RISK FACTORS AND ECONOMIC IMPACT OF POSTPARTUM DISEASES IN DAIRY COWS

By

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To God and all my other loved ones…
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>10</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>13</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>16</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>18</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>20</td>
</tr>
<tr>
<td>2 LITERATURE REVIEW</td>
<td>22</td>
</tr>
<tr>
<td>Dry Matter Intake and Factors that affect Intake</td>
<td>23</td>
</tr>
<tr>
<td>Control of Intake during the Transition Period</td>
<td>26</td>
</tr>
<tr>
<td>Control of Intake During Disease</td>
<td>29</td>
</tr>
<tr>
<td>Immunity during Transition Period</td>
<td>30</td>
</tr>
<tr>
<td>Calving Disorders</td>
<td>31</td>
</tr>
<tr>
<td>Causes and Predisposing factors of Calving Disorders</td>
<td>33</td>
</tr>
<tr>
<td>Calving disorders and Dry Matter Intake</td>
<td>34</td>
</tr>
<tr>
<td>Retained Placenta</td>
<td>35</td>
</tr>
<tr>
<td>Causes and Predisposing Factors of Retained Placenta</td>
<td>36</td>
</tr>
<tr>
<td>Retained Placenta and Dry Matter Intake</td>
<td>37</td>
</tr>
<tr>
<td>Metritis</td>
<td>38</td>
</tr>
<tr>
<td>Causes and Predisposing Factors of Metritis</td>
<td>39</td>
</tr>
<tr>
<td>Metritis and Dry Matter Intake</td>
<td>41</td>
</tr>
<tr>
<td>Ketosis</td>
<td>42</td>
</tr>
<tr>
<td>Causes and Predisposing Factors of Ketosis</td>
<td>43</td>
</tr>
<tr>
<td>Ketosis and Dry Matter Intake</td>
<td>44</td>
</tr>
<tr>
<td>Mastitis</td>
<td>44</td>
</tr>
<tr>
<td>Causes and Predisposing Factors of Mastitis</td>
<td>45</td>
</tr>
<tr>
<td>Mastitis and Dry Matter Intake</td>
<td>47</td>
</tr>
<tr>
<td>Digestive Disorders</td>
<td>47</td>
</tr>
<tr>
<td>Causes and Predisposing Factors for Digestive Disorders</td>
<td>48</td>
</tr>
<tr>
<td>Digestive disorders and Dry Matter Intake</td>
<td>49</td>
</tr>
<tr>
<td>Lameness</td>
<td>50</td>
</tr>
<tr>
<td>Causes and Predisposing Factors of Lameness</td>
<td>51</td>
</tr>
<tr>
<td>Lameness and Dry Matter Intake</td>
<td>52</td>
</tr>
<tr>
<td>Economics of Metritis</td>
<td>52</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3-1</td>
<td>Frequency table of calving and uterine disorders diagnosed during the first 21 days postpartum.</td>
</tr>
<tr>
<td>3-2</td>
<td>Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with metritis according to multivariable analysis.</td>
</tr>
<tr>
<td>3-3</td>
<td>Effect of each 0.1 percentage point decrease in the average of dry matter intake as a percentage of BW (DMI%BW) and each unit decrease in the average of energy balance (EB) in the last 3 days prepartum on diseases or disorders in the first 28 days postpartum.</td>
</tr>
<tr>
<td>3-4</td>
<td>Cut-offs of DMI as percentage of BW (DMI%BW) and energy balance (EB) to predict metritis postpartum.</td>
</tr>
<tr>
<td>3-5</td>
<td>Association of prepartum (-21 to -1 d) dry matter intake as percentage of BW (DMI%BW) and energy balance (EB) with postpartum disorders.</td>
</tr>
<tr>
<td>4-1</td>
<td>Frequency table of ketosis and mastitis by study diagnosed during the first 28 days postpartum.</td>
</tr>
<tr>
<td>4-2</td>
<td>Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with ketosis (Ket) postpartum according to multivariable analysis.</td>
</tr>
<tr>
<td>4-3</td>
<td>Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with clinical mastitis (Mast) postpartum according to multivariable analysis.</td>
</tr>
<tr>
<td>4-4</td>
<td>Effect of each 0.1 percentage point decrease in the average DMI as a percentage of BW (DMI%BW), and each unit decrease in the average of energy balance (EB) in the last 3 days prepartum on postpartum ketosis and clinical mastitis (CM) in the first 28 days postpartum.</td>
</tr>
<tr>
<td>4-5</td>
<td>Cut-offs of dry matter intake as percentage of BW (DMI%BW) and energy balance (EB) to predict ketosis postpartum.</td>
</tr>
<tr>
<td>4-6</td>
<td>Cut-offs of dry matter intake as percentage of BW (DMI%BW) and energy balance (EB) to predict mastitis postpartum.</td>
</tr>
<tr>
<td>5-1</td>
<td>Frequency table of digestive disorders (DDZ) and lameness by study diagnosed during the first 28 days postpartum.</td>
</tr>
</tbody>
</table>
5-2 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with digestive disorders (DDZ) postpartum according to multivariable analysis. ................................................................. 136

5-3 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with lameness postpartum according to multivariable analysis. ................................................................. 137

5-4 Association between each 0.1 percentage point decrease in the average of dry matter intake as percentage of BW (DMI%BW), and each unit decrease in the average of energy balance (EB) in the last 3 d prepartum on digestive disorders and lameness in the first 28 d postpartum. ....................................................... 138

5-5 Cut-offs of dry matter intake as percentage of BW (DMI%BW), and energy balance (EB) to predict digestive disorders postpartum. ................................................................. 139

5-6 Cut-offs of dry matter intake as percentage of BW (DMI%BW), and energy balance (EB) to predict lameness postpartum in cows with BCS ≥ 3.75. ............ 140

6-1 Variables and measurements to estimate the cost of metritis. ......................... 164

6-2 Economics, productive, and reproductive parameters according to disease status (LSM ± SEM). ....................................................................................... 165

A-1 Correlation matrix showing Spearman’s rho correlation coefficient and (p-values). ........................................................................................................... 171

A-2 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake with calving disorders and metritis according to multivariable analysis. .......... 172

A-3 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with calving disorders according to multivariable analysis. ....................................................................................... 173

A-4 Association between pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with metritis according to multivariable analysis. ........................................................................................................... 174

A-5 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake with ketosis and mastitis according to multivariable analysis. ......................... 175

A-6 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with ketosis according to multivariable analysis. 176
A-7 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with mastitis according to multivariable analysis. .......................................................... 177

A-8 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with digestive disorders (DDZ) according to multivariable analysis. ............................................................................ 178

A-9 Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with lameness according to multivariable analysis. ........................................................................................................... 179
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1</td>
<td>Causal diagram showing the relationship of dry matter intake, energy balance with postpartum diseases and its consequences.</td>
</tr>
<tr>
<td>3-2</td>
<td>Association of calving disorders postpartum with (A) DMI as percentage of BW (DMI%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.</td>
</tr>
<tr>
<td>3-3</td>
<td>Association of metritis postpartum with (A) DMI as percentage of BW (DMI%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.</td>
</tr>
<tr>
<td>4-1</td>
<td>Association of ketosis postpartum (n = 189) with (A) dry matter intake (%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.</td>
</tr>
<tr>
<td>4-2</td>
<td>Interaction (P ≤ 0.01) between ketosis and parity on energy balance (Mcal/d) in (A) primiparous and (B) multiparous cows during the prepartum (from -21 d to -1 d).</td>
</tr>
<tr>
<td>4-3</td>
<td>Interaction (P &lt; 0.01) between ketosis and parity on energy corrected milk (kg/d) in (A) primiparous and (B) multiparous cows during the postpartum period (from 1 d to 28 d).</td>
</tr>
<tr>
<td>4-4</td>
<td>Association between mastitis (n = 79) and (A) dry matter intake (%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.</td>
</tr>
<tr>
<td>5-1</td>
<td>Association of digestive disorder postpartum (n = 120) with (A) dry matter intake (DMI, (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.</td>
</tr>
<tr>
<td>5-2</td>
<td>Association between lameness (n = 35) and (A) dry matter intake (DMI, %BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.</td>
</tr>
<tr>
<td>5-3</td>
<td>Interaction (P &lt; 0.01) between lameness and BCS on dry matter intake (% of BW) and energy balance (Mcal/d) in cows with BCS &lt; 3.75 (A, C) and BCS ≥ 3.75 (B, D) during the prepartum period (from -21 d to -1 d).</td>
</tr>
</tbody>
</table>
6-1 Kaplan-Meier survival curves for proportion of non-pregnant cows according to disease status: Metritis (n = 2624) and no metritis (n = 6388). .......................... 166

6-2 Kaplan-Meier survival curves for proportion of cows present in the herd according to disease status: Metritis (n = 2624) and no metritis (n = 6388). .... 167

B-1 Association of prepartum and postpartum dry matter intake (DMI, kg/d) with (A) calving disorders and (B) metritis.............................................................. 180

B-2 Association of calving disorders and healthy cows with (A) prepartum and postpartum DMI (kg/d), (B) DMI as percentage of BW (DMI%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum..................... 181

B-3 Association of metritis and healthy cows with (A) DMI (kg/d), (B) DMI as percentage of BW (DMI%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. ....................................................... 182

B-4 Association of prepartum and postpartum dry matter intake (DMI, kg/d) with (A) ketosis and (B) mastitis................................................................. 183

B-5 Interaction (P < 0.01) between ketosis and parity on dry matter intake (kg/d) on (A) primigravid and (B) multigravid cows during the postpartum period (from 1 d to 28 d). ................................................................. 184

B-6 Association of ketosis (n = 189) and healthy (n = 132) cows with (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.... 185

B-7 Interaction (P < 0.01) between ketosis and parity on dry matter intake (kg/d) and energy balance on (A, C; P = 0.02) primigravid and (B, D) multigravid cows during the prepartum period (from -21 d to -1 d). ..................................................... 186

B-8 Interaction (P < 0.01) between ketosis and parity on energy corrected milk (kg/d) in (A) primigravid and (B) multigravid cows during the postpartum period (from 1 d to 28 d). ......................................................... 187

B-9 Dry matter intake (% of BW) according to disease status related to ketosis during (A) prepartum (-21 to -1 d) and (B) postpartum period (from 1 d to 28 d). ................................................................................... 188

B-10 Association between mastitis (n = 79) and healthy (n = 132) cows and (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.... 189
B-11  Dry matter intake (% of BW) according to disease status related to mastitis during (A) prepartum (-21 to -1 d) and (B) postpartum period (from 1 d to 28 d). ............................................................................................................................. 190

B-12  Association of digestive disorder (n = 120) and healthy cows (n = 132) with (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (% BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum................................................................................................................................................ 191

B-13  Association between lameness (n = 35) and healthy (n = 132) cows and (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (% BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum.... 192
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
</tr>
<tr>
<td>AI</td>
<td>Artificial insemination</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BCS</td>
<td>Body condition score</td>
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<tr>
<td>BHB</td>
<td>β-hydroxybutyric acid</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>C</td>
<td>Celsius</td>
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<tr>
<td>DA</td>
<td>Displacement abomasum</td>
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<tr>
<td>DDZ</td>
<td>Digestive Disorder</td>
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<tr>
<td>DietNet</td>
<td>Energy of the diet</td>
</tr>
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<td>DIM</td>
<td>Days in milk</td>
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<tr>
<td>DMI</td>
<td>Dry matter intake</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>Dry matter intake as percentage of body weight</td>
</tr>
<tr>
<td>EB</td>
<td>Energy balance</td>
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<tr>
<td>ECM</td>
<td>Energy corrected milk</td>
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<tr>
<td>EV</td>
<td>Evaporative cooling</td>
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<tr>
<td>HOT</td>
<td>Hepatic oxidation theory</td>
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<tr>
<td>IL-β</td>
<td>Interleukin 1 beta</td>
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<td>IL-2</td>
<td>Interleukin 2</td>
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<tr>
<td>IL-5</td>
<td>Interleukin 5</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
</tr>
<tr>
<td>KET</td>
<td>Ketosis</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>LDA</td>
<td>Left displaced abomasum</td>
</tr>
<tr>
<td>Mast</td>
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<td>Mcal</td>
<td>Megacalorie</td>
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<td>MEq</td>
<td>Milliequivalent</td>
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<td>Metritis</td>
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<td>Mmol</td>
<td>Milimole</td>
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<tr>
<td>NDF</td>
<td>Neutral detergent fiber</td>
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<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
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<tr>
<td>NRC</td>
<td>National Research Council</td>
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<tr>
<td>PGF2α</td>
<td>Prostaglandin F2alpha</td>
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<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
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<tr>
<td>RDA</td>
<td>Right displaced abomasum</td>
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<tr>
<td>RP</td>
<td>Retained placenta</td>
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<tr>
<td>SCC</td>
<td>Somatic cell count</td>
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<tr>
<td>Th1</td>
<td>T helper cells 1</td>
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<tr>
<td>Th2</td>
<td>T helper cells 2</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
</tbody>
</table>
The objectives of the present dissertation were to establish the associations between dry matter intake (DMI) and postpartum diseases (i.e. calving disorders, metritis, ketosis, mastitis, digestive disorders, and lameness) in dairy cows and to estimate the cost of metritis in the dairy herd.

Chapter 3 shows that prepartum DMI as % of body weight (DMI%BW) and energy balance (EB) were not associated with and were not predictors of calving disorders (i.e. dystocia, twins, and stillbirths), but cows that had calving disorders had less postpartum DMI%BW, greater EB, and smaller energy corrected milk (ECM) than those without calving disorders. Metritis was associated with less prepartum DMI%BW and EB, and prepartum DMI%BW and EB were significant predictors of metritis. In addition, metritis was associated with smaller postpartum DMI%BW and EB, and less ECM yield.

Chapter 4 shows that ketosis and mastitis were associated with less prepartum DMI%BW, and ketosis with reduced prepartum EB, and prepartum DMI%BW and EB were predictors of ketosis and mastitis. In addition, ketosis was associated with reduced
DMI\%BW and EB, and increased ECM, whereas mastitis was associated with reduced DMI\%BW and ECM, and increased EB and lesser ECM during the postpartum period.

Chapter 5 shows that prepartum DMI\%BW and EB were associated with digestive disorders (i.e. left displaced abomasum, sand impaction, cecal dilatation, diarrhea, bloat, constipation, and indigestion), and with lameness in cows with a body condition score (BCS) $\geq$ 3.75. In addition, this chapter also shows that prepartum DMI\%BW and EB were predictors of digestive disorders in all cows and lameness in cows with BCS $\geq$ 3.75. In addition, digestive disorders in all cows and lameness in cows with BCS $\geq$ 3.75 were associated with reduced DMI\%BW and EB, and digestive disorder was associated with reduced ECM during the postpartum period.

Chapter 6 shows that the cost of a case of metritis in the dairy farm is $532.3. In addition, a greater percentage of cows with metritis left the study by 305 days in milk had increased replacement and reproduction costs, and reduced milk yield.
CHAPTER 1
INTRODUCTION

Improvements in dairy cow genetics and management have led to a steady increase in milk production per cow in the last 60 years (USDA-NASS, 2015). However, greater milk yield requires more nutrients that have to be supplied by the diet consumed or body reserves.

During prepartum there is a decline of dry matter intake (DMI) that occurs around the last ten to seven days before calving being more pronounced in the last week of this period (Hayirli et al., 2002). Conversely, after calving, the cow increases her feed intake in an attempt to compensate the nutrients needed for maintenance, activity, lactogenesis, and lactopoiesis. However, this increment in intake is not able to supply the demands for nutrients needed during the first weeks of the new lactation, leading cows to mobilize lipid tissue in order to supply the energy and nutrients needed for this stage.

The decrease in DMI and associated increased concentrations in metabolites as non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) in plasma because of lipid mobilization, which is associated with immune deficit (Hammon et al., 2006), thereby leading to increased susceptibility to diseases and disorders during the postpartum period. Diseases that affect the uterine tract and the mammary gland, and metabolic disorders are the most common health disorders during this period. Among these diseases or disorders it can mention calving disorders (i.e. dystocia, twins, and stillbirths), metritis, ketosis, mastitis, digestive disorders (i.e. left displaced abomasum, sand impaction, cecal dilatation, diarrhea, bloat, constipation, and indigestion), and lameness.
As a consequence of the occurrence of these disorders, there will be economic losses. For example, cows with metritis can be present in up to 40% of the dairy cows, and it is associated with reduced milk production, impairment in reproduction performance, and increased in culling, thus having a negative impact in the profitability of the dairy herd.

Chapter 3 evaluates the association of DMI as percentage of BW (DMI\%BW), and energy balance (EB) with calving disorders and metritis. Chapter 4 evaluates the association of DMI\%BW and EB with ketosis and mastitis. Chapter 5 evaluates the association of DMI\%BW and EB with digestive disorders and lameness. Chapter 6 evaluates the impact of metritis on milk production, reproduction and survival and estimates the cost of metritis in dairy herds.
CHAPTER 2
LITERATURE REVIEW

Transition period, also called periparturient period, is the period from 3 weeks before parturition to 3 weeks after parturition. During this period, dairy cows will experience physiological and metabolic changes that prepare them for parturition and for the onset of lactation. As gestation reaches its final stage, nutrient demands increase in order to supply nutrients for the growing calf, the calving event, and lactogenesis.

Despite all physiological changes that occur in the cow to have a successful transition from a dry to a lactating cow, some metabolic imbalances occur putting cows at risk of having diseases or disorders during the first weeks of postpartum. Nutrient demands are not met during the transition period. During the final days of gestation, the DMI typically declines (Hayirli et al., 2002), hence, resulting in increased adipose tissue mobilization and, consequently, leading to increased concentrations of lipid metabolites such as NEFA and BHB in blood (Drackley, 1999; Grummer et al., 2004; French, 2006). Even though the process of lipid mobilization during the transition period is a normal adaptation in the cow, the increment in concentrations in NEFA and BHB in blood in the first weeks of lactation has been associated with reduced immune function (Hammon et al., 2006; Moyes et al., 2009), and with an increment in the incidence of diseases such as retained placenta (RP), metritis, and displaced abomasum (DA) (Ospina et al., 2010a).

Other disorders and diseases such as ketosis, mastitis, and digestive disorders also have high incidence during the first weeks postpartum, thus, causing economic losses by decreasing milk production, impairing reproduction, and increasing culling.
Therefore, a better understanding of all these diseases and disorders and the cow’s biology during the transition period may help to provide solutions that leads dairy cows to have a healthier transition from gestation to lactation and consequently reducing the economic impact in the dairy farm.

**Dry Matter Intake and Factors that affect Intake**

The average DMI during the last three weeks before parturition is 1.69% and 1.88% of BW for primiparous and multiparous cows, respectively (Hayirli et al., 2002). During this period, cows have a decline in DMI being less pronounced in primiparous cows (Hayirli et al., 2002; Grummer et al., 2004). In the day after calving, DMI begins to increase; however, intake only peaks 10 to 14 weeks in lactation which is later when the milk yield peaks in most cows, typically 4 to 8 weeks postpartum (NRC, 2001). As a result, most cows experience negative nutrient balance because DMI is not sufficient to supply the nutrients needed to meet demands for maintenance and production in the first 4 to 8 weeks postpartum.

After fresh feed is delivered, cows will have their peak of intake within the first 60 min (Huzzey et al., 2006). However, this intake may be affected by several factors such as animal, nutritional, environmental, and management. As animal factors, it can mention animal behavior, parity, and body condition score (BCS). Cows that are either too aggressive or non-aggressive at all may have their intake affected (Chebel et al., 2016). Chebel et al., (2016) explained that cows that are too aggressive will be trying to maintain for their dominance in the feed bunk instead of eating whereas cows with little to non-aggressive behavior will leave the feed bunk after the first displacement in order to avoid future confrontations. This behavior is also related to parity. Primiparous cows are displaced more often when they are mingled in the same feed bunk with multiparous
cows (Chebel et al., 2016), which can affect their intake. However, multiparous cows will experience a greater and more continuous decrease in DMI (52% vs 25%) during the last week of prepartum (Grummer et al., 2004). Primiparous cows are still growing meaning that they need to maintain DMI in order to satisfy the energy needs for muscle growth in addition to maintenance and gestation energy requirements (NRC, 2001). In addition, the decrease in DMI during the last week prepartum will depend on BCS, which is negatively correlated with dry matter intake (Hayirli et al., 2002). Overconditioned cows have a larger decrease DMI (Grummer et al., 2004) and lose more BCS during the prepartum period than thin cows (Chebel et al., 2018). This decrease in DMI is mainly due to actions of leptin in overconditioned cows affecting the satiety center in the hypothalamus. More detail of this mechanism will be provided later in this chapter.

Dietary factors explain ~ 24% of the variation of DMI during the dry period (Hayirli et al., 2002). Low fiber diets lead to greater DMI than high fiber diets because of greater digestibility (Dado and Allen, 1995). Conversely, DMI is negatively correlated with concentrations of neutral detergent fiber (NDF) because of less digestibility and passage rate through the rumen, which cause the rumen to be distended for longer time (Dado and Allen, 1995; Hayirli et al., 2002). This distension sends signals of satiety though the vagus nerve; therefore, decreasing intake (Miner et al., 1990). Other components of the diet such as fat and protein also have an on DMI; however, they count only for 1.3% and 1.4% of the variation in DMI (Hayirli et al., 2002). Other dietary factors that can affect DMI is particle size. The reduction of particle size from short to long increase DMI by 8% because it reduces sorting; therefore, allowing cows to eat
greater amounts of high fiber particles, which reduces rumination time, which allows a faster passage rate through the rumen (Kononoff et al., 2003).

The environmental factors that can affect DMI are mainly temperature and humidity. Cows that calve in the summer, when temperature and humidity are greater, had lesser DMI during the first 4 wk postpartum compared with cows that calved during the winter (Cardoso et al., 2013). Thompson et al. (1963) showed that cows under heat stress increase levels of cortisol compared with cows when they were in cool conditions. Cortisol increases during heat stress, which has been shown to be negatively correlated with intake (Reshalaitihan and Hanada, 2019). However, it has been shown that the negative effect of heat stress on DMI during the dry period continued during the postpartum period even after cows were cooled (Tao et al., 2011; Fabris et al., 2019).

Herd management also affects DMI. Adequate bunk space, dividing groups by parity, and having fresh feed continuously available are essential to stimulating DMI during the transition period (Huzzey et al., 2006; Chebel et al., 2016). On the other hand, dry matter intake decreases as stocking density increases (Huzzey et al., 2006). Greater stocking density increases cow’s displacement and competition for feed thus affecting intake (Huzzey et al., 2006; Chebel et al., 2016). Increase in feed bunk space from 51 to 102 cm per cow reduced the number of aggressive interactions and increased the percentage of cows feeding during the 90 min following feed delivery (DeVries et al., 2005), whereas decreasing feed bunk space from 81 to 21 cm caused a reduction in feeding time and an increase in aggressive behavior (Huzzey et al., 2006). In addition, delivering food more frequently decreased the displacement of more subordinated cows thus allowing them to increase their intake (DeVries et al., 2005).
**Control of Intake during the Transition Period**

At the beginning of the transition period, hormonal changes start to occur in the periparturient cow in order to prepare her for parturition. Some of the changes on these hormones affect intake during the final days of prepartum which decreases 30% to 35% starting ~ 10 d before parturition (Hayirli et al., 2002). Among these hormones, it can mention fetal cortisol which start to increase ~7 d before parturition (Comline et al. 1974) and has been shown to have a negative correlation with DMI in dairy cows (Reshalaitihan and Hanada, 2019). High levels of cortisol increase estrogen biosynthesis at expenses of progesterone conversion to androstenedione and under the activity of aromatase turning it to estradiol leading to an increment of estradiol in the cow bloodstream and a decrease in progesterone levels (Senger, 2003). This elevation of estradiol and decrease in progesterone is necessary for parturition to occur; however, the changes in these hormones have a negative effect on intake. Bargeloh et al. (1975) administered subcutaneous progesterone and estradiol to pregnant cows 14 and 7 d, respectively, before expected calving and showed that cows that received estradiol had decreased DMI whereas cows that received progesterone had greater DMI.

Noradrenaline is also increased closer to parturition in dairy cows (Hydbring et al., 1999). Rukkwamsuk et al. (1998) showed that lipolytic rate in the adipose tissue increased when noradrenaline was added in vitro; therefore, increased lipolytic rate increases levels of NEFA in blood leading to decrease intake, the mechanism of NEFA decreasing intake will be discussed later in this chapter. Other hormones such as oxytocin and prolactin also increase when cows are very close to parturition but there was no negative effect of these hormones on intake (Yayou et al., 2011).
Because of the decline in DMI, the process of lipolysis of triglycerides under the actions of adipose triglyceride lipase and hormone-sensitive lipase is initiated in order to compensate for the energy deficit (Schweiger et al., 2006). As a result, triglycerides are broken down to NEFAs and these are released by adipocytes into the bloodstream to finally serve as a fuel in order to meet the energy demands that are not being met by the diet. Once NEFAs are released into the bloodstream they bind to albumin and when they reach the liver they are taken by the hepatocytes, which start the esterification process (Pownall, 2001). Once inside the hepatocyte, NEFAs are either converted to acetyl-CoA, completely oxidized to carbon dioxide, or can be re-esterified as triglycerides in the cytosol (Emery et al., 1992). The acetyl CoA derived from NEFA enter the tricarboxylic acid cycle to generate adenosine triphosphate (ATP) or they are turned into BHB and ketone bodies such as acetone and acetoacetate.

Previous research has shown how ATP levels regulates intake. Koch et al. (1998) showed that rats with decreased liver ATP after administration of 2,5-anhydro-D-mannitol, had greater feed intake 15 to 45 minutes after treatment administration compared with rats that received saline, these results suggest that a decrease in ATP increased hunger ~15 minutes after being depleted. This energy status of the hepatocytes is the base of the hepatic oxidation theory (HOT) (Allen et al., 2009). This theory describes how intake is controlled in dairy cows during the transition period using NEFAs as fuel, with the final goal of sparing glucose for milk synthesis. Allen et al. (2009) describes that hunger is suppressed because elevated NEFA will be the most available substrate by the liver. Consequently, the generation of ATP will create a hyperpolarization of the hepatocyte and because of this hyperpolarization there is a
decrease in the release of signaling molecules and a decrease in the firing rate of hepatic vagal afferent nerves thus inhibiting the hypothalamic feeding centers and stimulating the satiety center thus reducing feed intake.

According to the HOT, later in the postpartum period, after physiologic changes that occurred at the beginning of the transition period starts to normalize, feed intake stops being in control of hepatic oxidation and start to be regulated by distension of the reticulo-rumen, and other fuels such as propionate (Allen et al., 2009). Previous research has shown that rumen fill regulates DMI in dairy cows (Dado and Allen, 1995). The distension of the reticulo-rumen will stimulate receptors that are present in the muscle layer in the wall of the rumen, and these receptors will send information to the ventral-medial hypothalamus and cause meal cessation (Allen, 2000). However, propionate seems to be the primary satiety signal in dairy cows during postpartum. Stocks and Allen (2012) infused acetate and propionate intraruminally, and cows that were infused with propionate had decreased meal size, meal length and DMI compared with cows infused with acetate. Furthermore, they also found that after propionate infusion, liver acetyl CoA increased, DMI tended to decrease; therefore, showing the hypophagia effects of propionate. Propionate induce satiety through hepatic vagus nerve (Anil and Forbes, 1988) and by stimulating two gut hormones peptide YY and glucagon like peptide-1 which acutely suppress appetite (Psichas et al., 2015).

In addition, DMI during the prepartum declines linearly as BCS increase (Hayirli et al., 2005). Overconditioned cow lose more body condition than thinner cows during the prepartum period (Chebel et al., 2018). These decrease in DMI may be due to the actions of leptin. There is a positive linear relationship between plasma leptin and BCS
and between leptin and body fat (Ingvartsen and Boisclair, 2001) suggesting that adipose tissue secretes more leptin in overconditioned animals. Leptin is one of the main hormones in controlling intake by sending anorexic signals to the hypothalamus (Elmquist et al., 1998); therefore, increased production of leptin in prepartum cows with high BCS could exacerbate the anorexic actions of hormones that increase at parturition such as cortisol, estrogen, and noradrenaline, and reduce even further prepartum DMI, thus resulting in more weight loss than thinner cows.

Control of Intake During Disease

After pathogen invasion, innate immune cells such as neutrophils, detect pathogen-associated molecular patterns, and immediately after this recognition occurs and together with endothelial cells, intracellular signaling cascades are triggered and cytokines are released. Several cytokines have anorectic effects (Buchanan and Johnson, 2007); however, the main cytokines that inhibit feed intake are Interleukin-1β (IL-1β), Tumor Necrosis Factor (TNF-α), and Interleukin-6 (IL-6). Several research have shown the hypophagic effects of IL-1β in different animal species. Sonti et al., (1996) showed that infusion of IL-1β directly in the brain of rats suppressed food intake by reducing meal size, feeding rate, and increasing meal intervals. In ruminants, van Miert et al. (1992) showed that after injection of IL-1β to dwarf goats, feed intake and ruminal contraction decreased. In addition, tumor necrosis factor-α and IL-6 also suppresses food intake after being infused in directly in the brain from rats (Plata-Salamán et al., 1996; Wallenius et al., 2002). These cytokines act in the hypothalamus, which is the main site that controls intake; however, cytokines cannot pass the blood brain barrier. Buchanan and Johnson (2007) proposed that cytokines can communicate through 2 routes, one by acting at the level of the blood brain barrier where they interact with
POMC and AgRP/NPY containing neurons which send signals to the hypothalamus cells thus decreasing intake, and the other route is by activating vagal afferents that project via the nucleus tractus solitarius and stimulating microglia cells to produce cytokines that reach the hypothalamus leading to a decrease in intake.

Anorexia during disease seems to be part of the defense mechanism when there is a pathological agent in the organism. A previous study showed that intake was reduced by ~60% in mice in the first 6 to 12 hours after induction of lipopolysaccharide (LPS) and this anorexic effect continued after 24 h of induction (Becskei et al., 2008) which shows that anorexia is one of the first reactions that the organism has when an inflammatory response is induced. Anorexia may contribute to pathogen elimination by decreasing blood metabolites such as iron which is essential for bacterial multiplication (Exton, 1997). In addition, it has been shown that anorexia before infection also have a positive impact in survival.

**Immunity during Transition Period**

Cows with an impair immune system during the transition period can put them at greater risk of disease postpartum. Kimura et al. (2002) showed that cows with RP had lesser killing activity and chemotaxis than cows with no RP whereas Hammon et al., (2006) showed that cows with metritis had neutrophils with impaired killing activity compared with cows that did not develop metritis.

Neutrophils can be negatively affected during the transition period for several reasons such as metabolic imbalances and environmental stress. Hammon et al., (2006) showed that the killing activity of neutrophils decline as plasma NEFA increased. In addition, Martinez et al., (2012) showed that neutrophil phagocytosis and killing activity was reduced in cows with subclinical hypocalcemia compared with
normocalcemic cows. Furthermore, cows that developed metritis had neutrophils with lesser glycogen than cows that did not develop metritis (Galvão et al., 2010) which means that they had less energy for the neutrophil function. Environmental stress such as heat stress also have a negative impact on neutrophil function. Cows that experienced heat stress during the prepartum had lesser phagocytosis activity in neutrophils compared with the ones that received evaporating cooling during prepartum (do Amaral et al., 2011).

Furthermore, the impairment in immune function of cows that experience disease postpartum such as metritis can continue to be impaired during the first weeks after calving (Galvão et al., 2012), which can put dairy cows at risk of other diseases. An adequate immune function in dairy cows is essential for a successful transition period; therefore, finding methods to keep a good immune function during the transition period is key to assure a successful lactation.

**Calving Disorders**

Cows that have dystocia, twins and stillbirths are classified as having calving disorders. Meijering (1984) defined dystocia as a difficult parturition due to an impediment of the fetal passage through the birth canal thus requiring assistance. Unassisted births have been reported to have a duration of $45.2 \pm 24.5$ min; therefore, calving events that take longer than 70 min after amniotic sac presence could be considered dystocic (Schuenemann et al., 2011). Cows are often categorized by calving ease to differentiate between eutocia and the different degrees of dystocia but the scoring system varies among researchers (Dematawewa and Berger, 1997; Meyer et al., 2001) and can go from a calving ease score of 2 levels ($1 = $eutral, $2 = $ystocia) to a 5-point calving ease score in which a score of $1 = $ no assistance; $2 = $ assistance by
one person without the use of mechanical traction; 3 = assistance by 2 or more people; 4 = assistance with mechanical traction; 5 = fetotomy or cesarean-section. United States is one of the countries that has the highest prevalence of dystocia in dairy cattle (22.6% in heifers, and 13.7% in heifers and cows) compared with other countries (1.5% to 6.6%) (Mee, 2008). Stillbirth is defined as the birth of a dead calf or a calf that died within 24 h to 48 of birth (Bicalho et al., 2007b). Incidence of twins ranges from 2.2 to 6.9% with a mean of 4.9% (Silva Del Río et al., 2007) and 3.3% of stillborn calves (Vergara et al., 2014).

Some studies have demonstrated the effect of calving disorders on milk production (Dematawewa and Berger, 1997; Atashi et al. 2012). Cows that experienced dystocia can have a mean of 135 kg on 305-d lactation lesser milk yield than cows with unassisted births (Atashi et al. 2012) and this decrease in milk yield can be more pronounced as calving ease score increases (Dematawewa and Berger, 1997). In addition, it has been reported that primiparous cows that had twins produced 1.2 kg/d less milk than cows that gave birth to singletons whereas multiparous cows that had twins had 0.8 kg/d lesser milk than multiparous that had singletons (Bicalho et al., 2007b). Stillbirth also affects milk yield, cows that had stillbirth had 544 kg less milk on 305-d lactation compared with cows that had live calves (Mahnani et al., 2018).

Calving disorders also negatively affect fertility. Days open and number of services per conception are affected by dystocia. Dematawewa and Berger (1997) found that days open in primiparous cows could increase from 6 to 33 days from a calving score 2 to 5 with a calving ease score system that goes from 1 to 5, 1 being no assistance and 5 as an extreme difficult dystocia, whereas in multiparous cows
increased from 14 to 29 starting on calving score 3. Number of services per conception was also increased in cows that had a calving ease score \( \geq 2 \) or from 3 to 4 in primiparous and multiparous, respectively, compared with their peers that had a calving ease score of 1 (Dematawewa and Berger, 1997). In addition, twins increased calving to conception interval by \( \sim 45 \) days and decrease the hazard of pregnancy by 22% compared with cows that had singletons (Bicalho et al., 2007b). Stillbirth increased days open by \( \sim 15 \) days and increased service per conception by 0.22 (Mahnani et al., 2018).

Calving disorders also affect culling. The percentage of dead cows increased as the calving ease score increased, regardless of parity (Dematawewa and Berger, 1997). In addition, cows that had twins had a 42% increased hazard of leaving the herd by culling or dead, compared with cows that had singletons (Bicalho et al., 2007b).

**Causes and Predisposing factors of Calving Disorders**

Dystocia can be caused by maternal or fetal factors or a combination of both as is the case of the feto-maternal disproportion. Among maternal factors it can be mentioned incomplete dilation of the birth canal, uterine torsion, and uterine inertia. Failure in the dilation of the birth canal has been associated with the presentation of the calf at the moment of parturition. Cows that had calved calves in posterior presentation have had decreased cervical dilation compared with calving calves in anterior position (Breeveld-Dwarkasing et al., 2002, Breeveld-Dwarkasing et al., 2003). Uterine torsion occurs when the uterus rotates from 180 up to 360 degrees (Frazer et al., 1996) impeding the calf from entering the birth canal. Uterine inertia is when the uterus is relaxed with very few or no contraction during parturition, and is associated with hypocalcemia. Calcium is essential for uterine contractions and previous research showed that induced hypocalcemia in ewes in different stages of parturition decreases
uterine contractions thus predisposing cows to dystocia (Robalo Silva and Noakes, 1984).

Besides hypocalcemia there are other factors on the maternal side that predispose cows to having dystocia such as gestation length, and parity. Vieira-Neto et al. (2017) found that multiparous cows with short (~266 d) and long (~285 d) gestation period had greater incidence of dystocia than multiparous cows with average gestation length (~276 d); however, gestation length did not affect the incidence of dystocia in primiparous cows. Primiparous cows have ~2.5 and 2.4 times greater odds of experiencing dystocia and stillbirth compared with multiparous cows, respectively (Johanson and Berger, 2003). This greater odds of having dystocia in primiparous cows is likely related to a smaller pelvic size.

Among fetal factors it can be mentioned fetal size, fetal position, sex of the calf, twinning, and stillbirths. Cows that delivered male calves had greater odds of needing assistance compared with cows that delivered female calves (Johanson and Berger, 2003). This may be related to birth weight because male calves are heavier than female calves (Dhakal et al., 2013). For each kg increase in birthweight the odds of having dystocia increase by 13% (Johanson and Berger, 2003). Fetal position other than the normal position is a cause of dystocia. Dystocia also occurs in case of fetal maldisposition, the most common are if the calf comes with a posterior presentation, posterior orbreech malpresentation, foreleg malposture, or cranial malposture, if there is twin calving and in case of stillbirth (Mee, 2004).

**Calving disorders and Dry Matter Intake**

There is limited literature on prepartum DMI and calving disorders. Proudfoot et al. (2009) showed an association between DMI and dystocia. In this study is shown that
DMI decrease by 1.9 kg in cows that experienced dystocia 48 hours previous to parturition compared with cows with eutocia. In addition they showed that standing bouts and feeding time was also reduced in cows that experience dystocia. As far as it is known there is no literature presenting information about the association between DMI and twins or stillbirth. Liboreiro et al. (2015) showed that cows that had stillbirth experienced had decreased rumination time before parturition which may suggest a decreased DMI whereas Chebel et al (2018) found that cows with a gestation length ≥284 d that had stillbirth were associated with an excessive BCS loss during the dry period, suggesting that they decrease intake. However, Liboreiro et al. (2015) did not find any association with prepartum rumination time and twinning.

**Retained Placenta**

Retained placenta is defined as the failure to expel the fetal membranes. Normally, the expulsion occurs within 12 hours after calving (Attupuram et al., 2016). The period considered to diagnose RP varies in the literature some researchers consider a cow having retained placenta if the expulsion does not occur within the first 12 hours, others will consider it after 24h and others even after 48 from calving (Fourichon et al., 1999).

The incidence of RP has been previously reported to be 8.6% (Kelton et al., 1998); however, this incidence may vary among studies because of different definitions of RP. Retained placenta affects the profitability of the dairy farm. Cows that develop RP and retained placenta for more than 12 hours had 237 kg lesser milk yield than cows that did not develop RP during the first 100 days of lactation (van Werven et al., 1992). In addition, it has been reported that RP increases the days to first service and decrease the conception at first service by 10% (Fourichon et al., 1999), increases days
open in heifer by 10 days and greater percentage of heifers were not pregnant after 150
DIM (Goshen and Shpigel, 2006). However, culling rate seems to be affected only if
placental membranes were retained more than 71 hours (van Werven et al., 1992).

**Causes and Predisposing Factors of Retained Placenta**

Retained placenta can be caused by several factors such as hormonal
imbalances during the prepartum, cellular alterations and an altered inflammatory
response. Parturition is an event that is orchestrated by different hormones such as
cortisol, PGF$_{2\alpha}$, estrogens, oxytocin, relaxin, among others. Estrogen will induce
softening of the placentome, cervix, and vagina and together with relaxin will alter
collagen fiber for their detaching and consequent degradation, whereas oxytocin will
stimulate uterine contraction to help with the physical detachment of the placenta
(ATTUPURAM et al., 2016). It has been shown that cows with RP have lesser
concentration of PGF$_{2\alpha}$ and oxytocin compared with cows that did not develop RP
(TAKAGI et al. 2002) thus delaying the placenta release. On the other hand, it has been
observed that retention of the placenta was more common when there was MHC Class I
compatibility between the dam and the calf (JOOSTEN et al., 1991; DAVIES et al., 2004).
This deficiency in alloreactivity might impair activation of a cell mediated immune
response and impair the detachment of the placenta. Regarding the innate immune
response, it has been demonstrated that cows that developed RP had lesser
chemotactic activity of leukocytes toward cotyledon supernatant, lesser chemottractant
(IL-8) concentration in the blood, thus decreased neutrophil migration to the sites of
placental attachment, and decreased neutrophil phagocytosis and killing ability from the
period 2 weeks before and 2 weeks after calving (Gunnink, 1984; KIMURA et al., 2002).
Gestation length is a risk factor of RP. Kumari et al. (2015) showed that the odd of having RP were 4.3 in cows with short gestation length (i.e. <275) compared with cows that had a gestation length from 275-290. In addition, Vieira-Neto et al. (2017) showed that among primiparous and multiparous cows that had RP, cows with short gestation length (i.e. 266 days) had greater incidence of RP compared with their peers with RP that had average gestation length of 276 days (primiparous: 18.4 vs. 5.2%; multiparous: 35.5 vs. 5.1%). Cows with a short gestation length may not be able to complete the maturation process of the placenta, not giving time for the caruncle epithelium to change its morphology and composition in order to start the process of detachment. Dystocia is another important risk factor for RP. The delayed involution and damage of the uterine wall caused by the mechanical traction of the calf during a dystocia hinder the normal release of the fetal membranes. In addition, low calcium in plasma has also being associated with RP (Melendez et al., 2004a), which can be explained by a lesser phagocytosis and killing ability of neutrophils with low calcium availability (Martinez et al., 2012) hindering the degradation of the extracellular matrix for membrane separation.

**Retained Placenta and Dry Matter Intake**

Literature on the association between prepartum DMI and RP is very limited. Dervishi et al. (2016) reported that cows that developed RP had lesser DMI at week -8 relative to parturition, and at the diagnosis week, including data before of the days before and/or after parturition whereas Luchterhand et al. (2016) did not find any association between prepartum DMI and RP. Similarly, Liboreiro et al. (2015) did not report a difference in rumination time before parturition between cows that did and did
not develop RP. Therefore, more research is needed to assess if prepartum DMI has an association with RP.

**Metritis**

The definition of metritis varies among studies because it differs on time and characteristics of the disease. Kelton et al. (1998) considered a cow to have metritis if she had a postpartum condition characterized by an abnormal cervical discharge, vaginal discharge, or both or uterine content, whereas Sheldon et al., (2006) proposed that cows with an abnormally enlarged uterus, a fetid watery red-brownish uterine discharge, and a fever be classified as puerperal metritis, whereas the term, metritis should be used for cows with delayed uterine involution and a fetid discharge in the absence of a fever. However, more recently, several researchers (Jeon et al., 2016; Cunha et al., 2018) have used a 5 point scale uterine discharge that has been derived from Williams et al. (2005), the classification more used has been: 1, not fetid normal lochia, viscous, clear, red, or brown; 2, cloudy mucoid discharge with flecks of pus; 3, not fetid mucopurulent discharge with <50% pus; 4, not fetid mucopurulent discharge with ≥50% pus; 5, fetid red-brownish, watery discharge, and cows with a discharge score of ≤4 are being classified as healthy, and cows with a score of 5 and a fever (≥39.5°C) are being classified as metritic. Even though, it has been shown that cows with fever and no fever have similar bacterial communities and not associated with the total bacterial load or specific bacteria (Jeon et al., 2016; Cunha et al., 2018); therefore, defining metritis as cows with a fetid red-brownish, watery uterine discharge with or without the presence of fever.

The incidence of metritis goes from 20 – 40% (Curtis et al., 1985; Markusfeld, 1987;) and it occurs very early in lactation, with ~90% in the first 14 DIM and with a
peak around 5-7 days (Galvão, 2011). Metritis is associated with lesser milk yield, impairment of reproduction, and increased culling. Cows that have develop metritis have being associated 5 kg/d lesser milk yield compared with cows that did not develop metritis (Huzzey et al., 2007; Daetz et al., 2016). Cows with metritis have reduced pregnancy per artificial insemination (AI) and are less cyclic compared with healthy cows, an increased calving to conception interval by ~36.5 days (Giuliodori et al., 2013; Ribeiro et al., 2013; Goshen and Shpigel, 2006). In addition, cows with metritis are ~30% more likely to be culled than cows that did not develop metritis (Wittrock et al., 2011).

Causes and Predisposing Factors of Metritis

In past years, it was believed that gravid uterus was sterile and that metritis in the dairy cow was caused by the onset of *Escherichia coli (E. coli)* in the uterus in the moment of parturition or right after it, and that infection with *E. coli* will path the way for other bacteria to grow thus developing metritis (Sheldon et al., 2009). However, recent data have shown that healthy and metritic cows share most genera at day of parturition and at day 6 postpartum (Jeon et al., 2015) and it is in abundance of some of the species that these two groups differ. Uterine microbiota is mainly composed by *Bacteroidetes, Fusobacteria, Proteobacteria, Tenericutes,* and *Firmicutes,* regardless of infection (Santos and Bicalho, 2011; Jeon et al., 2015) and the shift in bacteria growth of *Bacteroides, Porphyromonas,* and *Fusobacterium* from day 0 to day 3 is associated with the development of metritis (Jeon et al., 2015; Bicalho et al., 2017b). In the case of *E. coli,* the relative abundance that has been found in the uterus of cows with metritis has been very low (>1%) (Jeon et al., 2015; Jeon et al., 2016), and its presence has been associated with the microbiome of healthy cows (Jeon et al., 2016). However,
Bicalho et al. (2012) found that *E. coli* with the virulent factor *fimH* was associated with metritis and cows contaminated with *E. coli* had 4.7 times greater odds of developing metritis.

It was believed that the route for uterine contamination was through the vagina due to contamination from feces (Sheldon et al., 2009). However, uterine microbiota is associated to bacteria that are present in vagina, feces, and blood (Bicalho et al., 2017; Jeon et al., 2017) suggesting that bacteria can also travel through blood from intestine to the uterus during pregnancy. Nevertheless, after parturition the uterus environment may change to favor bacterial growth, mainly anaerobes. A delayed uterine involution, stoppage of blood flow through the placenta, low oxygen tension, necrosis of the caruncular epithelium and the accumulation of blood and allantoic fluid are conditions where anaerobic bacteria can thrive thus changing the uterine lochia to a purulent fetid discharge.

Among risk factors of metritis, it can mention dystocia, stillbirth, twins, RP, calving season, gestation length, and parity. The odds of having metritis in cows that experienced dystocia, stillbirths and twins were 4.32, 6.26, and 6.57 times greater compared with cows that did not experienced these conditions (Hossein-Zadeh, 2011). Cows with abnormal calving (i.e. dystocia, stillbirths and twins) have delayed in uterine involution compared with normal cows (Fonseca et al., 1983). After the integrity of the uterine epithelium tissue is compromised, and mechanical barriers such as mucus production is interrupted, bacteria have an easy access to the tissue and can cause infection. The odds of having metritis were 27.74 times larger in cows that develop RP compared with cows that did not develop RP (Hossein-Zadeh, 2011). Calving in cold
season is also associated with metritis. Hossein-Zadeh (2011) showed that cows that calved in winter had 2.45 greater odds of having metritis when compared with the ones that calved in spring. Parity is another risk factor for metritis. Cows with short gestation length had greater incidence of metritis compared with cows with an average gestation length (Vieira-Neto et al., 2017). Primiparous cows have 1.68 greater odds of having metritis than multiparous cows (Hossein-Zadeh, 2011).

Furthermore, subclinical hypocalcemia is associated with metritis. Cows with subclinical hypocalcemia had 3.24 times greater risk of developing metritis, and an 11-fold increase in the risk of developing puerperal metritis (Martínez et al., 2012). Low concentrations of calcium in blood are associated with immune impairment (Martínez et al., 2012), and cause low contractility (Al-Eknah and Noakes. 1989) of the uterus thus disrupting the process of elimination of bacteria. In addition, Hammon et al. (2006) found that cows with metritis had neutrophils with impaired phagocytosis and killing ability compared with cows with no metritis suggesting an impair immune system increases the risk of metritis.

**Metritis and Dry Matter Intake**

Limited research exists showing the association between DMI and metritis during the transition period. Huzzey et al. (2007) showed that cows that develop metritis had lesser DMI during the transition period compared with cows that did not develop metritis and showed that for each kg decrease of DMI during the last week of prepartum period increased the odds of having metritis by 2.87. In addition, they showed that cows that developed metritis had less feeding time and a smaller number of feeding bouts. The drop in DMI during the prepartum period could support the findings of Ospina et al., (2010) who showed that cows with more than 0.37 mEq/L NEFA prepartum were 1.9
more likely to have metritis compared with cows with less or equal than 0.29 mEq/L of NEFA.

**Ketosis**

Ketosis or hyperketonemia is a condition characterized by abnormally elevated concentrations of NEFA, BHB and ketone bodies such as acetoacetic acid and acetone in the body tissues and fluids. Even though blood NEFA is the gold standard to identify cows with ketosis, measurements of BHB are the most used due to easy measurement in the farm. In addition, thresholds NEFA and BHB have been determined to identify cows with hyperketonemia based on their association between these metabolites with health and production (Ospina et al. 2010a; Ospina et al. 2010b). Hyperketonemia or ketosis can be clinical or subclinical. Subclinical ketosis is defined as a cow having a blood concentration of BHB ≥1.2 mmol/L and showing no clinical signs whereas clinical ketosis cows present clinical signs and it is associated with concentrations of BHB ≥3.0 mmol/L (Oetzel, 2004).

The incidence of ketosis varies due to different definition across studies (McArt et al., 2013b). However, using a threshold of BHB ≥1.2 mmol/L McArt et al. (2012) reported an incidence of ketosis of 44% with a peak incidence on day 5 postpartum. Ketosis has been reported to be associated with lesser milk yield and increase culling. The association between milk yield and ketosis varies among studies, some researchers have showed that cows with ketosis produced more milk whereas others have reported lesser milk yield the first weeks of postpartum (McArt et al., 2013b). Chapinal et al. (2012) showed that cows with concentration levels of BHB ≥600 μmol/L during the last week prepartum had 1.7 kg/d lesser milk yield compared with cows with lesser concentrations of BHB, whereas multiparous cows that had NEFA of ≥0.5 mEq/L
had 1.6 kg/d lesser milk yield compared with multiparous cows with lesser concentrations of blood NEFA. In addition, Chapinal et al. (2012) shows that hyperketonemia during the first 2 week postpartum was also associated with lesser milk yield. In addition, Mcart et al. (2012) reported that for each 0.1 mmol/L increase in BHBA concentration at first positive test the milk yield decrease by 0.5 kg/d. Other studies have reported an association of ketosis with greater milk yield and others a lesser milk yield only in multiparous cows and greater milk yield in primiparous cows (Ospina et al. 2010a; McArt et al., 2013b). This is probably because greater producer cows are more prone to have ketosis and primiparous cows could cope better with ketosis as they are in a different homeorhetic stage compared with multiparous cows, as they need to nutrients for growth and milk production. Ketosis also increases culling. Mcart et al. (2012) showed that cows that develop ketosis had 3 times greater risk of being culled compared with cows that did not develop ketosis.

**Causes and Predisposing Factors of Ketosis**

Ketosis is a consequence of the lipid mobilization, as was explained earlier in this chapter, mobilization of adipose tissue may result in high ketones bodies concentrations in the organism. When ketones bodies accumulate in blood, clinical symptoms such as gradual loss of appetite and decrease in milk production, weight loss, moderate depression, dry feces, and decreased rumen motility may occur, and in severe cases neurologic signs can occur such as circling, head pressing, blindness, excessive salivation, tremors, and tetany may be seen (Smith, 2002).

Increased body condition score during the dry period and parity are the main predictors for ketosis. McArt et al. (2013a) showed that cows with high BCS during the dry period had greater mean of BHB from day 3 to 6 and had 1.2 greater risk of
developing ketosis compared with cows that had low BCS during the dry period. In addition, cows with more than 3 lactations had greater mean of BHB from 3 to 6 days of postpartum.

**Ketosis and Dry Matter Intake**

Using a threshold of BHB $\geq 1,000$ μmol/L on the first week of postpartum and BHB $\geq 1,000$ μmol/L and less $\geq 1,400$ μmol/L in week two or more Goldhawk et al. (2009) classified cows of having subclinical ketosis, and showed that cows that develop subclinical ketosis had lesser dry matter intake compared with healthy cows starting on week -1 and continue to be lesser until week 2 postpartum and that for each 1 kg decrease in average daily dry matter intake increased the risk of subclinical ketosis by 2.2 times. In addition, they also showed that cows that developed subclinical ketosis had fewer visits to the feeder and spent less time at the feeder than healthy animals.

**Mastitis**

Mastitis is defined as inflammation of the udder that may or not being infectious. Clinical mastitis when a cow has a visually abnormal milk secretion (e.g., clots, flakes, or watery) in one or more quarters which might or might not be accompanied by signs of inflammation of the udder tissue (e.g., heat, swelling, or discoloration of the skin) (Kelton et al., 1998). In addition, threshold of somatic cell count (SCC) of $>200,000$ cells/ml also is used to identify mastitis because of the association of high levels of SCC with intramammary infections by major pathogens (Schepers et al., 1997).

The incidence of mastitis in dairy herds from USA has been reported to be 24.8% and more recently in 28.9% (National Animal Health and Monitoring System, 2014; Miles et al., 2019). Clinical mastitis has a major economic impact in the dairy herd. Santos et al. (2004) showed that the time of occurrence of the first clinical mastitis
diagnosis had different impacts on milk yield. They showed that cows that developed clinical mastitis before first postpartum AI had 2.2 kg/d lesser milk yield compare with cows that did not develop mastitis whereas cows diagnosed between first postpartum AI and pregnancy diagnosis had 1.4 kg/d lesser milk yield during that lactation. However, if the first clinical mastitis case was diagnosed after the cow was pregnant the milk yield did not show any statistical difference when compared with cows that did not develop mastitis. Mastitis also impairs reproduction. Santos et al. (2004) showed that mastitis diagnosed before first AI had lesser percentage of conception at first AI, lesser pregnancy rate, greater abortion incidence, and greater days open compared with cows that did not develop mastitis. In addition, greater percentage of cows that were diagnosed with mastitis at any point in the study left the study compared with cows that did not develop mastitis (Santos et al., 2004).

**Causes and Predisposing Factors of Mastitis**

Mastitis can be contagious or environmental. In contagious mastitis, the transmission occurs from infected quarter to other healthy quarter in the same cow or in a different cow through a fomite such as milking equipment or contaminated gloves or hands. In environmental mastitis the microorganisms come from the environment and get access to the mammary gland and the transmission can occur from feces, bedding, or fomites (Smith, 2002). *Staphylococcus aureus, E. coli, Enterobacter* spp. *Streptococcus dysgalactiae, Trueperella pyogenes*, and *Pseudomonas* spp. have been identified as the pathogens with greater incidence in cases of clinical mastitis (Levison et al., 2016).

Microorganisms enter through the teat sphincter and multiply in the mammary gland starting an inflammatory process. The magnitude of inflammation will vary
depending on the microorganism that invades the tissue and its virulence and also will depend on the host defense against the pathogen (Harmon, 1994).

Parity, calving season, and conformation of the teat are among the risk factors for clinical mastitis. Parity also plays a role in the risk of having mastitis. Elghafghuf et al. (2014) showed that the hazard of having clinical mastitis increased by increase parity. Older cows (i.e. 4 years old) had less lactoferrin concentration compared with younger cows (2 years old) (Hagiwara et al., 2003) thus predisposing multiparous cows to develop mastitis by not preventing the invasion of pathogens in the mammary gland. In addition, as cows age, the udder become more pendulous and the teat conformation changes putting them in greater risk of having mastitis. On the other hand, cows that calve in spring and summer have greater hazard of having clinical mastitis compared with cows that calved in fall or winter, this may be due to warm temperatures and high humidity which combined create the perfect environment for bacteria multiplication. In addition, udder and teat conformation also are a risk factor for metritis. Cows with a loose fore udder attachment, flat teat end shape, low rear udder height increased the odds of by clinical mastitis diagnosis by 3.7, 1.5, and 2.8, respectively, compared with cows that had strong fore udder attachment, round teat end shape, and an intermediate rear udder height (Miles et al., 2019). Metabolic imbalances such a subclinical ketosis also increase the chance for developing mastitis. Raboisson et al. (2014) showed that cows with subclinical ketosis had 1.64 times greater chance of developing clinical mastitis compared with cows without subclinical ketosis. Hammon et al. (2006) showed that cows with greater NEFA impaired immune function, which can put cows at risk of infectious diseases as not being able to eliminate the pathogen properly.
**Mastitis and Dry Matter Intake**

As far as it is known there is no literature showing the association between dry matter intake and clinical mastitis during the transition period. Indications that DMI is associated with intake are based literature showing as association between increased NEFA and BHB concentrations and clinical mastitis (Schwegler et al., 2013; Moyes et al., 2009), the association of these metabolites and reduced immune function (Hammon et al., 2016) and the impairment of immune function in cows with clinical mastitis (Suriyasathaporn et al., 1999). In addition, Stangaferro et al. (2016b) showed that cows that develop clinical mastitis had decreased rumination time one day before clinical diagnosis and the rumination time stayed decreased until day 5 after diagnosis with a steep decrease on the day -1 until day 1 after diagnosis when compared with cows that did not develop mastitis.

**Digestive Disorders**

Digestive disorder is any disorder that affect the gastrointestinal tract (GI). Digestive disorders may include cows with displacement of abomasum (DA) and indigestion. Displaced abomasum is defined as the movement of the fourth compartment of the stomach to an abnormal position on the right or left side (LDA or RDA) of the abdomen, detected by auscultation of a “ping” sound with finger percussion whereas indigestion has been previously defined as a scant manure and lack of appetite with rumen and intestinal stasis (Stangaferro et al., 2016a). Cases as diarrhea, bloat, constipation, sand impaction, and cecal dilation also could be referred as digestive disorders as they affect the GI tract.

The incidence of digestive disorder defined as cows having diarrhea, decreased rumen motility, bloat or DA has been reported to be 9.4% (Vercouteren et al., 2015)
whereas DA has been reported with an incidence of 5% (Gröhn et al., 1998). Digestive disorders affect milk yield, reproduction, and culling in the dairy farm. Cows that develop acidosis, gas, off feed, or bloat had lesser milk yield compared with healthy cows but cows that experienced DA had lesser milk yield compared with healthy and compared with cows with other indigestions (Edwards and Tozer. 2004). In addition, Van Winden et al. (2003) found that cows that experienced DA produced 6.5 kg/d less compared with healthy cows. Digestive disorders also impair reproduction. Cows that developed diarrhea, decreased rumen motility, bloat, or DA had lesser percentage of cyclic cows at 21 d postpartum (Vercouteren et al., 2015). In addition, Ribeiro et al. (2013) showed that cows that developed diarrhea, bloat, or DA had lesser odds (OR = 0.19) of resumption of estrous cyclicity by day 49 and had reduced pregnancy per AI on day 60 after first AI. The literature is very limited showing an association between digestive disorder and culling, except for cows that experienced DA, in which is has been shown that cows with DA has 5.3% greater risk of being culled that cows that have not experienced DA (Gröhn et al., 1998).

**Causes and Predisposing Factors for Digestive Disorders**

As digestive disorder includes several conditions that affect the GI tract, the causes vary according to the affection. There is limited literature assessing risk factors of digestive disorders in dairy cows. The condition that has been more investigated has been DA. However, digestive disorder has been shown to be correlated (Spearman’s rho = 0.15) with metabolic disorders (Vercouteren et al., 2015). Similarly, DA is associated with metabolic disorders. Cows with increased NEFA prepartum and BHB and low calcium had greater risk of DA (Ospina et al., 2010b; LeBlanc et al., 2005; Chapinal et al., 2011; Rodriguez et al., 2017). Ospina et al. (2010b) showed that the
critical thresholds of NEFA during prepartum and postpartum to predict DA were 0.27 and 0.72 mEq/L, respectively, whereas for BHB during postpartum was 10 mg/dL. However, the mechanism of NEFA and BHB to cause DA is not well understood. A possible mechanism may be that because cows are using mainly NEFA as a fuel, and there is depression of DMI in cows with ketosis (Goldhawk et al., 2009), the empty abomasum is filled with gas leading to its displacement.

Other factors that are associated with an increased risk of having DA are high BCS, winter and summer season, and parity (Cameron et al. 1998). However, mechanism of most of this risk factors to cause DA are not completely understood. Cameron et al (1998) suggested that winter and summer season are risk factors for DA because of stress during summer that will decrease DMI and greater demand of energy during winter that can increase NEFA and BHB. Ain the case of BCS, as discussed earlier, overconditioned cows have greater weight loss during the transition period therefore increasing NEFA and BHB metabolites; therefore, increasing the risk of metritis. In the case of parity, DA risk was greater in multiparous cows compared with primiparous cows, and this may be related to multiparous cows being more likely to have ketosis therefore, increasing NEFA and BHB in blood.

**Digestive disorders and Dry Matter Intake**

As far as it is known there is no literature showing the association between dry matter intake and digestive disorder. However, it is logical that digestive disorder has an association with lesser DMI but the degree of association has not been determined during the transition period. In case of DA, van Winden et al. (2003) showed that cows that experienced DA had ~6.5 kg/d DMI during the 10 days before diagnosis.
Lameness

Locomotion scores are usually used to indicate the severity of the lameness and these scores can be measured manually or automatically. Most of the manual scores systems used by researchers are based on the asymmetric gait, reluctance to bear weight, and arched back, and is usually scored on a five-level scale whereas automatic measures are based on the kinetics of movement and postures, and behavioral patterns and production variables that could indicate lameness (Schlageter-Tello et al., 2014). However, Bicalho et al., (2007a) evaluated a visual score system with an automated locomotion score system (i.e. Stepmetrix) and showed that the visual score system done by trained veterinarians performed better than the automated score system. In this same research Bicalho et al., (2007a) proposed a 5-level score system proposed by was 1 = normal gait, 2 = presence of a slightly asymmetric gait, 3 = cow clearly favors one or more limbs (moderately lame), 4 = severely lame, or 5 = extremely lame (non-weight-bearing lame).

The incidence of lameness has been reported to be 23% but increasing by increasing parity (Bicalho et al., 2008). Lameness is associated with milk loss, reproduction impairment, and culling. After controlling for the milk yield during the first 3 weeks postpartum Bicalho et al. (2008) showed that cows that developed lameness had a milk loss of 1.5 kg/d compared with non-lame cows. On the other hand, Melendez et al. (2003) reported that cows that were lame had 25 percentage point (pp) lesser conception rate at first service, 7 pp lesser pregnancy rate, and 14 pp greater ovarian cysts compared with non-lame cows, and they reported that lame cows had a lesser hazard (OR = 0.43) of getting pregnant compared with cows that were not lame. In addition, overall lameness is associated with culling. Booth et al. (2004) showed that
cows that were diagnosed lame from 61 to 120 days in milk (DIM) had 2 times greater hazard of being culled compared with cows that were not lame. In addition, they also showed that the hazard of being culled varied depending on the type of lameness. For example, cows diagnosed with foot rot during 61 to 120 DIM had 5 times greater hazard of being culled than non-lame cows whereas cows that were diagnosed with foot warts during the same period were not associated with culling (Hazard ratio (HR) = 0.7; CI: 0.1-5.4).

**Causes and Predisposing Factors of Lameness**

Lameness can be infectious or noninfectious. Among infectious lameness it can be mentioned digital dermatitis, interdigital necrobacillosis, and interdigital dermatitis and among noninfectious are claw horn disruption lesions such as sole ulcers and white line disease.

Risk factors for noninfectious lameness are BCS, and parity. Low body condition score has greater risk of having noninfectious lameness, this may be due to the association of BCS with digital cushion thickness (Machado et al., 2010). As cows lose weight there is a loss in digital cushion thickness leading to claw horn disruption lesions. On the other hand, multiparous cows have greater risk of having noninfectious lameness (Machado et al., 2010). A possible explanation is the weakening and decreased elasticity of the connective tissue between the hoof horn and the bone of the third phalanx leading to contusions inside the claw. Subacute ruminal acidosis is another risk factor for noninfectious lameness. Bicalho et al. (2013) discuss the hypothesis that toxins released from the rumen degrade collagen fibers in the claws allowing the distal phalanx to move freely inside the capsule thus causing concussions of the soft tissue thus leading to claw lesions.
Risk factors for digital dermatitis include housing, stage of lactation, hoof conformation, among others. Access to pasture, clean legs and floors, and textured floors are among housing characteristics that are associated with lesser risk of digital dermatitis. In addition, there is a decrease of lameness cases during the dry period compared with other stages of lactation probably due to management. Cows in dry period do not have to move to the milk parlor; therefore, decreasing the chances of slipping and are not being exposed to the stress of being moved and mingled with other cows probably of other social hierarchy. A decreased width of the interdigital cleft has been associated with digital dermatitis possibly because of a smaller interdigital space would produce ideal conditions for pathogens to reproduce leading to infection.

**Lameness and Dry Matter Intake**

As far as it is known there is no literature showing the association of DMI and lameness during the transition period. Bach et al. (2007) reported that lame cows spent less time eating postpartum than non-lame cows and that the DMI. In addition, hyperketonemia has also been correlated with lameness (Collard et al., 2000) suggesting that cows with lameness have decrease intake.

**Economics of Metritis**

The profitability of the farm gets affected when there is a negative impact in milk production, reproduction, and/or when there is an increase in culling rate. Metritis has a negatively associated with these 3 areas, causing economic loss to the farm.

In addition, to the association on these 3 areas mentioned above, metritis also has an extra cost in treatment, and labor and this can range from $46 to $101 dollars depending on parity and the antibiotic used in the therapy (Lima et al., 2019).
The cost of metritis has been estimated previously but the literature is very limited and the estimation of the cost of metritis is very variable. In 1986, Bartlett et al. estimated that the total cost per metritis case was $106.00, and 22 years later Overton and Fetrow (2008) estimated that a cost per case of metritis ranged from $329 to $386 based in incomes and cost in one farm. Others have also estimated the cost of metritis but the estimation of the cost of metritis has not been the main focus of their study (Drillich et al., 2001; McArt et al., 2015; Lima et al., 2019) or the estimation was based in prices from a country outside the United States of America (US) (Mahnani et al., 2015). Drillich et al. (2001) estimated that metritis cost ranged from $291.19 to $362.09 depending on the treatment used (i.e. ceftiofur vs ampicillin + cloxacilin), whereas McArt et al. (2015) estimated that the cost of a metritis case attributable to hyperketonemia was $396. Lima et al., (2019) also estimated that metritis cost ranged from $255 to $392, depending on the treatment that was administered and the final destiny of the treated cow’s milk (i.e. with or without milk withhold or milk fed to calves) and Mahnani et al. (2015) estimated that the cost of metritis averaged $162.3 per case in farms from Iran.

Given the importance of metritis in the dairy herd and the variability in the estimation of the cost of a case of metritis, further research with current prices including several herds from different regions in the USA and a large sample size is needed to evaluate the economic impact of metritis.
CHAPTER 3
ASSOCIATION OF DRY MATTER INTAKE AND ENERGY BALANCE PREPARTUM AND POSTPARTUM WITH HEALTH DISORDERS POSTPARTUM: CALVING DISORDERS AND METRITIS

Summary

The main objective was to determine the association of DMI%BW, and EB prepartum (-21 d relative to parturition) and postpartum (28 d) with calving disorders (CDZ; dystocia, twins, and stillbirths; n = 101) and metritis (n = 114). For this, DMI%BW and EB were the independent variables and CDZ and metritis were the dependent variables. A secondary objective was to evaluate prepartum DMI%BW, and EB as predictors of CDZ and metritis. For this, CDZ and metritis were the independent variables and DMI%BW, and EB were the dependent variables. Data from 476 cows from 9 experiments were compiled. Cows that developed CDZ had lesser postpartum DMI%BW from d 3 to 12, and lesser ECM than cows that did not develop CDZ. Dry matter intake as percentage of BW, and EB prepartum did not affect the odds of CDZ. Cows with metritis had lesser prepartum DMI%BW and EB. Each 0.1 pp decrease in the average DMI%BW, and each Mcal decrease in the average EB in the last 3 d prepartum increased the odds of having metritis by 8%. The average DMI%BW and EB during the last 3 d prepartum produced significant cut-offs to predict metritis postpartum, which were ≤ 1.6 %/d and ≤ 2.5 Mcal/d, respectively. Cows that developed metritis had lesser overall postpartum DMI%BW, and ECM, and lesser EB from d 2 to 5, and from d 7 to 11 than cows that did not develop metritis. The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is muddled and variable. In summary, prepartum DMI%BW and EB were associated with and were predictors
of metritis although the effect sizes were small for metritis, and calving disorders and metritis were associated with decreased DMI%BW and ECM postpartum.

**Introductory Remarks**

Transition dairy cows experience a decline in DMI in the last week of gestation and the early lactation period is typically characterized by an increase in the incidence of disorders that compromise production and survival. The decline in DMI during prepartum period occurs in the last 10 d of gestation, although it is more pronounced in the last 4 d before calving (Hayirli et al., 2002). The decrease in DMI prepartum and insufficient DMI postpartum lead to a state of negative nutrient balance characterized by lipid mobilization and an increase in circulating concentrations of NEFA and BHB (Drackley, 1999; Grummer et al., 2004; French, 2006). Concentrations of NEFA ≥ 0.3 mM within 2 wk prepartum have been shown to increase the risk of uterine disorders such as metritis (Ospina et al., 2010b; Sepúlveda-Varas et al., 2015). Metritis is associated with reduced milk yield, impaired reproductive performance, and increased culling, which taken together result in economic losses (Mahnani et al., 2015; Overton and Fetrow, 2008). Cows with calving disorders (CDZ) such as dystocia, twins, or stillbirths, which are major risk factors for metritis, also have increased risk of culling (Vergara et al., 2014). Therefore, given the relevance of CDZ and uterine disorders, it is important to understand the factors associated with increased risk of these disorders to be able to devise strategies to reduce their incidence and mitigate their negative impacts on reproductive performance, culling, and profitability.

Previous research has observed that cows with severe metritis had reduced prepartum DMI, more noticeably in the last week of gestation, compared with cows with no metritis (Huzzey et al., 2007). Furthermore, for each kg decrease in DMI in the last
week of gestation increased the odds of severe metritis by 2.9 times (Huzzey et al., 2007). Nonetheless, because cows with other disorders were excluded (e.g., mastitis, vaginal tears, digestive disorder, etc.), these estimates are from a subset of the population and may not represent the whole population. In addition, the contribution of DMI to a predictive model that includes other risk factors for metritis such as parity, CDZ, and RP has not been evaluated. Therefore, further evaluation of prepartum DMI as a risk factor for metritis is warranted. Although a few studies have evaluated the association of DMI with dystocia (Proudfoot et al., 2009) and metritis (Huzzey et al., 2007; Schirmann et al., 2016), a comprehensive study of the association of pre- and postpartum DMI%BW, and pre- and postpartum EB with calving disorders and metritis is also lacking.

The hypotheses of this study were that reductions in DMI%BW and EB during the transition period are associated with calving disorders and metritis (Figure 3-1). The main objective of this current study was to evaluate the association of prepartum and postpartum DMI%BW and EB with CDZ (dystocia, twins, and stillbirths) and metritis. A secondary objective was to evaluate the use of prepartum DMI%BW, and EB as predictors of calving disorders and metritis.

**Materials and Methods**

**Experimental Design and Sample Size**

A retrospective longitudinal study was performed using the data from a total of 476 cows (139 primiparous and 337 multiparous) from 9 different experiments conducted at the University of Florida research dairy unit, located in the city of Hague, Florida. This was a convenience sample; therefore, no a priori sample size calculation was performed. For continuous variables, approximately 100 cows in the affected group
(CDZ, n = 101; metritis, n = 114; Table 1) would be needed to detect significant differences with an effect size of 0.20 (e.g. difference in DMI of 0.1 %/d when prepartum SD is 0.5 %/d of DMI%BW), alpha of 0.05 and beta of 0.2. Individual experiments were approved by the University of Florida Animal Research Committee.

The University of Florida dairy unit milked approximately 500 Holstein cows twice daily with a rolling herd average of approximately 10,500 kg/cow with an average BW in primiparous and multiparous cows of 560 kg (371 – 793 kg) and 660 kg (459 – 898 kg), respectively. The freestall beds and walking alleys were cleaned twice daily. Clean and dry sand was added on the top of the freestall beds twice weekly. Fans with misters and sprinklers over the feed line were present in the barns and activated when environmental temperatures rose above 18°C. There were two pre- and postpartum pens. The capacity of each prepartum pen was 30 cows and the capacity of each postpartum pen was 28 cows. The stocking density was maintained between 80 and 100%. Cows were vaccinated and treated for common diseases or disorders according to the standard operating procedures developed with participation of the veterinarians from University of Florida, College of Veterinary Medicine, Food Animal Reproduction, and Medicine Service.

The experiments were conducted from 2007 to 2015. Six of the 9 experiments were conducted during the hot months (June to October) to evaluate the effect of evaporative cooling during the dry period on production measures (do Amaral et al., 2009; do Amaral et al., 2011; Tao et al., 2011; Tao et al., 2012; Gomes, 2014; Thompson et al., 2014). For these experiments, cows were provided with shade only or with shade plus evaporative cooling with fans and sprinklers. The average
environmental temperature during the three weeks before calving for these experiments was 26.9ºC ± 2.0 ºC and a temperature humidity index (THI) of 77.7 ± 2.8. Herein, the categorization of heat stress abatement applied in these 6 previous experiments was maintained, resulting in cows categorized as hot with evaporative cooling (n = 108) or hot without evaporative cooling (n = 106). In the remaining studies, prepartum cows were enrolled from December to May with an average environmental temperature of 16.6 ± 3.4 ºC and THI of 61.8 ± 8.9 (Greco, 2014; Martinez et al., 2018; Zenobi, M. G. et al., 2018), and cows were provided evaporative cooling with fans and sprinklers when temperatures rose above 20 ºC. Fans and sprinklers were turned on and off automatically based on thermostat reading. Fans stayed on while environmental temperature exceeded 20 ºC, but sprinklers were on cycles of one minute on and three minutes off. Cows enrolled in the experiments from December to May could still be exposed heat stress. As far as it is known, a heat stress THI cut-off for the dry period has not been established; therefore, a prepartum cut-off of THI ≥ 70 was chosen as the midpoint between the traditional (72) and revised (68) THI cut-offs for lactating dairy cows (Armstrong, 1994; Zimbelman et al., 2009). Hence, cows were categorized as hot with evaporative cooling when the average THI during the last three weeks prepartum was ≥ 70 (n = 58 cows) and cool when the average THI for the last three weeks prepartum was < 70 (n = 204). Hence, to account for any conditional effect of heat abatement, the variable heat stress abatement was created: cool, hot without evaporative cooling, and hot with evaporative cooling. The following formula was used to calculate the THI, according to Dikmen and Hansen (2008):
\[ \text{THI} = 0.8^\circ \text{ ambient temperature} + ((\text{relative humidity}/100) \times (\text{ambient temperature} - 14.3)) + 46.4 \]

The meteorological data obtained from Weather Underground, Inc. (The Weather Underground, Inc., 2016) for the city of Hague, Florida was used to calculate THI.

**Measurement of Dry Matter Intake**

Cows had their DMI daily recorded using a system with individual feeding gates (Calan gates, American Calan Inc., Northwood, NH). For this study, DMI was collected from d -21 to -1 prepartum and from d 1 to 28 postpartum. Dry matter intake on the day of calving (d 0) was not included because upon parturition the diet changed from a prepartum to a lactating diet and intake could not be accurately recorded.

**Milk Yield and Energy Corrected Milk**

Cows were milked twice a day, and milk production was recorded automatically using milk meters (AfIFlo; S.A.E. Afikim). Data for milk components such as concentrations of fat, true protein, and lactose were available either daily (n = 356), or weekly (n = 120). For cows sampled weekly, daily measurements were estimated by interpolation. Milk fat % decrease linearly from week 1 to week 4 of lactation (Gross et al., 2011); therefore, interpolation would be an acceptable method for estimating daily fat %. As an example, when fat % was available for d 7 (Fat % = 3.12) and d 14 (Fat % = 3.55) postpartum, fat % on each subsequent day from d 7 to d 14 was calculated using the formula: Fat % subsequent day = [(Fat % d 14 – Fat % d 7) / 7] + Fat % previous day. For d 8, Fat % d 8 = [(3.55 – 3.12) / 7] + Fat % d 7 = 0.06 + 3.12 = 3.18%. The energy corrected milk (ECM) was calculated as follows, derived from NRC (2001):

\[ ECM = [(0.3246 \times \text{kg of milk}) + (12.86 \times \text{kg of fat}) + (7.04 \times \text{kg of protein})] \]
Body Weight and Body Condition Score

Cows were weighed daily (n = 232 cows) or weekly (n = 230 cows) using a digital scale (AfiWeight, S.A.E. Afikim). For cows weighed weekly, daily BW were estimated by interpolation. The prepartum BW was not available for 14 cows. Body weight was used to calculate DMI%BW and EB. Body condition was scored weekly by a member of the research team in each experiment during prepartum (range: d -21 to -1 relative to parturition) and postpartum (range: d 0 to 28 relative to parturition) using a 1 to 5 scale (from 1 = emaciated to 5 = obese) according to Ferguson et al. (1994). Agreement among BCS observers was not evaluated.

Energy Balance

The EB was calculated using NRC (2001) equations for energy requirements as follows:

For the prepartum EB:

\[ EB_{prepartum} = NE_{L} \text{(i.e. net energy lactation) intake} \] \[ - (NE_{L \text{ pregnancy}} + NE_{L \text{ maintenance}}) \]

For the postpartum EB:

\[ EB_{postpartum} = NE_{L} \text{ intake} \] \[ - (NE_{L \text{ maintenance}} + NE_{L \text{ milk}}) \]

Where

\[ NE_{L} \text{ intake}, NE_{L} \text{ maintenance}, NE_{L} \text{ pregnancy}, \text{ and } NE_{L} \text{ milk were calculated as follows:} \]

\[ NE \text{ intake} = DMI \times NE_{L} \text{ of the diet} \]

\[ NE_{L} \text{ maintenance} = (BW^{0.75} \times 0.08) \]

\[ NE_{L} \text{ pregnancy} = \left(0.00318 \times \text{day of gestation} - 0.0352 \times \text{calf BW/45}\right)/0.218. \]
\[NE_L \text{ milk} = (9.35 \times \text{milk yield} \times \text{fat \%} / 100) + (5.35 \times \text{milk yield} \times \text{protein \%} / 100) + (3.95 \times \text{milk yield} \times \text{lactose \%} / 100)\]

**Health Disorders**

Detailed paper and electronic health records were recorded for each cow. Each cow underwent a complete physical examination before enrollment in the initial trials, and cows showing signs of disease or disorders such as mastitis, lameness, digestive disorders, or pneumonia were not enrolled in the trials. Additionally, each cow underwent scheduled complete physical examinations by a trained herdsman or by a veterinarian from the College of Veterinary Medicine Food Animal Reproduction and Medicine Service (FARMS) at University of Florida on d 4, 7, and 12 postpartum. Furthermore, cow’s attitude was monitored daily prepartum by a member of the research team when cows were individually fed at 6:00 and 18:00 h and throughout the day when feed was pushed manually using a shovel every 2 h from 8:00 to 20:00 h. Any cow showing signs of depression, inappetence, lethargy, altered stride, or inflammation of the mammary gland underwent a physical examination by a trained herdsman or by a FARMS veterinarian. Cows that became sick during the prepartum period were excluded. In addition to cow’s attitude, daily milk yield was also monitored postpartum, and cows with a drop greater than 10% in milk yield underwent a physical examination by a trained herdsman or by a FARMS veterinarian. The veterinarians from FARMS performed physical examinations and provided supervision and training of herd personnel performing clinical diagnosis and treatment of postpartum cows at least once a week. Additionally, FARMS veterinarians were called to assist or confirm clinical diagnosis or treatment of postpartum cows throughout the weekdays and weekends. Only disease events occurring during the first 28 DIM were used in this study. The
electronic health records were first retrieved, and then confirmed the information using the paper health records. Cows with mismatched information or with a disease diagnosis prepartum were excluded from the study. The health disorders evaluated were calving disorders (dystocia, twins, stillbirths), metritis, ketosis, and mastitis. Calving difficulty was scored using a scale from 1 to 5; 1 = no assistance; 2 = assistance by one person without the use of mechanical traction; 3 = assistance by 2 or more people; 4 = assistance with mechanical traction; 5 = fetotomy or cesarean-section. Cows that required assistance (score ≥ 2) were considered to have dystocia. Stillbirth was defined as the birth of a dead calf or a calf that died within 24 h of birth. Metritis was characterized by presence of red-brownish watery fetid vaginal discharge within 21 DIM regardless of rectal temperature (Benzaquen et al., 2007). Cows suffering from metritis, ketosis, or mastitis were treated according to the farm standard operating procedure (http://animal.ifas.ufl.edu/facilities/du/).

**Statistical Analysis**

To evaluate the association of prepartum and postpartum DMI%BW, and EB with CDZ and metritis, the data were analyzed using ANOVA for repeated measures using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The data were divided into two periods, prepartum and postpartum. The dependent variables were prepartum DMI%BW or EB, and postpartum DMI%BW, EB, and ECM. The independent variable was one of the two disorders (CDZ or metritis), and they were modeled separately; cows that developed CDZ were compared with cows that did not develop CDZ, and cows that developed metritis were compared with cows that did not develop metritis. Cows that did not develop CDZ could have developed any other disorder including metritis. Likewise, cows that did not develop metritis could have developed
any other disorder. Other studies have used healthy cows as the comparison group (Huzzey et al., 2007). However, this would introduce selection bias; therefore, this could artificially increase the differences in the measures of DMI%BW between the groups and inflate the estimates in a prediction model. Although the focus of this study was the comparison between cows affected with calving disorders and metritis and unaffected cows, a comparison with healthy cows (i.e. cows that did not develop any disorder or disease diagnosed in the first 28 DIM) was also performed for comparison with the previous literature. Correlations between variables considered for inclusion in the models were assessed using Spearman’s correlation using the CORR procedure in SAS and are presented in Table A-1. The models also included the fixed effects of parity (primiparous vs. multiparous), BCS in the last week prepartum (< 3.75 vs. ≥ 3.75; Gearhart et al., 1990), day relative to calving (prepartum: d -21 to -1; postpartum: d 1 to 28), heat stress abatement (cool vs. hot without evaporative cooling vs. hot with evaporative cooling), and two-way interactions between disorder and other covariates, and cow was nested within experiment as a random effect. First order autoregressive, compound symmetry, and unstructured covariance structures were tested, and the first order autoregressive was selected because it resulted in the smallest Aikaike’s information criterion.

As an example, the initial model to evaluate the association between prepartum DMI%BW and metritis was:

\[ \text{DMI}\%\text{BW prepartum} = \text{metritis} + \text{day} + \text{heat stress abatement} + \text{BCS} + \text{parity} + \text{metritis x day} + \text{metritis x heat stress abatement} + \text{metritis x BCS} + \text{metritis x parity} + \text{cow (experiment)} \]
The disorder of interest was forced into the model, but other variables were removed from the model by stepwise backward elimination according to Wald-statistics criterion when \( P > 0.05 \). When an interaction was detected (\( P \leq 0.05 \)), then mean separation was assessed using the SLICE statement in the MIXED procedure, and multiple comparisons were performed using the Tukey-Kramer adjustment method in SAS.

Dystocia was first evaluated using all the calving scores (1 - 5), but was then dichotomized (score 1 = eutocia, score ≥ 2 = dystocia) because the pattern of DMI%BW and EB were similar among cows with calving ease score ≥ 2 (data not shown). Dystocia, twins, and stillbirths were first evaluated separately, but because the pattern of DMI%BW, and EB was similar (data not shown) they were combined into the variable CDZ to limit the number of variables presented in the paper. In addition, absolute DMI was analyzed as dependent variable and the results are shown in the appendix section.

An additional ANOVA for repeated measures was performed to evaluate which disease or disorder had the strongest association with prepartum DMI%BW and EB. The dependent variables were prepartum DMI%BW or EB. As independent variables all the diseases or disorders evaluated postpartum were included (i.e. calving disorders, metritis, ketosis, digestive disorders, mastitis, and lameness), and other covariates such as parity, BCS, day relative to parturition, and heat stress abatement. Cow was nested within experiment as a random effect. Independent variables were removed from the model by stepwise backward elimination according to Wald-statistics criterion when \( P > 0.05 \).
To evaluate the use of prepartum DMI%BW and EB as predictors of CDZ and metritis, each disease or disorder was considered the dependent variable, and DMI%BW and EB as independent variables. These data were analyzed by logistic regression with the GLIMMIX procedure of SAS. In this case, each disease or disorder was the dependent variable and the measures of prepartum DMI%BW or EB were assessed separately in different models as independent variables. For this purpose, the variables average DMI%BW or EB in the last 14, 7, and 3 d prepartum, and reduction from d -8 to -1 and from d -4 to -1 were created. Univariable and multivariable models were performed. The univariable models included cow nested within experiment as a random variable. Measures of DMI%BW or EB with $P < 0.20$ were selected for inclusion in the multivariable logistic regression models. Multivariable models also included parity (primiparous vs. multiparous), prepartum BCS (< 3.75 vs. ≥ 3.75), and heat stress abatement (cool vs. hot without evaporative cooling vs. hot with evaporative cooling), and cow nested within experiment as a random effect. The model for metritis also included the fixed effects of CDZ and RP. Two-way interaction terms between significant measures of DMI%BW and EB and other covariates were tested. A stepwise backward elimination was performed and explanatory variables with $P > 0.05$ according to the Wald-statistics criterion were removed from the model.

When a measure of DMI%BW, or EB prepartum was found to be significant ($P \leq 0.05$) after addition to the logistic regression model containing other covariates, the contribution to the predictive ability of the logistic regression model was assessed by comparing the area under the curve (AUC) of a receiver operating characteristic curve (ROC) of the model with and without the significant measures of DMI%BW or EB using
the ROCCONTRAST statement of the LOGISTIC procedure of SAS as previously reported (Vergara et al., 2014). The AUC ≤ 0.50 was considered noninformative, AUC between 0.50 and 0.70 was considered with low accuracy, AUC between 0.70 and 0.90 was considered accurate, and AUC between 0.9 and 1.0 was considered highly accurate (Swets J., 1988). Finally, cut-off values were determined for significant measures of DMI%BW and EB prepartum for predicting calving disorders and metritis using ROC, and the cut-off with the greatest Youden's J statistic which combines the values for sensitivity (Se) and specificity (Sp) was chosen. The Se, Sp, positive predicted value (PPV), negative predictive value (NPV) and overall accuracy of applying the cut-off to predict clinical mastitis and lameness were calculated. Statistical significance was considered when \( P \leq 0.05 \). The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is muddled and variable.

**Results**

The frequency of calving and uterine disorders diagnosed during the first 21 DIM is presented in Table 3-1. Results for the comparison between cows that developed calving disorders and metritis and healthy cows are presented in the appendix section and the Spearman correlation coefficient is shown in Table A-1.

**Association of Prepartum DMI%BW and EB with CDZ**

Calving disorders were not associated with prepartum DMI%BW (Figure 3-2A), or EB (Figure 3-2B) (Table 3-2).

**Prepartum DMI%BW and EB as Predictors of CDZ**

None of the measures of DMI%BW and EB were found to be significant predictors of CDZ in the univariable or multivariable models (\( P > 0.10 \); Table 3-3).
Association of Postpartum DMI%BW EB, and ECM with CDZ

The association between CDZ and postpartum DMI%BW, and postpartum EB were dependent on time (Table 3-2). Cows that developed CDZ had lesser DMI%BW from d 3 to 12 ($P \leq 0.05$; Figure 3-2A), but greater EB on d 15 ($P = 0.03$), 18 ($P < 0.01$), and 23 ($P = 0.05$) compared with cows that did not develop CDZ (Figure 3-2B). The ECM for cows that developed CDZ was lesser ($P < 0.01$) compared with cows that did not develop CDZ (Table 3-2), and an interaction ($P < 0.01$) between CDZ and day on ECM showed that cows that developed CDZ had lesser ECM compared with cows that did not develop CDZ from d 4 to 27 postpartum ($P \leq 0.05$; Figure 3-2C).

Association of Prepartum DMI%BW and EB with Metritis

Metritis was associated with prepartum DMI%BW, and EB (Table 3-2). Cows that developed metritis had lesser prepartum DMI%BW ($P = 0.01$) compared with cows that did not develop metritis (Table 3-2; Figure 3-3A) and had lesser EB ($P = 0.02$) during prepartum period compared with cows that did not develop metritis (Table 3-2; Figure 3-3B).

Prepartum DMI%BW, and EB as Predictors of Metritis

Of the covariates evaluated, CDZ and RP were the only significant predictors of metritis. Cows with CDZ had increased odds of developing metritis compared with cows that did not had CDZ (OR: 2.7; CI: 1.5-4.7; $P < 0.01$). Cows with RP had increased odds of developing metritis compared with cows that did not had RP (OR: 46.3; CI: 10.2-208.9; $P < 0.01$). The average of DMI%BW and EB during the last 3 d prepartum were significant predictors ($P \leq 0.05$) of metritis in both the univariable and multivariable models. In the multivariable model, for each 0.1 percentage point decrease in the average DMI%BW in the last 3 d prepartum and for each Mcal
decrease in the average EB in the last 3 d prepartum there was an increase in the odds of having metritis of 8% (Table 3-3).

When the average DMI%BW, and EB in the last 3 d prepartum were included individually in the metritis-predicting models containing RP and CDZ, the AUC increased from 0.67 (95% CI: 0.62 – 0.71) to 0.72 (95% CI: 0.68 – 0.76), and from 0.67 to 0.72 (95% CI: 0.68 – 0.76), respectively, and the differences between the areas were statistically significant ($P \leq 0.05$).

The average DMI%BW, and EB during the last 3 d prepartum produced significant ($P < 0.01$) cut-offs to predict metritis, which were $\leq 1.6 \%$/d, and $\leq 2.5$ Mcal/d, respectively (Table 3-4).

**Association of Postpartum DMI%BW, EB, and ECM with Metritis**

Cows that developed metritis had lesser postpartum DMI%BW ($P < 0.01$) compared with cows that did not develop metritis (Table 3-2), and an interaction ($P < 0.01$) between metritis and day showed that postpartum DMI%BW for cows that developed metritis was lesser than for cows that did not develop metritis from d 1 to 26 and on d 28 postpartum (Figure 3-3A). The association between metritis and postpartum EB was dependent on time (Table 3-2). Cows that developed metritis had greater negative EB from d 2 to 5, and from d 7 to 11 and lesser negative EB from d 25 to 27 compared with cows that did not develop metritis (Figure 3-3B). The ECM for cows that developed metritis was lesser ($P < 0.01$) compared with cows that did not develop metritis (Table 3-2), and an interaction ($P < 0.01$) between metritis and day on ECM showed that cows that developed metritis had lesser ECM from d 3 to 28 postpartum than cows that did not develop metritis ($P \leq 0.05$; Figure 3-3C).
Association of Prepartum DMI\%BW and EB with Postpartum Disorders

When all diseases or disorders were included in the model, ketosis, metritis, and digestive disorders remained associated \((P \leq 0.05)\) with prepartum DMI\%BW and EB. Additionally, day relative to parturition, BCS, and heat stress abatement were associated \((P \leq 0.05)\) with prepartum DMI\%BW and EB, and parity was associated \((P < 0.01)\) with prepartum DMI\%BW. The direction of the association was the same as reported herein or in other chapters of this dissertation (Table 3-5).

Discussion

The objectives of this study were to evaluate the association of prepartum and postpartum DMI\%BW, and EB with calving disorders and metritis, and to evaluate prepartum DMI\%BW, and EB as predictors of calving disorders and metritis. It was hypothesized that DMI\%BW, and EB during the transition period would be associated with calving disorders and metritis. Herein, it was shown that prepartum DMI\%BW or EB were not associated with CDZ, but cows that developed metritis had decreased overall DMI\%BW and EB prepartum. Furthermore, the average of DMI\%BW and EB in the last 3 d prepartum were significant explanatory variables for metritis, and the addition of DMI\%BW, and EB in the last 3 d prepartum to a model containing CDZ and RP significantly increased the predictive ability of the model. Postpartum DMI\%BW and ECM was decreased for cows that developed CDZ and metritis. In addition, cows that developed metritis had greater negative EB from d 2 to 9.

Previous studies have evaluated the association of prepartum absolute DMI with dystocia (Proudfoot et al., 2009), and metritis (Huzzey et al., 2007; Schirmann et al., 2016). Proudfoot et al. (2009) found that cows that had dystocia consumed 12\% less DMI during the last 2 d prepartum compared with cows with eutocia. Conversely, it was
observed that DMI (Table A-2, Figure B-1A), DMI%BW, and EB was similar among cows that did and did not have CDZ (dystocia, twins and stillbirths) throughout the entire prepartum period. Likewise, measures of DMI%BW, or EB prepartum were not significant explanatory variables for CDZ. It is not clear why the discrepancy between the findings presented in this study and previous findings (Proudfoot et al., 2009). One possibility is differences in the definition of dystocia. In the study by Proudfoot et al. (2009), cows that required two or more people to deliver the calf were compared with cows that required no assistance, whereas cows that required only one person to deliver the calf were excluded. Nonetheless, even when all the calving scores were compared, an effect of greater calving scores (> 2) on prepartum DMI could not observed, DMI%BW, and EB, had similar results when it was compared with healthy cows (Table A-3). Herein, a large number of cows with dystocia (n = 79) were included; which provides considerable confidence in the association of prepartum DMI%BW, and EB with dystocia. Moreover, no association of prepartum DMI%BW and EB with twins or stillbirths was found when compared with cows that did not develop twins or stillbirths. Nonetheless, when cows that had CDZ were compared with healthy cows, cows that had CDZ had lesser prepartum DMI%BW and EB (Table A-3; Figure B-2B, B-2C). When evaluated separately, cows that had twins showed lesser prepartum DMI%BW and EB than healthy cows carrying singletons (data not shown), and cows that had dystocia and stillbirths had similar prepartum DMI%BW and EB compared with healthy cows (data not shown). This finding, although not observable at the herd level because cows that do not have twins may have other disorders that affect DMI, may be a consequence of greater discomfort in cows carrying twins compared with cows carrying
singletons. As far as it is known, this is the first study to evaluate DMI%BW, and EB prepartum in cows that developed twins or stillbirths. Liboreiro et al. (2015) compared daily rumination time prepartum in cows that had twins and cows that had singletons, and found no difference, but cows that had stillbirths had lesser rumination time during the majority of the prepartum period than cows that had live calves, which may indicate a decrease in DMI prepartum in cows that had stillbirths. Nonetheless, rumination time was found not to be correlated with DMI because of the negative correlation between rumination time and feeding time (Schirmann et al., 2012); therefore, rumination time may be affected without affecting DMI.

During postpartum, cows that had CDZ had lesser DMI%BW and greater negative EB than cows that did not have CDZ. Proudfoot et al. (2009) showed that cows that had dystocia had similar DMI during the first 24 h and 48 h after calving and no differences in meal sizes after calving. Although, there was no difference between groups in DMI%BW on d 1 after calving, the DMI%BW was lesser starting on d 3, and DMI starting on d 2 (Figure B-2A), and such difference continued until d 12. The negative association between CDZ and DMI%BW may be a consequence of pain associated with dystocia and the predisposition of cows with dystocia to have vulvovaginal lacerations (Vieira-Neto et al., 2016), which may decrease the cow’s appetite in the first few DIM because of the associated pain and discomfort. In addition, CDZ are a risk factor for metritis, which may further decrease DMI%BW because of the associated clinical signs of the disease such as fever and lethargy, and the associated pain (Stojkov et al. 2015). Postpartum EB deficit in cows that had CDZ was similar to cows that did not have CDZ during the first day of postpartum and then the deficit was
lesser in some days after 2 weeks related to parturition. This was mainly because cows that had CDZ had lesser ECM throughout the observation period but recovered DMI after 14 DIM. Nonetheless, when cows that had CDZ were compared with healthy cows, there was a greater EB deficit postpartum in cows that had CDZ in the first two weeks postpartum because the differences in DMI%BW were more pronounced.

Cows that developed metritis had decreased DMI%BW and EB prepartum compared with cows that did not develop metritis. Nonetheless, after including all diseases in the model, DMI%BW lost significance but metritis was still associated with prepartum EB. Huzzey et al. (2007) reported that cows with severe metritis [putrid vaginal discharge with a red/brown color, watery, foul smelling and one recording of fever (≥39.5°C)] had decreased DMI during the last 2 weeks prepartum with a clear divergence from healthy cows starting on d -7. Schirmann et al. (2016) observed that cows that developed metritis concurrent with subclinical ketosis had a decrease in DMI from d -7 to -2 compared with healthy cows. Similar to previous literature, when cows that developed metritis were compared with healthy cows, prepartum DMI was lesser for metritic cows than healthy cows (Table A-3), with a clear divergence starting on day -5 (Figure B-3A). As mentioned previously, the present study is focused on the observable differences in prepartum DMI%BW and EB between cows that did and did not have metritis. In a herd, cows that do not develop metritis could have developed any other disease or disorder that are associated with decreased DMI, DMI%BW, and EB prepartum such as metabolic and digestive disorders; therefore, excluding cows that develop other conditions could inflate the measures of association. This is of particular relevance for the prediction model. Huzzey et al. (2007) reported that for each kg
decrease in DMI during the last week prepartum the odds of severe metritis increased by 2.87 times. In the present study, the effect sizes were small; 8% increase in the odds of having metritis with each unit decrease in the measures of both DMI%BW and EB, and the addition of DMI%BW, and EB in the last 3 d prepartum to a metritis-predicting model significantly increased the predictive ability of the models as evaluated by the AUC, indicating that DMI%BW and EB prepartum were contributors to the development of metritis even when accounting for other variables such as CDZ and RP. In addition, cut-offs for DMI%BW, and EB were determined to see if they could be used solely as a predictor of metritis, and the cut-offs resulted in moderate Se (78 - 83%) and low Sp (37 - 38%). In a previous study, Ospina et al. (2010b) reported low Se (37%) and moderate Sp (80%) for a prepartum NEFA cut-off of 0.37 mEq/L to predict metritis, RP or both. Therefore, the cut-off presented in this study is more useful for identifying the majority of the cows that will develop metritis, whereas Ospina’s is more useful for identifying cows that will not develop metritis. Perhaps a combination of DMI%BW or EB and NEFA prepartum could results in improved accuracy of predicting metritis. Others have used a health index score based on rumination time and physical activity during the postpartum period (i.e. d -5 to 2 relative to clinical diagnosis) to identify cows with metritis, and observed a Se of 55% but Sp was not reported (Stangaferro et al., 2016c). Huzzey et al. (2007) suggested that the decrease in DMI prepartum could be attributed to decreased cow’s dominance at the feed bunk. Nonetheless, it was later observed that cows that develop metritis are actually more dominant at the feed bunk, which may affect their DMI because the time spent protecting the feed bunk may reduce eating time (Chebel et al., 2016).
Cows with metritis also had lesser DMI%BW, EB, and ECM during postpartum period. Previous research has shown a decrease in DMI during postpartum period (Huzzey et al., 2007). The onset of infection leads to the release of cytokines and chemokines like IL-1β, IL-6, and TNF alpha that will induce anorexia (Plata-Salamán et al., 1996). Anorexia may worsen the energy balance and lead to body fat mobilization and ketone body production which may further decrease feed intake (Visinoni et al., 2012). Cows with metritis had greater negative EB during postpartum compared with cows that did not develop metritis from d 2 until d 9, then EB recovered, and was actually less negative for metritic cows from d 25 to 27. This pattern of EB occurred because cows with metritis had lesser ECM throughout the observation period but recovered DMI at the end of the observation period. It is interesting that the recovery in DMI%BW for cows with CDZ and metritis was not accompanied by a comparable increase in ECM. This finding indicates that CDZ and metritis are associated with long term changes in nutrient partitioning. During infection and inflammation peripheral insulin resistance decreases, and mammary glands reduce glucose uptake, which makes glucose more available to leukocytes to fight infection but leads to decreased milk yield (Bradford et al., 2015, Baumgard et al., 2017). It is interesting that the changes in the metabolism of the mammary gland persisted long after the initial trauma (i.e. calving disorders) or infection (i.e. metritis) had occurred, which deserves further investigation to better understand the effect of infection and inflammation on long term nutrient partitioning.

A limitation from this present study is that data were collected from different experiments over the years. This meant that different observers were collecting
subjective data such as BCS in each trial, agreement among observers could not be compared, and treatments had been applied in each trial (i.e. heat stress abatement), which had to be controlled for in the statistical analysis. Another limitation is that the association of metritis with DMI%BW, EB and ECM during postpartum period could not be evaluated before and after metritis diagnosis because dividing the data would have resulted in reduced sample size per day; therefore, increasing the standard errors or producing unreliable standard errors. As an example, cows that developed metritis on day 2 would have only one day of DMI before the disease diagnosis but 26 days of DMI after the disease diagnosis. For cows diagnosed with metritis from day 3 to 12 the number of days of DMI before the disease diagnosis would increase but the number of days of DMI after the disease diagnosis would decrease. In addition, the day cows developed metritis could not ascertain because cows were not examined daily. Lastly, even if the data of this study was divided before and after disease diagnosis, it could not infer causation because cows were not randomly assigned to develop metritis. Hence, herein it was presented the association between metritis development and DMI%BW and EB pre- and postpartum.

**Chapter Summary**

In summary, CDZ was not associated with prepartum DMI%BW or EB but was associated with a decrease in postpartum DMI%BW and ECM. Metritis was associated with a decrease in prepartum DMI%BW and EB. The average DMI%BW, and EB in the last 3 d prepartum were significant explanatory variables for metritis, and DMI%BW and EB in the last 3 days prepartum significantly increased the predictive ability of metritis-predicting models. Prepartum cut-offs for DMI%BW and EB to predict metritis were established, and had moderate Se and low Sp. In addition, metritis was associated with
a decrease in postpartum DMI%BW and ECM, and a greater negative EB. The results of this study give a better understating of the role DMI plays during the transition period; namely that when DMI%BW decrease and the deficit of EB increases, the risk of metritis increases, although the increase was small. In summary, DMI%BW and EB prepartum are significant but minor contributors to metritis development postpartum and cannot be used reliably to identify cows that will develop metritis postpartum.
<table>
<thead>
<tr>
<th>Disease/Disorder</th>
<th>Frequency</th>
<th>Percentage (%)</th>
<th>(^b)MPP (min - max)</th>
<th>(^c)IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^a)CP</td>
<td>101</td>
<td>21.2</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Dystocia</td>
<td>79</td>
<td>16.6</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Calving score 5</td>
<td>4</td>
<td>0.8</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Calving score 4</td>
<td>13</td>
<td>2.7</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Calving score 3</td>
<td>35</td>
<td>7.4</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Calving score 2</td>
<td>27</td>
<td>5.7</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Calving score 1</td>
<td>397</td>
<td>83.4</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Twins</td>
<td>13</td>
<td>2.7</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>25</td>
<td>5.3</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>Metritis</td>
<td>114</td>
<td>24.0</td>
<td>5 (2 - 12)</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\)Calving problems = twins, stillbirth, dystocia (cows with calving ease score ≥ 2 were considered to have dystocia). Calving ease score: 1 = no assistance, 2 = assistance by one person without the use of mechanical traction, 3 = assistance by two or more people, 4 = assistance with mechanical traction, and 5 = fetotomy or Cesarean-section. Sixteen cows with calving problems had more than one condition.

\(^b\)MPP = Average of days postpartum when the disorder was diagnosed.

\(^c\)IQR = Interquartile range.
Table 3-2. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with metritis according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET</td>
<td>No MET</td>
<td>MET</td>
<td>Day</td>
<td>MET x D</td>
<td>MET</td>
<td>No MET</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.55 ± 0.04</td>
<td>1.65 ± 0.02</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0.33</td>
<td>2.39 ± 0.06</td>
<td>2.80 ± 0.03</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.90 ± 0.4</td>
<td>2.90 ± 0.2</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td>-5.2 ± 0.6</td>
<td>-4.6 ± 0.4</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29.0 ± 1.0</td>
<td>34.5 ± 0.6</td>
</tr>
</tbody>
</table>

aMET: developed metritis.
bDay: day relative to parturition.
cMET x D: interaction between metritis and Day.
Table 3-3. Effect of each 0.1 percentage point decrease in the average of dry matter intake as a percentage of BW (DMI%BW) and each unit decrease in the average of energy balance (EB) in the last 3 days prepartum on diseases or disorders in the first 28 days postpartum.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>DMI (%BW)</th>
<th>OR</th>
<th>95% CI</th>
<th>P - value</th>
<th></th>
<th>EB (Mcal/d)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCDZ</td>
<td></td>
<td>1.04</td>
<td>0.98 - 1.09</td>
<td>0.17</td>
<td></td>
<td>1.02</td>
<td>0.96 - 1.07</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Metritis</td>
<td></td>
<td>1.08</td>
<td>1.02 - 1.14</td>
<td>&lt;0.01</td>
<td></td>
<td>1.08</td>
<td>1.03 - 1.14</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

*aCalving disorders; twins, stillbirth, dystocia
Table 3-4. Cut-offs of DMI as percentage of BW (DMI%BW), and energy balance (EB) to predict metritis postpartum.

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Acc (%)</th>
<th>AUC</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI%BW ≤ 1.6</td>
<td>78</td>
<td>37</td>
<td>28</td>
<td>84</td>
<td>46</td>
<td>0.58</td>
<td>0.01</td>
</tr>
<tr>
<td>EB, Mcal/d ≤ 2.5</td>
<td>83</td>
<td>38</td>
<td>29</td>
<td>88</td>
<td>48</td>
<td>0.59</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

aSensitivity; bSpecificity; cPositive predicted value; dNegative predictive value; eAccuracy; fArea under the curve.
Table 3-5. Association of prepartum (-21 to -1 d) dry matter intake as percentage of BW (DMI%BW) and energy balance (EB) with postpartum disorders.

<table>
<thead>
<tr>
<th></th>
<th>Metritis</th>
<th>Ketosis</th>
<th>Digestive Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>P - value</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.51 ± 0.04</td>
<td>1.59 ± 0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.39 ± 0.39</td>
<td>2.31 ± 0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*P - value ≤ 0.05 considered significant.
Figure 3-1. Causal diagram showing the relationship of dry matter intake, energy balance with postpartum diseases and its consequences. Based on the work of Beam and Butler 1999\textsuperscript{1}, Correa et al., 1993\textsuperscript{2}, Gröhn et al., 1998\textsuperscript{3}; Suriyasathaporn et al., 2000\textsuperscript{4}, Kimura et al., 2002\textsuperscript{5}, Melendez et al., 2003\textsuperscript{6}; Santos et al., 2004\textsuperscript{7}, Hammon et al., 2006\textsuperscript{8}, Proudfoot et al., 2009\textsuperscript{9}, Ospina et al., 2010a\textsuperscript{10}, Ospina et al., 2010b\textsuperscript{11}, Martinez et al., 2012\textsuperscript{12}, McArt et al., 2012\textsuperscript{13}, Ribeiro et al., 2013\textsuperscript{14}, Raboisson et al., 2014\textsuperscript{15}, and Sepúlveda-Varas et al., 2018\textsuperscript{16}. 

\[\text{Reduced Dry Matter Intake} \rightarrow \text{Negative Energy Balance} \rightarrow \text{Ketosis} \rightarrow \text{Metritis} \rightarrow \text{Calving Disorders} \rightarrow \text{Immune Dysfunction} \rightarrow \text{Mastitis} \rightarrow \text{Milk Fever} \rightarrow \text{Digestive Disorders} \rightarrow \text{Retained Placenta} \rightarrow \text{Culling} \rightarrow \text{Reproductive Failure} \rightarrow \text{Lameness} \]
Figure 3-2. Association of calving disorders postpartum with (A) DMI as percentage of BW (DMI%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI%BW: calving disorders - $P = 0.99$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P = 0.12$. Prepartum EB: calving disorders - $P = 0.62$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P = 0.25$. Postpartum DMI%BW: calving disorders - $P = 0.07$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P < 0.01$. Postpartum EB: calving disorders - $P = 0.56$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P < 0.01$. ECM: calving disorders - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P < 0.01$. 

- Figure A: Plot showing DMI percentage of BW by calving disorders status.
- Figure B: Plot showing energy balance by calving disorders status.
- Figure C: Plot showing energy corrected milk by calving disorders status.
Figure 3-3. Association of metritis postpartum with (A) DMI as percentage of BW (DMI%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI%BW: metritis - $P = 0.01$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day - $P = 0.33$. Prepartum EB: metritis - $P = 0.02$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day - $P = 0.09$. Postpartum DMI%BW: metritis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day - $P < 0.01$. Postpartum EB: metritis - $P = 0.37$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day - $P < 0.01$. ECM: metritis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day - $P < 0.01$. 


CHAPTER 4
ASSOCIATION OF DRY MATTER INTAKE AND ENERGY BALANCE PREPARTUM AND POSTPARTUM WITH HEALTH DISORDERS POSTPARTUM: KETOSIS AND MASTITIS

Summary

The main objective was to determine the association of DMI%BW and EB prepartum (-21 d relative to parturition) and postpartum (28 d) with ketosis (n = 189) and clinical mastitis (n = 79). For this, DMI%BW and EB were the independent variables and ketosis and clinical mastitis were the dependent variables. A secondary objective was to evaluate prepartum DMI%BW, and EB as predictors of ketosis and clinical mastitis. For this, ketosis and clinical mastitis were the independent variables and DMI%BW and EB were the dependent variables. Data from 476 cows from 9 experiments were compiled. Clinical mastitis was diagnosed if milk from one or more quarters was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the quarter, or generalized illness during the first 28 d postpartum. Ketosis was defined as the presence of acetoacetate in urine which resulted in any color change [5 mg/dL (trace) or greater] in the urine test strip (Ketostix®, Bayer). Cows that developed ketosis had lesser DMI%BW, and lesser EB on d -5, -3, -2, and -1 than cows without ketosis. Each 0.1 pp decrease in the average DMI%BW, and each Mcal decrease in the average of EB in the last 3 d prepartum increased the odds of having ketosis by 8%, and by 5%, respectively. Cut-offs for DMI%BW, and EB during the last 3 d prepartum to predict ketosis were established and were ≤ 1.5 %/d and ≤ 1.1 Mcal/d, respectively. Cows that developed ketosis had lesser postpartum DMI%BW, and EB, and greater ECM than cows without ketosis. Cows that developed clinical mastitis had lesser DMI%BW, but similar prepartum EB compared with cows without clinical mastitis. Each
0.1 pp decrease in the average DMI%BW, and each Mcal decrease in the average EB in the last 3 d prepartum increased the odds of having clinical mastitis by 10% and 8%, respectively. The average DMI%BW and EB during the last 3 d prepartum produced significant cut-offs to predict clinical mastitis postpartum, which were ≤ 1.2 %/d and ≤ 1.0 Mcal/d, respectively. Cows that developed clinical mastitis had lesser postpartum DMI%BW from d 3 to 15 and on d 17, greater EB on d 18, from d 21 to 23, and on d 26, and lesser ECM. The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is muddled and variable. In summary, measures of prepartum DMI were associated with, and were predictors of ketosis and clinical mastitis postpartum, although the effect sizes were small.

**Introductory Remarks**

The decrease in prepartum DMI and the insufficient DMI postpartum lead to a state of negative nutrient balance characterized by increased lipid mobilization in the form of NEFA and an increase in ketone bodies such as BHB (Drackley, 1999; Grummer et al., 2004; French, 2006). Several studies have found a negative association of ketosis on milk yield (Rajala-Schultz et al., 1999; Ospina et al., 2010a; Chapinal et al. 2012); however, the association was found to be conditional on parity, the day of onset of subclinical ketosis, and the peak BHB concentration in blood (Ospina et al., 2010a; Chapinal et al., 2012; McArt et al., 2012). Postpartum hyperketonemia has also been associated with postpartum diseases such as displaced abomasum and metritis, and with decreased fertility and increased culling, which incur in significant economic losses to dairy producers (Ospina et al., 2010a; Chapinal et al., 2011; McArt et al., 2013b). Several risk factors for postpartum ketosis have been determined. Mcart et al. (2013a) showed that cows with increased BCS, calf sex (male),
increased prepartum NEFA (≥ 0.30 mEq/L), decreased calving ease, stillbirth, and increased parity had greater risk of developing ketosis during the first 16 d postpartum. Moreover, a previous study observed a negative correlation between prepartum DMI and subclinical ketosis postpartum, and 1 kg decrease in the average daily DMI prepartum increased the risk of subclinical ketosis by 2.2 times (Goldhawk et al., 2009). Nonetheless, a comprehensive study of the association of prepartum DMI%BW, and prepartum EB with ketosis postpartum is still lacking.

Furthermore, clinical mastitis in one of the main diseases in dairy farms of the United States with an incidence that can range from 16% to 27% (USDA-NAHMS, 2018). Clinical mastitis is associated with reduced milk yield and fertility and increased culling, causing substantial economic losses to dairy farms (Santos et al., 2004; Hadrich et al., 2018) with an average cost per case of clinical mastitis that ranges from US$ 95 to 211, depending on the etiology (Bar et al., 2008; Cha et al., 2011).

Cows with ketosis have greater odds of having clinical mastitis (Raboisson et al., 2014). Immune cells that are exposed to high NEFA and BHB concentrations during the first weeks of lactation, which has been shown to be associated with decreased neutrophil function (Suriyasathaporn et al., 1999; Hammon et al., 2006). Suriyasathaporn et al., (2000) proposed that hyperketonemia affects the udder defense by affecting leukocytes’ phagocytosis, cytokine production, and migration. This is in agreement with the results from Hammon et al., (2006) who showed that the killing ability of neutrophils was negatively correlated with NEFA concentrations in the week of calving. In addition, clinical mastitis postpartum has been linked to decreased glucose
and increased NEFA and BHB concentrations prepartum (Schwegler et al., 2013; Moyes et al., 2009; Jánosi et al., 2003).

Given the relationship of NEFA and BHB with clinical mastitis and the association between DMI%BW, EB, NEFA, and BHB, it was hypothesized that a reduction in DMI%BW, and EB during the transition period would be associated with ketosis and clinical mastitis postpartum. Therefore, the main objective of this study was to evaluate the association of pre- and postpartum DMI%BW, and EB with ketosis and clinical mastitis postpartum. A secondary objective was to evaluate the use of prepartum DMI%BW, and EB as predictors of ketosis and clinical mastitis postpartum.

**Materials and Methods**

Study design, housing, measurement and calculation of DMI, milk yield, BW, BCS, and EB are described in detailed in Chapter 3. In summary, data from a total of 476 cows (139 primiparous and 337 multiparous) from 9 different experiments conducted at the University of Florida dairy unit, located in the city of Hague, Florida. This was a convenience sample; therefore, no a priori sample size calculation was performed. For continuous variables, approximately 200 cows in the affected group (ketosis, n = 198; Table 1) would be needed to detect statistical differences with an effect size of 0.2 (e.g. difference in DMI of 0.8 kg/d when prepartum SD is 4 kg/d of DMI), alpha of 0.05 and beta of 0.2. In the case of clinical mastitis (n = 79; Table 1), it would be able to detect statistical differences with an effect size of 0.3 (e.g. difference in DMI of 1 kg/d when prepartum SD is 4 kg/d of DMI), alpha of 0.05 and beta of 0.2.

**Health Disorders**

Detailed paper and electronic health records were recorded for each cow. Each cow underwent a complete physical examination before enrollment in the initial trials,
and cows showing signs of disease or disorders such as mastitis, lameness, digestive disorders, or pneumonia were not enrolled in the trials. Additionally, each cow underwent scheduled complete physical examinations by a trained herdsman or by a veterinarian from FARMS at University of Florida on d 4, 7, and 12 postpartum. Furthermore, cow’s attitude was monitored daily prepartum by a member of the research team when cows were individually fed at 6:00 and 18:00 h and throughout the day when feed was pushed manually using a shovel every 2 h from 8:00 to 20:00 h. Any cow showing signs of depression, inappetence, lethargy, altered stride, or inflammation of the mammary gland underwent a physical examination by a trained herdsman or by a FARMS veterinarian. Cows that became sick during the prepartum period were excluded. In addition to cow’s attitude, daily milk yield was also monitored postpartum, and cows with a drop greater than 10% in milk yield underwent a physical examination by a trained herdsman or by a FARMS veterinarian. The veterinarians from FARMS performed physical examinations and provided supervision and training of herd personnel performing clinical diagnosis and treatment of postpartum cows at least once a week. Additionally, FARMS veterinarians were called to assist or confirm clinical diagnosis or treatment of postpartum cows throughout the weekdays and weekends. Only disease events occurring during the first 28 DIM were used in this study. First, the electronic health records were retrieved, and then confirmed the information using the paper health records. Cows with mismatched information or with a disease diagnosis prepartum were excluded from the study. The health disorders recorded were ketosis, clinical mastitis, calving disorders (dystocia, twins, stillbirths), and metritis. Ketosis was defined as presence of acetoacetate in urine which resulted in any color change [5
mg/dL (trace) or greater] in the urine test strip (Ketostix®, Bayer). The test strip has 90% sensitivity and 86% specificity using blood BHB concentration ≥ 1.4 mmol/L (Carrier et al., 2004). This means that after each test, 10% of the cows with ketosis would not be diagnosed with ketosis and 14% of the cows not having ketosis would be diagnosed with ketosis. The chance of classifying cows as not having ketosis was decreased even further because cows were systematically tested at 4, 7 and 12 d postpartum. Of the cows never diagnosed with ketosis (n = 287), 17.4, 15.3, and 25.4% never produced a urine sample at 4, 7, and 12 d postpartum, respectively. Nine cows (3.1%) never produced a urine sample; therefore, they were removed from the analysis. Misclassification in this scenario would bias the estimates towards the null hypothesis. Cows were also tested for ketosis if they showed signs of depression, inappetence, lethargy, or a drop greater than 10% in milk yield. On average, cows were tested 2.4 ± 0.72 (range: 0 – 3) times during the first four weeks of lactation. Herein, no attempt was made to distinguish between subclinical and clinical ketosis. Clinical mastitis was diagnosed if milk from one or more quarters was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the quarter, or generalized illness. Trained farm employees actively diagnosed clinical mastitis during forestripping at each milking, and it was confirmed by the herdsman and/or by the FARMS veterinarians. Cows diagnosed with mild or moderate clinical mastitis were treated with intramammary antibiotics. Cows with severe clinical mastitis also received intramuscular antibiotics, intravenous non-steroidal anti-inflammatory and hypertonic saline solution in addition to intramammary antibiotics. Cows suffering
from ketosis, clinical mastitis, or metritis were treated according to the farm standard operating procedure (http://animal.ifas.ufl.edu/facilities/du/).

**Statistical Analysis**

To evaluate the association of prepartum and postpartum DMI%BW and EB with ketosis and clinical mastitis, the data was analyzed using ANOVA for repeated measures using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The data were divided into two periods, prepartum and postpartum. The dependent variables were prepartum DMI%BW or EB, and postpartum DMI%BW, EB, and ECM. The independent variable was one of the two disorders (ketosis or clinical mastitis), and they were modeled separately; cows that developed ketosis were compared with cows that did not develop ketosis, and cows that developed clinical mastitis were compared with cows that did not develop clinical mastitis. Cows that did not develop ketosis could have developed any other disorder including clinical mastitis. Likewise, cows that did not develop clinical mastitis could have developed any other disorder. Other studies have used healthy cows as the comparison group (Huzzey et al., 2007). However, this would introduce selection bias; therefore, this could artificially increase the differences in the measures of DMI%BW between the groups and inflate the estimates in a prediction model. Although the focus of this study was the comparison between cows affected with ketosis and clinical mastitis and unaffected cows, a comparison with healthy cows (i.e. cows that did not have any disorder diagnosed in the first 28 d postpartum) was also performed for comparison with the previous literature. The models also included the fixed effects of parity (primiparous vs. multiparous), BCS in the last week prepartum (< 3.75 vs. ≥ 3.75), day relative to calving (prepartum: d -21 to -1; postpartum: d 1 to 28), heat stress abatement (cool vs. hot...
without evaporative cooling vs. hot with evaporative cooling), and two-way interactions between disorder and other covariates, and cow was nested within experiment as a random effect. First order autoregressive, compound symmetry, and unstructured covariance structures were tested, and the first order autoregressive was selected because it resulted in the smallest Aikaike’s information criterion.

As an example, the initial model to evaluate the association between prepartum DMI%BW and ketosis was:

\[
\text{DMI}\%\text{BW prepartum} = \text{ketosis} + \text{day} + \text{heat stress abatement} + \text{BCS} + \text{parity} + \text{ketosis} \times \text{day} + \text{ketosis} \times \text{season} + \text{ketosis} \times \text{BCS} + \text{ketosis} \times \text{parity} + \text{cow (experiment)}
\]

The disorder of interest was forced into the model, but other variables were removed from the model by stepwise backward elimination according to Wald-statistics criterion when \( P > 0.05 \). When an interaction was detected, then mean separation was assessed using the SLICE option in the MIXED procedure, and multiple comparisons were performed using the Tukey-Kramer adjustment method in SAS.

To evaluate the use of prepartum DMI%BW, and EB as predictors of ketosis and clinical mastitis, each disorder was considered the dependent variable, and DMI%BW, and EB as independent variables. These data were analyzed by logistic regression with the GLIMMIX procedure of SAS. The objective was to assess if measures of prepartum DMI%BW or EB were associated with the odds of ketosis or clinical mastitis. In this case, each disease or disorder was the dependent variable and the measures of prepartum DMI%BW or EB were assessed separately in different models as independent variables. For this purpose, the variables average DMI%BW or EB in the last 14, 7, and 3 d prepartum, and the reduction from d -8 to -1 and from d -4 to -1 were
created. Univariable and multivariable models were performed. The univariable models included cow nested within experiment as a random variable. Measures of DMI%BW or EB with $P < 0.20$ were selected for inclusion in the multivariable logistic regression models. Multivariable models also included parity (primiparous vs. multiparous), prepartum BCS ($< 3.75$ vs. $\geq 3.75$; Gearhart et al., 1990), and heat stress abatement (cool vs. hot without evaporative cooling vs. hot with evaporative cooling), and cow nested within experiment as a random effect. Two-way interaction terms of measures of DMI%BW and EB with $P \leq 0.05$ and other covariates were tested. A stepwise backward elimination was performed and explanatory variables with $P > 0.05$ according to the Wald-statistics criterion were removed from the model.

When a measure of DMI%BW or EB prepartum was found to be significant ($P \leq 0.05$) after addition to the logistic regression model containing other covariates, the contribution to the predictive ability of the logistic regression model was assessed by comparing the AUC of a receiver operating characteristic curve of the model with and without the measures of DMI%BW or EB using the ROCCONTRAST statement of the LOGISTIC procedure of SAS as previously reported (Vergara et al., 2014). The AUC ≤ 0.50 was considered noninformative, AUC between 0.50 and 0.70 was considered with low accuracy, AUC between 0.70 and 0.90 was considered accurate, and AUC between 0.9 and 1.0 was considered highly accurate (Swets J., 1988). Finally, cut-off values for measures of DMI%BW and EB prepartum were determined with $P \leq 0.05$ for predicting ketosis and clinical mastitis postpartum using ROC, and the cut-off with the greatest Youden’s J statistic which combines the values for sensitivity and specificity was chosen. The sensitivity, and specificity and overall accuracy of applying the cut-off to
predict ketosis and clinical mastitis were calculated. Statistical significance was considered when $P \leq 0.05$. The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is muddled and variable. A limitation of the current study is that external validation of the predictive models were not performed; therefore, future validation studies are needed.

**Results**

The frequencies of ketosis and clinical mastitis are depicted in Table 1. Results for the comparison between cows that developed ketosis or clinical mastitis and healthy cows are presented in the supplementary file.

**Association of Prepartum DMI%BW and EB with Ketosis**

Ketosis was associated with a lesser DMI%BW ($P < 0.01$) in the last 3 weeks prepartum (Table 4-1). There was an interaction ($P < 0.01$) with day, which showed that DMI%BW for cows that developed ketosis was lesser compared with cows that did not develop ketosis from d -17 to -1 ($P \leq 0.01$) (Figure 4-1A). There was an interaction ($P < 0.01$) between ketosis and day on EB prepartum (Table 4-2). Cows with ketosis had lesser EB on d -5, -3, -2, and -1 (Figure 4-1B). There was an interaction ($P = 0.04$) between ketosis and parity on EB prepartum. The EB for primiparous cows that developed ketosis was similar to primiparous cows that did not develop ketosis (3.4 ± 0.7 vs. 2.9 ± 0.4 Mcal/d; $P = 0.56$; Figure 4-2A), whereas the EB for multiparous cows that developed ketosis was lesser than for multiparous cows that did not develop ketosis (1.6 ± 0.3 vs. 2.9 ± 0.3 Mcal/d; $P < 0.01$; Figure 4-2B).

**Prepartum DMI%BW and EB as Predictors of Ketosis**

Of the variables evaluated, parity and heat stress abatement were the only predictors of ketosis postpartum. Multiparous cows had increased odds of
developing ketosis postpartum compared with primiparous cows (OR: 4.7; CI: 2.3 - 7.9; \( P < 0.01 \)). Cows that were in heat stress without evaporating cooling during the prepartum period had lesser odds of developing ketosis postpartum compared with cows that were in heat stress with evaporative cooling (OR: 0.39; CI: 0.2 - 0.7; \( P < 0.01 \)) but the odds of having ketosis were not different between cows that were not in heat stress and cows that were in heat stress with evaporating cooling (OR: 1.5; CI: 0.9 - 2.4; \( P = 0.10 \)). The average DMI%BW, and EB during the last 3 d prepartum were explanatory variables for ketosis. For each 0.1 pp decrease in the average DMI%BW in the last 3 d prepartum, the odds of having ketosis increased by 8%. For each Mcal decrease in the average EB in the last 3 d prepartum, the odds of having ketosis increased by 5% (Table 4-4).

The AUC from the model containing parity and heat stress abatement was 0.63 (95% CI: 0.58 – 0.68) and increase to 0.72 (95% CI: 0.68 – 0.76) when the average DMI%BW in the last 3 d prepartum was included in the ketosis-predicting model. The AUC increased from 0.63 to 0.72 (95% CI: 0.67 – 0.76) when EB was included in the ketosis-predicting model. The AUC was different \( (P \leq 0.05) \) in both model comparisons. The average DMI%BW, and EB during the last 3 d prepartum produced \( (P < 0.01) \) cut-offs to predict ketosis postpartum, which were \( \leq 1.5 \%/d \), and \( \leq 1.1 \) Mcal/d, respectively (Table 4-5).

**Association of Postpartum DMI%BW, EB, and ECM with Ketosis**

During postpartum, cows that developed ketosis had lesser DMI%BW than cows that did not develop ketosis \( (P < 0.01; \text{Table 4-2}) \). Although there was an interaction \( (P < 0.01) \) between ketosis and day on postpartum DMI%BW, the DMI%BW for cows that developed ketosis was lesser than for cows that did not develop ketosis throughout the
entire postpartum period with the difference being more pronounced from d 5 to 17 (Figure 4-1A).

Cows that developed ketosis had lesser EB ($P < 0.01$) compared with cows that did not develop ketosis (Table 4-2). Although there was an interaction ($P < 0.01$) between ketosis and day on postpartum EB, the EB for cows that developed ketosis was lesser ($P < 0.01$) than for cows that did not develop ketosis throughout the entire postpartum period with the difference being more pronounced from d 1 to 8 (Figure 4-1B).

The ECM for cows that developed ketosis was greater ($P < 0.01$) than for cows that did not develop ketosis (Table 4-2). There was an interaction ($P < 0.01$) between ketosis and day on ECM, which showed that cows that developed ketosis had greater ECM compared with cows that did not develop ketosis from throughout the entire postpartum period with the difference being more pronounced from d 1 to 7 ($P \leq 0.05$; Figure 4-1C). There was an interaction ($P < 0.01$) between ketosis and parity on ECM. The ECM for primiparous cows that developed ketosis was greater than primiparous cows that did not develop ketosis (38.1 ± 1.8 vs. 28.6 ± 0.9 kg/d; $P < 0.01$; Figure 4-3A), whereas the ECM for multiparous cows that developed ketosis was similar to multiparous cows that did not develop ketosis (36.2 ± 0.8 vs. 36.6 ± 0.8 kg/d; $P = 0.72$; Figure 4-3B).

**Association of Prepartum DMI%BW and EB with Clinical Mastitis**

During the prepartum, cows that developed clinical mastitis had lesser DMI%BW compared with cows that did not develop clinical mastitis (1.56 ± 0.04 vs. 1.65 ± 0.02 %/d; $P = 0.05$; Table 4-3). Clinical mastitis was not associated with prepartum EB,
although cows that had clinical mastitis had numerically lesser EB than cows that did not have clinical mastitis (1.8 ± 0.4 vs. 2.6 ± 0.2; \( P = 0.08 \) Mcal/d; Table 4-3).

**Prepartum DMI\%BW and EB as Predictors of Clinical Mastitis**

Of the covariates evaluated, parity and heat stress abatement were the only significant predictors of clinical mastitis postpartum. Multiparous cows had increased odds of developing clinical mastitis postpartum compared with primiparous cows (OR: 2.5; CI: 1.1-4.9; \( P = 0.03 \)). Cows that were not in heat stress prepartum had decreased odds of developing clinical mastitis postpartum compared with cows in heat stress with (OR: 0.3; CI: 0.1-0.6; \( P < 0.01 \)) or without evaporating cooling (OR: 0.2; CI: 0.1-0.5; \( P < 0.01 \)). There was no difference between cows that were in heat stress with and without evaporating cooling (OR: 0.8; CI: 0.5-1.5; \( P = 0.56 \)). Of the measures of DMI evaluated, the average DMI\%BW and EB in the last 3 d prepartum were the only significant explanatory variables for clinical mastitis. For each 0.1 pp decrease in the average DMI\%BW in the last 3 d prepartum, the odds of developing clinical mastitis increased by 10%. For each Mcal decrease in the average EB in the last 3 d prepartum, the odds of having clinical mastitis increased by 8% (Table 4-4).

When the average DMI\%BW, or EB in the last 3 d prepartum were included in the clinical mastitis-predicting model containing only parity and heat stress abatement as explanatory variables, the AUC increased from 0.69 (95% CI: 0.64 – 0.73) to 0.71 (95% CI: 0.68 – 0.76), and from 0.69 to 0.72 (95% CI: 0.63 – 0.76), respectively. The differences between the AUCs were not statistically significant (\( P \geq 0.10 \)).

The average DMI\%BW, and EB during the last 3 d prepartum produced significant (\( P < 0.01 \)) cut-offs to predict clinical mastitis postpartum, which were \( \leq 1.2 \%/d \), and \( \leq 1.0 \) Mcal/d, respectively (Table 4-6).
Association of Postpartum DMI%BW, EB, and ECM with Clinical Mastitis

During postpartum, cows that developed clinical mastitis had lesser postpartum DMI%BW ($P < 0.01$) compared with cows that did not develop clinical mastitis (2.55 ± 0.07 vs. 2.76 ± 0.03 %/d; $P < 0.01$; Table 4-3). The interaction ($P < 0.01$) between clinical mastitis and day relative to calving was significant, which showed that postpartum DMI%BW for cows that developed clinical mastitis was lesser than for cows that did not develop clinical mastitis from d 3 to 15 ($P \leq 0.05$) and on d 17 ($P < 0.01$) postpartum (Figure 4-4A). Cows that developed clinical mastitis had similar postpartum EB compared with cows that did not develop clinical mastitis (-3.8 ± 0.7 vs. -4.9 ± 0.4 Mcal/d; $P = 0.17$; Table 4-3). However, the interaction ($P < 0.01$) between clinical mastitis and day relative to calving was significant, which showed that cows that developed clinical mastitis had greater postpartum EB on d 18 ($P = 0.05$), from d 21 to 23 ($P \leq 0.05$), and on d 26 ($P = 0.05$) compared with cows that did not develop clinical mastitis (Figure 4-4B). The ECM for cows that developed clinical mastitis was lesser ($P < 0.01$) compared with cows that did not develop clinical mastitis (28.7 ± 1.3 vs. 33.6 ± 0.6 kg/d; $P < 0.01$; Table 4-3). The interaction ($P < 0.01$) between clinical mastitis and day on ECM was significant, which showed that cows that developed clinical mastitis had lesser ECM throughout the entire postpartum period, being more pronounced from d 5 to 10, ($P \leq 0.05$) compared with cows that did not develop clinical mastitis (Figure 4-4C).

Discussion

In this study it was shown that cows that developed ketosis or mastitis had decreased prepartum DMI%BW, and that the average DMI%BW and EB in the last 3 d prepartum were predictive of ketosis and clinical mastitis, although the effect sizes were
small. Furthermore, cut-offs for prediction of ketosis and clinical mastitis were
established, although the accuracy was low. Postpartum DMI%BW and EB were
decreased in cows that developed ketosis, whereas ECM was increased in primiparous
cows that developed ketosis. Postpartum DMI%BW and ECM were decreased in cows
that developed clinical mastitis. Previous studies showed that cows with subclinical
ketosis had reduced DMI during the last week prepartum (Goldhawk et al., 2009). Dry
matter intake as percentage of BW prepartum was lesser for cows that developed
ketosis postpartum starting from d -17 prepartum but when the association between
ketosis and absolute DMI was analyzed, DMI was similar in cows with and without
ketosis (Figure B-4). The fact that cows that developed ketosis had similar prepartum
DMI but lesser DMI%BW compared with cows that did not develop ketosis was because
cows with ketosis were 72 kg heavier than cows that did not develop ketosis (729 ± 87
vs. 657 ± 104), and this was true for primiparous and multiparous cows (data not
shown); therefore, after controlling for BW, DMI was reduced in cows that developed
ketosis compared with the ones that did not develop ketosis. Furthermore, other
researchers have shown that overconditioned cows are known to have a greater
decrease in DMI prepartum and postpartum, and to be predisposed to ketosis
postpartum (Rukkwamsuk et al., 1999; Gillund et al., 2001).

Although DMI%BW prepartum was lesser for cows that developed ketosis, and
the average DMI%BW in the last 3 d prepartum was a significant explanatory variable
for ketosis, the effect size was quite modest; for each 0.1 pp decrease in the average
DMI%BW in the last 3 d prepartum, there was an increase of 8% in the odds of having
ketosis postpartum. The effect size for EB was similarly modest; for each 1 Mcal
decrease in the average of EB in the last 3 d prepartum, there was an increase of 5% in the odds of having ketosis. This contrasts with the results by Goldhawk et al. (2009), which observed that for each kg decrease in DMI in the last week prepartum, there was an increase of 120% in the odds of having subclinical ketosis. It is not clear why the discrepancy but a few differences between studies are worth discussing. First, Goldhawk et al. (2009) only included cows with ketosis (BHB ≥ 1.0 mmol/L) in the first week of lactation but excluded cows from the study if BHB remained above 1.4 mmol/L in the second week postpartum. In the present study, any cow with positive ketones in the urine during the first 4 weeks of lactation was included, although 81% (153/189) developed ketosis in the first week of lactation. The stated purpose for removing cows with BHB ≥ 1.4 mmol/L in the second week of lactation in the study by Goldhawk et al. (2009) was to exclude cows with more severe or chronic ketosis, which it was not done in this current study; therefore, if anything, differences in DMI in this study should have been larger and not smaller. In the study by Goldhawk et al. (2009), cows that developed ketosis were compared with healthy cows whereas in this study, cows that developed ketosis were compared with cows that did not develop ketosis but could have had any other disease. Nonetheless, when cows that developed ketosis were compared with healthy cows, it was observed that DMI prepartum decreased only on d -3 and -2 in cows that developed ketosis (Figure B-6A). In addition, in the study by Goldhawk et al. (2009), 90% (9/10) of the cows were multiparous, whereas in this current study 71% (337/476) were multiparous. Multiparous cows had a greater decrease in absolute DMI prepartum (Figure B-5B) meaning that a greater proportion of multiparous cows in the study could increase the differences between cows that did and did not develop ketosis.
Another difference between studies is the sample size; therefore, the small sample size in Goldhawk et al. (2009) could have led to common issues with small sample size such as inflated effect size and low reproducibility (Button et al., 2013).

An interesting finding was a decrease in the odds of ketosis in cows that were under heat stress without evaporating cooling during the prepartum period compared with cows that were under heat stress but with evaporative cooling and cows that were not under heat stress. This may be because cows that were under heat stress without evaporating cooling during the prepartum period produced ~5kg/d less milk than cows that were under heat stress with evaporative cooling (Fabris et al., 2017).

In this present study, it was also evaluated the predictive ability of DMI%BW and EB. The predictive ability of the models for ketosis increased modestly, although significantly when the average DMI%BW and EB in the last 3 d prepartum were independently included in the models containing other covariates. Therefore, although not as robust as previously reported, DMI%BW and EB in the last 3 d prepartum were shown to be predictors of ketosis. In addition, cut-offs values for prepartum DMI%BW and EB were determined to see if they could be used solely as a predictor of ketosis postpartum. The cut-offs had accurate to low sensitivity (71 - 51%), low specificity (51 - 53%), low accuracy (64 - 61%), and low AUC (0.63 - 0.60). Similar to the results presented in this current study, the cut-offs for prepartum NEFA (i.e. 0.26 mEq/L; sensitivity/specificity 53/61%; AUC 0.6) that were determined by Ospina et al., (2010b) to predict clinical ketosis postpartum had low sensitivity, specificity, and AUC. Therefore, although significant, these cut-offs are of limited applicability. In summary, DMI%BW and EB prepartum are significant but minor contributors to ketosis
development postpartum and cannot be used reliably to identify cows that will develop ketosis postpartum.

During postpartum, cows that developed ketosis had lesser DMI%BW compared with cows that did not develop ketosis. Goldhawk et al. (2009) reported that cows that developed subclinical ketosis had on average 23% lesser DMI in the first 2 weeks postpartum compared with healthy cows. In this current study, cows that developed ketosis had 12% (15.4 ± 4.6 vs. 13.8 ± 4.7 kg/d) lesser DMI during the first 2 weeks postpartum compared with cows that did not develop ketosis (Figure B-4A). Cows that developed ketosis with healthy cows were also compared and it was shown that the decrease in DMI during the first 2 weeks postpartum in cows that developed ketosis was 19% (16.8 ± 4.0 vs. 13.8 ± 4.7 kg/d), which is more similar to what was shown by Goldhawk et al. (2009). Another important point is that there was an interaction between ketosis and parity on ECM, showing that primiparous cows that developed ketosis produced 9.5 kg/d more ECM than primiparous cows that did not develop ketosis, whereas multiparous cows that developed ketosis had similar ECM compared with cows that did not develop ketosis. Therefore, primiparous cows that developed ketosis should be eating ~ 4.75 kg/d of dry matter assuming a 1:2 feed conversion of marginal milk per kg of DMI. Hence, when EB was evaluated, there was no interaction between parity and ketosis, and cows that developed ketosis had lesser EB than cows that did not develop ketosis.

In this study it was shown that cows that developed clinical mastitis had decreased prepartum DMI%BW, and that the average of DMI%BW and EB in the last 3 d prepartum were predictors of clinical mastitis. Nonetheless, the effect sizes were
small; 10% and 8% increase in the odds of having clinical mastitis postpartum with each unit decrease in the measures of DMI%BW and EB, respectively. Moreover, the addition of DMI%BW and EB in the last 3 d prepartum to a clinical mastitis-predicting model did not significantly increased the predictive ability of the models as evaluated by the AUC, indicating that although DMI%BW and EB prepartum are significant predictors of clinical mastitis postpartum, their contribution is minor when accounting for other variables such as parity and heat stress prepartum. In addition, cut-offs for DMI%BW and EB were determined to see if they could be used solely as a predictor of clinical mastitis postpartum, and the cut-offs resulted in low to moderate Se (50 - 67%), Sp (66 - 50%), overall accuracy (64 - 53%), and AUC (0.61 - 0.59), which indicate that, although significant, these cut-offs have limited applicability. In summary, DMI%BW and EB prepartum are significant but minor contributors to clinical mastitis development postpartum and cannot be used reliably to identify cows that will develop clinical mastitis postpartum.

Furthermore, cows that develop clinical mastitis postpartum have been shown to have decreased glucose and increased NEFA and BHB concentrations prepartum (Schwegler et al., 2013; Moyes et al., 2009), which indicates a decreased DMI%BW and EB prepartum; however, as far as it is known, this is the first time that prepartum DMI%BW and EB were compared between cows that did and did not develop clinical mastitis. Previous research has shown that hyperketonemia impairs phagocytic, chemotactic, and killing ability of neutrophils, and that immunosuppression peripartum is a predisposing factor for mastitis postpartum (Suriyasathaporn et al., 1999). Without a
good chemotactic response, neutrophils may be less able to reach the mammary gland to fight infections; therefore, predisposing cows to clinical mastitis.

During the postpartum period, the decrease in DMI%BW seen in cows that developed clinical mastitis may be explained by the inflammatory process and its association with pro-inflammatory cytokines such as TNF-α, IL-1 and IL6, which lead to swelling, pain, fever, loss of appetite, and decreased feed intake (Dantzer et al., 1993; Swiergiel and Dunn, 1999; Alluwaimi, 2004). Interestingly, clinical mastitis was associated with greater EB on d 18, 21 to 23 postpartum compared with cows that did not have clinical mastitis, although both groups were still in negative EB. This finding may be mainly because cows that developed clinical mastitis completely recovered DMI%BW after the second week of lactation but remained producing less ECM than cows that did not develop clinical mastitis. The persistent reduction in ECM among cows that developed clinical mastitis despite the recovery in DMI%BW is likely due to loss of function in the infected quarter/quarters caused by the infectious agent and by the inflammatory response against the infectious agent (Bradford et al., 2015).

A limitation from this study is that data were collected from different experiments over the years. This meant that different observers were collecting subjective data such as BCS in each trial, agreement among observers could not be compared, and treatments had been applied in each trial (i.e. heat stress abatement), which had to be controlled for in the statistical analysis. Another limitation is that the association of ketosis or mastitis with DMI%BW, EB and ECM during postpartum period could not be evaluated before and after ketosis or mastitis diagnosis because dividing the data would have resulted in reduced sample size per day; therefore increasing the standard errors
or producing unreliable standard errors. As an example, cows that developed ketosis or mastitis on day 1 would have zero days of DMI before the disease diagnosis and 27 days of DMI after the disease diagnosis. For cows diagnosed with ketosis or mastitis from day 2 to 28 the number of days of DMI before the disease diagnosis would increase but the number of days of DMI after the disease diagnosis would decrease. Lastly, even if the data of this present study was divided before and after disease diagnosis, causation could not be inferred because cows were not randomly assigned to develop ketosis or mastitis. Hence, herein it was presented the association between ketosis or mastitis development and DMI%BW and EB pre- and postpartum.

Chapter Summary

This study showed that ketosis and mastitis were associated with prepartum DMI%BW. The average DMI%BW and EB in the last 3 d prepartum were significant explanatory variables for ketosis and clinical mastitis postpartum and, the average DMI%BW and EB in the last 3 d prepartum significantly increased the predictive ability of ketosis-predicting models, although the effect sizes were small. Prepartum cut-offs for DMI%BW and EB to predict ketosis and clinical mastitis were established, although with low sensitivity, specificity, and overall accuracy. In addition, postpartum DMI%BW and EB were decreased in cows that developed ketosis, whereas ECM was increased in primiparous cows that developed ketosis. Postpartum DMI%BW and ECM were decreased in cows that developed clinical mastitis. The results of this study give a better understating of the role DMI%BW plays during the transition period; namely that when DMI%BW decrease, the risk of ketosis and clinical mastitis increase, although the increase was small. The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is muddled and variable. In summary, DMI%BW, and EB
prepartum are significant but minor In summary, DMI%BW, and EB prepartum are significant but minor contributors to ketosis and clinical mastitis development postpartum and cannot be used reliably to identify cows that will develop ketosis and clinical mastitis postpartum.
Table 4-1. Frequency table of ketosis and mastitis by study diagnosed during the first 28 days postpartum.

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease</th>
<th>Frequency</th>
<th>Percentage (%)</th>
<th>aMPP (min-max)</th>
<th>bIQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Mastitis</td>
<td>3</td>
<td>0.6</td>
<td>6 (2 – 14)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>38</td>
<td>8.0</td>
<td>7 (2 – 28)</td>
<td>8</td>
</tr>
<tr>
<td>Study 2</td>
<td>Mastitis</td>
<td>9</td>
<td>1.9</td>
<td>9.5 (1 – 28)</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>17</td>
<td>3.6</td>
<td>4 (4 – 12)</td>
<td>3</td>
</tr>
<tr>
<td>Study 3</td>
<td>Mastitis</td>
<td>4</td>
<td>0.8</td>
<td>4.5 (1 – 9)</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>3</td>
<td>0.6</td>
<td>7 (4 – 13)</td>
<td>4.5</td>
</tr>
<tr>
<td>Study 4</td>
<td>Mastitis</td>
<td>4</td>
<td>0.8</td>
<td>2 (1 – 7)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>7</td>
<td>1.5</td>
<td>7 (3 – 12)</td>
<td>3</td>
</tr>
<tr>
<td>Study 5</td>
<td>Mastitis</td>
<td>17</td>
<td>3.6</td>
<td>6 (1 – 26)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>9</td>
<td>1.9</td>
<td>7 (1 – 9)</td>
<td>3</td>
</tr>
<tr>
<td>Study 6</td>
<td>Mastitis</td>
<td>5</td>
<td>1.1</td>
<td>4 (2 – 8)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>16</td>
<td>3.4</td>
<td>7 (4 – 13)</td>
<td>4</td>
</tr>
<tr>
<td>Study 7</td>
<td>Mastitis</td>
<td>20</td>
<td>4.2</td>
<td>8 (1 – 22)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>19</td>
<td>4.0</td>
<td>7 (1 – 12)</td>
<td>3</td>
</tr>
<tr>
<td>Study 8</td>
<td>Mastitis</td>
<td>9</td>
<td>1.9</td>
<td>4 (1 – 26)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>36</td>
<td>7.6</td>
<td>6 (1 – 26)</td>
<td>7.3</td>
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<tr>
<td>Study 9</td>
<td>Mastitis</td>
<td>8</td>
<td>1.7</td>
<td>13 (4 – 27)</td>
<td>11</td>
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<tr>
<td></td>
<td>Ketosis</td>
<td>44</td>
<td>11.7</td>
<td>7 (1 – 27)</td>
<td>3</td>
</tr>
<tr>
<td>Total cases</td>
<td>Mastitis</td>
<td>79</td>
<td>16.5</td>
<td>7 (1 – 28)</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Ketosis</td>
<td>189</td>
<td>39.7</td>
<td>7 (1 – 28)</td>
<td>5</td>
</tr>
</tbody>
</table>

*aMPP = Median of days postpartum when the disease was diagnosed.

bIQR = Interquartile range.
Table 4-2. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with ketosis (Ket) postpartum according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum Ket</th>
<th>Prepartum No Ket</th>
<th>Postpartum Ket</th>
<th>Postpartum No Ket</th>
<th>Postpartum Ket x Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI%BW</td>
<td>1.54 ± 0.03</td>
<td>1.67 ± 0.02</td>
<td>2.45 ± 0.04</td>
<td>2.84 ± 0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>2.6 ± 0.4</td>
<td>2.9 ± 0.2</td>
<td>-8.1 ± 0.5</td>
<td>-3.2 ± 0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>37.2 ± 1.0</td>
<td>32.6 ± 0.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*aDay: day relative to parturition.

bKet x Day: interaction between ketosis and Day.
Table 4-3. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with clinical mastitis (Mast) postpartum according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th>P -value</th>
<th>Postpartum</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mast</td>
<td>No Mast</td>
<td>Mast</td>
<td>aD</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.56 ± 0.04</td>
<td>1.65 ± 0.02</td>
<td>0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.8 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

aD: day relative to parturition.
bM x D: interaction between clinical mastitis and Day.
Table 4-4. Effect of each 0.1 percentage point decrease in the average dry matter intake as a percentage of BW (DMI%BW), and each unit decrease in the average of energy balance (EB) in the last 3 d prepartum on postpartum ketosis and clinical mastitis (CM) in the first 28 days postpartum.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>DMI, %BW</th>
<th></th>
<th>EB, Mcal/d</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P-value</td>
<td>OR</td>
</tr>
<tr>
<td>Ketosis</td>
<td>1.08</td>
<td>1.03-1.13</td>
<td>&lt;0.01</td>
<td>1.05</td>
</tr>
<tr>
<td>CM</td>
<td>1.10</td>
<td>1.03-1.16</td>
<td>&lt;0.01</td>
<td>1.08</td>
</tr>
</tbody>
</table>
Table 4-5. Cut-offs of dry matter intake as percentage of BW (DMI%BW) and energy balance (EB) to predict ketosis postpartum.

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>aSe (%)</th>
<th>bSp (%)</th>
<th>cPPV (%)</th>
<th>dNPV (%)</th>
<th>eAcc (%)</th>
<th>fAUC</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, %BW</td>
<td>≤ 1.5</td>
<td>71</td>
<td>51</td>
<td>48</td>
<td>74</td>
<td>64</td>
<td>0.63</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>≤ 1.1</td>
<td>65</td>
<td>53</td>
<td>47</td>
<td>71</td>
<td>61</td>
<td>0.60</td>
</tr>
</tbody>
</table>

aSensitivity; bSpecificity; cPositive predicted value; dNegative predictive value; eAccuracy; fArea under the curve.
Table 4-6. Cut-offs of dry matter intake as percentage of BW (DMI%BW) and energy balance (EB) to predict mastitis postpartum.

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>aSe (%)</th>
<th>bSp (%)</th>
<th>cPPV (%)</th>
<th>dNPV (%)</th>
<th>eAcc (%)</th>
<th>fAUC</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI%BW ≤ 1.2</td>
<td>50</td>
<td>66</td>
<td>23</td>
<td>87</td>
<td>64</td>
<td>0.61</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d ≤ 1.0</td>
<td>67</td>
<td>50</td>
<td>21</td>
<td>88</td>
<td>53</td>
<td>0.59</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

aSensitivity; bSpecificity; cPositive predicted value; dNegative predictive value; eAccuracy, fArea under the curve.
Figure 4-1. Association of ketosis postpartum (n = 189) with (A) dry matter intake (%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI (%BW): ketosis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between ketosis and day - $P < 0.01$. Prepartum EB: ketosis - $P = 0.29$, day relative to parturition - $P < 0.01$, and the interaction between ketosis and day $P < 0.01$. Postpartum DMI (%BW): ketosis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between ketosis and day - $P < 0.01$. Postpartum EB: ketosis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between ketosis and day - $P < 0.01$. ECM: ketosis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between ketosis and day - $P < 0.01$. 
Figure 4-2. Interaction ($P \leq 0.01$) between ketosis and parity on energy balance (Mcal/d) in (A) primiparous and (B) multiparous cows during the prepartum (from -21 d to -1 d). Values are least squares means +/- SEM.
Figure 4-3. Interaction ($P < 0.01$) between ketosis and parity on energy corrected milk (kg/d) in (A) primiparous and (B) multiparous cows during the postpartum period (from 1 d to 28 d). Values are least squares means +/- SEM.
Figure 4-4. Association between mastitis (n = 79) and (A) dry matter intake (%BW), (B) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (C) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI (%BW): mastitis ($P = 0.05$), day relative to parturition ($P < 0.01$), and the interaction between mastitis and day ($P = 0.56$). Prepartum EB: mastitis ($P = 0.08$), day relative to parturition ($P < 0.01$), and the interaction between mastitis and day ($P = 0.39$). Postpartum DMI (%BW): mastitis ($P < 0.01$), day relative to parturition ($P < 0.01$), and the interaction between mastitis and day ($P < 0.01$). Postpartum EB: mastitis ($P = 0.17$), day relative to parturition ($P < 0.01$), and the interaction between mastitis and day ($P < 0.01$). ECM: mastitis ($P < 0.01$), day relative to parturition ($P < 0.01$), and the interaction between mastitis and day ($P < 0.01$).
CHAPTER 5
ASSOCIATION OF DRY MATTER INTAKE AND ENERGY BALANCE PREPARTUM AND POSTPARTUM WITH HEALTH DISORDERS POSTPARTUM: DIGESTIVE DISORDERS AND LAMENESS

Summary

The main objective was to determine the association of DMI%BW, and EB prepartum (-21 d relative to parturition) and postpartum (28 d) with digestive disorders (n = 120) and lameness (n = 35) postpartum. For this, DMI%BW and EB were the independent variables and digestive disorder and lameness were the dependent variables. A secondary objective was to evaluate prepartum DMI%BW, and EB as predictors of digestive disorder and lameness. For this, digestive disorder and lameness were the independent variables and DMI%BW, and EB were the dependent variables. Data from 476 cows from 9 experiments were compiled. Cows that developed digestive disorders had lesser prepartum DMI%BW and lesser EB from d -10 to -7, and from d -5 to -1 than cows without digestive disorders. Each 0.1 pp decrease in the average DMI%BW and each Mcal decrease in the average EB in the last 3 d prepartum increased the odds of having digestive disorders by 7% and 6%, respectively. Cut-offs for DMI%BW, and EB during the last 3 d prepartum to predict digestive disorders were established and were ≤ 1.4 %/d and ≤ 1.5 Mcal/d, respectively. During postpartum, cows that developed digestive disorders had lesser DMI%BW, EB, and ECM than cows without digestive disorders. The association of prepartum DMI%BW and EB with lameness was conditional on BCS. Among cows with BCS ≥ 3.75, those that were lame had lesser prepartum DMI%BW and EB than sound cows. Each 0.1 pp decrease in the average DMI%BW, and each Mcal decrease in the average EB in the last 3 d prepartum increased the odds of having lameness in cows with BCS ≥ 3.75 by 39% and
29%, respectively. Cut-offs for DMI%BW and EB during the last 3 d prepartum to predict lameness in cows with a BCS ≥ 3.75 were established and were ≤ 1.1 %/d and ≤ -1.9 Mcal/d, respectively. Cows that were lame had lesser postpartum DMI%BW. In summary, measures of prepartum DMI%BW and EB were associated with, and were predictors of digestive disorders in all cows and lameness in cows with BCS ≥ 3.75, although the effect sizes were small for digestive disorders and moderate for lameness.

**Introductory Remarks**

During the transition period, 3 weeks before to 3 weeks after parturition, dairy cows experience a decline in DMI and an increase in the incidence of disorders that compromise production and survival. Two of these disorders are digestive disorder and lameness. The decline in DMI during the prepartum could be associated with digestive disorders. Ospina et al. (2010a) observed an association between high blood NEFA during prepartum and displaced abomasum. In addition, indigestion has been associated with loss of BCS during the dry period (Chebel et al., 2018); therefore, it is likely that cows with digestive disorders postpartum would have experienced a more severe drop in DMI and EB prepartum. Hyperketonemia has also been correlated with lameness (Collard et al., 2000). Bach et al. (2007) reported that lame cows spent less time eating postpartum than non-lame cows and that the DMI decreased with increasing locomotion score which may explain the association of hyperketonemia with greater odds of having lameness (Raboisson et al., 2014).

The importance of digestive disorders in the dairy farm is due to its negative association with reproduction and milk that affect farm profitability. Digestive disorders (i.e. displaced abomasum, indigestion, diarrhea, rumen stasis, or bloat) have been associated with delayed resumption of ovarian cyclicity (Vercouteren et al., 2015),
impaired reproduction (Ribeiro et al., 2013), and decreased milk yield (Kirchman et al., 2017), thus causing economic losses to the farm. Similarly, lameness causes milk loss, decreases fertility, and increases culling (Melendez et al., 2003; Bicalho et al., 2008) leading to economic losses to the dairy farms ranging from US$ 132 to 216 per case, depending on the type of lesion causing lameness (Cha et al., 2010). Given the relevance of digestive disorders and lameness to the dairy farm, it is important to understand the factors associated with increased risk of these disorders in order to devise strategies to prevent them and mitigate their negative associations on milk yield, reproductive performance, culling and profitability.

Given the relationship of NEFA and BHB with clinical mastitis and lameness, and the association between DMI, EB, NEFA, and BHB, it was hypothesized that a reduction in DMI%BW and EB during the transition period would be associated with clinical mastitis and lameness postpartum. Therefore, the main objective of this current study was to evaluate the association of pre- and postpartum DMI%BW, and EB with digestive disorders and lameness postpartum. A secondary objective was to evaluate the use of prepartment DMI%BW, and EB as predictors of digestive disorder and lameness postpartum.

**Materials and Methods**

Study design, housing, measurement and calculation of DMI, milk yield, BW, BCS, and EB are described in detailed in chapter 3. In summary, data from a total of 476 cows (139 primiparous and 337 multiparous) was collected from 9 different experiments conducted at the University of Florida dairy unit, located in the city of Hague, Florida. This was a convenience sample; therefore, no a priori sample size calculation was performed. For continuous variables, with 120 cows were in the affected
group (digestive disorders n = 120; Table 1) would be able to detect statistical differences with an effect size of 0.25 (μ1 - μ2 = 1 kg/d of DMI; SD = 4 kg/d of DMI). In the case of lameness (n = 35; Table 1), only differences with an effect size of 0.4 (μ1 - μ2 = 1.6 kg/d of DMI; SD = 4 kg/d of DMI) would be found significant. Individual experiments were approved by the University of Florida Animal Research Committee.

Health Disorders

Detailed paper and electronic health records were recorded for each cow. Each cow underwent scheduled complete physical examinations by a trained herdsman or by a veterinarian from the College of Veterinary Medicine, FARMS at University of Florida on d 4, 7, and 12 postpartum. Furthermore, milk yield and cow’s attitude were monitored daily pre-and postpartum and milk yield was monitored postpartum. Any cow showing signs of depression, inappetence, lethargy, altered stride, inflammation of the mammary gland, or a drop greater than 10% in milk yield underwent a physical examination by a trained herdsman or by a FARMS veterinarian. The veterinarians from FARMS performed physical examinations and provided supervision and training of herd personnel performing clinical diagnosis and treatment of postpartum cows at least once a week. Additionally, FARMS veterinarians were called to assist or confirm clinical diagnosis or treatment of postpartum cows throughout the weekdays and weekends. Only health events occurring during the first 28 DIM were used in this study. First, the electronic health records were retrieved, then confirmed the information using the paper health records. Cows with mismatched information were excluded from the study. The health disorders recorded were calving disorders (dystocia, twins, stillbirths), metritis, ketosis, digestive disorders (indigestion and displaced abomasum), clinical mastitis, and lameness. Digestive disorders included left displaced abomasum, sand impaction, cecal
dilatation, diarrhea, bloat, constipation, and indigestion. Left displaced abomasum was diagnosed by a characteristic ping over the 9th to 13th ribs on left side and was confirmed during surgery. None of the cows followed were diagnosed with right displaced abomasum. Sand impaction was diagnosed during surgery in some cows that were suspected to have left displaced abomasum. Cecal dilatation was diagnosed via rectal palpation and was characterized by a caudal displacement of the dilated cecum as previously described (Braun et al., 2012). Cows with cecal dilatation usually present with abnormal demeanor decreased ruminal motility, scant feces, and colic. Cecal dilatation may evolve into volvulus and lead to death (Braun et al., 2012). Diarrhea was diagnosed in cows with watery feces that would sift through bedding (Priestley et al., 2013). Bloat was diagnosed in cows with gas distended rumen. Constipation was diagnosed in cows with very dry feces. Indigestion was diagnosed in cows with undigested feces (presence of large amount of undigested fiber and grain in feces), scant pasty malodorous feces, rumen stasis (< 1 rumen contraction per minute), or a combination of two or more of these signs. The diagnosis of some of the clinical signs of digestive disorders such as undigested feces, scant pasty malodorous feces and constipation can be subjective; therefore, a potential for misdiagnosis exists. Lameness was diagnosed daily by a herdsman or by a FARMS veterinarian by observing cows walking to and from the milking parlor. Cows with a visual locomotion score of 3 (short strides with one or more legs and slight sinking of dew-claws in limb opposite to the affected limb) or worse, in a 5-point scale were considered lame (Sprecher et al., 1997). Because cows were not systematically scored, there is a chance that only cows with more severe lameness were diagnosed. Prevalence of lameness vary widely in North
America but has been recently reported to average 9.6% (Adams et al., 2017). Lame cows were examined and trimmed by a trained hoof trimmer or by a FARMS veterinarian. The type of lesion found during the exam was recorded and entered in the computer management software. Training was provided to all new employees and new veterinarians by an experienced veterinarian. Training usually involved one hour of in-class training followed by one hour of hands-on training. Agreement among observers was not evaluated. Cows suffering from ketosis, digestive disorders, metritis, mastitis or lameness were treated according to the farm standard operating procedure (http://animal.ifas.ufl.edu/facilities/du/).

Statistical Analysis

Data were analyzed using ANOVA for repeated measures using the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The data were divided into two periods, prepartum and postpartum. The dependent variables were prepartum DMI%BW or EB, and postpartum DMI%BW, EB, and ECM. The independent variable was one of the two disorders (digestive disorders or lameness), and they were modeled separately; cows that developed digestive disorders were compared with cows that did not develop digestive disorders, and cows that developed lameness were compared with cows that did not develop lameness. Cows that did not develop digestive disorders could have developed any other disorder including lameness. Likewise, cows that did not develop lameness could have developed any other disorder. Other studies have used healthy cows as the comparison group (Huzzey et al., 2007). However, this would introduce selection bias; therefore, this could artificially increase the differences in the measures of DMI%BW between the groups and inflate the estimates in a prediction model. Although the focus of this study was the comparison between cows affected with
digestive disorders and lameness and unaffected cows, a comparison with healthy cows (i.e. cows that did not have any disorder diagnosed in the first 28 d postpartum) was also performed for comparison with the previous literature. Variables showing a Spearman’s rho correlation greater than 0.6 should not be included in the same model as independent variables; however, none of the variables showed a Spearman’s rho correlation greater than 0.4; therefore, they could be included in the same model as independent variables. The models also included the fixed effects of parity (primiparous vs. multiparous), BCS in the last week prepartum (< 3.75 vs. ≥ 3.75), day relative to calving (prepartum: d -21 to -1; postpartum: d 1 to 28), heat stress abatement (cool vs. hot without evaporative cooling vs. hot with evaporative cooling), and two-way interactions between disorder and other covariates, and cow was nested within experiment as a random effect. First order autoregressive, compound symmetry, and unstructured covariance structures were tested, and the first order autoregressive was selected because it resulted in the smallest Aikake’s information criterion.

As an example, the initial model to evaluate the association between prepartum DMI and digestive disorders was:

\[
\text{DMI}\%\text{BW prepartum} = \text{digestive disorders} + \text{day} + \text{heat stress abatement} + \text{BCS} + \text{parity} + \text{digestive disorders} \times \text{day} + \text{digestive disorders} \times \text{heat stress abatement} + \text{digestive disorders} \times \text{BCS} + \text{digestive disorders} \times \text{parity} + \text{cow} \ (\text{experiment})
\]

The disorder of interest was forced into the model, but other variables were removed from the model by stepwise backward elimination according to Wald-statistics criterion when \( P > 0.05 \). When an interaction was detected (\( P \leq 0.05 \)), then mean separation was assessed using the SLICE statement in the MIXED procedure, and
multiple comparisons were performed using the Tukey-Kramer adjustment method in SAS.

Categorical data were analyzed by logistic regression with the GLIMMIX procedure of SAS. In this case, each disorder was the dependent variable. The objective was to assess if measures of prepartum DMI%BW or EB were associated with the odds of digestive disorders or lameness. In this case, each disease was the dependent variable and the measures of prepartum DMI%BW or EB were assessed separately in different models as independent variables. For this purpose, the variables average DMI%BW or EB in the last 14, 7, and 3 d prepartum, and the difference between d -8 and -1 and between d -4 and -1 were created. Univariable and multivariable models were performed. The univariable models included cow nested within experiment as a random variable. Measures of DMI%BW or EB with \( P < 0.20 \) were selected for inclusion in the multivariable logistic regression models. Multivariable models also included parity (primiparous vs. multiparous), BCS in the last week prepartum (< 3.75 vs. ≥ 3.75; Gearhart et al., 1990), and heat stress abatement (cool vs. hot without evaporative cooling vs. hot with evaporative cooling), and cow nested within experiment as a random effect. Two-way interaction terms of significant measures of DMI%BW and EB with other covariates were tested. A stepwise backward elimination was performed and explanatory variables with \( P > 0.05 \) according to the Wald-statistics criterion were removed from the model.

When a measure of DMI%BW or EB prepartum was found to be significant after addition to the logistic regression model containing other covariates, their contribution to the predictive ability of the logistic regression model was assessed by comparing the
AUC of a ROC of the model with and without the significant measures of DMI%BW or EB using the ROCCONTRAST statement of the LOGISTIC procedure of SAS as previously reported (Vergara et al., 2014). The AUC ≤ 0.50 was considered noninformative, AUC between 0.50 and 0.70 was considered with low accuracy, AUC between 0.70 and 0.90 was considered accurate, and AUC between 0.9 and 1.0 was considered highly accurate (Swets J., 1988). Finally, cut-off values for significant measures of DMI%BW and EB prepartum for predicting digestive disorders and lameness postpartum were determined using ROC, and the cut-off with the greatest Youden's J statistic which combines the values for Se and Sp was chosen. The Se, Sp, positive PPV, NPV and overall accuracy of applying the cut-off to predict digestive disorders and lameness were calculated. Statistical significance was considered when $P \leq 0.05$.

**Results**

The frequencies of digestive disorders and lameness are depicted in Table 5-1. Results for the comparison between cows that developed digestive disorders or lameness and healthy cows is presented in the supplementary file.

**Association of Prepartum DMI%BW and EB with Digestive Disorders**

Digestive disorders in the first 4 weeks after calving was associated with lesser ($P = 0.02$) prepartum DMI%BW (Table 5-2). There was an interaction ($P < 0.01$) between digestive disorders and day, which showed that DMI%BW for cows that developed digestive disorders were lesser than for cows that did not develop digestive disorders from d -10 to -7, and from d -5 to -1 (Figure 5-1A). There was an interaction between digestive disorders and day ($P < 0.01$) on prepartum EB (Table 5-2). Digestive
disorders were associated with lesser prepartum EB from d -10 to -7, and from d -5 to -1 (Figure 5-2B).

Prepartum DMI%BW and EB as Predictors of Digestive Disorders

Of the covariates evaluated, parity was the only predictor of digestive disorders postpartum. Multiparous cows had increased odds of developing digestive disorders postpartum compared with primiparous cows (OR: 2.5; CI: 1.4-4.4; \( P < 0.01 \)). The average DMI%BW and EB during the last 3 d prepartum were explanatory variables for digestive disorders. For each 0.1 pp decrease in the average DMI%BW in the last 3 d prepartum, the odds of having digestive disorders increased by 7%. For each Mcal decrease in the average EB in the last 3 d prepartum, the odds of having digestive disorders increased by 6% (Table 5-4).

When the average DMI%BW and EB in the last 3 d prepartum were included individually in the digestive disorders-predicting models containing only parity, the AUC increased from 0.58 (95% CI: 0.54 – 0.63) to 0.63 (95% CI: 0.58 – 0.68) and from 0.58 to 0.62 (95% CI: 0.58 – 0.67), respectively; and the AUC were different (\( P < 0.05 \)) between the models.

The average DMI%BW and EB during the last 3 d prepartum produced (\( P < 0.01 \)) cut-offs to predict digestive disorders postpartum, which were ≤ 1.4 %/d and ≤ 1.5 Mcal/d, respectively (Table 5-5).

Association of Postpartum DMI, DMI%BW, EB, and ECM with Digestive Disorders

During postpartum, cows that developed digestive disorders had lesser DMI%BW (\( P < 0.01 \)) than cows that did not develop digestive disorders (Table 5-2). There was an interaction (\( P = 0.03 \)) between digestive disorders and day on postpartum DMI%BW, which showed that DMI%BW for cows that developed digestive disorders
were lesser than for cows that did not develop digestive disorders from d 3 to 28 (Figure 5-1A). Cows that developed digestive disorders had lesser EB ($P = 0.05$) than cows that did not develop digestive disorders (Table 5-2). The ECM for cows that developed digestive disorders were lesser ($P = 0.03$) than cows that did not develop digestive disorders (Table 5-2).

**Association of Prepartum DMI%BW and EB with Lameness**

Cows that developed lameness had lesser prepartum DMI%BW compared with cows that did not develop lameness ($1.50 \pm 0.07$ vs. $1.65 \pm 0.02 \%$/d; $P = 0.03$; Table 5-3; Figure 5-2A) but similar prepartum EB ($1.4 \pm 0.4$ vs. $2.7 \pm 0.2$ Mcal/d; $P = 0.07$; Table 5-3; Figure 5-2B). However, there were interactions ($P < 0.01$) between lameness and BCS on DMI%BW and EB, which showed that DMI%BW ($1.20 \pm 0.12$ vs. $1.59 \pm 0.03 \%$/d; $P < 0.01$; Figure 5-3B) and EB ($-1.2 \pm 1.2$ vs. $2.4 \pm 0.3$ Mcal/d; $P < 0.01$; Figure 5-3D) in cows with BCS ≥ 3.75 that developed lameness were lesser than for cows with BCS ≥ 3.75 that did not develop lameness. On the other hand, DMI%BW ($1.80 \pm 0.07$ vs. $1.71 \pm 0.02 \%$/d; $P = 0.24$; Figure 5-3A) and EB ($4.0 \pm 0.8$ vs. $3.0 \pm 0.2$ Mcal/d; $P = 0.23$; Figure 5-3B) were similar in cows with BCS < 3.75 that did and did not develop lameness.

**Prepartum DMI%BW and EB as Predictors of Lameness**

Of the covariates evaluated, parity was the only significant predictor of lameness postpartum. Multiparous cows had increased odds of developing lameness postpartum compared with primiparous cows (OR: 4.3; CI: 1.2-14.9; $P = 0.02$). None of the measures of DMI%BW, and EB were found to be significant predictors of lameness in the univariable or multivariable models ($P > 0.05$); however, there were interactions ($P < 0.01$) of the average DMI%BW and EB in the last 3 d prepartum with BCS on lameness
Each percentage point decrease in the average DMI%BW in the last 3 d prepartum increased the odds of having lameness by 39% in cows with BCS ≥ 3.75 (OR = 1.39, CI = 1.10 – 1.77; P < 0.01). Each percentage point decrease in the average EB in the last 3 d of prepartum increased the odds of having lameness by 29% in cows with BCS ≥ 3.75 (OR = 1.29, CI = 1.06 – 1.56; P = 0.01). However, neither DMI%BW (OR = 1.03, CI = 0.94 – 1.13; P = 0.55) or EB (OR = 1.02, CI = 0.93 – 1.12; P = 0.63) were significant predictors of lameness in cows with BCS < 3.75.

When the average DMI%BW or EB in the last 3 d prepartum were included in the lameness-predicting models for cows with BCS ≥ 3.75 containing parity, the AUC increased from 0.57 (95% CI: 0.49 – 0.65) to 0.81 (95% CI: 0.75 – 0.88) and from 0.57 to 0.78 (95% CI: 0.71 – 0.85), respectively. All the differences between the AUCs were statistically significant (P < 0.05).

The average DMI%BW and EB during the last 3 d prepartum produced significant (P < 0.01) cut-offs to predict lameness in cows with a BCS ≥ 3.75 postpartum, which were ≤ 1.1 %/d and ≤ -1.9 Mcal/d, respectively (Table 5-6).

**Association of Postpartum DMI%BW, EB, and ECM with Lameness**

Postpartum, lameness was associated with lesser lesser DMI%BW (2.43 ± 0.11 vs. 2.75 ± 0.03 %/d; P < 0.01; Table 5-3; Figure 5-2A); however, lameness was not significantly associated with postpartum EB (-6.7 ± 1.1 vs. -4.6 ± 0.3 Mcal/d; P = 0.06; Table 5-3; Figure 5-2B) or ECM (32.1 ± 2.0 vs. 32.8 ± 0.6 kg/d; P = 0.76; Table 5-3; Figure 5-2C).

**Discussion**

In this study it was shown that cows that developed digestive disorders in all cows and lameness in cows with BCS ≥ 3.75 had decreased DMI%BW and EB during
the transition period, and lesser ECM during the postpartum period. Furthermore, the average DMI%BW, and EB in the last 3 d prepartum were predictive of digestive disorders in all cows and lameness in cows with BCS ≥ 3.75, although the effect sizes were small. Furthermore, cut-offs for prediction digestive disorders in all cows and lameness in cows with BCS ≥ 3.75 were established, although the accuracy was low.

Cows with digestive disorders had decreased prepartum DMI%BW and EB from d -5 to -1. Furthermore, DMI%BW and EB were significant explanatory variables for digestive disorders postpartum and the predictive ability of the models for digestive disorders increased modestly, although significantly when the average DMI%BW and EB in the last 3 d prepartum were independently included in the models containing other covariates. This indicates that DMI%BW and EB prepartum are predictors of digestive disorders postpartum, but their contribution is minor when accounting for other variables such as parity. In addition, cut-offs for DMI%BW and EB were determined to see if they could be used solely as a predictor of digestive disorders postpartum, and the cut-offs resulted in low to moderate sensitivity (69 - 73%), specificity (48 - 55%), overall accuracy (53 - 58%), and AUC (0.59 - 0.61). Therefore, although significant, these cut-offs are of limited applicability. In summary, DMI%BW and EB prepartum are significant but minor contributors to digestive disorders development postpartum and cannot be used reliably to identify cows that will develop digestive disorders postpartum. As far as it is known, the association of DMI%BW, and EB prepartum with digestive disorders postpartum had not been evaluated. Previous work had shown that increased NEFA prepartum is a risk factor for displaced abomasum postpartum (Ospina et al., 2010a), which indicated that prepartum DMI%BW and EB could have been compromised in
cows that developed left displaced abomasum postpartum. Therefore, it was confirmed herein that prepartum DMI%BW was indeed compromised in cows that developed left displaced abomasum and other digestive disorders postpartum.

It was not surprising that, during postpartum, cows that developed digestive disorders had lesser DMI%BW and lesser milk yield compared with cows that did not develop digestive disorders. Cows with digestive disorders show reduced feed intake and milk yield (Østergaard and Gröhn, 1999, Edwards and Tozer, 2004). Edwards and Tozer (2004) found that sick cows (i.e. at least of the following: ketosis, RP, milk fever, left DA, indigestion, acidosis, and bloating, reduced feed intake or hardware disease) had on average 2.11 kg/d lesser milk yield compared with healthy cows. In this present study, when cows that developed digestive disorders were compared with cows that did not develop digestive disorders the decrease in ECM in cows with digestive disorders were on average of 2.43 kg/d; however, when cows that developed digestive disorders were compared with healthy cows the difference increased to an average of 3.65 kg/d (Table A-8). The EB was also decreased in cows that developed digestive disorders compared with cows that did not developed digestive disorders, which might be as a consequence of lesser DMI%BW and the onset of lactation.

Lame cows with BCS ≥ 3.75 had decreased DMI%BW and EB prepartum, whereas there were no differences between lame and sound cows with BCS < 3.75. Furthermore, the average DMI%BW and EB in the last 3 d prepartum were predictive of lameness in cows with BCS ≥ 3.75, and the addition of DMI%BW and EB in the last 3 d prepartum to a lameness-predicting model for cows with BCS ≥ 3.75 significantly increased the predictive ability of the models as evaluated by the AUC. The effect sizes
were moderate, indicating that DMI%BW and EB prepartum were important contributors to the development of lameness postpartum in cows with BCS ≥ 3.75, even when accounting for other variables such as parity. A possible explanation for this observation is that cows that were clinically lame postpartum were subclinically lame prepartum, and that overconditioned cows with subclinical lameness prepartum would have and exacerbation of their expected suppression of DMI compared with thinner cows (Rukkwamsuk et al., 1999). The AUC observed herein (0.78 to 0.81) are comparable to what was observed by Machado et al. (2011) (0.76 to 0.77) in three different models including a combination of digital cushion thickness, BCS, age, and claw horn disruption lesions (CHDL) at cessation of lactation. Their simplest model only included BCS, age and CHDL at cessation of lactation; therefore, at this point, using measures of DMI%BW to predict lameness postpartum would be less efficient than evaluating CHDL at the end of lactation because it would be limited to only cows with BCS ≥ 3.75 and it would not result in a substantial improvement in AUC even for this group of cows. The cut-offs for DMI%BW and EB to predict lameness in cows with BCS ≥ 3.75 had high Se (90%) and NPV (99%), low Sp (60-62%), PPV (13-14%) and overall accuracy (62-64%), and moderate AUC (0.78-0.82). Therefore, these cut-offs could be used as a screening test for postpartum lameness in cows with BCS ≥ 3.75 because of the high Se and NPV, although it would result in a high number of false positives because of the low PPV.

During the postpartum period, lame cows had significantly lesser DMI%BW and numerically lesser EB. Because lameness is a painful condition, it is not surprising that lame cows had lesser DMI%BW postpartum. Bach et al (2007) showed that number of visits to feed through, time spent eating, and DMI decreased with increasing locomotion.
score. Previous research has shown that lameness throughout the lactation was correlated with the severity of negative EB and with the total energy deficit during the first weeks of lactation (Collard et al., 2000).

Energy-corrected milk was not associated with lameness. Similar to the results presented in this current study, Hernandez et al. (2005) reported no significant difference in milk yield among cows that developed moderate lameness, lameness and non-lame cows. However, Hernandez et al. (2005) found that milk yield was lesser in multiparous cows that were lame compared with multiparous non-lame cows. Using a much larger sample size (1224 primiparous and 2399 multiparous), Bicalho et al. (2008) showed that cows that were lame had similar unadjusted milk yield throughout the lactation but showed a loss of 1 kg/d after controlling for milk yield during the first three weeks of lactation. Others have shown that milk yield is associated with the type of lameness. Amory et al. (2008) showed that cows with non-infectious lameness (i.e. sole ulcer and white line disease) had lesser milk yield after they were diagnosed as lame than non-lame cows but cows with infectious lameness (i.e. digital dermatitis) had similar milk yield to non-lame cows after they were diagnosed as lame. On the other hand, Hernandez et al. (2002) found that cows that developed white line disease or sole ulcers had similar milk yield compared with non-lame cows, whereas cows that developed infectious lameness (i.e. interdigital necrobacillosis) had lesser milk yield than non-lame cows. It is not clear why the differences in results; therefore, more research is needed to evaluate milk yield and milk losses in cows with different types of lameness.
As mentioned previously, in this present study is focused on to the observable differences in prepartum DMI% BW and EB between cows that did and did not have mastitis or lameness. In a herd, cows that do not develop digestive disorders or lameness could develop any other disease or disorder that are associated with decreased DMI% BW, and EB prepartum such as metritis, ketosis and clinical mastitis; therefore, excluding cows that develop other conditions could inflate the measures of association, which is of particular relevance for the prediction model. Indeed, a pattern of greater differences was observed when comparing affected cows with healthy cows than when comparing affected cows with unaffected cows. For instance, it was observed that cows that developed lameness had significantly lesser prepartum DMI% BW and EB (Table A-9), whereas the differences between cows that lameness and cows that did not develop lameness were not significant.

Chapter Summary

This study shows that digestive disorders were associated with prepartum DMI% BW and EB. The average DMI% BW and EB in the last 3 d prepartum were significant explanatory variables for digestive disorders, and the average DMI% BW and EB in the last 3 d prepartum increased the predictive ability of digestive disorders, although the effect sizes were small. Prepartum cut-offs for DMI% BW and EB to predict digestive disorders postpartum were established, although with low sensitivity, specificity, and overall accuracy. In addition, digestive disorders were associated with postpartum DMI% BW, EB, and ECM. It was also shown that the average DMI% BW and EB in the last 3 d prepartum were significant explanatory variables for lameness in cows with BCS ≥ 3.75, and the average DMI% BW and EB in the last 3 d prepartum significantly increased the predictive ability of lameness in cows with BCS ≥ 3.75.
Prepartum cut-offs for DMI%BW and EB to predict lameness postpartum in cows with BCS ≥ 3.75 were established, and had high Se and low Sp. In addition, lameness was associated with postpartum DMI%BW. The results of this study give a better understating of the role DMI plays during the transition period; namely that when measures of DMI decrease, the risk of digestive disorders in all cows, and the risk of lameness in cows with BCS ≥ 3.75 increase, although the increase in the risk of digestive disorders was small and the increase in the risk of lameness was moderate. In summary, DMI%BW and EB prepartum are significant but minor contributors to digestive disorders and lameness development postpartum and cannot be used reliably to identify cows that will develop digestive disorders and lameness postpartum.
Table 5-1. Frequency table of digestive disorders (DDZ) and lameness by study diagnosed during the first 28 days postpartum.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDZ</td>
<td>120</td>
<td>25.2</td>
</tr>
<tr>
<td>Lameness</td>
<td>35</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Table 5-2. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with digestive disorders (DDZ) postpartum according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th>Postpartum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Prepartum P</td>
<td></td>
<td>Postpartum P</td>
</tr>
<tr>
<td></td>
<td>aDDZ</td>
<td>No DDZ</td>
<td>DDZ</td>
<td>No DDZ</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.55 ± 0.04</td>
<td>1.65 ± 0.02</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.9 ± 0.4</td>
<td>2.7 ± 0.2</td>
<td>0.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

aDigestive disorders include left displaced abomasum, sand impaction, cecal dilatation, diarrhea, bloat, constipation, and indigestion.
bD: day relative to parturition.
cDDZ x Day: interaction between DDZ and Day.
Table 5-3. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with lameness postpartum according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lame</td>
<td>Not lame</td>
</tr>
<tr>
<td></td>
<td>aLame</td>
<td>bDay</td>
</tr>
<tr>
<td>DMI%BW</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.50 ± 0.07</td>
<td>1.65 ± 0.02</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.4 ± 0.4</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Lame: developed lameness postpartum.

*b* Day: day relative to parturition.

*c* L x D: interaction between Lame and Day.
Table 5-4. Association between each 0.1 percentage point decrease in the average of dry mater intake as percentage of BW (DMI%BW), and each unit decrease in the average of energy balance (EB) in the last 3 d prepartum on digestive disorders and lameness in the first 28 d postpartum.

<table>
<thead>
<tr>
<th>Disease</th>
<th>DMI%BW OR</th>
<th>95% CI</th>
<th>P-value</th>
<th>EB, Mcal/d OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive disorders</td>
<td>1.07</td>
<td>1.02-1.13</td>
<td>&lt;0.01</td>
<td>1.06</td>
<td>1.01-1.11</td>
<td>0.03</td>
</tr>
<tr>
<td>aLame BCS &lt; 3.75</td>
<td>1.03</td>
<td>0.94-1.13</td>
<td>0.55</td>
<td>1.02</td>
<td>0.93-1.12</td>
<td>0.63</td>
</tr>
<tr>
<td>Lame BCS ≥ 3.75</td>
<td>1.39</td>
<td>1.10-1.77</td>
<td>&lt;0.01</td>
<td>1.29</td>
<td>1.06-1.56</td>
<td>0.01</td>
</tr>
</tbody>
</table>

aInteraction between average DMI%BW and EB in the last 3 d prepartum and body condition score (BCS) in the last week prepartum on lameness; P < 0.05.
Table 5-5. Cut-offs of dry matter intake as percentage of BW (DMI%BW), and energy balance (EB) to predict digestive disorders postpartum.

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>aSe (%)</th>
<th>bSp (%)</th>
<th>cPPV (%)</th>
<th>dNPV (%)</th>
<th>eAcc (%)</th>
<th>fAUC</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI%BW</td>
<td>≤ 1.4</td>
<td>69</td>
<td>55</td>
<td>32</td>
<td>85</td>
<td>58</td>
<td>0.61</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>≤ 1.5</td>
<td>73</td>
<td>48</td>
<td>30</td>
<td>85</td>
<td>53</td>
<td>0.59</td>
</tr>
</tbody>
</table>

aSensitivity; bSpecificity; cPositive predicted value; dNegative predictive value; eAccuracy; fArea under the curve.
Table 5-6. Cut-offs of dry matter intake as percentage of BW (DMI%BW), and energy balance (EB) to predict lameness postpartum in cows with BCS ≥ 3.75.

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>aSe (%)</th>
<th>bSp (%)</th>
<th>cPPV (%)</th>
<th>dNPV (%)</th>
<th>eAcc (%)</th>
<th>fAUC</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI%BW</td>
<td>≤ 1.1</td>
<td>90</td>
<td>62</td>
<td>14</td>
<td>99</td>
<td>64</td>
<td>0.82</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>≤ -1.9</td>
<td>90</td>
<td>60</td>
<td>13</td>
<td>99</td>
<td>62</td>
<td>0.78</td>
</tr>
</tbody>
</table>

aSensitivity; bSpecificity; cPositive predicted value; dNegative predictive value; eAccuracy; fArea under the curve.
Figure 5-1. Association of digestive disorder postpartum (n = 120) with (A) dry matter intake (DMI, (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI (%BW): digestive disorder - P = 0.02, day relative to parturition - P < 0.01, and the interaction between digestive disorder and day - P < 0.01. Prepartum EB: digestive disorder - P = 0.06, day relative to parturition - P < 0.01, and the interaction between digestive disorder and day - P < 0.01. Postpartum DMI (%BW): digestive disorder - P < 0.01, day relative to parturition - P < 0.01, and the interaction between digestive disorder and day - P = 0.03. Postpartum EB: digestive disorder - P = 0.05, day relative to parturition - P < 0.01, and the interaction between digestive disorder and day - P = 0.44. ECM: digestive disorder - P = 0.03, day relative to parturition - P < 0.01, and the interaction between digestive disorder and day - P = 0.09.
Figure 5-2. Association between lameness (n = 35) and (A) dry matter intake (DMI, %BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI (%BW): lameness ($P = 0.03$), day relative to parturition ($P < 0.01$), and the interaction between lameness and day ($P = 0.52$). Prepartum EB: lameness ($P = 0.07$), day relative to parturition ($P < 0.01$), and the interaction between lameness and day ($P = 0.33$). Postpartum DMI: lameness ($P = 0.02$), day relative to parturition ($P < 0.01$), and the interaction between lameness and day ($P = 0.69$). Postpartum EB: lameness ($P = 0.06$), day relative to parturition ($P < 0.01$), and the interaction between lameness and day ($P = 0.22$). ECM: lameness ($P = 0.76$), day relative to parturition ($P < 0.01$), and the interaction between lameness and day ($P = 0.13$).
Figure 5-3. Interaction ($P < 0.01$) between lameness and BCS on dry matter intake (% of BW) and energy balance (Mcal/d) in cows with BCS < 3.75 (A, C) and BCS ≥ 3.75 (B, D) during the prepartum period (from -21 d to -1 d). Values are least squares means +/- SEM.
CHAPTER 6
ECONOMICS Of METRITIS

Summary

The objective of this study was to estimate the cost of metritis in dairy herds in the USA. Data of 11,733 dairy cows (4,102 primiparous and 7,631 multiparous) from 16 different farms located in 4 different regions of the US were compiled for up to 10 months after the calving date. Individual information such as type of reproductive management, number of inseminations, parity (primiparous and multiparous), calving date, days open, survival in the herd, milk production, and health status were collected. Data was analyzed with PROC MIXED from SAS. The dependent variables were 305 d milk production, percentage (%) of cows pregnant, % of cows that left the herd, milk sales ($/cow), cow sales ($/cow), replacement costs ($/cow), cost of reproduction ($/cow), feeding cost ($/cow), and profit ($/cow). All models had metritis, parity, and the interaction between covariates as independent variables, and farm as random variable. Variables were considered significant when $P \leq 0.05$. Metritis cost was calculated by subtracting the profit of cows with metritis from the profit of cows without metritis. Milk production and % of cows pregnant were less in cows that developed metritis than cows that did not develop metritis. The % of cows leaving the herd was greater in cows that developed metritis than cows that did not develop metritis. Milk sales, feeding costs, and profit were all less in cows that developed metritis than cows that did not develop metritis. Cow sales and replacement cost were greater in cows that developed metritis than cows that did not develop metritis. The cost of metritis in dairy cows was $532.3. In conclusion, metritis affected production, reproduction, and survival causing economic losses to the dairy herd.
Introductory Remarks

Metritis is one of the postpartum disorders with greatest incidence during the fresh period in dairy cows. The incidence of metritis has been reported to be around 20% in dairy farms and be present in up to 40% of the dairy cows (Curtis et al., 1985; Markusfeld, 1987). Although, this incidence may vary due to the large variation and inconsistency in the definition of metritis across herds and studies (Espadamala et al., 2016; Sannmann et al., 2012), which may be underestimating the real impact of metritis in the dairy farm (Espadamala et al., 2016; McCarthy and Overton, 2018).

Metritis is associated with reduce milk production, impairment in reproduction performance, and increased culling, thus having a negative impact in the profitability of the dairy herd. Previous research have shown that cows with metritis had reduced milk yield compared with cows that did not have metritis (Rajala and Gröhn, 1998; Dubuc et al., 2011; Goshen and Shpigel, 2006) and this impact in milk yield could be even greater as the association of metritis on milk yield is affected by misclassification bias (McCarthy and Overton, 2018). Using model simulation, McCarthy and Overton (2018) showed that farms with an inconsistent recording of metritis could have ~60 kg of milk loss in the 305-d mature-equivalent milk projection that will not be attributable to metritic cases, suggesting that inconsistency in the report of metritis is underestimating the impact of metritis on milk yield.

Moreover, metritis has been associated with impairment in the reproduction performance in dairy cows (Santos et al., 2010; Ribeiro et al., 2013). Cows with metritis have been shown to have reduced pregnancy per AI (i.e. ~ 14% less) compared with healthy cows (Santos et al., 2010), an increased calving to conception interval (i.e. ~36.5 days) (Giuliodori et al., 2013), and are less likely to have resumption of estrus
cyclicity at ~60 DIM (Santos et al., 2010; Ribeiro et al., 2013). However, some discrepancies exist in the association between metritis and culling (Grönh et al., 1998; Dubuc et al., 2011; Wittrock et al., 2011). Grönh et al. (1998) and Dubuc et al. (2011) did not find any association between metritis and culling, whereas Wittrock et al. (2011) showed that cows with metritis were ~30% more likely to be culled. Nevertheless, metritis is associated with reduced milk yield and impairment in fertility, which both represent major culling reasons in dairy farms (Pinedo et al., 2010). Increased culling would lead to increased replacement costs. In addition, metritis incur in costs due to veterinary services and treatment. Lima et al. (2019) calculated that the cost of treatment for metritis can range from $46 to $101 dollars depending on parity and the antibiotic used. Therefore, taking all together it can be inferred that metritis is one of the most costly diseases for the dairy herd but a comprehensive study including several herds with a large sample size is still missing.

Researchers have estimated the cost of metritis in the United States of America (USA) (Bartlett et al., 1986; Overton and Fetrow, 2008; McArt et al., 2015) ranging from $106 to $358. Bartlett et al. (1986) showed that the total costs of metritis was $106. However, Bartlett et al. (1986) compared lactations with and without metritis and did not find any difference in milk production between lactations that had metritis cases and lactations that did not; therefore, they did not take in account milk production as an input for the cost of metritis. Overton and Fetrow (2008) used inputs were based on data from only one farm and showed that a total cost of a case of metritis in a large dairy herd from California was $358 after including culling, milk loss, reproduction cost, and treatment due to metritis, whereas McArt et al. (2015) calculated the cost of metritis
cases that were attributed to ketosis. In addition, outside US, Mahnani et al., (2015) also estimated that a case of metritis in dairy farms of Iran costed around $162/case by reducing 305-d milk yield, increasing days open by 16 days, and increasing number of inseminations by 0.1 per cow per lactation. However, this research was done using prices and other parameters that are true for Iran and not US. Therefore, using a convenient sample size of 11,412, the objective of this study is to estimate the cost of metritis in dairy herds using data collected from 16 dairy herds located in 4 regions of US.

**Materials and Methods**

**Farms and Cows**

This study was performed using the data of 11,733 dairy cows (4,102 primiparous and 7,631 multiparous) from 16 different farms located in 4 different regions of the United States of America (i.e. Southeast, Midwest, Southwest, and Northeast). Data was collected from November 2012 to October 2014 for up to 10 months after the calving date. Information for individual calving events were available in the dataset, including herd identification, cow identification, type of reproduction management (i.e. estrus or timed artificial insemination), number of inseminations, inseminations dates, calving date, parity (i.e. primiparous and multiparous), and days open.

Cows were removed from the study if they did not get a metritis diagnosis (n = 44; 13 primiparous and 31 multiparous), or if they were categorized as having a metritis score of 4 at 5 ± 3 DIM and not being recheck for metritis diagnosis at day 12 ± 3 DIM (2682; 1,602 primiparous and 1,080 multiparous). Because 41% of the cows that were checked at d 5 and had a score of 4 had a score of 5 at 12 DIM it was no certain if cows
with a score 4 did not develop metritis. Therefore, the final dataset for this study included a total of 9,007 cows (3,272 primiparous and 5,735 multiparous).

**Health Disorders**

Metritis was diagnosed by examining the uterine discharge at 5 ± 3 and 12 ± 3 d postpartum using a 5-point scale as previously described (Chenault et al. 2004): 1 = not fetid normal lochia, viscous, clear, red, or brown; 2 = cloudy mucoid discharge with flecks of pus; 3 = not fetid mucopurulent discharge with < 50% pus; 4 = not fetid mucopurulent discharge with ≥ 50% pus; 5 = fetid red-brownish, watery discharge. Cows with a discharge score ≤ 3 were classified as healthy (n = 6266) and cows with a score of 5 were classified as metritic cows (n = 2741). In addition, the occurrence of dystocia, twins, stillbirth, retained placenta, and metritis were also recorded. Dystocia was defined as the cow receiving assistance by one or more people with or without the use of mechanical traction, having a fetotomy or cesarean-section. Stillbirth was defined as the birth of a dead calf or a calf that died within 24 h of birth. Retained placenta was characterized by failure to release the placenta within 24 h of parturition.

**Milk Production and Milk Income Calculation**

Milk production was measured monthly by the Dairy Herd Improvement Association (DHIA) for a total of 10 months. The total milk yield was calculated averaging the milk production of the 10 months and multiplying this average by the amount of days that the cow was in the study. If the cow was not culled and it was present at the end of the study (i.e. 305 DIM), then it was multiplied by 305 days. Milk income was calculated by multiplying the total milk yield for each cow by $0.40, which is the mean price per kg of milk sold from 2008 to 2018 (Agricultural prices, USDA, NASS).
Reproductive Management

Reproductive management varied among farms but usually included a combination of estrus detection and timed AI (TAI) for first and subsequent AI; therefore, it was assumed that all the cows were enrolled in a Presynch-Ovsynch program (Moreira et al., 2001), but with the option of being AI in estrus after the second PGF$_{2\alpha}$ of the presynchronization. It was assumed that all cows received a treatment of 25 mg PGF$_{2\alpha}$ intramuscular 14 days before the first AI, and at the end of the voluntary waiting period (average of days of first AI 75 ± 16). Cows had their tailheads painted and that the removal of the paint was an indication of estrus. Cows not observed in estrus were assumed to be enrolled in a 5-d timed AI program at 81 DIM and timed AI performed at 89 DIM. Data for first service was available for 8,156 cows, if cows were AI after estrus detection (n = 3,819) or if they were timed artificial inseminated (TAI) (n = 4,337). Data for 851 cows was not available, 92% (787/851) left the study by 56 DIM (SD ± 55), 6.8% (58/851) cows did not leave the study, but were marked as do not breed and the rest 0.7% (6/851) did not leave the study, but did not have any data on first service.

Pregnancy diagnosis was performed using transrectal ultrasonography at 32 ± 3, 60 ± 3, and at ~200 d after AI. Therefore, cows that were pregnant at the end of the study were assumed to have 3 pregnancy diagnoses, and cows that were not inseminated, and were not pregnant at the end of the study were assumed to not having been evaluated for pregnancy. A cow was diagnosed as pregnant by visualization of an amniotic vesicle containing an embryo with a heartbeat during ultrasonography.
Costs of Reproductive Management

The cost of reproductive management for cows that were AI after detected estrus for first service included the cost of pre-synchronization, labor cost for hormonal administration, cost of daily heat detection, labor cost of AI, cost of AI, and cost of pregnancy check. The cost of the reproduction management for cows that were serviced with TAI were calculated assuming that they received the same treatment as a cow that received AI after estrus detection plus the cost of an ovulation synchronization (Ovsynch) program. The cost for Pre-synch and the Presynch-Ovsynch program were calculated assuming a cost of $2.70/dose for PGF$_{2\alpha}$ and $2.00/dose for GnRH (Southwest Florida Milk, https://www.floridamilk.com/). The cost of labor for employees that administered the hormones and performed detection of estrus was assumed to be $10.80/h. Assuming that a person can administer 60 injections/h, the cost per injection was $0.18, and to this price it was added $0.05 of supplies for injections thus having a total cost of $0.23 for labor and supplies. For detection of estrus, it was assumed that each cow had 24 estrus checks and that a person can check 120 cows/h, thus resulting in a value of $0.09 per check. The cost of an AI technician was $120/h, and assuming that a technician can inseminate 30 cows/h, the cost per cow was $4.00/cow. The AI cost was calculated assuming a semen cost of $15.00/dose, and supplies for each insemination including AI sheath, sleeve, semen applicator, water bath, and chalk was assumed to be $0.50/AI. Therefore, the cost of AI in cows that were inseminated after showing estrus was $27.52/cow and the ones that were inseminated with TAI was $34.91/cow. In cows that received more than one AI, the cost per extra breeding was calculated averaging the AI cost in cows that were inseminated after showing estrus.
and the ones that were inseminated with TAI, excluding the cost of Presynch, resulting in $25.82.

The cost of pregnancy diagnosis was calculated assuming that each pregnant cow had 3 pregnancy checks; therefore, cows that received one AI and became pregnant were assumed to have 3 pregnancy checks. For cows that received several AIs, the cost of pregnancy check was multiplied by 0.5 to assumed half of the cost of pregnancy diagnosis, for each extra AI that the cow received and did not end in pregnancy. In addition, per each pregnancy loss, it was assumed one extra pregnancy check. The veterinarian cost for pregnancy diagnosis was $140.00/h, and assuming that a veterinarian can do 30 pregnancies diagnoses/h by ultrasonography, thus resulting in a cost per pregnancy check of $4.67/cow.

**Survival and Residual Cow Value**

Survival in the herd was observed until 305 DIM. Cows that survived until 305 DIM were censored at that point. A cow that died or was sold before 305 DIM was replaced by a first lactation cow, and DIM when dead or sold was recorded. Cows that were not pregnant by 305 DIM were considered to have been sold at 305 DIM, and were replaced by a first lactation cow. The value of a cow sold was calculated by multiplying the mean BW at the DIM when it was sold by the average beef price in the US of $1.65/kg between 2008 and 2017 (NASS, 2017, USDA). The change in BW during lactation was calculated based on the data of 175 cows, 81 primiparous and 94 multiparous, from the dairy research unit at the University of Florida. The BW of a certain day of lactation was calculated using a quadratic regression formula for primiparous and multiparous. For example, to calculate the BW for a cow on d 18, the following formula was used:
BW kg = β0 + β1 *18 DIM + β2 (18 DIM)^2

The average price for a replacement heifer from 2008 to 2018 was $1,576.2 (Agricultural prices 2009 to 2017, NASS, USDA,). The cow value from first to fourth lactation was calculated using the following formula:

Cow value = replacement cost – ((replacement cost – salvage value)/ 4)

Where salvage value = (% of primiparous cows sold x price per primiparous cow sold) + (% of multiparous cows sold x price per multiparous cow sold)

Where % of primiparous cows sold = 20.5%, % of multiparous cows sold = 79.5%, price primiparous cows = beef price x primiparous BW, and price multiparous cows = beef price x multiparous BW.

First value obtained with this formula was set for cows on lactation 1; for cows in lactation 2 until 4, it was calculated it by subtracting the replacement cost from the cow value on the previous lactation divided by 4, as an example, this formula was used to calculate the cow value on lactation 2:

Cow value lactation 2 = cow value on lactation 1 – (replacement cost – salvage value for cows on lactation 1/4).

Cost of Therapy

It was assumed that cows with metritis were treated with 6.6 mg of ceftiofur/kg of BW administered subcutaneously (Excede sterile suspension, 200 mg/250 mL of ceftiofur as ceftiofur crystalline free acid, Zoetis, Kalamazoo, MI) twice, with the second dose administered 72 hours after the initial dose. Body weights used to calculate the dose for primiparous and multiparous cows were 595 kg and 720 kg, respectively, which is the mean of BW during the first 14 days postpartum for each parity level. The cost of Excede box was set at $523 according to market price, resulting in a cost of $2.09/ml. In
addition to medication, the costs associated with therapies included the supplies for therapy administration and the cost of the time for cow restrain and administration of injectable, which was assumed to be 4 minutes per cow. Labor cost for performing treatments was priced at $10.80 per hour (USDA ERS, 2014), which resulted in $0.72 for injectable therapy. Cost of supplies for injectable treatments such as needles and syringes were $0.62 per treatment. Therefore, the total cost per treatment for a case of metritis for primiparous and multiparous was $84.9 and $102.1, respectively.

**Estimated Feed Costs**

For calculation of feed costs, it was assumed that all lactating cows were fed a TMR with a NE\textsubscript{L} density of 1.60 Mcal/kg. The milk NE was assumed at 0.69 Mcal/kg based on milk containing 3.5% fat, 4.8% lactose, and 3.2% protein. Therefore, each kg of marginal DM consumed supported 2.32 kg of milk. Primiparous cows had an average BW during the entire lactation of 615 kg, whereas multiparous cows had an average BW of 740 kg and the NE\textsubscript{L} needs for maintenance was calculated as 0.08 Mcal/kg of BW\textsuperscript{0.75}. It was assumed that the DM consumed by cows was able to meet the nutrient needs for maintenance and milk yield, with no changes in BW. The DMI required to support maintenance was calculated by multiplying each daily maintenance needs in kg of DMI by the number of days in lactation up until the cow died, was sold, or at 305 DIM, whichever came first. The DMI required to support milk production was calculated as the total milk yield in the lactation multiplied by 0.69 and then divided by 1.60. The costs associated with feed for each lactating cow were calculated by multiplying the total DMI (maintenance plus production) by the average cost of a lactating cow TMR from 2010 to 2017 of $0.26/kg of DM (ERS 2018, USDA).
Herd Budget Calculator and Statistical Analysis

The profit for each cow was calculated using an Excel spreadsheet and included the income and expenses during their lactation. Each cow generated income from the sale of milk and from their salvage when they were culled. Additional income for each cow was generated by the residual cow value at the end of the 305-d lactation. Expenses for each cow included those incurred with replacement cost, costs associated with reproductive management, therapeutic costs of metritis, and feed cost. The mean value for cows with and with no metritis were compared using PROC MIXED from SAS version 9.4 (SAS Institute Inc., Cary, NC), using farm as a random variable, parity and metritis as fixed variable, and the interaction between covariates. As dependent variables were milk production (kg/cow), pregnant cows (%), cows leaving the herd (%), cows sold (%), and of dead cows (%) by 305 DIM, milk sales ($/cow), cow sales ($/cow), replacement costs ($/cow), cost of reproduction ($/cow), breeding costs ($/cow), pregnancy checks costs ($/cow), feeding costs ($/cow), and profit ($/cow).

All models had parity, metritis, and the interaction between parity and metritis as fixed parameters. A stepwise backward elimination was performed and explanatory variables with $P > 0.10$ were removed from the model. Variables were considered significant when $P \leq 0.05$, and tendency when variables were between $P > 0.05$ and $P \leq 0.10$. The cost of metritis was the difference between the profit in cows with no metritis and cows with metritis. Distribution of residuals and homogeneity of variance were evaluated for each variable analyzed. The Kenward-Roger approximation method was used to calculate the denominator degrees of freedom for the F tests in the mixed models. The LSM and SEM were used to express the results.
Interval to pregnancy and to leaving the herd were analyzed by survival analysis with the Cox’s proportional hazard model using MedCalc version 18 (MedCalc software, Ostend, Belgium). For interval to pregnancy, those that did not become pregnant were censored when dead, sold, or at 305 DIM, whichever occurred first. For leaving the herd, cows that survived were censored at 305 DIM. As explanatory variables were metritis, calving season, and parity. Calving season was categorized in 4 seasons: spring (i.e. March, April, and May), summer (i.e. June, July, and August), fall (i.e. September, October, and November), and winter (i.e. December, January, and February). The adjusted HR and respective 95% CI were calculated, and the Kaplan-Meier option was used to generate the survival curves and compute the LSM ± SEM and median days to event.

Results

The proportion of cows with metritis was 30.4% (2,741/9,007). The inputs used to estimate the cost of metritis are depicted on Table 6-1.

Milk Production, Pregnancy Percentage, and Leaving the Herd by 305 Days in Milk

The milk production was less ($P < 0.01$) in cows that developed metritis compared with cows that did not develop metritis (9,752.6 ± 396.4 vs. 10,605.0 ± 393.1 kg/cow). The percent of cows that became pregnant was less ($P < 0.01$) in cows that developed metritis compared with cows that did not develop metritis (69.3 ± 1.5 vs. 80.4 ± 1.4 %). The percent of cows that left the study was greater ($P< 0.01$) in cows that developed metritis compared with cows that did not develop metritis (35.3 ± 1.5 vs. 24.7 ± 1.3 %). The percent of cows sold was greater ($P < 0.01$) in cows that developed metritis compared with cows that did not develop metritis (31.0 ± 1.5 vs. 22.5 ± 1.3 %).
The percent of cows that died was greater (P < 0.01) in cows that developed metritis compared with cows that did not develop metritis (4.3 ± 0.6 vs. 2.2 ± 0.6 %; Table 6-2).

**Milk and Cow Sales by 305 Days in Milk**

The total value of milk sale was less (P < 0.01) in cows that developed metritis compared with cows that did not develop metritis (3,901.1 ± 158.6 vs. 4,242.0 ± 157.2 $/cow). Income with cow sale was greater (P < 0.01) in cows that developed metritis compared with cows that did not develop metritis (345.8 ± 16.8 vs. 251.9 ± 15.4 $/cow) (Table 6-2).

**Cow Replacement and Reproduction Costs**

The cost of replacement was greater (P < 0.01) in cows that developed metritis compared with cows that did not develop metritis (530.4 ± 21.8 vs. 372.93 ± 19.7 $/cow) (Table 6-2).

The cost of reproduction did not differ (P = 0.91) between cows that developed metritis and cows that did not develop metritis (81.4 ± 1.4 vs. 81.6 ± 1.2 $/cow). There was an interaction (P = 0.04) between metritis and parity for the cost of breeding, because in primiparous cows that developed metritis, the cost of breeding was greater (P = 0.03) compared with primiparous cows that did not develop metritis (69.9 ± 1.7 vs. 65.8 ± 1.5 $/cow, whereas in multiparous cows, there was no difference (P = 0.54) in the cost of breeding between cows that developed metritis and those that did not develop metritis (65.4 ± 1.5 vs. 66.3 ± 1.1 $/cow). The cost of pregnancy check was less (P < 0.01) in cows that developed metritis compared with cows that did not develop metritis (13.9 ± 0.3 vs. 15.1 ± 0.3 $/cow, P < 0.01).
Feed Costs and Profit

The cost of feed was less ($P < 0.01$) in cows that developed metritis compared with cows that did not develop metritis ($1,536.2 \pm 48.1 \text{ vs. } 1,660.6 \pm 47.5 \text{ $/cow}$). The profit was less ($P < 0.01$) in cows that developed metritis compared with cows that did not develop metritis ($2,914.4 \pm 106.3 \text{ vs. } 3,437.8 \pm 103.9 \text{ $/cow}$) (Table 6-2).

Survival Analysis

Cows that developed metritis had decreased ($P < 0.01$) hazard of pregnancy compared with cows that did not develop metritis ($HR = 0.78, 95\% \text{ CI } = 0.74 \text{ – } 0.82,$). The mean ($155.0 \pm 1.7 \text{ vs. } 138.9 \pm 1.0 \text{ d})$ and median ($113, 95\% \text{ CI } = 111 \text{ – } 115 \text{ vs. } 127, 95\% \text{ CI } = 123 \text{ – } 131 \text{ d})$ days to pregnancy were greater in cows that developed metritis than in cows that did not develop metritis (Figure 6-1).

Cows that developed metritis had greater ($P < 0.01$) hazard of leaving the herd than cows that did not develop metritis ($HR = 1.48, 95\% \text{ CI } = 1.37 \text{ – } 1.60,$). The mean of days of cows leaving the herd were less in cows that developed metritis compared with cows that did not develop metritis ($259.2 \pm 1.7 \text{ vs. } 270.5 \pm 1.0 \text{ d})$ (Figure 6-2).

Discussion

The objective of this study was to estimate the cost of metritis in dairy herds in the USA. A case of metritis case had a mean cost of $532. Cows diagnosed with metritis had less milk production and reduced percent pregnant but had greater risk of culling by 305 DIM than cows not diagnosed with metritis.

Cows that developed metritis produced 852.4 kg less milk throughout the lactation than cows that did not develop metritis. This result agrees to previous research (McCarthy and Overton, 2018). McCarthy and Overton (2018) compared 305-d mature equivalent milk yield, which was estimated based on the milk production measured in
the second DHIA test, and showed that a case of metritis defined as a cow that had a flaccid uterus containing fetid fluids or possessing a foul watery discharge within 14 d of calving produced 847 kg less 305-d mature equivalent milk yield than cows that did not develop metritis. The decrease in milk yield could be because of the onset of inflammation. During infection and inflammation, peripheral insulin resistance decreases, and mammary gland reduces glucose uptake, which will lead to more availability of energy for immune cells to be able to fight infection, but it will compromise milk production (Bradford et al., 2015; Baumgard et al., 2017). However, as it was shown in Chapter 3, even after cows with metritis recovered their reduced DMI and EB after d 20 when compared with cows with no metritis, milk yield was still reduced in cows with metritis, which suggest that the changes in the metabolism of the mammary gland persisted even after cows with metritis increased DMI and EB. Therefore, more research should be done to understand better the long term effect of inflammation on the mammary gland.

Fertility was affected by metritis. The proportion of pregnant cows at the end of lactation was 11.1 percentage point less in cows that developed metritis compared with cows that did not develop metritis. In addition, cows that developed metritis had lesser hazard of getting pregnant and a mean of 17 d more days open than cows that did not develop metritis. The results presented in this current study are in agreement with previous research (Mahnani et al., 2015; Giuliodori et al. 2013). Mahnani et al. (2015) showed that cows that developed metritis had 16 d more days open than cows that did not develop metritis, whereas Giuliodori et al. (2013) showed that cows with metritis had lesser hazard rate (HR: 0.75, 95%CI 0.62-0.91) of pregnancy. Metritis has been
associated with delayed uterus involution (Melendez et al., 2004b; Heppelmann et al., 2015), delayed ovarian cyclicity and prolonged luteal phases (Opsomer et al., 1999; Ribeiro et al., 2016); which are factors that increase days open in dairy cows. In addition, it has been shown that metritis have negative carry over effect on fertilization and conceptus development. Ribeiro et al. (2016) showed that cows with uterine disease (i.e. retained placenta and metritis) had reduced proportion of cleaved, live and high-quality embryos than cows that did not have uterine disease; therefore, affecting the proportion of pregnant cows. In addition, the cost of breeding was greater in primiparous cows that developed metritis compared with primiparous cows that did not develop metritis, the cost of breeding did not differ between multiparous cows with and without metritis. The reason for the difference is because a larger proportion of primiparous cows that developed metritis were inseminated using timed AI than primiparous cows that did not develop metritis (data not shown), thus increasing the cost of breeding in primiparous cows that develop metritis. It has been reported that a smaller proportion of primiparous cows resume estrous cyclicity compared with multiparous by the end of the voluntary waiting period (Santos et al. 2009) and that metritis also increases the proportion of anovular cows (Ribeiro et al., 2016) thus decreasing the proportion of primiparous cows showing estrus leading to a larger proportion of them being subjected to a TAI program.

Metritis increased the proportion of cows that left the study by 10.6 percentage points compared with cows that did not have metritis. Previous research has shown contradictory results in the association of metritis and culling. Dubuc et al. (2011) showed that cows with metritis did not increased risk of culling at 30, 63, or 300 DIM,
whereas Ribeiro et al. (2016) showed that the proportion of cows that left the herd increased by 5 percentage points in those with uterine diseases. The differences in results could be explained by different statistical approaches. Dubuc et al. (2011) included retained placenta, twins, dystocia, metritis, and uterine health status (i.e. subclinical endometritis and purulent vaginal discharge) as predictors in their statistical model, and then preformed a stepwise backward elimination, whereas in the model of this present study risk factors in the model were not included to avoid multicollinearity. Retained placenta, dystocia, and twins are associated with metritis; therefore, in the model by Dubuc et al. (2011), metritis could have lost significance when modeled with the correlated predictors and thus removed from the model. Conversely, Ribeiro et al. (2016) did find that the proportion of cows that left the study was affected by uterine disease, however, the magnitude of the association was half of what was reported herein.

The cost or metritis presented in this study was greater than values previously reported. In 1986, Bartlett et al. estimated that the total cost per metritis case was $106.00 in dairy herds in the State of Michigan, which is less than half of what it was estimated to be the cost of metritis in this present study. The differences between studies may be because of the differences in how the estimation of metritis cost was calculated. Bartlett et al. (1986) did not find difference in milk production between lactations that had metritis cases and lactations that did not have metritis cases; therefore they did not take in account milk production as an input for the calculation of metritis cost; instead, they just inputed the loss of milk that was withhold due to the use of medication. Herein, one of the main components in the estimated
metritis cost is the loss in milk income during the lactation, which may explain a large portion of the differences between values.

A more recent study (Overton and Fetrow, 2008) estimated that a case of metritis cost ranged from $329 and $386 depending on the treatment used, which the largest value was $137 less than what was reported herein. Among the differences between studies that may explain these discrepancies in metritis cost, it can be mentioned that the study of Overton and Fetrow (2008) was based on costs and incomes of a single farm, whereas in the current study the data from 16 farms across the US were used. In the study by Overton and Fetrow (2008), the loss in milk income attributable to metritis was $258 less than what was reported in the present study, and the price of milk used by Overton and Fetrow (2008) was $0.11 less than what used herein. Another difference is that metritis treatment values varied from $53 to 109, whereas in the present study, the cheapest treatment was $32 greater than their highest value; however, their highest treatment cost was $7 greater than what was used herein. In addition, in the study done by Overton and Fetrow (2008) the salvage value was $554 to $393 less for primiparous and multiparous cows, respectively, than what was used herein.

On the other hand, Mahnani et al. (2015) estimated the cost of metritis being on average $162.3 per case, which is substantially less compared with what is reported herein. Some differences between studies might explain the differences in results. In the study of Mahnani et al. (2015), the differences in 305-d milk yield in cows that developed metritis and cows that did not develop metritis was less than in the present study (129.8 vs. 852.4 kg) and so was milk price (0.32 $/kg vs 0.40 $/kg), which led to a
difference of $300 in milk income. In addition, in the study of Mahnani et al. (2015), treatment cost was between $61 to $79 less than in the current study. The main reason is that the therapy cost is based on prices and antibiotics used in each respective country; however, is not clear which antibiotics were used in Mahnani et al. (2015). Finally, Mahnani et al. (2015) reported that the main losses caused by metritis was fertility cost, whereas no statistical difference was observed in overall reproductive cost herein. In the study of Mahnani et al. (2015), fertility cost was calculated based on the cost of days open, whereas the present study used a similar approach to that of Lima et al. (2019) and took in account breeding pregnancy diagnoses costs during the lactation. Similar to the present findings, Lima et al. (2019) also showed no differences in reproductive costs between cows not diagnosed with metritis and those diagnosed with metritis and treated with two different antimicrobials.

The cost of metritis has been estimated by others (Drillich et al., 2001; McArt et al., 2015; Lima et al., 2019). Drillich et al. (2001) estimated that the mean cost of metritis ranged from $291.19 to $362.09 depending on the treatment used; however, Drillich et al. (2001) evaluated the cost of metritis only taking in account the cost of treatments and the costs per pregnancy and did not take in account cost of DMI and cow value. In addition, in the study of Drillich et al. (2001) it was not clear how the cost of pregnancy was calculated, and the values used for replacement and veterinary fees were not indicated which makes very difficult the comparison between studies. McArt et al. (2015) estimated that the cost of a metritis case attributable to hyperketonemia was $396. In this study, McArt et al. (2015) used parameters to calculate the cost of a metritis case that also were attributable to hyperketonemia (e.g. death and culled
percentage) which could misestimate the true value of a case of metritis. Other differences between studies that are worth mentioning are that in the study from McArt et al. (2015), the cost of metritis estimation was based on inputs that were taken from the literature, and the estimation of a metritis case was based on a farm with 1,000 calving per year, not using values from observed from data collected from individual cows as did herein. In addition, Lima et al. (2019) also estimated that metritis cost ranged from $255 to $392, depending on the treatment that was administered and the final destiny of the treated cow’s milk (i.e. with or without milk withhold or milk fed to calves). Among the differences between studies, Lima et al. (2019) main focus was to compare the cost between two treatments, one that required milk withhold and one that did not require milk withhold; therefore, all cost for treatments are more detailed than in the present study.

Chapter Summary

Metritis causes economic losses to the dairy farm by affecting milk production, reproduction, and survival in the herd. Cows with metritis had reduced milk production and proportion of pregnancy, and a greater percentage of them left the herd by culling or death than cows that did not develop metritis. A case of metritis had an average cost of $532.3.
Table 6-1. Variables and measurements to estimate the cost of metritis.

<table>
<thead>
<tr>
<th>Input variable</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of cows with metritis, %</td>
<td>30.4</td>
</tr>
<tr>
<td>Average BW for primiparous cows, kg</td>
<td>615.0</td>
</tr>
<tr>
<td>Average BW for multiparous cows, kg</td>
<td>740.0</td>
</tr>
<tr>
<td>aMilk price, $/kg</td>
<td>0.40</td>
</tr>
<tr>
<td>Cost of leaving the herd</td>
<td></td>
</tr>
<tr>
<td>bBeef price, $/kg BW</td>
<td>1.65</td>
</tr>
<tr>
<td>Replacement cost, $/cow</td>
<td>1,576.21</td>
</tr>
<tr>
<td>Value of a cow Lact = 1 at the end of lactation, $/cow</td>
<td>1,476.84</td>
</tr>
<tr>
<td>Value of a cow Lact = 2 at the end of lactation, $/cow</td>
<td>1,377.47</td>
</tr>
<tr>
<td>Value of a cow Lact ≥ 3 at the end of lactation, $/cow</td>
<td>1,278.09</td>
</tr>
<tr>
<td>Labor, $/hour</td>
<td>10.8</td>
</tr>
<tr>
<td>Treatment cost</td>
<td></td>
</tr>
<tr>
<td>bExcede, $/mL</td>
<td>2.09</td>
</tr>
<tr>
<td>Excede dose, mL/100 kg</td>
<td>3.3</td>
</tr>
<tr>
<td>Excede primiparous, $/dose</td>
<td>41.08</td>
</tr>
<tr>
<td>Excede multiparous, $/dose</td>
<td>49.71</td>
</tr>
<tr>
<td>Labor and supplies, $/dose</td>
<td>1.34</td>
</tr>
<tr>
<td>Number of doses per treatment regimen</td>
<td>2</td>
</tr>
<tr>
<td>Metritis treatment in primiparous cows, $/cow</td>
<td>84.85</td>
</tr>
<tr>
<td>Metritis treatment in multiparous cows, $/cow</td>
<td>102.11</td>
</tr>
<tr>
<td>Reproduction cost</td>
<td></td>
</tr>
<tr>
<td>Labor estrus detection (120 detections/hour), $/estrus</td>
<td>0.09</td>
</tr>
<tr>
<td>Technician for AI (30 AI/hour), $/AI</td>
<td>4.00</td>
</tr>
<tr>
<td>Semen straw, $/straw</td>
<td>15.00</td>
</tr>
<tr>
<td>AI supplies, $/AI</td>
<td>0.50</td>
</tr>
<tr>
<td>Cost of PGF2α, $/unit</td>
<td>2.70</td>
</tr>
<tr>
<td>Cost of GnRH, $/unit</td>
<td>2.00</td>
</tr>
<tr>
<td>Cost of labor and supplies for hormones injections</td>
<td>0.23</td>
</tr>
<tr>
<td>Feed cost</td>
<td></td>
</tr>
<tr>
<td>Diet net energy, Mcal/kg</td>
<td>1.60</td>
</tr>
<tr>
<td>Milk net energy, Mcal/kg</td>
<td>0.69</td>
</tr>
<tr>
<td>Marginal feed, kg DM/kg milk</td>
<td>0.43</td>
</tr>
<tr>
<td>Maintenance for primiparous, kg DM/cow</td>
<td>6.18</td>
</tr>
<tr>
<td>Maintenance for multiparous, kg DM/cow</td>
<td>7.09</td>
</tr>
<tr>
<td>cFeed cost, $/kg</td>
<td>0.26</td>
</tr>
</tbody>
</table>

aSource: Agricultural prices (NASS 2017, USDA); bExcede 250 ml, $523.08 per bottle; cSource: Economic Research Service (2018, USDA).
Table 6-2. Economics, productive, and reproductive parameters according to disease status (LSM ± SEM).

<table>
<thead>
<tr>
<th>Item</th>
<th>No metritis</th>
<th>Metritis</th>
<th>aDiff</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk by 305 DIM, kg</td>
<td>10,605.0 ± 393.1</td>
<td>9,752.6 ± 396.4</td>
<td>-852.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pregnant by 305 DIM, %</td>
<td>80.4 ±1.4</td>
<td>69.3 ± 1.5</td>
<td>-11.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Left the herd by 305 DIM, %</td>
<td>24.7 ± 1.3</td>
<td>35.3 ± 1.5</td>
<td>10.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sold, %</td>
<td>22.5 ± 1.3</td>
<td>31.0 ± 1.5</td>
<td>8.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Death, %</td>
<td>2.2 ± 0.6</td>
<td>4.3 ± 0.6</td>
<td>2.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk sale by 305 DIM, $/cow</td>
<td>4,242.0 ± 157.2</td>
<td>3,901.1 ± 158.6</td>
<td>-340.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Residual cow’s value</td>
<td>1054.6 ± 18.1</td>
<td>909.7 ± 20.0</td>
<td>-144.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cow sale, $/cow</td>
<td>251.9 ± 15.4</td>
<td>345.8 ± 16.8</td>
<td>93.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Replacement costs, $/cow</td>
<td>389.3 ± 20.9</td>
<td>555.6 ± 23.0</td>
<td>166.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cost of reproduction, $/cow</td>
<td>81.6 ± 1.2</td>
<td>81.4 ± 1.4</td>
<td>-0.2</td>
<td>0.91</td>
</tr>
<tr>
<td>*Breeding cost, $/cow</td>
<td>66.1 ± 1.1</td>
<td>67.6 ± 1.3</td>
<td>1.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Pregnancy check cost, $/cow</td>
<td>15.1 ± 0.3</td>
<td>13.9 ± 0.3</td>
<td>-1.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Feed cost by 305 DIM, $/cow</td>
<td>1,660.6 ± 47.5</td>
<td>1,536.2 ± 48.1</td>
<td>-124.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Profit, $/cow</td>
<td>3,421.8 ± 103.0</td>
<td>2,888.9 ± 105.4</td>
<td>-532.3</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

aDiff = Difference between cows that developed metritis and cows that did not develop metritis;
*Interaction metritis with parity.
Figure 6-1. Kaplan-Meier survival curves for proportion of non-pregnant cows according to disease status: Metritis (n = 2624) and no metritis (n = 6388).
Figure 6-2. Kaplan-Meier survival curves for proportion of cows present in the herd according to disease status: Metritis (n = 2624) and no metritis (n = 6388).
CHAPTER 7
CONCLUSIONS

The studies in this dissertation report the association of DMI%BW and EB during the transition period with postpartum diseases, the use of DMI%BW as a predictor of disease postpartum, and the economic impact of metritis in the dairy farm. Dry matter intake was the missing link in the association with high metabolites such as NEFA and BHB, which indicated low energy balance, and postpartum diseases. Chapters 3, 4, and 5 of this dissertation showed the association of DMI%BW and EB in cows affected and not affected by calving disorders, metritis, ketosis, mastitis, digestive disorders, and lameness, which are the most frequent disorders that occur during the first weeks of postpartum. In addition, in Chapter 6 of this dissertation, it was evaluated the economic impact of metritis on dairy farms.

In Chapter 3, it was shown that DMI%BW and EB prepartum do not have an association with calving disorders and neither the average of DMI or EB in the last 3 days prepartum can be used to predict calving disorders. However, it is shown that during postpartum, cows that experienced calving disorders had less DMI%BW and ECM compared with cows that did not experience calving problems. These results suggest that cows that experienced calving disorders had signs of illnesses and developed other pathologies such as retained placenta and metritis that could have also impacted DMI and ECM. In addition, Chapter 3 also shows that cows that developed metritis had less prepartum DMI%BW and EB compared with cows that did not develop metritis and that for each unit decrease in these measurements during the last 3 days prepartum increased the odds of having metritis by 8%. In addition, it was shown that DMI%BW and EB have a predictor value although very modest. During the postpartum
period, DMI%BW, EB, and ECM were all lesser in cows that developed metritis compared with cows that did not develop metritis suggesting that the effect of the uterine infection impacted DMI%BW and EB and consequently also affected ECM.

In Chapter 4, it is shown that DMI%BW and EB prepartum are lesser in cows that developed ketosis compared with cows that did not develop ketosis and that these measures had predicted value, although modest. In addition, during postpartum, cows that developed ketosis had lesser DMI%BW and EB; however, they had increased ECM cows compared with cows that did not develop ketosis, and this association was mainly observed in primiparous cows. These results suggest that primiparous cows with greater milk potential mobilize more body tissue to cope with the nutrient needs for milk production and that cows with greater potential from milk production are more susceptible to develop ketosis than cows with less milk potential. In addition, in Chapter 4 it is also shown that cows that developed mastitis had lesser DMI%BW during prepartum compared with cows that did not develop mastitis and that prepartum DMI%BW and EB were predictors of mastitis although with very modest predictive values. During postpartum, cows that developed mastitis had lesser DMI%BW and ECM but greater EB suggesting that the low milk production caused by less functional quarters spared nutrients after DMI%BW recovered.

In Chapter 5, it is shown that cows that developed digestive disorders had lesser prepartum DMI%BW and EB and that these measures could predict digestive disorders postpartum although with modest predictive value. During postpartum, cows that developed digestive disorders had lesser DMI%BW, EB, and ECM. These results suggest that a low prepartum DMI%BW could predispose cows to digestive disorders.
and, after parturition, digestive disorders affect intake and consequently EB and ECM are affected. In addition, Chapter 5 shows that overconditioned cows that developed lameness had smaller prepartum DMI\%BW and EB and these measures could predict lameness in overconditioned cows. During postpartum, all cows that were lame had lesser DMI\%BW suggesting that after becoming lame, these cows were less able to walk and spend time in the feedbunk eating.

In Chapter 6, it is shown that metritis causes economic losses by affecting milk production, reproduction, and survival in the herd and that the cost of a case of metritis is $532.3.

In summary, DMI\%BW and EB prepartum were associated with most of the postpartum diseases evaluated and finding ways of maintaining intake during the prepartum period might decrease the incidence of these postpartum diseases. However, DMI\%BW and EB were not good predictors of postpartum diseases even when intake and disease were associated because of the low accuracy, i.e. inability to correctly identify cows that will have disease and those that will no have disease based on DMI\%BW. In addition, this dissertation quantified the economic impact of metritis which is one of the most prevalent diseases affecting dairy cows. Collectively, identifying ways of improving prepartum DMI\%BW will likely reduce the risk of diseases and the impact they have on the economics of dairy production.
Table A-1. Correlation matrix showing Spearman’s rho correlation coefficient and (p-values).

<table>
<thead>
<tr>
<th>Parity</th>
<th>¹BCS</th>
<th>²THI</th>
<th>³Dyst</th>
<th>⁴SB</th>
<th>Twin</th>
<th>⁵RP</th>
<th>⁶Met</th>
<th>⁷MF</th>
<th>⁸Ket</th>
<th>DA</th>
<th>Ind</th>
<th>⁹Mast</th>
<th>¹⁰Lame</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>1.00</td>
<td>0.07</td>
<td>0.13</td>
<td>-0.03</td>
<td>-0.01</td>
<td>0.05</td>
<td>0.06</td>
<td>0.04</td>
<td>0.13</td>
<td>0.27</td>
<td>0.12</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>BCS</td>
<td>0.07</td>
<td>1.00</td>
<td>-0.10</td>
<td>-0.05</td>
<td>-0.09</td>
<td>-0.02</td>
<td>-0.05</td>
<td>-0.02</td>
<td>0.10</td>
<td>0.15</td>
<td>0.001</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>THI</td>
<td>0.13</td>
<td>-0.10</td>
<td>1.00</td>
<td>0.10</td>
<td>0.12</td>
<td>-0.03</td>
<td>0.03</td>
<td>0.09</td>
<td>0.01</td>
<td>-0.10</td>
<td>-0.05</td>
<td>0.02</td>
<td>0.24</td>
</tr>
<tr>
<td>Dyst</td>
<td>-0.03</td>
<td>-0.05</td>
<td>0.10</td>
<td>1.00</td>
<td>0.25</td>
<td>0.002</td>
<td>0.07</td>
<td>0.15</td>
<td>0.12</td>
<td>-0.04</td>
<td>0.02</td>
<td>0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td>SB</td>
<td>-0.01</td>
<td>-0.09</td>
<td>0.12</td>
<td>0.25</td>
<td>1.00</td>
<td>0.13</td>
<td>0.02</td>
<td>0.20</td>
<td>0.09</td>
<td>-0.06</td>
<td>-0.01</td>
<td>0.03</td>
<td>-0.01</td>
</tr>
<tr>
<td>Twins</td>
<td>0.05</td>
<td>-0.02</td>
<td>-0.03</td>
<td>0.002</td>
<td>0.13</td>
<td>1.00</td>
<td>0.12</td>
<td>0.15</td>
<td>0.03</td>
<td>0.05</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>RP</td>
<td>0.06</td>
<td>-0.05</td>
<td>0.03</td>
<td>0.07</td>
<td>0.02</td>
<td>0.12</td>
<td>1.00</td>
<td>0.40</td>
<td>-0.01</td>
<td>0.14</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.07</td>
</tr>
<tr>
<td>Met</td>
<td>0.04</td>
<td>-0.02</td>
<td>0.09</td>
<td>0.15</td>
<td>0.20</td>
<td>0.15</td>
<td>0.40</td>
<td>1.00</td>
<td>0.13</td>
<td>0.18</td>
<td>-0.01</td>
<td>0.002</td>
<td>0.05</td>
</tr>
<tr>
<td>MF</td>
<td>0.13</td>
<td>0.10</td>
<td>0.01</td>
<td>0.12</td>
<td>0.09</td>
<td>0.03</td>
<td>-0.01</td>
<td>0.13</td>
<td>1.00</td>
<td>0.07</td>
<td>-0.05</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Ket</td>
<td>0.27</td>
<td>0.15</td>
<td>-0.10</td>
<td>-0.04</td>
<td>-0.06</td>
<td>0.05</td>
<td>0.14</td>
<td>0.18</td>
<td>0.07</td>
<td>1.00</td>
<td>0.27</td>
<td>0.09</td>
<td>-0.02</td>
</tr>
<tr>
<td>DA</td>
<td>0.12</td>
<td>0.001</td>
<td>-0.05</td>
<td>0.02</td>
<td>-0.01</td>
<td>0.02</td>
<td>0.12</td>
<td>-0.01</td>
<td>-0.05</td>
<td>0.27</td>
<td>1.00</td>
<td>0.12</td>
<td>-0.02</td>
</tr>
<tr>
<td>Ind</td>
<td>0.13</td>
<td>0.04</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>-0.01</td>
<td>0.002</td>
<td>0.01</td>
<td>0.09</td>
<td>0.12</td>
<td>1.00</td>
<td>0.13</td>
</tr>
<tr>
<td>Mast</td>
<td>0.15</td>
<td>0.02</td>
<td>0.24</td>
<td>-0.03</td>
<td>-0.01</td>
<td>0.02</td>
<td>-0.07</td>
<td>0.05</td>
<td>0.01</td>
<td>-0.02</td>
<td>-0.02</td>
<td>0.13</td>
<td>1.00</td>
</tr>
<tr>
<td>Lame</td>
<td>0.13</td>
<td>-0.04</td>
<td>0.02</td>
<td>-0.07</td>
<td>-0.03</td>
<td>0.002</td>
<td>0.03</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.12</td>
<td>0.13</td>
<td>0.06</td>
<td>0.10</td>
</tr>
</tbody>
</table>

¹THI = temperature – humidity index. ²BCS = body condition score. ³Dyst = dystocia. ⁴SB = stillbirth. ⁵RP = retained placenta. ⁶Met = metritis. ⁷MF = milk fever. ⁸Ket = ketosis. ⁹DA = displaced abomasum. ¹⁰Ind = indigestion (diarrhea, cecal dilatation, bloat). ¹¹Mast = mastitis. ¹²Lame = lameness.

APPENDIX A
SUPPLEMENTARY TABLES
Table A-2. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake with calving disorders and metritis according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P - value</td>
<td></td>
<td></td>
<td>P - value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Disorder</td>
<td>Day</td>
<td>Dis x D</td>
<td>Yes</td>
</tr>
<tr>
<td>aCDZ</td>
<td>10.5 ± 0.2</td>
<td>10.6 ± 0.1</td>
<td>0.59</td>
<td>&lt;0.01</td>
<td>0.67</td>
<td>15.1 ± 0.4</td>
</tr>
<tr>
<td>bMET</td>
<td>10.3 ± 0.2</td>
<td>10.7 ± 0.1</td>
<td>0.10</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>14.1 ± 0.3</td>
</tr>
</tbody>
</table>

aCDZ: Yes = cows developed calving disorders; No = cows did not develop calving disorder but could have developed other disorders.
bMET: Yes = cows developed metritis; No = cows did not develop metritis but could have developed other disorders.
cDay: Day relative to parturition.
dDis x D: interaction between disease or disorder and Day.
Table A-3. Association of pre-( -21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with calving disorders according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCDZ</td>
<td>bHealthy</td>
<td>P-value</td>
<td>aCDZ</td>
<td>bHealthy</td>
<td>P-value</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.4 ± 0.3</td>
<td>10.9 ± 0.2</td>
<td>0.08</td>
<td>&lt;0.01</td>
<td>0.44</td>
<td>15.2 ± 0.4</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.6 ± 0.04</td>
<td>1.7 ± 0.03</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.88</td>
<td>2.6 ± 0.06</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>2.5 ± 0.4</td>
<td>3.6 ± 0.3</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>0.77</td>
<td>-4.4 ± 0.6</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.5 ± 1.3</td>
<td>34.3 ± 1.0</td>
</tr>
</tbody>
</table>

aCDZ: developed calving disorders.
bHealthy: did not develop any disorder postpartum.
cDay: day relative to parturition. d CDZ x D: interaction between CDZ and Day.
Table A-4. Association between pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with metritis according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th></th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1MET</td>
<td>2Healthy</td>
<td>MET</td>
<td>3Day</td>
<td>4MET x D</td>
<td>MET</td>
<td>Healthy</td>
<td>P - value</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.2 ± 0.2</td>
<td>10.9 ± 0.2</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>14.1 ± 0.3</td>
<td>18.0 ± 1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.6 ± 0.04</td>
<td>1.7 ± 0.03</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>2.4 ± 0.05</td>
<td>3.0 ± 0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.7 ± 0.1</td>
<td>3.4 ± 0.3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>-5.3 ± 0.6</td>
<td>-3.0 ± 0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28.2 ± 1.0</td>
<td>34.0 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*MET*: developed metritis.
*Healthy*: did not developed any disorder postpartum.
*Day*: day relative to parturition.
*MET x D*: interaction between MET and Day.
Table A-5. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI) with ketosis and mastitis according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>aKet</td>
<td>10.3 ± 0.2</td>
<td>10.8 ± 0.1</td>
</tr>
<tr>
<td>bMast</td>
<td>10.3 ± 0.3</td>
<td>10.6 ± 0.1</td>
</tr>
</tbody>
</table>

aKet: Yes = cows developed ketosis; No = cows did not develop ketosis but could have developed other disorders.
bMast: Yes = cows developed mastitis; No = cows did not developed mastitis but could have developed other disorders.
cDay: Day relative to parturition.
dDis x D: interaction between disease or disorder and Day.
Table A-6. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with ketosis according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th>Postpartum</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aKet</td>
<td>Healthy</td>
<td>P - value</td>
<td>bDay</td>
<td>cKetxD</td>
<td>Ket</td>
<td>Healthy</td>
<td>Ket</td>
<td>Healthy</td>
<td>Ket</td>
<td>Day</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>11.1 ± 0.3</td>
<td>11.1 ± 0.2</td>
<td>0.89</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>15.3 ± 0.3</td>
<td>17.9 ± 0.3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.6 ± 0.03</td>
<td>1.8 ± 0.04</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>2.5 ± 0.04</td>
<td>3.0 ± 0.04</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>2.7 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>0.09</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>-8.7 ± 0.5</td>
<td>-3.6 ± 0.5</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36.4 ± 1.1</td>
<td>33.7 ± 0.9</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

aKet: developed ketosis postpartum.
bDay: day relative to parturition.
cKet x D: interaction between ketosis and Day.
Table A-7. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with mastitis according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th>P - value</th>
<th>Postpartum</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mastitis</td>
<td>Healthy</td>
<td>Mast</td>
<td>aDay</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.1 ± 0.3</td>
<td>10.8 ± 0.2</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.53 ± 0.05</td>
<td>1.72 ± 0.03</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.8 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

aDay: day relative to parturition.

bM x D: interaction between Lame and Day.
Table A-8. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with digestive disorders (DDZ) according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th></th>
<th>Postpartum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DDZ</td>
<td>Healthy</td>
<td>DDZ</td>
<td>Healthy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aDay</td>
<td>bDDZ x D</td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.2 ± 0.2</td>
<td>11.0 ± 0.2</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.6 ± 0.04</td>
<td>1.8 ± 0.03</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>2.0 ± 0.4</td>
<td>3.6 ± 0.3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*aDay: day relative to parturition.

bDDZ x D: interaction between digestive disorders and Day.
Table A-9. Association of pre- (-21 to -1 d) and postpartum (1 to 28 d) dry matter intake (DMI), DMI as percentage of BW (DMI%BW), energy balance (EB), and energy corrected milk (ECM) with lameness according to multivariable analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prepartum</th>
<th>Postpartum</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lame</td>
<td>Healthy</td>
<td>(^1)Lame (^2)Day (^3)LxD</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.0 ± 0.4</td>
<td>10.8 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>DMI%BW</td>
<td>1.47 ± 0.10</td>
<td>1.72 ± 0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EB, Mcal/d</td>
<td>1.1 ± 0.7</td>
<td>3.3 ± 0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\)Lame: developed lameness postpartum.
\(^2\)Day: day relative to parturition.
\(^3\)L x D: interaction between Lame and Day.
Figure B-1. Association of prepartum and postpartum dry matter intake (DMI, kg/d) with (A) calving disorders and (B) metritis. Values are least squares means +/- SEM. Prepartum DMI: calving disorders - $P = 0.59$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P = 0.67$. Postpartum DMI: calving disorders - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day $P < 0.01$. Prepartum DMI: metritis - $P = 0.10$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day $P = 0.08$. Postpartum DMI: metritis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day $P < 0.01$. 

APPENDIX B
SUPPLEMENTARY FIGURES
Figure B-2. Association of calving disorders and healthy cows with (A) prepartum and postpartum DMI (kg/d), (B) DMI as percentage of BW (DMI%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI: calving disorders - $P = 0.08$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P = 0.44$. Prepartum DMI%BW: calving disorders - $P = 0.05$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P = 0.88$. Prepartum EB: calving disorders - $P = 0.04$, day relative to parturition - $P < 0.01$, interaction between calving disorders and day - $P = 0.77$. Postpartum DMI: calving disorders - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day $P < 0.01$. Postpartum DMI%BW: calving disorders - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day $P < 0.01$. Postpartum EB: calving disorders - $P = 0.04$, day relative to parturition - $P < 0.01$, and the interaction calving disorders and day - $P < 0.01$. ECM: calving disorders - $P < 0.01$, day relative to parturition $P < 0.01$, and the interaction between calving disorders and day - $P < 0.01$. 


Figure B-3. Association of metritis and healthy cows with (A) DMI (kg/d), (B) DMI as percentage of BW (DMI%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI: metritis - *P* = 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01. Prepartum DMI%BW: metritis - *P* < 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01. Prepartum EB: metritis - *P* < 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01. Postpartum DMI: metritis - *P* < 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01. Postpartum DMI%BW: metritis - *P* < 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01. Postpartum EB: metritis - *P* < 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01. ECM: metritis - *P* < 0.01, day relative to parturition - *P* < 0.01, and the interaction between metritis and day *P* < 0.01.
Figure B-4. Association of prepartum and postpartum dry matter intake (DMI, kg/d) with (A) ketosis and (B) mastitis. Values are least squares means +/- SEM. Prepartum DMI: ketosis - $P = 0.96$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day - $P < 0.01$. Postpartum DMI: ketosis - $P < 0.01$, day relative to parturition - $P < 0.01$, and the interaction between calving disorders and day $P < 0.01$. Prepartum DMI: mastitis - $P = 0.22$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day $P < 0.01$. Postpartum DMI: mastitis - $P = 0.04$, day relative to parturition - $P < 0.01$, and the interaction between metritis and day $P < 0.01$. 
Figure B-5. Interaction ($P < 0.01$) between ketosis and parity on dry matter intake (kg/d) on (A) primigravid and (B) multigravid cows during the postpartum period (from 1 d to 28 d). Values are least squares means +/- SEM.
Figure B-6. Association of ketosis (n = 189) and healthy (n = 132) cows with (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI: ketosis - P = 0.89, day relative to parturition - P < 0.01, and the interaction between ketosis and day - P < 0.01. Prepartum DMI (%BW): ketosis - P < 0.01, day relative to parturition - P < 0.01, and the interaction between ketosis and day - P < 0.01. Prepartum EB: ketosis - P = 0.09, day relative to parturition - P < 0.01, and the interaction between ketosis and day - P < 0.01. Postpartum DMI: ketosis - P < 0.01, day relative to parturition - P < 0.01, and the interaction between ketosis and day - P < 0.01. Postpartum DMI (%BW): ketosis - P = 0.01, day relative to parturition - P < 0.01, and the interaction between ketosis and day - P < 0.01. Postpartum EB: ketosis - P = 0.01, day relative to parturition - P < 0.01, and the interaction between ketosis and day - P < 0.01. ECM: ketosis - P = 0.05, day relative to parturition - P < 0.01, and the interaction between ketosis and day P < 0.01.
Figure B-7. Interaction ($P < 0.01$) between ketosis and parity on dry matter intake (kg/d) and energy balance on (A, C; $P = 0.02$) primigravid and (B, D) multigravid cows during the prepartum period (from -21 d to -1 d). Values are least squares means +/- SEM.
Figure B-8. Interaction ($P < 0.01$) between ketosis and parity on energy corrected milk (kg/d) in (A) primigravid and (B) multigravid cows during the postpartum period (from 1 d to 28 d). Values are least squares means +/- SEM.
Figure B-9. Dry matter intake (% of BW) according to disease status related to ketosis during (A) prepartum (-21 to -1 d) and (B) postpartum period (from 1 d to 28 d). Values are least squares means +/- SEM. Prepartum: Ketosis plus other disorders (OD) (n = 132) vs. healthy (n = 132): 1.49 ± 0.04 vs. 1.73 ± 0.03; P < 0.01. Cows with only ketosis (n = 57) vs. healthy: 1.57 ± 0.04 vs. 1.73 ± 0.03; P < 0.01. Postpartum: cows with ketosis plus other disorders vs. healthy: 2.26 ± 0.05 vs. 2.99 ± 0.05; P < 0.01. Cows with only ketosis vs. healthy: 2.66 ± 0.07 vs. 2.99 ± 0.05; P = 0.01.
Figure B-10. Association between mastitis (n = 79) and healthy (n = 132) cows and (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI: mastitis (P = 0.04), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01). Prepartum DMI (%BW): mastitis (P < 0.01), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01). Prepartum EB: mastitis (P < 0.01), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01). Postpartum DMI: mastitis (P < 0.01), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01). Postpartum DMI (%BW): mastitis (P < 0.01), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01). Postpartum EB: mastitis (P = 0.59), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01). ECM: mastitis (P < 0.01), day relative to parturition (P < 0.01), and the interaction between mastitis and day (P < 0.01).
Figure B-11. Dry matter intake (% of BW) according to disease status related to mastitis during (A) prepartum (-21 to -1 d) and (B) postpartum period (from 1 d to 28 d). Values are least squares means +/- SEM. Prepartum: Mastitis plus other disorders (OD) (n = 68) vs. healthy (n = 132): 1.53 ± 0.05 vs. 1.73 ± 0.03; P < 0.01. Cows with only mastitis (n = 17) vs. healthy: 1.48 ± 0.08 vs. 1.73 ± 0.03; P < 0.01. Postpartum: cows with mastitis plus other disorders vs. healthy: 2.43 ± 0.07 vs. 2.99 ± 0.05; P < 0.01. Cows with only mastitis vs. healthy: 2.46 ± 0.16 vs. 2.99 ± 0.05; P < 0.01.
Figure B-12. Association of digestive disorder (n = 120) and healthy cows (n = 132) with (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI: digestive disorder \( P = 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P < 0.01 \). Prepartum DMI (%BW): digestive disorder \( P < 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P < 0.01 \). Prepartum EB: digestive disorder \( P < 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P < 0.01 \). Postpartum DMI: digestive disorder \( P = 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P < 0.01 \). Postpartum DMI (%BW): digestive disorder \( P = 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P < 0.01 \). Postpartum EB: digestive disorder \( P < 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P = 0.03 \). ECM: digestive disorder \( P < 0.01 \), day relative to parturition \( P < 0.01 \), and the interaction between digestive disorder and day \( P < 0.01 \).
Figure B-13. Association between lameness (n = 35) and healthy (n = 132) cows and (A) prepartum and postpartum dry matter intake (DMI, kg/d), (B) DMI (%BW), (C) energy balance (EB, Mcal/d) during the transition period (from -21 d to 28 d); and (D) energy corrected milk (ECM, kg/d) during the first 28 d postpartum. Values are least squares means +/- SEM. Prepartum DMI: lameness (P = 0.81), day relative to parturition (P < 0.01), and the interaction between lameness and day (P < 0.01). Prepartum DMI (%BW): lameness (P = 0.71), day relative to parturition (P < 0.01), and the interaction between lameness and day (P = 0.08). Prepartum EB: lameness (P = 0.84), day relative to parturition (P < 0.01), and the interaction between lameness and day (P < 0.01). Postpartum DMI: lameness (P < 0.01), day relative to parturition (P < 0.01), and the interaction between lameness and day (P < 0.01). Postpartum DMI (%BW): lameness (P < 0.01), day relative to parturition (P < 0.01), and the interaction between lameness and day (P < 0.01). Postpartum EB: lameness (P < 0.01), day relative to parturition (P < 0.01), and the interaction between lameness and day (P < 0.01). ECM: lameness (P = 0.30), day relative to parturition (P < 0.01), and the interaction between lameness and day (P < 0.01).
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BIOGRAPHICAL SKETCH

Johanny Maribel Pérez Báez was born in Santo Domingo, the capital of Dominican Republic in 1982. She is the third of four siblings. Her mother and father were born in rural areas from the north part of Dominican Republic. Johanny pursue her degree in Veterinary Medicine in the Universidad Autónoma de Santo Domingo (UASD) in 2005. On 2009 she joined UASD staff working in two research studies done by this university. During the development of these studies she received several trainings in reproduction biotechnologies in the Virginia-Maryland College of Veterinary Medicine. On 2011 she started to work as a lecturer and as a teacher assistant in reproduction courses in the Veterinary School of UASD. Working in UASD has taught Johanny how to work with students and have given her experience in projects development. On 2012, Johanny was accepted in the Fulbright program and in 2013 as a student at the College of Veterinary Medicine of University of Florida where she completed her Master of Science. In 2015 was awarded a fellowship the College of Veterinary Medicine of University of Florida to pursue her doctoral degree under the supervision of Dr. Klibs Galvão.