HEMATOLOGICAL PROFILES ASSOCIATED WITH PRODUCTIVE PERFORMANCE AND ESTRUS STATE IN GRASS-FED BEEF HEIFERS

by

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ABSTRACT

Feeding expenses and reproductive success are major drivers impacting profitability in the cow-calf sector. Similarities between feed efficient phenotypes and estrus state may be supported by markers of energetic shifts. Hematological measures associated with energy metabolism were investigated as proxies of feed efficiency and estrus in forage-fed beef heifers. Experiment 1 evaluated blood cell parameters, immunoglobulins and metabolites in relation to feed efficiency and heifer physiological state; Experiment 2 assessed metabolites to serve as proxies for estrus. Efficient heifers had greater lymphocytes, immunoglobulin M response, and lower alkaline phosphatase (ALP) concentrations. Efficient pregnant heifers had lower concentrations of cholesterol and globulin. Estrus state was strongly associated with fluctuations of ALP, aspartate aminotransferase, beta-hydroxybutyric acid, creatine kinase and triiodothyronine concentrations. However, age, body size and composition may influence such associations. There is potential for hematological parameters to serve as proxies of metabolic shifts related to feed efficiency and estrus state.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ADG	Average daily gain
ADP	Adenosine diphosphate
AG	Albumin globulin ratio
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUS	Australia
BHBA	Beta-hydroxybutyric acid
BKFT	Average back fat
BKT	Average back fat
BW	Average body weight
Ca	Calcium
CBC	Complete blood cell count
CF	Correction factor
CK	Creatine kinase
cl	Critical limit
CL	Corpus luteum
cm	Centimeter
cm^2	Centimeters squared
Co	Cobalt
CO_2	Carbon dioxide
CPEC	Cattle Performance Enhancement Company
Cu	Copper
CYL	Cattlemen Young Leaders
DIG	Days in gestation
dL	Deciliter
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization, United Nations
Fe	Iron
FG	Feed to gain ratio
fL	Femtolitre
Fl	Fluoride
FSH	Follicle stimulating hormone
g	Gram
GGT	Gamma glutamyl transferase

GHG	Greenhouse gas emissions
GLDH	Glutamate dehydrogenase
GLM	General linear model
GnRH	Gonadotropin releasing hormone
Ig	Immunoglobulin
IgG1	Immunoglobulin G1
IgM	Immunoglobulin M
IU	International units
K^+	Potassium ion
kg	Kilogram
L	Liter
LH	Luteinizing hormone
m	Meter
MAB	Average marbling
MARB	Average marbling
Mcal	Megacalorie
MCH	Mean cell hemoglobin
MCV	Mean corpuscular value
mg	Milligram
Mg	Magnesium
MHz	Megahertz
mL	Milliliter
mm	Millimeter
mmol/L	Millimole per liter
Mn	Manganese
Na^+	Sodium ion
Na^+/K^+ ATPase	Sodium-potassium adenosine triphophatase
NEFA	Non-esterified fatty acid
ng/ml	Nanograms per mililiter
nmol/L	Nanomoles per liter
NRC	Nutrient Requirements of beef Cattle
OD	Optical density
OVA	Ovalbumin
OSM	Calculated osmolality
PBS	Phosphate buffered saline
pg	Picogram
PG	Prostaglandin F2α
R^2	Coefficient of determination
RBC	Red blood cell count
REA	Average rib eye area
RFI	Residual feed intake

RIA	Radioimmunoassay
RMP	Average rump fat
RUMP	Average rump fat
SAS	Statistical Analysis Software
s.d.	Standard deviation
SD	Standard deviation
SNP	Single nucleotide polymorphism
T3	Triiodothyronine
T4	Thyroxine
TP	Total protein
TPV	Total plasma volume
UK	United Kingdom
U/L	Units per liter
USA	United States of America
USDA	United States Department of Agriculture
WBC	White blood cell count
Zn	Zinc
50K	50,000
°C	Degrees Celsius
%	Percent
µl	Microliter
µmol/L	Micromole per liter

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Chapter 1

INTRODUCTION

The beef industry is an important component of the Canadian economy, contributing to the gross domestic product and labor demand (Canfax, 2011). The industry is made up of a wide network of producers who operate cow-calf, background and finishing operations and numerous abattoirs and packaging plants that contribute to the production of a diversity of products (Field 2007), including the 23.4 kg of beef that each Canadian consumes yearly (Statistics Canada 2010). The Canadian beef industry has been working towards the goal of ensuring the economic, environmental and societal sustainability of the industry. Among other traits, improvements in feed efficiency is an important component of achieving this goal. Moreover, to remain profitable in the cow-calf sector, breeding females must raise a calf every year and remain on a yearly calving interval (Short and Bellows 1971). Improvements in assess feeding efficiency and estrus detection will contribute to the improvement of management practices in the beef industry, specifically within the cow-calf sector as researched in this thesis.

Feed costs are a large expense to beef producers, which can be reduced by improvements made in feed efficiency. Improvement in the efficiency of feed utilization in the cow herd may lead to an overall improvement of feed efficiency across all sectors, since this trait is heritable (Pitchford 2004). Reproduction is also important in defining profitability in the cow-calf sector. For lifetime reproductive success, heifers should be cycling prior to the breeding season and conceive again following the

first calf (Fields and Sand 1994). Reproductive losses in the beef industry have been attributed to issues such as failure to identify the estrus state (Roelofs *et al.* 2015), along with nutritional anestrus, low conception rates and neonatal losses (Short and Bellows 1971).

Biomarkers associated with physiological processes can be used to make inferences about the metabolic state of the animal (Richardson and Herd 2004). Positive relationships between feed inefficiency and blood cell measures, such as mean corpuscular volume and mean cell hemoglobin have been associated with greater energy metabolism in steers (Richardson *et al.* 2002). Superior adaptive immune function may be related to improved overall productive performance (Dhurandhar *et al.* 2000; Magnusson *et al.* 1999). Greater workload of visceral organs, such as in the liver have been associated with differences in feed efficiency in heifers. Some relationships observed have been between greater plasma liver enzyme concentrations of aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH; Gonano *et al.* 2014).

Physiological states including estrus and pregnancy are associated with metabolic shifts which can affect energetic efficiency (Parker *et al.* 2001; Bauman and Currie 1980). The increasing use of reproductive technologies in the beef industry requires successful estrus detection strategies, which may be complemented by using hematological profiling. Blood serum ions, including sodium, have been positively associated during the estrus day with conception rates in heifers (Small *et al.* 1996). Enzymes such as creatine kinase (CK) are associated with energy metabolism in cattle (Schlattner *et al.* 2006) and strenuous muscle activity in beef heifers during estrus

(Kenny and Tarrant 1988). Metabolic hormones, such as triiodothyronine (T3) have profound effects in the regulation of changes in metabolism (Villanueva *et al.* 2013). In addition, female body composition has strong associations with reproductive traits including the estrus state (Short and Bellows 1971). Biomarkers associated with adipose and muscle tissue metabolism are linked to shifts in energy metabolism during estrus (Parker *et al.* 2001).

The objectives of this thesis were to 1) evaluate complete blood count (CBC) parameters, IgG1 and IgM responses and blood plasma metabolic profiles relative to feed efficiency classification in forage-fed replacement beef heifer calves and pregnant beef heifers; 2) characterize blood plasma analytes in beef heifers during estrus and non-estrus states; and 3) compare the relationship between metabolic profile during estrus with age, feed efficiency, body size, and body composition.

Chapter 2

LITERATURE REVIEW

1. Introduction

1.1. The Canadian Beef Industry

1.1.1. Beef industry and the Canadian economy

The Canadian beef industry is an economic driver that contributes to the gross domestic product and provides income for over 200,000 Canadians (Canfax, 2011). Canada accounts for approximately 2% of international beef production and it is largely integrated into the global market. In 2014, 35% of the domestic beef production was exported to countries such as the United States, Hong Kong, Mexico, China and Japan while 26% of the beef consumed was imported mainly from the United States (Canfax 2015). In 2014, Canada exported over $1.7 billion of beef to the global market (Canfax 2014). On average the Canadian beef industry annually contributes $33 billion, directly and indirectly, to the Canadian economy (Canfax 2015) and compete with other meat supply chains, such as pork and chicken, which contributes $9.28 billion and $6.5 billion to the economy, respectively.

Provincially, the western provinces account for a significant portion of the national cattle herd which also encompasses a significant portion of the feedlot industry. In the Maritime Provinces, the majority of producers operate forage-based cow-calf operations, which provide calves to feedlots outside the region. In 2015, the Maritime beef industry produced $27 million in farm cash receipts to the local economy, making beef production a moderate contributor to the local agriculture sector

in comparison to sectors such as dairy ($305 million) and egg production ($72 million; Statistics Canada, 2015).

1.2. Environmental aspects related to the beef industry

A common perception of the general public is that current beef production has a greater environmental footprint than the beef production systems during the green revolution (Capper 2011). In general, agricultural practices play an important role in environmental issues, such as climate change, land degradation, water pollution, biodiversity loss and waste production (Gerber *et al.* 2013). A comprehensive report concluded that livestock production contributed 18% of all greenhouse gas (GHG) emissions caused by human activity (FAO 2006). In contrast to this study, Pitesky *et al.* (2009) determined GHG by the beef industry contributed 2.6% of all GHG emissions. A comparison of GHG emissions by Canadian beef production between 1981 and 2011 indicated 14.0% lower CO_2 emissions and significantly lower GHG over the last three decades (Legesse *et al.* 2016). Genetic and feed quality improvements have contributed indirectly to minimize GHG emissions (Capper 2011). In fact, these improvements are also linked to the advances in herd management, health, extension services and general husbandry practices that continue to contribute to optimize the efficiency of the beef cattle production systems (Field 2007).

With the growing world population it is predicted that an increase in the middle class will escalate the demand for meat production (FAO 2006). To increase beef production in a sustainable manner, it must make use of the current available land, water and nutrients, while also reducing waste and GHG (Gerber *et al.* 2013). One

suggested method to improve the use of natural resources in beef production is through the use of permanent pastures and grasslands which through effective management, can reduce inputs of mineral fertilization, grain and silage and improve biodiversity (Dumont *et al.* 2013). As detailed by de Haan *et al.* (1997), grazing can be visualized as cows in lush pastures in harmony with nature. Livestock can improve soil, and vegetation cover as well as plant and animal biodiversity. Grazing animals, such as cattle, can improve plant species composition by: removing biomass, which otherwise shades out plants and might provide the fuel for bush fires; controlling shrub growth and; dispersing seeds through their hoofs and manure. In addition, trampling can stimulate grass tilling, improve seed germination and break up hard soil crusts (Savory and Parsons 1980). The benefits of forage based systems are also sustained from the GHG emissions stand point. A life cycle assessment modelling investigation supports substantial reductions in GHG in forage based production systems (Pelletier *et al.* 2010).

1.2.1. Social aspects related to the beef industry

By the year 2050, the world population will have reached 9 billion people that, along with rising incomes and urbanization, will provide a challenge to agricultural systems (Gerber *et al.* 2013). Of the current 5 billion people living in developing countries, 4 billion live in rural settings where they have a lifestyle close to the land, with the ability to produce their own food (Hodges 2005). Within these societies, urban dwellers spend up to 90% of income on food versus 10% in Canada (Statistics Canada 2014). As the world population increases, a sustainable world food system must ensure

that people living in rural areas do not become hungry and make better use of their available resources (Hodges 2005). Considering that 45% of the world population is under the age of 24 years, there is an opportunity to support young farmers and to educate this generation about the importance of sustainable agriculture. Programs such as the youth development program 4-H, have widespread organizations across the globe to serve as a platform to educate the next generation of farmers and consumers (National 4-H Council 2015). In Canada, the number of beef farms has decreased and the average age of operators has increased to 55 years or older (Statistics Canada 2011). Encouraging the younger generation to enter into the beef sector could improve quality of life, provide income and contribute to the sustainability and resilience of the Canadian beef industry.

1.2.2. The Canadian and Maritime beef herd

The Canadian beef industry consists 68,500 beef farms managing various production systems including cow-calf, background and feedlot. By region, the industry breaks down to 85.5% in the west, 13.2% centrally, and 1.3% on the east coast (Statistics Canada 2016). The cow-calf sector is comprised of 3.8 million beef cows (Statistics Canada 2016) and is responsible for the production of both feeder cattle and breeding stock (Field 2007). In Canada, there are approximately 1 million animals in backgrounding operations (Statistics Canada 2014), which purchase calves from cow-calf producers after weaning and prepare these animals to enter a finishing operation, by feeding mainly roughage-based diets (Schmitz *et al.* 2003). The Canadian finishing cattle sector contains 2 million cattle (Statistics Canada 2016) purchased from

background or cow-calf operations; they are raised using high energy diets until they have reached market size (Field 2007). There are 3.8 million beef cows and 531,000 replacement heifers in Canada.

Since 2007, there has been a reduction in cattle inventories in the Maritime Provinces, by the end of 2007 the Maritime cattle population consisted of 63,700 cows and 8,100 replacement heifers and as of January 1, 2016, there were 42,300 cows and 6,200 replacement heifers in the Maritime Provinces (Statistics Canada, 2014). The Maritime beef industry is largely comprised of cow-calf operations that maintain replacement heifers and market feeder calves to other provinces, including Quebec, Ontario and Manitoba. Cow populations by province include: New Brunswick (15,600), Nova Scotia (17,400) and Prince Edward Island (9,300). Practices to increase profits within the cow-calf sector could be beneficial to both the Maritime and Canadian beef industry, as the effects would be felt throughout the entire production system. One area with potential for improvement is the reduction of feed costs associated with beef production.

1.2.3. Feed efficiency and beef industry

Feed costs are a large expense to beef producers, accounting for 70% of total production expenses (Herd *et al.* 1998). Approximately 65 to 85% of feed costs can be attributed to the cow-calf herd (Gregory 1972; Montano-Bermudez *et al.* 1990), with 70% of the consumed energy used to supply background maintenance requirements (Hill 2012). A decrease in such requirements, through improvement of feed efficiency, will be beneficial to increase the profitability and competitiveness of the beef industry

(Herd and Arthur, 2008). Improvements in feed efficiency can be accomplished through management practices, genetic selection and dietary manipulation. The indirect characterization of feed efficient phenotypes is of great assistance for all these strategies. It has been suggested that a 5% improvement in feed efficiency could be equivalent to reducing diet costs on a dry matter basis by $8 per ton (Field and Taylor, 2003), which would reduce input costs and environmental footprint (Hegarty *et al.* 2007).

Improvements in feed efficiency have been accomplished using various management practices, including progress in cattle husbandry. It has been suggested that factors, such as bedding depth can have a positive impact on average daily gain in growing cattle. Feeder cattle kept in cold, wet conditions result in nearly double the cost of gain per day than feeders kept in dry conditions (Mader 2011). Better handling practices can improve daily gains, feed efficiency and greater margins of profit in the livestock production sector (Belk *et al.* 2002). Feedlot managers reported that as a consequence of reduced electric prod usage in feedlots and quieter handling encouraged cattle to resume feed intake and reduced death loss due to respiratory sickness (Grandin 1998).

Growth promotants, such as ionophores, hormonal implants and beta adrenergic agonists, have been used to improve feed efficiency (Goodrich *et al.* 1984; Trenkle 1976). Ionophores are antimicrobials that improve feed efficiency, reduce methane production and the incidence of bloat and acidosis (Field 2007). Utilizing ionophores in heifers can reduce the age of puberty, which may have positive effects on lifetime productivity (Purvis *et al.* 1996). Hormonal implants, such as estrogenic

compounds, boost the deposition of protein, while reducing the deposition of fat. Implanted heifers have yielded a higher percentage of dark cutters, when implanted a second time with an estrogenic implant (Scanga *et al.* 1998). Beta adrenergic agonists promote muscle tissue growth by redirecting blood carrying nutrients towards muscle tissue and away from digestive organs and have been shown to reduce protein turnover (Field 2007). However, slight increases in mortality has been observed in feeders with certain types of beta adrenergic agonists (Loneragan *et al.* 2014). It has been estimated that the improved performance can provide an extra return of up to $10 for each dollar spent for the growth implant (Gould 2007). Although these technologies have been shown to increase beef industry profits, these practices have received considerable criticism, which has resulted in lost markets, creating a need to identify alternative methods to improve feed efficiency.

2. Feed efficiency historical perspective

2.1. Measures of feed efficiency

Energetic efficiency is the ratio of the output energy, such as performance traits, to the given form of energy (Brody 1945). Measuring efficiency on an individual animal basis within a production system is a challenging task. In the past, comparisons made between animals were accomplished by determining the feed intake and production outputs of the entire group over the complete production cycle, which had limited application for livestock species with more heterogeneous animal types, as is commonly observed in the beef industry. A number of measures of feed efficiency have been utilized in cattle including: gross efficiency (VeerKamp *et al.* 1998), maintenance

efficiency, partial efficiency of growth (Archer *et al.* 1999) and residual feed intake (Koch *et al.* 1963; Montanholi *et al.* 2009). Gross efficiency is calculated as a ratio of inputs (feed) to outputs (growth or weight gain) over a certain length of time (Kleiber 1961). This measure of efficiency favors animals with the potential for high growth rates and high mature body weights (Blaxter 1962), since selection can produce animals with larger ADG and increase feed costs. Maintenance efficiency is the ratio of body weight to feed intake to have zero weight change and is not a practical indicator of feed efficiency in growing cattle (Ferrell and Jenkins 1985). Partial efficiency of growth is the ratio of weight gain to feed after the expected maintenance requirements have been removed; however, this measurement assumes there is no variation in the efficiency of feed use for maintenance (Archer *et al.* 1999). There are practical limitations to determining any measure of feed efficiency, since the assessment can be laborious, time consuming (Herd *et al.* 2003), and expensive due to the equipment and labor required to evaluate individual feed intake, body weight (Moore *et al.* 2009) and body composition (Montanholi *et al.* 2010).

2.2. Residual feed intake

A measure of feed efficiency that has been widely discussed (i.e. Arthur *et al.* 2005; Cartsens *et al.* 2006; Montanholi *et al.* 2009) and to some extent applied in the beef industry (i.e. Angus Sire Benchmarking Project, Australian Angus Association, Armidale, AUS; USDA Feed Efficiency in Beef Cattle, USDA, Ames, USA), is residual feed intake (RFI). This trait represents the residual portion of feed intake that deviates from the expected level of feed intake on an individual animal basis, based on

the difference between actual daily feed intake and expected feed intake adjusted for body weight, growth, (Koch *et al.* 1963) and body composition (Montanholi *et al.* 2009), with efficient animals having a negative RFI.

Since RFI in general, is considered to be independent of level of production (Richardson *et al.* 2001), it also represents an interesting trait for studying the biological factors associated with feed efficiency. A few examples of biological phenomena related to RFI that may contribute to the indirect assessment of RFI include: hepatic mitochondrial function (Lancaster *et al.* 2014), body heat dissipation (Montanholi *et al.* 2010), blood plasma metabolites (Gonano *et al.* 2014), feeding behavior (Kelly *et al.* 2010) and visceral organs metabolism (Wang *et al.* 2009). Additionally, RFI is important for monitoring responses to selection for improved feed efficiency, such as those related to reproduction (Fontoura *et al.* 2016), milk composition (Montanholi *et al.* 2013a) and meat quality (Gomes *et al.* 2012).

3. Sources of variation on energy metabolism

3.1. Effect of physiological state

The biological processes involved in maintenance and growth are energetically and structurally varied, in response to metabolic demand (Kleiber 1961) Basal metabolism is the minimum energy necessary for mechanisms such as circulation, respiration, excretion and muscle work, which require constant effort even at rest (Brody 1945). According to Baldwin *et al.* (1980), it has been estimated 35 to 50% of the basal energy metabolism in cattle can be accounted for by service functions, including kidney work, heart work, respiration, integrative nerve functions and liver

functions. The remaining 45 to 50% account for the cellular functions such as protein synthesis, lipid synthesis and ion transport.

In growing animals, there is an additional energetic cost to support the work of growth and development. According to Short and Bellows (1971), stages of development including puberty onset, estrus and pregnancy rates are greatly influenced by the level of dietary energy. These authors observed that heifers fed low, medium and high levels of dietary energy reached puberty at 433, 411 and 388 days of age, respectively. Prior to breeding season, the percentage of heifers in estrus was 83, 34 and 7% for the heifers receiving the high, medium and low energy diets, respectively. The pregnancy rates were also affected, with 63% of the heifers in the low energy diet, compared to 90% of the heifers bred in the higher plane of nutrition following the breeding season.

During the estrus cycle and pregnancy, the ovarian steroids, estradiol and progesterone, induce coordinated changes in the procurement, ingestion, metabolism, storage, and expenditure of metabolic fuels (Wade and Schneider 1992). During estrus, estradiol can act in the brain to alter regulatory behaviors, such as feed intake and voluntary exercise (Fields and Sand 1994). The shift in energy metabolism during estrus is the result of ovarian hormones acting on peripheral tissues, such as adipose tissue, muscle, and liver, influencing metabolism, partitioning and storage of metabolic fuels (Wade and Schneider 1992). Consequently, female metabolism varies according to the stage of the estrus cycle. In women, a 9% elevation in 24 hours energy expenditure in the postovulatory luteal phase of the menstrual cycle, is thought to be caused by the increased secretion of progesterone (Webb 1986).

In the beef cow, maintenance requirements are of particular importance as maintenance energy costs represent approximately 70 to 75 % of the total annual energy requirements (Ferrell and Jenkins 1985). It has been suggested that increased metabolizable energy consumption during gestation is used by maternal tissues involved in service functions (Ferrell 1991). Nutrient requirements of a pregnant cow at the end of gestation are estimated to be approximately 75% greater than those in a non-pregnant cow of similar weight (Bauman and Currie 1980). Partitioning of nutrients for pregnancy falls into two types of regulation: homeostasis; which is responsible for maintaining equilibrium within the body and; homeorhesis, the coordinated control of metabolism throughout tissues used to support a physiological process, such as pregnancy or lactation (Bauman and Currie 1980). Due to the metabolic shifts during stages of gestation, the measurement of RFI in pregnant females can be challenging, and require the modelling of the gestation and fetus development stages through indirect measures (Wood *et al.* 2014).

3.2. Effect of body composition and Genetics

The determination of body composition is ideally assessed by determining chemical composition of the ground, empty body (Blaxter 1962). Measuring body composition is critical in the assessment of feed efficiency to understand the underlying biological differences in energy metabolism associated with differences in tissue deposition. For example, the accretion of lean tissue requires additional energy expenditure due to the continual and rapid protein turnover, in comparison to fat tissue deposition (Lobley *et al.* 1990). Based on the efficiency of energy expenditure, fat

accretion has shown greater efficiency and lower heat loss than protein accretion (Owens *et al.* 1995). Determining feed efficiency without accounting for body composition (Koch *et al.* 1963) can have adverse consequences. The determination of feed efficiency on feed intake and ADG alone can result in carcasses with variations in fatness, as shown by steers whose parents were selected for improved feed efficiency (based on RFI) and indicated lean body compositions (Richardson and Herd 2004).

Some cattle breeds, such as the Holstein, have greater abdominal fat deposition and less carcass fat (Dolezal *et al.* 1993). Cattle breeds have been selected partly based on their ability to adapt to different nutritional, environmental, and production systems (Solis *et al.* 1987). Feedlot cattle with various breed compositions have also been reported to have differences in carcass traits and body gain composition (Parish *et al.* 2014). Residual feed intake is considered a heritable trait, with values ranging from 0.25 to 0.45 (Pitchford 2004). Combining phenotypic and genotypic information from purebred breeds is a potential way to increase the size of the reference population and improve the accuracies of genomic estimated breeding values. These inferences can be used to develop genomic predictions for RFI in animals that are genotyped, but do not have phenotypic data (Meuwissen *et al.* 2001).

3.3. Effects of plane of nutrition

Differences in plane of nutrition alter energy metabolism and organ workload (Ramsey 2006). Visceral organs are a major contributor to whole-body energy expenditure and have been shown to differ in size, according to plane of nutrition and workload, such as organ size in feed restricted steers (Sainz and Bentley 1997). Feed

intake level is directly related to both protein synthesis and degradation, with protein synthesis having more intense responses than degradation (McBride and Kelly 1990). Organs differ greatly in their contribution to whole body protein synthesis, especially when the relative contribution to the body protein mass is taken into consideration. For example, gut and liver together comprise 7 to 9% of the body protein mass and account for more than 30% of the whole body protein synthesis, which represents a higher rate of synthesis per gram of tissue than that observed in muscle (Lobley 2003). Plane of nutrition has been positively associated with fractional muscle synthesis rate and negatively associated with fractional muscle breakdown rate in beef steers (Lobley *et al.* 2000). Changes in the energy expenditure of visceral organs, including the liver and gastrointestinal tract, have accounted for an estimated 5% of the variation in RFI (Richardson and Herd 2004). Plane of nutrition has been shown to influence intermediary metabolism. For example, feed withdrawal effects in lactating dairy cows mirrored the hepatic activities experienced during spontaneous ketosis (Baird *et al.* 1972).

3.4. Immune system and metabolism

The maintenance of the immune system is metabolically demanding, requiring shifts in nutrient and energy allocation from growth, reproduction, and other metabolic sinks (Sheldon and Verhulst 1996). For instance, response to an infection can reduce protein accretion in muscle as amino acids are mobilized to supply energy demands, resulting in decreased productive performance (Klassing 1988). The maintenance of a fully functional immune system comes with metabolic cost (Owens and Wilson 2000).

In fact, the selection for superior adaptive immune systems has been shown to improve productive performance in other species, including poultry (Dhurandhar *et al.* 2000) and swine (Magnusson *et al.* 1999). Adaptive immunity functions in a series of steps that take place following a cell-mediated or antibody-mediated immune response (Thrall 2004). The antibody-mediated immune response can be measured against a specific antigen, making an antibody response an appropriate means to measure an immune response.

Immunoglobulins (Ig) M and G1 are primary responders of the humoral immune system in ruminants and are produced in the lymphoid tissues (Thrall 2004). Adaptive immunity requires repetitive prompting to improve the capacity of immune response (Lawman *et al.* 1986). In a typical primary immune response, IgM is the first isotype produced and this is followed by production of IgG, as a result of immunoglobulin class-switching (Jain 1993). In cattle, the secondary response to an antigen, the production of IgM, tends to be equal or lower than during the primary response, whereas the production of IgG1 tends to have a greater response (Mahon *et al.* 2015).

4. Indirect assessments of feed efficiency

4.1. Blood components

Blood plasma serves as an appropriate medium to assess measures associated with metabolic processes, since the systemic circulation of blood throughout the entire body comes into contact with various tissues and it is the location of several biochemical reactions related to energy metabolism (Jones and Allison 2007). In

addition, these assessments can provide an indication of the health status of an animal, which influences metabolic rate and may mask the indirect assessment of feed efficiency or other types of biological phenomena. Plasma metabolites, including metabolic ions, metabolites, enzymes, and hormones, can be associated with energetically demanding functions of organ systems that influence efficiency of feed utilization (Blaxter 1962). In addition to metabolic processes, plasma metabolites may be related to differences in physiological state including growth, estrus and pregnancy, which have been shown to influence metabolite concentrations in females (Doornenbal *et al.* 1988; Small *et al.* 1996; Gonano *et al.* 2014).

4.1.1. Blood cells and other components

The complete blood cell count (CBC) is an assessment routinely used to evaluate health in ruminants (Jones and Allison 2007). The erythron assesses red blood cell parameters, including red blood cell count, hemoglobin, mean corpuscular volume, and mean cell hemoglobin. The leukon assessment includes white blood cell counts (segmented neutrophils, lymphocytes and monocytes), while platelet content relates to blood coagulation properties.

The CBC analysis has been evaluated as a potential indirect assessment of feed efficiency in cattle and sheep (Richardson *et al.* 2002; Rincon-Delgado *et al.* 2011). Positive relationships between feed inefficiency and blood cell measures, including mean corpuscular volume and mean cell hemoglobin have been observed in steers (Richardson *et al.* 2002). These associations have also been noted in other species, with higher concentrations of red and white blood cells and reduced mean corpuscular

volume in feed inefficient ewes (Rincon-Delgado *et al.* 2011). Parameters, such as red blood cell indices, could be related to physiological functions, including oxygen consumption and transport, and may differ as a result of changes in metabolic rate (Riedesel and Engen 2015).

4.1.2. Specific immune response

Specific immune response has been shown to be affected by physiological state. From as early as birth, development of gut microbial flora has been reported to be essential for the development of gut function and antibody production that provide a degree of protection against disease (Cebra 1999). Strategies used during the weaning process have impacts on the immune responsiveness of weaning calves, particularly the timing and the excess stressors involved during the weaning process (MacKenzie *et al.* 1997). Age and pregnancy have been shown to affect adaptive immune response in pregnant heifers (Hine *et al.* 2011), although, no relationships were observed with RFI (Lawrence *et al.* 2011). The responsiveness of the immune system can be objectively evaluated in cattle by measuring the specific immunoglobulin response to unfamiliar proteins, such as ovalbumin (OVA) (Cartwright *et al.* 2011; Wagter *et al.* 1999). Challenging an immune response using OVA has proven to be effective for the study of health traits in dairy cows (Mallard *et al.* 1997).

4.1.3. Blood plasma metabolites

Identification of physiological markers, as a means of selection for feed efficiency, may be used as a phenotypic indicator in combination with direct feed

efficiency measurement, or potentially used as a pre-test screen to identify animals for feed efficiency testing. Concentrations of blood plasma metabolic enzymes, including alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase, creatine kinase, glutamate dehydrogenase; compounds including albumin, beta-hydroxybutyric acid, carbon dioxide, cholesterol, creatinine, globulin, glucose, haptoglobin, non-esterified fatty acid, urea; ions including calcium, phosphorus, magnesium, sodium, potassium, chloride and anion gap and; hormone triiodothyronine, may be associated with metabolic processes associated with energy metabolism.

4.1.3.1. Triiodothyronine

Triiodothyronine (T3) is produced by the conversion of thyroxine (T4) to its bioactive form T3 by 5'- deiodinase in the thyroid gland and other tissues (Moreno *et al.* 2008). The correlation between circulating thyroid hormone concentrations and energy balance with increased energy metabolism is well known in cattle (Rauw 1998). Triiodothyronine increases energy expenditure by reducing metabolic efficiency by inactivating the mitochondrial oxidative phosphorylation pathway (de Lange *et al.* 2001). Lower concentration of T3 has been associated with lower feed intake in steers (Christopherson *et al.* 1979) and to greater feed efficiency in beef cows (Walker *et al.* 2015). In pullets, higher concentration of T3 has been associated with feed inefficiency (Van Eerden *et al.* 2006). In terms of reproduction, T3 increases the basal metabolic rate and impacts the release of sex hormones by the pituitary gland (Goff 2015). In females, T3 has been shown stimulate bovine thecal cell steroidogenesis *in vitro* (Spicer

et al. 2001). Additionally, T3 has profound effects on the regulation of metabolism (Villanueva *et al.* 2013), similar to those observed during the estrus state.

4.1.3.2. Compounds

Metabolic products resulting from different biological processes provide insight into the fluctuations in energy metabolism of animals with different feed efficiencies (Richardson *et al.* 2004). Beta-hydroxybutyric acid (BHBA) is produced by the breakdown of lipids for energy (Gomez *et al.* 2002), by rumen butyrate production (Van Soest 1994) and has been suggested as a product of lipolysis in animals under stress (Warriss, 1995), which is oxidized in muscle tissue (Hocquette *et al.* 1998). It has been suggested that increased BHBA concentrations suppress luteinizing hormone pulses, creating negative energy signals which inhibit gonadotropin secretion (Iwata *et al.* 2011). During estrus, greater concentrations of BHBA may be related to an increased demand of energy sources during periods of reduced feed intake (Fernández-Foren *et al.* 2011), increased physical activity and intermediary metabolism (Parker *et al.* 2001).

During periods of reduced energy intake relative to requirement, cattle may experience a state of negative energy balance (Grummer 1995). Elevated serum NEFA and BHBA, along with reduced glucose can indicate a negative energy balance state (Adewuyi *et al.* 2005). Unlike monogastric animals, the ability of the ruminant to ferment carbohydrates into volatile fatty acids provides an energy store that is readily absorbable for energy production (Serjrsen and Neimann-Sorensen 1981). Concentrations of NEFA are elevated during specific physiological states, including

late pregnancy, even when energy requirements are met (Petterson *et al.* 1994; McGee *et al.* 2006). Greater concentrations of NEFA have been observed in grass-fed primiparous beef cows during postpartum with improved feed efficiency as a result of increased lipolysis (Lawrence *et al.* 2011).

Carbon dioxide (CO_2) is produced by oxidative metabolism in the mitochondria (Reece, 2004). The amount of CO_2 produced depends on the rate of metabolism and the relative amounts of carbohydrate, fat and protein metabolized (Kleiber 1961). In the blood, CO_2 is present in the body as either bicarbonate or carbonic acid (Reece 2004). Plasma CO_2 can provide an indication of the metabolic rate in cattle (Brody 1945). Greater concentrations of CO_2 have been observed in open, feed inefficient heifers, indicating greater oxygen consumption and heat production in less efficient heifers (Gonano *et al.* 2014).

Cholesterol is synthesized primarily by the liver (Christie 1981) through an energetically expensive process (Van Soest 1994). Cholesterol is a precursor for steroid hormone production, including oestrogens and corticosteroids (Cheeke and Dierenfeld 2010). Additionally, cholesterol is considered amphipathic, which allows the compound to be a constituent of cell membranes and the outer layer of lipoproteins (Cheeke and Dierenfeld 2010). Cholesterol can be an indicator of increased liver workload, in association with greater AST levels (Gonano *et al.* 2014). Heifers fed supplemental oil, with the intention to increase serum cholesterol, had greater body weight gains and improved ovarian luteal activity (Vann *et al.* 2003).

Creatinine is a breakdown product produced by the loss of phosphoric acid from creatine phosphate during muscle catabolism and is a marker of muscle mass in

cattle (Reece 2015). Higher concentrations of creatinine may be related to greater protein turnover in the muscle tissue (Lobley 1990). In the same way, CK is an enzyme located in skeletal, smooth, and cardiac muscle that is involved in muscle catabolism to supply amino acids for protein synthesis and gluconeogenesis (Thrall *et al.* 2004).

Glucose is primarily synthesized in the liver via gluconeogenesis in ruminants from C3 and C5 compounds (Reece 2015). In addition to the mammary glands, other tissues in the body use glucose, primarily through oxidation or triglyceride synthesis in adipose and other tissues (Reynolds, 1995). A positive correlation between RFI and glucose concentration in feedlot steers was found by Richardson *et al.* (2004). Glucose is the only energy source used by the neural system (Reece 2015). Since the neural-endocrine system is involved in the control of reproduction and hormone secretion it has been suggested that glucose is an essential metabolic substrate for proper reproductive processes in beef cows (Short and Adams 1988).

In the rumen, nitrogenous compounds can be processed by microorganisms into microbial protein (Cheeke and Dierenfeld 2010). The majority of the amino acids utilized by the rumen microbes are deaminated and used as energy sources, producing ammonia, branched chain volatile fatty acids, glucogenic and lipogenic compounds, CO_2 and methane (Van Soest 1994). Urea is the product of ammonia degradation in the liver which may be sourced from ruminal absorption, or amino acid catabolism from feed or body tissues (Berg *et al.* 2002). Increased plasma urea nitrogen indicates greater amino acid catabolism or increased protein intake (Mortimore and Poso 1987) and has been used to evaluate nitrogen use efficiency in livestock (Kohn *et al.* 2005). Lower plasma urea values have been observed in low-RFI, pregnant heifers during late

pregnancy, which may be associated with greater liver metabolism suggested for feed efficient animals (Gonano *et al.* 2014) and differences in protein turnover (Lobley 1990). However, the diminished feed intake of feed efficient animals may be related to the difference in feed (nitrogen) intake (Sath *et al.* 2012).

4.1.3.3. Ions

Calcium is the most abundant mineral in the body, which provides strong bone structure and supports muscle activity (Reece 2015). The free ion form of calcium that is bound to serum proteins and is essential for nerve conduction and cell signaling (Suttle 2010). The active absorption of calcium by the small intestine is controlled by the parathyroid and calcitriol hormones (Suttle 2010). Calpastatin is a specific inhibitor of the calcium activated protease calpain system, and thus inhibits protein degradation, has been reported to differ for feed efficient cattle (McDonagh *et al.* 2001). Greater concentrations of calcium in the bovine oviduct during estrus may be associated with sperm viability due to the requirement of calcium for the binding of oviduct proteins to spermatozoa (Hugentobler *et al.* 2007).

Potassium plays a major role in the control of cellular membrane potential and is involved in fluid balance, heart function, blood pressure, and blood pH (Reece 2015). Potassium absorption is an unregulated process in the small intestine and rumen (Suttle 2010). Potassium transport through the cellular membrane consists of a number of mechanisms which maintain high intracellular potassium concentrations (Suttle 2010). Potassium concentration in the blood decreases when the rate of protein synthesis within the cell increases; this may suggest increased protein synthesis due to increased

protein deposition, mobilization and turnover (Lobley 1990). During stages of growth, particularly in young cattle, potassium may act as an indicator of protein synthesis. Serum potassium was not associated with differences in RFI of pregnant beef heifers (Dias *et al.* 2016).

Phosphorus is essential for cellular biology and energy metabolism with implications for protein synthesis (Suttle 2010). Higher concentrations of phosphorus in young cattle have been directly related to growth hormone activity, which promotes intestinal phosphate absorption and renal phosphate re-absorption (Meyer and Harvey 2004). Phosphorus also contributes to the production of the muscle storage molecules, creatine phosphate and ATP (Huber and Breves 1999). Beyond one year of age, concentration of phosphorus has been shown to decrease in cattle (Doornenbal *et al.* 1988). Phosphorus was also shown not to be associated with feed efficiency in pregnant heifers (Dias *et al.* 2016).

Sodium absorption occurs primarily in the large intestine and by active transport across the rumen epithelium (Suttle 2010). It is estimated that 40% of the energy used by the body is used by the Na^+/K^+ - ATPase pumps to maintain intracellular sodium concentrations (Goff 2015). The continuous exchange of Na^+ and K^+ via the ATP-dependent Na^+-K^+ pump provides the basis for glucose and amino acid uptake, while maintaining high intracellular concentrations (Milligan and Summers 1986). Sodium is a major cation of the extracellular fluid that plays a critical role in osmotic pressure and water content of the circulating blood (Goff 2015). Plasma sodium revealed no differences in terms of feed efficiency in pregnant beef heifers

(Dias *et al.* 2016), but may vary according to the stage of the estrus cycle in nulliparous beef heifers (Small *et al.* 1996).

4.1.3.4. Enzymes

Metabolic enzymes such as ALP, aspartate aminotransferase (AST), creatine kinase (CK), gamma glutamyltransferase (GGT), and glutamate dehydrogenase (GLDH), play active roles in biological processes that impact energy metabolism. Studies have related circulating concentrations of enzymes to variability in feed efficiency in cattle. Alkaline phosphatase catalyzes the liberation of inorganic phosphate from phosphate esters and is present in many body tissues, which produce different isoforms of ALP including liver, bone and placenta, in relation to distinct metabolic states (She *et al.* 2000). It has been suggested that ALP may be involved in the transport of choline (Milne 1985) and phosphates (Corathers 2006) across the cell membrane. Studies have shown an indirect correlation with ALP and feed intake (Richardson *et al.* 2004) and with growth rate in cattle (Walawski *et al.* 1980). Physiological state may also play a role in the circulating concentrations of ALP. During pregnancy, the uterine production of the placental isoform of ALP intensifies (Cheung 2007), which continues to increase with length of gestation in cows (Yokus and Cakir 2006).

Aspartate aminotransferase is found in mitochondria (80%) and plasma (20%) with isoenzymes identifying each site (Rej 1978). This enzyme produces oxaloacetate, used as a substrate for the citric acid cycle and gluconeogenesis (Doornenbal *et al.* 1988). Aspartate aminotransferase is a key enzyme in amino-acid metabolism (Stryer

1988). Studies have associated AST with feed inefficiency in beef steers, which was suggested to be the result of higher levels of protein catabolism in the liver (Richardson *et al.* 2004). Conversely, greater concentrations of AST has been associated with improved feed efficiency in open beef heifers (Gonano *et al.* 2014).

Gamma glutamyltransferase (GGT) is synthesized in most body tissues, with the highest concentrations occurring in the pancreas and kidney and lower concentrations in hepatocytes (Thrall *et al.* 2004). It has been suggested that GGT plays an adaptive role in reacting to oxidative stress in a number of cell types, tissues, and organs (Whitfield 2001). The gamma-glutamyl cycle utilizes GGT and ATP to break down glutathione at the cell membrane into its constituent amino acids that can be readily used by cells (Whitfield 2001). Beef cattle with reduced feed efficiency have been reported to have greater serum GGT concentrations and a higher percentage of liver periportal inflammation (Alexandre *et al.* 2015). In addition, during periods of low energy intake, GGT can indicate lipid mobilization in cattle (González *et al.* 2011).

Glutamate dehydrogenase is a leakage enzyme free in the cytoplasm that is present in high concentrations in the liver of ruminants (Thrall *et al.* 2004). The GLDH reaction in the cytosol for the production of glutamate, is a result of increased ammonia concentrations in the mitochondria, to be used as a source of nitrogen (Lehninger 1975). In the study by Gonano *et al.* (2014), it was suggested that GLDH in pregnant heifers followed a similar pattern as urea, which could be associated with reduced amino acid catabolism to allow partitioning of amino acids for fetal growth.

Creatine kinase buffers cellular ATP and ADP concentrations by catalyzing the reversible exchange of high energy phosphate bonds between phosphocreatine and

ADP produced during muscle contraction (Brancaccio *et al.* 2007). Creatine kinase is a controller of cellular energy homeostasis that creates a large pool of phosphocreatine from creatine, to serve as an energy source for tissues with fluctuating energy demands, such as muscle (Schlattner *et al.* 2006). Greater concentration of plasma CK during estrus is likely the result of increased estrus behaviors, such as mounting and restlessness (Ball and Peters 2007). The greater concentration of CK in heifers during estrus could suggest greater energetic demands of the muscle tissue to support physical estrus behavior and this behavior relies on CK production of phosphocreatine. This implies that CK could be considered an indicator of feed efficiency during estrus, as well as a potential indicator of reproductive performance.

4.1.3.5. Blood transport proteins

Albumin is a major carrier protein in the plasma which accounts for 80% of the ontonic pressure of the blood (Thrall 2004). Albumin is synthesized by the liver and plays an active role in the transport of free fatty acids, bile acids, calcium and hormones (Thrall 2004). Globulins include various enzymes and transport proteins involved in metabolic processes related to energy metabolism; the abundance of these proteins is affected by such factors as age and pregnancy (Kanecko 2008). Stressors such as extreme weather (Cozzi *et al.* 2011), high altitude (Kumar and Kumar 2000), and stocking rate (Tarrant and Grandin 2000), are capable of increasing the globulin concentration in cattle which may be result of higher metabolic rate due to stress.

5. Estrus cycle

5.1. Estrus cycle and body composition

For optimal reproductive performance, beef heifers should enter puberty at approximately 9 to 12 months of age, which enables two to three estrus cycles prior to the first breeding season (Kinder *et al.* 1994). Puberty is a process whereby animals become capable of reproducing and it can be affected by factors such as environment, nutritional status, and season (Schillo *et al.* 1992). The initiation of puberty is characterized by a series of hormonal changes, which lead to the maturation of ovarian follicles, resulting in increasing concentrations of estradiol.

The bovine estrus cycle has been described by Amstalden and Williams (2015) briefly; standing estrus is a period of 6 to 24 hours where a female will stand for breeding, followed by ovulation. After ovulation, the luteal cells form the corpus luteum (CL), which secretes progesterone until around day 17 when the CL begins to regress allowing the cow to enter standing heat again by day 21. During the 21 day cycle, follicles in response to the surge and regression of follicle stimulating hormone (FSH), develop in wavelike patterns (2 to 3 per cycle) after every ovulation. In the absence of progesterone, the dominant follicle becomes the ovulatory follicle and is regulated by LH. In the presence of progesterone, the dominant follicle does not ovulate but undergoes cell death, and a new follicular wave is initiated. When maternal recognition of pregnancy does not occur, the uterus releases prostaglandin F2α (PG) to induce luteolysis. When progesterone is not present, a high concentration of estradiol produced by the follicles causes standing estrus and the behavioral changes associated with it. The high concentrations of estradiol stimulate the surge of gonadotropin

releasing hormone (GnRH). This surge of GnRH results in an LH spike, causing ovulation of the ovulatory follicle.

Nutritional management is a major factor controlling reproduction. Insufficient energy and nutrient intake delays puberty in heifers, lengthens postpartum anestrus in cows and causes anestrus in cows and post-pubertal heifers (Short and Adams 1988). Day *et al.* (1986) observed that the typical LH increase prior to puberty was blocked in pre-pubertal, feed restricted heifers. The effect of energy restriction has been seen in estrus cycling heifers, where blood plasma LH and progesterone concentrations were reduced significantly in feed restricted heifers, compared to the control group, in the second and third estrus (Gombe and Hansel 1973). Post-pubertal heifers, with greater body composition and greater empty body fat, remained cyclic longer than heifers in moderate condition prior to feed restriction (Cassady *et al.* 2009).

5.2. Estrus detection methods

In general, a female in estrus displays signs of estrus including: standing for mounting, Flehmen response, mounting other females and increased activity that is best observed in the early morning and evening (Roelofs *et al.* 2010) as well as; reduced feed intake (Reith *et al.* 2014). Bovine females in estrus or approaching estrus may also show vaginal mucus discharge (Rae 2002) and vulvar edema (Hopkins 2003).

Some practical methods of estrus detection used in the beef industry, especially by breeding programs using artificial insemination, include visual indicators, such as chin-ball markers mounted on a teaser animal, adhesive tail patches, tail head chalking (Hopkins 2003) and pressure sensing devices (Dalton *et al.* 2001). Other estrus

detection tools include pedometers (Silper *et al.* 2015) and neck mounted collars (Stevenson *et al.* 2014), which sense the fluctuations in activity that is related to the estrus state (Roelofs *et al.* 2015). Infrared imaging (Hurnik *et al.* 1985) has also been proposed as a technique to assess signs of estrus in cattle, based on the body surface changes in response to the estrus state (Talukder *et al.* 2014).

Clinical methods of estrus detection can provide information on the internal development of reproductive organs, such as reproductive tract palpation and scoring (Anderson *et al.* 1991) and ultrasonography (Pierson and Ginther 1988). Less popular approaches to determine estrus cycle status include blood concentrations of progesterone (McCarthy *et al.* 2012), luteinizing hormone (Mondal *et al.* 2006) and estradiol (Vynckier *et al.* 1990). In addition to blood hormones, blood plasma metabolites, such as minerals, are demonstrated to be associated with estrus state (Small *et al.* 1996).

Chapter 3

ASSOCIATIONS BETWEEN BLOOD CELLS, IMMUNE RESPONSE AND PLASMA METABOLIC PROFILE WITH PRODUCTIVE PERFORMANCE IN GRASS-FED REPLACEMENT BEEF HEIFERS

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ABSTRACT: Indicators of feed efficiency such as hematological measures may be used to optimize the identification of cattle with superior feed efficiency. The objectives were to evaluate in the relationships between blood cell parameters, immunoglobulins (Ig) and plasma metabolites with feed efficiency in beef heifer calves and pregnant heifers. Heifers were fed grass silage during a 124-day performance test where repeated blood samples were collected. The specific immune response of IgG1 and IgM to ovalbumin was assessed. Residual feed intake was used to categorize each population of heifers into feed efficiency categories (low and high RFI) and blood analytes were compared between these classes. Efficient heifer calves exhibited lower mean cell hemoglobin, lower mean corpuscular volume, greater abundance of lymphocytes and reduced segmented neutrophil abundance on day 1. Efficient pregnant

heifers had greater leukocyte abundance on day 1 and greater abundance of lymphocytes with fewer segmented neutrophils on day 124. Efficient heifer calves exhibited an increased IgM response compared to inefficient heifer calves, but no differences in IgG1 response were found. Throughout the test, efficient heifer calves had elevated plasma potassium and phosphorus, reduced alkaline phosphatase (ALP) and had a trend toward lower triiodothyronine ($P = 0.06$) compared to inefficient heifer calves. Efficient pregnant heifers had greater concentrations of ALP, non-esterified fatty acids and creatinine than inefficient pregnant heifers. Inefficient pregnant heifers had greater concentrations of cholesterol and globulin than efficient pregnant heifers. There is potential for using hematological measures as proxies for feed efficiency. However, attention must be given to the distinct associations according to physiological state.

Key Words: alkaline phosphatase; hemoglobin; immunoglobulin; leukocyte; potassium; residual feed intake.

INTRODUCTION

Feed costs are a major expense of beef production where a large portion of these costs are due to the breeding cow herd, responsible for 70% of the energy background requirements (Hill and Ahola 2012). Understanding the physiological basis underlying the variation in feed efficiency may assist in identifying proxies to improve feed efficiency in order to facilitate genetic selection and nutritional manipulation.

Improvements in feed efficiency can be achieved by the determination and selection for residual feed intake (RFI; kg DM/day) in individual animals. However, this assessment is laborious, time consuming and requires expensive equipment. Studies have identified potential proxies for RFI using traits associated with energy metabolism, including body heat dissipation (Montanholi *et al.* 2009), hepatic mitochondrial function (Lancaster *et al.* 2014) and visceral organ metabolism (Wang *et al.* 2009). Research has also identified hematological measures as potential proxies for feed efficiency (Richardson *et al.* 2002; Kelly *et al.* 2010; Lawrence *et al.* 2011; Gonano *et al.* 2014). However, there is a need to further evaluate this broad class of assessments in cattle differing in physiological stages, such as heifers at peri-pubertal and at pregnancy stages.

Blood cell parameters, measured using the complete blood cell (CBC) analysis, provide information about health status and metabolic state. Parameters such as red blood cell indices could be related to physiological functions, including oxygen consumption and transport, and may differ as a result of changes in metabolic rate (Riedesel and Engen 2015). Furthermore, white blood cell subpopulations can be associated with differences in immune function, which may also influence energy partitioning (Lochmiller *et al.* 2000). Positive relationships between feed efficiency and blood cell measures, including mean corpuscular volume, mean cell hemoglobin, and lymphocyte count have been observed in steers (Richardson *et al.* 2002). These associations have also been observed in other species, with higher concentrations of red and white blood cells and reduced mean corpuscular volume in feed inefficient ewes (Rincon-Delgado *et al.* 2011).

The maintenance of the immune system is metabolically demanding and may require shifts in nutrient and energy allocation from growth, reproduction, and basal metabolism (Sheldon and Verhulst 1996). For instance, response to an infection can reduce protein accretion in muscle as amino acids are mobilized toward energy production, resulting in decreased productive performance (Klassing 1988). Immunoglobulin M (IgM) and immunoglobulin G1 (IgG1) are primary responders of the humoral immune system in ruminants (Thrall 2004). The responsiveness of the immune system can be objectively evaluated in cattle by measuring the specific immunoglobulin response to unfamiliar proteins, such as ovalbumin (OVA) (Cartwright *et al.* 2011; Wagter *et al.* 1999). This approach has proven to be effective for the study of health traits (Mallard *et al.* 1997) and may serve to relate immune response to feed efficiency in the bovine.

Plasma metabolites, including metabolic ions, compounds, enzymes and hormones, are associated with energetically demanding functions of organ systems that influence efficiency of feed utilization (Blaxter 1962). Metabolic products such as cholesterol (Rauw *et al.* 2007), creatinine and non-esterified fatty acids (NEFA) (Lawrence *et al.* 2011) are thought to be related to feed efficiency in cattle. Similarly, ions related to cellular potential and energy metabolism, including phosphorus, are associated with differences in feed efficiency (Ternouth *et al.* 1990). Lower concentration of the metabolic hormone triiodothyronine (T3) has been related to lower feed intake in steers (Christopherson *et al.* 1979) and to improved feed efficiency in beef cows (Walker *et al.* 2015).

Physiological state impacts hematological measures in cattle during stages of development, including growth and gestation. Blood cell measures that appear to differ based on physiological state include mean cell hemoglobin and mean corpuscular volume in heifer calves (Richardson *et al.* 1996) and white blood cell count in pregnant heifers (Sattar and Mirza 2009). Immunoglobulins also vary according to the physiological stages of beef females, including IgM during weaning (Mackenzie 1997) and IgG during late pregnancy (Mallard *et al.* 1998). Additionally, metabolite profiles of heifers differ in relation to age (Doornenbal *et al.* 1988) and stage of pregnancy (Gonano *et al.* 2014; Yokus and Chakir 2005).

Considering that hematological measures are associated with changes in feed efficiency and stage of developmental, the evaluation of such parameters in beef females from distinct stages of development (peri-pubertal and pregnant) will broaden the understanding of the biology underlying differences in feed efficiency; this may lead to the identification of proxies for feed efficiency. Therefore, the objectives of this study were to evaluate the association between CBC parameters, specific IgG1 and IgM responses and plasma metabolic profiles relative with feed efficiency rank in forage-fed replacement beef heifer calves and pregnant beef heifers.

MATERIAL AND METHODS

Animals and experimental design

Experimental procedures involving animals were performed in accordance with the recommendations of the Canadian Council on Animal Care guidelines (2009).

Two groups of crossbred replacement heifers consisted of the following: 107 heifer calves (mean ± s.d., 287 ± 27.6 days of age, 253 ± 37.8 kg at the start of the trial), and 36 pregnant heifers (557 ± 93.5 days of age, 406 ± 41.7 kg) were consigned from 20 producers from the Atlantic region of Canada and housed at the Maritime Beef Test Station (Nappan, Nova Scotia). Due to the differing farms and husbandry practices under which the heifers were raised, the following prophylactic treatments were given to all heifers prior to the performance evaluation: ivermectin (0.01 mL/kg Bimectin® Bimedia, Oakbrook Terrace, USA), vitamin E and selenium supplement (0.01 mL/kg Dystocel®, Zoetis, Kirkland, Canada), tulathromycin (2.5 mg/kg Draxxin®, Zoetis, Lirkland, Canada), bovine rhinotracheitis-virus diarrhea-parainfluenza-3-respiratory Syncytial virus vaccine (2 mL Bovi-Shield GOLD FP 5, Zoetis, Kirkland, Canada) and clostridium chauvoei-septicum-haemolyticum-novyi-sordellii-perfringens types C & D-haemphilus somnus bacterin-toxoid (2 mL Vision 8 Somnus with SPUR, Merck Animal Health, Summit, USA).

The 107 heifer calves were divided into two pens of 36 and one pen of 35 and the 36 potentially pregnant heifers were housed in a single pen. Pens included access to an indoor feeding area (20.5 by 12.9 m) and bedding area supplied with wheat straw (12.9 by 5.5 m), as well as access to an outdoor yard area (15.5 by 9.1 m). Heifers were fitted with a visual identification tag (Allflex® Global Maxi Female ear tag, Allflex, Dallas, USA) and an automated feeding system identification tag (Allflex® Full Duplex electronic ID ear tag, Allflex, Dallas, USA). Heifers had a 14-day adaption period to adjust to the automated feeding system, facilities and pen mates. Heifers were tested for feed intake and productive performance for 124 days, from mid-June to the end of

October (2014). Individual feed intakes were recorded continuously using an automated feeding system (GrowSafe® Feed Intake System, Airdrie, Canada). Heifers were weighed and scanned by ultrasound for body composition every 30.6 ± 3.0 days days on four consecutive days (one pen per day). The pregnancy status of the 36 potentially pregnant heifers was assessed by blood test on day 28 of the performance evaluation (BioPRYN®; Biotracking, Moscow, USA) and 31 were found to be pregnant. The 5 open heifers were kept with the pregnant heifers to maintain the animal to bunk ratio. Heifers were also evaluated for immune response to OVA during days 56 to 77 of the productive performance evaluation.

Heifers were fed for *ad libitum* intake a grass silage diet predominantly comprised of timothy (*Phleum pretense*), meadow fescue (*Festuca pratensis*), Kentucky bluegrass (*Poa pratensis*), reed canary grass (*Phalaris arundinacea*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). The total mixed ration was composed of (dry matter basis) 99.5% haylage and 0.5% mineral and vitamin premix[1]. Feed samples collected weekly were pooled by month, then monthly determinations were averaged over the entire experiment. Chemical composition was (dry matter basis): crude protein 15.5%, acid detergent fiber 29.6%, neutral detergent fiber 53.7%, starch 6.5%, total digestible nutrients 70.29% and digestible energy 2.92 Mcal/kg determined by near infrared (NIR) analyzer.

Breed composition was determined via hair follicle DNA extraction and 50K single-nucleotide polymorphism (SNP) sequencing (ADMIXTURE® software,

[1] Contains 7.8% Na, 27% Ca, 0.02% P, 2.5% Mg, 2,400 mg/kg Fe, 900 mg/kg Cu, 75 mg/kg iodine, 2,300 mg/kg Mn, 2,400 mg/kg Zn, 13 mg/kg Co, 3000 mg/kg Fl, 200,000 IU/kg Vitamin A, 27,000 IU/kg Vitamin D-3, 4000 IU/kg Vitamin E

University of California, Los Angeles, USA), followed by estimation of individual and population breed allele frequencies from the SNP data, using pairwise comparison (Connolly *et al.* 2014). The average breed composition of heifer calves was 28.5% Angus, 18.5% Simmental, 17.8% Limousine, 11.9% Hereford, and 23.3% other European breeds. For the pregnant heifers, the breed composition was 30.8% Simmental, 26.1% Hereford, 16.0% Angus, 8.6% Shorthorn, and 18.5% other European breeds.

Productive performance and biometrics

Body weights were measured in the morning prior to feeding, using a livestock scale (CattleMaster, E. S. Martin Welding, Linwood, Canada). Heifers were restrained in a squeeze chute (Pearson Livestock Equipment, Thedford, USA) for ultrasound image scanning. Ultrasound imaging was performed using an Aloka SSD-500 ultrasound unit (model 5044; 172 mm; 3.5 MHz; Corometrics Medical Systems, Wallingford, USA) equipped with a long probe. The minimum subcutaneous fat depth over the longissimus muscle in the fourth quadrant distal to the spine was measured from this image, along with longissimus muscle area. Six sagittal scans across the 11th, 12th and 13th ribs were collected using the long probe and marbling score was estimated using CPEC software (Cattle Performance Enhancement Company, Oakley, USA). Subcutaneous fat thickness was measured at the junction of the coxal and ischiatic tuber. Ultrasound images were used to determine back fat thickness (BKT, mm), rib eye area (REA, cm^2), marbling (MAB, score 1: devoid; 11: prime) and rump fat thickness (RMP; mm). Average ultrasound traits over the performance evaluation

were determined using linear regression. Feed to gain ratio (FG) was calculated by dividing the dry matter intake (DMI; g/day) by average daily gain (ADG; kg/day). The age of the heifers at the end of the performance evaluation was determined using the records obtained from the producers (AGE; days). Days in gestation (DIG; days) of pregnant heifers was determined using the calf birth date, assuming a typical gestation length of 273 days (Andersen and Plum 1965).

The RFI models were developed in a similar manner to those described previously (Montanholi *et al.* 2009; Gonano *et al.* 2014). The model with the highest R^2, while showing the lowest Bayesian information criteria, was selected for each population of heifers. The RFI of the heifer calves was calculated using the following model ($R^2 = 0.53$):

$$\text{DMI} = -10.291 + 8.071\,(\text{ADG}) + 0.040\,(\text{BW}) - 0.103\,(\text{REA}) + 0.118\,(\text{RMP}) + 0.059\,(\text{AGE}) + \text{RFI}$$

Similarly, the pregnant heifer RFI was calculated using the following model ($R^2 = 0.49$):

$$\text{DMI} = -2.88 + 2.66\,(\text{ADG}) + 0.017\,(\text{BW}) - 0.018\,(\text{REA}) + 0.081\,(\text{BKT}) - 0.150\,(\text{RMP}) - 0.11\,(\text{MAB}) + 0.007\,(\text{AGE}) + 0.005\,(\text{DIG}) + \text{RFI}$$

The predicted dry matter intake (DMI; kg/day) was subtracted from the average feed intake to determine the RFI values.

Blood sampling

Blood sampling was performed between 8:30 and 11:30 prior to feed distribution. Heifers were restrained in a squeeze chute and secured with a nylon rope halter in order to expose the jugular vein region. The jugular area was disinfected with 70% isopropyl alcohol and blood samples were collected using 0.9 x 25 mm blood collection needles (BD Vacutainer® Precision Glide, BD Inc., Franklin Lakes, USA) into the following tubes: sodium heparin blood collection tubes (BD Vacutainer®, BD Inc., Franklin Lakes, USA) for the metabolite profile; EDTA blood collection tube (Monoject™ Blood Collection Tube, Kendall Healthcare, Mansfield, USA) for the CBC analysis; serum separator tubes (BD Vacutainer®, BD Inc., Franklin Lakes, USA) for the specific antibody response. Samples for CBC analysis were taken at the start and end of the performance evaluation and stored at 4 °C prior to analysis. Samples for plasma metabolite profile analysis were taken every 30.6 ± 3.0 days during the performance evaluation, then stored at 4°C until further processing. Blood samples for immune response were collected and kept at room temperature for 25 minutes to allow clotting and then further processed. Both the metabolite profile and the immune response samples were centrifuged at 4°C at 3000g for 25 minutes, then the supernatant was decanted into micro centrifuge tubes and kept frozen until analysis.

Complete blood cell profile

Blood cell parameters were measured with a hematology analyzer (Sysmex XT-20001 V Hematology Analyzer, Sysmex Canada Inc., Mississauga, Canada). Red blood cell parameters included red blood cell count (RBC; 10^6 cells/µl), hemoglobin

(g/dL), mean corpuscular volume (MCV; FL), mean cell hemoglobin (MCH; pg) and platelets (10^3 cells/µl). White blood cell (WBC) parameters consisted of total white blood cell count (WBC; 10^3 cells/µl), segmented neutrophils (%WBC), lymphocytes (%WBC), and monocytes (%WBC).

Plasma metabolic profile determination

Concentrations of blood plasma metabolic enzymes, including alkaline phosphatase (ALP; U/L), gamma-glutamyl transferase (GGT; U/L), aspartate aminotransferase (AST; U/L), creatine kinase (CK; U/L), glutamate dehydrogenase (GLDH; U/L); compounds including albumin (g/L), albumin globulin ratio (AG), cholesterol (mmol/L), carbon dioxide (mmol/L), creatinine (µmol/L), globulin (g/L), glucose (mmol/L), haptoglobin (g/L), non-esterified fatty acid (NEFA; mmol/L), urea (mmol/L) and ions including calcium (mmol/L), phosphorus (mmol/L), magnesium (mmol/L), sodium (mmol/L), potassium (mmol/L), chloride (mmol/L) and anion gap (mmol/L) were determined, using an automated analyzer (Cobas® c 311/501 analyzer, Roche Diagnostics GmbH, Indianapolis, USA). Determination of β-hydroxybutyrate was carried out using a commercial kit (Randox ®, RANDOX Laboratories Ltd., Ireland, UK). Plasma concentration of triiodothyronine (T3; nmol/L) was quantified using an ELISA test (IMMULITE 1000, Siemens Healthcare Diagnostic Products, Malvern, USA).

Plasma globulin was determined by subtracting the albumin concentration from the total protein concentration. Calculated osmolality (OSM; mmol/L) is the sum

of separately estimated solute concentrations (Dormandy 1967) and was determined using the following formula (Bhagat *et al.* 1984):

OSM = 1.86 (sodium (mmol/L) + potassium (mmol/L)) + glucose (mmol/L) + urea (mmol/L)

Immune response to ovalbumin (OVA) vaccination

The specific antibody response was established by evaluating the specific antibody response to OVA injection, following the methodology adapted from You *et al.* (2008). Briefly, the vaccines were prepared by dissolving 15 mg of Quil-A (Purified Saponin Quil-A® Brenntag Biosector, Frederikssund, Denmark) and 15 mg OVA (Chicken egg white albumin, Sigma Catalogue #A5503-5G, Sigma-Aldrich, Spruce Street, USA) in 30 mL of physiological saline. The solution was vortexed until dissolved. Two mL were transferred to 3 mL syringes and placed on ice. The injection was delivered intramuscularly, in the neck region, on days 0 and 14 of the immune response evaluation. To determine the baseline, primary, and secondary responses of OVA specific IgM and IgG1, blood samples were taken prior to vaccination on day 0, prior to the booster on day 14 and at day 21, respectively.

The baseline and specific antibody response was quantified using a modified antigen specific IgM and IgG1 enzyme-linked immunosorbent assays (ELISA) (Cartwright *et al.* 2011 and Heriazon *et al.* 2011). The bovine IgG1 response to OVA was evaluated by modified ELISA (Cartwright *et al.* 2011; Heriazon *et al.* 2011). In summary, high binding 96-well plates (Corning, Acton, USA) were coated with 100 μl/well of 1.44 mg/ml of OVA (Sigma–Aldrich, St. Louis, USA) dissolved in

carbonate-bicarbonates coating buffer (pH 9.6) overnight at 4 ºC. All plates were blocked (200 µl/well) with fresh made phosphate buffered saline (PBS) containing 3% Tween- 1.5% BSA-1.5% fetal bovine serum for 1 hour at room temperature after washing 3 times. Pooled positive control sera from day 21 samplings, and individual serum samples were diluted to 1:1600 and 1:3200 in sample conjugate buffer [PBS containing 1.5% Tween 20 and 0.3M NaCl] and incubated (100 ul/well) for 2 hours at room temperature. All control and individual samples were analyzed in triplicate. Alkaline phosphatase-conjugated sheep anti-bovine IgG1 (Cedarlane, Burlinton, Canada) was diluted to 1:4000 in sample conjugate buffer and incubated for 1 hour at room temperature. Then 80ul/well of alkaline phosphate yellow liquid substrate (pNPP; Sigma–Aldrich, St. Louis, USA) was added into each well and incubated for 30 minutes in the dark. The plates were read on the Wallac1420 VICTOR 3 Multilabel Counter (PerkinElmer, Waltham, USA) at 405 nm, and the optical density (OD) was obtained.

The OVA-specific bovine IgM was measured similarly to IgG1, except the control sera, individual serum samples and the alkaline phosphatase conjugated rabbit anti-bovine IgM detection antibody (Cedarlane, Burlinton, Canada) were diluted to 1:100, 1:200, and 1:2000, respectively, in sample conjugate buffer. Coated buffer wells containing serum were also included for each individual sample to account for non-specific IgM binding.

All OD readings were normalized across plates using the correction factor (CF) (You *et al.* 2008) calculated as:

$$CF = \frac{\text{overall mean OD of positive control from all tested plates (100x + 200x for IgM OR 1600x + 3200x for IgG1)}}{\text{actual mean OD of positive control from individual plate}}$$

To measure the true OD for IgM, the non-specific binding OD was subtracted from individual sample OD readings. Therefore, all OD readings for each sample were corrected before statistical analysis. For OVA-specific antibody ELISA, the respective inter-assay coefficients of variation for all plates was 7.6% for IgG1 and 6.9% for IgM. The adjusted primary and secondary response values of IgG1 and IgM were calculated for each immunoglobulin by subtracting the baseline immunoglobulin concentration from the corresponding primary or secondary response concentration.

Statistical Analyses

Data was analyzed using SAS software (SAS version 9.4, SAS Institute Inc., Cary, USA). Normality was tested by the univariate procedure and transformations were completed where necessary. Heifer calf CBC and blood plasma parameters were transformed by natural logarithm (MCH final, cholesterol), negative four exponent (MCH initial), square root (BHBA, T3), negative two exponent (glucose) and reciprocal (ALP and CK). Pregnant heifer CBC and blood plasma parameter transformations included natural logarithm (WBC initial and final, potassium, GLDH, glucose, and urea), exponent negative four (MCH initial and final, initial hemoglobin), reciprocal (ALP, initial MCV), square root (BHBA) and squared (anion gap). Heifer calf immune response parameters were transformed by natural logarithm (IgM baseline, IgG1 primary), exponent negative two (IgG1 base line), and sin (IgM primary).

The general linear model (GLM) select procedure was used to determine the optimal linear model for the productive performance, CBC, and immune parameters for both the heifer calves and pregnant heifers. This procedure determined that breed composition was an effect to be included in the evaluation of productive performance measures but not for CBC and immune response parameters in either population of heifers. Heifer calves were split into two groups based on RFI: efficient (n = 54; RFI = -0.83 kg/day), and inefficient (n = 53; RFI = 0.85 kg/day). Similarly, pregnant heifers were split into efficient (n = 16; RFI= -1.02 kg/day), and inefficient (n = 15; RFI = 1.06 kg/day) groups. All parameters were compared across feed efficiency groups in each population of heifers. Preliminary results for immunoglobulin response suggested a potential distinction between extreme groups for feed efficiency when populations were divided into thirds. Therefore, heifer calves were also classified as efficient (n = 36; RFI = -1.15 kg/day), average (n = 36; RFI = 0.01 kg/day), and inefficient (n = 35; RFI = 1.14 kg/day), and the pregnant heifers as efficient (n = 11; RFI = -1.18 kg/day), average (n = 10; RFI = -0.39 kg/day), and inefficient (n = 10; RFI = 1.64 kg/day).

The GLM procedure model used for productive performance traits between feed efficiency groups was the following:

$$Y_{ijk} = \mu + \text{FEgroup}_i + \text{Breed}_j + e_{ijk}$$

where Y_{ijk} is the k-th trait measured on the i-th feed efficiency group, μ is the overall mean of the trait, FEgroup_i is the feed efficiency group, Breed_i is the breed composition and e_{ijk} is the residual random effect associated with the k-th measure. A model excluding breed composition was used for the CBC and immune response parameters. Similarly, the least square means of all the hematological measures were also

determined to compare categories of the two populations of heifers (heifer calf or pregnant heifer) upon averaging the repeated assessments.

Means between feed efficiency groups were also compared for repeated measures, as part of the metabolic profile dataset, using the mixed procedure. Preliminary analysis also confirmed a lack of significant effect of breed composition and, therefore, the fixed effect of breed was not included in the model below:

$$Y_{ijtkl} = \mu + FEgroup_i + \sum_{k=0}^{nf} \varphi_{jtk}\beta_k + \sum_{k=0}^{nr} \varphi_{jtk}\gamma_{jk} + \varepsilon_{ijtkl}$$

where Y_{jtkl} is the *k*-th blood parameter measured on the *t*-th day (t = 1-5 sampling) of the *j*-th heifer from the *i*-th feed efficiency group; μ is the overall mean effect for the blood parameter; $FEgroup_i$ is the fixed effect of the *i*-th feed efficiency group; $\sum_{k=0}^{nf} \varphi_{jtk}\beta_k$ are fixed regression coefficients; $\sum_{k=0}^{nr} \varphi_{jtk}\gamma_{jk}$ are the *k*-th random regression for the *j*-th heifer; φ_{jtk} is the *k*-th linear polynomial for blood parameter of heifer *j* at day *t*; *nf* and *nr* are the order of the linear polynomial for fixed and animal effects regressions; and ε_{ijkl} is the residual random effect associated with the assessment on the *t*-th day on the *j*-th heifer.

The least square means comparisons for analysis conducted using the general linear model and mixed model were performed using the Scheffé test. Transformed data was back-transformed and confidence limits of the means were calculated using 95% intervals. For all analyses, data were considered statistically significant when $P \leq 0.05$ and a trend towards significance when $0.10 \geq P > 0.05$.

RESULTS

The descriptive statistics and least square means of productive performance traits by feed efficiency groups for heifer calves and pregnant heifers are shown in Tables 3.1 and 3.2, respectively. In both populations of heifers, feed efficient animals demonstrated reduced DMI and FG without affecting indicators of body fatness and leanness. Efficient heifer calves and pregnant heifers have the potential to consume 303 kg and 358 kg less feed (dry matter basis) yearly, respectively, in comparison to inefficient heifers.

The CBC parameters at the start and end of the performance evaluation, by feed efficiency groups for the heifer calves and pregnant yearlings, are shown in Tables 3.3 and 3.4, respectively. The CBC parameters by physiological state is shown in Table 3.6. Feed efficient heifer calves tended to have greater MCH (P = 0.08) at the start and a higher MCH at the end of the testing period. Efficient heifer calves also tended to have lower MCV (P = 0.07) at the end of the performance test. CBCs from efficient pregnant heifers at the start of the performance test were higher WBC (P = 0.07). Inefficient pregnant heifers tended to have higher MCV (P = 0.07). Efficient heifer calves and pregnant yearlings had greater abundance of lymphocytes and fewer segmented neutrophils, compared to the inefficient heifers. Total red blood cell count, hematocrit and hemoglobin content did not differ by feed efficiency group for either heifer calves or pregnant heifers.

Immunoglobulin responses to OVA of the heifer calves and the pregnant heifers as well as heifer calves' immunoglobulin response according to feed efficiency

grouping in thirds is shown in Figure 3.1. When comparing the heifer populations, the secondary response of IgM in the pregnant heifers was greater than that observed in heifer calves, with no differences for IgG1 [Fig. 3.1(a)]. The secondary IgM response was 60.90% lower in the heifer calves and 13.86% lower in the pregnant heifers, compared to the primary IgM response to OVA. Conversely, the secondary IgG1 response was 98.25% higher in the heifer calves and 86.73% higher in the pregnant heifers, in comparison to the primary IgG1 response to OVA. Immunoglobulin response, compared by halves, revealed no differences according to feed efficiency (Table 3.5). When compared by thirds, the efficient heifer calves had a greater secondary IgM response than the inefficient heifer calves [Fig. 3.1(b)].

The plasma metabolite concentrations by heifer category is shown in Table 3.6. Heifer calves had greater hematocrit, platelets, RBC, WBC, CK, cholesterol, glucose, potassium and phosphorus than pregnant heifers. Pregnant heifers had greater MCH, MCV, ALP, CO_2, creatinine, globulin and chloride than the heifer calves. The metabolite concentrations by feed efficiency group for heifer calves and pregnant heifers are shown in Tables 3.7 and 3.8. Efficient heifer calves had decreased ALP and greater concentrations of phosphorus and potassium over the performance evaluation compared to the inefficient heifer calves (Fig. 3.2). Efficient heifer calves tended to have lower concentration of T3 during the performance evaluation (P = 0.06), compared to the efficient heifer calves. Efficient pregnant heifers had increased concentrations of alkaline phosphatase, NEFA (Fig. 3.3), creatinine (Fig. 3.4) and a trend for increased AG ratio (P = 0.07), compared to the inefficient pregnant heifers. Inefficient pregnant heifers had greater concentrations of cholesterol (Fig. 3.3) and

globulin (Fig. 3.4) and a trend for greater concentrations of calcium (P = 0.06) than efficient heifers.

DISCUSSION

The difference in feed intake for the same rate of body weight gain and body composition observed when comparing efficient and inefficient heifers illustrates the possibility of reducing feed costs without hindering productivity, which has also been reported elsewhere (Kelly *et al.* 2010; Lawrence *et al.* 2011; Gonano *et al.* 2014). Improvements in efficiency of feed utilization within the cow herd are important to trigger the improvement of feed efficiency across all production stages given the moderate heritability of RFI (Pitchford 2004). Hematological measures are related to energy metabolism and appear to be associated with feed efficiency in replacement heifers (Kelly *et al.* 2010; Lawrence *et al.* 2011; Gonano *et al.* 2014). Therefore, the evaluation of these traits may culminate in the identification of practical proxies for feed efficiency. In fact, there is a growing demand for new phenotypes to enhance the improvement of feed efficiency through genetic selection (Gonzalez-Reico *et al.* 2014) and also through nutritional manipulation (Patience 2012) to fulfill the societal demands for efficient beef production (Gerber *et al.* 2013).

The results regarding productive performance traits observed in our study were comparable to those from heifers in similar physiological states and fed forage-based diets. Heifer calf RFI and FG were comparable to those found by Bingham *et al.* (2009), ADG was of similar magnitude to that observed by Bodine *et al.* (2001), and rib eye

area and DMI were in line with the results observed by Black *et al.* (2015). Pregnant heifer RFI and BKT were similar to those found by Lawrence *et al.* (2011) and FG, DMI, ADG and MAB results were comparable to the results reported by Gonano *et al.* (2014).

The CBC and metabolic profile measures are routinely used to assess health status in cattle. The values observed for individual heifers (data not shown) indicated that our heifers were healthy according to recommended ranges in cattle (Jones and Allison 2007; University of Guelph Animal Health Lab Guidelines, 2015). Considering that disease states are associated with a reduced productive efficiency (Morton *et al.* 2008) due to the impact on energetic partitioning (Brody 1945), it is important to ensure the health of the heifers when assessing feed efficiency.

Other studies have identified parameters of the CBC analysis as potential indirect assessments or biomarkers of RFI in beef heifers (Lawrence *et al.* 2011). The outcome of a suggested reduction of MCH and MCV was also found in feed efficient crossbred steers (Richardson *et al.* 2002). It has been shown that inefficient animals produce more radiant heat than efficient animals (Montanholi *et al.* 2009), which is associated with increased oxygen consumption (Montanholi *et al.* 2008). This phenomenon is typically seen in athletic animals, including Standardbred horses and Greyhounds, which exhibit greater resting MCH and MCV due to the increased oxygen requirements for athletic performance (Riedesel and Engen 2015). This suggests that animals with greater oxygen demands have a greater concentration of hemoglobin and higher corpuscular volume per erythrocyte. Since there were no differences in RBC between feed efficiency groups yet the inefficient heifers tended to have higher MCH

and MCV, this may imply inefficient heifers have greater oxygen requirements. This increased oxygen demand may be due to increased basal metabolism and, consequently, reduced feed efficiency (Brody 1945). The greater MCH and MCV and reduced RBC shown by the pregnant heifers in comparison to heifer calves may be due to greater oxygen requirement related to the metabolic demands of pregnancy (Bauman and Currie 1980). This is also supported by the observation of higher plasma concentration of CO_2 by the pregnant heifers.

The greater concentration of lymphocytes in the efficient heifers may be related to the presence of more readily available oxygen as a result of decreased energy requirements. The oxidation of protein, lipid and carbohydrate by reactive oxygen species can increase energy expenditure while decreasing cell-mediated immunity as demonstrated in lymphocyte homogenates of inefficient steers (Bottje and Cartsens 2008). During their quiescent state, lymphocytes depend on oxidative phosphorylation as the primary means of ATP production, a process which relies on oxygen as an electron receptor (Kominsky *et al.* 2010). Thus, feed efficient heifers with lower oxygen demands may have more oxygen available for oxidative phosphorylation and a preferable condition for lymphocytes, leading to an increased lymphocyte abundance.

Higher abundance of segmented neutrophils in inefficient heifers could be related to susceptibility to stress. Increased segmented neutrophil abundance in mildly stressed cattle is thought to be a result of cortisol release, causing demargination of neutrophils from blood vessel walls following β-adrenergic stimulation (Colditz 2002) that is associated with increased heart rate (Hessing *et al.* 1994) and blood pressure (Fokkema *et al.* 1995). This explanation is supported by another study where beef

heifers experiencing stress exhibited a reduced FG and a greater abundance of neutrophils (Mitlöhner *et al.* 2002).

Variation within white blood cell subclasses is also related to the cellular immune system (Tizard 2012). Monocytes such as neutrophils participate in inflammatory responses via phagocytosis while lymphocytes are involved in acquired immunity and immunoglobulin production (Thrall *et al.* 2004). The differences revealed between leukocyte subpopulations could be related to mechanisms involved with the innate immune system. During a neutrophil inflammatory response the increased oxygen consumption results in increased energy expenditure (Colditz 2002). Thus, the higher abundance of segmented neutrophils in both inefficient heifer calves and pregnant heifers may be related to increased energy requirements to support the background energy requirements, consequently impacting productive performance.

Adaptive immunity requires repetitive prompting to improve the capacity of immune response (Lawman *et al.* 1986). In a typical primary immune response IgM is the first isotype produced and this is followed by production of IgG as a result of immunoglobulin class-switching (Jain 1993). In response to a second vaccination with the same antigen, IgM production tends to be equal or lower than during the primary response; this was replicated in our study by the pregnant heifers and heifer calves, respectively. Conversely, the secondary response of IgG1 tends to be greater (Mahon *et al.* 2015) as also shown by both populations of heifers in our study. Our results for IgG1 primary and secondary responses were similar to those noted by Wagter *et al.* (1999) in dairy cows. Similar to our results, Lawrence *et al.* (2011) also found no differences between phenotypic RFI and IgG1 concentration in pregnant heifers. The

distinct developmental stages of the pregnant heifers and heifer calves could be related to the differences in IgM. As the cow reaches maturity, there is an increased capacity to produce immunoglobulins, which is observed by the abundance of immunoglobulin in the colostrum when younger and older dairy cows were compared (McGee *et al.* 2006). Moreover, the higher secondary response of IgM in the feed efficient heifers over the inefficient heifers, demonstrated when populations were evaluated comparing thirds based on RFI (Fig. 3.1), could be the result of a superior adaptive immune system and may be related to improved overall productive performance, which has been shown in other species including poultry (Dhurandhar *et al.* 2000) and swine (Magnusson *et al.* 1999).

Alkaline phosphatase catalyzes the liberation of inorganic phosphate from phosphate esters and is present in many body tissues including liver, bone and placenta (She *et al.* 2000). It has been suggested that ALP may be involved in the transport of choline (Milne 1985) and phosphates (Corathers 2006) across the cell membrane. Previous studies have shown an indirect correlation with ALP and feed intake (Richardson *et al.* 2004) and with growth rate in cattle (Walawski *et al.* 1980). Since the heifer calves in our study were at a stage of rapid growth and development, the increased concentration of ALP in feed inefficient heifer calves may be related to the increased transport of phosphates, suggesting an increased metabolic demand towards background metabolism. Conversely, the higher concentration of ALP in the efficient pregnant heifers may be related to the uterine production of the placental isoform of ALP (Cheung 2007). It has been demonstrated that insulin-like growth factors that regulate fetal growth become more active following de-phosphorylation by placental

ALP (Solomon *et al.* 2014). In addition, the transport of phosphate across cell membranes may be elevated during pregnancy due to the stimulus of placental ALP (Seleen 1978). This suggests the efficient pregnant heifers may be better equipped to deliver provisional resources to the growing fetus, an idea which requires further investigation. Moreover, the placental production of ALP increases with length of gestation in cows (Yokus and Cakir 2006); thus, the difference shown in ALP concentrations according to heifer category may be related to shifts in energy metabolism during pregnancy to support fetal growth.

Lipogenesis and lipolysis are two reciprocal mechanisms involved in the regulation of energy balance (Bauman and Currie 1980). Non-esterified fatty acids are products of the rapid mobilization of lipid and protein stores which act to increase the supply of fatty acids available for fetal growth (Adewuyi *et al.* 2005). Circulating concentrations of NEFA are elevated during late pregnancy, even when energy requirements are met (Petterson *et al.* 1994). The greater concentrations of NEFA demonstrated by the efficient pregnant heifers towards the end of the performance evaluation (Fig. 3.3) could be related to differences in fat mobilization required to support oxidative metabolism in the maternal tissues (Bell 1995). On the other hand, lower cholesterol concentrations in the efficient pregnant heifers may be related to differences in cholesterol metabolism and diminished lipogenesis. Cholesterol is synthesized primarily by the liver (Christie 1981) and its synthesis is an energetically expensive process (Van Soest 1994). The lower concentration of cholesterol in efficient pregnant heifers could indicate reduced lipogenesis, suggesting lower energetic demands by the liver. The overall cholesterol pattern shown in Fig 3.3 indicates reduced

serum cholesterol concentration in the pregnant heifers by the end of the performance evaluation, which was also observed during later stages of gestation in beef cows (Guédon *et al.* 1999).

Globulins include various enzymes and transport proteins involved in metabolic processes related to energy metabolism; their abundance can be affected by age and pregnancy (Kanecko 2008). The higher globulin concentration for the inefficient pregnant heifers may be associated with stress. For instance, stressors such as extreme weather (Cozzi *et al.* 2011), high altitude (Kumar and Kumar 2000), and stocking rate (Tarrant and Grandin 2000) are capable of increasing the plasma globulin concentration in cattle. The pregnant heifers of this study may have endured stressors including hot weather as well as a relatively high stocking rate, which could explain the greater levels of globulin. Moreover, cattle experiencing heat stress (Mitlöhner *et al.* 2002) and high stocking density (Mader and Colgan 2007) have demonstrated reduced feed efficiency. This may suggest that the inefficient pregnant heifers have less ability to cope with stressors, which may impact productivity (Koolhaas *et al.* 1999).

Creatinine is a breakdown product produced by the loss of phosphoric acid from creatine phosphate during muscle catabolism and is a marker of muscle mass in cattle (Reece 2015). The higher concentration of creatinine in efficient pregnant heifers could be related to age of the pregnant heifers (Cozzi *et al.* 2011). Greater concentration of creatinine in feed efficient pregnant heifers was also observed by Lawrence *et al.* (2011). The overall pattern for creatinine (Fig 3.4) indicated higher concentrations during the earlier sampling dates, which could be related to a warmer environment and increased amino acid catabolism (Abeni *et al.* 2007). The higher concentrations of

creatinine observed in the efficient pregnant heifers could indicate greater protein turnover in the muscle tissue (Lobley 1990). In the same way, CK is an enzyme located in the skeletal, smooth and cardiac muscle which is involved in muscle catabolism to supply amino acids for protein synthesis and gluconeogenesis (Thrall *et al.* 2004). The highest concentration of CK observed in the heifer calves may be related to the age of the heifers, and was also observed by Gonano *et al.* (2014).

Ion transport contributes 20% of the variation in basal energy expenditure between animals (Bottje and Cartsens 2008). Efficient heifer calves and the overall heifer calf group exhibited greater concentrations of plasma metabolic ions phosphorus and potassium, which may be associated with stage of growth. Potassium plays a major role in the control of cellular membrane potential and is involved in fluid balance, heart function, blood pressure, and blood pH (Suttle 2010). Potassium concentration in the blood decreases when the rate of protein synthesis within the somatic cell increases. This has been suggested by potassium deficiency inhibiting effect on growth and protein synthesis in rat muscle tissue *in vitro* (Harris and Manchester 1966) and skeletal muscle *in vivo* (Dørup and Clausen 1988). Considering that feed inefficient heifer calves exhibited a reduced concentration of potassium, this may suggest increased protein synthesis possibly due to increased protein deposition, mobilization and turnover (Lobley 1990).

Phosphorus is essential for cellular biology and energy metabolism with implications for protein synthesis (Suttle 2010). The greater concentration of phosphorus in efficient heifer calves may indicate a greater availability of phosphorus for growth and energy metabolism. Higher concentrations of phosphorus in young

cattle has been directly related to growth hormone activity, which promotes intestinal phosphate absorption and renal phosphate re-absorption (Meyer and Harvey 2004). Plasma phosphorus also contributes to the production of the muscle storage molecules including creatine phosphate and ATP (Huber and Breves 1999), providing a more readily available energy source in the post absorptive state of the efficient heifer calves. Beyond one year of age, plasma concentration of phosphorus has been shown to decrease in beef cattle (Doornenbal *et al.* 1988) which may explain the higher phosphorus concentrations for the heifer calves relative to the pregnant yearlings.

Triiodothyronine is associated with increased energy metabolism (Rauw 1998). Higher concentration of T3 is related to feed inefficiency in pullets (Van Eerden *et al.* 2006) and in non-lactating cows (Walker *et al.* 2015). Thyroid hormones increase anabolism and catabolism that result in increased heat production (Villanueva *et al.* 2013). The suggested higher concentration of T3 in the feed inefficient heifer calves and greater abundance of MCH suggest an increased metabolic rate, therefore increasing the oxygen demands and reducing feed efficiency. Triiodothyronine may also be related to the greater abundance of ALP in the feed inefficient heifer calves. When applied to rat osteoblasts in-vitro, T3 increased the activity of ALP (Sato *et al.* 1987), suggesting triiodothyronine may also be related to the greater abundance of ALP in feed inefficient heifer calves. It is also documented that, during growth, T3 has a synergetic relationship with growth hormone in heifers (Cabello and Wrutniak 1989), supporting the argument of metabolic rate differences between heifers of distinct feed efficiency.

CONCLUSION

Hematological measures have the potential to be used as indirect assessments of feed efficiency in peri-pubertal heifer calves and pregnant yearlings. Feed efficient heifer calves appear to have lower oxygen requirements, which is consonant to increased energy conservation. Results support a lack of antagonistic associations between improved feed efficiency and humoral defense as indicated by IgG1 concentrations, and a potential synergistic association as indicated by IgM in heifer calves. Cellular defense is linked with feed efficiency, as indicated by the direct and indirect associations between lymphocytes and segmented neutrophils, respectively, in both populations of heifers. Higher concentration of potassium and phosphorus in the efficient heifer calves may explain the greater efficiency during stages of growth and development. Efficient pregnant heifers appear to have reduced metabolic demands of the liver during pregnancy, as indicated by lower concentration of cholesterol and globulin. Physiological state should be taken into consideration when measuring hematological measures identified as proxies for feed efficiency.

Conflict of interest: The authors declare that they have no conflict of interest

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Table 3.1. Heifer calves descriptive statistics and least square means of productive performance traits (mean, confidence limit) by feed efficiency group.

Variable (abbreviation; unit)	Mean	SD	Efficient (n = 54)	Inefficient (n = 53)	*P*-value
Residual feed intake (RFI; kg DM/day)	0.00	1.07	-0.83 (-1.01, -0.66)	0.85 (0.67, 1.03)	<0.01
Average daily gain (ADG; kg/day)	0.73	0.16	0.71 (0.67. 0.76)	0.74 (0.70, 0.79)	0.35
Dry matter intake (DMI; kg DM/day)	6.22	1.29	5.45 (5.17, 5.73)	7.01 (6.73, 7.30)	<0.01
Feed to gain ratio (FG)	8.80	2.18	7.92 (7.38, 8.46)	9.70 (9.15, 10.24)	<0.01
Initial body weight (kg)	264.31	38.40	261.20 (250.82, 271.57)	267.47 (257.00, 277.94)	0.40
Final body weight (kg)	343.69	42.35	339.00 (327.55, 350.37)	348.51 (336.99, 360.02)	0.25
Initial rib eye area (cm^2)	32.62	4.35	32.62 (31.44, 33.80)	32.63 (31.44, 33.82)	0.99
Final rib eye area (cm^2)	48.70	6.98	47.76 (45.89, 49.64)	49.66 (47.77, 51.55)	0.16
Initial back fat (mm)	0.48	0.97	0.54 (0.28, 0.81)	0.42 (0.15, 0.68)	0.51
Final back fat (mm)	2.33	0.73	2.31 (2.12, 2.51)	2.35 (2.15, 2.55)	0.82
Initial rump fat (mm)	0.82	1.22	0.83 (0.49, 1.15)	0.82 (0.50, 1.16)	0.97
Final rump fat (mm)	2.41	1.27	2.21 (1.87, 2.55)	2.61 (2.27. 2.95)	0.11
Initial marbling[A] (score 1-11)	7.08	0.43	7.07 (6.95, 7.18)	7.10 (6.99, 7.22)	0.64
Final marbling[A] (score 1-11)	6.91	0.52	6.92 (6.78, 7.06)	6.91 (6.77, 7.05)	0.93

[A] 1: devoid; 11: prime according to Canadian beef quality grade

Table 3.2. Pregnant heifer descriptive statistics and least square means of productive performance traits (mean, confidence limit) by feed efficiency group.

Variable (abbreviation; unit)	Mean	SD	Efficient (n = 18)	Inefficient (n = 18)	*P*-value
Residual feed intake (RFI; kg DMI/day)	0.00	1.24	-1.02 (-1.45, -0.59)	1.06 (0.61, 1.50)	<0.01
Average daily gain (ADG; kg/day)	0.99	0.17	1.01 (0.93, 1.10)	1.02 (0.94, 1.11)	0.86
Dry matter intake (DMI; kg/day)	9.68	1.73	8.64 (7.89, 9.39)	10.93 (10.15, 11.70)	<0.01
Feed to gain ratio (FG)	3.00	0.62	2.63 (2.36, 2.90)	3.24 (2.96, 3.52)	<0.01
Days in gestation (DIG; days)	137.35	78.31	118.25 (78.88, 157.62)	157.73 (117.08, 198.39)	0.16
Initial body weight (kg)	418.39	45.88	416.46 (392.60, 440.32)	432.28 (407.64, 456.92)	0.35
Final body weight (kg)	542.11	53.22	545.05 (516.67, 569.44)	560.22 (532.97, 587.46)	0.36
Initial rib eye area (cm^2)	38.51	8.52	37.50 (33.04, 41.96)	41.40 (36.80, 46.00)	0.22
Final rib eye area (cm^2)	63.74	6.50	63.06 (59.52, 66.61)	65.00 (61.32, 68.65)	0.45
Initial back fat (mm)	1.31	1.06	1.08 (0.52, 1.63)	1.64 (1.06, 2.22)	0.16
Final back fat (mm)	2.65	1.15	2.56 (1.94, 3.18)	2.90 (2.27, 3.54)	0.44
Initial rump fat (mm)	2.55	1.90	2.41 (1.12, 3.08)	2.59 (1.22, 3.34)	0.79
Final rump fat (mm)	3.84	2.35	3.67 (2.18, 4.26)	4.13 (2.36, 4.72)	0.62
Initial marbling[A] (score 1-11)	7.44	0.46	7.43 (7.19, 7.67)	7.42 (7.18, 7.67)	0.98
Final marbling[A] (score 1-11)	6.38	0.70	6.49 (6.17, 6.82)	6.38 (6.04, 6.71)	0.60

[A] 1: devoid; 11: prime according to Canadian beef quality grade

Table 3.3. Complete blood cell count least square means comparison (mean, confidence limit) in heifer calves at the start and end of the performance evaluation by feed efficiency group.

Analyte (abbreviation; unit)	Start of performance evaluation			End of performance evaluation		
	Efficient	Inefficient	*P*-value	Efficient	Inefficient	*P*-value
Fibrinogen (g/dL)	4.26 (4.00, 4.52)	4.19 (3.93, 4.45)	0.70	3.80 (3.50, 4.09)	3.85 (3.55, 4.15)	0.81
Hematocrit (%)	35.93 (34.86, 36.99)	35.87 (34.79, 36.95)	0.94	35.37 (34.37, 36.37)	36.09 (35.08, 37.11)	0.32
Hemoglobin (mg/dL)	120.48 (117.1, 124.5)	120.81 (116.8, 124.1)	0.90	122.35 (122.0, 129.2)	125.62 (118.8, 125.9)	0.20
Lymphocytes (% WBC)	67.07 (64.35, 69.76)	63.30 (60.59, 66.01)	0.05	62.24 (59.41, 65.07)	60.17 (57.29, 63.05)	0.31
Mean cell hemoglobin (MCH; pg)	14.55 (14.39, 14.71)	14.75 (14.59, 14.91)	0.08	16.01 (15.79, 16.24)	16.43 (16.20, 16.65)	0.01
Mean corpuscular value (MCV; fL)	43.39 (42.85, 43.93)	43.88 (43.34, 44.42)	0.21	46.29 (45.60, 47.00)	47.22 (46.51, 47.92)	0.07
Monocytes (% WBC)	3.61 (3.07, 4.16)	3.53 (3.00, 4.08)	0.83	4.10 (3.44, 4.75)	3.71 (3.04, 4.39)	0.42
Platelets (10^3 cells/µl)	514.33 (473.16, 555.01)	486.3 (444.74, 527.86)	0.34	416.63 (384.55, 448.71)	430.42 (398.04, 462.80)	0.55
Red blood cell count (RBC; 10^6 cells/µl)	8.29 (8.05, 8.54)	8.19 (7.94, 8.44)	0.56	7.65 (7.43, 7.87)	7.67 (7.44, 7.89)	0.92
Segmented neutrophils (% WBC)	27.11 (24.51, 29.71)	30.85 (28.22, 33.48)	0.05	26.89 (24.51, 29.27)	26.98 (24.56, 29.40)	0.96
White blood cell count (WBC; 10^3 cells/µl)	10.31 (9.74, 10.90)	10.28 (9.70, 10.90)	0.94	10.13 (9.50, 10.80)	9.81 (9.17, 10.45)	0.48

Table 3.4. Complete blood cell count least square means comparison (mean, confidence limit) in pregnant heifers at the start and end of the performance evaluation by feed efficiency group.

Analyte (abbreviation; unit)	Start of performance evaluation			End of performance evaluation		
	Efficient	Inefficient	*P*-value	Efficient	Inefficient	*P*-value
Fibrinogen (g/dL)	4.94 (4.35, 5.54)	4.38 (3.69, 5.08)	0.22	3.78 (2.99, 4.57)	4.23 (3.30, 5.16)	0.45
Hematocrit (%)	32.38 (30.71, 34.05)	31.67 (29.95, 33.40)	0.49	35.88 (34.33, 37.42)	36.27 (34.67, 37.86)	0.92
Hemoglobin (mg/dL)	117.81 (111.21, 124.42)	114.53 (107.71, 121.36)	0.49	121.00 (115.46, 126.54)	122.53 (116.82, 128.25)	0.72
Lymphocytes (% WBC)	62.31 (57.78, 66.84)	62.13 (57.45, 66.81)	0.96	68.44 (64.48, 72.40)	62.53 (58.45, 66.62)	0.04
Mean cell hemoglobin (MCH; pg)	15.28 (14.70, 15.85)	16.04 (15.44, 16.64)	0.15	36.33 (7.42, 65.23)	17.50 (-12.35, 47.35)	0.36
Mean corpuscular value (MCV; fL)	41.94 (40.30, 43.70)	43.94 (42.10, 45.96)	0.07	50.04 (48.38, 51.70)	51.88 (49.94, 53.83)	0.23
Monocytes (% WBC)	3.67 (2.30, 5.03)	3.23 (1.63, 4.84)	0.68	4.65 (3.34, 5.96)	4.46 (2.96, 5.96)	0.85
Platelets (10^3 cells/µl)	396.75 (332.65, 460.85)	340.93 (272.40, 409.45)	0.23	306.56 (252.59, 360.54)	310.87 (255.12, 366.61)	0.91
Red blood cell count (RBC; 10^6 cells/µl)	7.72 (7.21, 8.23)	7.13 (6.68, 7.74)	0.17	7.23 (6.86, 7.59)	7.03 (6.65, 7.41)	0.45
Segmented neutrophils (% WBC)	30.50 (26.36, 34.64)	31.13 (26.85, 35.41)	0.83	20.00 (16.67, 23.33)	26.60 (23.16, 30.04)	0.01
White blood cell count (WBC; 10^3 cells/µl)	8.94 (8.18, 9.78)	7.96 (7.26, 8.73)	0.07	8.24 (7.33, 9.26)	8.06 (7.14, 9.10)	0.80

Table 3.5. Immunoglobulin response to OVA (mean, confidence limit) in heifer calves and pregnant heifers grouped by high and low feed efficiency.

Immunoglobulin response	Heifer calves			Pregnant heifers		
	Efficient	Inefficient	*P*-value	Efficient	Inefficient	*P*-value
IgM primary	0.31 (0.25, 0.37)	0.35 (0.29, 0.41)	0.36	0.30 (0.16, 0.44)	0.19 (0.05, 0.34)	0.30
IgM secondary	0.30 (0.21, 0.37)	0.28 (0.22, 0.36)	0.71	0.52 (0.31, 0.73)	0.60 (0.38, 0.82)	0.61
IgG1 primary	0.21 (0.17, 0.24)	0.24 (0.20, 0.27)	0.20	0.25 (0.18, 0.32)	0.21 (0.13, 0.28)	0.39
IgG1 secondary	0.84 (0.77, 0.90)	0.85 (0.79, 0.91)	0.76	0.82 (0.70, 0.94)	0.80 (0.68, 0.92)	0.82

Table 3.6. Least square means comparison for CBC parameters and plasma metabolites (mean, confidence limit) in heifer calves and pregnant heifers.

Traits (abbreviation; unit)	Heifer calves	Pregnant heifers	*P*-value
CBC parameters			
Fibrinogen (g/dL)	4.03 (3.87, 4.19)	4.34 (4.04, 4.64)	0.07
Hematocrit (%)	35.81 (35.22, 36.40)	34.05 (32.98, 35.14)	0.01
Hemoglobin (mg/dL)	122.31 (120.16, 124.45)	118.98 (115.00, 122.97)	0.15
Lymphocytes (% WBC)	63.29 (61.69, 64.89)	63.90 (60.93, 66.87)	0.72
Mean cell hemoglobin (MCH; pg)	15.44 (15.31, 15.57)	16.16 (15.89, 16.42)	<0.01
Mean corpuscular value (MCV; fL)	45.05 (44.70, 45.41)	46.06 (45.35, 46.79)	0.01
Monocytes (% WBC)	3.75 (3.42, 4.09)	4.10 (3.47, 4.72)	0.34
Platelets (10^3 cells/µl)	461.95 (440.32, 483.58)	336.45 (296.26, 376.64)	<0.01
Red blood cell count (RBC; 10^6 cells/µl)	7.95 (7.81, 8.10)	7.30 (7.03, 7.57)	<0.01
Segmented neutrophils (% WBC)	27.90 (26.50, 29.29)	27.00 (24.40, 29.60)	0.55
White blood cell count (WBC; 10^3 cells/µl)	9.95 (9.60, 10.32)	8.33 (7.79, 8.91)	<0.01
Plasma analytes			
Enzymes (U/L)			
Alkaline phosphatase (ALP; U/L)	95.07 (88.87, 102.20)	114.81 (99.28, 136.11)	0.02
Aspartate aminotransferase (AST; U/L)	54.95 (53.65, 56.24)	56.85 (54.48, 59.22)	0.17
Creatine kinase (CK; U/L)	170.97 (163.90, 178.34)	153.56 (141.95, 166.12)	0.02
Gamma glutamyl transferase (GGT; U/L)	16.20 (15.76, 16.64)	16.22 (15.41, 17.03)	0.96
Glutamate dehydrogenase (GLDH; U/L)	18.12 (16.95, 19.36)	16.06 (14.23, 18.12)	0.09
Compounds			
Albumin (g/L)	33.98 (33.70, 34.26)	33.81 (33.30, 34.32)	0.56
Albumin : globulin ratio	1.03 (1.01, 1.05)	0.99 (0.96, 1.03)	0.08
Beta hydroxy butarate acid (BHBA; µmol/L)	160.72 (154.24, 167.20)	167.62 (155.58, 179.66)	0.32
Carbon dioxide (CO_2 ; mmol/L)	23.40 (23.07, 23.73)	26.24 (25.62, 26.85)	<0.01
Cholesterol (mmol/L)	3.42 (3.31, 3.53)	3.05 (2.85, 3.25)	<0.01
Creatinine (µmol/L)	114.45 (111.47, 117.42)	125.89 (120.36, 131.41)	<0.01
Globulin (g/L)	33.50 (33.02, 33.98)	34.53 (33.65, 35.40)	0.04
Glucose (mmol/L)	4.36 (4.29, 4.43)	3.96 (3.85, 4.08)	<0.01
Non-esterified fatty acids (NEFA: mmol/L)	0.26 (0.25, 0.28)	0.28 (0.25, 0.32)	0.34
Urea (mmol/L)	2.65 (2.58, 2.72)	2.76 (2.63, 2.88)	0.15
Ions (mmol/L)			
Anion gap (mmol/L)	27.37 (26.92, 27.83)	27.84 (27.00, 28.71)	0.33
Calcium (mmol/L)	2.40 (2.38, 2.41)	2.39 (2.37, 2.42)	0.84
Chloride (mmol/L)	94.60 (94.33, 94.87)	96.56 (96.05, 97.06)	<0.01
Magnesium (mmol/L)	0.87 (0.86, 0.88)	0.89 (0.87, 0.91)	0.19
Osmolality (mmol/L)	277.50 (277.01, 278.00)	277.04 (276.12, 277.97)	0.39
Potassium (mmol/L)	4.30 (4.26, 4.34)	4.21 (4.13, 4.28)	0.03
Phosphorus (mmol/L)	2.19 (2.15, 2.23)	2.00 (1.93, 2.07)	<0.01
Sodium (mmol/L)	141.12 (140.86, 141.37)	141.14 (140.66, 141.61)	0.94
Hormone			
Triiodothyronine (T3; nmol/L)	1.86 (1.80, 1.91)	1.77 (1.67, 1.87)	0.15

Table 3.7. Plasma analytes least square means comparison (mean, confidence limit) in heifer calves by feed efficiency group.

Traits (abbreviation; unit)	Efficient	Inefficient	*P*-value
Enzymes (U/L)			
Alkaline phosphatase (ALP; U/L)	86.43 (93.89, 121.36)	98.62 (103.77, 131.47)	0.03
Aspartate aminotransferase (AST; U/L)	56.10 (54.27, 57.93)	55.15 (53.30, 57.00)	0.24
Creatine kinase (CK; U/L)	160.46 (170.17, 196.23)	154.73 (165.40, 191.76)	0.22
Gamma glutamyl transferase (GGT; U/L)	16.48 (15.85, 17.12)	16.35 (15.71, 16.99)	0.76
Glutamate dehydrogenase (GLDH; U/L)	20.44 (17.87, 23.02)	21.55 (18.95, 24.16)	0.55
Compounds			
Albumin (g/L)	33.76 (33.34, 34.17)	33.80 (33.38, 34.23)	0.87
Albumin globulin ratio	1.01 (0.99, 1.04)	1.02 (1.00, 1.05)	0.18
Beta hydroxy butarate acid (BHBA; µmol/L)	155.95 (153.17, 168.85)	156.02 (152.51, 168.33)	0.99
Carbon dioxide (CO_2 ; mmol/L)	23.34 (22.91, 23.77)	23.45 (23.01, 23.88)	0.73
Cholesterol (mmol/L)	3.32 (3.27, 3.54)	3.36 (3.31, 3.57)	0.72
Creatinine (µmol/L)	115.70 (112.20, 119.20)	113.21 (109.68, 116.74)	0.32
Globulin (g/L)	34.07 (33.31, 34.83)	33.71 (32.94, 34.47)	0.50
Glucose (mmol/L)	4.43 (4.30, 4.57)	4.47 (4.33, 4.61)	0.32
Non-esterified fatty acids (NEFA: mmol/L)	0.35 (0.31, 0.38)	0.31 (0.28, 0.35)	0.15
Urea (mmol/L)	2.64 (2.57, 2.71)	2.65 (2.58, 2.73)	0.79
Ions (mmol/L)			
Anion Gap (mmol/L)	27.71 (27.12, 28.31)	27.23 (26.63, 27.83)	0.26
Calcium (mmol/L)	2.39 (2.38, 2.41)	2.40 (2.38, 2.42)	0.55
Chloride (mmol/L)	94.49 (94.18, 94.80)	94.71 (94.39, 95.03)	0.33
Magnesium (mmol/L)	0.870 (0.856, 0.883)	0.877 (0.864, 0.890)	0.44
Osmolality (mmol/L)	277.53 (276.91, 278.14)	277.46 (276.84, 278.07)	0.87
Potassium (mmol/L)	2.23 (2.18, 2.27)	2.16 (2.11, 2.20)	0.03
Phosphorus (mmol/L)	4.36 (4.31, 4.41)	4.24 (4.19, 4.29)	<0.01
Sodium (mmol/L)	141.12 (140.81, 141.43)	141.11 (140.80, 141.43)	0.98
Hormone			
Triiodothyronine (T3; nmol/L)	1.81 (1.75, 1.88)	1.90 (1.83, 1.96)	0.06

Table 3.8. Plasma metabolite least square means comparison (mean, confidence limit) in pregnant yearlings by feed efficiency group.

Traits (abbreviation; unit)	Efficient	Inefficient	*P*-value
Enzymes (U/L)			
Alkaline phosphatase (ALP; U/L)	119.90 (97.94, 154.54)	71.17 (62.58, 82.58)	<0.01
Aspartate aminotransferase (AST; U/L)	55.42 (52.60, 58.24)	58.42 (55.50, 61.34)	0.14
Creatine kinase (CK; U/L)	131.84 (122.25, 143.06)	143.76 (132.03, 157.75)	0.14
Gamma glutamyl transferase (GGT; U/L)	16.23 (15.10, 17.35)	16.21 (15.04, 17.37)	0.98
Glutamate dehydrogenase (GLDH; U/L)	14.34 (12.14, 16.95)	16.25 (13.67, 19.31)	0.30
Compounds			
Albumin (g/L)	33.71 (33.15, 34.27)	33.92 (33.34, 34.50)	0.61
Albumin globulin ratio	1.02 (0.98, 1.06)	0.97 (0.93, 1.01)	0.07
Beta hydroxy butarate acid (BHBA; µmol/L)	161.38 (147.48, 175.90)	165.66 (151.05, 180.94)	0.67
Carbon dioxide (CO_2 ; mmol/L)	22.90 (22.22, 23.58)	22.30 (21.59, 23.01)	0.22
Cholesterol (mmol/L)	2.68 (2.40, 2.97)	3.42 (3.13, 3.72)	<0.01
Creatinine (µmol/L)	131.45 (123.35, 139.55)	119.71 (111.34, 128.09)	0.05
Globulin (g/L)	33.69 (32.45, 34.69)	35.56 (34.42, 36.70)	0.02
Glucose (mmol/L)	3.96 (3.87, 4.05)	3.93 (3.84, 4.02)	0.62
Non-esterified fatty acids (NEFA: mmol/L)	0.34 (0.30, 0.38)	0.24 (0.21, 0.28)	<0.01
Urea (mmol/L)	2.74 (2.59, 2.89)	2.57 (2.43, 2.72)	0.10
Ions (mmol/L)			
Anion Gap (mmol/L)	26.54 (25.80, 27.26)	26.55 (25.78, 27.29)	0.99
Calcium (mmol/L)	2.37 (2.34, 2.40)	2.42 (2.38, 2.45)	0.06
Chloride (mmol/L)	96.56 (95.80, 97.33)	96.53 (95.74, 97.32)	0.95
Magnesium (mmol/L)	0.88 (0.85, 0.91)	0.89 (0.87, 0.92)	0.54
Osmolality (mmol/L)	277.51 (276.45, 278.57)	276.53 (275.42, 277.63)	0.20
Potassium (mmol/L)	4.15 (4.06, 4.24)	4.24 (4.15, 4.34)	0.15
Phosphorus (mmol/L)	2.02 (1.91, 2.14)	1.97 (1.86, 2.09)	0.52
Sodium (mmol/L)	141.39 (140.83, 141.95)	140.87 (140.28, 141.45)	0.20
Hormone			
Triiodothyronine (T3; nmol/L)	1.83 (1.70, 1.95)	1.71 (1.58, 1.84)	0.20

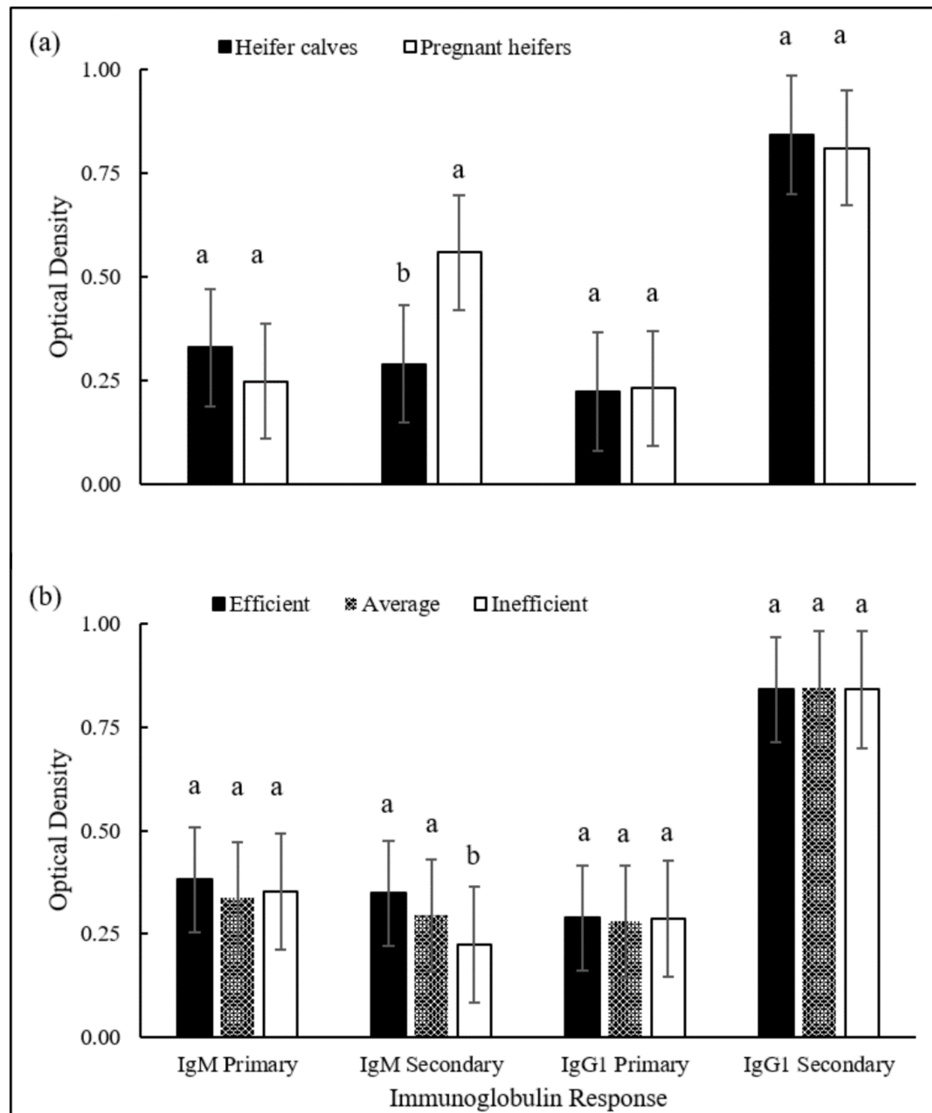

Figure 3.1. Specific immune response of immunoglobulins M (IgM) and G1 (IgG1) to ovalbumin in beef heifers (a) heifer calves and pregnant heifers; and (b) heifer calves by feed efficiency groups (efficient, average and inefficient). Differing superscript denotes $P \leq 0.05$, according to the Scheffé multiple comparison test and error bars are the 95% confidence interval.

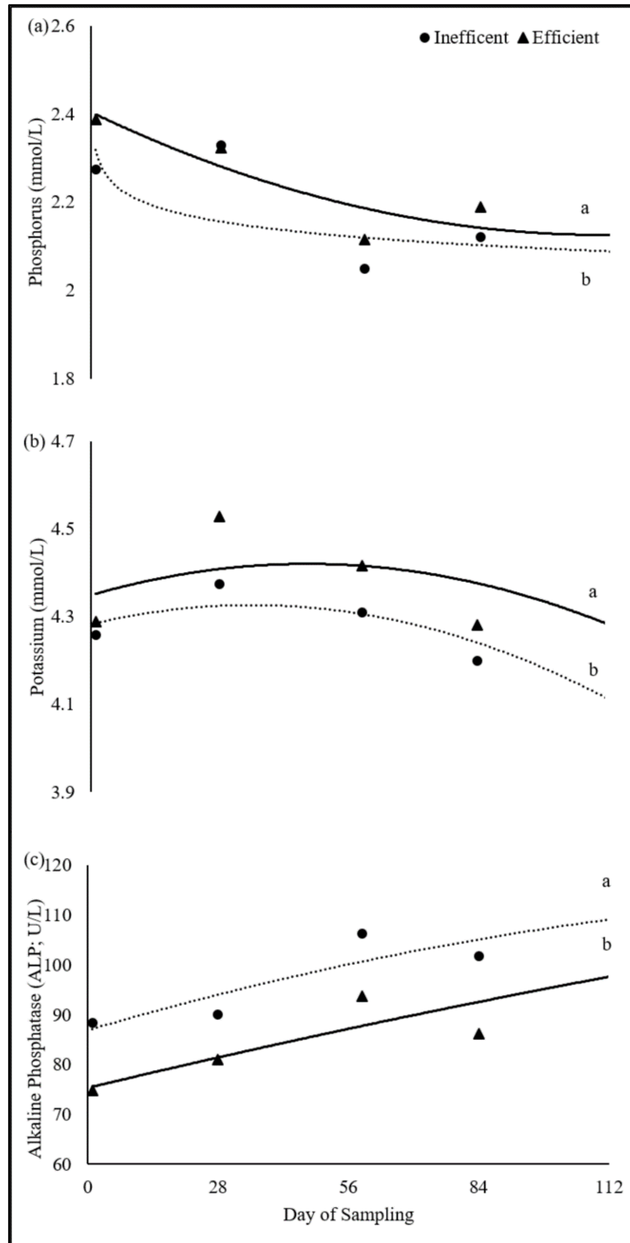

Figure 3.2. Plasma metabolites in heifer calves over the performance evaluation by feed efficiency group, (···●···) inefficient and (---▲---) efficient. (a) Phosphorus, (b) Potassium and (c) Alkaline phosphatase. Differing superscript denotes $P \leq 0.05$ within day of sampling, according to the Scheffé multiple comparison test.

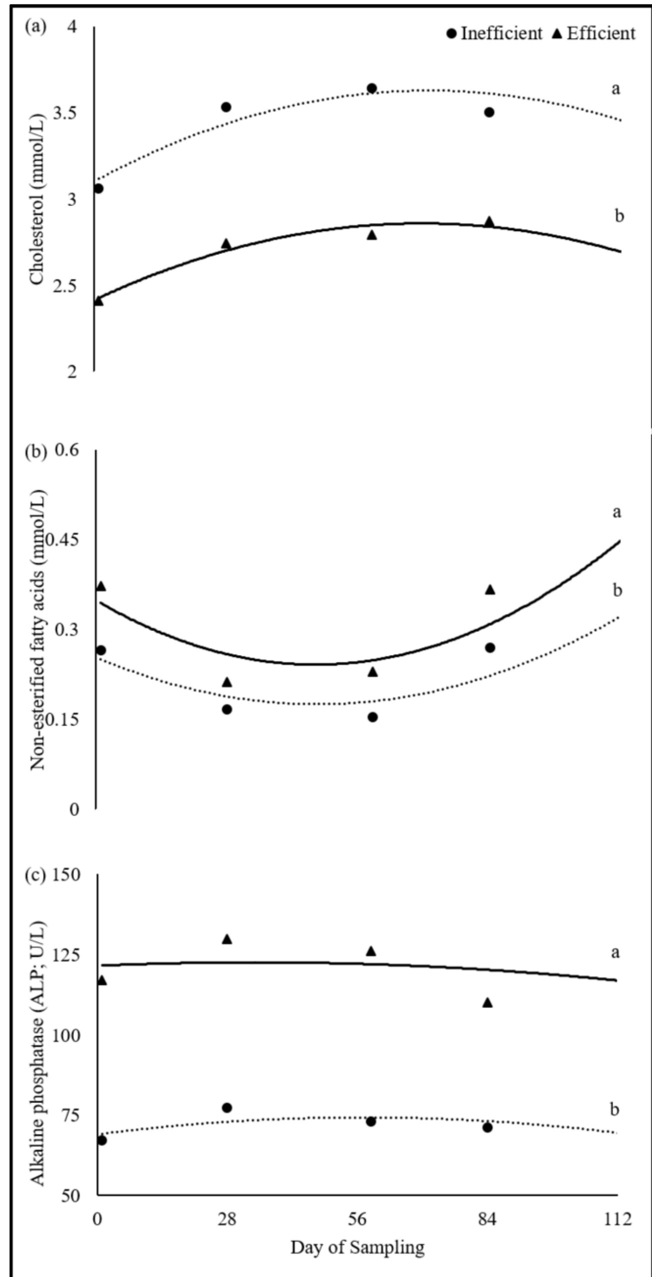

Figure 3.3. Plasma metabolites in pregnant heifers over the performance evaluation by feed efficiency group, (┈●┈) inefficient and (---▲---) efficient. (a) Cholesterol, (b) Non-esterified fatty acids and (c) Alkaline phosphatase. Differing superscript denotes $P \leq 0.05$, according to the Scheffé multiple comparison test.

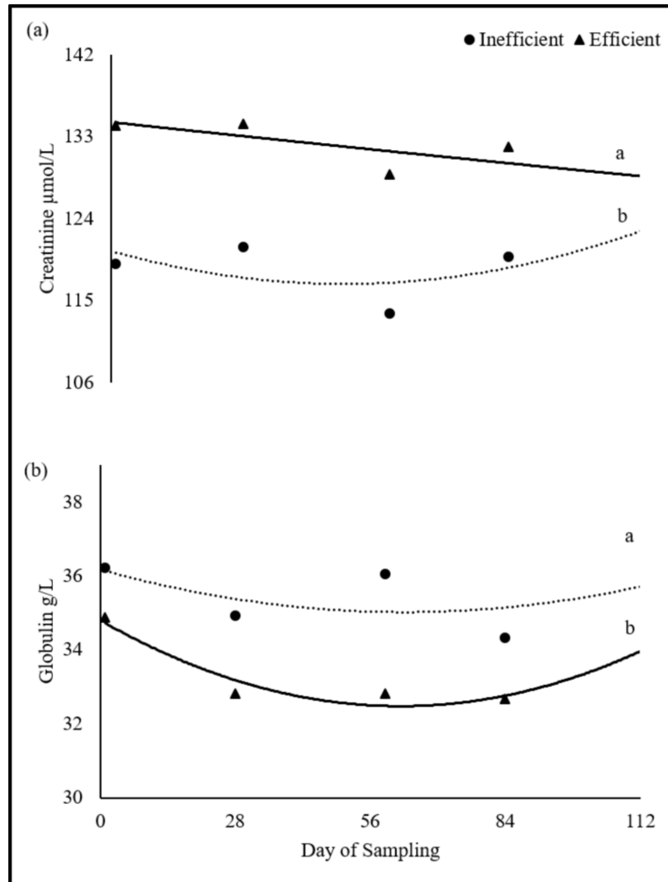

Figure 3.4. Plasma metabolites in pregnant heifers over the performance evaluation by feed efficiency group, (┈●┈) inefficient and (---▲---) efficient. (a) Creatinine and (b) Globulin. Differing superscript denotes $P \leq 0.05$, according to the Scheffé multiple comparison test.

Chapter 4

METABOLIC PROFILE OF BEEF HEIFERS DURING ESTRUS AND NON-ESTRUS STATES

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Abstract: Hematological metabolic profiles in heifers could contribute to the development of proxies for estrus detection and provide clues to further understand female biology in response to the estrus state. One-hundred-and-seven beef heifers were observed for estrus behaviour twice daily for 124 days. Feed intake and productive performance (body weight and composition) traits were measured and feed efficiency was determined using residual feed intake (RFI; kg DM/day). Blood plasma samples were collected when signs of estrus were observed and every 30±2 days. Heifers were considered in estrus (n = 71) when plasma progesterone concentrations were <0.6 ng/ml. Least square means of metabolic profiles were compared between estrus and non-estrus state and within estrus state by groupings heifers according to performance traits and age. Heifers in estrus exhibited higher concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), beta-hydroxybutyric acid (BHBA), creatine kinase (CK) and triiodothyronine (T3) than non-estrus state. Heifers

in estrus revealed lower osmolality and concentrations of calcium, sodium, and total protein than during non-estrus state. Younger (and smaller) heifers had greater concentrations of CK, gamma-glutamate transferase (GGT), glucose and sodium than the older heifers. Heifers with reduced fatness had increased osmolality and concentrations of cholesterol, CK, phosphorus, sodium and reduced T3 levels. Feed efficient heifers had greater levels of AST, cholesterol and GGT than inefficient heifers. Plasma metabolites may be complementary to estrus detection upon further validation and effects of age, feed efficiency, body size and body composition should be considered to optimize this hematological assessment.

Keywords: body composition, creatine kinase, estrus detection, fatness, feed efficiency, triiodothyronine

INTRODUCTION

Reproduction is recognized as an important factor which influences profitability in the cow-calf sector. The increasing use of reproductive technologies in the beef industry relies on successful estrus detection. Despite the variety of estrus detection methods available (Roelofs *et al.* 2015), there is a possibility of identifying complementary indicators through blood plasma metabolic profiling. In fact, previous studies have illustrated the association of blood plasma with reproductive performance in heifers (Small *et al.* 1996), such as improved conception rates in heifers with greater phosphorus concentration during estrus. Additionally, the biology of the estrus state

may be better understood by evaluating associations between blood plasma analytes during estrus with body weight and composition, age and feed efficiency.

During estrus, metabolic shifts occur in association with the preparation of the female reproductive tract for breeding (Ball and Peters 2007). Blood serum ions including sodium have been positively associated during estrus day with conception rates in heifers (Small *et al.* 1996). Similarly, calcium levels have been positively related to oviduct ion concentrations in beef heifers during estrus (Hugentobler *et al.* 2007). Enzymes such as creatine kinase (CK) have been associated with energy metabolism in cattle (Schlattner *et al.* 2006) and strenuous muscle activity in beef heifers during estrus (Kenny and Tarrant 1988). Greater ALP concentration during estrus in the bovine has been attributed to increased uteral ALP isoform production during estrus (Moss *et al.* 1954). Markers of energy status, such as beta-hydroxybutyric acid (BHBA), have been positively related to energy intake during estrus in ewes (Fernández-Foren *et al.* 2011). Triiodothyronine (T3) have association with reproduction, such as the effect on follicle-stimulating hormone to amplify differentiation of granulosa cells during ovulation in women (Maruo *et al.* 1992). Additionally, T3 has profound effects in the regulation of changes in metabolism (Villanueva *et al.* 2013), similarly as those observed during estrus.

Body condition can affect components of the hypothalamic-pituitary-gonadal-axis (Pradhan and Nakagoshi, 2008). Fatness has shown a positive association with reproductive traits, including age of puberty (Brooks *et al.* 1985) and the return to estrus following calving (Short and Bellows 1971). Biomarkers related to adipose tissue metabolism, such as non-esterified fatty acids (NEFA) and BHBA, have been

negatively associated with reproductive performance in cattle (Ospina *et al.* 2010). Lean body content has been positively related to extended anestrus state (Short and Bellows 1971). Biomarkers related to lean tissue, such as CK, can indicate increased metabolism in muscle tissue (Schlattner *et al.* 2006). Moreover, it has been established that body composition can be affected by such factors as age (Brody 1945), which has also been shown to influence the metabolite profile (Doornenbal *et al.* 1988). Likewise, the energetic shifts associated with estrus state (Parker *et al.* 2001) suggests that indicators of energy metabolism, such as residual feed intake (RFI), may be complementary to understand the biology underlying the estrus state.

The estrus state is associated with metabolic shifts, which are also related to body composition and metabolic rate. Plasma metabolite profiling during this state may further the understanding of estrus physiology and potentially identify proxies for estrus. The objectives of this study were to: a) characterize blood plasma analytes in beef heifers during estrus and non-estrus states; and b) compare the metabolic profile during estrus in relation to age, feed efficiency, body size, body fatness and leanness categories.

MATERIAL AND METHODS

Animals and experimental design

The experimental procedures involving animals were performed according to the recommendations of the Canadian Council on Animal Care guidelines (2009). A group of 107 crossbred replacement heifers (290±25 days (mean±SD) of age, 253±37.8

kg of body weight (BW) at the start of the testing period and 418±25 days of age, 343.69±42.4 kg of BW at the end of testing period) were consigned from 14 producers from the Atlantic region of Canada and housed at the Maritime Beef Testing Society (Nappan, Canada). Breed composition of individual heifers was determined via hair follicle DNA extraction and 50K SNP sequencing (ADMIXTURE® software, University of California, Los Angeles, USA) (Connolly *et al.* 2014). The average breed composition was 31.13% Angus, 18.74% Simmental, 13.98% Limousine, 10.49% Maine Anjou, 9.94% Hereford and 15.72% other European beef breeds. Heifers were fed a total mixed ration (dry matter basis) composed of 99.5% haylage and 0.5% mineral and vitamin premix[2] with digestible energy of 2.92 Mcal/kg. Individual feed intakes were recorded using a feeding system (GrowSafe® Feed Intake System, Airdrie, Canada). Heifers were tested for productive performance and observed for signs of estrus for 124 days, from mid-June to the end of October (2014).

Productive performance evaluation

In order to calculate RFI, body weight and ultrasound measures were taken every 30±2 days. Body weight was recorded in the morning of the performance evaluation prior to feeding. Heifers were ultrasound scanned using an Aloka SSD-500 (model 5044; 172 mm; 3.5 MHz; Corometrics Medical Systems, Wallingford, USA) equipped with a long probe. Ultrasound images were used to determine back fat thickness (BFKT, mm), rib eye area (REA, cm^2), intramuscular fat (marbling) score

[2] Contains 7.8% Na, 27% Ca, 0.02% P, 2.5% Mg, 2,400 mg/kg Fe, 900 mg/kg Cu, 75 mg/kg iodine, 2,300 mg/kg Mn, 2,400 mg/kg Zn, 13 mg/kg Co, 3000 mg/kg Fl; Vitamins: 200,000 IU/kg A, 27,000 IU/kg D3, 4000 IU/kg E.

(MARB, score 1: devoid; 11: prime) and rump fat (RUMP, mm). Average ultrasound traits over the performance evaluation were determined using linear regression. Residual feed intake was defined as the difference between predicted feed intake and actual feed intake (Koch *et al.* 1963). Predicted feed intake was determined as a multiple linear regression of DMI on ADG, BW, BKFT, REA, MARB, RUMP and age ($R^2 = 0.43$), similarly, as detailed by Montanholi *et al.* (2009).

Estrus detection and blood sampling

Heifers were fitted with estrus detection patches (Estrotect™ Heat Detector, MAI Animal Health, Elmwood, USA) and observed in the morning and evening for estrus behaviours, such as bawling, restlessness, and mounting (Roelofs *et al.* 2010). Heifers with a scratched patch and/or displaying estrus signs were brought into the handling facility. Heifers were then examined for signs of estrus, including edematous labia majora of the vulva, pink to red colour of the vaginal mucosa and presence of viscous mucus secretion in the vulva, as well as mounting marks in the lumbar region and around the flanks (Roelofs *et al.* 2010). Heifers identified in estrus state were blood sampled (jugular venipuncture) and plasma was extracted, as described by Montanholi *et al.* (2013b). To confirm an estrus state, plasma samples were analyzed for progesterone content, using a radioimmunoassay (Immunochem™ Coated Tube Progesterone RIA Kit, ICN Pharmaceuticals, Costa Mesa, USA), validated by Rawlings *et al.* 1984. Heifers with progesterone concentrations <0.6 ng/ml were considered in estrus, similar to McCarthy *et al.* (2012). Blood samples were also collected during the productive performance evaluation every 30 ± 2 days. The non-

estrus sample used for plasma metabolite profile comparison was selected from the productive performance sampling within 12±8 days of the estrus day for each heifer. The approximate 21 day duration of the bovine estrus cycle (Amstalden and Williams 2015) was considered to ensure that the performance sample was a non-estrus sample.

Blood plasma analytes

Estrus and non-estrus plasma samples were assessed for the metabolite profile. Concentrations of blood plasma metabolic ions: calcium (mmol/L), phosphorus (mmol/L), sodium (mmol/L) and potassium (mmol/L); and compounds: albumin (g/L), globulin (g/L), total protein (TP; g/L), carbon dioxide (CO_2; mmol/L)cholesterol (mmol/L), creatinine (μmol/L), glucose (mmol/L), non-esterified fatty acid (NEFA; mmol/L), urea (mmol/L); and; enzymes: alkaline phosphatase (ALP; U/L), aspartate aminotransferase (AST; U/L),), creatine kinase (CK; U/L), gamma-glutamyl transferase (GGT; U/L) and glutamate dehydrogenase (GLDH; U/L) were determined using an automated analyzer (Cobas® c 311/501 analyzer, Roche Diagnostics GmbH, Indianapolis, USA). Determination of beta-hydroxybutyric acid (BHBA) was done using colorimetry (Randox ®, RANDOX Laboratories Ltd., Ireland, UK). Plasma globulin was determined by subtracting albumin from the total protein concentration. Osmolality (mmol/L) was determined as described by Bhagat *et al.* 1984. Changes in total plasma volume (TPV; %) was calculated as described by Boyd (1981) using estrus and non-estrus TP concentrations. Blood plasma concentrations of triiodothyronine (T3; nmol/L) was quantified using an ELISA assay (IMMULITE 1000, Siemens Healthcare Diagnostic Products, Malvern, USA).

Statistical Analyses

Data was analyzed using the SAS software (v 9.4, SAS Institute Inc., Cary, USA). Normality was tested using the univariate procedure and required the following transformations: natural logarithm (ALP, globulin, GLDH and T3), square root (BHBA and glucose), and reciprocal (CK). The GLM procedure was used to compare the plasma metabolite means during estrus and non-estrus states according to the model:

$$Y_{ijkl} = \mu + \text{Group}_i + \text{Age}_j + \text{Breed}_k + e_{ijkl}$$

where Y_{ijkl} is the l-th trait measured on the k-th breed and at j-th age for the i-th trait grouping, μ is the overall mean of the trait, Group_i is the metabolite or performance trait group, Breed_k is the breed composition and e_{ijkl} is the residual random effect associated with the l-th measure. Plasma metabolites were compared across the extremes of the phenotypic classifications (for age, body weight, REA, BKFT, RUMP, MARB and RFI) within the estrus samples by comparing the 25.35% (n = 18) most divergent phenotypes in each tail of the normal distributions. These categorical groupings were different as indicated in Tables 4.2 and 4.3. The odds ratio of identifying an estrus state by plasma metabolite measures was verified using the logistic regression procedure by fitting all the analytes that differed in least square means between estrus and non-estrus states. The variation of the plasma metabolites in relation to RFI, was determined by the variance importance analysis procedure also by including the analytes that differed between estrus and non-estrus states. For all analyses, results were considered significant when $p \leq 0.05$ and a trend towards significance when $0.10 \geq p > 0.05$.

RESULTS

Figure 4.1 shows the dispersion of progesterone concentrations of the heifers found with signs of estrus. Metabolic profiles during estrus and non-estrus states are shown in Table 4.1. Estrus state was associated with greater concentrations of ALP, AST, BHBA, CK and T3 and a 4.10% increase in blood plasma volume, in relation to the non-estrus state. Moreover, estrus state was associated with reduced osmolality and levels of calcium, GLDH, globulin, sodium and total protein, in comparison to the non-estrus state. The global analysis of these metabolic differences revealed a 91.5% probability of identifying estrus state based on ALP, AST, BHBA, CK and T3, with 76% accounted solely by T3. For every one unit increase of ALP, AST, BHBA, CK and T3, the certainty of the heifer being in estrus increases by a factor of 1.001, 1.036, 1.032, 1.001 and 3.420, respectively.

The arrangement of heifers in the estrus state, according to age, body size and composition, and feed efficiency, was also associated with differences in the metabolic profile. Younger and older heifers differed between GGT and glucose concentrations (Table 4.2). Heavier and lighter heifers had differences in CK and sodium concentrations (Table 4.2). Leanness was related to plasma globulin and TP (Table 4.2). Fatness was associated with differences in CK, osmolality and sodium concentrations, based on MARB (Table 4.3), and with differences in cholesterol, phosphorus and T3, based on RUMP (Table 4.3). In relation to feed efficiency, heifers with a low RFI (feed efficient) revealed greater cholesterol (3.96 vs. 3.56; $p \leq 0.05$), compared to high RFI heifers. Additionally, cholesterol indicated the strongest

variation in relation to RFI, followed by GLDH, potassium, phosphorus, T3, glucose, TPV, ALP, GGT, AST, calcium and AG (Fig. 4.2).

DISCUSSION

Detection of estrus remains an important topic due to the global trend of increasing individual herd size (Gerber *et al.* 2013) and the rise in productivity that impacts reproductive performance (Rauw 1998). Estrus state in beef heifers can be characterised by behavioural and physiological signs along with hormonal concentrations. In light of the intricate hormonal regulation of the estrus cycle, progesterone level is considered to be a practical determinant of the estrus cycle stage in the bovine (Amstalden and Williams 2015). Based on the signs of estrus and the blood plasma progesterone concentrations (Fig. 4.1), the 71 heifers found with estrus signs were confirmed to be in estrus state at the occasion of blood collection for metabolic profiling. Moreover, the metabolite profile of individual heifers were within the ranges for healthy cattle (University of Guelph Animal Health Lab Guidelines, 2015), ensuring that the associations found between these and estrus were not affected by sickness states.

Calcium and sodium have roles in energy metabolism and reproductive function in cattle (Small *et al.* 1996). In our study, estrus state was associated with lower concentrations of calcium and sodium, as well as reduced osmolality. The increase in TPV during estrus is anticipated with the reduction of calcium and sodium in the blood plasma (Vrijens and Rehrer 1999) and associated with the vulva swelling,

as frequently observed during the estrus state (Roelofs *et al.* 2015). The lower concentration of sodium during estrus is also likely associated with lower osmolality, based on determination of this measure (Dormandy 1967). Additionally, the physiological increase in estradiol concentration during estrus is thought to stimulate the calcium regulating hormones leading to increased calcium absorption and greater ALP concentration (Brommage *et al.* 1993).

Alkaline phosphatase is an enzyme involved in the active transport of phosphates across the cell membrane (Nandi *et al.* 2007) and is present in many tissues, including liver, bone and placenta (She *et al.* 2000). Greater ALP concentration during estrus in the bovine has been attributed to increased uterine ALP isoform production (Moss *et al.* 1954). In other species, greater ALP concentration during estrus has been observed in does (Yaqub *et al.* 2013) and ewes (Hassanein *et al.* 1999). Conversely, lower serum ALP concentrations have been reported during estrus in lactating cows (Schultz *et al.* 1971), which may be attributed to physiological differences including age, parity and stage of lactation (Sato *et al.* 2005) that influence the release of different ALP isoforms (She *et al.* 2000).

The greater concentration of AST during the estrus state represent another example of anabolic response and has been reported elsewhere (Yaqub *et al.* 2013). This enzyme catalyzes the production of oxaloacetate, gluconeogenesis and has been associated with muscle activity (Doornenbal *et al.* 1988). The greater AST concentration during the estrus state could be associated with greater physical activity during estrus in heifers, similar to CK. Creatine kinase is a controller of cellular energy homeostasis and creates a pool of phosphocreatine from creatine to serve as an energy

source for tissues with fluctuating energy demands, such as muscle (Schlattner *et al.* 2006). Interestingly, heifers in estrus present increased incidence of dark cuts due to increased muscle activity (Kenny and Tarrant 1988).

Beta-hydroxybutyric acid is a product of the mobilization of lipids to counter an energy demand (Van Soest 1994). The greater concentration of BHBA during estrus may be related to an increased demand of energy sources during periods of reduced feed intake (Fernández-Foren *et al.* 2011), and due to increased physical activity, as well as intermediary metabolism (Parker *et al.* 2001) in association with estrus. In high producing dairy cows, increased BHBA indicates negative energy balance (Mann *et al.* 2015) and has been related to ovarian dysfunction (Opsomer *et al.* 1998). In our heifers, increased BHBA levels are likely indicating a shift in fat metabolism in response to the functional workload related to the estrus state.

Triiodothyronine participates in the regulation of metabolic rate and impacts the release of sex hormones (Goff 2015). In other species, greater concentration of plasma T3 during the estrus day has also been observed in buffalo heifers (Dalvi 2013) and gilts (Toniollo *et al.* 1998). Changes in sodium and other ions in response to increased levels of T3 may have contributed to the surge in TPV during estrus (Basu and Mohapatra 2012). Greater liver production of AST has been associated with elevated concentrations of T3 (Malik and Hodgson 2002). Concentration of T3 is positively correlated with ALP during estrus in goats (Bhooshan and Kumar 2007). Fat mobilization, as indicated by increased BHBA during estrus in dairy cows, was also accompanied by greater T3 concentrations (Mohebbi-Fani *et al.* 2012). Furthermore, the administration of estradiol has been shown to increase the concentration of T3 in

women (Bisschop *et al.* 2006), which supports the increase of T3 during the estrus state. Therefore, these examples of strong ties between T3 and intermediary metabolism supports the key relevance of T3 as a predictor of estrus state.

Besides being leaner, younger heifers had greater globulin and TP during estrus that is the opposite of the overall pattern for the estrus state discussed above. Augmented globulin and TP suggest a greater rate of protein turnover (Lobley *et al.* 1990) in the leaner heifers during estrus. This is further supported by the increased CK in the leaner heifers during estrus, as CK is also an indicator of protein metabolism (Schlattner *et al.* 2006). Additionally, heavier heifers had overall greater fatness, with greater BW and MARB being associated with reduced osmolality and sodium during estrus. Considering that adipose tissue has a lower water content in comparison to muscle tissue (Sheng 2013), heifers with greater body fatness have diminished capacity for sodium dissociation. To date, changes in body composition from lean to fat tissue have shown a negative association with electrolyte and fluid balance in women (Metheny 2011). This evidence supports the adjustment for fatness and leanness when comparing plasma sodium during estrus, in heifers varying in body condition.

Cholesterol synthesis is greatly dependent on the liver metabolic rate (Van Soest 1994). The fact that feed efficiency was associated with cholesterol levels and variability during estrus may be related to the inherent increased liver metabolism found in feed efficient animals, as indicated by AST levels (Gonano *et al.* 2014), which is further supported by the greater AST concentration during estrus. Since cholesterol is a precursor to steroid hormones (Cheeke and Diernenfeld 2010), this also relates to potential improved ovarian function (Grummer *et al.* 1988) in feed efficient heifers.

A strong association between RFI and GLDH variability was observed. This enzyme is present in the liver of ruminants and catalyzes the deamination of glutamate as part of ureagenesis (Kravos and Malesic 2008), suggesting a greater amino acid metabolism by the liver (Van Soest 1994) in efficient heifers during estrus. In ruminants, GGT can indicate increased liver activity and may be associated with amino acid availability (Thrall *et al.* 2004). The gamma-glutamyl cycle utilizes GGT and ATP to breakdown proteins into amino acids that can be readily taken up by cells (Whitfield 2001). In addition to ALP and AST, during periods of low energy intake, GGT can specify lipid mobilization in cattle (González *et al.* 2011). Feed efficient heifers may be better equipped to produce readily available energy sources for the greater energetic demands during the estrus state. Interestingly, minerals (Ca, P and K), ALP, AST and T3 are also strongly related to RFI variability (Fig. 4.2). In essence, these similarities reinforce the associations between anabolic energetic shifts during estrus and background metabolism, represented by RFI (Parker *et al.* 2001; Montanholi *et al.* 2010; Mohebbi-Fani *et al.* 2012).

CONCLUSION

The estrus state is strongly associated with the fluctuations of ALP, AST, BHBA, CK and T3. These analytes, especially T3, may increase the certainty of identifying the estrus state. The effects of age, feed efficiency, body size and composition should be taken into consideration when assessing metabolite profiles in heifers for estrus detection. Further research in regards to these measures to evaluate response to dietary

treatments and husbandry systems, and to verify the suitability of other matrices for metabolite quantification to assess estrus are warranted.

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AUTHOR CONTRIBUTIONS

EM Crane and YR Montanholi were responsible for the study design, data collection and analysis, and writing the manuscript. JC Munro and SL Bourgon contributed to data collection and editing of the manuscript. M Diel de Amorim, RV Ventura and AH Fredeen contributed to data interpretation and editing of the manuscript.

Table 4.1. Descriptive statistics (mean and standard deviation) and least square means of plasma metabolites during estrus and non-estrus states

Metabolite (abbreviation; unit)	Mean ± SD	Estrus	Non-estrus	p-value
Ions				
Calcium (mmol/L)	2.39±0.12	2.37	2.41	0.05
Phosphorus (mmol/L)	2.12±0.27	2.09	2.15	0.18
Sodium (mmol/L)	140.04±2.82	139.36	140.73	<0.01
Osmolality (mmol/L)	275.73±5.45	274.70	276.75	0.02
Compounds				
Albumin (g/L)	33.94±2.17	33.63	34.12	0.10
Albumin globulin ratio (AG)	1.05±0.15	1.07	1.03	0.13
Globulin (g/L)	32.81±4.12	31.47	33.25	<0.01
Total protein (TP; g/L)	66.75±3.96	65.37	67.93	<0.01
Beta-hydroxybutyric acid (BHBA; μmol/L)	214.37±84.08	262.56	157.60	<0.01
Carbon dioxide (CO_2; mmol/L)	23.79±2.88	23.55	24.02	0.32
Cholesterol (mmol/L)	3.63±0.62	3.59	3.66	0.48
Creatinine (μmol/L)	105.71±15.38	105.34	106.08	0.76
Glucose (mmol/L)	4.35±0.48	4.34	4.34	0.94
Urea (mmol/L)	3.03±0.93	3.12	2.94	0.27
Enzymes				
Alkaline phosphatase (ALP; U/L)	123.20±71.64	117.24	101.87	0.05
Aspartate aminotransferase (AST; U/L)	59.62±11.74	63.24	56.00	<0.01
Creatine kinase (CK; U/L)	246.13±230.90	205.28	159.58	<0.01
Gamma glutamate transferase (GGT: U/L)	16.58±3.14	16.35	16.27	0.87
Glutamate dehydrogenase (GLDH; U/L)	18.08±8.32	15.62	17.65	0.07
Hormone				
Triiodothyronine (T3; nmol/L)	2.12±0.45	2.30	1.86	<0.01

Table 4.2. Least square means of biotype and blood plasma metabolites during estrus state by age, body size and leanness

Trait (abbreviation; unit)	Age			Body weight			Ribeye area		
	Younger	Older	p-value	Lighter	Heavier	p-value	Low	High	p-value
Biotype									
Age (days)	319.41	410.98	<0.01	339.91	391.25	<0.01	332.11	387.89	<0.01
Ribeye Area (REA; cm^2)	41.81	49.09	<0.01	41.58	49.21	<0.01	37.57	53.18	<0.01
Back fat (BKFT; mm)	1.14	2.00	<0.01	1.14	2.16	<0.01	1.17	1.94	0.03
Rump fat (RUMP; mm)	2.11	1.64	0.17	1.06	2.98	<0.01	1.88	1.80	0.87
Marbling (MARB; score 1-11)	7.00	7.13	0.29	6.85	7.03	0.32	6.90	7.14	0.23
Ions									
Calcium (mmol/L)	2.36	2.36	0.97	2.41	2.35	0.38	2.39	2.39	0.94
Phosphorus (mmol/L)	2.07	2.11	0.57	1.97	2.18	0.14	2.04	2.23	0.23
Potassium (mmol/L)	4.28	4.34	0.47	4.36	4.24	0.40	4.28	4.41	0.25
Sodium (mmol/L)	139.70	139.01	0.50	141.18	138.93	0.03	140.44	138.94	0.17
Osmolality (mmol/L)	275.19	274.20	0.62	278.02	274.09	0.06	276.60	274.73	0.36
Total plasma volume (TPV; %)	1.69	4.01	0.72	3.59	4.15	0.87	2.74	5.72	0.45
Compounds									
Albumin globulin ratio (AG)	1.06	1.06	0.96	1.03	1.07	0.46	1.01	1.14	0.07
Globulin (g/L)	31.84	31.32	0.60	33.05	30.76	0.11	33.22	29.66	0.02
Total protein (TP; g/L)	65.71	64.68	0.33	67.09	64.13	0.07	66.72	63.66	0.05
Beta-hydroxybutyric acid (BHBA; µmol/L)	283.28	292.27	0.70	252.81	289.41	0.35	257.53	286.35	0.45
Cholesterol (mmol/L)	3.63	3.53	0.63	3.81	3.45	0.17	3.61	3.65	0.86
Creatinine (µmol/L)	104.01	104.43	0.93	107.39	101.66	0.26	106.35	101.04	0.40
Glucose (mmol/L)	4.34	4.11	0.04	4.22	4.45	0.40	4.53	4.29	0.40
Urea (mmol/L)	2.93	3.45	0.16	3.06	3.29	0.63	2.83	3.68	0.09
Enzymes									
Alkaline phosphatase (ALP; U/L)	115.49	128.59	0.45	118.66	116.18	0.90	111.23	107.54	0.87
Aspartate aminotransferase (AST; U/L)	66.10	63.17	0.43	68.05	61.05	0.17	64.43	61.23	0.54
Creatine kinase (CK; U/L)	193.43	197.67	0.88	231.54	165.26	0.03	227.89	193.04	0.42
Gamma glutamate transferase (GGT; U/L)	17.45	15.19	0.03	16.32	17.20	0.48	16.01	16.31	0.83
Glutamate dehydrogenase (GLDH; U/L)	16.93	14.40	0.24	17.68	13.16	0.19	13.66	14.77	0.67
Hormone									
Triiodothyronine (T3; nmol/L)	2.40	2.39	0.95	2.38	2.42	0.85	2.52	2.26	0.29

Table 4.3. Least square means of biotype and blood plasma profile during estrus state by fatness classifications

Trait (abbreviation; unit)	Back fat			Marbling			Rump fat		
	Low	High	p-value	Low	High	p-value	Low	High	p-value
Biotype									
Age (days)	340.90	395.21	<0.01	349.15	389.40	0.02	377.55	364.17	0.19
Ribeye Area (REA; cm^2)	40.71	48.46	<0.01	41.85	46.85	0.07	43.26	44.94	0.40
Back fat (BKFT; mm)	0.78	2.66	<0.01	1.64	1.56	0.84	1.37	1.96	<0.01
Rump fat (RUMP; mm)	1.66	2.82	0.06	1.87	1.92	0.93	0.72	3.54	<0.01
Marbling (MARB; score 1-11)	7.02	6.94	0.74	6.41	7.48	<0.01	6.96	6.94	0.92
Ions									
Calcium (mmol/L)	2.39	2.38	0.90	2.36	2.37	0.87	2.32	2.35	0.50
Phosphorus (mmol/L)	2.13	2.07	0.68	2.04	2.12	0.56	2.24	1.94	<0.01
Potassium (mmol/L)	4.29	4.35	0.71	4.31	4.24	0.58	4.35	4.20	0.11
Sodium (mmol/L)	139.97	139.03	0.37	140.51	137.60	0.05	140.03	140.25	0.83
Osmolality (mmol/L)	272.57	274.76	0.71	276.65	271.18	0.04	275.86	276.03	0.93
Total plasma volume (TPV; %)	3.50	1.29	0.49	4.64	5.13	0.89	2.88	4.98	0.40
Compounds									
Albumin globulin ratio (AG)	1.03	1.11	0.33	1.06	1.05	0.99	1.07	1.07	0.99
Globulin (g/L)	32.74	30.21	0.13	32.03	31.38	0.70	32.07	31.49	0.63
Total protein (TP; g/L)	66.61	64.00	0.12	66.03	64.74	0.39	66.72	65.72	0.47
Beta-hydroxybutyric acid (BHBA; µmol/L)	271.03	287.41	0.70	284.49	260.90	0.52	229.60	265.23	0.31
Cholesterol (mmol/L)	3.37	3.65	0.29	3.48	3.75	0.44	3.86	3.40	0.05
Creatinine (µmol/L)	108.44	101.33	0.34	106.74	108.32	0.84	110.01	106.21	0.50
Glucose (mmol/L)	4.24	4.46	0.27	4.51	4.17	0.37	4.27	4.52	0.28
Urea (mmol/L)	3.06	3.42	0.50	2.90	3.08	0.67	3.07	2.77	0.40
Enzymes									
Alkaline phosphatase (ALP; U/L)	122.75	129.45	0.77	96.95	125.15	0.15	98.59	127.29	0.06
Aspartate aminotransferase (AST; U/L)	62.43	64.40	0.63	66.13	58.26	0.15	59.88	61.00	0.80
Creatine kinase (CK; U/L)	181.88	211.02	0.44	227.59	154.88	0.04	189.52	202.98	0.70
Gamma glutamate transferase (GGT; U/L)	16.56	16.07	0.69	16.86	16.10	0.62	15.95	16.63	0.59
Glutamate dehydrogenase (GLDH; U/L)	15.62	13.61	0.55	14.47	14.69	0.94	15.97	12.20	0.15
Hormone									
Triiodothyronine (T3; nm)	2.34	2.49	0.52	2.30	2.28	0.95	2.09	2.57	0.02

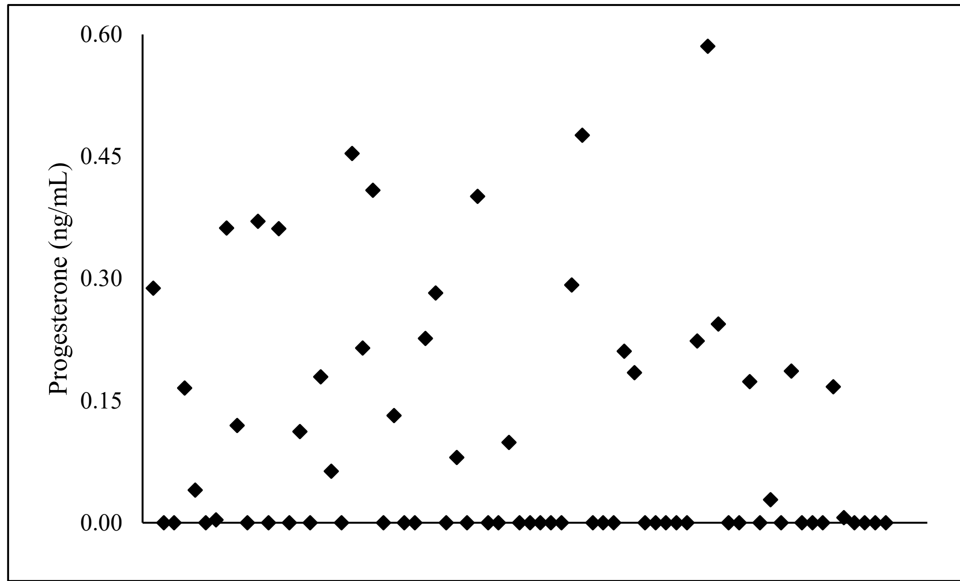

Figure 4.1. Plasma progesterone of heifers found with estrus signs (0.10±0.15 ng/mL; mean±SD).

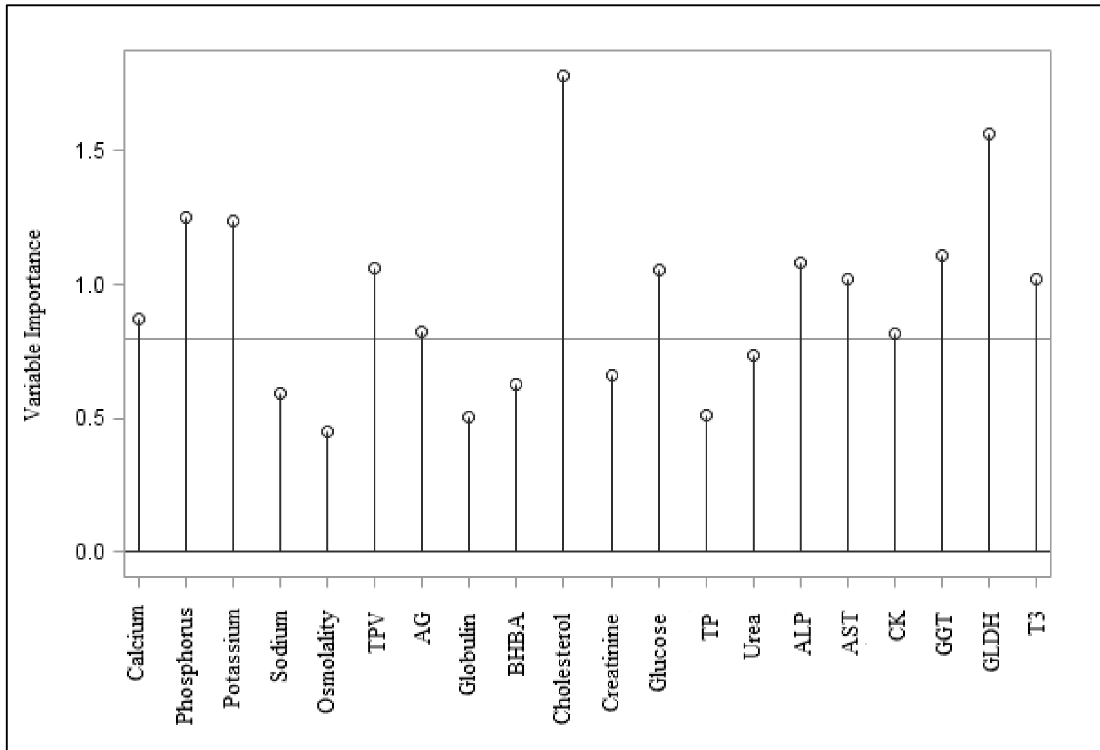

Figure 4.2. Variable importance of blood plasma metabolites with residual feed intake (RFI). Vertical lines above the horizontal line indicate a significant influence on the explanation of the RFI variance. Albumin globulin ratio (AG), total protein (TP), total plasma volume (TPV), alkaline phosphatase (ALP), gamma-glutamate transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK), glutamate dehydrogenase (GLDH), beta-hydroxybutyric acid (BHBA), and triiodothyronine (T3)

Chapter 5

DISCUSSION

Research was completed in the search of biomarkers of energy metabolism to assess feed efficiency and estrus state through hematological measures using pregnant yearlings and peri-pubertal crossbred beef heifers fed on a grass-based ration as experimental units. More specifically, this research was based on three models, namely animal category (to compare heifer calves and pregnant heifers); feed efficiency (to compare classes of feed efficiency within categories of heifers); and estrus state (to compare estrus and non-estrus states). Understanding fluctuations in metabolism may assist in the identification of proxies for productive efficiency and the estrus state. These efforts in phenotyping may result in complementary tools to serve genetic and nutrition programs, as well as general beef cattle husbandry.

The variations in hematological measures according to heifer category were shown in Chapter 3. The greater MCH, MCV and plasma concentration of CO_2 and reduced RBC demonstrated by the pregnant heifers in comparison to the heifer calves (Figure 5.1) appear to be related to greater oxygen requirement to meet the metabolic demands of pregnancy (Bauman and Currie 1980). Moreover, the heifer calves had the highest concentration of CK, which is related to the age and growth of the heifers (Thrall *et al.* 2004). Greater concentrations of CK were observed in open heifers in comparison to pregnant heifers by Gonano *et al.* (2014); these researchers evaluated the same individuals as open heifers, and during early and late pregnancy, which indicates that differences in CK are primarily influenced by physiological state. This

illustrates an increased metabolic rate of the pregnant yearling heifers over heifer calves.

Hematological parameters evaluated as potential indirect assessments of feed efficiency were reported in Chapter 3. The greater concentration of lymphocytes in both efficient heifer calves and pregnant yearlings may be related to the presence of more readily available oxygen as a result of decreased energy requirements, in comparison to inefficient counterparts. Feed efficient pregnant heifers appear to have increased metabolic demands by the liver as suggested by greater plasma cholesterol and globulin concentrations (Figure 5.1). Indicators of energy balance, such as NEFA, can be affected by factors including days in gestation (Bell 1995). Efficient heifer calves exhibited greater concentrations of plasma metabolic ions phosphorus and potassium, which may be associated with stage of growth. The differences in metabolites shown between the feed efficiency groupings of heifer calves and the pregnant yearlings illustrates differences in energy requirements during differing physiological stages such as pre-pubertal growth and pregnancy (NRC 2000). Therefore, in practice utilizing hematological parameters as an indirect assessment of feed efficiency should take into consideration the physiological state of the animal.

The metabolite profile of heifers in estrus was assessed in Chapter 4 and summarized in Figure 5.1. Differences in the metabolite profiles during the estrus and non-estrus states suggest an overall increase in metabolic rate during the estrus state. Indicators of fat mobilization including BHBA suggested an energetic shift in response to the functional workload during the estrus state. Similarly, the profile of the enzymes ALP, AST and CK suggested an increased metabolic rate in several tissues including

the liver and muscle during estrus, which was also supported by the substantial increase T3 levels in such state.

During estrus, T3 concentrations were about 21% higher than during the non-estrus state. The same comparison in efficient and inefficient heifers revealed an increased concentration of 4.85% in the inefficient heifers. The magnitude of differences for each of the metabolite categories measured for this study, in comparison to the other models, suggests that studying the estrus state can provide an extreme view of shifts in energy metabolism. This can provide more clarity about the underlying biological differences associated with the feed efficiency groupings, or indicating greater challenges to establish hematological proxies for feed efficiency.

This Thesis contributes to the knowledge concerning energy metabolism in the bovine female. Measures of the CBC analysis associated with feed efficiency provides new connections between the underlying biology of feed efficiency in heifers. These measures may be used as indicators of RFI upon further validation. Further research to validate these assessments in heifers may consider reducing sampling intervals and early in life assessments to establish a protocol to use hematological measures as an indirect assessment of feed efficiency in beef heifers. This study also indicates that growing heifers with improved feed efficiency also have a more responsive immune system function, which is an example of desirable collateral association between complex traits (health and feed efficiency). Similarly, this study identified differences in plasma metabolite profiles according to the estrus and non-estrus state. Use of the five key plasma metabolites (ALP, AST, BHBA, CK and T3) may complement current estrus detection methods. Further advances on evaluation of the suitability of other

matrices for metabolite quantification (i.e. milk, saliva, urine and feces) and the evaluation of dietary and weather conditions on such metabolic associations are warranted.

In essence, the application of hematological measures to predict feed efficiency and the estrus state may contribute to the indirect assessment of these complex traits. This complementary role is extremely relevant to the sustainability of the beef industry, and in integration of precision agriculture in beef cow barns across the country.

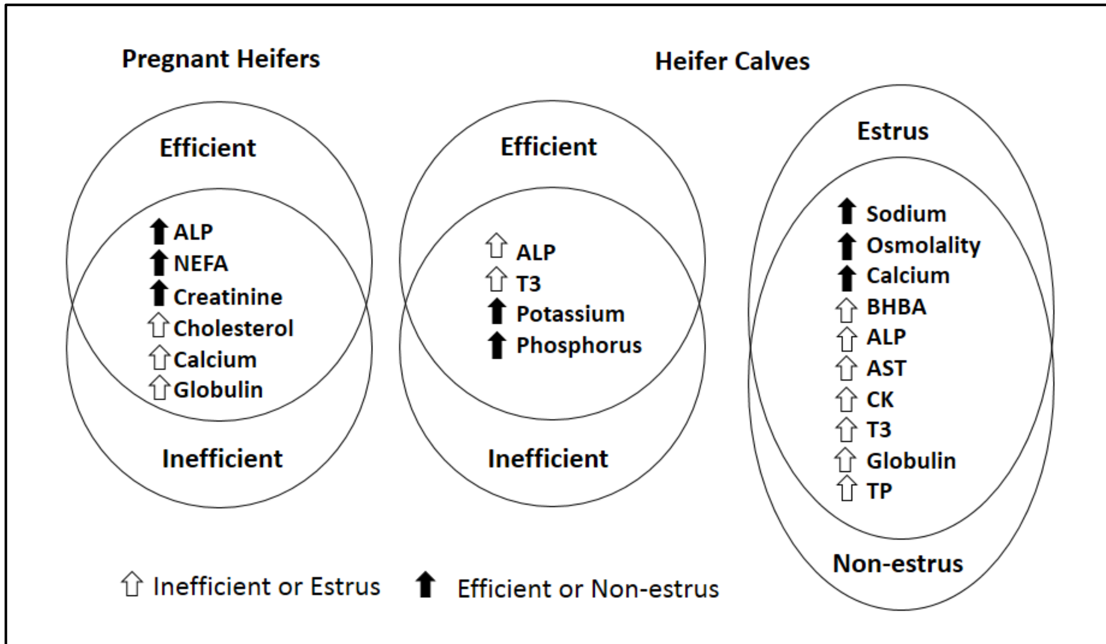

Figure 5.1. Associations of plasma metabolites and metabolic rate fluctuations according to animal categories, feed efficiency and estrus state.

REFERENCES

Abeni F, Calamari L, and Stefanini L (2007) Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. *International Journal Biometeorology* **52**, 87–96.

Adewuyi AA, Gruys E and van Eerdenburg FJCM (2005) Non-esterified fatty acids (NEFA) in dairy cattle. A review. *Veterinary Quarterly* **27**, 117-126.

Alexandre PA, Kogelman LJA, Santana MHA, Passarelli D, Pulz LH, Fantinato-Neto P, Silva PL, Leme PR, Strefezzi RF, Coutinho LL, Ferraz JBS, Eler JP, Kadarmideen HN and Fukumasu H (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. *BMC Genomics* **16**, 1-13.

Amstalden M and Williams G (2015) Neuroendocrine control of estrus and ovulation. In: Bovine Reproduction. Wiley Blackwell, 203-218.

Andersen H and Plum M (1965) Gestation length and birth weight in cattle and buffaloes: a review. Journal Series Nebraska Agricultural Experiment Station, Lincoln, NE.

Anderson, KJ, Lefever DG, Brinks JS and Odde KG (1991) The use of reproductive tract scoring in beef heifers. *Agricultural Practice and Science Journal* **12**, 123-128.

Archer JA, Richardson, EC, Herd, RM and Arthur PF (1999) Potential for selection to improve efficiency of feed use in beef cattle: A review. *Australian Journal of Agricultural Research* **50**, 147–161.

Arthur PF, Herd RM, Wilkins JF and Archer JA (2005) Maternal productivity of Angus cows divergently selected for post-weaning residual feed intake. *Australian Journal of Experimental Agriculture* **45**, 985–993.

Baird GD, Heitzman and Hibbitt KG (1972) Effects of starvation on intermediary metabolism in the lactating cow. A comparison with metabolic changes occurring during bovine ketosis. *Biochemistry Journal* **5**, 1311-1318.

Baldwin RL, Smith NE, Taylor J and Sharp M (1980) Manipulating metabolic parameters to improve growth rate and milk secretion. *Journal of Animal Science* **51**, 1416-1428.

Ball PJH and Peters AR (2007) The ovarian cycle. In: Reproduction in Cattle. Blackwell Publishing Oxford, pp. 40-55.

Basu G and Mohapatra A (2012) Interactions between thyroid disorders and kidney disease. *Indian Journal of Endocrinology Metabolism* **16**, 204-213.

Bauman DE and Currie B (1980) Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science* **63**, 1514-1529.

Belk K, Scanga JA, Smith GC and Grandin T (2002) The relationship between good handling/stunning and meat quality in beef, pork, and lamb. Available at http://www.grandin.com/meat/hand.stun.relate.quality.html

Bell AW (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* **73**, 2804-2819.

Berg JM, Tymoczko JL and Stryer L (2002) Biochemistry 5th ed. (WH Freeman).

Bhagat CI, Garcia-Webb P, Fletcher E, Beilby JP (1984) Calculated osmolality vs. measured plasma osmolalities revisited. *Clinical Chemistry* **30**, 1703-1705.

Bhooshan N and Kumar P (2007) Profile of ovarian and thyroid hormones, alkaline phosphatase and cholesterol at oestrus cycle, gestation and early lactation in blood plasma of Marwari goats. *Indian Journal of Animal Science* **77**, 1233-1237.

Bingham GM, Friend TH, Lancaster PA, Cartsens GE (2009) Relationship between feeding behavior and residual feed intake in growing Brangus heifers. *Journal of Animal Science* **87**, 2685- 2689.

Bisschop PH, Toorians AW, Endert E, Wiersinga WM, Gooren LJ and Fliers E (2006) The effects of sex-steroid administration on the pituitary-thyroid axis in transsexuals. *European Journal of Endocrinology* **155**, 11-16.

Black TE, Bischoff KM, Mercadante VRG, Marquezini GHL, DiLorenzo N, Chase CC, Coleman SW, Maddock TD and Lamb GC (2015) Relationships among performance, residual feed intake, and temperament assessed in growing beef heifers and subsequently as 3-year-old, lactating beef cows. *Journal of Animal Science* **91**, 2254-2263.

Blaxter KL (1962) The Energy Metabolism of Ruminants (Hutchinson and Co. LTD).

Bodine TN, Purvis II HT and Lalman DL (2001) Effects of supplement type on animal performance, forage intake, digestion, and ruminal measurements of growing beef cattle. *Journal of Animal Science* **79**, 1041- 1051.

Bottje WG, and Carstens GE (2008). Association of mitochondrial function and feed efficiency in poultry and livestock species. *Journal of Animal Science* **87**, 48–63.

Boyd JW (1981) The relationships between blood haemoglobin concentration, packed cell volume and plasma protein concentration in dehydration. *British Veterinary Journal* **137**, 166 – 172.

Brancaccio P, Maffulli N, Limongelli FM (2007) Creatine kinase monitoring in sport medicine. British Medical Bulletin 81, 209- 230.

Brody S (1945) Bioenergetics and Growth with Special Reference to the Efficiency Complex in Domestic Animals (Hafner Press).

Brommage R, Binacua C and Carrié A (1993) Ovulation-associated increase in intestinal calcium absorption during the rat estrus cycle is blunted by ovariectomy. *Biology of Reproduction* **49**, 544-548.

Brooks AL, Morrow RE, Youngquist RS (1985) Body composition of beef heifers at puberty. *Theriogenology* **24**, 235-250.

Cabello G and Wrutniak C (1989) Thyroid hormone and growth: relationships with growth hormone effects and regulation. *Reproduction, Nutrition, Development* **29**, 387-402.

Cameron ND (1992) Correlated physiological responses to selection for carcass lean content in sheep. *Livestock Production Science* **30**, 53- 68.

Canfax. (2011). Cycle indicators and heifer retention. 1-8. Available online at: http://www.canfax.ca/Samples/Cycle%20Indicators%20%20Heifer%20Retention%20 May %2020 11.pdf

Canfax (2014) 2014 Annual Outlook. A Publication of the Canadian Cattlemen's Association. pp 2-36

Canfax (2015): 2015 Annual Outlook. A Publication of the Canadian Cattlemen's Association. pp 2-45.

Capper JL (2011) The environmental impact of beef production in the United States: 1977 compared with 2007. *Journal of Animal Science* **89**, 4249–4261.

Carstens, GE, and Tedeschi LO (2006) Defining Feed Efficiency in Beef Cattle. Proceedings from the Beef Improvement Federation 38th Annual Research Symposium and Annual Meeting, Choctaw, MS, United States, 12–21.

Cartwright SL, Begley N, Schaeffer LR, Burnside EB, Mallard BA (2011) Antibody and cell-mediated immune responses and survival between Holstein and Norwegian Red x Holstein Canadian calves. *Journal of Dairy Science* **94**, 1576–1585.

Cassady JM, Maddock TD, DiCostanzo A and Lamb GC (2009) Body composition and estrus cyclicity responses of heifers of distinct body conditions to energy restriction and repletion. *Journal of Animal Science* **87**, 2255-2261.

Cebra JJ (1999) Influences of microbiota on intestinal immune system development. *The American Journal of Clinical Nutrition* **69**, 1046-1051.

Cheeke PR and Dierenfeld ES (2010) Comparative animal nutrition and metabolism. CAB International, Nosworthy Way, UK.

Cheung J (2007) 'Amniotic fluid alkaline phosphatase as a biomarker of fetal growth and development.' MSc thesis, McGill University, Montreal.

Christie WW (1981) Lipid metabolism in ruminant animals (Pergamon Press).

Christopherson RJ, Gonyou HW and Thompson JR (1979) Effects of temperature and feed intake on plasma concentration of thyroid hormones in beef cattle. *Canadian Journal of Animal Science* **59**, 655-661.

Colditz IG (2002) Effects of the immune system on metabolism: implications for production and disease resistance in livestock. *Livestock Production Science* **75**, 257-268.

Connolly S, Fortes MRS, Piper EK, Seddon JM, Kelly MJ (2014) Determining the number of animals required to accurately determine breed composition using genomic data. In 'Proceedings, 10th World Congress of Genetics Applied to Livestock Production'.

Corathers SD (2006) Focus on diagnosis: the alkaline phosphatase level: nuances of a familiar test. *Pediatric Review* **27**, 382-384.

Cozzi G, Ravarotto L, Gottardo F, Stefani AL, Contiero B, Moro L, Brscic M and Dalvit P (2011) Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. *Journal of Dairy Science* **94**, 3895-3901.

Dalton JC, Nadir S, Bame JH, Noftsinger M, Nebel RL, Saacke RG (2001) Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. *Journal of Dairy Science* **84**, 2413-2418.

Dalvi SH, Deshmukh BT and Kumarasamy J (2013) Thyroid hormone levels during oestrus cycle in pandharpuri buffalo. *Buffalo Bulletin* **32**, 380-384.

Day ML, Imakawa K, Zalesky DD, Kittok RJ and Kinder JE (1986) Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. *Journal of Animal Science* **62**, 164l-1648.

de Haan, C (1996) Rangeland Development in a Developing World. In: Proceedings of the Fifth International Rangeland Congress. Society for Range Management, Denver, Colorado.

De Lange P, Lanni A, Beneduce L, Moreno M, Lombardi A, Silvestri E and Goglia F (2001) Uncoupling Protein-3 Is a Molecular Determinant for the Regulation of Resting Metabolic Rate by Thyroid Hormone. *Endocrinology* **142**, 3414-3420.

Dhurandhar NV, Israel BA, Kolesar JM, Mayhew GF, Cook ME, Atkinson RL (2000) Increased adiposity in animals due to a human virus. *International Journal of Obesity* **24**, 989-996.

Dias RS, Montanholi YR, Lopez S, Smith BA, Miller SP, France J (2016) Utilization of macro-minerals and trace elements in pregnant beef heifers with distinct feed efficiencies. *Journal of Dairy Science* [JDS-15-10796.R1; accepted March 14th, 2016].

Dolezal HG, Tatum JD, Williams FL (1993) Effects of Feeder Cattle Frame Size, Muscle Thickness and Age Class on Days Fed, Weight, and Carcass Composition. *Journal of Animal Science* **71**, 2975-2985.

Doornenbal H, Tong AKW, Murray NL (1988) Reference values of blood parameters in beef cattle of different ages and stages of lactation. *Canadian Journal of Veterinary Research* **52**, 99-105.

Dormandy TL (1967) Osmometry. *Lancet* **303**, 301-304.

Dørup I and Clausen T (1988) Effects of potassium deficiency on growth and protein synthesis in skeletal muscle and the heart of rats. *British Journal of Nutrition* **62**, 269-284.

Dumont B, Fortun-Lamothe L, Jouven M, Thomas M and Tichit M (2013) Prospects from agroecology and industrial ecology for animal production in the 21st century. Animal **7**, 1028-1043.

FAO (2006) The State of food insecurity in the world. Eradicating world hunger – taking stock ten years after the World Food Summit, pp 1-44.

Fernández-Foren A, Abecia JA, Vázquez MI, Forcada F, Sartore I, Carriquiry M, Meikle A and Sosa C, 2011: Food restriction in sheep : response endocrine - metabolic body reserves as dependent. ITEA **107**, 257-271.

Ferrell CL (1991) Maternal and fetal influences on uterine and conceptus development in the cow: II. Blood flow and nutrient flux. *Journal of Animal Science* **69**, 1954-1965.

Ferrell CL, and TG Jenkins (1985) Cow type and the nutritional environment: Nutritional aspects. *Journal of Animal Science* **61**, 725-741.

Field TG and Taylor RE (2003) Beef Production and Management Decisions. Prentice Hall, Upper Saddle River, New Jersey.

Field TG (2007) Beef Production and Management Decisions 5th ed. Pearson Prentice Hall, Columbus.

Fields MJ and Sand RS (1994) Factors Affecting Calf Crop. CRC Press, Boca Raton, USA.

Fokkema DS, Koolhaas JM, Gugten JVD (1995) Individual characteristics of behavior, blood pressure, and adrenal hormones in colony rats. *Physiological Behavior* **57**, 857-862.

Fontoura ABP, Montanholi YR, Diel de Amorim M, Foster RA, Chenier T and Miller SP (2016) Associations between feed efficiency, sexual maturity and fertility-related measures in young beef bulls. *Animal* **10**, 96-105.

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A and Tempio G (2013) Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome.

Goff JP (2015) The endocrine system. In: Dukes' Physiology of Domestic Animals 13th ed. Wiley Blackwell, 617-726.

Gombe S and Hansel W (1973) Plasma leutinizing hormone (LH) and progesterone levels in heifers on restricted energy intakes. *Journal of Animal Science* **37**, 728-733.

Gomes RC, Sainz RD, Silva SL, César MC, Bonin MN and Leme PR (2012) Feedlot performance, feed efficiency reranking, carcass traits, body composition, energy requirements, meat quality and calpain system activity in Nellore steers with low and high residual feed intake. *Livestock Science* **150**, 265-273.

Gomez E, Duque P, Diaz E, Facal N, Antolin I, Hidalgo C and Diez C (2002) Effects of acetoacetate and D-β-hydroxybutyrate on bovine in vitro embryo development in serum-free medium. *Theriogenology* **57**, 1551-1562.

Gonano CV, Montanholi YR, Schenkel FS, Smith BA, Cant JP, and Miller SP (2014) The relationship between feed efficiency and the circadian profile of blood plasma analytes measured in beef heifers at different physiological stages. *Animal* **10**, 1-15.

González FD, Muino R, Pereira V, Campos R and Benedito JL (2011) Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. *Journal of Veterinary Science* **12**, 251-255.

Gonzalez-Reico O, Coffey MP and Pryce JE (2014) On the value of the phenotypes in the genomic era. *Journal of Dairy Science* **97**, 7905-7915.

Goodrich RD, Garrett JE, Gast DR, Kirick MA, Larson DA, Meiske JC (1984) Influence of monensin on the performance of cattle. *Journal of Animal Science* 58, 1484–1498.

Gould R (2007) Nutrition and Management: Growth Implants for Beef Cattle – Economic Implications. Alberta Agriculture and Forestry available at http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/beef11700

Grandin, T (1998) Reducing handling stress improves both productivity and welfare. *Professional Animal Scientist* **4**, 1 -10.

Gregory, KE (1972) Beef cattle type for maximum efficiency "Putting it all together". *Journal of Animal Science* **34**, 881-884.

Grummer RR and Carroll DJ (1988) A review of lipoprotein cholesterol metabolism: importance to ovarian function. *Journal of Animal Science* **66**, 3160-3173.

Grummer RR (1995) Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science* **73**, 2820-2833.

Guédon L, Saumande J, Dupron F, Couquet C and Desbals B (1999) Serum cholesterol and triglycerides in postpartum beef cows and their relationship to the resumption of ovulation. *Theriogenology* **51**, 1405-1415.

Harris EJ and Manchester KL (1966) The effects of potassium ions and denervation on protein synthesis and the transport of amino acids in muscle. *Biochemistry* **101**, 135-145.

Hassanein MRR, Hussein SA and Hayat H (1999) Some biochemical studies during estrus cycle and after synchronization in Barki ewes. *Journal of Biochemistry Molecular Biology* **17**, 281-299.

Hegarty RS, Goopy JP, Herd RM, McCorkell B (2007) Cattle selected for lower residual feed intake have reduced daily methane production. *Journal of Animal Science* **85**, 1479–1486.

Herd RM, Richardson EC, Hegarty RS, Woodgate R, Archer JA and Arthur PF (1998) Pasture intake by high versus low net feed efficient Angus cows. *Animal Production in Australia* **22**, 137–140.

Herd RM, Archer JA, and Arthur PF (2003) Reducing the cost of beef production through genetic improvement in residual feed intake: opportunity and challenges to application. *Journal of Animal Science* **81**, 9–17.

Herd, RM and Arthur PF (2008) Physiological basis for residual feed intake. *Journal of Animal Science* **87**, 64–71.

Heriazon A, Hamilton K, Huffman J, Wilkie BN, Sears W, Quinton M, Mallard BA (2011) Immunoglobulin isotypes of lactating Holstein cows classified as high, average, and low type-1 or -2 immune responders. *Veterinary Immunology and Immunopathology* **144**, 259-269.

Hessing MJC, Hagelso AM, Schouten WGP, Wiepkema PR, Van Beek JAM (1994) Individual behavioral and physiological strategies in pigs. *Physiological Behavior* **55**, 39-46.

Hill RA and Ahola JK (2012) Feed Efficiency in the Beef Industry (Wiley-Blackwell).

Hine BC, Cartwright SL and Mallard BA (2011) Effect of age and pregnancy status on adaptive immune responses of Canadian Holstein replacement heifers. *Journal of Dairy Science* **94**, 981-991.

Hocquette JF, Ortigues-Marty I, Pethick D, Herpin P, Fernandez (1998) Nutritional and hormonal regulation of energy metabolism in skeletal muscle of meta-producing animals. *Livestock Production Science* **56**, 115-143.

Hodges J (2005) Cheap food and feeding the world sustainably. *Livestock Production Science* **92**, 1-16.

Hopkins SM (2003) Reproductive Patterns of Cattle. Pages 395-409 in M. H. Pinead and M. P. Dooley eds. McDonald's Veterinary Endocrinology and Reproduction Blackwell Publishing Ltd.

Huber K and Breves G (1999) Influence of dietary phosphorus depletion on central pathways of intermediary metabolism in rats. *Archives of Animal Nutrition* **52**, 299-309.

Hugentobler SA, Morris DG, Sreenan JM and Diskin MG (2007) Ion concentrations in oviducts and uterine fluid and blood serum during the estrus cycle in the bovine. *Theriogenology* **68**, 538-548.

Hurnik JF, Webster AB and DeBoer S (1985) An investigation of skin temperature differentials in relation to estrus in dairy cattle using a thermal infrared scanning technique. *Journal of Animal Science* **61**, 1095-1102.

Iwata K, Kinoshita M, Susaki N, Uenoyama Y, Tsukamura H and Maeda K (2011) Central injection of ketone body suppresses luteinizing hormone release via the catecholaminergic pathway in female rats. *Journal of Reproduction and Development* **57**, 379–384.

Jain NC (1993) Essentials of Veterinary Hematology (Lea & Febiger).

Jones ML, Allison RW (2007) Evaluation of the ruminant complete blood cell count. *Veterinary Clinics Food Animal Practice* **23**, 377-402.

Kaneco JJ, Harvey JW, and Bruss M (2008) Clinical Biochemistry of Domestic Animals (Academic Press/Elsevier).

Kelly AK, McGee MM, Crews DH, Fahey AG, Wylie AR and Kenny DA (2010) Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. *Journal of Animal Science* **88**, 109-123.

Kenny FJ and Tarrant PV (1988) The effect of oestrus behaviour on muscle glycogen concentration and dark-cutting in beef heifers. *Meat Science* **22**, 21-31.

Kinder JE, Robertson MS, Wolfe MW and Stumf TT (1994) Management factors affecting puberty in the heifer. In Factors Affecting Calf Crop. CRC Press Inc. Fields MJ and Sands RS (eds) 69-90.

Klassing KC (1988) Nutritional aspects of leukocytic cytokines. *Journal of Nutrition* **118**, 1436-1446.

Kleiber, M (1961) The fire of life: An introduction to animal energetics. John Wiley and Sons, Incorporated, New York, NY, United States, 1-454.

Koch RM, Swiger LA, Chambers D and Gregory KE (1963) Efficiency of feed use in beef cattle. *Journal of Animal Science* **22**, 486–494.

Kohn RA, Dinneen MM and Russek-Cohen E (2005) Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *Journal of Animal Science* **83**, 879-889.

Kominsky DJ, Campbell EL, and Colgan SP (2010) Metabolic shifts in immunity and inflammation. *The Journal of Immunology* **184**, 4062-4068.

Koolhaas KM, Korte SM, De Boer SF, Van Der Veqt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MA and Blokhuis HJ (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Behavioral Reviews* **23**, 925-935.

Kravos M and Malesic I, (2008) Kinetics and isoforms of serum glutamate dehydrogenase in alcoholics. *Alcohol & Alcoholism* **43**, 281–286.

Kumar B and Kumar N (2000) Effects of altitude on some blood biochemical profile of 15 months old non-pregnant crossbred heifers. *Indian Journal of Animal Science* **34**, 108-111.

Lancaster PA, Carstens GE, Michal JJ, Brennan KM, Johnson KA and Davis ME (2014) Relationships between residual feed intake and hepatic mitochondrial function in growing beef cattle *Journal of Animal Science* **92**, 3134-3141.

Lawman MP, Gauntlett D, and Gallery F (1986) Antibody-dependent cellular cytotoxicity and natural killer cell activity in cattle: mechanisms of recovery from infectious diseases. In 'The Rumminant Immune System in Health and Disease' (Ed Morrison WI) pp. 346-373 (Cambridge University Press, Cambridge).

Lawrence P, Kenny DA, Earley B, Crews DH, McGee M (2011) Grass silage intake, rumen and blood variables, ultrasonic and body measurements, feed behavior, and activity in pregnant beef heifers differing in phenotypic residual feed intake. *Journal of Animal Science* **89**, 3248-3261.

Legesse G, Beauchemin KA, Ominski KH, McGeough EJ, Krobel R, MacDonald D, Little SM and McAllister TA (2016) Greenhouse gas emissions of Canadian beef production in 1981 as compared with 2011. Animal Production Science **56**, 153-168.

Lehninger AL (1975) Biochemistry, the molecular basis of cell structure and function 2nd ed. Worth Publishers, Inc. pp. 1104.

Lobley GE (1990) Energy metabolism reactions in ruminant muscle: responses to age, nutrition and hormonal status. *Reproduction Nutrition Development* **30**, 13-34.

Lobley GE, Sinclair KD, Grant CM, Miller L, Mantle D, Calder AG, Warkup CC and Maltin CA (2000) The effects of breed and level of nutrition on whole-body and muscle protein metabolism in pure-bred Aberdeen angus and Charolais beef steers. *The British Journal of Nutrition* **84**, 275-284.

Lobley GE (2003) Protein turnover – what does it mean for animal production? *Canadian Journal of Animal Science* **83**, 327-340.

Lochmiller RL and Deerenberg C (2000) Trade-offs in evolutionary immunology: What is the cost of immunity? *Oikos* **88**, 87-98.

Loneragan GH, Thomson DU and Scott HM (2014) Increased mortality in groups of cattle administered the β-Adrenergic agonists ractopamine hydrochloride and zilpaterol hydrochloride. *PloS ONE* **9**, pp. 1-13.

Mackenzie AM (1997) Effect of transportation and weaning on humoral immune responses of calves. *Research in Veterinary Science* **63**, 227-230.

Mader TL (2011) Mud effects on feedlot cattle. Nebraska Beef Cattle Report, 1-2.

Mader TL and Colgan SL (2007) Pen density and straw bedding during feedlot finishing. *Nebraska Beef Cattle Reports* Paper 70.

Magnusson U, Wilkie B, Artusson K, Mallard B (1999) Interferon-alpha and haptoglobin in pigs selectively bred for high and low immune response and infected with *Mycoplasma hyorhinis*. *Veterinary Immunology and Immunopathology* **68**, 131-137.

Mahon CR, Lehman DC, and Manuselis G (2015) Textbook of Diagnostic Microbiology 5th ed (Elsevier).

Malik R and Hodgson H (2002) The relationship between the thyroid gland and the liver. *QJM* **95**, 559-569.

Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Lacey Vankampen C, Wagter L and Wilkie BN (1998) Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *Journal of Dairy Science* **81**, 585-595.

Mallard BA, Wagter LC, Ireland MJ, and Dekkers, JCM (1997) Effects of growth hormone, insulin-like growth factor I, and cortisol on periparturient antibody response profiles of dairy cattle. *Veterinary Immunology and Immunopathology* **60,** 61–76.

Mann S, Leal Yepes FA, Overton TR, Wakshlag JJ, Lock AL, Ryan CM and Nydam DV (2015) Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. *Journal of Dairy Science* **98**, 3366-3382.

McBride BW and Kelly JM (1990) Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. *Journal of Animal Science* **68**, 2997-3010.

McCarthy SD, Roche JF and Forde N (2012) Temporal changes in endometrial gene expression and protein localization of members of the IGF family in cattle: effects of progesterone and pregnancy. *Physiological Genomics* **44**, 130-140.

McDonagh MD, Herd RM, Richardson EC, Oddy VH, Archer JA and Arthur PF (2001) Meat quality and the calpain system of feedlot steers following a single generation of divergent selection for residual feed intake. *Australian Journal of Experimental Agriculture* **41**, 1013.–1021.

McGee M, Drennan MJ and Caffrey PJ (2006) Effect of age and nutrient restriction pre partum on beef suckler cow serum immunoglobulin concentrations, colostrum yield, composition and immunoglobulin concentration and immune status of their progeny. *Irish Journal of Agricultural and Food Research* **45**, 157-171.

Metheny NM (2011) Fluid and electrolyte balance in the aged. In: Fluid and Electrolyte Balance 5th ed. Jones and Bartlett Learning, 355-357.

Meuwissen TH, Hayes BJ and Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* **157**, 1819-1829.

Meyer, DJ, and Harvey JW (2004) Veterinary Laboratory Medicine: Interpretation & Diagnosis 3rd ed (Saunders).

Milligan LP and Summers M (1986) The biological basis of maintenance and its relevance to assessing responses to nutrients. *Proceedings of the Nutrition Society* **45**, 185-193.

Milne EM (1985) The diagnostic value of alkaline phosphatase in canine medicine: a review. *Journal of Small Animal Practice* **26**, 267–278.

Mitlöhner FM, Galyean ML and McGlone JJ (2002) Shade effects on performance, carcass traits, physiology, and behavior of heat-stressed feedlot heifers. *Journal of Animal Science* **80**, 2043-2050.

Mohebbi-Fani M, Nazifi S, Bahrami S and Jamshidi O (2012) Circulating thyroid hormones and indices of energy and lipid metabolism in normal and hormonally induced oestrus cows. *Journal of Basic and Applied Sciences* **8**, 270-274.

Mondal M, Rajkhowa C and Prakash BS (2006) Oestrus behaviour and timing of ovulation in relation to onset of oestrus and LH peak in Mithun (*Bos frontalis*) cows. *Reproduction in Domestic Animals* **41**, 479-484.

Montanholi YR, Odongo NE, Swanson KC, Schenkel FS, McBride BW and Miller SP (2008) Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (*Bos taurus*). *Journal of Thermal Biology* **33**, 468-475.

Montanholi YR, Swanson KC, Schenkel FS, McBride BW, Caldwell TR and Miller SP (2009) On the determination of residual feed intake and associations of infrared thermography with efficiency and ultrasound traits in beef bulls. *Livestock Science* **125**, 22-30.

Montanholi YR, Swanson KC, Palme R, Schenkel FS, McBride BW, Lu D, Miller SP (2010) Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. *Animal* **4**, 692-701.

Montanholi YR, Lam S, Veripolli V, Vander Voort G and Miller SP (2013a) Short Communication: Associations between chemical composition and physical properties of milk and colostrum with feed efficiency in beef cows. *Canadian Journal of Animal Science* **93**, 487-492.

Montanholi YR, Palme R, Haas LS, Swanson KC, Vander Voot G and Miller SP (2013b) On the relationships between glucocorticoids and feed efficiency in beef cattle. *Livestock Science* **155**, 130-136.

Montano-Bermudez M, Nielsen MK and Deutscher GH (1990) Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. *Journal of Animal Science* **68**, 2279-2288.

Moore SS, Mujibi FD and Sherman EL (2009) Molecular basis for residual feed intake in beef cattle. *Journal of Animal Science* **87**, 41–47.

Moreno M, de Lange P, Lombardi A, Silvestri E, Lanni A and Goglia F (2008) Metabolic effects of thyroid hormone derivatives. *Thyroid* **18**, 239–253.

Mortimore GE, Poso AR (1987) Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annual Review of Nutrition* **7**, 539- 564.

Morton J, Coles B, Wright K, Gallimore A, Morrow JD, Terry ES, Anning PB, Morgan BP, Dioszeghy V, Kuhn H, Chaitidis P, Hobbs AJ, Jones SA and O'Donnell VB (2008) Circulating neutrophils maintain physiological blood pressure by suppressing bacteria and IFNγ-dependent iNOS expression in the vasculature of healthy mice. *Blood* **111**, 5187- 5193.

Moss S, Wrenn TR, Sykes JF (1954) Alkaline phosphatase, glycogen, and periodic acid-Schiff positive substances in the bovine uterus during the estrus cycle. *Endocrinology* **55**, 261-273.

Nandi S, Kumar VG, Manjunatha BM, Gupta PS (2007) Biochemical composition of ovine follicular fluid in relation to follicle size. *Development, Growth and Differentiation* **49**, 61-66.

National 4-H Council (2015) 4-H Around the World available at http://www.4-h.org/about/global-network/

NRC, Nutrient Requirements of Beef Cattle 7th ed (2000) National Academy Press, Washington, DC.

Opsomer G, Coryn M, Deluyker H and de Kruit A (1998) An Analysis of Ovarian Dysfunction in High Yielding Dairy Cows after Calving Based on Progesterone Profiles. *Reproduction in Domestic Animals* **33**, 193-204.

Ospina PA, Nydam DV, Stokol T and Overton TR (2010) Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *Journal of Dairy Science* **93**, 1596-1603.

Owens FN, Gill DR, Secrist DS and Coleman SW (1995) Review of some aspects of growth and development of feedlot cattle. *Journal of Animal Science* **73**, 3152-3172.

Owens IP and Wilson K (1999) Immunocompetence: a neglected life history trait or conspicuous red herring? *Trends in Ecological Evolution* **14**, 170-172.

Parish JA, Karisch BB, Vann RC and Riley DG (2014) Effects of steer breed composition on feedlot performance and carcass traits. *The Professional Animal Scientist* **30**, 43-50.

Parker GC, McKee ME, Bishop C and Coscina DV (2001) Whole-body metabolism varies across the estrus cycle in Sprague-Dawley rats. *Physiology & Behavior* **74**, 399-403.

Patience JF (2012) 5. The influence of dietary energy on feed efficiency in grow-finish swine. In Feed Efficiency in Swine (Wageningen Academic Publishers).

Pelletier N, Pirog R and Rasmussen R (2010) Comparative life cycle environmental impacts of three beef production strategies in the Upper Midwestern United States. *Agricultural Systems* **103**, 380-389.

Petterson JA, Dunshea FR, Ehrhardt RA, and Bell AW (1994) Pregnancy but not moderate undernutrition attenuates insulin suppression of fat mobilization in sheep. *Journal of Nutrition* **124**, 2431-2436.

Pierson RA and Ginther OJ (1988) Ultrasound imaging of the ovaries and uterus in cattle. *Theriogenology* **29**, 21-37.

Pitchford WS (2004) Genetic improvement of feed efficiency of beef cattle: what lessons can be learnt from other species? *Australian Journal of Experimental Agriculture* **44**, 371-382.

Pitesky ME, Stackhouse KR and Mitloehner FM (2009) Chapter 1 – Clearing the Air: Livestock's Contribution to Climate Change. *Advances in Agronomy* **103**, 1-40.

Pradhan R and Nakagoshi N (2008) Reproductive disorders in cattle due to nutritional status. *Journal of International Development Cooperation* **14**, 45-66.

Purvis HT and Whittlier JC (1996) Effects of ionophore feeding and anthelmintic administration on age and weight at puberty in spring-born beef heifers. *Journal of Animal Science* **74**, 736-744.

Rae DO (2002) Bovine Estrus: Tools for detection and understanding, in: Factors Affecting Calf Crop Biotechnology of Reproduction. Fields MJ, Sand RS and Yelich JV eds. CRC Press Ltd. Baca Raton, Florida.

Ramsey JJ and Hagopian K (2006) Energy Expenditure and Restriction of Energy Intake: Could Energy Restriction Alter Energy Expenditure in Companion Animals? *The Journal of Nutrition* **136**, 19585-19665.

Rauw WM, Kanisb E, Noordhuizen-Stassenc EN and Grommersc FJ (1998) Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* **56**, 15-33.

Rauw WM, Portolés O, Corella D, Soler J, Reixach J, Tibau J, Prat JM, Diaz I and Gómez-Raya L (2007) Behavior influences cholesterol plasma levels in a pig model. *Animal* **1**, 865-871.

Rawlings NC, Jeffcoate IA and Rieger DL (1984) The influence of estradiol 17β and progesterone on peripheral serum concentrations of luteinizing hormone and follicle stimulating hormone in the ovarietomized ewe. *Theriogenology* **22**, 473-488.

Reece WO (2015) Body water: properties and functions. In 'Dukes' Physiology of Domestic Animals' (Eds Reece WO, Erickson HH, Goff JP, Uemura EE) pp. 103-154. (Wiley Blackwell: Danvers)

Reith S, Pries M, Verhulsdonk C, Brandt H and Hoy S (2014) Influence of estrus on dry matter intake, water intake and BW of dairy cows. *Animal* **8**, 748-753.

Rej R (1978) Aspartate aminotransferase activity and isoenzyme proportions in human liver tissues. *Clinical Chemistry* **24**, 1971- 1979.

Reynolds CK (1995) Quantitative aspects of liver metabolism in ruminants in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction: Proc. 8th International Symposium Ruminant Physiology W. v. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, ed. Ferdinand Enke Verlag, Stuttgardt, Germany. Pages 351-371.

Richardson EC, Herd RM, Arthur PF, Wright J, Xu G, Dibley K and Oddy VH (1996) Possible physiological indicators for net feed conversion efficiency in beef cattle. *The Australian Society of Animal Production* **21**, 103-106.

Richardson EC, Herd RM, Oddy VH, Thompson JM, Archer JA and Arthur, PF (2001) Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. *Australian Journal of Experimental Agriculture* **41**, 1065–1072.

Richardson EC, Herd RM, Colditz IG, Archer JA, Arthur PF (2002) Blood cell profiles of steer progeny from parents selected for and against residual feed intake. *Australian Journal of Experimental Agriculture* **42**, 901-908.

Richardson EC and Herd RM (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. *Australian Journal of Experimental Agriculture* **44**, 431-440.

Richardson EC, Herd RM, Archer JA and Arthur PF (2004) Metabolic differences in Angus steers divergently selected for residual feed intake. *Australian Journal of Experimental Agriculture* **44**, 441-452.

Riedesel DH and Engen RL (2015) The heart and vasculature: Gross structure and basic properties. In 'Dukes' Physiology of Domestic Animals' (Eds Reece WO, Erickson HH, Goff JP, Uemura EE) pp. 287-463. (Wiley Blackwell: Danvers)

Rincon-Delgado RM, Gutierrez-Banuelos H, Perez-Vazquez ED, Muro-Reyes A, Diaz-Garcia LH, Banuelos-Valenzuela R, Gutierrez-Pina FJ, Medina-Flores CA, Escareno-Sanchez LM, Aguilera-Soto JI, Lopez-Carlos MA and Arechiga-Flores CF (2011) Relationship of residual feed intake on specific hematological and biochemical parameters in Rambouillet sheep. *Journal of Animal and Veterinary Advances* **10**, 1112-1116.

Roelofs JB, López-Gatius, Hunter RHF, van Eerdenburg FJCM and Hanzen CH (2010) When is a cow in estrus? Clinical and practical aspects. *Theriogenology* **74**, 327-344.

Roelofs JB, Van Erp-van der Kooij E (2015) Estrus detection tools and their applicability in cattle: recent and perspectival situation. *Animal Reproduction Science* **12**, 498-504.

Sainz RD and Bentley BE (1997) Visceral organ mass and cellularity in growth-restricted and refed beef steers. *Journal of Animal Science* **75**, 1229-1236.

Sath K, Sokun K, Pauly T and Holtenius K (2012) Feed intake, digestibility, and N retention in cattle fed rice straw and para grass combined with different levels of protein derived from cassava foliage. *Asian-Australasian Journal of Animal Sciences* **25**, 956-961.

Sato K, Han DC, Fujii Y, Tsushima T, Shizume K (1987) Thyroid hormone stimulates alkaline phosphatase activity in cultured rat osteoblastic cells (ROS 17/2.8) through 3,5,3'-triiodo-L-thyronine nuclear receptors. *Endocrinology* **120**, 1873-1881.

Sato J, Kanata M, Yasuda J, Sato R, Okada K, Seimiya Y and Naito Y (2005) Changes of serum alkaline phosphatase activity in dry and lactational dairy cows. *The Journal of Veterinary Medical Science* **67**, 813-815.

Sattar A and Mirza RH (2009) Haematological parameters in exotic cows during gestation and lactation under subtropical conditions. *Pakistan Veterinary Journal* **29**, 129-132.

Savory A and Parsons SD (1980) The Savory Grazing Method. Rangelands **2**, 234-237.

Scanga JA, Belk KE, Tatum JD, Grandin T and Smith GC (1998) Factors contributing to the incidence of dark cutting beef. *Journal of Animal Science* **76**, 2040- 2047.

Schlattner U, Tokarska-Schlattner M, Wallimann T (2006) Mitochondrial creatine kinase in human health and disease. Mitochondrial creatine kinase in human health and disease. *Biochimica et Biophysica Acta* **1762**, 164-180.

Schmitz A, Ayars M, Highmoor T and Perillat B (2003) An Assessment of Western Canadian Beef Development, 1-193.

Schultz RH, Fahning ML and Graham EF (1971) A chemical study of uterine fluid and blood serum of normal cows during the oestrus cycle. *Journal of Reproduction and Fertility* **27**, 355-367.

Schillo KK, Hall JB and Hileman SM (1992) Effects of nutrition and season on the onset of puberty in the beef heifer. *Journal of Animal Science* **70**, 3994-4005.

Seleen JC (1978) Alkaline phosphatase activity in amniotic fluid in the second half of pregnancy. Amniotic Fluid: Research and Clinical Application (Elservier/North-Holland Biomedical Press).

Serjrsen K and Neimann-Sorensen A (1981) Current topics in Veterinary Medicine and Animal Science. Factors influencing fertility in the postpartum cows. *Nutritional Physiology and Feeding of the Cow around Parturition* **20,** 325-357

She QB, Mukherjee JJ, Huang JS, Crilly KS and Kiss Z (2000) Growth factor-like effects of placental alkaline phosphatase in human fetus and mouse embryo fibroblasts. *FEBS Letters* **469**, 163-7.

Sheldon BC and Verhulst S (1996) Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. *Trends in Ecology Evolution* **11**, 317-321.

Short RE and Bellows RA (1971) Relationships amoung weight gains, age at puberty and reproductive performance in heifers. *Journal of Animal Science* **32**, 127-131.

Short RE and Adams DC (1988) Nutritional and hormonal interrelationships in beef cattle reproduction. *Canadian Journal of Animal Science* **68**, 29–39.

Silper BF, Robles I, Madureira AMI, Burnett TA, Reis MM, de Passillé AM, Rushen J and Cerri RLA (2015) Automated and visual measurements of estrus behavior and their sources of variation in Holstein heifers. I: Walking activity and behavior frequency. *Theriogenology* **84**, 312-320.

Small JA, Charmley E, Rodd AV and Fredeen AH (1996) Serum mineral concentrations in relation to estrus and conception in beef heifers and cows fed conserved forage. *Canadian Journal of Animal Science* **77**, 55-62.

Solis JC, Byers FM, Schelling GT, Long CR and Greene LW (1987) Maintenance requirements and energetic efficiency of cows of different breed types. *Journal of Animal Science* **66**, 764-773.

Solomon AL, Siddals KW, Baker PN, Gibson JM, Aplin JD and Westwood M (2014) Placental alkaline phosphatase de-phosphorylates insulin-like growth factor (IGF)-binding protein-1. *Placenta* **35**, 520-522.

Spicer LJ, Alonso J and Chamberlain CS (2001) Effects of Thyroid Hormones on Bovine Granulosa and Thecal Cell Function In Vitro: Dependence on Insulin and Gonadotropins. *Journal of Dairy Science* **84**, 1069-1076.

Statistics Canada (2010) Food Statistics available at http://www.statcan.gc.ca/pub/21-020-x/2009001/part-partie1-eng.htm

Statistics Canada (2011) Demographic Changes in Canadian Agriculture available at http://www.statcan.gc.ca/pub/96-325-x/2014001/article/11905-eng.htm#a3

Statistics Canada (2014) Average household food expenditure, by province (Canada) available at http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/famil132a-eng.htm

Statistics Canada (2015) Farm cash receipts, by province (quarterly) (Canada) available at http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/agri04a-eng.htm

Statistics Canada (2016) Cattle inventories, by province (Canada) available at http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/prim50a-eng.htm

Stevenson JS, Hill SL, Nebel RL, DeJarnette JM (2014) Ovulation time and conception risk after automated activity monitoring in lactating dairy cows. *Journal of Dairy Science* **97**, 4296-4308.

Stryer L (1988) ´Biochemistry by Lubert Stryer.´ (W H Freeman, New York)

Suttle NF (2010) Mineral Nutrition of Livestock 4th ed (CAB International).

Talukder S, Kerrisk KL, Ingenhoff L, Thompson PC, Garcia SC and Celi P (2014) Infrared technology for estrus detection and as a predictor of time of ovulation in dairy cows in a pasture-based system. *Theriogenology* **15**, 925-935.

Tarrant V and Grandin T (2000) Cattle Transport. In ‘Livestock Handling and Transport’ (Ed. Grandin T) pp. 151-173 (CABI Publishing, New York, NY).

Ternouth JH (1990) Phosphorous and beef production in northern Australia 3. Phosphorous in cattle- A review. *Tropical Grasslands* **24**, 159-169.

Thrall MA, Baker DC, Campbell TW, DeNicola D, Fettman MJ, Lassen ED, Rebar A, Weiser G, Troy DB (Eds) (2004) Veterinary Hematology and Clinical Chemistry (Lippincott Williams & Wilkins).

Tizard IR (2012) Veterinary Immunology 9th ed (Saunders).

Toniollo GH, Vicente WRR, Oliveria CA, Malheiros EB, Carvalho MB (1998) Triiodothyronine (T3) and thyronine (T4) serum level evaluation during the oestrus cycle in guilts. *Brazilian Journal of Veterinary Research and Animal Science* **35**, 210-214.

Trenkle A (1976) The anabolic effect of estrogens on nitrogen metabolism of growing and finishing cattle and sheep. *Environmental Quality and Safety* **5**, 79-88.

University of Guelph (2015) Animal Health Laboratory Guidelines, Guelph, Ontario.

Van Eerden E, Van Den Brand H, Heetkamp MJW, Decuypere E and Kemp B (2006) Energy partitioning and thyroid hormone levels during Salmonella enteritidis infections in pullets with high or low residual feed intake. *Poultry Science* **85**, 1775-1783.

Van Soest PJ (1994) Nutritional Ecology of the Ruminant (Cornell University Press).

Vann RC, Tucker SV, Ray RA and Baker JF (2013) Case study: supplemental fat effects on cholesterol profiles of nulliparous beef heifers. *The Professional Animal Scientist* **19**, 35-38.

Veerkamp RF (1997) Selection for economic efficiency of dairy cattle using information on live weight and feed intake: A review. *Journal of Dairy Science* **81**, 1109-1119.

Villanueva I, Alva-Sánchez C and Pacheco-Rosado J (2013) Review Article. The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. *Oxidative Medicine and Cellular Longevity* **2013**, 1-15.

Vrijens DMJ and Rehrer NJ (1999) Sodium-free ingestion decreases plasma sodium during exercise in the heat. *Journal of Applied Physiology* **86**, 1847-1851.

Vynckier L, Debackere M, De Kruif A and Coryn M (1990) Plasma estradiol-17 beta concentrations in the cow during induced estrus and after injection of estradiol-17 beta benzoate and estradiol-17 beta cypionate--a preliminary study. *Journal of Veterinary Pharmacology and Therapeutics* **13**, 36-42.

Wade GN and Schneider JE (1992) Metabolic fuels and reproduction in female mammals. *Neuroscience & Behavioral Reviews* **16**, 235.

Wagter LC, Mallard BA, Wilkie BN, Leslie KE, Boettchert PJ, and Dekkers JCM (1999) A quantitative approach to classifying Holstein cows based on antibody responsiveness to its relationship to peripartum mastitis occurrence. *Journal of Dairy Science* **83**, 488-498.

Walawski K, Majewski A, Lenzy Z, Dabrowska MJ and Ruszczynska B (1980) Relationship between the polymorphism and activity of alkaline phosphatase in the blood serum and the rate of growth in young black-and-white cattle. *Genetica Polonica* **21**, 195-201.

Walker RS, Martin RM, Gentry GT, Gentry LR (2015) Impact of cow size on dry matter intake, residual feed intake, metabolic response, and cow performance. *American Society of Animal Science* **93**, 672-684.

Wang YJ, Ko M, Holligan S, McBride BW, Fan MZ, Swanson KC (2009) Effect of dry matter intake on visceral organ mass, cellularity, and the protein expression of ATP synthase, Na/K-ATPase, proliferating cell nuclear antigen and ubiquitin in feedlot steers. *Canadian Journal of Animal Science* **89**, 253-262.

Warriss, PD, Brown SN, Knowles TG, Kestin SC, Edwards JE, Dolan SK and Phillips AJ (1995) Effects on cattle of transport by road for up to 15 hours. *Veterinary Record* **136**, 319-323.

Webb P (1986) 24-hour energy expenditure and the menstrual cycle. *American Journal of Clinical Nutrition* **44**, 614-619.

Whittfield JB (2001) Gamma glutamyl transferase. *Critical Reviews in Clinical Laboratory Sciences* **38**, 263-355.

Wood KM, Montanholi YR, Fitzsimmons CF, Miller SP, McBride BW and Swanson KC (2014) Characterization and evaluation of residual feed intake measured in mid- to late-gestation mature beef cows and relationships with circulating serum metabolites and linear body measurements. *Canadian Journal of Animal Science* **94**, 1-10.

Yaqub LS, Kawu MU, Ayo JO, Ambali SF, Habibu B (2013) Effect of oestrus cycle on serum electrolytes and liver enzymes in Red Sokoto goats. *African Journal of Biochemistry Research* **7**, 174-178.

Yokus B and Cakir UD (2006) Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. *Biological Trace Element Research* **109**, 255-266.

You Q, Karrow NA, Quinton M, Mallard BA, Boermans J (2008) Enhanced cutaneous hypersensitivity reactions are associated with ovine high and low cortisol responsiveness to acute endotoxin challenge. *Veterinary Dermatology* **19**, 174-183.