOWN
Bt.395.1.S1_at	COX8B	1.88	0.53	1.00	7.81E-05	DOWN
Bt.11199.1.S1_at	MYOZ1	1.86	0.53	0.99	1.83E-04	DOWN
Bt.17415.3.A1_at	ERRFI1	1.86	1.02	1.89	2.46E-04	DOWN
Bt.19284.1.A1_at	---	2.46	0.56	1.38	3.19E-04	DOWN
Bt.17777.1.S1_at	OPTN	2.69	0.50	1.34	3.21E-04	DOWN
Bt.27891.1.S1_at	LARS2	2.53	0.73	1.84	3.49E-04	DOWN
Bt.16448.2.A1_at	SFRS2IP	1.82	0.69	1.24	3.79E-04	DOWN
Bt.11918.1.A1_at	---	2.62	0.59	1.54	3.79E-04	DOWN
Bt.29581.1.A1_at	---	2.10	0.94	1.97	5.54E-04	DOWN
Bt.20091.1.S1_at	TCF20	2.10	0.76	1.60	5.93E-04	DOWN
Bt.154.1.S1_at	CCL8	2.56	0.43	1.10	6.21E-04	DOWN
Bt.20427.2.S1_at	UTP6	5.44	0.57	3.10	8.08E-04	DOWN
Bt.12704.1.S1_at	LOC514801	3.76	0.40	1.51	8.72E-04	DOWN
Bt.19274.1.A1_at	C1QTNF7	1.57	1.01	1.59	1.15E-03	DOWN
Bt.28744.1.S1_at	GBP4	10.23	0.22	2.27	1.33E-03	DOWN
Bt.12285.3.S1_a_at	NMI	2.14	0.48	1.03	1.48E-03	DOWN
Bt.8920.1.S1_at	---	1.78	0.91	1.62	1.68E-03	DOWN
Bt.24361.1.S1_at	ESF1	2.18	0.65	1.42	1.70E-03	DOWN
Bt.12638.1.S1_at	PML	2.33	0.47	1.09	1.86E-03	DOWN
Bt.17229.1.A1_at	ZNFX1	1.55	0.65	1.00	1.92E-03	DOWN
Bt.28798.1.A1_at	ANKRD22	1.97	0.53	1.04	1.96E-03	DOWN
Bt.12760.1.S1_at	INHBA	16.97	0.45	7.61	2.11E-03	DOWN

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.869.1.S1_at	DPM1	1.70	0.72	1.23	2.31E-03	DOWN
Bt.29823.1.S1_at	BOLA	10.09	0.16	1.64	2.38E-03	DOWN
Bt.26562.2.S1_at	CCDC86	1.60	0.63	1.01	2.97E-03	DOWN
Bt.27759.2.S1_at	IDO1	2.63	0.49	1.30	2.97E-03	DOWN
Bt.4937.1.S1_at	LOC505941	2.05	0.47	0.96	3.33E-03	DOWN
Bt.22116.1.A1_at	IL18BP	3.98	0.28	1.13	3.33E-03	DOWN
Bt.21798.1.S1_at	GIMAP6	2.26	0.50	1.13	3.44E-03	DOWN
Bt.18116.1.S1_at	PARP12	2.27	0.64	1.44	4.24E-03	DOWN
Bt.24492.1.S1_at	STAT2	2.12	0.47	0.99	4.50E-03	DOWN
Bt.21839.1.A1_at	TOP1	1.67	0.68	1.13	4.60E-03	DOWN
Bt.1817.1.S1_at	ETV1	2.25	0.75	1.68	4.60E-03	DOWN
Bt.22021.1.S1_at	IFI16	5.28	0.37	1.94	4.60E-03	DOWN
Bt.8436.1.S1_at	IFI6	19.54	0.17	3.38	4.71E-03	DOWN
Bt.29960.1.S1_at	---	2.24	0.55	1.23	4.74E-03	DOWN
Bt.19792.1.A1_at	---	3.11	0.33	1.02	5.56E-03	DOWN
Bt.27590.1.A1_at	SMARCA4	2.15	0.55	1.19	5.89E-03	DOWN
Bt.21981.3.S1_at	ANTXR1	2.08	0.79	1.64	6.17E-03	DOWN
Bt.27889.1.S1_at	DLD	1.62	0.98	1.59	6.46E-03	DOWN
Bt.11379.1.S1_at	IFT52	2.06	0.66	1.37	6.69E-03	DOWN
Bt.29823.1.S1_x_at	BOLA	8.73	0.15	1.27	6.69E-03	DOWN
Bt.22980.1.S1_at	TRIM21	2.70	0.34	0.92	6.79E-03	DOWN
Bt.13257.2.A1_at	LTV1	2.43	0.60	1.46	6.93E-03	DOWN
Bt.18440.2.S1_at	LOC510382	2.74	0.89	2.44	7.02E-03	DOWN
Bt.14054.1.A1_at	IFRD1	1.91	0.62	1.18	7.20E-03	DOWN
Bt.12141.2.S1_a_at	ZCCHC6	2.55	0.49	1.25	7.25E-03	DOWN
Bt.9098.1.A1_at	---	1.55	0.65	1.01	7.40E-03	DOWN
Bt.27143.1.A1_at	ODF2L	1.70	0.80	1.36	7.56E-03	DOWN
Bt.5197.1.S1_at	G3BP1	1.80	0.67	1.21	7.64E-03	DOWN
Bt.11475.1.A1_at	PDLIM5	1.82	0.61	1.10	8.07E-03	DOWN
Bt.15854.1.A1_at	FUBP1	1.96	0.64	1.26	8.15E-03	DOWN
Bt.28523.1.S1_at	DTX3L	4.55	0.28	1.26	8.49E-03	DOWN
Bt.9391.2.S1_at	BIRC3	1.58	0.87	1.37	8.57E-03	DOWN
Bt.18873.1.A1_at	---	4.46	0.27	1.19	8.57E-03	DOWN
Bt.8054.1.S1_at	SYAP1	1.56	0.86	1.34	8.95E-03	DOWN
Bt.24779.2.S1_at	CREM	1.84	1.17	2.15	8.95E-03	DOWN
Bt.22413.1.A1_at	TLE4	1.71	0.79	1.36	1.01E-02	DOWN
Bt.12665.1.A1_at	LOC531600	1.65	0.80	1.31	1.02E-02	DOWN
Bt.14054.2.S1_at	IFRD1	2.96	0.62	1.85	1.02E-02	DOWN
Bt.11043.1.S1_a_at	BCL2L12	1.45	0.71	1.03	1.05E-02	DOWN
Bt.12300.1.S1_at	MYH1	1.53	0.67	1.02	1.08E-02	DOWN
Bt.29924.1.S1_at	---	1.55	0.72	1.11	1.08E-02	DOWN
Bt.24211.1.A1_at	ASPN	1.92	0.68	1.31	1.20E-02	DOWN
Bt.28764.1.A1_at	LOC787057	2.07	0.79	1.64	1.20E-02	DOWN
Bt.20416.1.S1_at	TAP1	2.48	0.50	1.25	1.21E-02	DOWN
Bt.17614.1.S1_at	RBM25	3.07	0.60	1.84	1.21E-02	DOWN
Bt.23941.1.A1_at	ZFP161	1.59	0.80	1.27	1.24E-02	DOWN
Bt.26926.1.S1_at	---	1.51	1.01	1.53	1.27E-02	DOWN
Bt.29432.1.A1_at	PKHD1	3.05	0.47	1.44	1.28E-02	DOWN
Bt.28577.1.S1_at	SENP6	1.59	0.76	1.20	1.29E-02	DOWN
Bt.15687.1.S1_at	HERC4	1.83	0.80	1.46	1.33E-02	DOWN
Bt.24095.1.A1_at	USP1	1.58	0.80	1.26	1.36E-02	DOWN
Bt.2294.1.S1_a_at	UBA7	6.61	0.20	1.34	1.44E-02	DOWN
Bt.28139.1.S1_at	LOC614107	3.54	0.32	1.12	1.50E-02	DOWN

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.10692.1.S1_at	---	1.82	0.75	1.36	1.50E-02	DOWN
Bt.12663.1.S1_at	KRT19	2.30	0.56	1.29	1.50E-02	DOWN
Bt.20785.2.S1_at	IFI44	5.37	0.28	1.49	1.50E-02	DOWN
Bt.26364.1.A1_at	BTBD8	1.74	0.80	1.40	1.54E-02	DOWN
Bt.1927.1.S1_at	CRISPLD2 /// TIMM13	2.35	0.63	1.47	1.54E-02	DOWN
Bt.27320.1.A1_at	SGOL2	1.67	0.88	1.47	1.56E-02	DOWN
Bt.13777.2.S1_at	GIMAP7	4.90	0.91	4.44	1.56E-02	DOWN
Bt.25471.1.S1_at	ATXN3	2.33	0.74	1.73	1.56E-02	DOWN
Bt.16739.1.A1_at	---	3.79	0.82	3.11	1.66E-02	DOWN
Bt.7576.1.S1_at	---	1.45	0.75	1.09	1.69E-02	DOWN
Bt.22683.1.S1_at	RBM10	1.93	0.55	1.06	1.73E-02	DOWN
Bt.25103.1.S1_at	TDRD7	1.86	0.56	1.04	1.78E-02	DOWN
Bt.20785.1.A1_at	IFI44	5.49	0.26	1.44	1.78E-02	DOWN
Bt.6636.1.S1_at	---	3.25	0.14	0.46	1.80E-02	DOWN
Bt.26415.1.A1_at	---	1.57	0.84	1.31	1.91E-02	DOWN
Bt.8053.1.S1_at	ATAD1	1.57	0.68	1.07	1.99E-02	DOWN
Bt.27589.1.A1_at	DNAH12L /// LOC781795	1.71	0.71	1.22	1.99E-02	DOWN
Bt.8206.1.S1_at	SFRS7	1.52	0.82	1.25	2.00E-02	DOWN
Bt.26408.1.A1_at	SFRS2IP	1.56	0.98	1.53	2.08E-02	DOWN
Bt.2186.1.S1_at	ZNFX1	4.23	0.23	0.99	2.08E-02	DOWN
Bt.22064.2.S1_at	RSRC2	1.76	0.78	1.38	2.13E-02	DOWN
Bt.27830.1.A1_at	SP140	2.21	0.61	1.34	2.26E-02	DOWN
Bt.19620.1.A1_at	IFI44	6.10	0.22	1.34	2.54E-02	DOWN
Bt.27876.1.A1_at	ZCCHC10	1.43	0.60	0.86	2.75E-02	DOWN
Bt.21565.1.S1_at	IWS1	1.69	0.69	1.17	2.75E-02	DOWN
Bt.4507.1.S1_at	C4A	1.88	0.54	1.00	2.75E-02	DOWN
Bt.22737.1.S1_at	ERBB2IP	1.60	0.84	1.35	2.77E-02	DOWN
Bt.16234.2.S1_at	SFRS18	2.27	1.10	2.51	2.83E-02	DOWN
Bt.9705.1.S1_at	NKTR	1.52	0.86	1.31	3.00E-02	DOWN
Bt.25832.1.S1_at	---	2.01	0.80	1.60	3.00E-02	DOWN
Bt.26232.2.A1_at	---	2.11	0.49	1.04	3.03E-02	DOWN
Bt.8997.1.S1_at	RANGAP1	3.09	0.31	0.96	3.03E-02	DOWN
Bt.6225.2.A1_at	PRKD3	1.77	0.76	1.34	3.07E-02	DOWN
Bt.19339.1.S1_at	---	1.54	0.98	1.51	3.13E-02	DOWN
Bt.17777.3.S1_at	OPTN	2.99	0.55	1.65	3.13E-02	DOWN
Bt.15971.1.S1_at	CCAR1	1.78	0.76	1.36	3.16E-02	DOWN
Bt.11791.2.S1_at	---	1.42	0.91	1.29	3.17E-02	DOWN
Bt.25196.1.A1_at	---	1.45	0.91	1.32	3.18E-02	DOWN
Bt.21801.2.S1_at	HNRNPL	1.50	0.79	1.19	3.18E-02	DOWN
Bt.4079.2.S1_a_at	TARDBP	1.41	0.90	1.27	3.19E-02	DOWN
Bt.22869.1.S2_at	FABP5	3.02	0.63	1.92	3.20E-02	DOWN
Bt.13189.1.A1_at	ORC4L	1.46	0.83	1.21	3.23E-02	DOWN
Bt.17612.2.S1_at	CFHR4	1.81	0.53	0.96	3.26E-02	DOWN
Bt.6822.1.S1_at	RNF150	1.82	0.72	1.30	3.29E-02	DOWN
Bt.20270.1.S1_at	MSL1	1.69	0.70	1.19	3.43E-02	DOWN
Bt.24098.1.A1_at	IFIH1	5.49	0.24	1.31	3.63E-02	DOWN
Bt.8736.1.S1_at	LOC520588	1.40	0.98	1.37	3.64E-02	DOWN
Bt.28626.2.S1_at	LOC521363	1.72	0.93	1.61	3.64E-02	DOWN
Bt.1736.1.A1_at	SOCS1	1.44	0.66	0.96	3.76E-02	DOWN
Bt.5360.1.S1_a_at	PAPOLA	1.89	0.81	1.52	3.85E-02	DOWN
Bt.27403.1.S1_at	LOC540987	1.49	0.95	1.41	3.98E-02	DOWN

Appendix E. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FAT-HLA	Ave. Exp. FAT-LLA	Adjusted P value	Regulation
Bt.17717.1.A1_at	USPL1	2.04	0.75	1.52	3.98E-02	DOWN
Bt.27071.1.S1_at	TRIM38	1.90	0.58	1.09	3.99E-02	DOWN
Bt.18116.2.A1_at	PARP12	2.44	0.45	1.10	3.99E-02	DOWN
Bt.16350.2.A1_s_at	GBP5	1.46	0.69	1.00	4.01E-02	DOWN
Bt.2465.1.S1_at	---	2.41	0.55	1.32	4.01E-02	DOWN
Bt.24940.1.A1_at	---	8.34	0.50	4.17	4.01E-02	DOWN
Bt.24767.1.S1_at	INTS3	1.67	0.87	1.45	4.05E-02	DOWN
Bt.14464.1.A1_at	GPHN	2.01	0.73	1.47	4.08E-02	DOWN
Bt.24317.1.A1_at	SOX6	1.64	0.86	1.40	4.14E-02	DOWN
Bt.18045.1.S1_at	MTPAP	1.76	0.80	1.41	4.18E-02	DOWN
Bt.19107.1.S1_at	---	2.25	0.51	1.15	4.18E-02	DOWN
Bt.27118.1.A1_at	LOC510651	2.40	0.54	1.30	4.18E-02	DOWN
Bt.17777.2.S1_at	OPTN	2.71	0.62	1.68	4.18E-02	DOWN
Bt.22283.1.S1_at	PLEKHA2	1.44	0.73	1.05	4.19E-02	DOWN
Bt.4758.1.S1_at	FABP3	1.86	0.59	1.10	4.19E-02	DOWN
Bt.25537.1.A1_at	UXS1	2.65	0.65	1.71	4.19E-02	DOWN
Bt.22626.1.A1_at	ANKRD12	1.92	0.70	1.34	4.26E-02	DOWN
Bt.29194.1.S1_at	PLIN4	1.65	0.97	1.60	4.30E-02	DOWN
Bt.5240.1.S1_at	CTGF	2.54	0.65	1.66	4.30E-02	DOWN
Bt.11259.1.S1_at	ISG12(A)	7.05	0.25	1.73	4.30E-02	DOWN
Bt.26804.1.S1_at	LOC100847122	2.28	0.66	1.51	4.35E-02	DOWN
Bt.17848.2.S1_at	ZMYND8	1.91	0.77	1.48	4.37E-02	DOWN
Bt.26892.1.S1_at	NBN	1.73	0.69	1.20	4.46E-02	DOWN
Bt.20110.1.S1_at	PSMF1	2.16	0.42	0.90	4.59E-02	DOWN
Bt.17432.1.S1_at	ARL5B	1.42	0.84	1.20	4.60E-02	DOWN
Bt.4898.1.S1_at	BASP1	1.54	0.80	1.24	4.60E-02	DOWN
Bt.7349.1.S1_at	---	2.05	0.68	1.41	4.60E-02	DOWN
Bt.12854.1.S1_at	---	2.17	0.69	1.50	4.63E-02	DOWN
Bt.8323.1.S1_at	DDX21	1.41	0.72	1.01	4.65E-02	DOWN
Bt.22335.1.S1_a_at	---	1.42	0.84	1.19	4.65E-02	DOWN
Bt.13489.1.S1_at	ZMIZ1	1.67	0.73	1.22	4.65E-02	DOWN
Bt.13777.1.S1_at	GIMAP7	2.25	0.77	1.72	4.65E-02	DOWN
Bt.18080.2.S1_at	LOC787094	2.44	0.67	1.63	4.65E-02	DOWN
Bt.6686.1.S1_at	CASK	1.76	0.75	1.32	4.77E-02	DOWN
Bt.25111.1.A1_at	LOC508347	5.60	0.21	1.20	4.79E-02	DOWN
Bt.23306.1.S1_at	---	1.57	0.82	1.29	4.90E-02	DOWN
Bt.11237.1.S1_at	YTHDC1	1.57	0.79	1.24	4.93E-02	DOWN
Bt.25084.1.S1_at	---	2.08	0.78	1.62	4.93E-02	DOWN

APPENDIX F
DIFFERENTIALY EXPRESSED FOR THE INTERACTION FATTY ACID BY MILK
REPLACER

List of differential expressed genes in liver of Holstein males at 30 d of age. Effect of feeding essential fatty acids prepartum and high linoleic acid in milk replacer (Interaction of contrasts FA by MR). Calves were fed a high or low linoleic acid milk replacer from 1 – 30 d of age and were born from dams fed diets supplemented with no fat (Control), saturated fatty acids (SFA), or essential fatty acids (EFA) starting at 8 wk before expected calving date.

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA by HLA	Ave. Exp. FA by LLA	Adjusted P value	Regulation
Bt.23696.1.A1_at	LOC509457	80.98	80.96	1.00	2.15E-13	UP
Bt.26769.1.S1_at	GIMAP8	20.67	19.50	0.94	1.63E-08	UP
Bt.17415.3.A1_at	ERRFI1	3.64	1.03	0.28	3.19E-06	UP
Bt.11918.1.A1_at	---	4.96	2.01	0.41	8.63E-05	UP
Bt.9655.2.S1_at	LOC790332	6.58	6.17	0.94	1.28E-04	UP
Bt.27940.1.A1_at	RHBG	5.58	2.04	0.37	1.47E-04	UP
Bt.29581.1.A1_at	---	2.83	0.99	0.35	4.21E-04	UP
Bt.2858.1.S1_at	ABHD6	2.71	1.14	0.42	1.01E-03	UP
Bt.12910.1.S1_at	OGDH	2.84	1.25	0.44	1.01E-03	UP
Bt.17073.1.S1_at	---	3.46	2.03	0.58	1.37E-03	UP
Bt.13381.1.S1_at	CIDEC	1.91	1.92	1.00	1.46E-03	UP
Bt.12508.1.S1_at	DCTPP1	3.14	1.70	0.54	1.46E-03	UP
Bt.29194.1.S1_at	PLIN4	3.33	1.25	0.37	1.46E-03	UP
Bt.26926.1.S1_at	---	2.16	0.94	0.43	2.16E-03	UP
Bt.3248.1.S1_at	ALDH4A1	2.28	1.07	0.47	2.16E-03	UP
Bt.27286.2.S1_at	ECD	2.57	1.98	0.77	2.20E-03	UP
Bt.11411.1.S1_at	CIAPIN1	2.81	1.32	0.47	2.30E-03	UP
Bt.11270.2.S1_at	VAR5	3.28	1.31	0.40	2.82E-03	UP
Bt.7413.1.S1_at	GRN	1.90	1.19	0.63	3.14E-03	UP
Bt.13376.1.S1_at	DHRS1	3.15	1.56	0.49	5.54E-03	UP
Bt.22533.1.S1_at	ALDOA	2.80	1.75	0.62	5.69E-03	UP
Bt.13641.1.S1_at	GSTZ1	1.85	1.14	0.62	5.86E-03	UP
Bt.21467.1.S1_at	COG4	2.27	1.44	0.63	6.34E-03	UP
Bt.17537.1.A1_at	SAA4	3.77	1.86	0.49	6.59E-03	UP
Bt.6020.1.S1_at	DNAJC11	2.15	0.97	0.45	8.76E-03	UP
Bt.4643.1.S1_at	LMAN2	1.76	1.21	0.69	8.81E-03	UP
Bt.24793.1.S1_at	MN1	1.97	1.98	1.00	8.81E-03	UP
Bt.11256.1.S1_at	CNOT1	2.18	0.99	0.45	8.81E-03	UP
Bt.16525.1.A1_at	---	2.91	1.34	0.46	8.81E-03	UP
Bt.1946.1.S1_at	NSFL1C	1.89	1.11	0.58	9.06E-03	UP
Bt.4141.1.S1_at	COPE	1.95	1.25	0.64	9.19E-03	UP
Bt.24662.1.S1_at	AKT1S1	2.19	1.28	0.58	9.19E-03	UP
Bt.26538.1.S1_at	LOC509420	2.78	1.49	0.54	9.19E-03	UP
Bt.12980.3.S1_a_at	CL43	2.99	1.02	0.34	9.28E-03	UP
Bt.21021.1.S1_at	TBC1D7	2.28	1.48	0.65	9.99E-03	UP
Bt.7237.2.S1_a_at	HADHA	4.15	1.74	0.42	1.04E-02	UP
Bt.10880.1.S1_at	TIMM50	1.83	1.15	0.63	1.06E-02	UP
Bt.6556.1.S1_at	LOC504773	3.18	1.37	0.43	1.10E-02	UP
Bt.23735.1.A1_s_at	---	2.45	2.31	0.95	1.15E-02	UP
Bt.28934.1.S1_at	AREG	10.53	10.74	1.02	1.31E-02	UP
Bt.5399.1.S2_at	NADK	1.87	0.91	0.49	1.32E-02	UP
Bt.4880.1.S1_at	SLC25A3	2.00	1.16	0.58	1.32E-02	UP

Appendix F. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA-HLA	Ave. Exp. FA-LLA	Adjusted P value	Regulation
Bt.21216.1.S1_at	CXorf56	2.15	1.26	0.59	1.32E-02	UP
Bt.1983.1.S1_at	EMR1	3.54	1.60	0.45	1.32E-02	UP
Bt.8775.1.S1_at	AP1B1	1.62	1.05	0.65	1.32E-02	UP
Bt.1753.1.S1_at	ATP6V1E1	1.77	1.24	0.70	1.32E-02	UP
Bt.805.1.S1_at	ADIPOR2	2.68	1.46	0.54	1.32E-02	UP
Bt.3736.1.A1_at	PDE4DIP	1.92	1.18	0.62	1.34E-02	UP
Bt.4937.1.S1_at	LOC505941	2.03	0.98	0.48	1.36E-02	UP
Bt.13588.2.S1_at	PSAT1	4.59	2.01	0.44	1.64E-02	UP
Bt.5096.1.S1_at	CCT3	3.21	1.46	0.45	1.64E-02	UP
Bt.25957.1.S1_at	MAVS	2.64	1.35	0.51	1.69E-02	UP
Bt.5083.1.S1_at	SLC27A4	7.31	1.71	0.23	1.73E-02	UP
Bt.8121.1.S1_x_at	BOLA	2.58	1.42	0.55	1.84E-02	UP
Bt.18914.1.S1_at	---	1.76	1.39	0.79	1.98E-02	UP
Bt.13486.1.A1_at	GLDC	2.36	1.08	0.46	2.06E-02	UP
Bt.11279.1.A1_at	CLCN4	2.58	1.55	0.60	2.06E-02	UP
Bt.23366.1.S1_at	CDIPT	2.68	1.42	0.53	2.07E-02	UP
Bt.24007.1.A1_at	SLC15A2	2.89	1.79	0.62	2.07E-02	UP
Bt.23171.2.S1_at	PCBD1	2.01	1.08	0.53	2.11E-02	UP
Bt.1207.1.S1_at	SLC16A13	2.64	1.57	0.59	2.14E-02	UP
Bt.20997.1.S1_at	C2H1orf144	3.65	1.18	0.32	2.18E-02	UP
Bt.12030.2.S1_at	ACTN4	2.08	1.23	0.59	2.26E-02	UP
Bt.3023.1.S1_at	NIT1	2.49	1.50	0.60	2.26E-02	UP
Bt.23169.1.S1_at	SIRPA	2.89	1.49	0.51	2.26E-02	UP
Bt.10387.1.S1_at	ABCF1	2.91	1.42	0.49	2.26E-02	UP
Bt.20265.1.A1_at	ECD	1.85	1.54	0.83	2.28E-02	UP
Bt.10361.1.S1_at	---	1.51	0.97	0.64	2.34E-02	UP
Bt.20361.2.A1_at	FBXL20	3.56	2.81	0.79	2.37E-02	UP
Bt.8730.1.S1_at	RAPGEF2	1.87	1.04	0.56	2.41E-02	UP
Bt.5334.1.S1_at	RPSA	1.73	1.16	0.67	2.53E-02	UP
Bt.282.1.S1_at	VDAC1P5	1.69	1.08	0.64	2.59E-02	UP
Bt.28586.1.S1_at	ERMP1	1.56	1.27	0.81	2.59E-02	UP
Bt.1332.1.S1_a_at	COX10	1.97	0.98	0.50	2.66E-02	UP
Bt.26568.2.S1_a_at	LOC531049	2.25	1.33	0.59	2.67E-02	UP
Bt.13705.1.S1_at	SSR2	2.18	1.22	0.56	2.70E-02	UP
Bt.4902.1.S1_at	CTSZ	1.90	1.52	0.80	2.78E-02	UP
Bt.121.1.S1_at	FRZB	3.29	2.58	0.78	2.78E-02	UP
Bt.18847.1.A1_at	---	4.48	1.44	0.32	2.78E-02	UP
Bt.8090.2.S1_at	MYBBP1A	2.17	0.80	0.37	2.78E-02	UP
Bt.19899.1.A1_at	HGD	1.76	1.24	0.71	2.80E-02	UP
Bt.653.1.S1_at	NEK6	2.00	1.13	0.56	2.80E-02	UP
Bt.8078.1.S1_at	ARPC4	2.35	1.02	0.43	2.81E-02	UP
Bt.20281.3.S1_a_at	PGM1	2.19	1.42	0.65	2.89E-02	UP
Bt.27204.1.S1_at	LPCAT3	6.25	1.42	0.23	2.99E-02	UP
Bt.6460.1.S1_at	PDIA6	1.97	1.43	0.72	3.04E-02	UP
Bt.2580.1.S1_at	GALM	2.05	1.58	0.77	3.04E-02	UP
Bt.23164.1.S1_at	UQCRC1	2.22	1.23	0.55	3.04E-02	UP
Bt.1987.1.S1_at	TAX1BP3	1.86	1.28	0.69	3.05E-02	UP
Bt.5399.1.S1_at	NADK	2.11	1.11	0.53	3.09E-02	UP
Bt.12370.1.S1_at	MLF2	3.83	1.41	0.37	3.27E-02	UP
Bt.4475.1.S1_at	NDUFS2	1.54	1.03	0.67	3.40E-02	UP
Bt.5771.1.S1_at	---	1.96	0.98	0.50	3.40E-02	UP
Bt.16137.1.S1_at	ALDH9A1	2.74	1.35	0.49	3.40E-02	UP
Bt.24597.1.S1_at	GLG1	4.35	1.37	0.31	3.40E-02	UP

Appendix F. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA-HLA	Ave. Exp. FA-LLA	Adjusted P value	Regulation
Bt.9632.2.S1_at	DMBT1	2.71	1.37	0.50	3.54E-02	UP
Bt.15334.2.A1_at	STAT3	3.95	1.12	0.28	3.54E-02	UP
Bt.14207.1.S1_at	GCAT	2.26	1.02	0.45	3.57E-02	UP
Bt.15886.1.S1_at	ACSL5	2.38	1.10	0.46	3.59E-02	UP
Bt.12957.1.A1_at	TNRC6B	1.55	0.83	0.54	3.69E-02	UP
Bt.4431.1.S1_a_at	ATP5B	1.56	1.26	0.81	3.69E-02	UP
Bt.12586.1.A1_at	LOC508439	1.71	1.21	0.71	4.01E-02	UP
Bt.13633.1.A1_at	---	4.00	1.70	0.42	4.07E-02	UP
Bt.20207.1.A1_at	ALG12	1.70	1.14	0.67	4.09E-02	UP
Bt.227.3.A1_x_at	GSTA1	1.96	1.38	0.70	4.09E-02	UP
Bt.4604.1.S1_a_at	ACSM1	1.97	1.08	0.55	4.09E-02	UP
Bt.20145.1.S1_at	PRELID1	2.04	1.22	0.60	4.09E-02	UP
Bt.2113.1.S1_at	CNDP2	2.30	1.26	0.55	4.09E-02	UP
Bt.11167.1.S1_at	GLRX5	2.54	1.59	0.63	4.09E-02	UP
Bt.2110.1.S1_at	DPP3	2.99	1.29	0.43	4.09E-02	UP
Bt.3487.1.S1_at	TPI1	3.11	1.55	0.50	4.09E-02	UP
Bt.23902.1.A1_at	---	1.40	1.08	0.77	4.15E-02	UP
Bt.20229.1.S1_at	TBRG4	1.47	0.93	0.64	4.25E-02	UP
Bt.20711.1.S1_at	RDH16	1.68	1.12	0.67	4.26E-02	UP
Bt.20322.3.S1_a_at	WDR18	2.87	1.43	0.50	4.27E-02	UP
Bt.19922.1.S1_at	HPD	3.45	1.98	0.58	4.27E-02	UP
Bt.7915.1.S1_at	MDH2	1.63	1.12	0.69	4.35E-02	UP
Bt.1059.3.S1_a_at	ATP2A2	2.86	1.22	0.43	4.48E-02	UP
Bt.23179.1.S1_at	HSP90AA1	2.94	1.46	0.50	4.48E-02	UP
Bt.227.2.A1_at	GSTA1	2.01	1.07	0.53	4.50E-02	UP
Bt.22783.1.S1_at	ENO1	3.58	1.42	0.40	4.56E-02	UP
Bt.17219.1.A1_at	MPDU1	1.78	1.08	0.60	4.56E-02	UP
Bt.15691.1.S1_at	KCNK5	2.12	1.08	0.51	4.64E-02	UP
Bt.18479.1.A1_at	ZNF608	2.09	1.48	0.71	4.71E-02	UP
Bt.5183.1.S1_at	TUBA4A	3.32	1.30	0.39	4.71E-02	UP
Bt.5196.1.S1_at	WDR55	1.74	1.15	0.66	4.72E-02	UP
Bt.3811.1.S1_at	MRPS18B	2.04	1.35	0.66	4.72E-02	UP
Bt.23605.2.S1_at	THRA	2.87	1.30	0.45	4.74E-02	UP
Bt.1552.1.S1_at	SARS	2.07	1.27	0.61	4.75E-02	UP
Bt.13588.3.A1_at	PSAT1	5.39	2.16	0.40	4.88E-02	UP
Bt.22543.1.S1_at	---	1.49	0.79	0.53	4.90E-02	UP
Bt.9298.1.S1_at	AARSD1	2.56	1.37	0.54	4.90E-02	UP
Bt.4404.1.A1_at	PRSS2	32.28	0.03	1.08	2.07E-08	DOWN
Bt.841.1.S1_at	---	2.90	0.74	2.16	1.43E-05	DOWN
Bt.19274.1.A1_at	C1QTNF7	2.51	1.01	2.53	2.87E-05	DOWN
Bt.17034.1.A1_at	---	3.63	0.26	0.96	3.05E-05	DOWN
Bt.21721.1.A1_at	USP2	3.82	0.27	1.05	4.09E-05	DOWN
Bt.26364.1.A1_at	BTBD8	4.13	0.99	4.10	8.63E-05	DOWN
Bt.27889.1.S1_at	DLD	2.30	1.07	2.47	1.53E-04	DOWN
Bt.18003.1.S1_at	CUL3	3.33	0.73	2.44	2.73E-04	DOWN
Bt.26650.1.S1_at	---	1.91	1.27	2.43	7.94E-04	DOWN
Bt.10084.1.S1_at	CASP3	1.93	0.83	1.60	1.01E-03	DOWN
Bt.3549.1.A1_at	VAMP4	2.67	0.83	2.23	1.37E-03	DOWN
Bt.29324.1.S1_at	---	3.37	0.64	2.15	1.46E-03	DOWN
Bt.18440.2.S1_at	LOC510382	5.20	0.79	4.11	1.46E-03	DOWN
Bt.20977.3.S1_at	CCPG1	2.11	0.97	2.05	1.51E-03	DOWN
Bt.26308.2.A1_at	RAD18	2.56	0.82	2.09	1.51E-03	DOWN
Bt.18026.1.A1_at	ERBB2IP	1.94	0.78	1.51	1.78E-03	DOWN

Appendix F. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA-HLA	Ave. Exp. FA-LLA	Adjusted P value	Regulation
Bt.6645.1.S1_at	RNPC3	2.18	0.71	1.55	1.78E-03	DOWN
Bt.21952.1.A1_at	---	1.99	0.81	1.62	2.16E-03	DOWN
Bt.9974.1.S1_at	CCL3	5.53	0.38	2.11	2.29E-03	DOWN
Bt.16425.1.A1_at	---	2.09	0.63	1.32	2.38E-03	DOWN
Bt.27320.1.A1_at	SGOL2	2.46	0.80	1.98	2.71E-03	DOWN
Bt.2962.1.S1_at	---	4.90	0.74	3.62	3.80E-03	DOWN
Bt.24892.1.A1_at	RIT1	2.14	1.02	2.19	4.63E-03	DOWN
Bt.8169.1.S1_at	SLC39A6	1.86	1.00	1.86	4.68E-03	DOWN
Bt.24249.1.S1_at	SUV420H1	2.34	0.68	1.59	4.68E-03	DOWN
Bt.22483.1.S1_at	SEC31B	1.68	0.71	1.20	5.50E-03	DOWN
Bt.9391.2.S1_at	BIRC3	1.97	0.75	1.48	5.50E-03	DOWN
Bt.12290.1.S1_at	PSIP1	2.71	0.79	2.14	5.50E-03	DOWN
Bt.29879.1.S1_at	KAT2B	3.07	0.79	2.41	5.69E-03	DOWN
Bt.29587.1.S1_at	WAC	1.95	0.78	1.52	5.78E-03	DOWN
Bt.17352.1.A1_at	LOC785119	1.89	0.80	1.50	5.86E-03	DOWN
Bt.20677.1.S1_at	NSL1	2.44	1.04	2.53	5.86E-03	DOWN
Bt.13743.1.A1_at	RFK	2.42	1.01	2.45	6.09E-03	DOWN
Bt.16789.1.A1_at	C5H12orf11	1.98	0.89	1.77	6.33E-03	DOWN
Bt.19575.1.S1_at	HSPA14	1.73	0.66	1.15	6.59E-03	DOWN
Bt.2424.1.S1_at	DPYD	1.81	0.87	1.58	6.59E-03	DOWN
Bt.17364.1.A1_at	---	8.43	0.46	3.88	6.59E-03	DOWN
Bt.26410.1.A1_at	MTERF	2.26	0.82	1.85	7.53E-03	DOWN
Bt.6802.1.S1_at	RGS5	2.77	2.08	5.76	7.53E-03	DOWN
Bt.20206.1.A1_at	ATP11B	2.09	0.80	1.67	7.64E-03	DOWN
Bt.9140.1.S1_at	GMNN	1.96	1.17	2.31	7.64E-03	DOWN
Bt.5692.1.S1_at	LOC100425208	2.59	0.63	1.62	7.87E-03	DOWN
Bt.15299.1.A1_at	---	1.87	0.99	1.84	8.03E-03	DOWN
Bt.24203.1.S1_at	ANGPTL3	1.66	0.93	1.55	8.07E-03	DOWN
Bt.22150.1.A1_at	LZTFL1	1.77	0.97	1.72	8.39E-03	DOWN
Bt.21957.1.S1_at	---	2.54	0.69	1.75	8.39E-03	DOWN
Bt.29107.1.S1_at	---	2.11	0.83	1.76	8.76E-03	DOWN
Bt.6275.1.S1_at	TGFBR1	2.89	0.74	2.14	8.76E-03	DOWN
Bt.22044.1.S1_at	---	3.33	0.85	2.84	8.76E-03	DOWN
Bt.6397.2.S1_at	HMGB2	2.22	1.14	2.54	8.81E-03	DOWN
Bt.5129.1.S1_a_at	NNAT	6.26	0.67	4.18	8.81E-03	DOWN
Bt.25471.1.S1_at	ATXN3	3.42	0.75	2.57	9.19E-03	DOWN
Bt.18792.1.S1_at	DCTN6	3.81	1.07	4.09	9.19E-03	DOWN
Bt.16672.1.A1_at	LOC698727	9.04	0.51	4.65	9.19E-03	DOWN
Bt.24506.2.A1_at	CHIC2	1.79	1.04	1.86	9.28E-03	DOWN
Bt.19906.1.A1_at	---	4.16	0.64	2.67	9.28E-03	DOWN
Bt.22524.2.A1_at	BBS5	1.89	0.89	1.69	9.62E-03	DOWN
Bt.19339.1.S1_at	---	1.96	0.70	1.38	1.00E-02	DOWN
Bt.28187.1.S1_at	WEE1	2.37	0.67	1.58	1.04E-02	DOWN
Bt.20758.1.S1_at	LOC541014	1.96	0.76	1.49	1.04E-02	DOWN
Bt.5542.2.S1_at	NAP1L1	2.02	0.89	1.79	1.15E-02	DOWN
Bt.7327.2.S1_a_at	MGC133692	1.84	0.91	1.68	1.28E-02	DOWN
Bt.16580.1.S1_at	CD2AP	3.54	0.60	2.11	1.32E-02	DOWN
Bt.26318.1.S1_a_at	FAIM	4.91	0.62	3.05	1.32E-02	DOWN
Bt.11751.1.A1_at	KLHL23	2.78	0.94	2.61	1.32E-02	DOWN
Bt.17883.2.A1_at	---	3.96	0.79	3.12	1.34E-02	DOWN
Bt.22730.1.S1_at	FGFR1OP2	1.72	0.79	1.36	1.35E-02	DOWN
Bt.26416.1.A1_at	---	4.93	0.72	3.56	1.56E-02	DOWN
Bt.24361.1.S1_at	ESF1	1.92	0.88	1.69	1.58E-02	DOWN

Appendix F. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA-HLA	Ave. Exp. FA-LLA	Adjusted P value	Regulation
Bt.13768.1.S1_at	DYNLT3	1.88	0.90	1.69	1.64E-02	DOWN
Bt.5635.1.S1_at	TCEAL1	2.36	0.75	1.77	1.64E-02	DOWN
Bt.18220.1.A1_at	CCDC112	3.74	0.74	2.77	1.64E-02	DOWN
Bt.17653.1.A1_at	UPP2	4.15	1.18	4.91	1.64E-02	DOWN
Bt.26408.1.A1_at	SFRS2IP	1.77	0.83	1.47	1.81E-02	DOWN
Bt.28101.1.S1_at	---	1.57	0.97	1.53	1.82E-02	DOWN
Bt.6289.1.S1_at	SPTLC1	1.89	0.89	1.67	1.82E-02	DOWN
Bt.25471.2.A1_at	ATXN3	2.50	0.85	2.13	1.82E-02	DOWN
Bt.23178.1.S2_at	DCN	1.70	1.01	1.73	1.84E-02	DOWN
Bt.11445.1.A1_at	BCL10	2.68	0.85	2.27	1.84E-02	DOWN
Bt.842.1.A1_at	TOR1AIP1	2.05	0.79	1.62	1.98E-02	DOWN
Bt.13981.1.S1_at	TM2D2	2.99	0.59	1.75	2.01E-02	DOWN
Bt.9774.1.S1_a_at	MGC165862	3.10	1.00	3.10	2.05E-02	DOWN
Bt.835.1.A1_at	SNTB1	2.13	0.80	1.70	2.06E-02	DOWN
Bt.22421.1.A1_at	LOC530325	3.01	0.80	2.41	2.07E-02	DOWN
Bt.4405.1.S1_s_at	CCDC104	1.51	0.97	1.47	2.11E-02	DOWN
Bt.27403.1.S1_at	LOC540987	1.84	0.78	1.43	2.14E-02	DOWN
Bt.27099.1.A1_at	SEC62	2.30	0.81	1.86	2.25E-02	DOWN
Bt.2859.1.A1_at	LOC540253	3.32	0.84	2.78	2.26E-02	DOWN
Bt.19723.1.A1_at	ACTR10	2.03	0.93	1.90	2.35E-02	DOWN
Bt.19839.1.A1_at	Ppig	1.72	0.91	1.56	2.35E-02	DOWN
Bt.26150.1.A1_at	L2HGDH	1.61	1.34	2.16	2.37E-02	DOWN
Bt.6180.1.S1_at	FRG1	2.15	0.96	2.05	2.37E-02	DOWN
Bt.22656.2.S1_at	---	2.78	0.70	1.95	2.41E-02	DOWN
Bt.367.1.S1_at	OLR1	6.07	0.22	1.35	2.55E-02	DOWN
Bt.18577.2.A1_at	LOC472962	2.54	0.85	2.15	2.59E-02	DOWN
Bt.3599.1.S1_at	NPM1	1.52	0.93	1.40	2.59E-02	DOWN
Bt.8054.1.S1_at	SYAP1	1.59	1.04	1.66	2.59E-02	DOWN
Bt.23900.1.A1_at	---	1.86	0.96	1.79	2.59E-02	DOWN
Bt.26992.1.A1_at	ADAM10	1.89	0.87	1.65	2.59E-02	DOWN
Bt.17846.1.A1_at	---	3.56	0.73	2.59	2.59E-02	DOWN
Bt.15685.1.A1_at	MOSC2	1.57	0.87	1.36	2.66E-02	DOWN
Bt.19212.1.S1_at	KLHL9	1.53	0.95	1.46	2.70E-02	DOWN
Bt.29175.1.A1_at	ZUFSP	1.74	0.73	1.27	2.70E-02	DOWN
Bt.27187.1.S1_at	MPHOSPH10	2.97	0.61	1.80	2.77E-02	DOWN
Bt.13815.1.S1_at	---	1.72	0.87	1.50	2.78E-02	DOWN
Bt.21268.1.S2_at	RPS6KB1	1.81	0.80	1.45	2.78E-02	DOWN
Bt.25832.1.S1_at	---	2.40	0.79	1.91	2.80E-02	DOWN
Bt.8905.1.S1_at	ITCH	2.02	0.70	1.41	2.85E-02	DOWN
Bt.6341.1.S1_at	DNAJC1	2.15	0.53	1.15	2.85E-02	DOWN
Bt.22563.1.A1_s_at	CSDE1	1.47	0.93	1.37	2.89E-02	DOWN
Bt.9974.1.S1_a_at	CCL3	2.89	0.48	1.39	2.90E-02	DOWN
Bt.11233.1.S1_at	LOC787143 ///	1.47	0.87	1.27	2.92E-02	DOWN
	TOP2B					
Bt.2048.1.S1_at	AGPS	1.59	0.96	1.52	2.96E-02	DOWN
Bt.812.1.S1_at	---	2.09	0.91	1.89	2.96E-02	DOWN
Bt.19218.2.S1_at	CNOT6	1.73	0.90	1.56	2.99E-02	DOWN
Bt.21099.1.A1_at	BRMS1L	1.75	1.02	1.78	2.99E-02	DOWN
Bt.26828.1.S1_at	CNTLN	5.22	0.62	3.21	2.99E-02	DOWN
Bt.6899.1.S1_at	LOC784769	1.77	0.80	1.41	3.01E-02	DOWN
Bt.14124.2.S1_at	USP33	3.13	0.75	2.33	3.01E-02	DOWN
Bt.9069.1.S1_at	ANKRD10	1.51	0.72	1.08	3.04E-02	DOWN
Bt.24095.1.A1_at	USP1	1.71	0.96	1.65	3.04E-02	DOWN

Appendix F. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA-HLA	Ave. Exp. FA-LLA	Adjusted P value	Regulation
Bt.20932.1.S1_at	NSA2	2.21	0.74	1.64	3.04E-02	DOWN
Bt.19339.3.A1_at	SOCS6	2.73	0.81	2.21	3.04E-02	DOWN
Bt.16614.1.A1_s_at	SYNCRIP	2.31	0.54	1.24	3.05E-02	DOWN
Bt.18023.1.S1_at	ZNF322	1.77	0.79	1.40	3.09E-02	DOWN
Bt.19519.1.S1_at	HLTF	1.86	1.03	1.91	3.09E-02	DOWN
Bt.12664.2.S1_at	ZMYM5	3.26	0.64	2.09	3.10E-02	DOWN
Bt.19575.2.S1_at	HSPA14	2.40	0.68	1.63	3.28E-02	DOWN
Bt.14059.1.A1_at	AUH	1.60	1.05	1.68	3.31E-02	DOWN
Bt.22350.1.A1_at	GMCL1	2.11	0.99	2.08	3.36E-02	DOWN
Bt.17517.1.S1_at	MGC134574	1.90	1.02	1.94	3.40E-02	DOWN
Bt.14075.1.S1_at	ARHGAP5	1.99	0.92	1.83	3.40E-02	DOWN
Bt.9527.2.S1_at	KLF10	2.95	0.87	2.57	3.53E-02	DOWN
Bt.27042.1.S1_at	CENPC1	2.52	0.81	2.05	3.55E-02	DOWN
Bt.27322.1.S1_at	AP1AR	2.61	0.84	2.18	3.55E-02	DOWN
Bt.1738.1.S1_at	HIBCH	1.64	0.95	1.56	3.57E-02	DOWN
Bt.23998.1.A1_a_at	CUX2	4.19	0.59	2.49	3.59E-02	DOWN
Bt.14129.1.S1_at	LACTB2	1.64	0.99	1.62	3.65E-02	DOWN
Bt.23960.1.S1_at	CA5B	2.52	0.77	1.94	3.72E-02	DOWN
Bt.3678.1.S1_at	MKI67IP	1.65	0.76	1.25	3.83E-02	DOWN
Bt.6993.2.A1_a_at	NME7	1.92	0.99	1.90	3.83E-02	DOWN
Bt.14283.1.A1_at	---	2.16	0.98	2.13	3.83E-02	DOWN
Bt.8039.2.S1_a_at	---	2.48	0.83	2.06	3.83E-02	DOWN
Bt.15306.1.A1_at	PHF3	1.62	0.79	1.28	3.98E-02	DOWN
Bt.28207.1.S1_at	RNF19A	2.43	0.79	1.92	3.98E-02	DOWN
Bt.15872.1.S1_at	SLU7	2.52	1.53	3.86	3.98E-02	DOWN
Bt.22064.2.S1_at	RSRC2	1.84	0.72	1.32	4.00E-02	DOWN
Bt.22976.1.S1_at	SMC4	2.86	0.91	2.60	4.00E-02	DOWN
Bt.28577.1.S1_at	SENP6	1.54	0.96	1.48	4.01E-02	DOWN
Bt.13556.1.S1_at	CFH	2.98	0.52	1.55	4.05E-02	DOWN
Bt.13332.1.S1_at	SLC25A46	1.67	0.74	1.23	4.07E-02	DOWN
Bt.16052.2.A1_at	TSPYL1	1.94	0.84	1.63	4.07E-02	DOWN
Bt.15706.1.A1_at	---	2.34	0.85	2.00	4.07E-02	DOWN
Bt.27143.1.A1_at	ODF2L	1.65	1.00	1.65	4.09E-02	DOWN
Bt.21869.1.S1_at	LOC537017	2.31	0.84	1.93	4.09E-02	DOWN
Bt.18928.1.A1_at	EIF4E3	2.43	1.09	2.65	4.09E-02	DOWN
Bt.29506.1.S1_at	CCDC82	3.21	0.66	2.13	4.09E-02	DOWN
Bt.24749.1.S1_at	LOC100430496	3.21	0.63	2.04	4.09E-02	DOWN
Bt.23992.1.A1_at	---	4.78	0.39	1.87	4.09E-02	DOWN
Bt.19232.1.A1_at	---	5.35	1.06	5.68	4.11E-02	DOWN
Bt.13989.1.A1_at	CAV2	1.99	1.00	1.99	4.15E-02	DOWN
Bt.19994.1.S1_at	LOC789597	1.94	0.87	1.69	4.25E-02	DOWN
Bt.25190.1.A1_at	---	2.16	0.90	1.93	4.26E-02	DOWN
Bt.18440.3.A1_at	LOC510382	16.56	0.62	10.24	4.27E-02	DOWN
Bt.24205.1.A1_at	FGB	2.05	0.85	1.73	4.30E-02	DOWN
Bt.20934.1.S1_at	LOC100137763	4.04	0.94	3.80	4.32E-02	DOWN
Bt.28945.1.A1_at	LOC100440461	1.98	0.80	1.59	4.48E-02	DOWN
Bt.20666.1.S1_at	---	2.98	0.72	2.15	4.48E-02	DOWN
Bt.16828.1.A1_at	---	1.85	0.68	1.26	4.56E-02	DOWN
Bt.13336.1.A1_at	SMC4	2.82	0.86	2.42	4.56E-02	DOWN

Appendix F. Continued

Affimetrix ID	Gene symbol	Fold change	Av. Exp. FA-HLA	Ave. Exp. FA-LLA	Adjusted P value	Regulation
Bt.5916.1.S1_at	PGCP	2.01	0.74	1.48	4.61E-02	DOWN
Bt.22676.1.A1_at	GPN3	1.86	0.93	1.73	4.75E-02	DOWN
Bt.16000.1.S1_at	ENTPD4	2.02	0.56	1.13	4.75E-02	DOWN
Bt.444.1.S1_at	PDE6C	8.50	0.57	4.81	4.75E-02	DOWN
Bt.2765.1.S1_at	---	1.98	0.85	1.67	4.75E-02	DOWN
Bt.5188.1.S1_at	ABTB1	2.65	0.49	1.31	4.75E-02	DOWN
Bt.22672.1.A1_at	HPGD	3.34	0.93	3.11	4.75E-02	DOWN
Bt.2899.1.S2_at	FOS	4.65	0.34	1.57	4.75E-02	DOWN
Bt.16276.1.A1_at	ARSK	2.45	0.94	2.29	4.88E-02	DOWN
Bt.22069.1.A1_at	CCPG1	1.89	0.89	1.67	4.90E-02	DOWN
Bt.2190.1.S1_at	FUBP3	2.06	0.84	1.73	4.90E-02	DOWN
Bt.8039.1.S1_at	TMEM170A	2.22	0.72	1.61	4.91E-02	DOWN

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BIOGRAPHICAL SKETCH

Miriam Garcia Orellana earned her bachelor degree in animal sciences at Universidad Nacional Agraria la Molina in December 1997 and her engineer degree in animal sciences in December 1999. From August 1999 to September 2003, Miriam worked for two government agencies, first in a project called “Implementation of small animal units in public schools” where she served until the end of 2000. After, she moved to the highlands of Peru to work as an animal production consultant assisting low income farmers. In 2004, Miriam was awarded a scholarship from Consejo Nacional de Ciencia y Tecnologia in Peru and returned to Universidad Nacional Agraria la Molina, where she got a master in ruminant nutrition after defending the thesis “Effect of feeding two diets with different nutritive value on milk production and composition and metabolic profile of native Peruvian and Brown Swiss cows”. Starting in 2006 to August 2008 Miriam worked with Dr. Carlos A. Gomez at Universidad Nacional Agraria la Molina as an associated researcher where she gained enormous experience in dairy cattle nutrition. In fall 2008, she was awarded the University of Florida CALS Alumni Graduate Award to pursue her PhD in animal sciences under the guidance of Dr. Charles R. Staples. During her program Miriam has lead two projects and participated in several others, she also has served as teaching assistant and invited instructor. She is impassioned for teaching and research, after graduating; she is planning to pursue a career that would allow her to stay on the same path.