EFFECTS OF SUPPLEMENT TYPE ON PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF BRAHMAN-CROSSBRED CATTLE

By

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This thesis is dedicated to my wife Flavia, and also to my entire family.

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EFFECTS OF SUPPLEMENT TYPE ON PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF BRAHMAN-CROSSBRED CATTLE

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Two studies were conducted to compare the performance and physiological responses of forage-fed beef cattle supplemented with either molasses-based or citrus pulp-based supplements. The first study consisted in two separate experiments. In Exp. 1, we compared BW gain, reproductive efficiency, and plasma concentration of BUN, glucose, insulin, IGF-I, GH, and progesterone (P4) of 60 Brahman x British heifers supplemented 3x/wk with either a molasses-based (ML) or citrus pulp-based supplement (CT). Supplement intakes were formulated to be iso-caloric and iso-nitrogenous. Reproductive performance was not affected by treatments; however, mean BW gain was greater ($P < 0.05$) for heifers fed CT (0.30 and 0.40 kg/d for ML and CT groups; SEM = 0.02). Mean plasma concentrations of glucose, insulin and IGF-I were greater ($P < 0.05$) for heifers fed CT, whereas BUN was greater for heifers fed ML. Mean plasma P4 concentration did not differ between treatments. In Exp. 2, we compared forage DMI and plasma concentration of BUN, glucose, insulin, IGF-I, and P4 of 24 Brahman x British

mature cows supplemented with the same treatments of Exp. 1. Forage DMI did not differ between treatments; however, a day and treatment x day interaction ($P < 0.05$) were detected. No differences were detected in any blood metabolite/hormone. For blood P4 analysis, the two treatments were further separated into one of two sub-treatments, in a 2 x 2 factorial arrangement: A) exogenous source of P4 (CD) or B) natural source of P4 (NCD). Only the results from the CD group were reported because cows from the NCD group were at random stages of the estrous cycle. No differences in plasma P4 concentration were detected between CT and ML-fed cows, but a treatment x period interaction ($P < 0.01$) was detected because cows fed CT had greater plasma P4 concentrations during the beginning of the experiment. The second study compared BW gain, forage DMI, and plasma concentrations of BUN, glucose, insulin, IGF-I, and GH of 24 yearling Brahman x British steers supplemented 3x/wk with either CT or ML, or every day with a citrus pulp-based supplement (CD). Supplement intakes were again formulated to be iso-caloric and iso-nitrogenous. Forage DMI did not differ among treatments; however, a day and treatment x day interaction were detected ($P < 0.05$). Mean BW gain was greater ($P < 0.05$) for CD vs. ML, tended to be greater for CD vs. CT ($P = 0.13$), but did not differ for CT vs. ML (0.30, 0.18 and 0.10 kg/d for CD, CT and ML, respectively; SEM = 0.05). Mean plasma concentration of glucose was lesser ($P < 0.05$) for CD vs. ML and CT. Mean insulin was greater ($P < 0.05$) for CT vs. CD and ML. We concluded that forage-fed cattle offered a citrus pulp-based supplement had increased BW gain compared to cattle offered a molasses-based supplement. Additionally, BW gain was further increased if citrus pulp-based supplements were offered daily.

CHAPTER 1
INTRODUCTION

The beef industry in Florida primarily consists of cow-calf enterprises with forage as the main component of cattle diets. The reproductive performance of the herd is one of the most important factors for the overall efficiency of these operations, whereas energy is the first nutritional concern for cattle reproduction. Forages grown in Florida usually are lacking in energy density, and consequently, energy supplementation is often required.

A variety of feedstuffs can be used as ingredients for energy supplements, but price and availability are highly variable. Citrus pulp and molasses are energy concentrated byproducts that originate from citrus and sugarcane production, respectively. Both enterprises are important and extensive agricultural systems in Florida. Despite its moderate DM content (approximately 75 %), molasses is classified as a liquid feed, whereas citrus pulp is commonly processed and fed as dry pellets. The different physical characteristics of these products lead to differences in intake behavior, which have an important effect in operations where cattle are supplemented with low frequency.

Molasses and citrus pulp also differ in their carbohydrate profile. Sucrose is the main carbohydrate of molasses, whereas pectin is the main carbohydrate of citrus pulp. Pectin and sucrose are fermented differently in the rumen, potentially impacting forage intake, diet digestibility, and energy utilization.

For these reasons, experiments were conducted to compare the effects of supplement type on performance and physiologic responses of Brahman-crossbred cattle.

CHAPTER 2
LITERATURE REVIEW

Supplementation for Forage-Based Diets

Forage is the cheapest and most available source of nutrition for beef cattle in the state of Florida. With the purpose of minimizing costs of production, it is the main dietary component of the cow-calf operations located in the state. However, the forages grown in Florida usually do not have adequate nutrient density to meet the nutrient requirements of cattle consuming typical amounts of DM (Moore et. al., 1991), particularly animals with greater nutrient demands. According to the NRC (1996), nutritional requirements of beef cattle increase with lactation, pregnancy, and growth rates. Consequently, growing cattle and lactating beef cows require a high plane of nutrition to maintain adequate performance. When their diets are based on low-quality forages, as in the case of Florida, nutrient supplementation is often required.

Energy Supplementation

Energy supplementation is required to maintain optimal animal performance in areas where energy availability from grazed forages is limited (Caton and Dhuyvetter, 1997). In Florida, even when protein supplements are fed and forage intake is potentially increased, energy requirements usually are not met because of the lack in forage quality. Bodine and Purvis (2003) also indicated that although protein is typically considered the major limiting nutrient for cattle, its supplementation may not result in adequate energy supply to the animals. Consequently, supplemental energy is required.

Contrary to protein supplements, energy supplements often decrease forage intake and digestibility (Caton and Dhuyvetter, 1997; Kunkle et al., 1999; Bodine and Purvis, 2003). A data base containing 66 publications concluded that forage intake was decreased when supplemental TDN intake was greater than 0.7 % of BW (Moore et al., 1999). These effects are even more pronounced when supplements contain high quantities of nonstructural carbohydrates (NSC) such as starch and sugars (Sanson et al., 1990; Olson et al., 1999). The potential mechanisms by which high NSC supplements depress forage intake and/or digestibility include a decrease in ruminal pH, decrease in synthesis and activity of cellulolytic enzymes (Martin et. al., 2001), impaired bacterial attachment to fibrous digesta (Hiltner and Dehority, 1983), and an increase in lag time for fiber digestion (Mertens and Loften, 1980). Bowman and Sanson (2000) compiled 116 publications and observed that supplements with high quantities of NSC do not have a negative impact on forage intake when fed less than 0.3 % of BW or when the NSC fraction of the supplement does not exceed 0.1 % of BW. However, restricting energy supplementation to these levels may impair animal performance, particularly when animals are fed low-quality forages and have high nutritional demands.

An alternative to prevent depressed forage intake when energy supplementation is necessary is the utilization of fibrous by-products feedstuffs as energy sources in the diet. These feedstuffs usually have reduced NSC with moderate TDN content, are rapidly digestible in the rumen and also are low-priced when compared to the costs of high NSC feedstuffs. Some authors have reported no differences in performance when animals were fed energy supplements with high or low NSC content (Sunvold et al., 1991; Horn et al., 1995). These data imply that high NSC supplements can be replaced by highly digestible

fibrous supplements without a depression in animal performance. Other authors reported a small negative effect on forage intake when supplements with low NSC content were fed (Bowman and Sanson, 1996; Caton and Dhuyvetter, 1997; Royes et al., 2001). A review by Bowman and Sanson (2000), however, concluded that low NSC energy supplements, contrary to those with high NSC concentration, increased low-quality forage intake and digestibility.

According to the NRC (2001), the NSC fraction of a feedstuff is found inside the plant cells, and is composed of sugars, starches, organic acids and other carbohydrates such as fructans, serving as the major source of energy for cattle. The NSC fraction is highly soluble in the ruminal environment. This fraction is degraded rapidly by the microbial population, and because of this, increases the rate and amount at which VFAs are formed. Increasing the NSC content or availability in diets typically results in elevated VFA production/accumulation in the rumen with a decreased acetate:propionate molar ratio. This subsequently leads to a decrease in ruminal pH that may be detrimental to fibrolytic bacteria, resulting in impaired forage digestibility (Aldrich et al., 1993; Joy et al., 1997; Knowlton et al., 1998).

The energy provided by feedstuffs with a low NSC content usually originates either from NDF or pectin. According to the NRC (2001), NDF is the measurement that best differentiates structural from nonstructural carbohydrates and constitutes most of the structural components in plant cells: cellulose, hemicellulose and lignin. On average, NDF is less digestible than NSC and its concentration in feedstuffs is negatively associated with energy (NRC, 2001). The chemical profile of NDF (ratio of cellulose:hemicellulose:lignin) affects its digestibility. Cellulose and hemicellulose are

potentially but incompletely digested and utilized as an energy source by the microbial population in the rumen. In contrast, lignin is poorly or even not digested at all (Jung and Allen, 1995). Therefore, feedstuffs with a similar content of NDF will not necessarily have similar energy concentration. Depending on the lignin concentration within the NDF fraction, feeds with greater NDF may have more available energy than feeds with lesser NDF content (NRC, 2001). The concentration of NDF in the diet is positively associated with ruminal pH since the microbes generally ferment the NDF fraction slower. Therefore, lesser VFA production/accumulation and a greater acetate:propionate molar ratio in the rumen may result.

Pectins are found within the plant cell wall, but are not linked to lignin. Pectins are well digested by the ruminal microbes, but not by mammalian enzymes (Ariza et al., 2001). Although classified as a dietary fiber, pectins are not represented in the NDF fraction, and are classified as non-fiber carbohydrates (NFC; Arthington et al., 2002). The NFC fraction is similar to the NSC. It includes all carbohydrates not included in NDF, but also includes pectins, which are not classified as part of the NSC fraction (NRC, 2001). Compared to NDF, pectins are rapidly and extensively digested in the rumen, as reported by Ben-Ghedalia et al. (1989), where the apparent digestibility was 98.7 % for pectin and 79.4 % for cell wall constituents by sheep fed high-pectin based feed. Although the digestibility of pectins and NSC components is similar, there are some major differences between them. When pectin escapes ruminal fermentation, it cannot be digested in the small intestine, whereas NSC can be digested. Further, when pectins are fermented in the rumen, they yield greater acetate:propionate ratios compared to NSC fermentation.

Energy supplementation is used to correct deficiencies in order to maintain or enhance animal performance. A variety of different feedstuffs can be used as energy sources in ruminant diets. Supplements with low NSC concentration are a viable alternative to substitute traditional and costly supplements with high NSC concentration. When designing and formulating a supplementation program, the source of energy should be carefully chosen, since the differences in energy fermentation and digestion among carbohydrate sources may impact animal performance.

Molasses-Based Supplements

According to Curtin (1983), the term molasses referred specifically to the final effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugarcane or sugar beets. Nowadays, current feed nomenclature characterizes molasses as any liquid feedstuff containing more than 43 % sugar (Curtin, 1983). Sugarcane molasses is a by-product of the sugarcane refining process to obtain sucrose, and must not contain less than 46 % total sugar expressed as invert. Invert is a 50:50 mixture of glucose and fructose, obtained when sucrose is hydrolyzed. If molasses moisture content exceeds 27 %, its density is determined by double dilution and must not be less than 79.5° Brix (Curtin, 1983). The industry uses the term Brix as a measurement of molasses total solid content. This term originally indicated the concentration of sucrose in pure sucrose solutions on a weight basis, but molasses also contains glucose, fructose, raffinose and numerous non-sugar organic materials (Curtin, 1983). Therefore, the Brix value for molasses often will differ from actual sugar or total solid content and will represent nothing more than a number denoting specific gravity, which cannot be related to either sucrose or DM content (Curtin, 1983).

The chemical composition of sugarcane molasses varies widely, similar to that observed in many other industrial by-products. It is influenced by several factors such as soil type, temperature, moisture, season of production, plant variety, production practices at a particular processing plant, and by storage variables (Curtin, 1983). These factors lead to significant variations in nutrient content, flavor, coloration, viscosity and total sugar content (Curtin, 1983).

Sugarcane molasses contains large quantities of simple sugars and other carbohydrates, contributing to its high-energy value. Molasses contains around 88 % of the TDN of corn (72 vs. 82 % on a DM basis, respectively), and the principal sugar is sucrose, constituting around 30 to 40 % of molasses on an as-fed basis (Austin, 2003). In contrast to energy, sugarcane molasses is low in CP content, and its nitrogenous material mainly consists of non-protein compounds. However, CP content can vary according to soil type as reported by Chapman et al. (1965), where molasses produced from sugarcane grown on soils high in organic matter contained 7 to 10 % of CP compared to 3 % for molasses from sugarcane grown on mineral rich soils. Regarding mineral content, when compared to other energy sources such as cereal grains, sugarcane molasses is relatively high in calcium, potassium, magnesium, sodium, chlorine and sulfur, but low in phosphorus (Curtin, 1983).

According to Kunkle et al. (1995), blackstrap molasses is the by-product of the sugar industries in south Florida and Louisiana. Most Florida sugarcane is grown on organic rich soils, resulting in a molasses with greater protein content than molasses derived from sugarcane grown on soils with low organic matter (Kunkle et al., 1995). Molasses production is increased during fall and winter, and its price is determined by the

world market. More than 500,000 tons of molasses are produced annually in south Florida and this makes the state a net exporter of the product (Kunkle et al., 1995). Blackstrap molasses typically contains 22 % moisture, 60 % TDN and 7 % CP on an as-fed basis (Kunkle et al., 1995). Molasses slurries are common on many Florida ranches. Slurries are created by blending molasses with dry ingredients, typically protein sources such as cottonseed meal or feather meal.

Therefore, molasses-based supplements are classified as energetic supplements, containing high quantities of NSC derived from its high sugar content.

Supplementing Molasses to Forage-Based Diets

A beneficial use for molasses is its addition to diets based on low quality forages or roughages, providing a readily available source of energy. However, molasses does not contain adequate protein to meet the CP requirements of cattle fed low quality forages; consequently a source of supplemental protein is often required.

Feeding molasses has been studied extensively over the past century. Pate (1983) compiled the results of several studies where molasses was supplemented to beef cattle diets based on forage. Supplementation usually resulted in decreased forage intake, but total DMI was increased. Animal performance was consequently increased, but not compared to when supplements based on other energy sources, such as cereal grains, were fed. However, when plant protein was added as a nitrogen source, molasses-based supplements were similar to corn-based supplements in terms of BW gain by beef cattle.

More recently, a review reported the effects of liquid supplements on the performance of cattle fed forage-based diets (Moore et al., 1995). Generally, BW gain was increased but responses were variable, and no consistent relationship between rates of supplementation and gain were observed. When molasses was the only ingredient of

the supplement, gains were sometimes decreased and at other times increased. However, when a major protein source was added, BW gains increased considerably. Supplementation of molasses only increased forage consumption when intake of forage was less than 1.75 % of BW without supplement; otherwise forage intake was decreased.

A recent study compared sources and rates of energy supplementation for yearling cattle fed ammoniated hay (Royes et al., 2001). The authors compared supplements based on sugarcane molasses, corn or soybean hulls, and reported reduced feed efficiency and consequent less BW gain for animals fed molasses compared to the other supplements, supporting the findings reported by Pate (1983).

Several authors reported that feeding cattle diets containing high quantities of sucrose or molasses resulted in a increase in the molar percentage of butyric acid in the rumen (Owen et al., 1967; Kellogg and Owen, 1969; Hatch and Beeson, 1972). In addition, Reyes (1974) and Silvestre et al. (1977) also noted a substantial increase in the molar percentage of butyric acid in the rumen of cattle fed increasing quantities of molasses. Olbrich and Wayman (1972) compared the molar percentage of butyric acid in the ruminal fluid of steers fed either molasses or corn, and reported a greater concentration for the animals fed molasses. Based on these reports, feeding molasses to cattle increases the molar percentage of butyric acid in the rumen, which usually is at the expense of propionic acid when molasses substitutes grain or at the expense of acetic acid when molasses is fed as a supplement in forage-based diets (Pate, 1983). This variation in VFA profile may be related to differences in ruminal microbial population. Ruminal protozoa population has been positively correlated with butyrate synthesis in the rumen (Hristov et al., 2001), whereas defaunation decreased the concentrations of butyrate in the

ruminal environment (Mendoza et al., 1993; Faciola et al., 2004). The inclusion of 35 % of molasses in beef cattle diets increased the ruminal protozoa population (Foreman and Herman, 1953). Therefore, an increased protozoa population in the rumen might be one of the causes to the greater butyrate synthesis observed in cattle fed with molasses-containing diets.

Most of the available information about the effects of VFA proportion on energy metabolism involves acetate:propionate ratios, which are negatively correlated to energy utilization and consequent animal performance. Given that butyrate, like acetate, is not a glucose precursor, a similar effect can be attributed to butyrate:propionate ratios. This could explain why animals fed molasses-based supplements typically have reduced performance compared to animals fed grain-based supplements (Pate, 1983).

In summary, although molasses supplementation usually has a negative impact on forage DMI, overall performance is often improved by supplementing molasses to cattle fed energy deficient diets. Further, molasses also can be used as an effective carrier to deliver other nutrients, such as protein, vitamins and minerals.

Citrus Pulp-Based Supplements

Florida produces more than 80 % of the total United States supply of citrus, and almost 90 % of this production is processed into juice (Hodges et al., 2001). Citrus pulp is the residue from citrus processing, and consists of a mixture of peel, rag and seeds. Annually, the Florida citrus industry produces more than 1 million tons of citrus pulp (Hodges et al., 2001) and most of this total has been exported to foreign countries throughout the past years, causing local prices to increase, and resulting in a decrease in domestic usage. However, recent declines in exports have made citrus pulp an affordable by-product feed for ruminants in the United States (Arthington and Pate, 2001).

Citrus pulp has been fed to ruminants since the beginning of 20th century, when it was suggested to have a potential value as a feedstuff. Walker (1917) made the initial observation that cattle readily consumed a mix of fresh citrus pulp and cull fruits, but the feed often spoiled before animals consumed it all. In the 1930s, dried citrus pulp began to be produced commercially after Scott (1926) dehydrated grapefruit and fed it to dairy cattle. Since then, the production and availability of dry citrus pulp has increased steadily (Arthington et al., 2002). The drying procedure consists of grinding or chopping and then dehydrating the fresh fruit residue. To aid in the dehydration process, calcium oxide or calcium hydroxide is mixed in the process (Arthington et al., 2002).

Citrus pulp can be fed wet, as a dry meal, or in dry pellets. The wet product is usually shipped directly from the juice plant, arranged as piles in the pastures and offered to cattle. Arthington and Pate (2001) suggested that it is typically uneconomical to feed wet pulp because of its high wasting rate (up to 30 %) and shipping costs associated to its low DM content (15 to 20 %). Cattle consume citrus pulp in either the wet or dry form, but dry citrus pulp is more easily transported and handled (Arthington et al., 2002). Dry citrus pulp is a bulky feedstuff, having a density of approximately 303 kg/m³. For this reason and because of economic and transport limitations, pelleting dry citrus pulp is favorable since it increases density, handling efficiency, and decreases dustiness and bridging in storage bins (Arthington et al., 2002).

Ammerman et al. (1967) compared performance of steers fed nonpelleted and pelleted citrus pulp added at different concentrations within the supplement. When fed at 66 % of the total supplement, steers fed pelleted citrus pulp gained more weight (1.38 kg/d) compared to those fed the nonpelleted pulp (1.19 kg/d). The authors attributed the

differences to the greater density of the pelleted form (211 to 374 kg/m³ for nonpelleted and pelleted form, respectively), increasing its consumption by the steers. A similar study conducted with lambs also indicated that feeding pelleted citrus pulp increased intake and animal performance (Loggins et al., 1964). Lambs fed pellets had numerical greater BW gain (0.12 kg/d) when compared to lambs fed the nonpelleted form (0.11 kg/d) and also had slightly greater intake (0.06 kg/d). Pinzon and Wing (1976) reported no differences in the ruminal pH, ruminal ammonia concentration, blood urea nitrogen concentration, and VFA concentration in the rumen of steers fed pelleted or nonpelleted citrus pulp at similar rates.

Chemical composition and physical characteristics of citrus pulp mostly depends on the type of fruit and processing methods used to produce it (Arthington et al., 2002). The same authors compared the nutritional value of samples collected in two different studies (Ammerman et al., 1968; Arosemena et al., 1995) and tabular values reported by NRC (1996). The authors concluded that regardless of some variations among the three sources - attributed to production site, citrus variety and proportion of seeds and peel - the nutritional values were similar.

According to the NRC (1996), citrus pulp usually contains 82 % TDN and 6 to 7 % CP (DM basis). Calcium content is particularly high in citrus pulp because of the addition of calcium salts to aid in the drying process. The carbohydrate fraction of citrus pulp is mainly composed of 25 to 44 % pectin, 12 to 40 % sugar and 1 % or less starch on a DM basis (Hall, 2000). Compared with common cereal grains such as corn, citrus pulp contains moderate amounts of NSC (sugars and starch) and high concentration of pectins. For that reason, citrus pulp contains a different carbohydrate profile compared to

traditional energy feeds. Citrus pulp-based supplements are classified as energy supplements, and despite the modest content of NSC, digestibility is great because of the large concentration of pectins.

Supplementing Citrus Pulp to Forage-Based Diets

Several studies have been conducted to investigate citrus pulp and subsequent pectin fermentation in the ruminal environment. Welch and Smith (1971) compared rumination patterns when sheep and cattle were fed pelletized citrus pulp, beet pulp or alfalfa meal in addition to mixed hay. Cell wall content was 27, 50, 48, and 68 % of DM for citrus pulp, beet pulp, alfalfa meal, and mixed hay, respectively. Citrus pulp decreased rumination time for sheep and cattle when compared to mixed hay fed alone (0.34 vs. 0.16 min/g of DM consumed for mixed hay and citrus pulp, respectively). However, when data were analyzed per unit of cell wall content, no difference in rumination time was found (0.50 vs. 0.56 min/g of cell wall content consumed for mixed hay and citrus pulp, respectively). Alternatively, beet pulp and alfalfa meal reduced rumination time when data were analyzed as total DM or cell wall content consumed. The authors attributed these effects to the lack of physical form required to stimulate rumination in beet pulp and alfalfa meal, which were greater in cell wall content compared to citrus pulp. The beef pulp and alfalfa meal were finely ground, and consequently did not contribute substantially to the rumination process. The authors concluded that addition of citrus pulp to forage-based diets did not influence rumination process and time, despite its low cell wall content compared to forages. Fiber digestibility increased when citrus pulp was added to sheep diets in a dose-dependent pattern (Sudweeks, 1977). Strobel and Russell (1986) added different sources of carbohydrate to cultures of ruminal bacteria and measured the fermentation process and products. The authors reported that carbohydrate

fermentation was decreased when pH became more acidic, particularly when pectin was the major carbohydrate source. Pectin utilization decreased by 53 % when culture pH dropped from 6.7 to 6.0, indicating that organisms that degrade pectins are pH sensitive. At neutral culture pH (around 6.7), the authors reported that addition of starch and sucrose increased lactic acid production, whereas little lactic acid was produced when pectin was added. When culture pH was lowered to 5.5, lactate production quadrupled for the starch, doubled for sucrose and did not change for pectin. Acetic acid production was always doubled for pectin compared to all other carbohydrates, even when pH dropped below 6.0. Therefore citrus pulp may be less likely to cause lactic acidosis and consequential impaired fiber digestibility when compared to these other feedstuffs high in NSC. Several other researches also have reported that feeding citrus pulp increased the concentrations of acetic acid in the rumen and also increased the acetic to propionic acid ratio when compared to traditional energy sources such as cereal grains (Loggins et al., 1964; Hentges et al., 1966; Drude et al., 1971). Although greater ruminal propionate production usually was associated with high concentrate diets and negatively associated with high forage diets, when forage (corn silage) was replaced with increasing amounts of citrus pulp (0 %, 33 % 67 %, and 100 %) in steers diets, only a moderate decrease in the ruminal pH (7.12, 6.97, 6.81, and 6.76, respectively) was reported (Schaibly and Wing, 1974). Furthermore, acetate synthesis was the greatest and propionate was the poorest when diets contained 100 % citrus pulp, even if compared to 100 % corn silage diets. The authors concluded that citrus pulp, though not considered a forage in the strictest sense, does contain some forage-like properties which promote less acidic ruminal pH values. All these data imply that citrus pulp, rich in pectin, can have less

detrimental effects on ruminal pH and fermentation products compared to other energy sources, such as high NSC containing feedstuffs.

Research was conducted in the 1950s and 1960s to investigate the impact of supplementing citrus pulp on the performance of beef cattle fed forage-based diets. Chapman et al. (1953) reported no significant differences in gain or feed efficiency when steers grazing St. Augustine pasture were supplemented with 2.3 kg/d of citrus pulp or snapped corn, or had free-choice access to sugarcane molasses. But in a similar study, steers on pasture fed citrus pulp tended to have greater gains (0.78 kg/d) compared to steers fed ground snapped corn (0.72 kg/d), sugarcane molasses (0.65 kg/d), or a mixture of corn, citrus pulp and cottonseed meal (0.75 kg/d) (Chapman et al., 1961).

Research resulting from several studies indicated positive results from feeding citrus pulp as an energy source to feedlot animals. Kirk and Davis (1954) fed steers for 120 d with either ground snapped corn or citrus pulp and reported similar gains (1.08 and 0.99 kg/d, respectively), but animals fed citrus pulp had greater feed efficiency (5.40 and 4.70 kg of required TDN / kg of BW gain, respectively). Peacock and Kirk (1959) conducted three 140 d trials in which steers were fed either corn or citrus pulp at 70 % of the total diet, and did not detect differences in gain or feed efficiency. Ammerman et al. (1963) replaced ground corn with citrus pulp at 22, 44 and 66 % of the total diet of feedlot steers and did not find differences in gain across treatments, but gains were numerically greater for the 22 and 44 % inclusion rate (1.35 and 1.48 kg of ADG, respectively). Kirk and Roger (1970) published a review that contained 23 separate trials conducted at the University of Florida – Range Cattle Research and Education Center in Ona, Florida, which used citrus products in beef finishing diets. Citrus pulp was the main

energy source in 20 of these studies. The authors concluded that the amount of citrus pulp fed was positively associated with feedlot performance.

Research demonstrates that citrus pulp is a good energy source for ruminants, particularly for animals fed forage-based diets, since it does not seem to interfere with fiber digestion and rumen health. With a large citrus industry in Florida, citrus pulp may provide an economical advantage for producers in the southeastern portion of the U.S.

Energy Metabolism

According to an extensive review by Bergman (1990), the VFAs, also known as short-chain fatty acids, are mainly produced in the rumen by microbial fermentation of carbohydrates, and contribute approximately 70 % to the caloric requirements of ruminants. The major VFAs are acetate, propionate and butyrate, produced in a ratio varying from approximately 75:15:10 to 40:40:20 (acetate:propionate:butyrate). After synthesis, VFAs are absorbed across the ruminal epithelium, from which they are either carried by ruminal veins to the portal vein and hence through the liver or are retained and metabolized for energy by the ruminal epithelial cells (Bergman, 1990). Acetate and butyrate are oxidized to produce energy whereas propionate is reserved for gluconeogenesis. Approximately 90 % of the butyrate formed in the rumen is retained by epithelial tissue and converted to ketone bodies or carbon dioxide whereas the remainder is removed by the liver (Bergman, 1990). Little acetate – approximately 30 % - is retained in the rumen compared to butyrate (90 %). Most of the acetate is oxidized throughout body tissues to generate ATP. Acetate also is the major source of acetyl CoA for lipogenesis (Bergman, 1990). Propionate is 50 % retained in the rumen and the remainder is removed from portal blood by the liver, where it serves as a major substrate for gluconeogenesis (Bergman, 1990). This process is essential to glucose status of the

ruminant animal because almost all dietary glucose is fermented in the rumen and does not reach the small intestine for absorption. In summary, propionate is directed to glucose synthesis whereas acetate and butyrate are oxidized for energy generation, but butyrate is highly retained in the rumen and poorly contributes as an energy source for other tissues.

Although acetate is the largest single energy source absorbed through the portal system, accounting for more than half of the total energy absorbed as VFA (Bergman et al., 1990; Baird et al., 1975), it is inefficiently utilized as an energy source by tissues when compared to glucose, which is effectively synthesized from propionate in the liver. Glucose is crucial for maintenance and productive functions in ruminants such as growing and lactating cattle (Reynolds, 2005), and propionate accounts for up to 76 % of the glucose synthesized in the liver (Reynolds et al., 1994). Ruminants eating forage-based diets depend on liver synthesis of glucose to meet their metabolic requirements (Huntington, 1997), and increased liver glucose synthesis is positively associated with energy balance and animal performance. Research by Aiello et al. (1989) also reported detrimental effects of butyrate on VFA metabolism by restraining hepatic propionate utilization, thus inhibiting the conversion of propionate to glucose. In addition, the inhibition is specific and not due to a general competition among VFAs for metabolism. High energy diets and supplements selected to enhance animal performance are typically associated with increased VFA production, particularly propionate, leading to increased glucose synthesis. Even when these are based on ingredients that are not propiogenic, such as citrus pulp or molasses, overall formation of propionate is increased in the rumen as a consequence of increased total VFA production.

Insulin is a hormone synthetized by beta-cells in the Islets of Langerhans in the pancreas, and primarly regulates glucose uptake by cells (Austgen et al., 2003). This hormone also has an important effect on lipogenesis and cellular uptake of amino acids and some electrolytes (Austgen et al., 2003). Although there always is a low rate of insulin secretion in the pancreas, the amount secreted into the blood increases as blood concentrations of glucose rises, and similarly decreases as blood concentrations of glucose falls. Therefore, insulin is important for body homeostasis because it maintains blood glucose under constant concentrations and also has an anabolic role because it increases cellular uptake of nutrients. The mechanism by which insulin promotes glucose uptake by cells consists in the activation of insulin receptors embedded in the plasma membrane, which regulates the number and operation of protein molecules also in the plasma membrane that transport glucose into the cell (Austgen et al., 2003). Once inside the cells, glucose enters glycolysis and the respiratory cycle to generate ATP molecules.

Insulin-like growth factor I is a protein hormone that resembles insulin on its structure, and is mainly synthetized by the liver in response to GH (Gluckman et al., 1987). However, several studies in cattle demonstrated that IGF-I concentrations in blood were positively correlated to feed intake (Bossis et al., 1999; Armstrong et al., 2001; Rausch et al., 2002) and blood insulin concentration (Keisler and Lucy, 1996; Webb et al., 2004). The IGF-I molecule binds to specific receptors present on many cell types in several tissues and has an anabolic function promoting cell growth and multiplication (Quin, 1992).

Growth hormone, also known as somatotropin, is a protein hormone synthesized and secreted by cells called somatotrophs in the anterior pituitary (Austgen et al., 2003).

It assists in the control of several physiologic processes, particularly stimulating anabolic processes such as cell growth and multiplication. Growth hormone can stimulate tissue growth by two different mechanisms; 1) direct effects by binding with receptors on target cells, or 2) indirect effects by stimulating IGF-I secretion by the liver. The majority of the growth-promoting effects of GH are actually due to IGF-I acting on target cells, especially in muscle and bone (Austgen et al., 2003). Growth hormone also affects nutrient metabolism since it stimulates protein anabolism in tissues, enhances the utilization of fat by stimulating triglyceride oxidation in adipocytes, and also is one of a series of hormones that serves to maintain blood glucose within a normal range (Austgen et al., 2003). Synthesis and secretion of GH are regulated primarily by two well-characterized hypothalamic peptides: stimulated by GHRH or inhibited by somatostatin. In addition, GH synthesis and secretion also are regulated by IGF-I concentrations in blood. Increased concentrations of IGF-I in blood decrease the secretion of GH by directly suppressing its synthesis in the somatotrophs and by stimulating the release of somatostatin from the hypothalamus (Austgen et al., 2003). Since insulin concentration and feed intake are positively correlated with IGF-I, they consequently are negatively associated with GH secretion and concentrations in blood. The integration of all the factors that affect GH synthesis and secretion lead to a pulsatile pattern of release of this hormone (Austgen et al., 2003). Studies with beef cattle demonstrated that frequency of GH pulses is not affected, but pulse height, amplitude and overall GH concentration in blood are inversely correlated with energy intake (Nolan et al., 1990; Hornick et al., 1998; Bossis et al., 1999).

Energy Metabolism and Beef Cattle Performance

Feed intake, blood metabolites associated with nutritional status such as the glucose/insulin/IGF-I/GH axis, and consequent animal performance are usually associated in cattle. Research by Vizcarra et al. (1998) analyzed the influence of BW gain on concentrations of blood glucose and insulin in primiparous beef cows. The authors fed cows in order to attain gains of 0.45 or 0.90 kg/d and reported that animals under a higher plane of nutrition had greater concentrations of glucose and insulin in blood. In a similar study with beef steers, blood glucose, insulin, and IGF-I typically increased with rate of gain (Hersom et al., 2004). Animals fed to 60, 100 or 160 % of maintenance energy and protein requirements also had increased blood concentrations of glucose, insulin and IGF-I when feeding level increased (Lapierre et al., 2000). Ellenberger et al. (1989) compared serum concentration of glucose, IGF-I and GH in steers fed either ad libitum gaining 1.40 kg/d, or restricted amounts gaining 0.37 kg/d. Mean serum concentrations of GH were elevated (45.6 vs. 23.4 ng/mL) and serum concentrations of IGF-I were decreased (108 vs. 167 ng/mL) for feed-restricted steers. Bossis et al. (1999) compared blood concentrations of glucose, insulin, IGF-I and also blood concentration, pulse frequency and pulse amplitude of GH from heifers fed either to maintain body condition score or restricted diets to induce negative energy balance. Decreased blood glucose (58.7 vs. 71.5 mg/dL), insulin (0.95 vs. 1.85 ng/mL), and IGF-I (15.35 vs. 94.1 ng/mL) concentrations but increased GH concentration (38.7 vs. 12.1 ng/mL) and pulse amplitude (36.3 vs. 17.6 ng/mL) for feed-restricted heifers were noted. Pulse frequency of GH did not change between groups.

It can be concluded that animal performance is associated with energy intake and metabolism. There is a direct relationship among diet and blood concentrations of

glucose, insulin, IGF-I and GH, and these metabolites also have direct effects on each other. Additionally, blood concentration of IGF-I is typically associated with BW gain and therefore, should be maximized.

Energy and Nitrogen Synchronization in the Rumen

Ruminants have a high rate of ammonia absorption from the rumen, which like peptides and amino acids, is a product of dietary protein degradation by the microbial population in the rumen (Huntington, 1990). Ammonia is a major precursor for microbial protein synthesis, and its excess is absorbed by ruminal epithelium and transported mostly to the liver. In liver, ammonia is converted into urea, which can be re-utilized as a nitrogen source in the rumen via saliva or circulatory system (blood urea nitrogen; BUN), or be excreted in urine by the kidneys. Hepatic ammonia metabolism and renal excretion of urea require energy. Therefore, elevated amounts of ammonia produced in and absorbed by the rumen increase the animal's requirement for energy (Huntington, 1990).

Microbial protein synthesis is optimal when both energy and nitrogen sources are available in the rumen (Hammond, 1997). Diets rich in protein content but lacking either in energy availability or synchrony between ruminal energy and nitrogen supplies may limit the utilization of available nitrogen by ruminal microorganisms (Huntington, 1997), and also increase BUN concentrations (Santos, 2004). Alternatively, diets with inadequate protein concentrations limit ruminal microbial growth and compromise fiber digestion. Therefore, to increase efficiency of nitrogen utilization in the rumen and consequent microbial protein synthesis, energy and nitrogen should be available in the rumen in a coordinated pattern. Ruminal degradability and digestibility of dietary carbohydrates and protein differ among feedstuffs (NRC, 2001) and these differences may influence microbial fermentation and protein synthesis in the rumen. Diets or

supplements containing highly degradable energy sources, such as high NSC feedstuffs, should also contain protein sources with high rumen degradability in order to grant a synchronized availability of energy and nitrogen to the microbial population in the rumen. Conversely, when fibrous ingredients such as citrus pulp are the main energy source, protein sources with lower degradation rates should be added.

Several studies examined the effects of synchronizing energy and nitrogen supplies in the rumen. When steers were infused with starch and casein in their rumen or abomasum, they had reduced ammonia absorption and increased nitrogen retention when both nutrients were delivered in the rumen (Taniguchi et al., 1995). In research with lambs, Matras et al. (1991) fed three different sources of energy varying in ruminal degradability (barley > steam-flaked corn > dry rolled sorghum) combined with three different sources of protein also varying in ruminal degradation rates (urea > 50:25:25 of urea, blood meal and corn gluten meal > 50:50 blood meal and corn gluten meal) in a 3 x 3 Latin square design. Nitrogen utilization was most efficient when rapidly degraded sources (barley and urea) and slowly degraded sources (sorghum and blood + corn gluten meal) of energy and protein were fed together. The authors concluded that it was advantageous to feed animals sources of protein and energy containing similar ruminal degradation rates. Spicer et al. (1986) fed feed ingredients differing in ruminal degradation rates to beef steers (barley > corn > sorghum) and reported that microbial protein synthesis and efficiency of nitrogen utilization was positively associated with protein and carbohydrate degradability of the feedstuff. Studies with dairy cattle indicated that synchronizing ruminal fermentability of carbohydrate and protein sources increased nitrogen utilization and consequent outflow of bacterial protein from the rumen. Herrera-

Saldana et al. (1990) fed lactating dairy cows barley or milo as energy sources (90.5 vs. 70.5 % of ruminal carbohydrate degradability, respectively) and cottonseed meal or brewers dried grains as protein sources (56.6 vs. 38 % of ruminal protein degradability, respectively). The authors reported increased microbial protein synthesis when animals were fed synchronized diets, particularly diets with highly degradable ingredients, which resulted in increased milk production. In a similar study, Aldrich et al. (1993) fed lactating dairy cows four diets formulated for high and low ruminal degradability of carbohydrate and protein. The authors reported that passage of bacterial N to the small intestine was the greatest (262 g/d) when highly ruminally-degradable nonstructural carbohydrate was combined with high rumen-available protein, and was the least when high rumen-available nonstructural carbohydrate was combined with low rumen-available protein (214 g/d).

Monitoring BUN is a technique that can be used for measuring protein and energy status in cattle and is highly correlated with the amount of ammonia produced and absorbed in the rumen (Hammond, 1997). Therefore, in healthy ruminants, BUN concentrations are indicative of the protein to energy ratio in the diet, and furthermore, synchrony between dietary energy and protein degradability in the rumen. Little work has been reported in beef cattle with regard to the influence of ruminal energy and nitrogen synchronization with BUN concentrations. Most of the work analyzed the effects of dietary protein concentration or degradability. Hammond (1983) fed steers isocaloric diets having three concentrations of CP and reported that BUN concentrations increased from 2.6 to 11.1 mg/dL as dietary CP increased from 6 to 18 % (DM basis). Burris et al. (1975) fed steers isocaloric and isonitrogenous corn-cottonseed hull based diets

supplemented with either urea or soybean meal. Urea increased BUN values (6.7 vs. 4.8 mg/dL). Similar to the studies examining ruminal synchrony of energy and nitrogen, Chase et al. (1993) fed bulls diets formulated to provide 75 or 150 % of maintenance energy requirements but equal CP intake. The concentrations of BUN averaged 5.6 mg/dL in bulls fed the highest energy diet and 19.7 mg/dL at the lowest energy intake, demonstrating that increasing dietary energy intake, while holding protein intake constant, decreased BUN concentrations. In other studies with beef cattle, BUN concentrations between 11 and 15 mg/dL were associated with maximum rates of gain for growing animals (Byers and Moxon, 1980) and concentrations of BUN of 7 to 8 mg/dL were associated with maximum performance of finishing steers (Preston et al., 1978) or mature cows (Hammond, 1997). The variance in BUN concentration reported in these studies may be related to the different protein requirements for each animal category. Optimal concentrations of BUN can be associated with maximized microbial protein synthesis, leading to increased performances. Concentrations that are less than these optimal values may indicate a deficiency in dietary protein relative to the energy intake, while excessive values of BUN may indicate unnecessary dietary protein and/or lack of either energy availability or synchrony between ruminal energy and nitrogen supplies, leading to increased ammonia formation and absorption in the rumen.

Nutrition and Reproduction in Beef Cattle

The primary nutrient consideration for optimum reproductive performance in beef cattle, particularly females, is energy (Mass, 1987). Inadequate energy intake is correlated to decreased reproductive performance, delayed onset of puberty, extended postpartum anestrous and decreased conception and pregnancy rates in cattle (Santos, 2004). Therefore, one important goal for beef cattle operations, particularly cow-calf

enterprises in Florida, is the development of nutrition programs that emphasize energy supplementation to maintain or enhance the reproductive efficiency of the herd.

Onset of Puberty in Beef Heifers

Age at puberty is a major determinant of lifetime reproductive performance of beef cows, and is defined as the age at which heifers have their first ovulation and consequential luteal phase (Santos, 2004). Sexual maturity is defined as the age at which animals reach their maximum reproductive potential and are definitely able to conceive and gestate. In cattle, sexual maturity is associated with onset of puberty, and heifers typically attain sexual maturity around three or four normal estrous cycles (around 60 to 90 d) after the onset of puberty (Santos, 2004). One of the most important goals for cow-calf operations is to develop heifers so that they are able to conceive at 14 to 16 mo of age and calve at approximately 2 yr of age (Schillo et al., 1992). Heifers should also experience two or three estrous cycles before the onset of the breeding season in order to be sexually mature. For that reason, replacement heifers should attain puberty around 12 mo of age to achieve optimal reproductive performance during their first breeding season (Schillo et al., 1992). Heifers that conceive early in their first breeding season and therefore calve early in their first calving season wean more and heavier calves during their productive lives (Lesmeister et al., 1973).

Age at puberty is directly associated with BW, body composition, and breed type. Furthermore, heifers with greater energy intake and consequent greater BW gain attain puberty earlier (Schillo et al., 1992). The onset of puberty seems to be associated with increased frequency and amplitude of LH pulses that stimulates development of ovarian follicles to the preovulatory stages. These LH pulses are clearly associated with nutritional status (Schillo, 1992). As explained previously, energy intake increases VFA

production in the rumen, particularly propionate, which increases glucose synthesis and consequent insulin and IGF-I secretion. These hormones and metabolites appear to increase gonadotropin secretion (LH and FSH) by influencing hypothalamic-hypophyseal secretory activity (Butler and Smith, 1989; Schillo et al., 1992), and also appear to amplify the effects of these hormones in ovarian cells (Spicer and Echternkamp, 1995). The onset of puberty is a gradual process. During the pre-pubertal period, ovarian follicles secrete basal quantities of estrogens which inhibit LH secretion. This negative feedback declines as puberty approaches due to increased pulsatile secretion of LH (Day et al., 1984). An increased nutritional plane may decrease the sensitivity to the negative feedback from estradiol and enhance concentrations of LH released (Foster, 1988).

In summary, the exact mechanism by which a higher plane of nutrition anticipates puberty is not well known, but the increase in the availability of metabolites and hormones (glucose, insulin, and IGF-I) that may enhance gonadotropin secretion/activity and decrease the sensitivity of the hypothalamic-hypophyseal tissues to the negative feedback from estrogens may be potential explanations.

Reproductive Performance in Postpartum Beef Cows

Postpartum interval, characterized as the period from parturition to resumption of estrus, largely determines the likelihood of cows becoming pregnant during the breeding season (Wiltbank, 1970). Calves derived from cows that faced long postpartum intervals tend to be younger and lighter at weaning (Ferrell, 1991). Therefore, decreased postpartum interval is a major goal to be attained in cow-calf enterprises in order to increase the efficiency of the operation.

The mechanisms associated with the acquisition and maintenance of reproductive capability in postpartum cows results from functional integration of the hypothalamic-

hypophyseal-ovarian axis (Hess et al., 2005). During late gestation, elevated production of steroids by placenta and maternal ovaries, particularly estradiol and progesterone (P4), exerts intense negative feedback on the hypothalamus, decreasing the release of GnRH (Short et al., 1990; Lucy, 2003) and therefore depleting hypophyseal reserves of LH (Wettemann et al., 2003). Despite the relatively fast restoration of LH supply in the hypophysis after calving, postpartum anestrous is sustained because of the sensitivity to negative feedback effects from estradiol (Short et al., 1990). Postpartum anestrous can be even prolonged due to the negative effects of calf-nursing (Williams, 1990) and severe negative energy balance on the amplitude and frequency of LH secretion (Schillo, 1992). During the early postpartum period, when the requisites for energy increase primarily due to lactation, energy intake often does not satisfy energy requirements. Consequently, decreased blood concentration of glucose, insulin, IGF-I, P4 and also GnRH and LH pulse frequency are observed (Santos, 2004). The mechanism by which energy affects GnRH and gonadotropins secretion is yet unknown, but blood concentrations of glucose, insulin and IGF-I seem to mediate this process. Glucose is the primary metabolic fuel for the central nervous system, and low glucose concentrations appear to reduce hypothalamic secretion of GnRH (Wettemann et al., 2003). Due to its impact in glucose metabolism, insulin may be an important link between nutritional and reproductive processes. Insulin combined with glucose may stimulate GnRH release from the hypothalamus (Arias et al., 1992). Insulin mediates follicular dynamics (Webb et al., 2004), stimulates cell proliferation and steroid synthesis in the ovary (Wettemann and Bossis, 2000) and also facilitates hepatic production of IGF-I (Keisler and Lucy, 1996; Webb et al., 2004). Blood concentrations of IGF-I were increased for beef cows that

resumed estrous cycles early postpartum, but not for those that remained in anestrous (Roberts et al., 1997). Other studies also reported the presence of IGF-I receptors in the ovaries and dominant follicles of beef cows (Armstrong and Benoit, 1996; Bao and Garverick, 1998). Therefore, IGF-I may be a positive signal to the hypothalamo-hypophyseal-ovarian axis to resume normal functionality after parturition.

Feed Intake and Reproductive Performance in Cattle

The endocrine events that lead to resumption of luteal activity in postpartum cows and the attainment of puberty in heifers are similar (Looper et al., 2003). Elevations in blood P4 concentrations were detected during the period that immediately preceded puberty in heifers (Gonzalez-Padilla et al., 1975), and this P4 is originated from ovaries (Berardinelli et al., 1979), specifically from compact luteal tissues embedded within the ovary. The same pattern was observed in postpartum beef cows, since first ovulation after parturition was usually followed by a temporary increase (4 to 5 d of duration) in P4 (Williams and Ray, 1980; Perry et al., 1991). The increase in concentrations of P4 appears to be a necessary factor for the resumption of normal estrous cycles. Looper et al. (2003) indicated that 81 % of cows that had a rise in P4 before first estrus had normal length estrous cycles (19 to 23 d) whereas only 36 % of cows that did not have increased P4 had normal estrous cycles. The rise in P4 before the onset of puberty in heifers and first estrus in cows may have a role in the endocrinal changes leading to the establishment of gonadotropins and gonadal hormone secretions, preparing the reproductive system to attain normal and fertile estrous cycles (Kinder et al., 1987).

Circulating concentration of P4 is also critical for the onset and maintenance of pregnancy, especially during the early phases (Spencer and Bazer, 2002). Progesterone prepares the uterine environment for gestation, and also inhibits the release of hormones

that may disrupt pregnancy. In cattle, blood concentration of P4 before or after breeding has been associated with conception rates. Conception rates were influenced by plasma concentrations of P4 before artificial insemination in dairy cows (Folman et al., 1990). Cows were estrus synchronized by two injections of PG, and cows with P4 concentration below 5 ng/mL 2 d before second PG administration had conception rates of 36 %, whereas cows with concentration of P4 above 5 ng/mL had conception rates of 75 %. Xu et al. (1997) also synchronized estrous in lactating dairy cows with two treatments of PG 13 d apart, but half of the animals were supplemented with exogenous P4 for 5 d before second injection. The authors reported improved estrous response (89.6 vs. 82.9 %) and conception rate from first AI (65.1 vs. 59.7 %) for animals that received exogenous P4. Robinson et al. (1989) supplemented dairy cows with exogenous P4 between d 5 to 12 or d 10 to 17 after insemination, and detected increased pregnancy rates compared to untreated cows (60 to 30 %). These findings are also supported by several other studies (Wiltbank et al., 1956; Folman et al., 1973; Fonseca et al., 1983), validating the importance of P4 for the onset and maintenance of pregnancy, and subsequent conception rates.

Several studies with dairy cattle reported that high feed intake may decrease circulating P4 concentrations, and therefore impair reproductive efficiency. Vasconcelos et al. (2003) suggested that feeding pattern may alter blood P4 concentration in pregnant lactating Holstein cows. In this study, feed was withheld 12 h before the experiment started and then cows were provided with either 100 % of a total mixed ration (TMR) at once, half of TMR every 12 h, a quarter of TMR every 6 h, or leaving animals unfed for an additional 12 h (control). After first feeding, blood was sampled hourly for 24 h. The

authors reported that supplying either 100 % or 50 % of TMR decreased circulating P4 concentration by 1 h after feeding and concentrations remained depressed until 8-9 h after feeding, whereas feeding 25 % of TMR did not reduce concentrations of P4. In a similar study, Sangsritavong et al. (2002) aspirated follicles and regressed the corpus luteum of non-lactating Holstein cows in order to synchronize the follicular wave, and then treated animals with no feed, feed intake at 0.5 of maintenance requirements (M) or at 1.5 M. The authors determined that blood P4 concentrations were inversely correlated with feeding regimen at 4 h after feeding (4.14, 3.33 and 2.70 ng/mL of P4 for unfed, 0.5 M and 1.5 M, respectively). The same authors compared circulating P4 concentrations in lactating cows either unfed or fed 0.5 M, 1.5 M, or 2.2 M. Again, feed intake decreased P4 concentrations between 1 h and 4 h after feeding, although no differences were found between cows fed 1.5 or 2.2 M. Research by Rabiee et al. (2001) compared P4 concentration of non-lactating grazing dairy cows with full or restricted access (2 h/d) to pasture. Animals were drenched 2x/d with chromic oxide capsules to allow daily feed intake estimation. Circulating P4 was supplied by exogenous sources and initial blood concentrations of P4 were similar among animals, since endogenous production was eliminated prior to the study via GnRH-agonist implants. The average daily DMI of pasture was greater for cows in the full access group (15.9 vs. 6.3 kg of DM) and their plasma P4 concentrations were less (1.08 vs. 1.71ng/mL) compared to the restricted group. In research with beef cows, Pescara et al. (2005) synchronized the ovulation of 364 cows. After AI, cows were fed daily 2 kg or 6 kg of corn (as-fed basis). Blood concentrations of P4 were greater for animals fed 2 kg of corn (1.7 vs. 1.3 ng/mL) on d 7 after AI, but conception rates did not differ between the treatments. Arthington et al.

(2004) supplemented beef heifers (3x/wk) with either a molasses/cottonseed meal slurry or range cubes. Heifers supplemented with range cubes readily consumed the entire amount of feed, whereas molasses-fed heifers had a longer period for total supplement consumption (48 h). Animals had similar rates of BW gain, but pregnancy rates were different (76.3 vs. 49.2 % for molasses and range cube-fed heifers, respectively). The authors attributed this effect to the different supplement intake pattern, associating faster feed intake with acute decreases in circulating P4 and consequently impaired pregnancy rates, although blood concentrations of P4 were not determined.

The mechanism by which feed intake decreases blood P4 concentration in cattle is well defined by Sangsritavong et al. (2002). After meal consumption, liver blood flow increases in order to transport digested nutrients from the gut through the liver and on to the rest of the body (Huntington, 1990). Liver is the main site of peripheral steroid inactivation and catabolism by enzymatic processes, converting biologically active hormones into inactive forms, which are then eliminated primarily in the urine (Steimer, 2003). Blood flow to the liver is substantially increased when large amounts of feed are ingested, therefore increasing the rate by which P4 is inactivated and catabolized, causing acute and chronic reductions in circulating concentrations of P4.

In summary, high planes of nutrition may be detrimental to reproductive efficiency if not managed correctly. During periods where P4 is particularly fundamental, either for priming the hypothalamus-hypophysis axis for the onset/re-attainment of estrous cycles or for the onset/maintenance of pregnancy, feed intake should be carefully controlled in order to avoid sharp decreases in circulating P4.

CHAPTER 3
EFFECTS OF SUPPLEMENT TYPE ON PERFORMANCE, REPRODUCTIVE AND PHYSIOLOGICAL RESPONSES OF BRAHMAN-CROSSBRED FEMALES

Introduction

The primary nutritional consideration for optimum reproductive performance of beef females is energy (Mass, 1987). With greater energy intake and consequent improved energy status, heifers attain puberty earlier (Schillo et al., 1992) whereas cows resume postpartum estrous cycles earlier (Roberts et al., 1997). Alternatively, inadequate energy intake is correlated highly with delayed onset of puberty, extended postpartum intervals and decreased conception and pregnancy rates (Santos, 2004). In Florida, where the beef cattle industry is primarily constituted of cow-calf operations with diets based on forages lacking in energy availability (Moore et al., 1991), nutritional programs with emphasis on energy supply are therefore required.

Citrus pulp and molasses are energy by-products originated from orange and sugarcane industries, respectively, two of the most important agricultural operations in Florida. Sucrose is the main carbohydrate of molasses, whereas pectin is the main carbohydrate of citrus pulp. Pectin and sucrose are fermented differently in the rumen, potentially impacting forage intake, diet digestibility, and energy utilization.

Molasses is classified as a liquid feed, whereas citrus pulp is processed and fed commonly as dry pellets. Previous research by our group (Arthington et al., 2004) reported that differences in physical form of the supplement led to variances in intake behavior and reproductive performance. Beef heifers supplemented with dry feed

consumed the entire amount of supplement faster than heifers supplemented with an equivalent amount of CP and energy from a liquid supplement. Although no differences in BW gain were detected, heifers fed the liquid supplement had greater overall pregnancy rates. Supported by studies that indicated that infrequent and/or acute feed intake is associated negatively with circulating progesterone (P4) concentration (Sangsritavong et al., 2002; Vasconcelos, et al., 2003), we hypothesized that heifers supplemented with dry feed had decreased circulating P4 and consequent impaired pregnancy rates.

The objective of the present experiments was to investigate the effects of supplement type on performance and reproductive efficiency of Brahman-crossbred cattle, including plasma metabolites and hormones.

Material and Methods

Two experiments were conducted at the University of Florida – IFAS, Range Cattle Research and Education Center, Ona. The first experiment was conducted from September to December 2004 and was divided into a sampling phase (September and October) and a breeding phase (November and December). The second experiment was conducted during the months of October and November 2004. The animals utilized in these experiments were cared for by acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals

Experiment 1. Sixty Brahman x British crossbred heifers (BW ± SD = 246 ± 23 kg; age ± SD = 10 ± 1 mo) were utilized in this experiment. For the sampling phase (d 0 to d 45), heifers were stratified by initial BW and age, and randomly allocated to 12 pens (5

heifers/pen). Pens were assigned randomly to one of two treatments: 1) molasses-based supplement (ML) or 2) citrus pulp-based supplement (CT). Pen was considered the experimental unit (6 pens/treatment) and each pen was a 1.3 ha pasture of bahiagrass (*Paspalum notatum*). For the breeding phase (d 46 to d 107), heifers were re-allocated by treatment into two bahiagrass pastures and exposed to bulls for a 60-d breeding season.

Experiment 2. Initially, 40 non-lactating and non-pregnant multiparous Brahman x British crossbred cows had follicular and luteal function synchronized (Figure 3-1) by treatment with GnRH (100 *u*g; Cystorelin; Merial Ltd., Duluth, GA) and insertion of an intra-vaginal P4 releasing device containing 1.38 g of P4 (Eazi-Breed CIDR; Pfizer Animal Health, New York, NY) on d -21, followed by treatment with of PGF2α (25 mg; Lutalyse; Pfizer Animal Health, New York, NY) and CIDR removal on d -14, and a second treatment with GnRH (100 *u*g) 48 h after the PGF2α injection (d -12). Transrectal ultrasonography was performed immediately and 48 h after the second GnRH injection (d -10). Cows that were detected at the same stage of the follicular wave were then chosen to be enrolled in the experiment (Figure 3-1). Thirty-four cows were selected and stratified by age, parity, BW, and BCS (1 = emaciated, 9 = obese; Wagner et al., 1988) and randomly assigned to one of the two treatments: 1) exogenous source of P4 (CD) or 2) natural source of P4 (NCD). In order to obtain 12 cows at the beginning of the estrous cycle in each group at the start the experiment (d 0), all cows were again treated with PGF2α (25 mg) on d -5 (Figure 3-1). At this time, two CIDRs were inserted in the vagina of the cows from group CD, and remained throughout the entire experiment. Two days later (d - 3), cows from NCD were treated with GnRH (100 *u*g) (Figure 3-1).

At the beginning of the experiment (d 0), both groups CD and NCD containing twelve cows each (BW ± SD = 502 ± 55 kg; age ± SD = 5 ± 2 yr) were stratified by BW and randomly allocated to 12 feedlot pens (2 cows/pen, 6 pens/group). Pens were then randomly assigned to one of the two treatments in a 2 x 2 factorial arrangement of treatments: A) molasses-based supplement (ML) or B) citrus pulp-based supplement (CT). Pen was considered the experimental unit.

Diets

Experiment 1. Pasture quality was estimated to be 56 % TDN and 7.4 % CP (DM basis) from samples analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). A complete mineral mix and water were offered ad libitum throughout the experiment. Stargrass (*Cynodon nlemfuensis*) hay was offered when pasture availability was limited. Hay quality was estimated to be 52 % TDN and 6.5 % CP (DM basis) from samples also analyzed by commercial laboratory (Dairy One Forage Laboratory). Treatments consisted of two energy supplements (Table 3-1), fed 3x/wk (Mondays, Wednesdays and Fridays) at a rate of 2.1 and 2.3 kg of DM per heifer daily for ML and CT, respectively. Supplement intakes were formulated to be iso-caloric and iso-nitrogenous (Table 3-2) and also balanced for calcium content, given the high concentration of calcium in citrus pulp.

Experiment 2. Limpograss (*Hemarthria altissima*) hay was offered ad libitum throughout the entire experiment, and hay quality was estimated to be 54 % TDN and 9.1 % CP (DM basis) from samples analyzed by commercial laboratory (Dairy One Forage Laboratory). Cows had free access to a complete mineral mix and water. Treatments consisted of two energetic supplements (Table 3-3), fed 3x/wk (Mondays, Wednesdays and Fridays) at a rate of 4.1 and 4.5 kg of DM per cow daily for ML and CT,

respectively. Supplement intakes were formulated to be iso-caloric and iso-nitrogenous (Table 3-4) and also balanced for calcium content, given the high concentration of calcium in citrus pulp.

Sampling

Experiment 1. One week before the start (d -7 and d -6) and at the end of the experiment (d 107 and d 108), heifers were weighed for 2 consecutive days to determine both full and shrunk (after 16 h of feed restriction) BW. Heifers also were weighed every other week to monitor average daily gain (ADG) rates, but overall ADG was calculated using initial and final shrunk BW values. Blood samples were collected weekly (on Wednesdays) throughout the entire experiment to determine onset of puberty using blood P4 concentrations. Heifers were considered pubertal if P4 value was greater than 1.5 ng/mL for 2 consecutive weeks.

During the sampling phase, in addition to the weekly collections, blood samples were obtained once a day during four consecutive days, every other week, starting at 4 h after supplements were offered for determination of glucose, blood urea nitrogen (BUN), insulin, IGF-I, GH, and P4 concentrations. These samples were collected from d 0 to d 3, d 14 to d 17, d 28 to d 31 and d 42 to d 45, which were classified as periods (P 1, P 2, P 3 and P 4, respectively) for further statistical analysis. Periods always started on Mondays and ended on Thursdays.

During the breeding season, heifers were exposed to mature Angus bulls from d 46 to d 107. Each group was exposed to 2 bulls at the same time (1:15 bull to heifer ratio), and bulls were rotated weekly between groups to account for potential effect of bull. Heifer pregnancy status was verified by presence of fetus using transrectal

ultrasonography (5.0 MHz transducer, Aloka 500V, Wallingford, CT) 70 d after the end of the experiment (d 177).

Random samples of feedstuff, pasture, and hay were collected during the trial and analyzed for nutrient composition at commercial laboratories (Dairy One Forage Laboratory for cottonseed meal and citrus pulp samples, and SDK Laboratories, Hutchinson, KS for molasses samples).

Experiment 2. During the first 3 weeks of the experiment (d 1 to d 21), blood samples were collected immediately prior and 4, 8, 24, 32 and 48 h after the first supplement feeding of the week (d 1, 8 and 15) for determination of glucose, BUN, insulin, IGF-I and P4 concentrations. Weeks were classified as periods (week 1 = P 1; week 2 = P 2; week 3 = P 3) for further statistical analysis.

During the second part of the experiment (d 22 to d 37), daily forage DMI was recorded. Hay was offered in ad libitum amounts, and refusal was collected and weighed daily. A sample of the offered hay was collected twice during this period for nutrient analysis, while samples of refusal were collected daily from each pen to determine DM content. Hay samples were dried for 96 h at 50º C in forced-air ovens. Random samples of the feedstuffs also were collected during the trial, and all the samples obtained for nutrient composition were analyzed at commercial laboratories (Dairy One Forage Laboratory for cottonseed meal and citrus pulp samples, and SDK Laboratories for molasses samples).

Blood Analysis

Blood samples were collected - via jugular venipuncture during Exp. 1 and from coccygeal vein or artery during Exp. 2 - into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin,

placed on ice immediately, and centrifuged at 855 g for 30 min for plasma collection. Plasma was frozen at -20°C on the same day of collection.

A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to determine both plasma glucose (Coulombe and Favreau, 1963; modified and described by Bran + Luebbe Industrial Method #339-01) and BUN (Gochman and Schmitz, 1972; modified and described by Bran + Luebbe Industrial Method #339-19). A double antibody radioimmunoassay (RIA) was used to determine plasma concentrations of insulin (Malven et al., 1987; Badinga et al., 1991), IGF-I (Badinga et al., 1991) and GH (Badinga et al., 1991). The extraction procedure used in the IGF-I assay was modified from Badinga et al. (1991) by using a ethanol:acetone:acetate ratio of 6:3:1. Concentrations of plasma P4 were determined using Coat-A-Count Kit (DPC® Diagnostic Products Inc., Los Angeles, CA) solid phase ^{125}I RIA. The intra- and inter-assay CV for Exp. 1 were, respectively, 9.66 and 10.05 % for insulin, 8.13 and 5.87 % for IGF-I, 5.95 and 18.71 % for GH, and 4.48 and 8.15 % for P4. The intra- and inter-assay CV for Exp. 2 were, respectively, 12.89 and 15.92 % for insulin, 9.90 and 11.00 % for IGF-I, and 2.41 and 11.19 % for P4.

Statistical Analysis

Experiment 1. Data were analyzed using the PROC MIXED procedure of SAS (SAS, 2001).

The statistical model used was:

$$Y = \mu + TRT_i + P_j + PEN_{k(i)} + HEIFER_{l(k)} + DAY_{m(j)} + TRTP_{ij} + TRTDAY_{im(j)} + E_{ijklm}$$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

P = fixed effect of period

PEN = random effect of pen within treatment

HEIFER = random effect of heifer within pen

DAY = fixed effect of day within period

TRTP = effect due to interaction of treatment and period

TRTDAY = effect due to interaction of treatment and day within period

E = residual error

Results are reported as least square means. Means were separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Only significant interactions are reported.

Experiment 2. Data were analyzed using the PROC MIXED procedure of SAS (SAS, 2001).

The statistical model used for forage DMI data analysis was:

$$Y = \mu + TRT_i + PEN_{j(i)} + DAY_k + TRTDAY_{ik} + E_{ijkl}$$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

PEN = random effect of pen within treatment

DAY = fixed effect of day

TRTDAY = effect due to interaction of treatment and day

E = residual error

The statistical model used for plasma hormones and metabolites data analysis was:

$$Y = \mu + TRT_i + P_j + PEN_{k(i)} + COW_{l(k)} + TIME_{m(j)} + TRTP_{ij} + TRTTIME_{im(j)} + E_{ijklm}$$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

P = fixed effect of period

PEN = random effect of pen within treatment

COW = random effect of cow within pen

TIME = fixed effect of time within period

TRTP = effect due to interaction of treatment and period

TRTTIME = effect due to interaction of treatment and time within period

E = residual error

Results are reported as least square means. Means were separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were classified at $P > 0.05$ and ≤ 0.10. Only significant interactions are reported. All plasma metabolites and hormones (but P4) data were analyzed comparing CT vs. ML without distinction between CD and NCD. Progesterone data compared CT vs. ML within CD and NCD groups.

Results and Discussion

Experiment 1

Heifers were provided supplement treatments on Mondays, Wednesdays and Fridays at 0700 h throughout the entire experiment. Heifers fed ML required approximately a 48 h period to completely consume the supplement, whereas heifers fed CT consumed the whole amount of supplement 24 to 36 h after supplements were offered. This intake behavior was unexpected, since previous research by our group (Arthington et al., 2004) reported that heifers in similar conditions of management, consumed entire amounts of dry supplement within 2 to 3 h after feeding, whereas liquid supplement also was entirely consumed in approximately 48 h. Therefore, we concluded that intake behavior did not differ between treatments as expected, and the results obtained for heifer performance and reproductive efficiency in this experiment should be primarily attributed to the nutritional differences between CT and ML.

For all plasma metabolites and hormone data, day(period) and period effects were significant ($P < 0.01$). The significance in day(period) effect can be explained by the daily variability in animal behavior and consequent supplement consumption, considerably affected by the environment and management activities, although supplement feeding and blood samplings always started at the same time throughout the entire experiment. Regarding the significance in period effect, it can be attributed to animal maturation and also to the adaptation of the animals to the experimental procedures such as feeding and handling management, since it was observed that both energy and protein status of the animals improved with the advance of the experiment without any modification in the treatments.

Heifers fed CT had greater ADG compared to heifers fed ML (0.40 vs. 0.30 kg/d, respectively; $P < 0.05$, SEM = 0.02; Table 3-5), concurring with previous data reporting that animals fed molasses-based supplements usually have inferior BW gain compared to animals fed supplements based on other energy sources, such as corn or soybean hulls (Pate, 1983; Royes et al., 2001). However, the superiority in performance from CT-fed heifers was not reflected in reproductive efficiency. There were no treatment effects on pregnancy rate, puberty rate and number of days required to attain puberty (Table 3-5).

Heifers fed ML had greater overall BUN concentrations ($P < 0.05$) compared to heifers fed CT (5.17 vs. 4.17 mg/dL, respectively; SEM = 0.24) (Table 3-6; Figure 3-2). However, this effect was mainly observed during the first two periods of the experiment. During periods 3 and 4, the significance was less apparent ($P = 0.07$ and $P = 0.60$, respectively). Therefore, a treatment x period interaction was detected ($P < 0.01$). A treatment x day(period) interaction also was detected ($P < 0.01$) and can be mainly attributed to the differences in supplement intake behavior and supplement ruminal degradability, leading to differences in daily BUN concentrations between the treatments. Monitoring BUN is a technique used for measuring protein status in cattle and is directly correlated with the amount of ammonia produced and absorbed in the rumen (Huntington, 1990). Ammonia is a product of dietary protein degradation by the microbial population in the rumen and is a major precursor for microbial protein synthesis. Concentrations of BUN should range between 11 and 15 mg/dL for growing animals to ensure maximum rates of gain (Byers and Moxon, 1980). This goal can be achieved by feeding animals with correct quantities of protein and energy (Hammond, 1997; Huntington, 1997), and also synchronizing the supply of these nutrients in the

rumen by feeding energy and protein sources with similar rates of ruminal degradability (Spicer et al., 1986; Matras et al., 1991; Taniguchi et al., 1995). In the current study, both treatments would be deficient in protein according to the BUN concentrations observed, since they were consistently below the optimum (Table 3-6). However, supplements were formulated to supply the protein requirements of developing heifers gaining similar weight as observed in this experiment, using the NRC model (NRC, 1996). Therefore, BUN might not be the most accurate indicator of the protein status in our case, since the BW gain of heifers were in an expected range and the negative effects of protein deficiency were thus not observed. The differences between treatments may be attributed to the faster rate of ruminal degradation of sucrose compared to pectin (NRC, 2001), optimizing synchrony of protein and energy supply in the rumen of heifers fed ML during the initial periods.

Plasma glucose (83.30 vs. 74.68 mg/dL for CT and ML, respectively; SEM = 2.46), insulin (0.89 vs. 0.75 ng/mL for CT and ML, respectively; SEM = 0.04) and IGF-I (121.53 vs. 108.91 ng/mL for CT and ML, respectively; SEM = 4.10) concentrations were greater for heifers fed CT ($P < 0.05$; Table 3-6; Figure 3-3, Figure 3-4 and Figure 3-5, respectively). The differences between the treatments increased with the advance of the experiment for glucose and insulin concentrations; therefore, treatment x period interactions were detected ($P < 0.01$). A treatment x day(period) interaction was only observed for glucose ($P < 0.01$), and similar to BUN, may also be attributed to the differences in supplement intake behavior. Several researchers have reported that feeding diets containing high molasses or sucrose inclusion rates resulted in an increase in butyric acid concentration in the rumen of cattle (Owen et al., 1967; Kellogg and Owen, 1969;

Hatch and Beeson, 1972), whereas diets rich in citrus pulp, consequently pectin, produced a greater proportion of acetic acid (Loggins et al., 1964; Hentges et al., 1966; Drude et al., 1971). Both butyric acid and acetic acid are VFAs that oxidized for energy production (Bergman, 1990). The epithelial tissue of the rumen retains approximately 90 % of the butyrate produced and converts it to ketone bodies or carbon dioxide, whereas only 30 % of acetate is metabolized by ruminal epithelium, and the remaining is oxidized throughout most of the body tissues to generate ATP (Bergman, 1990). In addition, butyrate also has a detrimental effect on VFA metabolism by restraining hepatic propionate utilization, thus inhibiting gluconeogenesis and restraining glucose synthesis and availability (Aiello et al., 1989). Ruminal pH and forage intake and/or digestibility were not measured in the present experiment. However, several authors reported that feeding supplements based on non-structural carbohydrates (NSC), such as sucrose, depressed forage intake (Sanson et al., 1990; Olson, et al., 1999) and digestibility by decreasing ruminal pH and consequent microbial activity (Mertens and Loften, 1980; Hiltner and Dehority, 1983; Martin et. al., 2001). Alternatively, when supplements based on fibrous by-products such as citrus pulp were fed, forage intake and digestibility was less negatively affected (Bowman and Sanson, 1996; Caton and Dhuyvetter, 1997; Royes et al., 2001) or even increased (Bowman and Sanson, 2000).

The positive relationship between glucose, insulin, and IGF-I concentrations with ADG, observed in this experiment, is also supported by others (Vizcarra et al., 1998; Lapierre et al., 2000; Hersom et al., 2004). Some authors also included analysis for plasma GH concentration and observed a significant inverse relationship between GH concentrations and animal performance/energy status (Ellenberger et al., 1989; Bossis et

al., 1999). In these studies, blood was sampled in serial collections (every 20 and 10 min, respectively) to accurately measure GH concentrations, which is released in a pulsatile pattern (10 to 15 pulses/d; Hornick et al., 1998; Bossis et al., 1999). This might be an explanation to why no differences were detected in GH concentrations between CT- and ML-fed heifers (41.45 vs. 39.27 ng/mL, respectively; $P = 0.62$, SEM = 3.20) (Table 3-6; Figure 3-6), given that blood was sampled once a day and we were not able to identify pulses or basal concentrations of this hormone. Therefore, GH analysis in this experiment was not accurate enough to be associated directly to the glucose/insulin/IGF-I axis. The correlation between all plasma metabolites and hormones with ADG also was determined with Pearson correlation coefficients (Table 3-7). The only significant correlation was observed for IGF-I ($P < 0.01$).

In order to verify if plasma P4 concentrations would be influenced by treatments and consequent intake behavior, only samples from pubertal heifers with plasma P4 greater than 2 ng/mL during the sampling period were used. In view of the fact that these heifers were at random stages of the estrous cycle and consequently concentrations of P4 varied significantly across heifers, we analyzed the difference of P4 concentration on a percentage basis, comparing the values from days that supplements were offered to values from days that supplements were not offered (set as 100 %). Both treatments had a decrease in P4 concentration during feeding days ($P < 0.01$; SEM = 2.9); however, no differences were observed between treatments (89.5 and 90.3 % for CT and ML, respectively; $P = 0.66$, SEM = 4.1). Elevated or infrequent feed intake may decrease circulating concentrations of P4, and therefore impair reproductive efficiency given that P4 is essential for the onset of puberty (Gonzalez-Padilla et al., 1975) and for the

recognition and maintenance of early pregnancy (Spencer and Bazer, 2002). Vasconcelos et al. (2003) fed pregnant lactating Holstein cows with either 100 % of the daily diet at once, half of the diet every 12 h, a quarter of diet every 6 h or left animals unfed. The authors reported that the first two treatments decreased circulating concentrations of P4 by 1 h after feeding and concentrations remained depressed until 8 to 9 h after feeding. An inverse relationship between feed intake and blood P4 concentrations also was reported by others (Rabiee et al., 2001; Sangsritavong et al., 2002; Pescara et al., 2005). In the current experiment, the differences observed in feed intake behavior (24 to 36 h and 48 h for total supplement consumption for CT and ML, respectively) were not as substantial as expected, and might explain why no differences were found in blood P4 concentrations and also reproductive performance between treatments.

Nutritional status, and consequent BW gain and composition, typically is considered the primary determinant of puberty attainment because it is highly correlated with frequency and amplitude of LH pulses, which stimulate development of ovarian follicles to preovulatory stages (Schillo et al., 1992). Furthermore, blood metabolites and hormones such as glucose, insulin, and IGF-I have been associated with reproductive performance. These substances seem to be the connection between nutritional status and the increase in LH secretion and activity, by influencing hypothalamic-hypophyseal secretory activity (Butler and Smith, 1989; Schillo et al., 1992) and also amplifying the effects of LH in ovarian cells (Spicer and Echternkamp, 1995). However, in the present study, neither BW gain nor the glucose/insulin/IGF-I axis were associated with reproductive performance. Heifers fed CT had greater ($P < 0.05$) ADG and blood concentrations of these metabolites/hormones, but similar reproductive performance

compared to heifers fed ML. When heifers were divided, disregarding treatments, by puberty attainment and pregnancy status, IGF-I was again the only measurement significantly associated with reproductive performance (Table 3-8). Concentrations of IGF-I are correlated positively with insulin concentrations (Table 3-7; Keisler and Lucy, 1996; Webb et al., 2004), that in turn are increased as blood concentrations glucose rises, and similarly decreased as blood concentrations of glucose declines. Therefore, in order to increase blood IGF-I concentrations to enhance BW gain and reproductive performance, glucose synthesis should be maximized. Feeding level is associated positively with blood glucose concentration (Vizcarra et al., 1998; Lapierre et al., 2000; Hersom et al., 2004). In addition, since propionate accounts for up to 76 % of the glucose synthesized in the liver (Reynolds et al., 1994), diets containing ingredients that produce a greater ratio of propionate in the rumen, such as starches, should be fed.

Experiment 2

The same supplement intake behavior from Exp. 1 was observed in Exp. 2. Cows were supplemented on Mondays, Wednesdays and Fridays at 0800 h, and cows fed ML required around 48 h to consume the entire amount of supplement, whereas cows fed CT required 24 to 36 h.

Overall forage DMI did not differ between treatments (1.16 vs. 1.20 % for CT and ML, respectively; $P = 0.52$, SEM = 0.08; Figure 3-7). However, a day effect and also a treatment x day interaction were detected ($P < 0.01$). Forage DMI was lesser for both groups during the days that supplements were fed (0.65 and 1.55 % for CT vs. 0.96 and 1.38 % of BW for ML during feeding and non-feeding days, respectively; $P < 0.01$, SEM = 0.09). This effect likely can be attributed to the increased consumption of the supplements during feeding days compared to non-feeding days, since forage DMI is

negatively associated with energy supplement intake (Caton and Dhuyvetter, 1997; Kunkle et al., 1999; Bodine and Purvis, 2003). Furthermore, forage DMI for cows fed CT was less than for cows fed ML during feeding days (0.65 vs. 0.96 % of BW, respectively, $P < 0.05$, SEM = 0.090) but not during non-feeding days ($P = 0.19$). This treatment x day interaction can be explained by the greater consumption of supplement during feeding days by CT-fed cows, since these cows consumed the entire amount of supplement sooner than cows fed ML (24 to 36 vs. 48 h, respectively). As cited previously, a greater depression in forage intake usually is observed when NSC-based supplements are fed (Sanson et al., 1990; Olson, et al., 1999). However, these results were obtained comparing supplements with similar physical form and intake behavior. In the current study, no differences in overall forage DMI were observed. The differences in supplement physical form and consequent consumption behavior led to the significant differences in forage intake pattern between treatments, characterized by the treatment x day interaction.

No differences between treatments were detected for BUN concentrations (4.46 vs. 4.19 mg/dL for CT and ML, respectively; $P = 0.52$, SEM = 0.29) (Table 3-9, Figure 3-8). A time(period) effect ($P < 0.01$) was observed because BUN concentrations usually increased after supplements were offered, illustrating the improvement in the protein status of the cows after supplements were consumed. A period effect also was observed ($P < 0.01$). Cows had greater BUN concentrations during the second period, intermediary during the third period, and lesser during the first period (3.45, 5.21 and 4.32 mg/dL, respectively; SEM = 0.23). A treatment x time(period) interaction was detected ($P < 0.01$). For the second and third periods, cows fed CT had comparable BUN

concentrations during the first 8 h after supplement feeding, but greater for the following hours compared to ML-fed cows. Although CT-fed cows had greater supplement consumption during feeding days and consequently during the first hours after feeding, citrus pulp undergoes slower ruminal degradation compared to molasses (NRC, 2001). This effect may have delayed the increase in BUN associated with the synchrony between ruminal energy and protein supply. A treatment x period interaction also was observed for BUN ($P < 0.01$). During the first period, cows fed ML tended to have greater BUN concentrations (3.02 vs. 3.87 mg/dL for CT and ML, respectively; $P = 0.06$, SEM = 0.33), but cows fed CT had greater BUN concentrations during the third period (4.79 vs. 3.86 mg/dL for CT and ML, respectively; $P < 0.05$, SEM = 0.33). According to Hammond (1997), BUN concentrations should range between 7 to 8 mg/dL for mature beef cows. Therefore, cows on both treatments would be deficient in protein intake according to their BUN concentrations, since they were often below this optimal range (Table 3-9). However, both supplements were formulated to supply the protein requirements of mature cows, using the NRC model (NRC, 1996)

Plasma glucose concentrations did not differ between treatments (84.85 vs. 85.95 mg/dL for CT and ML, respectively; $P = 0.88$, SEM = 5.12) (Table 3-9, Figure 3-9). A time(period) effect was observed ($P < 0.01$) and can be attributed to the fluctuation in glucose synthesis and availability in blood after supplements were offered and consumed. No differences were observed in plasma insulin (1.77 vs. 1.51 ng/mL for CT and ML, respectively; $P = 0.33$, SEM = 0.19) (Table 3-9, Figure 3-10) and plasma IGF-I concentrations (110.11 vs. 100.09 ng/mL for CT and ML, respectively; $P = 0.49$, SEM = 9.88) (Table 3-9, Figure 3-11). A time(period) effect was significant only for insulin ($P <$

0.01), and can be associated to the same effect observed for glucose, since the concentration of these substances in blood are mutually dependent. For both hormones, a period effect ($P < 0.01$) and a treatment x period interaction ($P < 0.05$) were detected. The significance in period effects indicates that the energy status of cows from both treatments increased with the advance of the experiment, and can be explained by the adaptation of the cows to the experimental procedures, particularly the frequent blood sampling, since treatments remained unchanged. The differences between treatments for insulin and IGF-I, increased with the advance of the experiment, explaining the significance of the treatment x period interaction. During the second period, CT-fed cows tended to have greater plasma insulin concentrations compared to ML-fed cows (1.97 vs. 1.50 ng/mL, respectively; $P = 0.09$, SEM = 0.19). According to the NRC (1996), the cows utilized in this experiment required around 8.5 Mcal/d of NE_M. Given that overall forage DMI was similar and treatments were iso-caloric, cows from both treatments consumed approximately 15.0 Mcal/d (175 % of the NE_M requirements). Supplements were fed at the same percentage of animal BW during Exp. 1 and Exp. 2. Because energy requirements for growing heifers and mature cows are different, the heifers in Exp. 1 were fed with moderate amounts of energy, whereas the cows in Exp. 2 were fed excessive amounts of supplemental energy. This might be an explanation for why the results observed during Exp. 1 were not replicated during Exp. 2.

When analyzing initial plasma P4 concentrations, cows from the NCD group were at various stages of the estrous cycle. However, all cows from the CD group had similar plasma P4 concentrations, mostly originating from the exogenous source. Therefore, only data from the CD group were used to compare if cows fed CT had different plasma P4

concentrations compared to cows fed ML. Plasma P4 concentration did not differ between treatments (4.08 vs. 3.14 ng/mL for CT and ML, respectively; $P = 0.12$, SEM = 0.34; Figure 3-12). A period effect was observed ($P < 0.01$) because plasma P4 concentrations decreased linearly for both groups from the first period to the last period, and this effect can be associated with the decrease in P4 release from the CIDRs. A time(period) effect also was observed ($P < 0.01$). Throughout the entire experiment, plasma P4 concentrations decreased for both groups after the supplements were offered. This decrease can be associated with: a) supplement intake, which may have increased the rate at which plasma P4 was metabolized in the liver, b) natural deterioration of the CIDRs, or c) the sum of both factors. A treatment x period interaction also was detected ($P < 0.01$). Cows fed CT had greater plasma P4 concentration during the first period (5.97 vs. 4.31 ng/mL for CT and ML, respectively; $P < 0.05$, SEM = 0.31), but not for the second and third periods ($P = 0.20$ and $P = 0.39$, respectively). Concentrations of BUN, insulin and IGF-I were numerically greater for CT-fed cows compared to ML-fed cows during the second and third periods, but similar or lesser during the first period. This may imply that during the first period, cows fed with CT although consumed the entire amount of supplement faster, possibly had lower nutrient absorption by the digestive tract, therefore blood flow to the liver and consequent hepatic P4 metabolism were decreased. In conclusion, differences were not observed in plasma P4 concentrations between cows fed CT or ML but for first period, even though blood was frequently collected from cows with similar basal plasma P4 concentrations. Further, the differences observed in the first period were contrary to our expectations.

Conclusions

The objective of the first experiment was to investigate if heifers fed a molasses-based supplement would experience greater reproductive performance compared to heifers fed with supplements based on dry feed (citrus pulp), as previously observed by our group. To further test our main hypothesis, we conducted a second experiment with cows, where blood was collected with greater frequency to verify if P4 was being metabolized at a greater extent in cows supplemented with citrus pulp. However, the expected supplement intake behavior was not observed in either experiment, and consequently no major differences between treatments were observed in plasma P4 concentrations of heifers and cows.

In the first experiment, heifers supplemented with CT had greater concentrations of plasma glucose, insulin and IGF-I, which resulted in greater ADG compared to ML-supplemented heifers; however, reproductive performance was not impacted by treatments. In addition, plasma concentration of IGF-I was the only plasma measurement significantly correlated with BW gain and positively associated with attainment of puberty and pregnancy.

In the second experiment, no major differences between treatments were found for plasma metabolites and hormones. We concluded that the cows were provided excessive supplemental energy, and any potential responses to the treatments were suppressed. In addition, although no differences where observed for overall forage DMI, cows supplemented with citrus pulp have a greater daily oscillation in forage intake compared to cows supplemented with molasses.

Table 3-1. Composition of supplements fed to heifers (Exp. 1).

Ingredients (%; as-fed)	**CT** [1]	**ML** [2]
Blackstrap molasses	0.00	78.30
Citrus Pulp	74.70	0.00
Cottonseed meal	25.30	20.20
Calcium carbonate	0.00	1.50

[1] CT = citrus pulp-based supplement fed 3x/wk (Mondays, Wednesdays and Fridays).
[2] ML = molasses-based supplement fed 3x/wk (Mondays, Wednesdays and Fridays).

Table 3-2. Nutrient composition and intake rate of supplements fed to heifers (Exp. 1) [1].

Nutrient Composition (%; DM)	**CT**	**ML**
TDN	70.00	77.00
CP	19.00	20.07
Calcium	1.70	1.80
Phosphorus	0.60	0.50
Supplement Intake (kg/head/d) [2]		
DM	2.30	2.10
TDN	1.61	1.61
CP	0.43	0.43
Calcium	0.04	0.04
Phosphorus	0.01	0.01

[1] Supplements fed on Mondays, Wednesdays, and Fridays (3x/wk).
[2] Estimated from the supplement consumption of the pen (5 heifers/pen).

Table 3-3. Composition of supplements fed to cows (Exp. 2).

Ingredients (%; as-fed)	**CT** [1]	**ML** [2]
Blackstrap Molasses	0.00	75.40
Citrus Pulp	74.75	0.00
Cottonseed Meal	25.25	23.41
Calcium Carbonate	0.00	1.19

[1] CT = citrus pulp-based supplement fed 3x/wk (Mondays, Wednesdays and Fridays).
[2] ML = molasses-based supplement fed 3x/wk (Mondays, Wednesdays and Fridays).

Table 3-4. Nutrient composition and intake rate of supplements fed to cows (Exp. 2) [1].

Nutrient Composition (%; DM)	**CT**	**ML**
TDN	69.00	76.00
CP	16.60	18.50
Calcium	1.50	1.40
Phosphorus	0.40	0.40
Supplement Intake (kg/head/d) [2]		
DM	4.53	4.10
TDN	3.13	3.20
CP	0.75	0.75
Calcium	0.06	0.06
Phosphorus	0.02	0.02

[1] Supplements fed on Mondays, Wednesdays, and Fridays (3x/wk).
[2] Estimated from the supplement consumption of the pen (2 cows/pen).

Table 3-5. Effect of treatments on ADG and reproductive performance of heifers in experiment 1.

Item	CT	ML	SEM	*P* =
ADG, kg/d [1]	0.40	0.30	0.02	0.008
Pregnancy rate, % [2]	60.0	57.5	8.05	0.830
Puberty rate, % [3,4]	80.0	76.6	9.94	0.817
Days to puberty [5]	72.4	69.3	5.05	0.650

[1] Calculated using initial and final shrunk BW.
[2] Pregnant heifers / total heifers.
[3] Cycling heifers / total heifers.
[4] Puberty = plasma progesterone concentration greater than 1.5 ng/mL for two consecutive weeks.
[5] Average days to puberty attainment after the beginning of the study.

Table 3-6. Effects of treatments on plasma metabolites and hormones of heifers (Exp. 1) fed citrus pulp- (CT) or molasses-based supplement (ML) [1,2].

	Period 1			Period 2				Period 3				Period 4					
(d)	Tu	Wed	Th	Mo	Tu	Wed	Th	Mo	Tu	Wed	Th	Mo	Tu	Wed	Th	SE	*P* =
BUN [3]																	0.015
CT	1.2	2.7	1.3	0.5	2.5	1.7	2.9	4.7	8.3	5.1	6.4	4.1	6.9	5.7	10.4	0.24	
M	2.7	3.7	4.7	2.7	2.6	2.6	4.3	6.7	6.9	6.5	7.1	7.1	6.5	6.4	7.9	0.24	
Glucose [3]																	0.032
CT	91.2	81.1	83.6	80.3	85.4	83.6	87.7	77.4	84.4	88.8	81.8	76.6	88.9	73.6	82.8	2.46	
ML	80.6	76.9	81.3	72.9	77.6	77.1	78.1	68.6	73.5	70.5	71.2	69.4	74.7	71.9	70.6	2.46	
Insulin [3]																	0.043
CT	0.83	0.86	0.92	0.86	0.96	0.77	0.86	0.99	0.97	0.96	0.84	0.90	0.97	0.80	0.86	0.04	
ML	0.72	0.89	0.87	0.87	0.74	0.74	0.77	0.75	0.70	0.70	0.71	0.68	0.69	0.64	0.64	0.04	
IGF – I [3]																	0.049
CT	121.8	114.5	120.0	118.3	117.6	109.7	117.3	124.2	127.9	113.2	118.8	125.5	140.3	125.5	130.7	4.03	
ML	110.5	102.5	106.0	110.3	107.1	102.6	104.9	104.3	114.5	104.5	106.2	109.5	123.9	113.6	115.3	4.15	
GH [3]																	0.629
CT	50.7	42.6	35.7	39.9	43.5	45.8	46.3	34.1	42.7	47.6	42.6	34.0	35.7	42.8	35.4	3.17	
ML	53.6	43.6	38.8	32.2	47.4	39.3	42.7	29.5	32.8	41.3	34.8	32.8	41.7	35.5	36.4	3.21	

[1] Blood samples not collected on Monday during the first period.
[2] Days at which supplements were offered are underlined.
[3] BUN and Glucose = mg/dL; Insulin, IGF – I, and GH = ng/mL.

Table 3-7. Correlations between plasma measurements and ADG of heifers (Exp. 1) [1,2].

Item	ADG	Glucose	Insulin	IGF-I
Glucose (mg/dL)	-0.065			
	0.62			
Insulin (ng/mL)	0.137	- 0.140		
	0.30	0.29		
IGF-I (ng/mL)	0.414	0.003	0.329	
	< 0.01	0.98	< 0.05	
GH (ng/mL)	0.102	0.240	0.031	0.128
	0.44	0.07	0.81	0.33

[1] Upper row = correlation coefficients.
[2] Lower row = *P*–values.

Table 3-8. Effects of performance and plasma concentration of metabolites and hormones on reproductive performance of heifers (Exp. 1).

	Puberty Attainment [1]			
	Pubertal	Non-Pubertal	SEM	*P - Value*
Number of animals	45	13	-	-
ADG (kg/d)	0.35	0.33	0.06	0.601
Glucose (mg/dL)	78.10	79.05	2.96	0.777
Insulin (ng/mL)	0.83	0.80	0.05	0.643
IGF-I (ng/mL)	120.11	99.10	4.21	< 0.001

	Pregnancy Status [2]			
	Pregnant	Open	SEM	*P - Value*
Number of animals	33	12	-	-
ADG (kg/d)	0.37	0.31	0.07	0.208
Glucose (mg/dL)	79.70	74.97	2.52	0.120
Insulin (ng/mL)	0.83	0.83	0.06	0.987
IGF-I (ng/mL)	123.35	111.38	4.44	0.054

[1] Puberty = plasma progesterone concentration greater than 1.5 ng/mL for two consecutive weeks.
[2] Pubertal heifers only.

Table 3-9. Effects of treatments on plasma metabolites and hormones of cows (Exp. 2) fed citrus pulp- (CT) or molasses-based supplement (ML) [1].

	Period 1						Period 2						Period 3							
(h)	0	4	8	24	32	48	0	4	8	24	32	48	0	4	8	24	32	48	SE	*P* =
BUN [2]																				0.520
CT	0.8	1.2	1.8	3.7	4.3	6.1	4.3	3.1	3.2	7.0	8.3	7.5	4.6	2.7	1.7	4.7	6.3	8.5	0.29	
ML	1.7	2.3	2.6	4.8	5.5	6.1	3.6	3.7	4.1	5.7	5.7	6.3	2.7	2.8	2.7	4.4	5.1	5.3	0.28	
Glucose [2]																				0.882
CT	89.0	81.5	98.5	84.4	80.9	79.3	88.1	87.5	88.4	86.3	78.1	77.2	84.4	85.2	79.0	88.3	89.6	81.0	5.21	
ML	93.8	88.5	96.2	86.1	83.5	79.9	89.9	80.4	90.7	88.8	78.6	76.4	85.4	83.0	86.3	85.0	90.8	83.2	5.04	
Insulin [2]																				0.339
CT	0.92	1.19	1.08	1.25	1.37	1.79	1.69	2.35	2.22	2.26	1.80	1.48	1.43	2.12	2.34	2.53	2.69	1.42	0.19	
ML	1.04	1.23	1.24	1.11	1.14	1.13	1.34	1.61	1.80	1.41	1.57	1.27	1.66	1.75	2.14	1.97	2.28	1.40	0.20	
IGF – I [2]																				0.483
CT	95.1	90.7	89.4	97.8	90.6	95.4	114.7	112.1	108.4	117.3	110.1	108.1	119.2	115.7	123.9	132.8	125.5	124.9	9.98	
ML	94.9	86.4	89.9	90.7	79.8	90.8	97.8	101.3	101.6	104.0	99.5	99.1	103.2	107.4	106.4	112.6	106.9	114.1	9.98	

[1] Supplements were offered after blood was sampled at 0 h.
[2] BUN and Glucose = mg/dL; Insulin, and IGF – I = ng/mL.

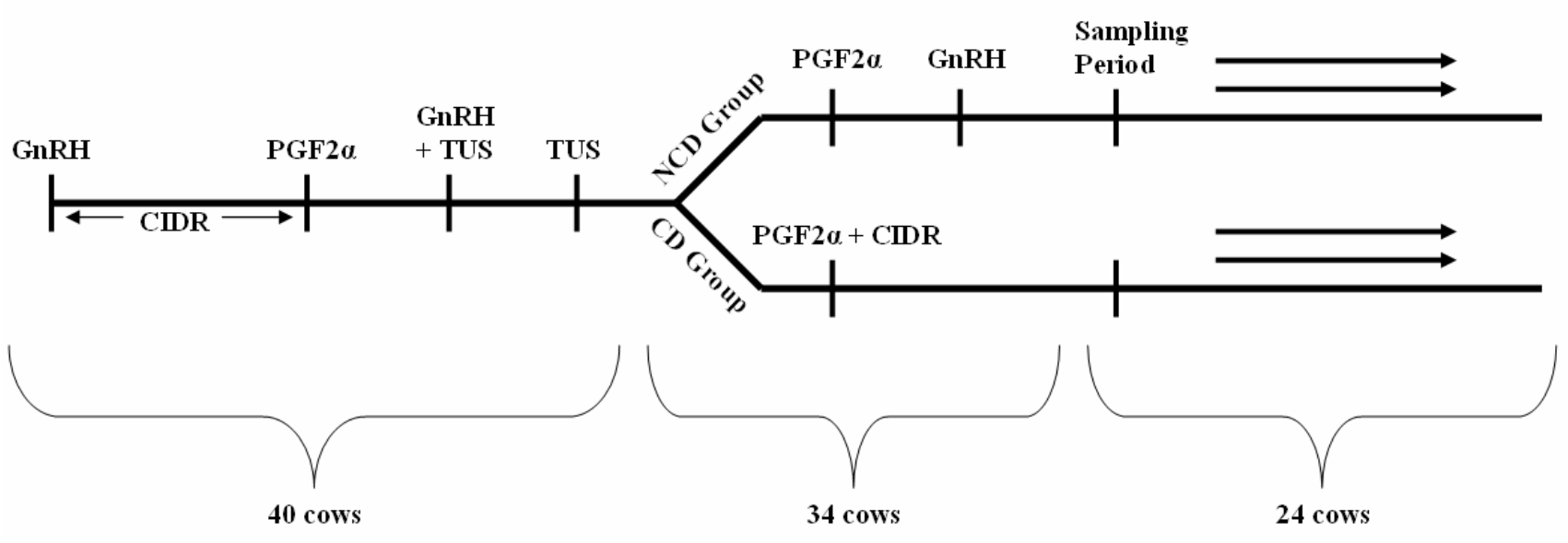

Figure 3-1. Outline of the follicular and luteal function synchronization protocol. Transrectal ultrasonography = TUS.

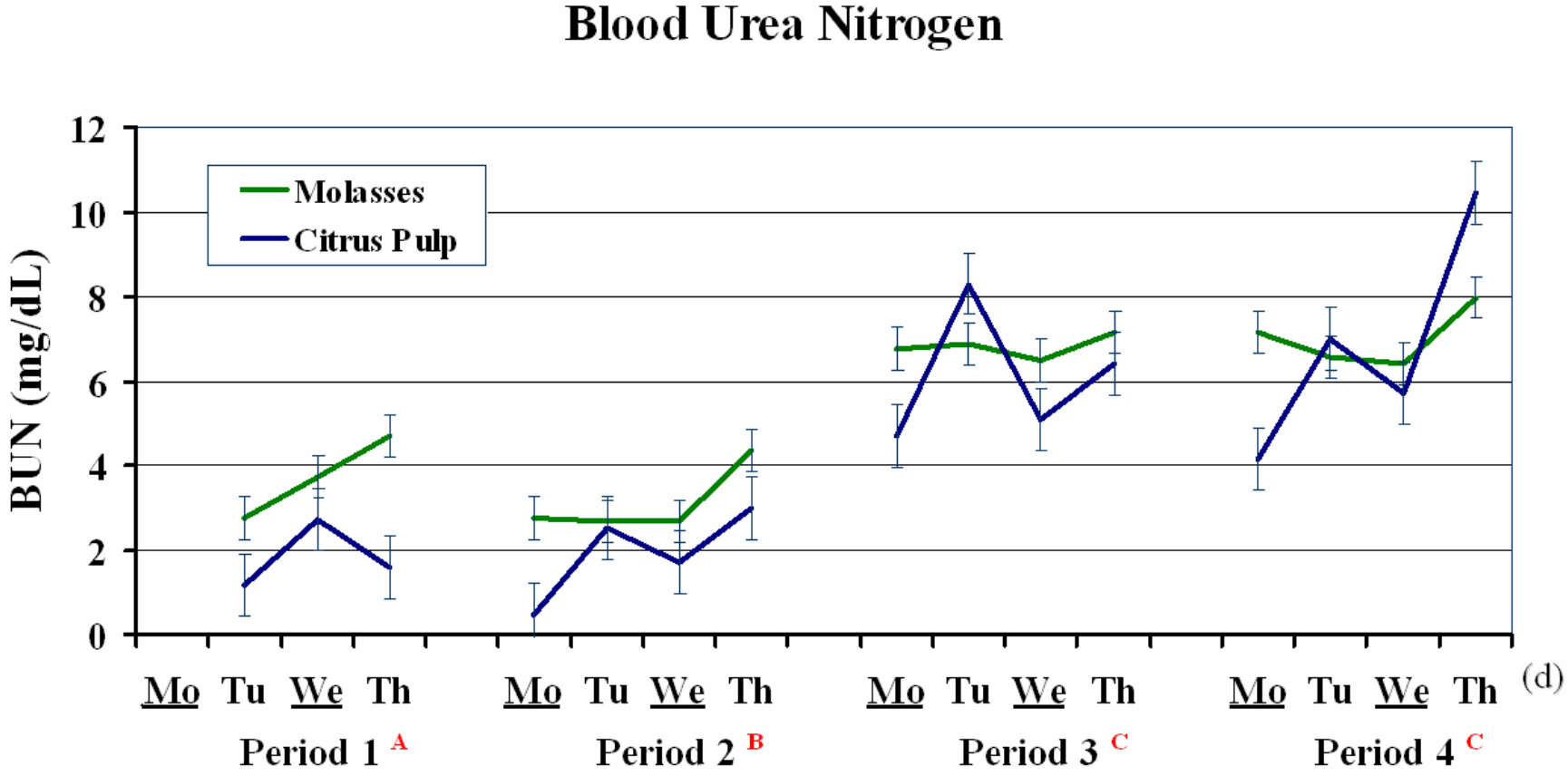

Figure 3-2. Blood urea nitrogen (BUN) concentrations of heifers supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Days at which supplements were offered are underlined. Heifers fed ML had greater mean BUN concentration ($P < 0.05$, SEM = 0.24). Period and day(period) effects were significant ($P < 0.01$). Treatment x period and treatment x day(period) interactions were also significant ($P < 0.01$), and periods with different letters differ ($P < 0.05$).

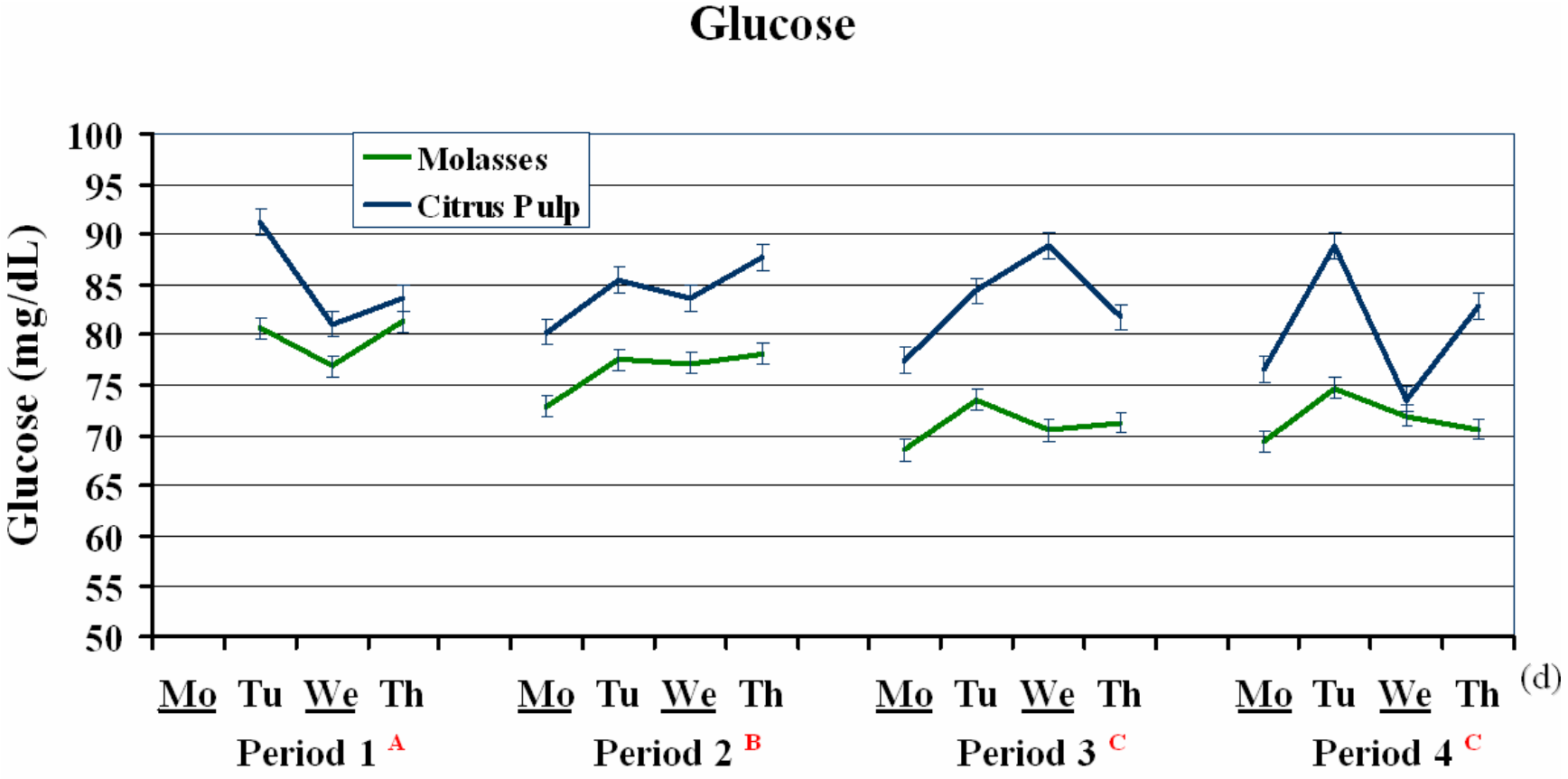

Figure 3-3. Plasma glucose concentrations of heifers supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Days at which supplements were offered are underlined. Heifers fed CT had greater mean glucose concentration ($P < 0.05$, SEM = 2.46). Period and day(period) effects were significant ($P < 0.01$). Treatment x period and treatment x day(period) interactions were also significant ($P < 0.01$), and periods with different letters differ ($P < 0.05$).

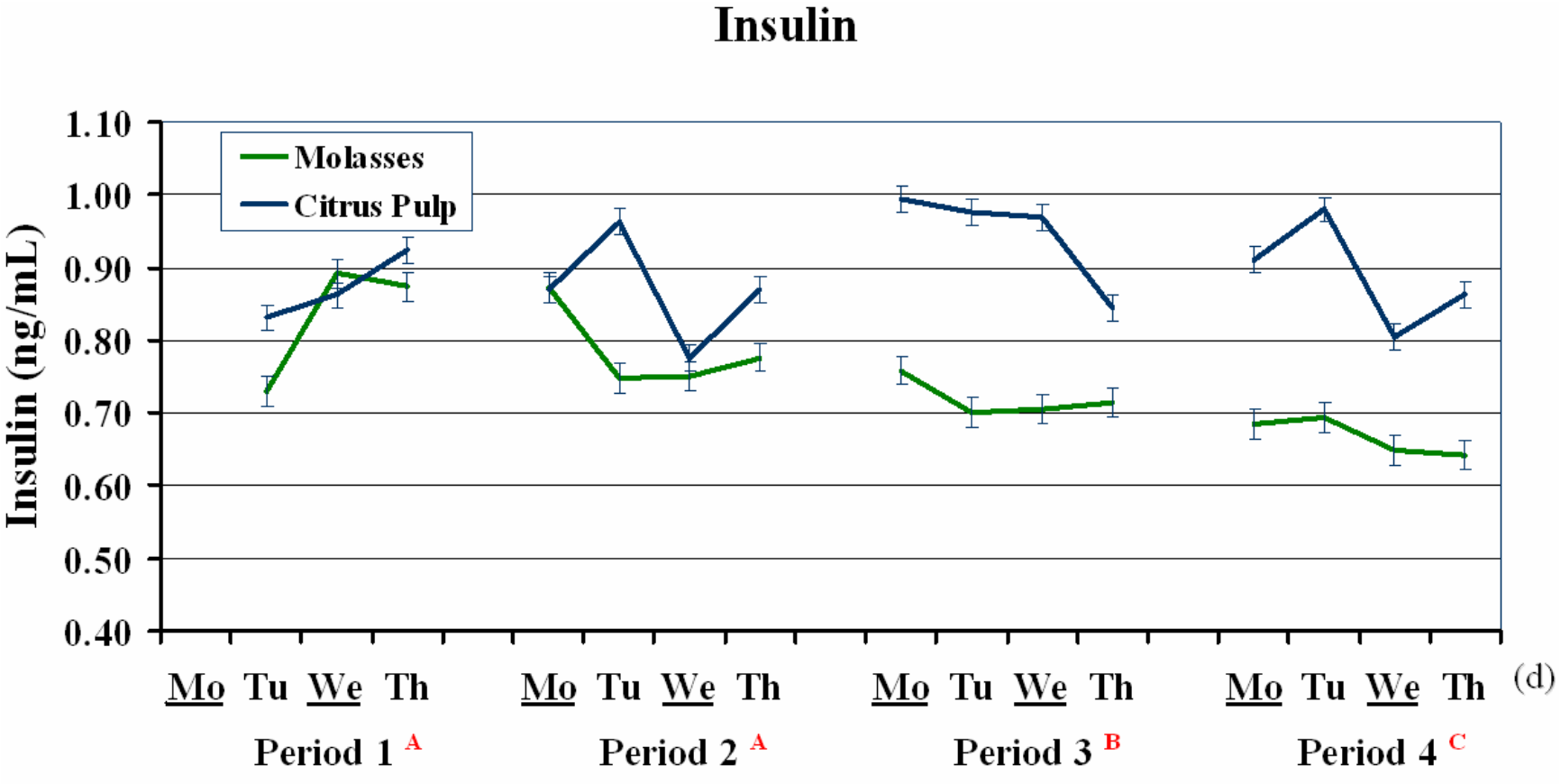

Figure 3-4. Plasma insulin concentrations of heifers supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Days at which supplements were offered are underlined. Heifers fed CT had greater mean insulin concentration ($P < 0.05$, SEM = 0.04). Period and day(period) effects were significant ($P < 0.01$). Treatment x period interaction was also significant ($P < 0.01$), and periods with different letters differ ($P < 0.01$).

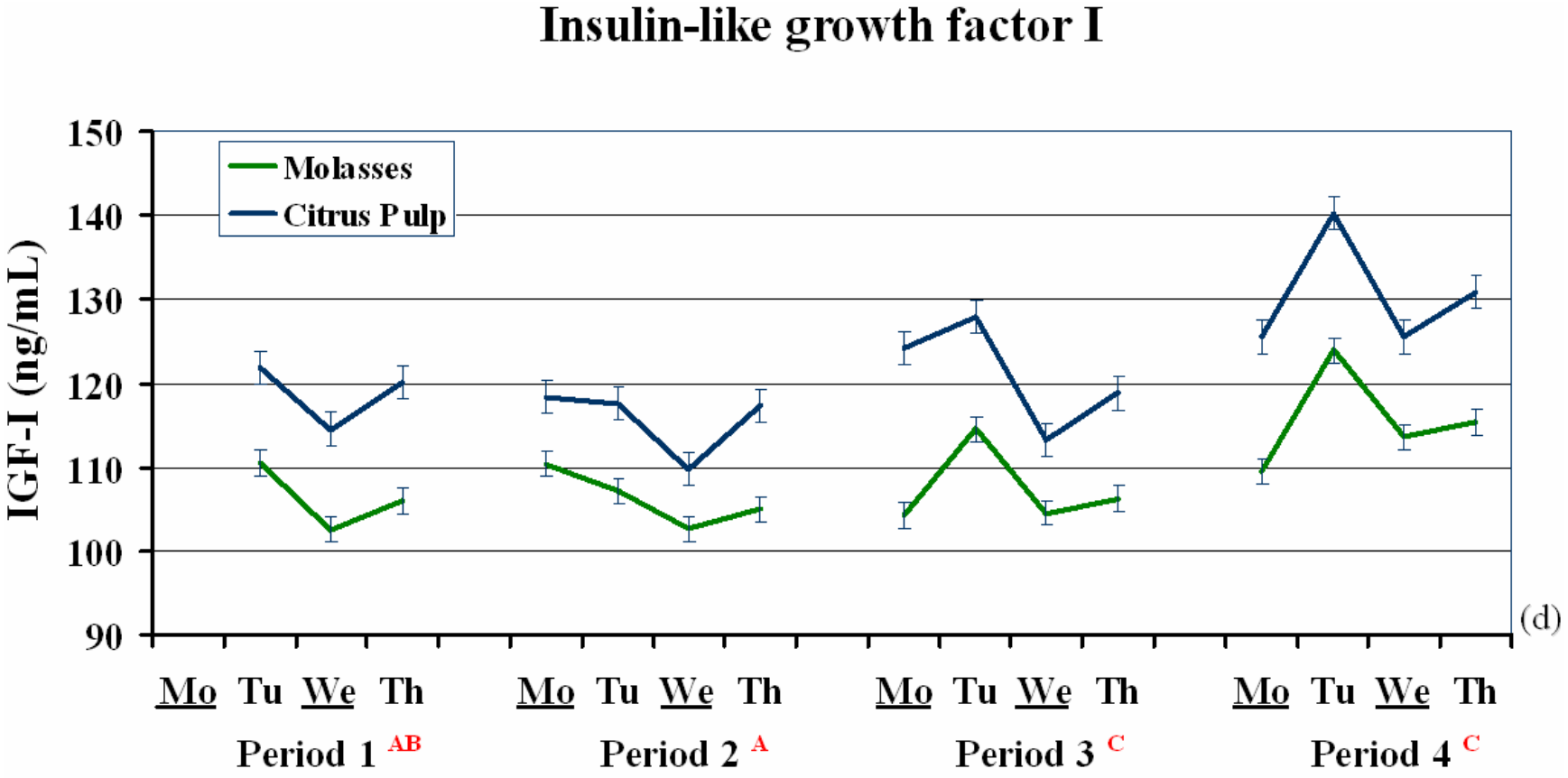

Figure 3-5. Plasma insulin-like growth factor I (IGF-I) concentrations of heifers supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Days at which supplements were offered are underlined. Heifers fed CT had greater mean IGF-I concentration ($P = 0.05$, SEM = 4.10). Period and day(period) effects were also significant ($P < 0.01$), and periods with different letters differ ($P < 0.05$).

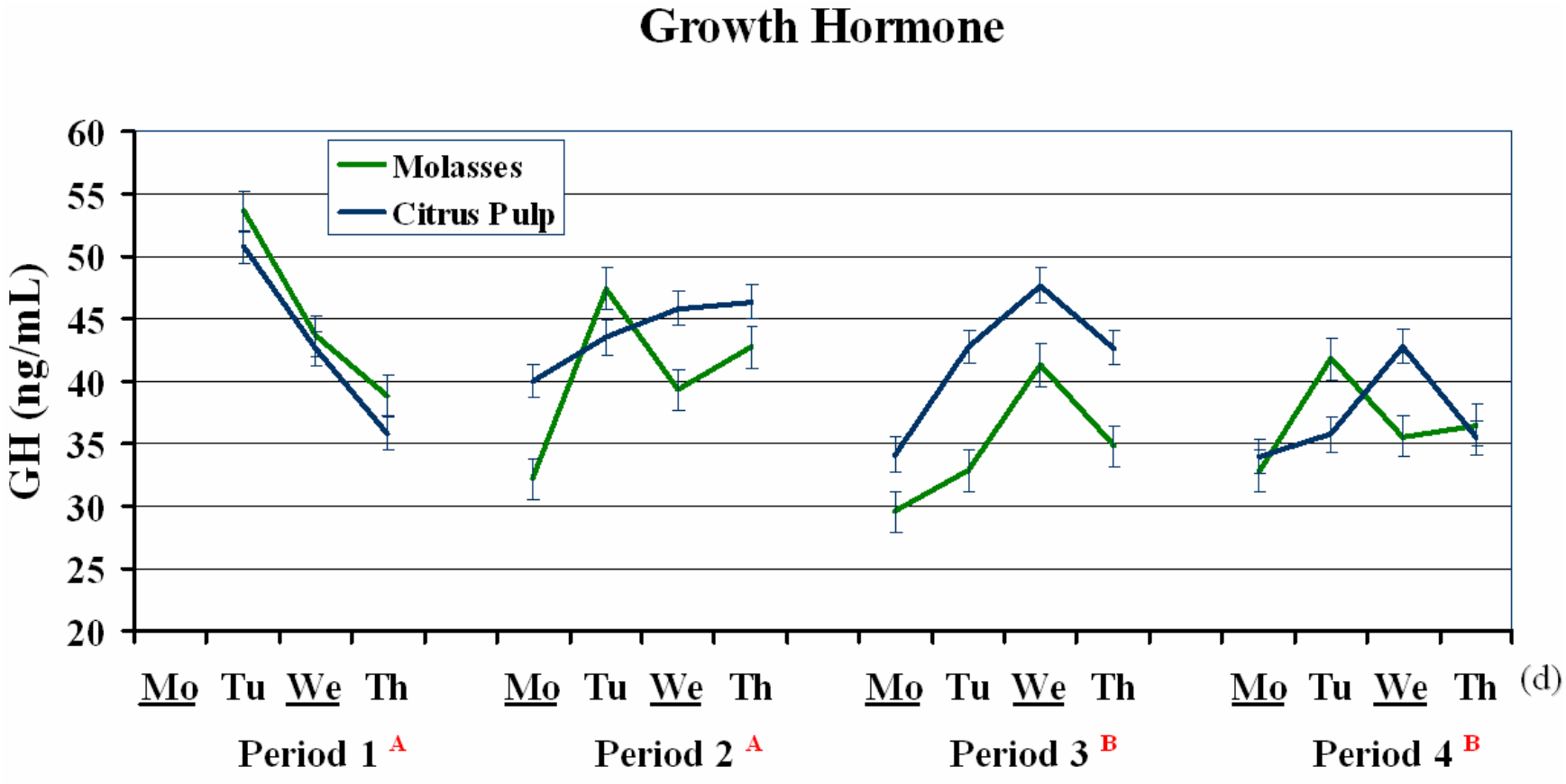

Figure 3-6. Plasma growth hormone (GH) concentrations of heifers supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Days at which supplements were offered are underlined. No significant differences were observed ($P = 0.62$, SEM = 3.20). Period and day(period) effects were significant ($P < 0.01$). Treatment x period interaction was also significant ($P < 0.05$), and periods with different letters differ ($P < 0.05$).

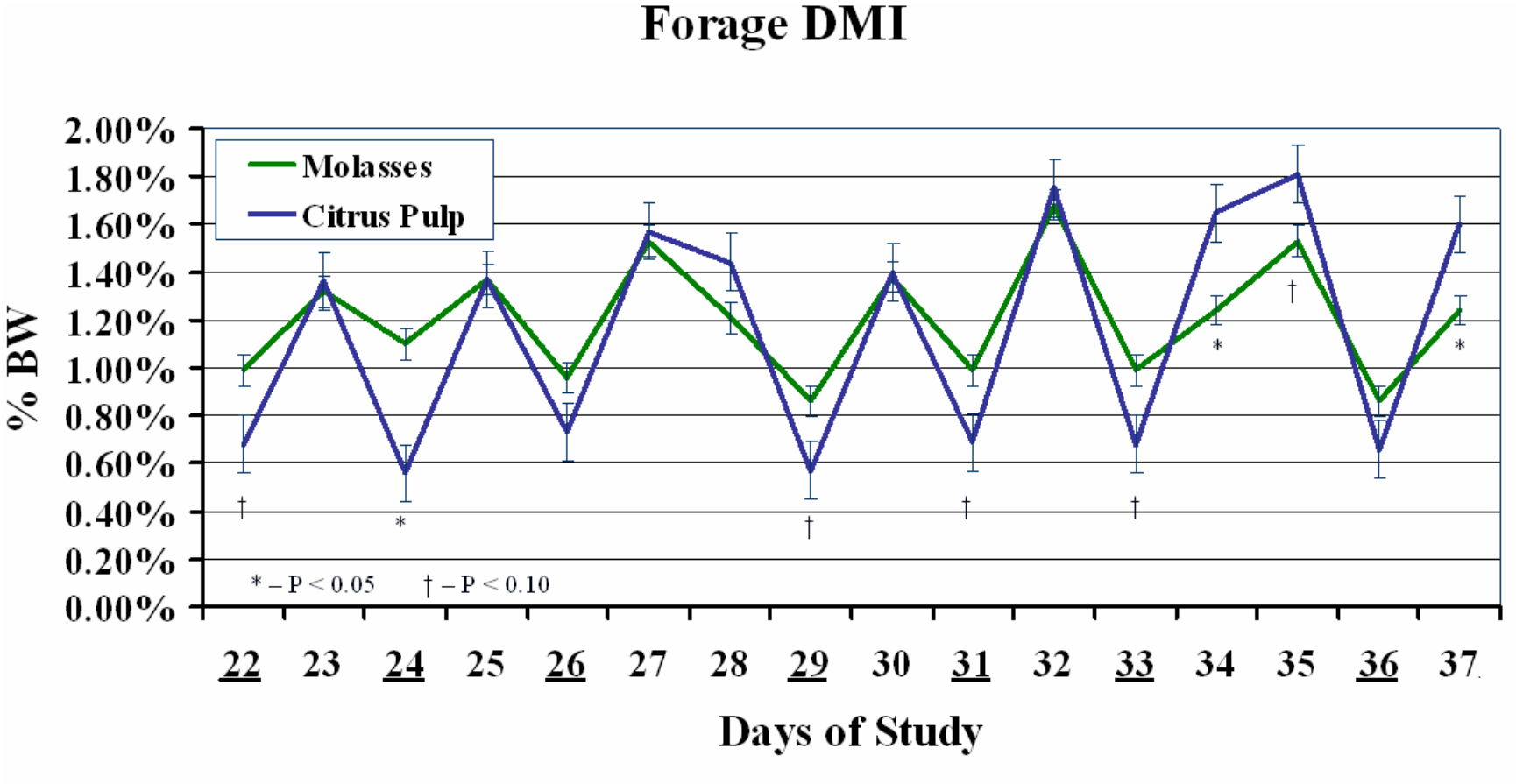

Figure 3-7. Forage DMI of cows supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Days at which supplements were offered are underlined. No significant differences were observed (1.16 vs. 1.20 % for CT and ML, respectively; P = 0.52, SEM = 0.08). Day effect and treatment x day interaction were significant (P < 0.01).

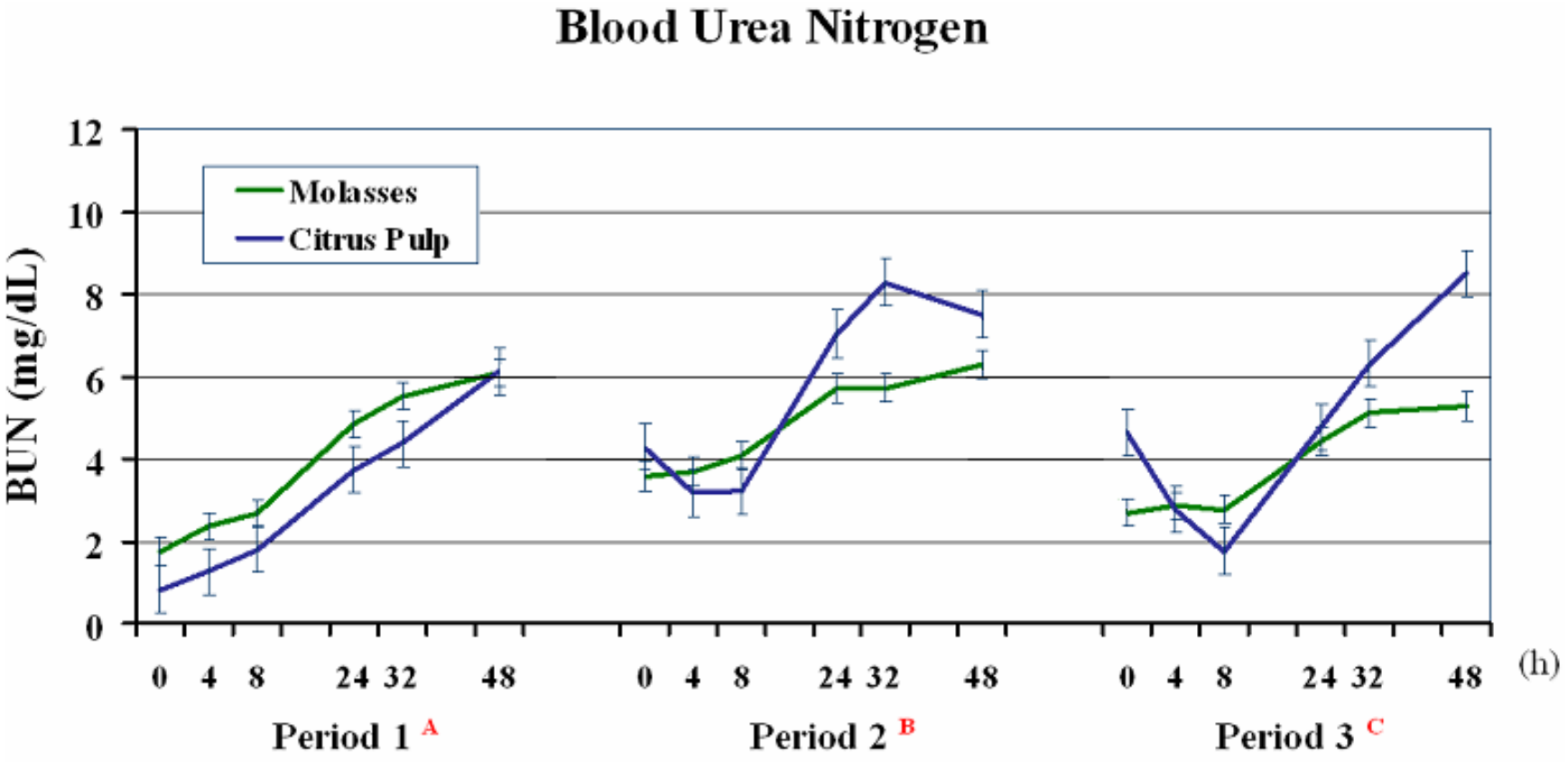

Figure 3-8. Blood urea nitrogen (BUN) concentrations of cows supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Supplements were fed after blood was sampled at 0 h. No significant differences were observed ($P = 0.52$, SEM = 0.29). Period and time(period) effects were significant ($P < 0.01$). Treatment x period and treatment x time(period) interactions were also significant ($P < 0.01$), and periods with different letters differ ($P < 0.01$).

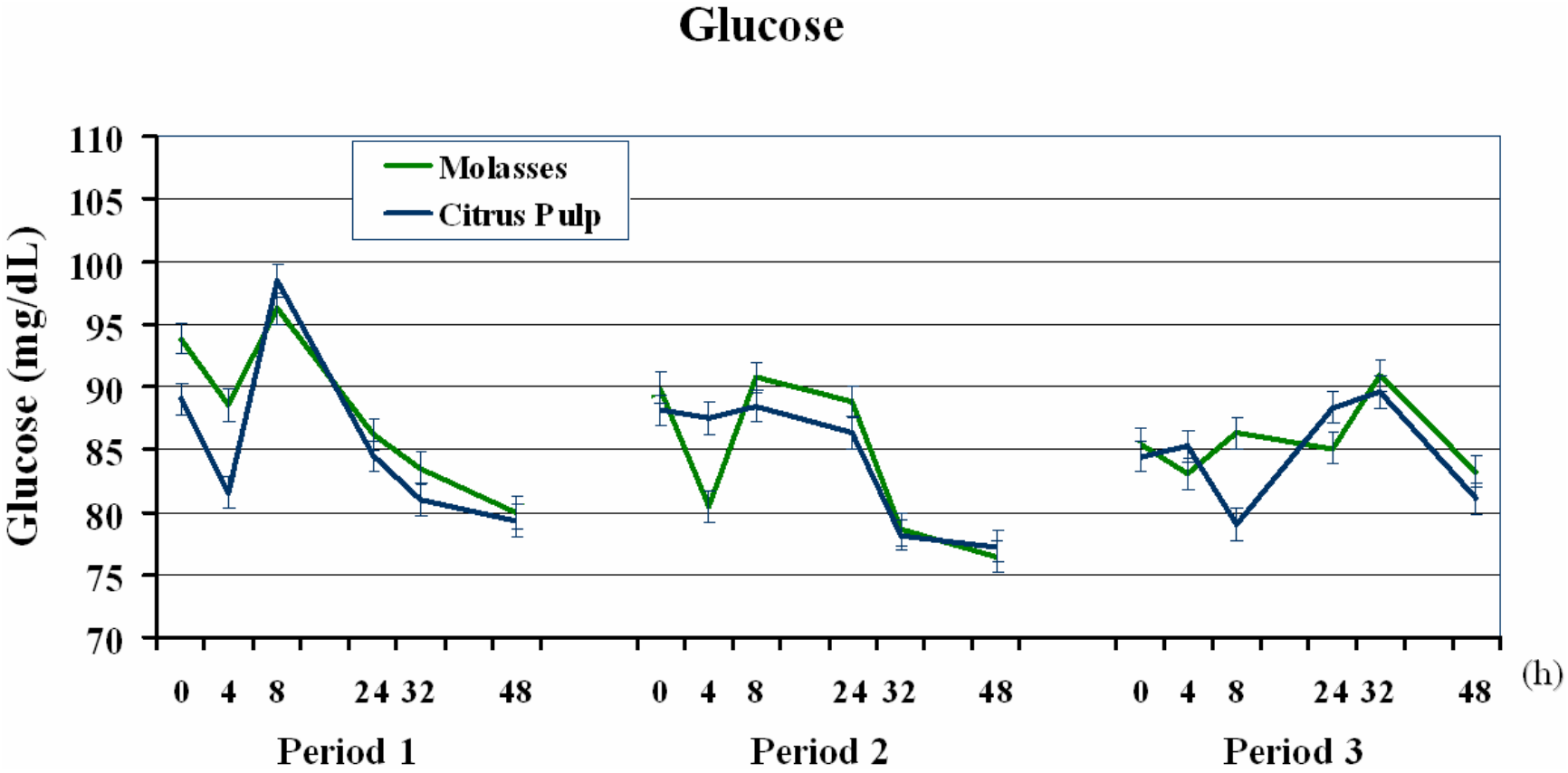

Figure 3-9. Plasma glucose concentrations of cows supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Supplements were fed after blood was sampled at 0 h. No significant differences were observed (P = 0.88, SEM = 5.12). Time effect was significant (P < 0.01).

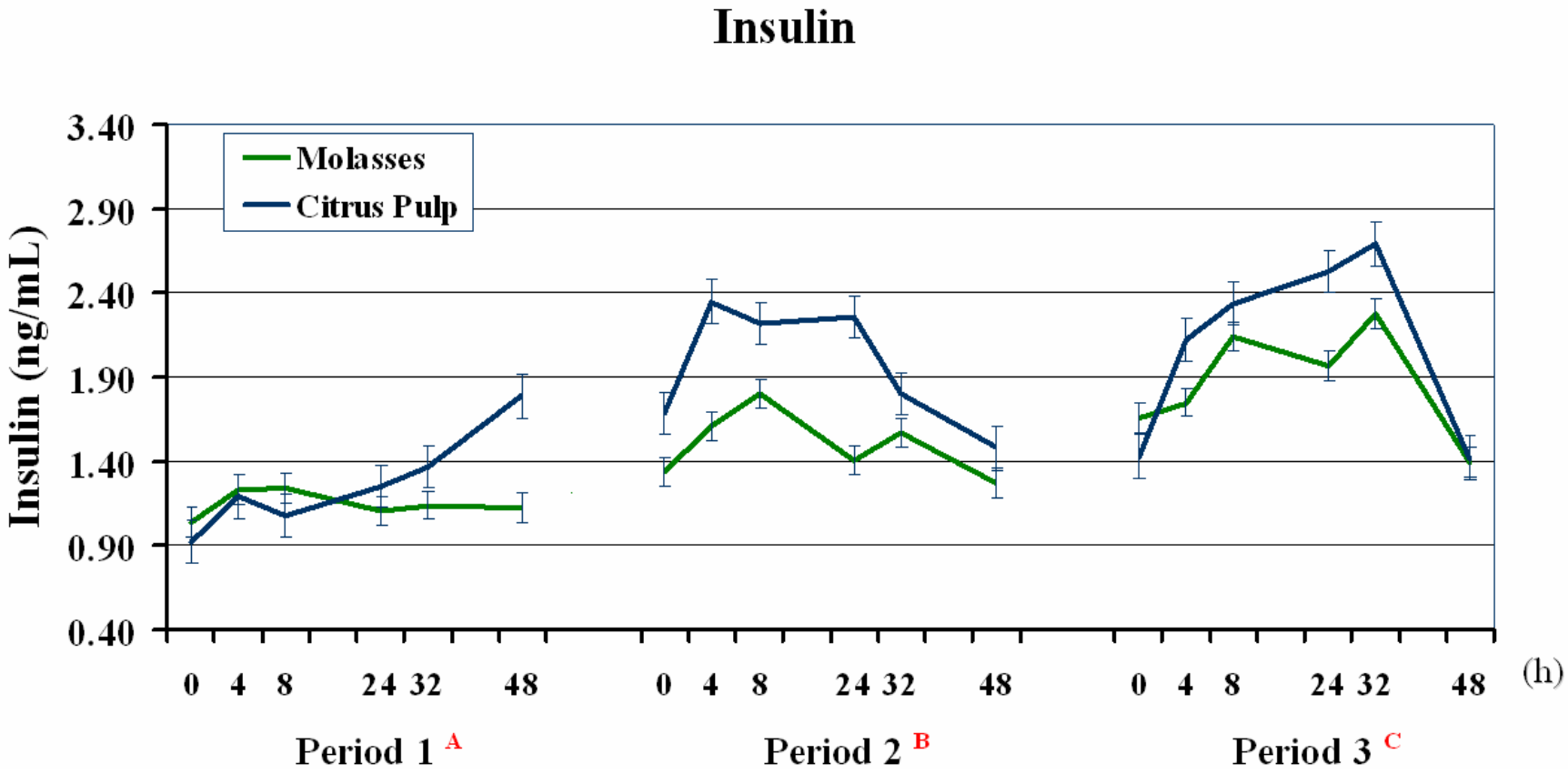

Figure 3-10. Plasma insulin concentrations of cows supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Supplements were fed after blood was sampled at 0 h. No significant differences were observed (P = 0.33, SEM = 0.19). Period and time(period) effects were significant (P < 0.01). Treatment x period interaction was also significant (P < 0.05), and periods with different letters differ (P < 0.01).

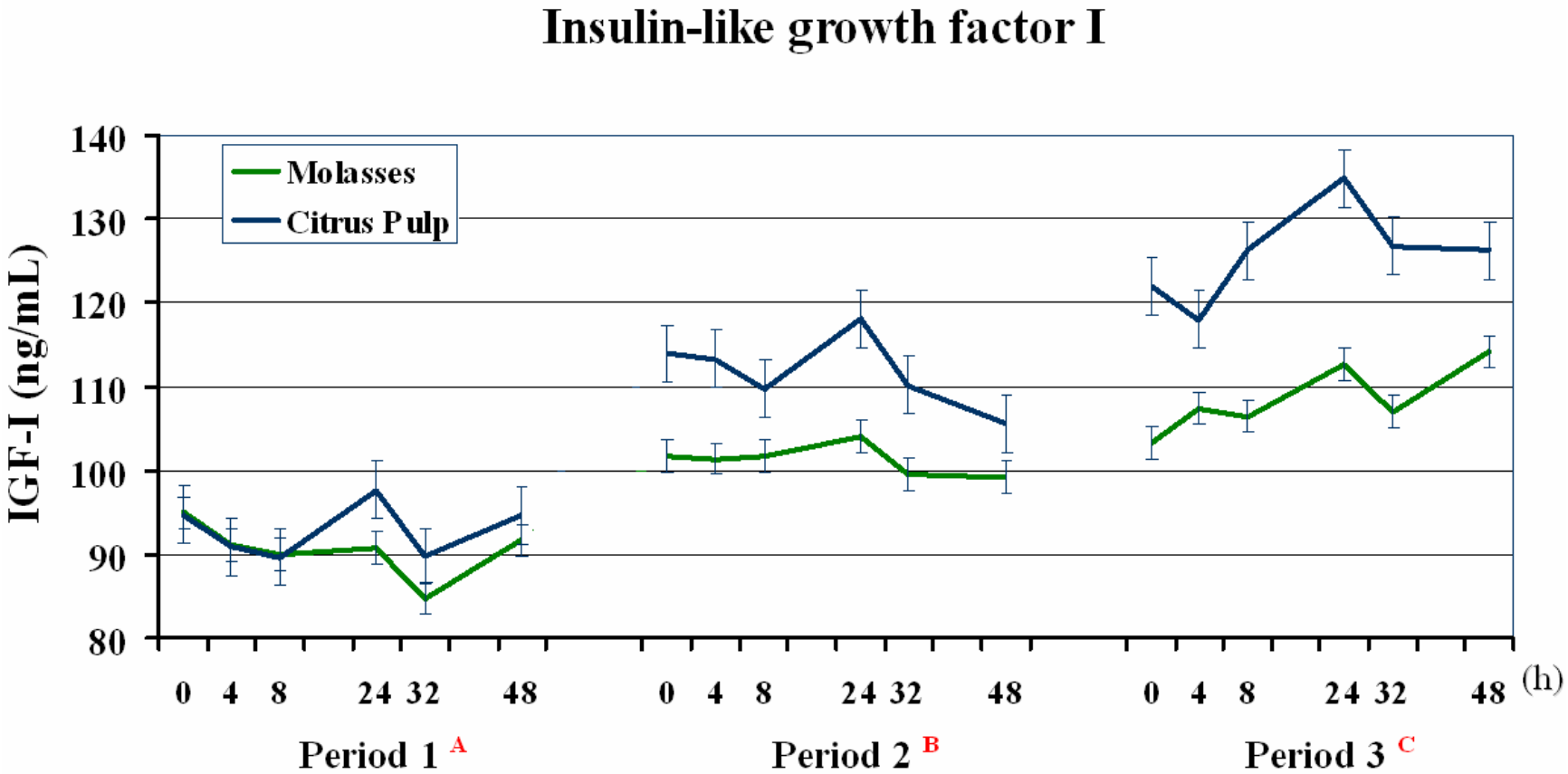

Figure 3-11. Plasma insulin-like growth factor I (IGF-I) concentrations of cows supplemented with citrus pulp- (CT) or molasses-based supplement (ML). Supplements were fed after blood was sampled at 0 h. No significant differences were observed ($P = 0.49$, SEM = 9.88). Period effect was significant ($P < 0.01$). Treatment x period interaction was also significant ($P < 0.01$), and periods with different letters differ ($P < 0.05$).

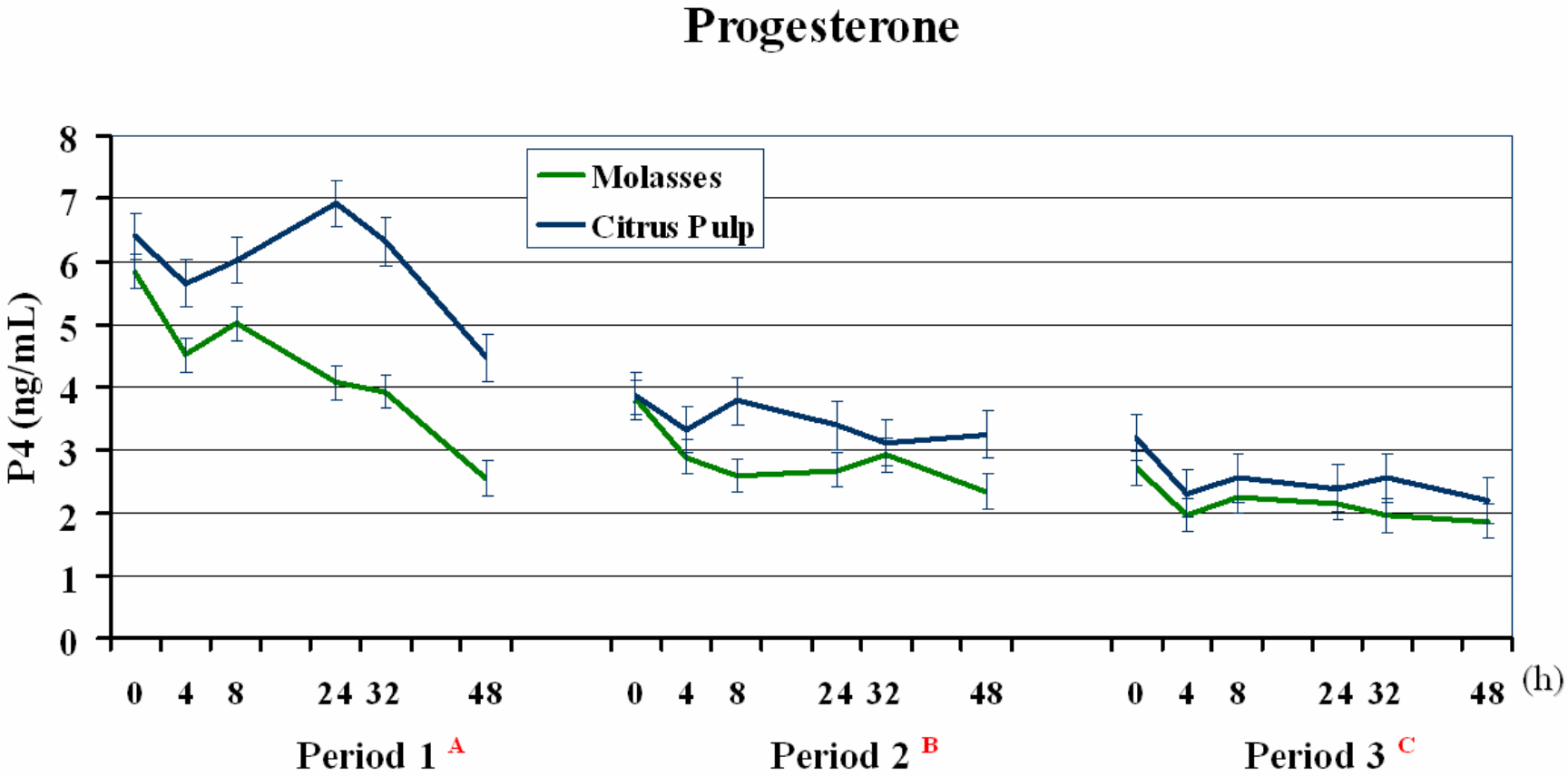

Figure 3-12. Plasma progesterone (P4) concentration of cows with CIDR and supplemented with citrus pulp- (CT) or molasses-based supplement (ML). No significant differences were observed (P = 0.12, SEM = 0.33). Period and time(period) effects were significant (P < 0.01). Treatment x period interaction was also significant (P < 0.01), and periods with different letters differ (P < 0.01).

CHAPTER 4
EFFECTS OF SUPPLEMENT TYPE AND FEEDING FREQUENCY ON PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF YEARLING BRAHMAN-CROSSBRED STEERS

Introduction

Energy supplementation is required in most of Florida cow-calf operations because the tropical perennial grasses grown in the state usually do not have adequate energy content to meet the requirements of the cattle (Moore et al., 1991). The frequency at which supplements are offered depends on the supplement type and also on the management system of the operation. According to a review compiled by Kunkle et al. (1999), cattle supplemented daily, 3x/wk, or 1x/wk have similar rates of BW gain. However, the majority of these publications referred to protein or grain-based supplements, and none evaluated energy supplements based on low-starch by-products, such as molasses or citrus pulp.

Citrus pulp and molasses originate from orange and sugarcane production, respectively, which are both important and abundant agricultural enterprises in Florida. For that reason, these feeds are widely used as energy supplements for beef cattle operations across the state. Molasses, despite its high DM content (approximately 75 %), is classified as a liquid feed, whereas citrus pulp is commonly processed and fed as dry pellets. Differences in physical form between citrus pulp and molasses may lead to differences in feed intake behavior (Arthington et al. 2004). Molasses and citrus pulp also differ in their carbohydrate profile. Although both are low-starch energy feedstuffs, sucrose is the main carbohydrate of molasses, whereas pectin is the main carbohydrate of

citrus pulp. Pectin and sucrose are fermented differently in the rumen, potentially impacting forage intake, diet digestibility, and energy utilization.

The objective of this experiment was to investigate the effects of supplement type and feeding frequency on performance, plasma metabolites and hormones, and voluntary forage intake of yearling steers.

Material and Methods

This study was conducted at the University of Florida – IFAS, Range Cattle Research and Education Center, Ona. The study was conducted during the months of November and December 2004. The animals utilized in this experiment were cared for by acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals

Twenty four Brahman x British crossbred steers (BW ± SD = 257 ± 26 kg; age ± SD = 12 ± 1 mo) were utilized in this experiment. Steers were stratified by initial BW and randomly allocated to 12 pens (2 steers/pen). Pens were then randomly assigned to one of three treatments: 1) molasses-based supplement fed 3x/wk (ML), 2) citrus pulp-based supplement fed 3x/wk (CT), or 3) citrus pulp-based supplement fed daily (CD). Pen was the experimental unit (4 pens/treatment).

Diets

Limpograss (*Hemarthria altissima*) hay (54 % TDN and 9.1 % CP; DM basis) was coarsely ground and offered at amounts to ensure ad libitum intake throughout the study. Steers also had free access to a complete mineral mix and water. Treatments consisted of three energy supplements (Table 4-1), fed either 3x/wk (Mondays, Wednesdays and Fridays for ML and CT) or every day (CD) at a daily rate of 2.3 kg of DM per heifer for

CT and CD and 2.1 kg of DM per heifer for ML. Supplement intakes were formulated to be iso-caloric and iso-nitrogenous (Table 4-2) and also balanced for calcium content, given the high concentration of calcium in citrus pulp.

Sampling

One week before the start and at the end of the study, steers were weighed for 2 consecutive days to determine both full and shrunk BW (after 16 h of feed restriction).

During the first 3 weeks of the study (d 1 to d 21), blood samples were collected immediately prior and 4, 8, 24, 32 and 48 h after the first supplement feeding of the week (d 1, 8 and 15) for determination of glucose, blood urea nitrogen (BUN), insulin, IGF-I and GH concentrations. Weeks were considered periods (week 1, P 1; week 2, P 2; week 3, P 3) for further statistical analysis.

For the second part of the study (d 22 to d 40), forage DMI was recorded daily. Hay refusal was collected and weighed before hay and supplement feeding. A sample of the hay was collected twice during this period for nutrient analysis, while samples of the non-consumed hay were collected daily from each pen to determine DM content. Hay samples were dried for 96 h at 50º C in forced-air ovens. Random samples of the feedstuffs also were collected during the trial, and all samples were analyzed for nutrient composition at commercial laboratories (Dairy One Forage Laboratory, Ithaca, NY for hay, cottonseed meal and citrus pulp samples, and SDK Laboratories, Hutchinson, KS for molasses samples).

Blood Analysis

Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing

sodium heparin, placed on ice immediately after sampling, and centrifuged at 855 g for 30 min for plasma collection. Plasma was frozen at -20°C at the same day of collection.

A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to determine both plasma glucose (Coulombe and Favreau, 1963; modified and described by Bran + Luebbe Industrial Method #339-01) and BUN (Gochman and Schmitz, 1972; modified and described by Bran + Luebbe Industrial Method #339-19). A double antibody radioimmunoassay (RIA) was used to determine plasma concentrations of insulin (Malven et al., 1987; Badinga et al., 1991), GH (Badinga et al., 1991) and IGF-I (Badinga et al., 1991). The extraction procedure used in the IGF-I assay was modified from Badinga et al. (1991) by using a ethanol:acetone:acetate ratio of 6:3:1. The intra- and inter-assay CVs were, respectively, 5.79 and 28.26 % for insulin, 7.80 and 7.86 % for IGF-I, and 13.47 and 18.06 % for GH.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS, 2001).

The statistical model used for forage DMI data analysis was:

$$Y = \mu + TRT_i + PEN_{j(i)} + DAY_k + TRTDAY_{ik} + E_{ijkl}$$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

PEN = random effect of pen within treatment

DAY = fixed effect of day

TRTDAY = effect due to interaction of treatment and day

E = residual error

The statistical model used for plasma metabolites and hormones data analysis was:

$Y = \mu + TRT_i + P_j + PEN_{k(i)} + STEER_{l(k)} + TIME_{m(j)} + TRTP_{ij} + TRTTIME_{im(j)} + E_{ijklm}$

where

Y = response variable

μ = mean

TRT = fixed effect of treatment

P = fixed effect of period

PEN = random effect of pen within treatment

STEER = random effect of steer within pen

TIME = fixed effect of time within period

TRTP = effect due to interaction of treatment and period

TRTTIME = effect due to interaction of treatment and time within period

E = residual error

Results are reported as least square means. Means were separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.15. Only significant interactions are reported.

Results and Discussion

Molasses is a viscous liquid feedstuff, often mixed with protein ingredients and fed as slurries. The majority of beef cattle operations offer molasses slurries one to three times per week; therefore we did not include a treatment with the molasses-based supplement fed daily. Supplement intake behavior was observed for all treatments. Steers

fed ML consumed the whole amount of supplement by approximately 48 h after feeding. Steers fed CT consumed their offer by 24 to 36 h, whereas CD-fed steers consumed their offer within 4 h.

Mean BW gain was greater ($P < 0.05$) for CD vs. ML, and tended to be greater ($P = 0.13$) for CD vs. CT (0.30, 0.18 and 0.10 kg/d for CD, CT and ML, respectively; SEM = 0.05). Similar to our previous experience with heifers, feeding citrus pulp-based supplements at the same frequency of molasses-based supplements numerically increased BW gain. However, differences became significant if supplementation frequency increased. Our findings concur with previous reports indicating inferior BW gain for animals fed molasses compared to animals fed other energy sources (Pate, 1983; Royes et al., 2001). Feeding citrus pulp-based supplements daily tended to increase BW gains compared to the same supplement fed 3x/wk. To our knowledge, no studies evaluating supplementation frequency with citrus pulp-based supplements have been reported.

Overall forage DMI did not differ among treatments (1.50, 1.29 and 1.36 % for ML, CT and CD, respectively; P = 0.16, SEM = 0.07; Figure 4-1), but ML tended to have greater forage DMI than CT ($P = 0.06$, SEM = 0.07). From d 31 to the end of the experiment, the collection of forage leftovers and the consequent forage DMI observation were debilitated by inclement weather. The differences between ML and CT became intensified during this period and therefore, the statistical tendency observed may not be realistic. A significant day effect and also a significant treatment x day interaction were detected ($P < 0.01$). Forage DMI was less for CT and ML during the days that these supplements were fed (0.99 and 1.56 for CT vs. 1.41 and 1.59 % BW for ML during feeding days and non-feeding days, respectively; $P \leq 0.01$, SEM = 0.08). A day effect

was not significant for CD-fed steers ($P = 0.35$). These findings concur with our previous experience with mature cows. Supplement consumption was greater for CT- and ML-fed steers during the days it was offered, and since forage DMI is negatively associated with energy supplement intake, forage DMI was reduced (Caton and Dhuyvetter, 1997; Bodine and Purvis, 2003). In addition, forage DMI for steers fed CT was less compared to steers fed ML during feeding days only (0.99 vs. 1.41 % BW, respectively, $P < 0.01$, SEM = 0.08). Greater supplement intake after feeding for CT steers might be the cause for the decreased forage consumption, since these steers consumed the entire amount of supplement sooner than steers fed ML (24 to 36 h vs. 48 h, respectively). Feeding energy supplements daily instead of 3x/wk decreased the oscillation, but not overall forage DMI. This effect may be explained by a reduction of the substitution effect. During the experiment, supplement consumption was mainly constant for CD-fed steers, but oscillated significantly for the steers fed CT. Several studies indicated that forage intake was decreased when non-structural carbohydrates-based supplements were fed (Sanson et al., 1990; Olson, et al., 1999). The same response were not realized when fibrous by-product supplements were fed (Bowman and Sanson, 2000). In the present study, however, this pattern was not observed. Steers fed ML tended ($P = 0.06$) to have greater forage intake than CT-fed steers, however, this result should be interpreted with caution.

Concentration of BUN tended to be greater for CD vs. ML (3.55 vs. 1.97 mg/dL, respectively; $P = 0.06$, SEM = 0.53) (Table 4-3, Figure 4-2), but did not differ for CD vs. CT (2.92 mg/dL for CT; $P = 0.42$) and CT vs. ML ($P = 0.23$). A significant time(period) effect ($P < 0.01$) was observed. For all treatments, BUN concentrations increased after feeding. A significant period effect also was detected ($P < 0.01$). Mean BUN

concentration of all treatments was greater ($P < 0.01$) during the first period compared to the second and third periods (4.02, 2.25 and 2.17, respectively; SEM = 0.33). Concentrations of BUN are directly correlated with the amount of ammonia produced and absorbed in the rumen (Huntington, 1990). Ammonia is a product of dietary protein degradation by the microbial population in the rumen and is a major precursor for microbial protein synthesis. Concentrations of BUN should range between 11 and 15 mg/dL for growing animals to ensure maximum rates of gain (Byers and Moxon, 1980). This goal can be achieved by feeding animals with correct quantities of protein and energy (Hammond, 1997; Huntington, 1997), and also synchronizing the supply of these nutrients in the rumen by feeding energy and protein sources with similar rates of ruminal degradability (Spicer et al., 1986; Matras et al., 1991; Taniguchi et al., 1995). In the current study, all treatments would be deficient in protein content according to the BUN concentrations observed, since they were always below the optimal range (Table 4-3). However, treatments were formulated to supply the requirements of growing steers, using the NRC model (NRC, 1996). Feeding citrus pulp-based supplements daily seemed to improve the protein status of steers when compared to molasses-based supplements fed 3x/wk. This response may be associated with an improved synchrony of protein and energy availability in the rumen.

Plasma glucose concentrations were decreased for CD vs. CT and ML (66.05, 76.24, and 76.55 mg/dL, respectively; $P < 0.05$, SEM = 3.02) (Table 4-3, Figure 4-3) but did not differ for CT vs. ML ($P = 0.94$). A time(period) effect was detected ($P < 0.01$) and can be attributed to the variance in glucose synthesis and availability in blood after supplements were offered and consumed. A period effect also was detected ($P < 0.01$).

Mean plasma glucose concentration of all treatments was greater ($P < 0.01$) during P 1 and P 2 vs. P 3 (73.85, 74.40 and 70.60 mg/dL, respectively; SEM = 1.80). Plasma insulin concentration was greater for CT vs. CD and ML (0.60, 0.46 and 0.43 ng/mL, respectively; $P < 0.05$, SEM = 0.04) (Table 4-3, Figure 4-4) but did not differ between CD vs. ML ($P = 0.63$). A significant time(period) effect was observed ($P < 0.01$) and can be associated with fluctuations of plasma glucose concentrations, since the concentration of these substances in blood are mutually dependent. A significant treatment x period interaction was detected ($P < 0.01$). From P 1 to P 3, plasma insulin concentration increased for CT ($P < 0.01$), was constant for ML, and decreased for CD ($P < 0.01$). Plasma IGF-I concentration did not differ among treatments (96.49, 102.71 and 78.65 ng/mL for CD, CT and ML, respectively; $P = 0.27$, SEM = 10.22) (Table 4-3, Figure 4-5). A period effect was significant ($P < 0.01$). Mean IGF-I concentration of all treatments increased from P 1 to P 3 (78.00, 97.37 and 102.49 ng/mL, respectively; SEM = 5.99). Additionally, steers fed CD had the greatest increase in plasma IGF-I concentrations, reflected in the treatment x period interaction ($P < 0.01$). Plasma GH concentrations did not differ among treatments (74.52, 74.85 and 76.21 ng/mL for CD, CT and ML, respectively; $P = 0.98$, SEM = 8.00) (Table 4-3, Figure 4-6). A period effect was observed ($P < 0.01$) since mean plasma GH concentration of all treatments decreased from the first to the third period (84.15, 76.85 and 64.59 ng/mL, respectively, SEM = 5.07). A significant time(period) effect also was detected ($P < 0.01$). This was likely due to a pulsatile pattern of GH release, ranging from 10 to 15 pulses/d (Hornick et al., 1998; Bossis et al., 1999). Although blood sampling was relatively frequent in this experiment, it was not frequent enough to identify pulses or basal concentrations of this hormone.

Several studies conducted with beef cattle reported a relationship between the glucose/insulin/IGF-I/GH axis and animal BW gain. Increased rates of BW gain have been associated with greater concentrations of blood glucose, insulin, and IGF-I, and decreased concentrations of GH (Vizcarra et al., 1998; Bossis et al., 1999; Lapierre et al., 2000). However, in the present study, steers fed CD had greater BW gain than CT- and ML-fed steers, but lesser concentrations of glucose and insulin (only compared to CT), and similar IGF-I and GH concentrations. We theorized that CD-fed steers had a more efficient utilization of energy compared to the other treatments. The lesser plasma glucose concentration may have been the result of enhanced cell uptake. Consequently, less insulin was required to activate the receptors and regulate the transport of glucose into the cells. This effect was mostly noted during the second and third period (Figure 4-4). However, IGF-I concentrations, which usually are positively correlated with insulin concentrations (Keisler and Lucy, 1996; Webb et al., 2004), rapidly increased from the P 1 to P 3 for CD-fed steers (Figure 4-5). This indicates that, althought glucose and insulin concentrations were not as great, energy status was improving at a greater rate for CD-fed steers compared to the other treatments, which may have resulted in the greater BW gain.

Conclusions

We concluded that feeding a citrus pulp-based supplement daily improved steer performance compared to the same supplement fed 3x/wk or an iso-caloric and iso-nitrogenous supplement based on liquid molasses also fed 3x/wk. However, these results were not reflected by the blood hormones and metabolites usually associated with animal performance, such as glucose, insulin, IGF-I and GH.

Table 4-1. Composition of supplements fed to steers.

Ingredients (%; as-fed)	**ML** [1]	**CT** [2]	**CD** [3]
Blackstrap molasses	78.30	0.00	0.00
Citrus pulp	0.00	74.70	74.70
Cottonseed meal	20.20	25.30	25.30
Calcium carbonate	1.50	0.00	0.00

[1] ML = citrus pulp-based supplement fed 3x/wk (Mondays, Wednesdays and Fridays).
[2] CT = molasses-based supplement fed 3x/wk (Mondays, Wednesdays and Fridays).
[3] CD = citrus pulp-based supplements fed daily.

Table 4-2. Nutrient composition and intake rate of supplements fed to steers [1].

Nutrient Composition (%; DM)	**ML**	**CT**	**CD**
TDN	77.00	70.00	70.00
CP	20.07	19.00	19.00
Calcium	1.80	1.70	1.70
Phosphorus	0.50	0.60	0.60
Supplement Intake (kg/head/d) [2]			
DM	2.10	2.30	2.30
TDN	1.61	1.61	1.61
CP	0.43	0.43	0.43
Calcium	0.04	0.04	0.04
Phosphorus	0.01	0.01	0.01

[2] Estimated from the supplement consumption of the pen (2 steers/pen).

Table 4-3. Effects of treatments on plasma metabolites and hormones of steer fed 3x/wk a citrus pulp- (CT) or molasses-based supplements (ML), or fed daily a citrus-pulp based supplement (CD) [1].

	Period 1						Period 2						Period 3							
(h)	0	4	8	24	32	48	0	4	8	24	32	48	0	4	8	24	32	48	SE	*P* =
BUN [2]																				0.161
CT	3.6	3.4	4.9	4.1	4.6	5.6	2.0	1.1	0.8	2.7	3.6	3.6	1.0	0.7	0.5	2.4	3.6	3.9	0.52	
ML	1.2	2.6	3.7	2.9	3.7	3.9	1.4	1.4	1.0	0.6	1.3	1.7	0.8	0.8	0.8	1.1	2.1	3.9	0.52	
CD	3.6	3.9	2.7	5.6	4.9	6.9	3.1	2.6	1.8	3.9	2.4	5.0	2.2	1.5	1.0	3.7	2.2	6.5	0.53	
Glucose [2]																				0.063
CT	72.7	73.7	76.0	75.2	80.1	81.7	73.9	79.8	76.8	78.2	77.4	81.8	72.2	70.9	74.0	77.9	82.5	67.0	2.89	
ML	75.9	75.6	72.6	79.5	81.0	82.1	78.3	78.6	72.7	72.2	79.3	83.7	74.1	71.7	74.6	76.5	76.9	72.1	3.10	
CD	59.1	65.6	70.7	66.9	69.4	70.7	68.1	68.3	68.0	63.2	65.7	72.6	65.8	60.8	63.5	62.6	68.1	59.3	3.08	
Insulin [2]																				0.034
CT	0.41	0.61	0.66	0.50	0.61	0.49	0.52	0.63	0.74	0.74	0.64	0.50	0.50	0.70	0.71	0.72	0.64	0.51	0.04	
ML	0.40	0.47	0.44	0.38	0.44	0.32	0.34	0.48	0.45	0.41	0.45	0.37	0.41	0.51	0.57	0.42	0.44	0.35	0.04	
CD	0.47	0.55	0.68	0.46	0.44	0.41	0.50	0.58	0.45	0.40	0.47	0.39	0.43	0.43	0.45	0.34	0.44	0.33	0.04	
IGF – I [2]																				
CT	86.6	87.2	84.0	90.2	86.6	93.2	107.0	106.9	106.6	111.7	107.2	101.5	110.5	115.1	107.1	112.5	123.0	111.5	10.21	
ML	76.4	76.0	73.1	66.4	63.6	65.2	82.6	81.1	81.8	86.7	80.5	81.2	84.0	85.4	76.8	86.9	87.0	80.4	10.21	
CD	85.5	82.6	70.5	74.2	67.8	74.2	105.5	104.6	95.7	100.8	104.8	105.9	108.3	110.3	106.4	110.3	111.2	117.7	10.23	
GH [2]																				0.987
CT	80.9	81.4	75.6	97.6	91.1	74.4	61.8	55.2	84.7	73.3	100.5	70.8	64.5	63.7	66.7	68.1	62.7	73.9	7.93	
ML	74.8	110.0	70.3	100.9	94.4	80.5	63.6	78.8	71.9	92.1	89.4	82.3	49.2	48.8	64.8	70.0	77.6	51.9	8.00	
CD	75.1	97.3	61.8	72.9	74.8	100.2	71.0	54.4	70.0	90.5	83.1	89.4	77.0	63.4	81.4	57.3	59.1	62.1	8.07	

[1] Supplements were offered after blood was sampled at 0 h.
[2] BUN and Glucose = mg/dL; Insulin, IGF – I, and GH = ng/mL.

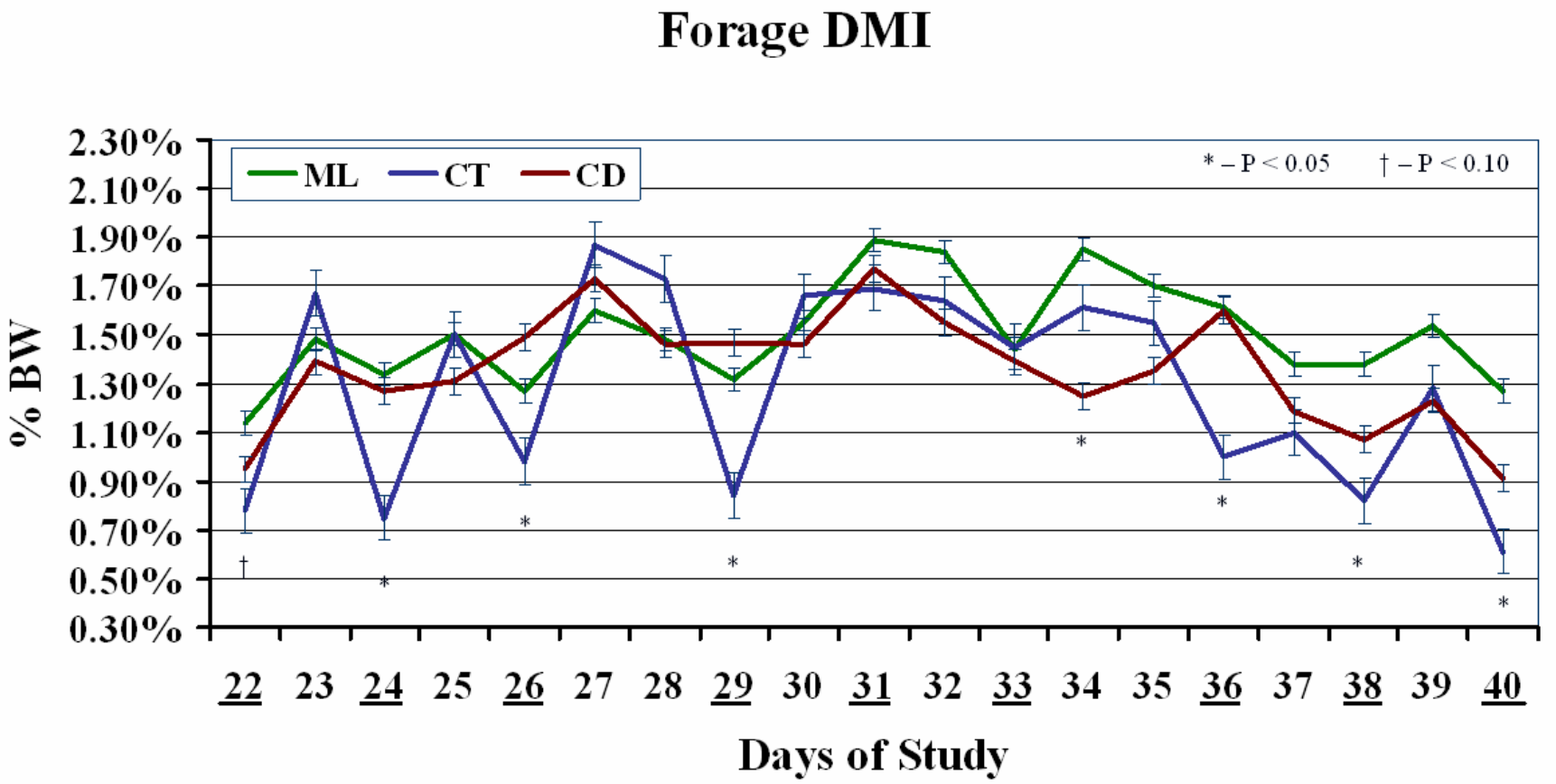

Figure 4-1. Forage DMI of steers supplemented 3x/wk with citrus pulp- (CT) or molasses-based supplement (ML), or daily with citrus pulp-based supplement (CD). Days at which supplements were offered are underlined. No significant differences were observed (1.50, 1.29 and 1.36 % for ML, CT and CD, respectively; P = 0.16, SEM = 0.07). Day effect and treatment x day interaction were significant (P < 0.01).

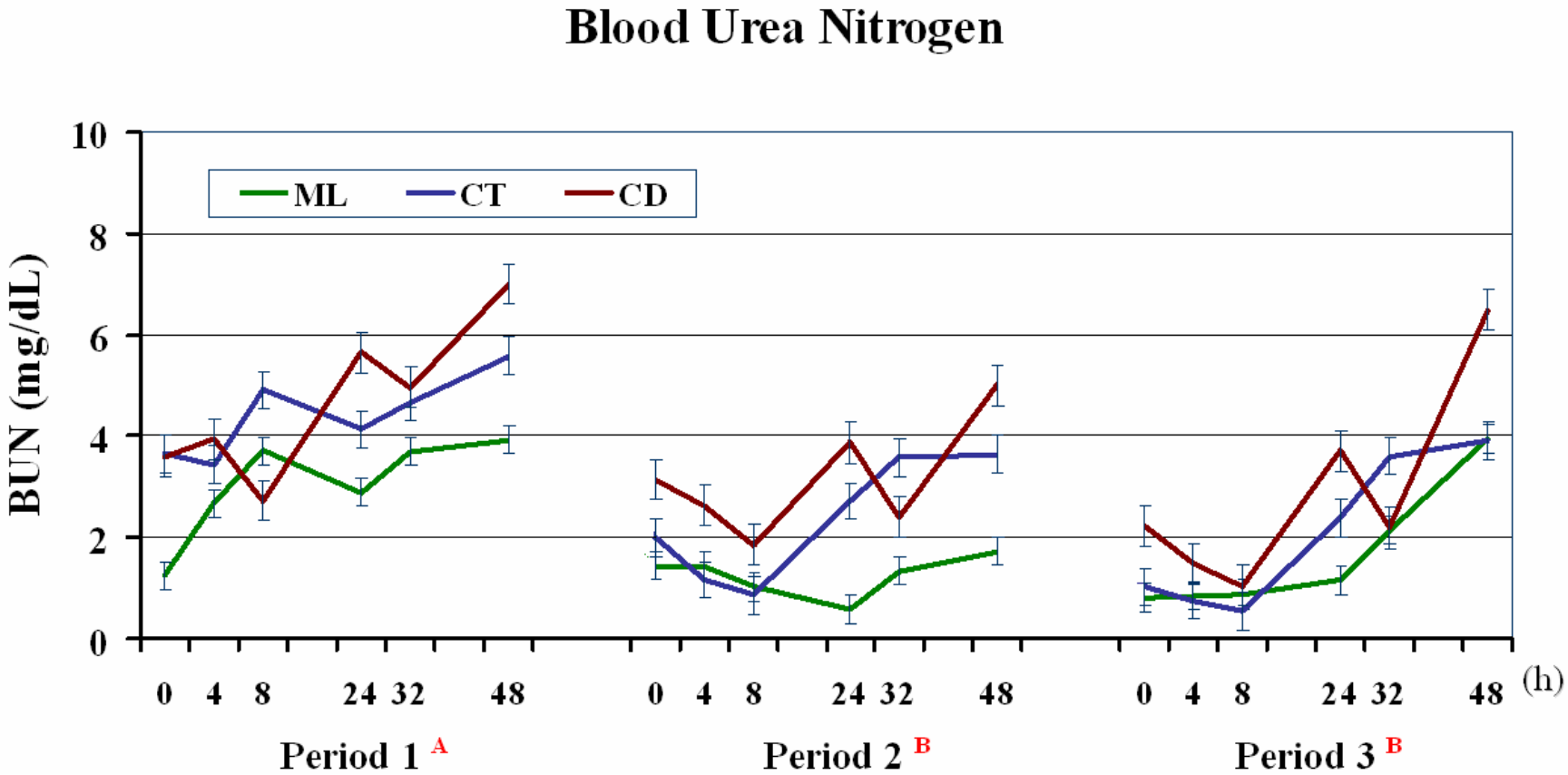

Figure 4-2. Blood urea nitrogen (BUN) concentrations of steers supplemented 3x/wk with citrus pulp- (CT) or molasses-based supplement (ML), or daily with a citrus pulp-based supplement (CD). Supplements were offered after blood was sampled at 0 h. Steers fed CD tended to have greater mean BUN concentration than steers fed ML ($P = 0.06$, SEM = 0.53). Period and time(period) effects were significant ($P < 0.01$). Periods with different letters differ ($P < 0.01$).

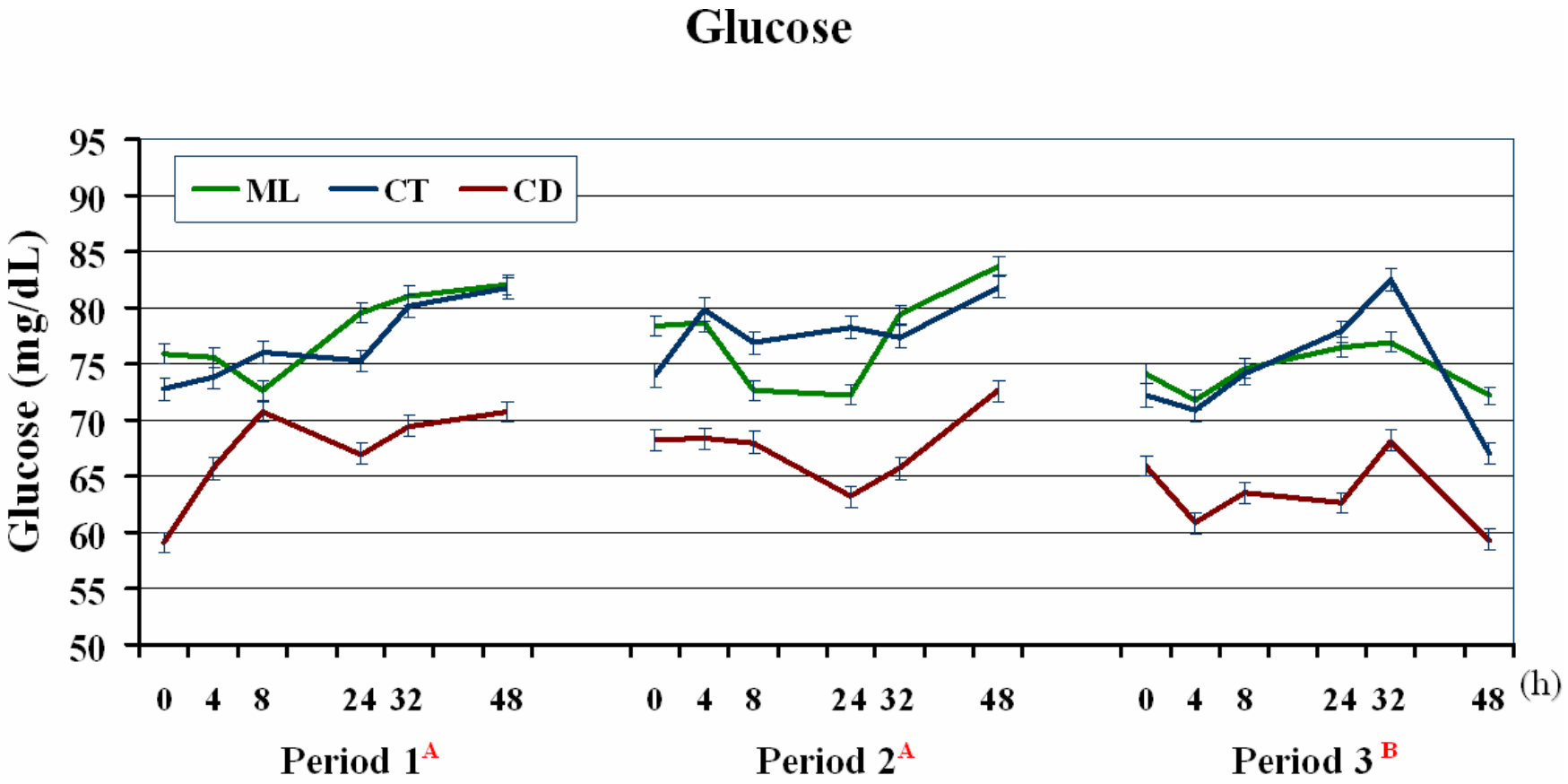

Figure 4-3. Plasma glucose concentrations of steers supplemented 3x/wk with citrus pulp- (CT) or molasses-based supplement (ML), or daily with a citrus pulp-based supplement (CD). Supplements were offered after blood was sampled at 0 h. Steers fed CD had lesser mean glucose concentration than steers fed either with ML and CT ($P < 0.05$, SEM = 3.02). Period and time(period) effects were significant ($P < 0.01$), and periods with different letters differ ($P < 0.01$).

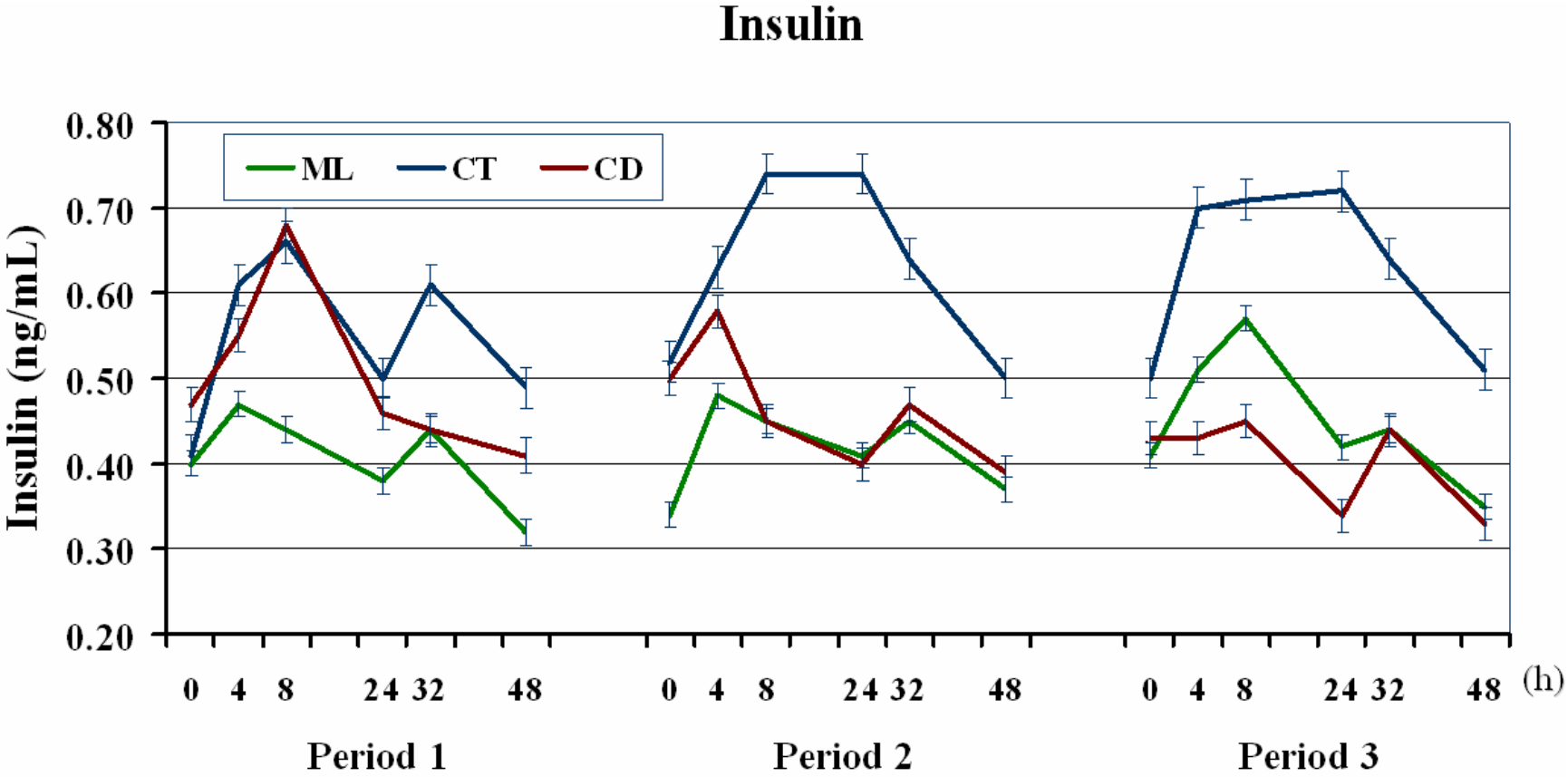

Figure 4-4. Plasma insulin concentrations of steers supplemented 3x/wk with citrus pulp- (CT) or molasses-based supplement (ML), or daily with a citrus pulp-based supplement (CD). Supplements were offered after blood was sampled at 0 h. Steers fed CT had greater mean insulin concentrations than steers fed ML and CD ($P < 0.05$, SEM = 0.04). A time(period) effect and a treatment x period interaction were significant ($P < 0.01$).

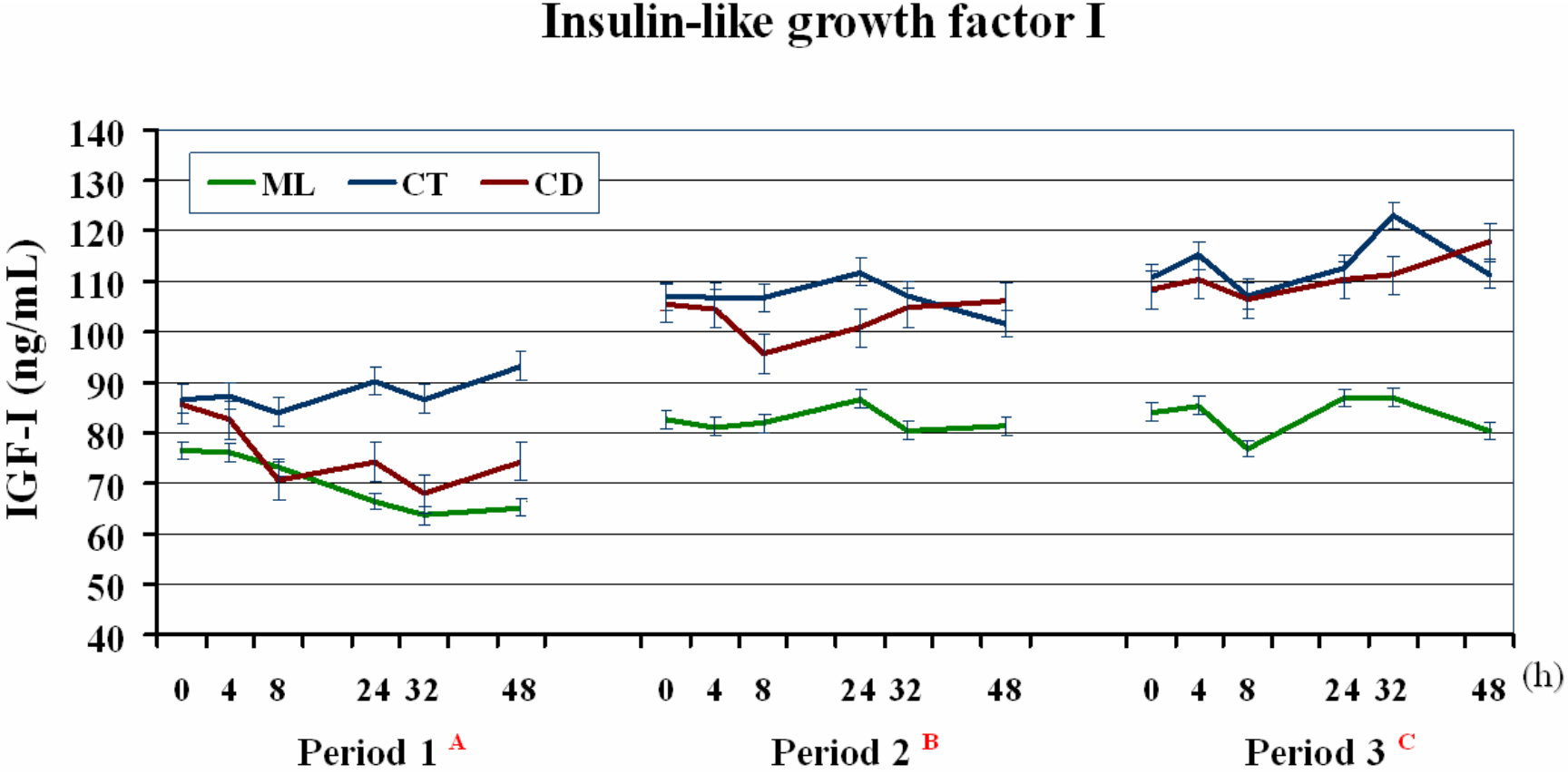

Figure 4-5. Plasma insulin like growth factor I (IGF-I) concentrations of steers supplemented 3x/wk with citrus pulp- (CT) or molasses-based supplement (ML), or daily with a citrus pulp-based supplement (CD). Supplements were offered after blood was sampled at 0 h. No significant differences were observed ($P = 0.27$, SEM = 10.22). Period effect was significant ($P < 0.01$). A treatment x period interaction was also significant ($P < 0.01$), and periods with different letters differ ($P < 0.01$).

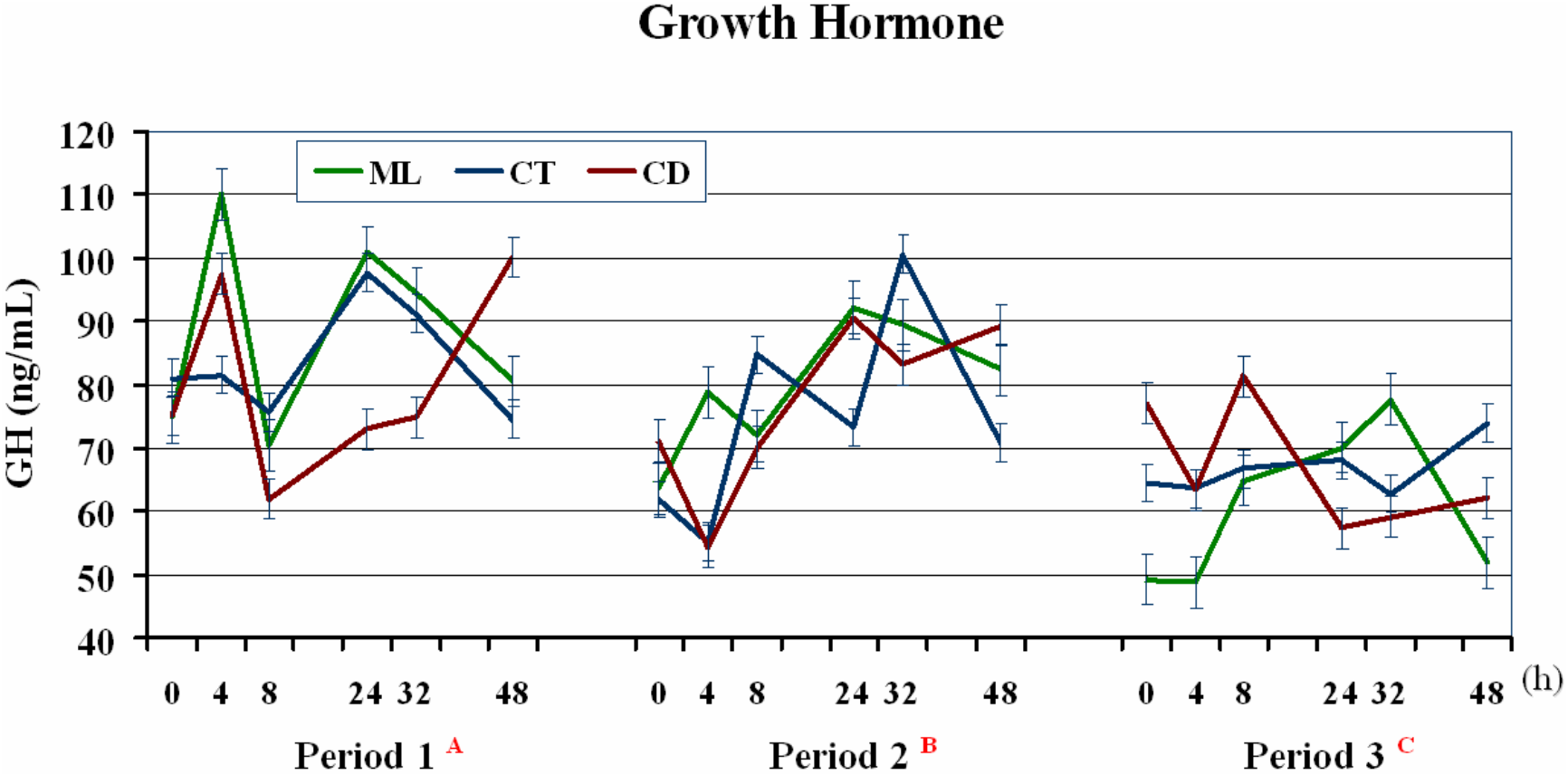

Figure 4-6. Plasma growth hormone (GH) concentrations of steers supplemented 3x/wk with citrus pulp- (CT) or molasses-based supplement (ML), or daily with a citrus pulp-based supplement (CD). Supplements were offered after blood was sampled at 0 h. No significant differences were observed (P = 0.98, SEM = 8.00). Period and time(period) effects were significant (P < 0.05), and periods with different letters differ (P < 0.05).

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BIOGRAPHICAL SKETCH

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