RELATIONSHIPS OF BLOOD PARAMETERS AND FERTILITY-RELATED TRAITS WITH AGE AND FEED EFFICIENCY IN YOUNG BEEF BULLS

by

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A million million spermatozoa
All of them alive;
Out of their cataclysm but one poor Noah
Dare hope to survive.
And among that billion minus one
Might have chanced to be
Shakespeare, another Newton, a new Donne—
But the One was Me.

-Aldous Huxley, 1920

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ABSTRACT

Feed efficiency and bull fertility are two major factors affecting profitability of the beef industry. However, there is concern of an antagonistic relationship between these two factors, highlighting the need to clarify the relationship between age, feed efficiency and sexual development. Two experiments were undertaken to evaluate associations of blood parameters and fertility-related measures with age and feed efficiency in yearling bulls. Firstly, 32 bulls were studied and among other results, younger and efficient bulls exhibited lower testosterone and triiodothyronine levels, respectively. Secondly, investigation of 158 bulls revealed that younger bulls had smaller scrotal circumference, higher scrotal radiant heat loss and fewer normal sperm while efficient bulls indicated lower scrotal circumference, scrotal radiant heat loss, and a trend towards lower testicular echogenicity and higher sperm head defects. Metabolic differences associated with variation in feed efficiency may impact reproductive function as illustrated by features of delayed sexual development in efficient bulls.

LIST OF ABBREVIATIONS AND SYMBOLS USED

$	Dollars
%	Percent
©	Copyright
®	Registered Trademark
°C	Degree Celsius
ADG	Average daily gain
AGE	Age
ALP	Alkaline phosphatase
AMH	Anti- Müllerian Hormone
AST	Aspartate aminotransferase
AVG	Average
BDNF	Brain-derived neurotrophic factor
BIC	Bayesian information criterion
BIO	Beef Improvement Opportunities
BKFT	Backfat thickness
BSE	Breeding soundness evaluation
BW	Body weight
CBC	Complete blood cell count
CCK	Cholecystokinin
Cells/L	Cells per liter
CK	Creatine kinase
cm	Centimeter
cm^2	Centimeter square
CO	Colorado
Co	Company
CO_2	Carbon dioxide
DM	Dry matter
DM/day	Dry matter intake per day
DMI	Dry matter intake
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FG	Feed to gain ratio
fL	Femtoliters
FSH	Follicle-stimulating hormone
g	Gram
g/L	Gram per liter
GnRH	Gonadotropin-releasing hormone
h	Hour
i.e.	Id est
IGF1	Insulin-like growth factor 1
Inc.	Incorporated
IU	International unit

IU/kg	International unit per kilogram
kg	Kilogram
kg/d	Kilogram per day
LH	Luteinizing hormone
Ltd.	Limited
m	Meter
MAX	Maximum
Max.	Maximum
MCH	Mean cell hemoglobin
MCV	Mean cell volume
MHz	Megahertz
Min.	Minimum
mL	Milliliter
mm	Millimeter
mmol/L	Millimole per liter
N	Nitrogen
ng/mL	Nanograms per milliliter
nmol/L	Nanomole per liter
NRC	National Research Council
P	P-value
pg/mL	Picogram per milliliter
r	Pearson's coefficient
R^2	Coefficient of determination
RBC	Red blood cells
RBEA	Ribeye area
RFI	Residual feed intake
RFI_{AGE}	Residual feed intake-age
RFI_{US}	Residual feed intake-ultrasound
RIA	Radioimmunoassay
RUMP	Rumpfat thickness
S.D.	Standard deviation
SAS	Statistical Analysis Software
SC	Scrotal circumference
STA	Scrotal temperature apex
STB	Scrotal temperature base
STG	Scrotal temperature gradient
STT	Scrotal temperature area
T_3	Triiodothyronine
T_4	Thyroxine
THI	Temperature humidity index
U/L	Units per liter
USA	United States of America
vs	Versus
WBC	White blood cells

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CHAPTER 1

INTRODUCTION

Canada is the fifth largest beef exporter in the world, making the beef industry an important economic driver that contributes around $33 billion annually to the Canadian economy (Canfax, 2016). Moreover, agriculture is the main economic driver in the Maritimes, with beef cattle contributing $20.2 million annually (Nova Scotia Department of Agriculture, 2013). Canada is also a major worldwide exporter of beef cattle genetics, mainly through the export of frozen semen (Agriculture and Agri-Food Canada, 2014). Beef cattle breeding programs have focused on the improvement of feed efficiency, which is being followed by recent research initiatives in the dairy industry (Varga and Dechow, 2013). The improvement of feed efficiency has a tremendous impact on the economics and environmental aspects of the beef industry (Food and Agriculture Organization of the United Nations, 2013). However, recent studies have demonstrated that improved feed efficiency seems to be associated with features of delayed sexual maturity in young bulls (Fontoura et al. 2016; Montanholi et al. 2016a).

Reproductive performance has greater impact on beef economic returns than does either growth rate or product quality (Trenkle and Willham, 1977). Effective bull selection is the main driver leading to genetic improvement of the herd, with each bull contributing nearly 90% of its genetic material to the herd (Field, 2007). Bulls that reach sexual maturity younger contribute more to the overall genetic gain by shortening the generation interval (Holroyd and McGowan, 2014). Additionally, lifetime cow fertility

can be improved by indirect selection in bulls (Mackinnon et al. 1990), leading to increased profitability by the cow-calf sector. Optimum conception rate at first service can only be achieved in cows with adequate body condition scores (Dunn et al. 1969) being bred to bulls selected for improved fertility (Wiltbank and Parish, 1986). This supports the importance of assessing yearling bulls for early sexual maturity and reproductive potential. It is of even greater importance in regions, such as the Maritimes, where the average cow-calf herd is 40 cows (Prince Edward Island Department of Agriculture and Fisheries, 2015) and natural service, using the same bull over multiple breeding seasons, is the principal mating system for beef cattle in Canada.

Efficiency is defined as the ability to achieve maximum productivity with minimum wasted effort or expense (Oxford Dictionary of English, 2010). Thus, in animal production, an efficient animal attains the greatest amount of output (i.e. meat, milk, egg), with minimum input, which is generally feed. In the beef industry, feed is the largest variable cost associated with production (Miller et al. 2001) and therefore, improving cattle efficiency results in considerable savings by the industry, while reducing the environmental footprint associated with beef production (Food and Agriculture Organization of the United Nations, 2013). Feed efficiency has moderate to high heritability (Pitchford, 2004) and genetic improvement for this complex trait relies on extensive phenotyping for indirect indicators. Hence, selection for feed efficiency in breeding bulls can contribute to a more efficient herd and a more profitable industry. However, selection for production efficiency can lead to undesirable effects on metabolism, health and reproduction of cattle and other livestock species (Rauw et al. 1998). Over the years, concerns regarding improvements in feed efficiency and potential

negative consequence in terms of reproduction traits in bulls have arisen. Presently, there is a growing body of evidence suggesting an antagonistic association between improved feed efficiency and fertility-related measures in young beef bulls, such as delayed sexual development (Awda et al. 2013; Fontoura et al. 2016; Montanholi et al. 2016a). Differences in energy metabolism between cattle with diverging feed efficiency (Montanholi et al. 2013a) may influence sexual development and reproductive function (Pruitt et al. 1986) in young bulls. Moreover, feed efficiency (Owens et al. 1995) and sexual development (Coe, 1999) are strongly influenced by age and therefore, age is an important factor to be considered, when assessing sexual development and fertility-related measures in the context of feed efficiency.

Even though studies have identified an association of feed efficiency with fertility-related measures in young bulls (Awda et al. 2013; Fontoura et al. 2016; Hafla et al. 2012; Montanholi et al. 2016a; Wang et al. 2012), further evaluation to investigate changes in sexual development over time, in a large population of bulls, is needed. Additionally, exploration of phenotypes, as possible proxies of feed efficiency, in young bulls is warranted to prevent selection for feed efficiency at the expense of reproductive function. This thesis was initiated in May 2014 to assess associations of blood parameters with age and feed efficiency models under two sampling routines and to characterize the categorical comparisons and variability in sexual development and fertility-related measures, in the context of age and feed efficiency in yearling bulls.

CHAPTER 2

LITERATURE REVIEW

2.1 Feed efficiency in the beef industry

Improvement in feed efficiency provides important economic benefits to the beef industry in terms of profitability and enhanced environmental sustainability (Ahola and Hill, 2012). Fox et al. (2001) reported that a 10% improvement in feed efficiency due to better use of metabolizable energy can lead to a 43% increase in profit. In the past, beef cattle improvement programs have focused on selection to improve output-related traits, such as body weight (BW), carcass and fertility traits (Ahola and Hill, 2012). Since feed is the largest variable cost associated with beef production (Miller et al. 2001), improvement programs have included cost-related traits, such as feed efficiency, in their breeding programs. Residual feed intake (RFI) is the preferred feed efficiency trait for genetic improvement as it is moderately heritable (Pitchford, 2004). Moreover, genetic variation in feed efficiency occur in most breeds of cattle (Crowley et al. 2010), suggesting that genetic selection for improvement of feed efficiency can occur through crossbreeding (Elzo et al. 2009) or within a breed (Herd and Bishop, 2000). Additionally, the expectation of improved feed efficiency in progeny of cattle selected for feed efficiency has been confirmed by others (Donoghue et al. 2011), supporting the premise that selection for improved feed efficiency is profitable for the beef industry.

Differences in the amount and type of feed ingested, sex and breed of animals, and environmental conditions in which animals are managed influences feed efficiency

(Brody, 1945); this suggests alternative means to improve feed efficiency, such as nutritional manipulations and improvement in husbandry systems. Nutritional approaches to improve feed efficiency have been previously investigated. Feeding diets containing whole corn with no added roughage tend to improve feed efficiency of beef steers (Turgeon et al. 2010). Similarly, a 22% rise in feed efficiency in steers was observed with the optimization of rumen degradable protein by removing roughage from a corn-base diet (Kerley, 2012). Moreover, the use of feed enzymes, such as exogenous fibrolytic enzymes, resulted in improved ruminal fiber digestion, leading to improved feed efficiency (Beauchemin et al. 2003). Additionally, cattle in intensive husbandry systems are exposed to greater abundance of stressors, which may result in lower performance or reduced feed efficiency (Fox, 1985). Higher stocking densities (Lee et al. 2012), muddy pens (DeRouchey et al. 2005) and low environmental temperature (Schake, 1996) resulted in reduced feed efficiency in cattle. Thus improvements in management practices can lead to an increase in feed efficiency, as well as better animal welfare (Abney and Galyean, 2006).

2.2 Assessment of feed efficiency

Many indices have been proposed to determine energetic efficiency of cattle. Gross feed efficiency can be useful for the evaluation of production efficiency in growing and finishing cattle (Archer et al. 1999). However, this trait has been strongly associated with growth traits (Arthur et al. 2001), leading to increased mature weight of breeding females, with minimal reduction in feed intake (Herd and Bishop, 2000).

Energy for maintenance requirements represents a large proportion of the total

energy requirements of cattle and is defined as a ratio of feed intake used for maintenance per unit of metabolic body size (Ferrell and Jenkins, 1985). However, measurements of maintenance efficiency can only be obtained in animals with steady liveweight and thus, cannot be measures in growing animals, plus this assessment relies on inaccurate estimates of maintenance (Archer et al. 1999).

Partial efficiency of growth is defined as the ratio of weight gain per unit of feed used for growth after subtracting requirements for maintenance (Kellner, 1909). Maintenance requirements can be estimated from feeding tables (i.e., NRC, 1996) or from population estimates of maintenance energy requirements (Archer et al. 1999). Although partial efficiency of growth offers advantages over gross feed efficiency due to lower associations with average daily gain (ADG), difficulties arise when using partial efficiency of growth as inherent animal variation in energetic efficiencies associated with maintenance are not captured (Berry and Crowley, 2013).

The concept of RFI was first described in poultry by Titus (1928) then applied to beef cattle by Koch et al. (1963). Feed intake is adjusted for BW and ADG, separating feed intake into two components; the expected feed intake for the given level of production and the residual portion (Koch et al. 1963). The residual portion is used to identify animals that deviate from the expected level of feed intake, with feed efficient animals having lower RFI. Residual feed intake is independent of BW and ADG, allowing for inherent comparison between animals at different stages of production. The residual portion is related to basal energy requirements and differences in efficiency of growth (Archer et al. 1999), supporting the association of feed efficiency with energy metabolism.

The computation of RFI requires the estimation of expected feed intake which can be obtained by using individual feed intake prediction models (i.e. NRC, 1996) or through phenotypic or genetic regression of actual feed test data (Kennedy et al. 1993). The original phenotypic regression model for RFI proposed by Koch et al. (1963) was designed for growing cattle, and can be adapted to other categories of cattle, such as pregnant heifers (Gonano et al. 2014). Furthermore, past studies (Montanholi et al. 2009), included measures of body composition to account for variation in carcass leanness and fatness. Measures of body composition may be obtained directly from the carcass or indirectly, using ultrasound scanning. Inclusion of either carcass or ultrasound body composition traits in the RFI determination model is important to avoid biases related to carcass composition (Montanholi et al. 2009).

2.3 Determinants and indicators of feed efficiency

Better understanding of physiological processes that regulate variation in feed utilization may substitute the need for measuring individual feed intake for a minimum of 84 days (Archer and Bergh, 2000), which is a limiting factor to expand the assessment of feed efficiency across the beef industry. Thus, there is a need for alternative measures of feed efficiency in the beef industry, such as genetic and biological indicators.

2.3.1 Genetics

The existence of genetic variation and heritability for RFI suggests potential for genetic improvement of feed efficiency (Arthur and Herd, 2008; Pitchford, 2004). Progeny from parents selected for improved efficiency had lower feed intake without negative impact on other performance traits (Donoghue et al. 2011). Additionally, RFI in

post-weaning heifers was observed to be moderately correlated with RFI measured when they became mature cows (Archer et al. 2002; Black et al. 2013; Hafla et al. 2013). This suggests that selection for improved post-weaning RFI has the potential to produce progeny that are efficient in all segments of the industry. The use of genomic information can be a strategy to improve the selection of phenotypes, such as RFI (Santana et al. 2014). Genome-wide association studies were useful to identify markers that explained important variations observed in RFI (Rolf et al. 2011). Information obtained from markers along the chromosome improved detection of young candidates for genetic selection, leading to genetic gain, while reducing generation interval (Lu et al. 2012). Genetic improvement for this complex trait relies on extensive phenotyping of indirect indicators. The success of genomic selection depends on accurate collection of phenotypic data and requires a significant effort, in order to build large reference populations of animals with desirable phenotypes and genotypes (Gonzalez-Recio et al. 2014).

Although genetic selection for improved RFI may lead to enormous benefits for the industry, selection for production efficiency can also result in undesirable effects on metabolism, health and reproduction of cattle and other livestock species (Rauw et al. 1998). Thus, genetic selection must be properly delimited to attain a balance between feed intake and other essential characteristics, such as gain, reproduction and meat quality (Santana et al. 2014).

2.3.2 Metabolic rate

Gross energy is the total amount of energy consumed by an animal, while net

energy is the energy available for an animal to use for background energy requirements and production sinks (Reynolds, 2000). Background energy requirements can be divided into cell maintenance functions such as mitochondrial respiration, ion pumping and protein turnover and service functions such as heart, brain, gastrointestinal tract, liver function and thermoregulation (Baldwin et al. 1980). Reduction of specific cell maintenance and service functions could considerably improve animal production efficiency. Mitochondria are the sites of energy transfer in the cells and it has been proposed that the rate of mitochondrial respiration is increased in efficient steers, compared to inefficient steers (Kolath et al. 2006; Lancaster et al. 2014). Other studies (Herd and Bishop, 2000; Kolath et al. 2006; Montanholi et al. 2013a), also provided further evidence to support an association between improved feed efficiency and background energy requirements. Service functions, such as nutrient absorption in the gastrointestinal tract, are also important energetic sinks. The gastrointestinal tract uses a large amount of energy, in proportion to its weight (Johnson et al. 1990). This suggests that animals with differing levels of feed intake, such as those with diverging feed efficiency, may vary in the amount of energy used in the gastrointestinal tract, as a response to differing functional workload imposed by differences in levels of feed intake between efficient and inefficient cattle (Montanholi et al. 2013a).

Body composition is also an important factor which influences energy metabolism and consequently, feed efficiency. Reduced rates of protein degradation are associated with an increased in lean body mass without a rise in maintenance energy needs (Lobley, 2003). Castro Bulle et al. (2006) observed that improved utilization of feed was associated with both lower rates of muscle breakdown and maintenance energy

requirements in cattle. Efficiency of fat accretion is approximately 1.7 times that of protein (Owens et al. 1995). However, more water is stored with retained protein than with retained fat and thus, lean tissue gain is four times as efficient as fat accretion (Owens et al. 1995). When assessing feed efficiency by using the original RFI model (Koch et al. 1963), disparities in body composition traits are observed such as greater lean tissue in efficient animals. However, when body composition traits are included in the RFI determination model (Montanholi et al. 2009), no difference in carcass composition is observed between efficiency groups (Awda et al. 2013). Therefore, the importance of the effect of body composition on RFI variation must be taken into consideration, in order to ensure that improved feed efficiency does not lead to undesirable changes in carcass composition.

Heat production associated with metabolism can be measured via gas exchange through indirect calorimetry (Kleiber, 1961) or radiant heat loss assessment, using infrared thermography (Montanholi et al. 2008). Infrared thermography offers a practical approach to the assessment of heat production in livestock species (Montanholi et al. 2008). However, this technology has many other implications in the beef industry, such as detection of health disorders (Hurnik et al. 1984), indication of tissue damage and discomfort (Schwartzkopf-Genswein and Stookey, 1997), estrus detection (Hellebrand et al. 2003) and testicular thermoregulation (Kastelic et al. 1995). In addition, studies have shown an association between infrared thermography and feed efficiency (Digiacomo et al. 2014; Montanholi et al. 2009; Montanholi et al. 2010), with feed efficient cattle displaying lower radiant heat dissipation. The association of radiant heat loss with RFI is likely associated with variation in energy metabolism according to feed efficiency

phenotypes (Montanholi et al. 2010).

2.4 Blood plasma parameters

Blood concentrations of metabolic products, ions, enzymes, proteins and hormones have been examined as potential indirect indicators of feed efficiency in cattle (Gonano et al. 2014; Kelly et al. 2010; Richardson et al. 2004; Walker et al. 2015). Metabolic products, such as carbon dioxide (CO_2), glucose, urea and acetate have been studied in the context of feed efficiency. Carbon dioxide has been observed to be lower in efficient heifers over the circadian period (Gonano et al. 2014). Mitochondrial respiration in tissue consumes approximately 95% of available oxygen, in order to produce substrates, including CO_2, which is transported in the blood (Pittman, 2011). As CO_2 is diffused into blood due to energetic processes within the tissue, it can be an indicator of energy utilization and efficiency. This suggests that feed efficient cattle are more efficient in energy utilization, by maximizing the use of energetic compounds and minimizing the production of CO_2, an end product of the energy cascade utilization (Kleiber, 1961).

Blood glucose was demonstrated to be higher in inefficient steers (Kolath et al. 2006); however, others (Kelly et al. 2010; Wood et al. 2014) observed no relationship between feed efficiency and blood glucose in beef cattle. Disparities between studies are likely due to differences in sex, stage of gestation and plane of nutrition. Glucose is a highly energetic substrate, utilized for maintenance functions by the liver in beef cattle, with liver function accounting between 5 to 10% of basal energy expenditure (Baldwin et al. 1980). Overall, glucose is utilized as an energetic fuel throughout the body, although studies disagree on the relationship between feed efficiency and glucose. This suggests

that efficient animals utilize less glucose to maintain basal energy expenditure compared to inefficient animals.

Higher levels of acetate (Karissa et al. 2014; Kelly et al. 2010), were observed in efficient cattle. Blood acetate clearance rate was shown to be related to feed intake in ruminants, as indicated by depressed feed intake associated with reduced clearance rate (Preston and Leng, 1987). Thus, higher blood acetate in efficient cattle may play a key role in the observed reduction in feed intake.

Ion transport, a cellular maintenance-related process, account for 30 to 40% of total basal energy expenditure in ruminants (Baldwin et al. 1980). The relevance of ion transport in metabolic function suggests that differences in ion transport could contribute to variation in feed efficiency (Richardson and herd, 2004). Furthermore, proper mineral supplementation was found to be essential for optimal cattle performance (Spears, 1996) and health (Suttle, 2010). In a recent study by Dias et al. (2016), absorption of copper, zinc, cobalt and manganese were increased in efficient pregnant heifers, suggesting differences in use of these ions between efficiency groups.

There has been growing research interest regarding the relationship of circulating enzymes and feed efficiency. Aspartate aminotransferase (AST) has been shown to be associated with feed efficiency (Gonano et al. 2014; Richardson et al. 2004). Aspartate aminotransferase is a key liver enzyme in amino acid metabolism (Stryer, 1988) and may reflect the differences in metabolic demand of organ function between efficiency groups.

Additionally, brain-derived neurotrophic factor (BDNF) also offers potential as an indirect indicator of feed efficiency. Brain-derived neurotrophic factor is a neurotrophin which is mainly involved in synaptic plasticity, neuronal maturation,

dendritic remodeling and formation of synaptic contacts (Schinder and Poo, 2010). However, recent studies demonstrated the metabolic role of BDNF through its implication in the neuroendocrine control of mammalian feeding behavior and energy homeostasis (Cordeira et al. 2010). Decrease in BDNF signaling and expression can influence eating behavior and energy expenditure by the regulation of appetite and energy balance in the hypothalamus as observed by increase feed intake and body weight in humans and mice (Xu et al. 2003). In cattle, BDNF has been scarcely studied but recently, mutations in the BDNF gene region were shown to be associated with variations in milk fat yield (Zielke et al. 2011). Additionally, expression of BDNF and its receptor in subcutaneous adipose tissue of lactating cows has been observed (Colitti et al. 2015), supporting its role in the regulation of energy balance in cattle.

In recent studies, testosterone has major anabolic effects on the physical maturation of males and is important in enhancing muscles mass by promoting protein metabolism (Sheffield-Moore, 2000). Additionally, testosterone levels have been associated with ADG and feed efficiency (Cook, R.B. et al. 2000), suggesting an association between feed efficiency and testosterone levels of young bulls.

Leptin is synthesized primarily by adipose tissue and has a functional role in reproduction, inflammation and whole body energy homeostasis (Friedman and Halaas, 1998) through its effects on appetite, body composition, energy expenditure and nutrient partitioning (Kershaw and Flier, 2004). Leptin has been observed to be greater in inefficient cattle (Richardson et al. 2004; Walker et al. 2015). Alternatively, other studies reported no association between feed efficiency and leptin levels (Kelly et al. 2010).

Moreover, regulation of leptin expression may be influenced by thyroid hormones (Ramos and Zamoner, 2014).

Thyroid hormones, thyronine (T_4) and triiodothyronine (T_3), are under the control of the hypothalamic-pituitary-thyroid axis and are crucial in the regulation of growth, differentiation, and metabolism in nearly all somatic tissues (Hulbert, 2000). While both are important, T_3 has been demonstrated to have a greater biological activity, compared to T_4 (Gross, 1993) and mainly function as an oxidative metabolism stimulant (Hulbert, 2000). Triiodothyronine has been associated with higher maintenance energy requirements (Iossa et al. 2001), increased energy expenditure and feed intake (Hulbert, 2000), all of which are consistent with the increase in feed intake of inefficient cattle. Elevated thyroid hormones affect thermogenesis through variations in heat production (Hulbert, 2000), and thus may explain part of the variation in heat production between animals with diverging feed efficiency.

2.5 Sexual development

The process of sexual development in bulls involves a complex maturation mechanism of the hypothalamus-pituitary-testes axis, consisting of three periods, namely the infantile, prepubertal, and pubertal (Amann, 1986), which involve changes in gonadotropins, blood hormones and reproductive tract maturation.

In *Bos taurus* bulls, the infantile period lasts from birth to approximately two months of age and is characterized by low production of gonadotropin-releasing hormone (GnRH), gonadotropins and testosterone (Amann, 1986). During this period, little change in testicular cell structure has been observed, with mesenchymal-like cells comprising the

majority of the testicular interstitial tissue (Brito, 2015a). The lumen-less seminiferous tubules are mainly comprised of undifferentiated Sertoli cells that undergo maximal cell multiplication between one and two months of age, in conjunction with gonocyte proliferation (Abdel-Raouf, 1960).

Research has indicated that maturation of the hypothalamus causes an increase in GnRH pulse secretion, driving the transition from the infantile period to the prepubertal period (Brito, 2015a). This stage extends from approximately two to six months of age and is characterized by an increase in gonadotropin secretion, a phenomenon called the early gonadotropin rise (Evans et al. 1995). This event is driven by increased GnRH secretion, resulting in a drastic increase in luteinizing hormone (LH) pulse frequency (Amann et al. 1986; Evans et al. 1995), which is important for Leydig cell proliferation and differentiation (Brito, 2015a). Initiation of Leydig cell steroidogenesis is characterized by increased secretion of androstenedione, which declines as the cells complete maturation and begin secreting testosterone (Brito, 2015a). During the first three to four months of age, testosterone concentrations are low, then the rise in LH pulse after four months of age is followed by an increase in testosterone pulses and mean testosterone concentrations (McCarthy et al. 1979). There is a progressive increase in the proportion of testicular parenchyma occupied by seminiferous tubules (Curtis and Amann, 1981). Greater follicle-stimulating hormone (FSH) concentrations stimulate the proliferation of undifferentiated Sertoli cells through FSH-binding sites in the seminiferous tubules (Brito, 2015a). It has been demonstrated that FSH secretion, Sertoli cells maturation and increased testosterone secretion are involved in the differentiation of

gonocytes into spermatogonia that enter meiosis around 4 to 5 months of age (Evans et al. 1996).

In *Bos taurus* cattle, the pubertal period occurs between 6 to 12 months of age and is characterized by reduced gonadotropin secretion, increased testosterone secretion, initiation of spermatogenesis, appearance of sperm into the ejaculate and rapid testicular growth (Amann et al. 1986). Puberty is defined as the process by which a bull becomes capable of reproducing and involves the development of the gonads and secondary sexual organs, as well as the development of the ability to breed (Brito, 2015a), which is defined as when an ejaculate contains 50 million or more sperm, with at least 10% sperm motility (Wolf et al. 1965). Testosterone pulse frequency does not increase between 6 to 10 months of age, but pulse amplitude rises with consequent elevation in testosterone mean concentrations until approximately 12 months of age (McCarthy et al. 1979). The drop in LH secretion is most likely the result of negative feedback of testosterone on the hypothalamus (Rawlings et al. 1995). Moreover, the production of inhibin produced by Sertoli cells, may act on the pituitary to limit FSH secretion (Kaneko et al. 1993). The elevation in testosterone concentration is also essential for initiation of spermatogenesis (McCarthy et al. 1979; Rawlings et al. 1978). Formation of the blood-testis barrier is observed after six to seven months of age in accordance with the formation of the tubular lumen (Abdel-Raouf, 1960). At this time, the appearance of primary spermatocytes and advanced germ cells is observed, followed by secondary spermatocytes and round spermatids (Brito, 2015a). Spermatogenesis reaches adult levels at approximately 12 months of age (Killian and Amann, 1972). At eight months of age, elongated spermatids appear and their number increase rapidly after 10 months of age (Abdel-Raouf, 1960).

Mature sperm appear in the seminiferous tubules at approximately 8 to 10 months of age (Wolf et al. 1965). Prior to puberty, semen quality is poor: however, a gradual increase in sperm motility and a decrease in sperm abnormalities is observed after reaching puberty (Lunstra et al. 1978). Scrotal circumference (SC) rapidly increases between 6 to 15 months of age, depending on breed, then plateaus (Lunstra et al. 1978). After reaching puberty, bulls proceed through a period of sexual maturation between 14 and 21 months of age, depending on breed and species, during which SC and semen quality continue to increase slowly (Holroyd and McGowan, 2014).

2.6 Factors influencing sexual development

2.6.1 Breed

The effect of breed must be taken into consideration when assessing reproductive development of young bulls, as significant variation in age at puberty exists between breeds of beef cattle (Lunstra and Echternkamp, 1982). In general, bulls from continental beef breeds (i.e. Charolais, Simmental and Limousin) attain puberty later than breeds from British breeds (i.e. Angus, Hereford and Shorthorn) (Lunstra et al. 1978; Wolf et al. 1965). Early maturing breeds tend to have lower BW and SC at puberty and higher concentrations of LH compared to late-maturing bulls (Evans et al. 1995). Additionally, variation in adipose and lean tissue accretion between breeds may result in disparity in sexual development. Istasse et al. (1990), investigated differences in blood parameters, sexual development and body composition in Holstein and Belgian Blue bulls, two cattle breeds which are extremely different in muscle mass production. The authors observed

higher carcass fat in the Holstein bulls, which was also associated with greater T_3 and earlier onset of sexual development. Therefore, variation in sexual development should be taken into account when evaluating bulls of different breeds (Barth, 2000).

2.6.2 Nutrition

In bulls, reduced energy intake during calfhood resulted in impaired sexual development of bulls via impact on the GnRH pulse generator in the hypothalamus, with low nutrition leading to a decrease in LH secretion (Brito et al. 2007). More recently, it was proposed, that reproductive function in bulls can be maximized by providing high nutrition during calfhood and ensuring an ADG greater than 1.2 kg/day (Barth et al. 2008). In general, low nutrition after weaning also has adverse effects on growth and sexual development. In a study by Mann et al. (1967), reduced nutrition resulted in lower vesicular gland weight, vesicular gland fructose and citric acid contents, and circulating testicular testosterone. Additionally, variation in diet composition has been demonstrated to impact sexual development. A study by Rekwot et al. (1988) observed greater BW, body condition score, SC, semen volume, sperm concentration, and sperm motility in yearling bulls receiving high protein diets, than in bulls receiving low protein levels.

2.6.3 Energy metabolism

Reproduction is an energy-draining process that may become unnecessary for immediate survival and thus when energy availability is limited, processes that ensure survival are favored over reproduction (Schneider et al. 2000). Energy allocation decisions between somatic tissue deposition, such as skeletal muscle and adipose, may

represent differences in investment towards survivorship or reproduction (Bribiescas, 2001). Additionally, although metabolic investment in male gametogenesis is thought to be minimal when compared to female gametogenesis (Bribiescas, 2001), energy metabolism has been shown to be a key factor supporting male reproductive functions such as sperm motility (Miki, 2007). Spermatogenesis is closely associated with metabolic regulations (Rato, 2012), as the metabolic needs of germ cells change throughout sexual development (Mazzaud-Guittot et al. 2010). Additionally, metabolic status is central to the regulation of the reproductive system, since even minor energetic disturbances can cause downregulation of the hypothalamus-pituitary-testis axis, which may lead to disturbance of the reproductive axis and a negative effect on Sertoli cell function (Petersen and Soder, 2006). Furthermore, past studies have shown that cattle diverging in feed efficiency differ in their metabolic profiles and energy metabolism (Gonano et al. 2014; Montanholi et al. 2013a; Richardson et al. 2004) suggesting that animals with diverging feed efficiency may also differ in their reproductive ability.

2.6.4 Body composition

In young bulls, lean tissue growth displays an isometric pattern, relative to other body measures, such as empty BW, during sexual development (Perry and Arthur, 2000). Lean tissue growth decreases as bulls become heavier and tends to stabilize when maturity is reached (Costa e Silva et al. 2013). On the other hand, fat is a late developing component of the body (Perry and Arthur, 2000), and tends to increase as animal reaches puberty (Costa e Silva et al. 2013). The stabilization of muscle mass with maturity coincides with the plateau in testosterone levels observed in sexually mature bulls (Brito,

2015a), supporting the association between testosterone and development of muscle mass (Sheffield-Moore, 2009). Leptin rises with age in young bulls (Brito et al. 2007), which agrees with higher release of leptin with increase fat mass (Ramos and Zamoner, 2014). The association of leptin with the onset of puberty is not clear; however, studies suggest that although leptin is not the primary factor for puberty initiation in males, lower levels of this hormone could be associated with delayed onset of puberty (Gill et al. 1999). Effect of body composition on reproductive performance of mature bulls has been scarcely studied. However, under-conditioning a bull prior to the breeding season can cause loss of strength in the hind limbs and difficulties mounting, leading to reduced service capacity (King, 2015). Whereas, over-conditioning can lead to detrimental effects on semen quality, likely a result of excess scrotal fat deposition (Barth et al. 2008).

2.7 Reproductive measures

2.7.1 Breeding soundness evaluation

Guidelines for the current breeding soundness evaluation (BSE) were adapted in 1992 by the Society for Theriogenology. A typical BSE includes a general physical examination, ending with the collection of semen (Hopper, 2015). During the physical examination, conformation of feet and legs as well as body condition score are assessed (Barth, 2000). Moreover, reproductive organs, including the testes and internal reproductive organs, are examined for any abnormalities (Alexander, 2015). In the beef industry, semen is typically collected via electroejaculation and sperm motility is assessed for gross and individual motility immediately following collection (Hopper and

King, 2015). Eosin-Nigrosin stained slides are also prepared for the assessment of sperm morphology, for which at least 100 sperm must be examined (Barth, 2000). A bull is considered to be a satisfactory potential breeder when minimum requirements of at least 30% sperm progressive motility and 70% morphologically normal sperm in the ejaculate, as well as a SC of at least 30 cm, depending on age and breed (Kastelic and Thundathil, 2008). Bulls may be classified as deferred and retested 60 days later if the minimum requirements are not met, due to immaturity, illness or stress whereas a bull that does not meet the standards of the BSE and is unlikely to meet them at a later date, is classified as unsatisfactory (Hopper, 2015).

2.7.2 Testes ultrasonography

Testis ultrasound is a cost-effective, easily repeatable, convenient and non-invasive technique, first reported in bulls by Pechman and Eilts (1987). This technique can be used for the detection of scrotal and testicular pathologies (Gnemmi and Lefebvre, 2010), male infertility (Patel and Pareek, 1989) and testicular development (Evans et al. 1996). Hyperechogenic structures appear bright within an ultrasound image, whereas anechoic structures, which do not reflect any ultrasound waves, appear black (Gayrard et al. 2010). The testes are suitable subjects for ultrasound scanning as they are easily accessible, and are readily compared with one another (Gnemmi and Lefebvre, 2010). The testis parenchyma appears homogenous with medium echogenicity and surrounds the more hyperechogenic mediastinum (Gnemmi and Lefebvre, 2010). The parenchyma is comprised primarily of convoluted seminiferous tubules that contain the Sertoli, germinal and Leydig cells, along with associated vascular, neural and stromal tissues (Momont and

Checura, 2015). Testicular echogenicity is correlated with several histomorphological and endocrine features throughout sexual development (Evans et al. 1996). In general, changes in the micro-environment of the testis, with age, are closely related to testicular development and lead to changes in the relative density of the testicular tissue (Aravindakshan et al. 2000), resulting in variations in testicular echogenicity (Arteaga et al. 2005). Minimal changes in testicular echogenicity are observed in post-pubertal bulls (Brito et al. 2012a). While testicular echogenicity have been positively correlated with the percentage of seminiferous tubule-cross sections, stage of development of seminiferous tubule, seminiferous tubule diameter and serum testosterone concentrations in pre-pubertal bulls (Evans et al. 1996). However, in post-pubertal bulls, testicular echogenicity was positively correlated with daily sperm production (Brito et al. 2012a) and negatively correlated with seminiferous tubule diameter (Gábor et al. 1998) and seminiferous epithelium area (Brito et al. 2012a). Interestingly, a recent study (Montanholi et al. 2016a) noted a positive correlation for testicular pixel intensity with seminal plasma protein 1 and Spermadhesin Z13, two highly abundant seminal proteins that may be associated with echogenic properties of the testis (Dogra et al. 2003). Thus, evaluation of testicular echogenicity is capable of detecting changes in testicular microstructure and endocrine secretion and may augment breeding soundness examination of bulls.

2.7.3 Scrotum surface temperature

Production of fertile sperm in bulls requires the testes to be kept 2 ˚C to 6 ˚C cooler than core body temperature, with increased testicular temperature leading to

reduced semen quality (Kastelic et al. 1995). Physical properties of the testes and scrotum work together to regulate testicular temperature. The thin, relatively hairless skin of the scrotum contains numerous subcutaneous blood vessels and sweat glands, which promote heat loss (Waites, 1970). The cremaster muscle and the *dartos tunic* allow testes to move away or stay close to the body, keeping them at desirable temperature (Robertshaw and Vercoe, 1980). The vascular cone, composed of the highly coiled testicular artery surrounded by the pampiniform plexus, acts as a counter-current heat exchanger, transferring heat from the artery to the vein (Cook et al. 1994) as it is vascularized from base to apex (Kastelic et al. 1997). On the other hand, the base of the scrotum is warmest and the apex is coolest, whereas, testes are cooler at the base and warmer at the apex following vascularization from apex to base (Kastelic et al. 1997).

Infrared thermography is a non-invasive technology that can be used to evaluate scrotal surface temperature and temperature patterns of bulls through radiated heat energy. Scrotal temperature of approximately 30 to 31 ˚C at the base of the scrotum and 28 to 29 ˚C at the apex of the scrotum have been reported in beef bulls (Cook et al. 1994; Kastelic et al. 1997). Normal thermographs display left to right symmetry, with a 4 ˚C to 6 ˚C decrease in temperature from the top to bottom of the scrotum, in the form of horizontal temperature bands (Coulter, 1988). Abnormal temperature patterns lack left to right symmetry and often display hot-spots (Coulter, 1988). In the past, scrotum thermography has been used to evaluate semen quality in bulls. Studies observed that nearly all bulls displaying abnormal temperature patterns had reduced semen quality (Coulter, 1988) and lower conception rates (Lunstra and Coulter, 1997) than bulls with normal temperature pattern. However, not every bull with reduced reproductive function

displayed abnormal temperature patterns (Coulter, 1988; Lunstra and Coulter, 1997). Therefore, scrotal thermography is a useful tool for screening bulls prior to the BSE, which may be complementary to analysis of semen quality.

2.7.4 Blood hormones

In bulls, the temporal pattern of blood concentration of sex hormones changes throughout the sexual development in bulls (Brito, 2015a). Thus, levels of sex hormones can be indicative of stage of sexual development. Studies reported associations between circulating levels of testosterone, leptin and T_3 on testicular development and reproductive function of males (Ramos and Zamoner, 2014).

Thyroid hormones play a key role in the metabolism of nearly all body tissues, including the testes (Ramos and Zamoner, 2014). There is evidence showing that T_3 regulates maturation and growth of the testis via regulation of Sertoli and Leydig cell proliferation and differentiation (Holsberger and Cook, 2005). Hypothyroidism is associated with delayed sexual maturity (Holsberger and cook, 2005) whereas hyperthyroidism is associated with earlier development of seminiferous tubules lumen (Zamoner et al. 2007). In young bulls, the influence of differing levels of thyroid hormones on reproductive function has not been extensively evaluated, although studies have indicated that thyroidectomy in male calves results in a complete absence of sexual drive during adulthood and that high T_4 resulted in a decline in spermatozoa respiration *in vitro* (Lardy and Philips, 1943; Petersen et al. 1941). Moreover, a recent study noted an association between thyroid hormone markers and age at puberty in young bulls (Fernández et al. 2014).

Leptin concentration increase rapidly 20 to 12 weeks prior to the onset of puberty, then gradually rises until 8 weeks post-puberty, in bulls (Brito et al. 2007). Testicular leptin receptors have been discovered in the germ cells and Leydig cells of mice (Caprio et al. 2003). Additionally, expression of leptin in the testis changes with age (Caprio et al. 2003), indicating that leptin may be an important factor in testicular development. In developing bulls, increase in leptin and testosterone levels were closely related (Brito et al. 2007), suggesting possible association regulating the secretion of these two hormones.

2.8 Feed efficiency and sexual development in bulls

A growing body of evidence suggests that selection for increased productivity and feed efficiency has a negative association with fertility measures in livestock (Rauw et al. 1998). Due to rising concern in the beef industry, studies have been investigating the relationship of feed efficiency and fertility related-measures in young beef bulls. So far, the evidence suggest an antagonistic association between improved feed efficiency and fertility-related traits, as observed by lower sperm motility (Awda et al. 2013; Fontoura et al. 2016; Wang et al. 2012) and viability (Awda et al. 2013), higher amount of sperm abnormalities (Fontoura et al. 2016; Hafla et al. 2013), smaller SC (Awda et al. 2013), reduced testicular echogenicity (Fontoura et al. 2016), larger diameter seminiferous tubules of immature stage (Fontoura et al. 2016) and variation in seminal plasma proteomics profile (Montanholi et al. 2016a).

Energy is a limited resource for all organisms which, once acquired, can be stored and be used for maintenance, growth or reproduction (Kozlowski and Wiegert, 1987). Cattle which differ in feed efficiency also differ in metabolic profile (Richardson et al.

2004; Montanholi et al. 2013a); this suggests that cattle diverging in feed efficiency allocate energetic resources differently. Trade-offs between fertility and production have been studied in dairy cows, where cows display different profile in the way they allocate energy between lactation, reproduction and survival (Ollion et al. 2016). Similarly, when cattle maximize feed efficiency, there may be costs associated with this increase in energy use efficiency. Thus bulls in which differ in feed efficiency may differ in energy partitioning for maintenance, storage, and sexual development.

Chapter 3

ASSOCIATIONS OF BLOOD PARAMETERS WITH AGE, FEED EFFICIENCY AND SAMPLING ROUTINE IN YOUNG BEEF BULLS

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Abstract: Utilization of blood parameters as proxies for feed efficiency is an avenue to maximize economic and environmental sustainability of the beef industry. Among other factors, age and sampling routine may impact the reliability of blood parameters used to assess feed efficiency. Thus, the objectives were to assess associations of blood parameters with age and different feed efficiency models under two sampling routines. Thirty-two crossbred bulls with an average body weight (BW) of 587 ± 86 kg and age of 369 ± 30 days at the end of performance test were studied. Residual feed intake-ultrasound (RFI_{US}) was calculated using average daily gain, BW and ultrasound traits for body composition. An alternative model was studied by including age in the RIF_{US} model (RFI_{AGE}). Seven blood samples for each bull were collected during a 32-day on-farm sampling period and an additional sample was collected at slaughter. Blood samples were analyzed for blood metabolites and hormones. Bulls were divided into younger (n=16; age=342 ± 1.0 days) and older (n=16; age=393 ± 1.0 days), and into efficient (n=16;

RFI_{US}=-0.56 ± 0.14 kg DM/day, RFI_{AGE}=-0.53 ± 0.13 kg DM/day) and inefficient (n=16; RFI_{US}=0.62 ± 0.14 kg DM/day, RFI_{AGE}=0.52 ± 0.13 kg DM/day) groups. Means of blood parameters were compared for age and feed efficiency groups using a mixed model for on-farm sampling and a general linear model for sampling at slaughter. During the on-farm sampling, glucose, potassium and insulin-like growth factor 1 were lower in older bulls while urea, osmolality, testosterone, follicle stimulating hormone and luteinizing hormone (LH) were higher in older bulls. At slaughter, albumin was higher in older bulls. Over the on-farm sampling, carbon dioxide concentration was higher in efficient bulls for RFI_{AGE}. Acetate, testosterone and LH were higher in efficient bulls for RFI_{US}, and triiodothyronine (T_3) was lower in efficient bulls for both RFI models. At slaughter, alkaline phosphatase was lower while leptin was higher in efficient bulls for RFI_{US}. Brain-derived neurotrophic factor and T_3 were higher and lower, respectively, in efficient bulls for both RFI models. Brain-derived neurotrophic factor, assessed at slaughter, is a potential proxy for feed efficiency. Triiodothyronine is strongly associated with feed efficiency, regardless of sampling routine and variation in phenotypic age. Overall, these results support the association of blood parameters with age and feed efficiency and illustrate the impact of on-farm versus slaughterhouse sampling on components of intermediary metabolism in bulls. These findings may be applied in the understanding of bovine physiology and in the development of proxies for productive performance.

Keywords: age, brain-derived neurotrophic factor, carbon dioxide, residual feed intake, slaughter, triiodothyronine

Introduction

Improvement of feed efficiency has received increased attention in the beef cattle sector as an opportunity to maximize economic and environmental sustainability of the beef industry. Measures of feed efficiency include feed to gain ratio (FG) and residual feed intake (RFI). Residual feed intake is a leading measure in the beef industry with applications in the identification of phenotypic proxies for feed efficiency. Despite studies evaluating indicators of feed efficiency in the bovine (Kolath et al. 2006; Montanholi et al. 2013b, Richardson et al. 2004), there is a shortage of indicators for RFI with application in commercial herds. Therefore, there is a need for alternative measures to assess feed efficiency in the bovine, and blood may be a feasible matrix as it provides insight into physiological, nutritional and metabolic processes (Russel and Wright, 1983). Blood parameters associated with energy metabolism have been related to RFI in beef cattle (Gonano et al. 2014; Kelly et al. 2011; Walker et al. 2015). However, it is known that factors such as age and stress can impact the profile of blood parameters (Doornenbal et al. 1988; Minka and Ayo, 2009) and thus may impact the determination of RFI and associations between blood analytes and feed efficiency.

The original model for RFI described in beef cattle by Koch et al. (1963) accounts for body weight (BW) and average daily gain (ADG). Past studies (Montanholi et al. 2009; Schenkel et al. 2004), included measures of body composition to account for variation in carcass leanness and fatness, which impacts metabolic rate (Baldwin et al. 1980). Similarly, variation in age is associated with changes in metabolism (Brody, 1945) and feed efficiency (Loyd et al. 2011) as well as shifts in composition of gain (Owens et al. 1995) providing evidence that age should be considered when modelling for RFI.

Although cattle within a contemporary group may be of similar age, they may differ in physiological maturity since puberty and sexual maturity occur between 280 and 400 days of age in beef bulls (Brito et al. 2012b) and can coincide with performance test evaluation as part of the routine in the beef industry. Variation in developmental stage are associated with changes in the profile of blood hormones and metabolites of bulls (Doornenbal et al. 1988); for example, increased testosterone, insulin-like growth factor 1 (IGF1) and leptin are associated with sexual development in beef bulls (Brito, 2015a). In fact, the 90-day age range allowed in a contemporary group of yearling bulls (BIO®, 2015) can result in a 20% difference in testosterone levels between animals (Lunstra et al. 1978). Phenotypes that are commonly recorded in performance evaluation programs of beef bulls are adjusted for age (Schenkel et al. 2004). However, the search for proxies for productive performance traits, such as blood parameters, may be affected by the age variation accepted in the industry.

Management practices and sampling routine can affect the levels of blood parameters; for instance, cortisol levels in cattle increase rapidly in response to a human approaching the animal (Hopster et al. 1999). Cattle become familiar with repeated non-aversive on-farm procedures, but novel experiences such as loading, transport and off-loading are strong stressors (Grandin, 1997). Exposure to such stressors prior to slaughter can cause animals to release varied levels of metabolites and hormones into the blood stream, leading to changes in energy metabolism, respiratory function, immune function and osmotic regulation (Minka and Ayo, 2009).

A shift in body composition occurs during sexual development (Brody, 1945). The importance of adjusting RFI for body composition has been shown by others

(Montanholi et al. 2009; Schenkel et al. 2004). Since hormones associated with sexual development can impact productive performance of bulls (Cook, R.B., 2000), inclusion of phenotypic age in RFI determination model can impact observed variation in dry matter intake (DMI) influencing the association of blood parameters with feed efficiency. Furthermore, physiological indicators are affected by variation in age and exposure to novel experiences including changes in blood hormones and metabolites levels (Matteri et al. 2000). Difference in feed efficiency impacts concentrations of blood parameters (Kelly et al. 2011) sexual maturity (Fontoura et al. 2016) and stress response (Montanholi et al. 2013b). Thus, one can hypothesize that variation in age, RFI determination model and sampling routine may impact the association of blood parameters with feed efficiency. The objectives of this study were to 1) investigate the fluctuation of blood parameters in relation to age; 2) compare blood parameters in relation to feed efficiency using two RFI determination models; and 3) compare blood parameters in relation to feed efficiency between repeated sampling during a 32-day on-farm period and sampling at slaughter.

Material and methods

Animals and management

The study was in accordance with the guidelines of the Canadian Council on Animal Care Guidelines (2009). A group of 32 crossbred bulls, initially weighing 359 ± 67 kg (mean $\pm$ standard deviation) and 256 ± 29 days of age were housed at the Elora Beef Research Centre (University of Guelph, Canada). The overall breed composition

based on pedigree records of the bulls consisted of 54% Angus, 31% Simmental and 15% other European breeds and crosses. Bulls were fed *ad libitum* a high-moisture corn-based diet (Table 3.1) once daily between 08:30 and 09:30 h. Bulls were housed and managed in an indoor pen (36 by 28 m) with 2/3 of the area bedded with wood shavings that were replenished at least once weekly. The pen contained eight automated feeding stations (Insentec, B.V., Marknesse, The Netherlands) used to record individual feed intake. Bulls underwent a 113-day performance test during which daily feed intake, ADG, BW and ultrasound measurements of body composition were assessed. Scrotal circumference (SC) was measured at the end of the test using a scrotal measuring tape (Lane Manufacturing Inc., Denver, CO, USA) as described by Awda et al. (2013). Average BW and age of the bulls at the end of the performance test were 544 ± 80 kg and 369 ± 29 days, respectively. Following the performance test, bulls were slaughtered on 8 different days in groups of 4 over a period of 23 days and at an average BW of 608 ± 81 kg and age of 404 ± 31 days.

Productive performance

Backfat thickness (BKFT; mm), rumpfat thickness (RUMP; mm) and ribeye area (RBEA; cm^2) were assessed using real-time ultrasound as described by Montanholi et al. (2009). Briefly, backfat thickness was determined by measuring the minimum subcutaneous fat depth over the *longissimus* muscle in the fourth quadrant distal to the spine; rumpfat thickness was measured at the juncture of the *gluteus medius* and superficial *gluteus medius* muscles; and ribeye area was determined by scanning the *longissimus* muscle area. Average BW, BKFT, RUMP and RBEA were calculated by

computing the animal's BW and performance traits intercept plus average daily increment on each trait multiplied by mid-duration (56.5 days) of test.

Feed intake data obtained from the automated feeding system was filtered to exclude outlier records or days where mechanical problems occurred, which accounted for less than 2% of the records. Dry matter intake over the testing period was calculated from the average of remaining records. Average BW, ultrasound measurements and individual DMI were used in the calculation of RFI. Several models were tested to calculate RFI, similar to the calculations by Montanholi et al. (2009). The most appropriate model explaining the variation in feed intake had a R^2 of 0.24 and was modelled as follows:

$$DMI_{US} = 4.74 + 0.32(ADG) + 0.01(BW) + 0.03(BKFT) - 0.13(RUMP) - 0.03(RBEA) + RFI_{US}$$

where RFI_{US} is the residual proportion of the model that represents the deviation of the observed feed intake from the expected feed intake. Subsequently, age (AGE) was added to the model above and the R^2 increased to 0.37 while Bayesian information criterion (BIC) obtained was similar to the previous model.

$$DMI_{AGE} = 9.81 - 0.27(ADG) + 0.02(BW) + 0.16(BKFT) - 0.17(RUMP) + 0.005(RBEA) - 0.03(AGE) + RFI_{AGE}$$

where RFI_{AGE} represents the residual proportion of the model that represents the deviation of the observed feed intake from the expected feed intake. Feed to gain ratio was also determined as a ratio of DMI to ADG.

Blood sampling and processing

Blood samples were obtained between 6:00 and 8:00 h on days 1, 5, 19, 22, 25, 27 and 32 of a 32-day on-farm sampling period, with the first collection occuring 14 days prior to the end of the performance test. During on-farm sampling, animals were restrained in a squeeze chute (Silencer®, Hydraulic Squeeze Chute, Moly Manufacturing Inc., Lorraine, USA) and blood was collected via jugular venipuncture using a 10 mL sodium heparin tube (Vacutainer ®, BD Inc., Franklin Lakes, USA) similarly described by Montanholi et al. (2013b). Blood samples were also collected at slaughter, starting 26 days following the end of the performance test, in 10 mL sodium heparin tubes for plasma and 10 mL silicone coated tubes (Vacutainer ®, BD Inc., Franklin Lakes, USA) for serum. After sampling, blood tubes were stored on ice for collection of plasma and for 25 minutes at room temperature to allow clotting and collection of serum. Samples were centrifuged (3000g for 25 minutes at 4 °C), then plasma and serum were harvested and stored at -80°C until further analysis.

Characterization of blood parameters

Blood plasma or serum samples were analyzed for metabolic products, ions, proteins, sex hormones and metabolic hormones. Plasma metabolic products included: carbon dioxide (CO_2; mmol/L) determined using an automated analyzer (Cobas 4000 c311, Roche Diagnostics GmbH, Mannheim, Germany); glucose (mmol/L), urea (mmol/L) and cholesterol (mmol/L) determined using a similar automated analyzer (Cobas® c 311/501 analyzer, Roche Diagnostics GmbH, Indianapolis, USA); and acetate

(g/L) determined via spectrophotometry using a commercial kit (K-ACETRM, Megazyme© International, Wicklow, Ireland).

Plasma ions included: sodium (mmol/L), potassium (mmol/L) and chloride (mmol/L) determined using an automated analyzer (Cobas® c 311/501 analyzer, Roche Diagnostics GmbH, Indianapolis, USA); and osmolality (mmol/L) calculated as the sum of individual solute concentrations: osmolality = 1.86 (sodium (mmol/L) + potassium (mmol/L)) + glucose (mmol/L) + urea (mmol/L) as suggested by Bhagat et al. (1984).

Plasma proteins included: aspartate aminotransferase (AST; U/L), alkaline phosphatase (ALP; U/L), creatine kinase (CK; U/L) and albumin (g/L) determined using an automated analyzer (Cobas® c 311/501 analyzer, Roche Diagnostics GmbH, Indianapolis, USA); and brain-derived neurotrophic factor (BDNF; ng/mL) measured in serum using a competitive ELISA (Brain-Derived Neurotrophic Factor (BDNF) Elisa Kit, Cloud-Clone Corp, Texas, USA).

Plasma sex hormones included: testosterone (ng/mL) analyzed by radioimmunoassay (Coat-A-Count, Siemens Healthcare Diagnostic Products, Malvern, USA); follicle stimulating hormone (FSH; ng/mL) and luteinizing hormone (LH; ng/mL) analyzed by validated radioimmunoassay (Rawlings and Evans, 1995); plasma anti-Müllerian hormone (AMH; pg/mL) measured using an ELISA (Anti-Müllerian Hormone (AMH) Sample Test Kit for Bovine Blood Serum, MOFA Global, Wisconsin, USA); and prolactin (ng/mL) analyzed by radioimmunoassay using methodology optimized for bovine samples (Prairie Diagnostic Services, Saskatoon, Canada).

Metabolic hormones included: plasma cortisol (ng/mL) obtained using a commercially available kit (Coat-A-Count, Diagnostic Products Corporation, Los

Angeles, USA); cholecystokinin (ng/mL) measured in serum using a competitive ELISA (Bovine Cholecystokinin (CCK) Elisa Kit, BlueGene Biotech Co Ltd, Shanghai, China); ghrelin (pg/mL) measured in serum using an ELISA assay (Bovine Ghrelin Elisa Kit, BlueGene Biotech Co Ltd., Shanghai, China), total thyroxine (T_4; nmol/L), total triiodothyronine (T_3; nmol/L) and insulin-like growth factor 1 (IGF1; ng/mL) quantified in plasma using ELISA tests (IMMULITE 1000, Siemens Healthcare Diagnostic Products, Malvern, USA); and leptin (ng/mL) analyzed in plasma by radioimmunoassay (Multi-Species Leptin RIA Kit, Millipore, Missouri, USA).

Statistical analysis

Data was analyzed using SAS software version 9.4 (2014) (SAS Institute Inc., Cary, USA). Normality was evaluated via evaluation of skewness, kurtosis and Anderson-Darling test for each trait using the univariate procedure (PROC UNIVARIATE). Data for the means comparison between age groups and feed efficiency groups included eight measures with significant skewness during the on-farm sampling period and were transformed by taking their logarithm (leptin, acetate, FSH, AMH), square root (testosterone, prolactin and cortisol) and reciprocal (AST) values. Seven parameters measured at slaughter showed significant skewness and were transformed using logarithm (AST, CK, CO_2, osmolality, urea), square root (CCK) or reciprocal transformation (testosterone). The profile of CK over the 32-day sampling period and for sodium and glucose at slaughter did not meet the requirements for normality. Ghrelin, CCK and BDNF were only measured at slaughter. Data from the seven sampling occasions during on-farm sampling was pooled for each animal to allow means

comparison between sampling routines (on-farm vs. slaughter). The data included twelve measures with significant skewness and were transformed by taking their logarithm (urea, testosterone, FSH, LH, prolactin, CCK, leptin), square root (cortisol) and reciprocal (acetate, potassium, AST, CK) values. Data for glucose is not presented for means comparison between sampling routines as requirements for normality were not met. On-farm data for AMH was only measured on day 25 of the 32-day sampling period. The back-transformed data and 95% confidence limit (lower limit, upper limit) are presented in the results.

Bulls were classified as either younger (n=16; age=342±1.0 days) and older (n=16; age=393±1.0 days) and into efficient (n=16; RFI_{US}=-0.56±0.14 kg DM/day, RFI_{AGE}=-0.53±0.13 kg DM/day) and inefficient (n=16; RFI_{US}=0.62±0.14 kg DM/day, RFI_{AGE}=0.52±0.13 kg DM/day) based on the obtained RFI values from each RFI model.

Repeated measures for plasma parameters during on-farm sampling were analyzed through random regression using the mixed procedure (PROC MIXED), according to the following model:

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

where Y_{jtkl} is the *k*-th blood parameter measured on the *t*-th day (*t*=1-7 sampling) of the *j*-th bull from the *i*-th age group (*i*= younger and older) from the l-th breed (*l*= Angus, Simmental and other European breeds and crosses); μ is the overall mean effect for the blood parameter; AGE_i is the fixed effect of the *i*-th age group; $Breed_l$ is the fixed effect of the *l*-th breed; $\sum_{k=0}^{nf} \varphi_{jtk}\beta_k$ are fixed regression coefficients; $\sum_{k=0}^{nr} \varphi_{jtk}\gamma_{jk}$ are the *k*-th random regression for the *j*-th bull; φ_{jtk} is the *k*-th linear polynomial for blood

parameter of bull *j* at day *t*; *nf* and *nr* are the order of the linear polynomial for fixed and animal effects regressions and; ε_{ijtkl} is the residual random effect associated with the assessment on the *t*-th day on the *j*-th bull. Additionally, a similar model was used to compare means between feed efficiency groups (RFI_{US} and RFI_{AGE}). The autoregressive covariance structure was selected based upon maximum likelihood and BIC.

The general linear model procedure (PROC GLM) was used to compare means of blood plasma and serum parameters between age groups on the day of slaughter fitting the following model:

$$Y_{ijkl} = \mu + AGE_i + Breed_l + \varepsilon_{ijkl}$$

where Y_{ijk} is the *k*-th trait measured on the *j*-th bull, belonging to the *i*-th age group; μ is the overall mean effect for the trait; AGE_i is the fixed effect of the *i*-th age group; $Breed_l$ is the fixed effect of the *l*-th breed breed (*l*= Angus, Simmental and other European breeds and crosses); and; ε_{ijkl} is the residual random effect associated with the assessment of the *j*-th bull. A similar model was used to compare means of age, SC and productive traits at the end of the performance test, as well as means of blood parameters between feed efficiency (RFI_{US} and RFI_{AGE}) groups at slaughter and between sampling routine (on-farm and slaughter). For all analyses, results 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 of age, SC and productive performance traits are presented in Table 3.2. Descriptive statistics of the blood parameters during on-farm sampling and at slaughter are presented in Table 3.3. As indicated in Table 3.4., the

productive performance measures indicate no difference in RFI_{US} and RFI_{AGE}, DMI, FG and ADG between age groups. As expected, older bulls had greater initial and final BW, BKFT, RBEA and RUMP. The productive performance measures for both RFI_{US} and RFI_{AGE} revealed no difference in age between efficiency groups (Table 3.5. For both RFI models, efficient bulls had lower RFI, DMI and FG while no difference between efficiency groups was observed for body composition traits.

Figures 3.1 to 3.4 depict the CO_2, acetate, testosterone and T_3 levels during on-farm sampling, separated by age and by feed efficiency groups for both RFI models. Figures 3.1A to 3.4A correspond to the mean analyte concentrations during on-farm sampling by age group, corresponding to overall means reported in Table 3.6. During on-farm sampling, no difference was observed between age groups for CO_2, acetate or T_3 concentration but younger bulls did demonstrate higher glucose and lower testosterone levels. Older bulls tended to have higher urea (P = 0.09), FSH (P = 0.05) and LH (P = 0.07) while potassium and IGF1 levels were higher in younger bulls. Mean comparison between age groups at slaughter are also depicted in Table 3.6.

Table 3.7 and Table 3.8 show the mean analyte concentrations during on-farm sampling by feed efficiency groups for RFI_{US} and RFI_{AGE} corresponding to Figures 3.1B to 3.4B and Figure 3.1C to 3.4C, respectively. The CO_2 curves for efficient and inefficient bulls differed between RFI_{AGE} groupings only, with greater levels of CO_2 in efficient bulls. The acetate and testosterone curves for efficient and inefficient bulls differed for RFI_{US} only, with greater levels of acetate and testosterone in efficient bulls. Levels of LH were numerically (P=0.09) increased in efficient bulls. The T_3 curves for efficient and inefficient bulls differed for both RFI models, with lower levels of T_3 in

efficient bulls. Means comparison between efficiency groups for RFI_{US} and RFI_{AGE} at slaughter are also depicted in Table 3.7 and Table 3.8, respectively. Lower ALP levels were suggested (P=0.08) in efficient bulls whereas leptin was higher in efficient bulls for RFI_{US}. Brain-derived neurotrophic factor was higher while T_3 was lower in efficient bulls for RFI_{US}; trend were suggested for these variables (BDNF: P=0.09, T_3: P=0.08) when considering RFI_{AGE}.

Mean comparison between sampling periods are presented in Table 3.9. Urea levels were increased at slaughter compared to on-farm sampling. At slaughter, chloride levels were lower while potassium levels were increased in comparison. Alkaline phosphatase was greater during on-farm sampling while CK and albumin were increased at slaughter. Luteinizing hormone levels were greater during on-farm sampling, while prolactin and cortisol levels increased at slaughter.

Discussion

Scrotal circumference

Values obtained for SC followed those observed in yearling beef bulls by Fontoura et al. (2016). Scrotal circumference is a measure that can be used to determine testicular development (Lunstra et al. 1978). Older bulls displayed larger SC, agreeing with results by Lunstra et al. 1978; where a difference in SC of approximately 2.5 cm between age groups in yearling crossbred bulls was suggested. The lack of relationship between SC and feed efficiency agrees with other studies (Fontoura et al. 2016; Hafla et al. 2012). In contrast, Awda et al. 2013 observed smaller SC in efficient bulls, thus

further research is needed to elucidate the relationship between feed efficiency and scrotal circumference in young bulls.

Productive performance

Values observed for DMI, FG, BW and ADG were comparable to studies where bulls were fed diets predominantly of high moisture corn during the finishing phase (Montanholi et al. 2009). Ultrasound traits were similar to results reported by Montanholi et al. (2009) for RBEA and BKFT and results by Fontoura et al. (2016) for RUMP in yearling beef bulls. Results observed when comparing performance traits between younger and older bulls are similar to results by Lee et al. (2014) reporting relationships between age, body weight and ultrasound traits. The lower feed intake of efficient bulls represents savings of approximately 204.4 kg and 193.5 kg annually for RFI_{US} and RFI_{AGE}, respectively, without penalizing productivity. This difference in feed intake for achieving the same BW, rate of gain and body composition in efficient bulls indicates the potential for reducing feed costs and environmental impact of the beef industry, as suggested elsewhere (Hegarty et al. 2007).

Metabolic products

The higher levels of CO_2 observed in efficient bulls for RFI_{AGE} differ from Gonano et al. (2014); these authors reported lower CO_2 in feed efficient heifers over the circadian cycle. Due to the role of CO_2 as an end-product of the energy cascade (Kleiber, 1961) the results by Gonano et al. (2014) suggest that feed efficient cattle are more efficient in energy utilization by maximizing their use of metabolic energy. However, in

the current study, contrasting results were observed, which was likely a response to differences in sampling routines. Blood collected hourly over the circadian period using jugular catheters (Gonano et al. 2014) reduces stress associated with sample collection when compared to restraint and handling in a chute (Cook, et al. 2000). Under stress and increased physical activity respiratory rate increases, resulting in increased alveolar ventilation and decreased blood CO_2 (Grossman, 1983). Thus, lower blood CO_2 in cattle undergoing handling stress may be explained by increased respiratory rate, suggesting that inefficient cattle are more reactive to stressful situations (Richardson et al. 2004) and have a fight or flight response to stress (Koolhaas et al. 1999).

Differences in blood CO_2 levels were only observed for RFI_{AGE}. Blood CO_2 levels rise with increased tissue activity (Pittman, 2011). Tissue activity and metabolism vary with growth and age, whereas ventilation rate varies with metabolic rate (Brody 1945). Thus, differences in CO_2 levels between efficiency groups for RFI_{AGE} may represent differences in stress response rather than differences in metabolism associated with age. Moreover, differences in blood CO_2 were not observed between feed efficiency groups at slaughter. This may be explained by differences in sampling technique; on the day of slaughter, animals were stunned using captive bolt and blood was collected during exsanguination. Following stunning, pulmonary respiration ceases and therefore any change in blood CO_2 immediately following captive bolt is possibly caused by cellular respiration and cellular metabolism rather than by changes in breathing frequency.

Glucose levels exceeded the normal clinical range (University of Guelph Animal Health Laboratory User Guide, 2015) obtained from lactating dairy cows. These differences may be due to variation in physiological stage of animals used, as metabolic

demand for glucose varies with lactation (Van Soest, 1982). The lower glucose levels in older bulls agrees with Ban-Tokuda et al. (2007); these authors also observed decreasing levels of plasma glucose with increasing age in bulls. This difference in glucose levels with age can be due to a rise in insulin caused by increased feed intake (Pavlík et al. 2010) or by increased fattening (Ban-Tokuda et al. 2007) in older bulls. In our study, feed intake did not differ between age groups but older bulls had greater BKFT and RUMP suggesting that glucose levels were affected by the disparity in fattening. Glucose has been shown to be increased in inefficient steers (Kolath et al. 2006). However, similar to our observations, Kelly et al. (2010) found no relationship between feed efficiency and blood glucose in beef cattle. Differences between results for circulating glucose may be due to variation in metabolic body size, age and fasting time (Van Soest, 1982).

The observed increase in urea levels in blood of older bulls is in agreement with Doornenbal et al. (1988). Lower blood urea in younger animals suggests a greater efficiency to convert nitrogen into amino acids and proteins, and correspondingly a faster growth rate (Pavlík et al. 2010). As cattle grow composition of gain shifts from protein accretion to fat deposition, since energetic efficiency of fat deposition is greater than protein accretion, efficiency of growth decreases as cattle mature (Owens et al. 1995). Contrary to another study (Richardson et al. 2004), no difference in urea between efficiency groups was observed. However, these authors (Richardson et al. 2004) used a RFI model which excluded body composition traits. Since urea is a product of protein degradation and is correlated with lean growth, differences in body composition could have contributed to the associations between blood urea and feed efficiency in other

studies. This further demonstrates the importance of adjusting for body composition measures when evaluating RFI in order to avoid potential biases.

Acetate levels during on-farm sampling and at slaughter were lower than those observed by Gonano et al. (2014) in beef heifers fed a roughage-based diet. Differences are most likely due to variation in diet composition, as a high grain diet results in lower acetate levels when compared to a high forage diet (Rust and Owens, 1981). The higher acetate observed in efficient bulls for RFI_{US} during the on-farm sampling period is in agreement with findings noted by Karisa et al. (2014) in feedlot steers fed a ration similar to that consumed by the bulls in our study. Blood acetate clearance rate is related to feed intake in ruminants, as indicated by depressed feed intake associated with reduced clearance rate (Preston and Leng, 1987). Thus, higher blood acetate in efficient bulls is consistent with reduction in feed intake observed in efficient cattle. However, there was no difference in acetate concentration between efficiency groups when comparing groups using RFI_{AGE}. Similarly, Gonano et al. (2014) did not observe differences in acetate between efficiency groups using a RFI model adjusted for age. Conversely, higher blood acetate levels in efficient cattle have been observed when using a RFI model with or without inclusion of body composition (Karisa et al. 2014; Kelly et al. 2010). This suggests that variation in blood acetate with RFI is mainly affected by variation in age rather than variation in body composition. Difference between efficiency groups was only observed during on-farm sampling. Transportation stress is associated with alterations in digestion and rumen function in cattle (Loerch and Fluharty, 1999) and may explain the lack of difference in acetate concentrations between feed efficiency groups at slaughter.

Ions

Mulei et al. (1988) observed decreasing levels of blood potassium with age in cows. This is in agreement with the lower blood potassium levels in older bulls in this study. No difference between age groups was observed for sodium; however, another study noted higher blood sodium with age in cows (Mulei et al. 1988). Ion transport, a cellular maintenance-related process, accounts for 30 to 40% of total basal energy expenditure in ruminants (Baldwin et al. 1980). The relevance of ion transport in energy metabolism and metabolic function suggests that differences in ion transport could contribute to variation in feed efficiency (Richardson et al. 2002). However, no difference in ion concentration or osmolality was observed between feed efficiency groups. Stress induces cell stimulation that causes cell potential to change from rest potential to the action potential (Minka and Ayo, 2009) resulting in significant changes in electrolyte balance of cattle (Schaefer et al. 1997). Similarly to our study, Nemec Svete et al. (2012) observed increased potassium in blood collected at exsanguination following transport and stunning of horses.

Proteins

In mature animals, ALP originates mainly from the liver; however, in growing animals the majority of ALP originates from bone tissues (Doornenbal, 1988). Therefore, higher blood ALP in younger yearling bulls is indicative of rapid skeletal growth (Doornenbal, 1988). Pavlík et al. (2010) observed a decrease in ALP levels from approximately 4 months to 12 months of age in bulls. In the present study, bulls were older than 12 months at the end of the performance test, which may explain the lack of

difference between age groups here. Alkaline phosphatase is positively correlated with average feed intake in cattle (Richardson et al. 2004). Thus, the higher feed intake observed in inefficient bulls may explain the trend towards higher levels of blood ALP. Alternatively, greater hepatic mitochondrial respiration has been associated with improved feed efficiency (Lancaster et al. 2014). In rats, reduced mitochondrial respiration of a diseased liver is related to increase ALP (Younes et al. 2007). Thus, this may suggest that greater hepatic mitochondrial respiration in efficient bulls is responsible for lower blood ALP. Alkaline phosphatase levels differed only between groups classified by RFI_{US}, demonstrating the impact of even a small variation in age on the assessment of ALP. Differences in ALP between efficiency groups were observed at slaughter, with an 11% decrease in mean levels. Transportation is associated with reduced alkaline phosphatase levels (Zhong et al. 2011) in ruminants, and may explain the difference in ALP between on-farm and at slaughter sampling.

The observed increase in albumin concentration in older bulls is in agreement with Pavlík et al. (2010). Lower blood albumin is associated in part with lower muscle mass (Thalacker-Mercer, 2007), explaining the lower albumin levels in younger bulls. Investigation of the relationship between albumin and feed efficiency revealed higher blood albumin in inefficient lambs (de Paula et al. 2013). However, similarly to Richardson et al. (2004) no difference in albumin levels was observed in the current study between efficiency groups in cattle. Albumin is an important circulating antioxidant that protects cells by scavenging reactive oxygen species (Minka and Ayo, 2009). Blood albumin increases during transportation in cattle, mainly due to an increase in stress-

related reactive oxygen species production (Minka and Ayo, 2009). This is in agreement with the 9% increase in albumin measured on day of slaughter.

Levels of BDNF were higher in efficient bulls for both models, suggesting that BDNF levels may be resistant to variation related to age. Brain-derived neurotrophic factor is a neurotrophin mainly involved in synaptic plasticity, neuronal maturation, dendritic remodeling and formation of synaptic contacts (Schinder and Poo, 2000). Studies demonstrated the metabolic role of BDNF in the neuroendocrine control of mammalian feeding behavior and energy homeostasis by describing increased feed intake and body weight with decreased BDNF signaling and expression (Rios et al. 2001). Expression of BDNF in subcutaneous adipose tissue of lactating cows has been observed (Colitti et al. 2015) supporting its role in the regulation of energy balance in cattle. Therefore, the proposed functionality of BDNF may explain the increased feed intake observed in inefficient bulls in this study.

Sex hormones

Anti-Müllerian hormone secretions by the Sertoli cells change with sexual development (Rota et al. 2002). In bulls, AMH levels start to decrease at 150 days of age and become steady following 330 days of age (Rota et al. 2002). Lack of difference between age groups for AMH may be attributed to limited sample numbers, as only one sample was analyzed towards the end of the on-farm sampling period while the increased age of the bulls at slaughter may explain the lack of difference between age groups for AMH.

Testosterone plays a key role in the development of male accessory organs, external sexual characteristics and male behavior (Moletta et al. 2014). In our study, bulls were progressing through the pubertal period, which is characterized by an increase in testosterone (Brito, 2015a) as observed here with greater testosterone levels in older bulls. Although FSH and LH levels are expected to plateau during this period, a slight increase in mean levels may still be observed (Brito, 2015a) explaining the increased FSH and LH observed in older bulls in the current study. The anabolic property of testosterone influences performance traits such as ADG and feed efficiency in bulls (Cook, R.B. et al. 2000). Thus, the greater levels of testosterone and LH observed in feed efficient bulls (RFI_{US}) suggests an association between sex hormones and feed efficiency. However, a greater variation in age was observed between efficiency groups for the RFI_{US} classification compared to the RFI_{AGE} classification and can explain the greater testosterone, FSH and LH observed in efficient bulls when no adjustment for age was performed. Stress induces a decrease in LH secretion (Welsh Jr and Johnson, 1981) and may be responsible for the 80% decrease in LH observed at slaughter. Moreover, response to stress also involves an increase in prolactin secretion in ruminants (Matteri et al. 2000), reflecting the 45% increase in prolactin levels observed at slaughter.

Metabolic hormones

In young bulls, IGF1 levels increase from 238 to about 350 days of age, followed by a decrease in concentration (Brito, 2015a), explaining the higher IGF1 results observed in older bulls during the on-farm sampling period and the lack of difference at slaughter. Studies have reported decreased IGF1 with improved efficiency in beef cattle

(Arthur et al. 2004; Moore et al. 2005). Despite the fact that IGF1 has been suggested to serve as a proxy for RFI (Davis and Simmen, 2006), others have observed minimal or no correlation between IGF1 and feed efficiency (Lancaster et al. 2008). Our study further supports the lack of relationship between RFI and IGF1 in beef cattle.

Thyroid hormones are under the control of the hypothalamic-pituitary-thyroid axis and are crucial for the control of growth, differentiation, and metabolism in nearly all somatic tissues (Hulbert, 2000). Levels of T_4 in the current study were not associated with feed efficiency, which differs from results by Walker et al. (2015). These authors observed higher plasma T_4 in inefficient heifers and a positive correlation between RFI and T_4. Disparity in results may be due to sex differences, as T_4 tends to be higher in bull calves (Kahl and Bitman, 1983). Similar to results reported by Walker et al. (2015), levels of plasma T_3 were lower in efficient bulls. Higher T_3 concentrations have been associated with higher maintenance energy requirements (Iossa et al. 2001). Triiodothyronine increases energy expenditure and feed intake (Hulbert, 2000), which is consistent with the increase in feed intake of inefficient bulls. Elevated thyroid hormones affect thermogenesis through stimulation of heat production (Hulbert, 2000), and thus may explain the increased radiant heat loss observed in inefficient steers (Montanholi et al. 2010). Differences in T_3 are consistent across RFI models and sampling periods, suggesting that variation in T_3 remains a robust indicator of feed efficiency despite variation in age and sampling routines.

Leptin is produced mainly by adipose tissue and is involved in the regulation of whole-body energy homeostasis through its effects on appetite, body composition, energy expenditure and nutrient partitioning (Kershaw and Flier, 2004). Leptin was higher in

efficient bulls at slaughter for RFI_{US} only. The results observed in this study are contrary to those of past studies that reported higher mean concentration of leptin in inefficient cattle (Walker et al. 2015) and a positive correlation between feed efficiency and leptin (Richardson et al. 2004). However, other studies also observed no difference in mean levels of leptin between efficiency groups (Kelly et al. 2010; Richardson et al. 2004) and no correlation between leptin and feed efficiency (Kelly et al. 2010; Walker et al. 2015). Disparity in RFI modelling may be a factor contributing to differences between studies. Previous studies used the RFI model proposed by Koch et al. (1963), which does not account for body composition traits and can result in large variation in body fat between efficiency groups (Fontoura et al. 2016; Kelly et al. 2011). Blood leptin is highly correlated with fat mass in cattle (Geary et al. 2003). In our study, both RFI models included adjustments for fatness levels, thereby reducing variation between efficiency groups. This suggests that metabolic differences independent of fatness levels may account for higher leptin levels in efficient bulls, which act to depress feed intake (Geary et al. 2003). Studies have shown that leptin is a stress-related hormone that can inhibit the hypothalamic-pituitary-adrenal axis activity and play an important role in acute stress response (Roubos et al. 2012), which may explain the observed difference between sampling periods for feed efficiency. Thus, further research is needed to understand the role of leptin on the stress response in cattle and its association with feed efficiency.

Residual feed intake modeling

Cattle with different feed efficiency differ in energy expenditure (Richardson and Herd, 2004). In the same manner, variation in age and physiological stage in cattle

influence whole-body energy expenditure (Brody, 1945) and can introduce bias when evaluating metabolic differences between efficiency groups. In this study, age was not significantly different between feed efficiency groups, but disparity in blood parameters between RFI models was still obtained after adjusting for variation in age. Furthermore, the fact that the RFI_{AGE} model had a greater R^2 than the RFI_{US} model, indicates that RFI_{AGE} was more appropriate to fit the variation associated with dry matter intake. The original model used to calculate RFI (Koch et al. 1963) can be improved by the inclusion of indicators of body composition (Montanholi et al. 2009; Schenkel et al. 2004). Inclusion of body composition reduces energetic efficiency differences associated with accretion of fat and protein (Owens, 1995). In the same manner, it was demonstrated that adjustment for age reduces the variation in metabolic processes related to age.

Sampling routines

Repeated blood sampling allows for greater statistical power through the increase in sample size and thus accuracy of results. Although a single blood sample obtained at slaughter has a lower statistical power, the variation in blood parameters observed at slaughter here is in accordance with those of stressed cattle, as observed elsewhere (Matteri et al. 2000; Minka and Ayo, 2009; Welsh Jr and Johnson, 1981). Pre-slaughter stress can cause significant changes in blood metabolite and hormone profiles (Minka and Ayo, 2009; Schaefer et al. 1997). For instance, differences in levels of certain blood parameters were observed between on-farm sampling and slaughter, such as a 62% increase in CK and a 31% increase in cortisol. Nonetheless, in the beef industry, harvesting tissues at slaughter allows for large and efficient collection of phenotypic data

that is not accessible on-farm in commercial settings. Thus, there is a need to identify blood parameters that are robustly associated with biologically-significant traits despite changes in sampling routine, as well as those which are better measured at slaughter. In this study, the majority of parameters showing variation between feed efficiency groups during the on-farm sampling period were not consistent at slaughter. Triiodothyronine was the only blood parameter that was consistently lower in efficient bulls between sampling periods. This emphasizes the need to understand the impact of pre-slaughter stress in order to more accurately evaluate variation in feed efficiency through blood parameters.

Conclusion

Differences in age of young beef bulls are associated with disparity in blood parameters related to intermediary metabolism, illustrating that variation in age impacts the background energy requirements of growing animals. Association of some blood parameters with feed efficiency was influenced by the inclusion of age in the RFI model. Our results demonstrate that even small variation in age can impact physiological and metabolic indicators in young bulls. This further supports the benefits of adjusting RFI model for age in order to more accurately evaluate differences in efficiency. Furthermore, association of blood parameters with feed efficiency was influenced by sampling routine. This highlights the impact of pre-slaughter stress on the metabolic profile of cattle and the need to identify proxies that remain predictive despite variation in sampling routine, as well as proxies that are better measured at slaughter. We observed the potential of T_3 as a blood parameter which may be robustly associated with feed efficiency in beef bulls.

This hormone appears to be predictive despite variation in age, on-farm handling and sampling at slaughter. Studies involving a larger population as well as cattle of different sexes and physiological stages are warranted.

Table 3.1 Ingredients and chemical composition of the diet

Ingredient composition (as fed)	Percentage
High moisture corn	52.20
Alfalfa silage	42.50
Soybean meal	3.65
Premix[a]	1.65
Chemical composition (% DM basis)	**Mean**
Dry matter (%)	53.89
Crude protein (N x 6.25)	16.28
Neutral detergent fiber	19.16
Acid detergent fiber	11.33
Total digestible nutrients	85.08

[a] Contains 93.6% soybean meal, 5.7% vitamin premix (4,400,000 IU/kg vitamin A, 1,100,000 IU/kg vitamin D, and 7700 IU/kg vitamin E), and 0.7% monensin premix (200 g monensin/kg).

Table 3.2 Descriptive statistics of age and performance traits of bulls over the 112-day performance evaluation

Traits (Unit)	Mean	S.D.	Min.	Max.
Age at end of performance test (days)	368.81	29.71	299.00	405.00
Age at slaughter (days)	403.63	31.62	346.00	448.00
Scrotal circumference at end of performance test (SC; cm)	37.23	2.53	33.00	43.00
Residual feed intake ultrasound (RFI_{US}; kg DM/day)	0.00	0.77	-1.37	1.83
Residual feed intake age (RFI_{AGE}; kg DM/day)	0.00	0.70	-1.34	2.00
Dry matter intake (DMI; kg/day)	7.03	0.88	5.05	9.13
Feed-to-gain ratio (FG)	3.90	0.52	2.80	4.82
Average daily gain (ADG; kg/day)	1.82	0.21	1.54	2.31
Initial body weight (initial BW; kg)	345.15	69.22	227.14	481.12
Final body weight (final BW; kg)	587.18	86.01	435.11	724.10
Initial backfat thickness (initial BKFT; mm)	3.18	1.22	1.26	5.65
Final backfat thickness (final BKFT; mm)	6.44	2.20	2.75	13.10
Initial rumpfat thickness (initial RUMP; mm)	2.41	1.36	0.77	6.04
Final rumpfat thickness (final RUMP; mm)	6.28	2.48	1.61	12.46
Initial ribeye area (initial RBEA; cm^2)	62.56	7.95	45.33	76.77
Final ribeye area (final RBEA; cm^2)	90.14	10.93	67.47	106.34

Table 3.3 Descriptive statistics of blood parameters of bulls when sampled during on-farm sampling period and at slaughter

Blood parameters (Abbreviation; Unit)	On-farm				Slaughter				Reference
	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	
Metabolic products									
Carbon dioxide (CO_2; mmol/L)	22.81	1.82	18.00	28.00	22.84	1.77	20.00	28.00	17.00-26.00[1]
Glucose (mmol/L)	4.67	0.41	3.60	5.70	5.72	0.96	4.60	8.20	2.50-4.30[1]
Urea (mmol/L)	4.03	0.84	2.20	7.10	4.56	1.25	2.60	10.20	3.00-8.00[1]
Cholesterol (mmol/L)	2.38	0.53	1.20	3.86	2.87	0.63	1.38	4.05	1.73-7.73[1]
Acetate (g/L)	33.70	7.81	16.48	61.93	36.81	13.60	19.00	79.06	49.95-53.15[2]
Ions									
Sodium (mmol/L)	136.41	7.17	116.00	152.00	136.90	5.22	123.00	150.00	132.00-145.00[1]
Potassium (mmol/L)	4.37	0.51	2.90	6.30	5.63	0.89	4.00	7.80	3.90-5.60[1]
Chloride (mmol/L)	95.12	4.58	83.00	108.00	92.45	3.00	83.00	98.00	90.00-113.00[1]
Osmolality (mmol/L)	270.51	14.17	230.00	303.00	275.26	10.10	253.00	297.00	265.00-295.00[1]
Proteins									
Aspartate amino transferase (AST; U/L)	61.26	16.82	30.00	168.00	81.52	28.93	44.00	197.00	44.00-153.00[1]
Alkaline phosphatase (ALP; U/L)	121.06	32.28	51.00	230.00	110.52	25.01	60.00	173.00	25.00-127.00[1]
Creatine kinase (CK; U/L)	121.69	66.96	41.00	539.00	422.68	243.76	119.00	992.00	44.00-211.00[1]
Albumin (g/L)	31.42	3.87	20.00	41.00	34.86	3.99	27.00	43.00	30.00-42.00[1]
Brain-derived neurotrophic factor (BDNF;	-	-	-	-	51.98	35.19	1.59	137.95	12.20-64.10[3]
Sexual hormones									
Anti-Müllerian hormone (AMH; pg/mL)	463.65	171.42	203.00	895.00	444.89	151.75	193.00	820.00	200.00-700.00[5]
Testosterone (ng/mL)	4.38	2.83	0.39	14.35	6.20	5.34	1.31	16.80	4.10-7.30[4]
Follicle stimulating hormone (FSH; ng/mL)	0.39	0.30	0.01	2.07	0.33	0.23	0.09	0.84	0.20-0.60[4]
Luteinizing hormone (LH; ng/mL)	0.66	0.85	0.00	5.64	0.13	0.10	0.00	0.39	0.10-0.30[4]
Prolactin (ng/mL)	65.97	56.33	1.60	402.00	125.67	87.24	14.00	403.00	68.10-98.2[6]
Metabolic hormones									
Cortisol (ng/g)	41.46	29.18	1.37	210.33	58.3	29.52	18.06	110.97	30.00-220.00[1]
Cholecystokinin (CCK; pg/mL)	-	-	-	-	51.22	29.25	9.92	145.93	15.00-35.00[7]
Ghrelin (pg/mL)	-	-	-	-	31.79	13.32	5.02	57.76	10.00-200.00[8]
Thyroxine (T_4; nmol/L)	86.42	12.57	51.50	125.00	97.59	12.77	68.50	127.00	59.04-93.55[9]
Triiodothyronine (T_3; nmol/L)	2.71	0.42	1.72	4.40	2.79	0.39	2.03	3.85	2.49-3.21[9]
Insulin-like growth factor 1 (IGF1; ng/mL)	500.48	89.68	300.00	799.00	395.45	71.90	246.00	540.00	350.00-550.00[4]
Leptin (ng/mL)	3.04	1.48	0.70	8.10	2.80	1.16	1.20	5.30	2.00-6.00[4]

[1]University of Guelph Animal Health Laboratory User Guide, 2015; [2]Gonano et al. 2014; [3]Bocchio-Chiavetto et al. 2010; [4]Brito, 2015a; [5]Rota et al. 2002; [6]Stolla et al. 1979; [7]Cappellozza et al. 2011; [8]Wertz-Lutz et al. 2006; [9]Pavlík et al. 2010.

Table 3.4 Age and productive performance mean (confidence limit) comparison between younger and older bulls during the performance test

Traits (Abbreviation; Unit)	Younger	Older
Age at end of performance test (Age; days)	341.6[b] (334.9, 348.4)	393.0[a] (382.9, 403.4)
Age at slaughter (days)	377.3[b] (369.3, 385.3)	429.8[a] (418.9, 440.7)
Scrotal circumference (SC; cm)	35.6[a] (34.5, 36.7)	37.7[b] (36.2, 39.1)
Residual feed intake ultrasound (RFI_{US}; kg	0.2 (-0.2, 0.6)	-0.3 (-0.8, 0.3)
Residual feed intake age (RFI_{AGE}; kg DM/day)	0.0 (-0.4, 0.4)	-0.1 (-0.6, 0.4)
Dry matter intake (DMI; kg/day)	7.0 (6.5, 7.4)	6.8 (6.2, 7.4)
Feed-to-gain ratio (FG)	4.0 (3.7, 4.3)	3.7 (3.3, 4.0)
Average daily gain (ADG; kg/day)	1.7 (1.6, 1.8)	1.8 (1.7, 2.0)
Initial body weight (initial BW; kg)	293.9[b] (268.0, 319.8)	374.4[a] (340.1, 408.7)
Final body weight (final BW; kg)	521.6[b] (493.2, 551.6)	617.1[a] (573.0, 664.6)
Initial backfat thickness (initial BKFT; mm)	2.3[b] (1.8, 2.7)	3.7[a] (3.1, 4.3)
Final backfat thickness (final BKFT; mm)	5.1[b] (4.2, 6.1)	7.0[a] (5.7, 8.3)
Initial rumpfat thickness (initial RUMP; mm)	1.6[b] (1.0, 2.2)	3.0[a] (2.2, 3.8)
Final rumpfat thickness (final RUMP; mm)	5.0[b] (3.8, 6.1)	7.2[a] (5.7, 8.7)
Initial ribeye area (initial RBEA; cm^2)	58.2[b] (54.9, 61.5)	69.4[a] (65.0, 73.8)
Final ribeye area (final RBEA; cm^2)	82.7[b] (78.4, 87.0)	97.3[a] (91.6, 103.0)

Subscripts (a,b) in the same row are different ($P<0.05$) based on T-test.

Table 3.5 Age and productive performance mean (confidence limit) comparison between efficient and inefficient bulls for RFI_{US} and RFI_{AGE} during the 112-day performance test

Traits (Abbreviation; Unit)	Efficient (RFI_{US})	Inefficient (RFI_{US})	Efficient (RFI_{AGE})	Inefficient (RFI_{AGE})
Age at end of performance test (Age; days)	364.6 (349.3, 380.6)	352.2 (337.4, 367.6)	358.5 (343.0, 374.7)	358.15 (342.7, 374.3)
Age at slaughter (days)	403.1 (386.2, 420.0)	386.6 (370.2, 403.0)	396.8 (379.2, 414.5)	392.4 (375.3, 409.6)
Scrotal circumference (SC; cm)	36.9 (35.6, 38.2)	35.8 (34.5, 37.0)	37.8 (36.7, 38.9)	36.7 (35.6, 37.8)
Residual feed intake ultrasound (RFI_{US}; kg DM/day)	-0.6^{b} (-0.9, -0.3)	0.6^{a} (0.3, 0.9)	-0.5^{b} (-0.8, -0.2)	0.6^{a} (0.3, 0.9)
Residual feed intake age (RFI_{AGE}; kg DM/day)	-0.5^{b} (-0.8, -0.1)	0.5^{a} (0.1, 0.8)	-0.5^{b} (-0.8, -0.3)	0.5^{a} (0.2, 0.8)
Dry matter intake (DMI; kg/day)	6.3^{b} (5.9, 6.7)	7.4^{a} (7.0, 7.8)	6.4^{b} (6.0, 6.8)	7.4^{a} (7.0, 7.8)
Feed-to-gain ratio (FG)	3.6^{b} (3.3, 3.8)	4.2^{a} (4.0, 4.5)	3.6^{b} (3.3, 3.8)	4.2^{a} (4.0, 4.5)
Average daily gain (ADG; kg/day)	1.8 (1.7, 1.9)	1.8 (1.7, 1.9)	1.8 (1.7, 1.9)	1.8 (1.6, 1.9)
Initial body weight (Initial BW; kg)	323.9 (287.9, 359.8)	318.9 (282.9, 354.8)	320.1 (284.2, 356.1)	322.6 (286.7, 358.6)
Final body weight (Final BW; kg)	555.8 (514.8, 600.1)	549.1 (508.6, 592.9)	552.8 (511.9, 596.9)	552.1 (511.3, 596.2)
Initial backfat thickness (initial BKFT; mm)	2.9 (2.3, 3.5)	2.6 (2.0, 3.3)	2.8 (2.1, 3.4)	2.8 (2.1, 3.4)
Final backfat thickness (final BKFT; mm)	5.7 (4.5, 6.9)	5.8 (4.7, 7.0)	5.9 (4.7, 7.0)	5.7 (4.5, 6.9)
Initial rumpfat thickness (initial RUMP; mm)	2.1 (1.3, 2.9)	2.1(1.4, 2.9)	2.1 (1.3, 2.8)	2.2 (1.4, 2.9)
Final rumpfat thickness (final RUMP; mm)	5.5 (4.1, 6.8)	6.0 (4.1, 6.8)	5.6 (4.2, 7.0)	5.8 (4.5, 7.2)
Initial ribeye area (initial RBEA; cm^2)	62.1 (57.3, 66.9)	62.0 (57.3, 66.9)	61.3 (56.6, 66.1)	62.7 (58.0, 67.5)
Final ribeye area (final RBEA; cm^2)	88.0 (81.8, 94.2)	87.3 (81.1, 93.5)	87.8 (81.7, 94.0)	87.5 (81.3, 93.7)

Subscripts (a,b) in the same row and within the same RFI classification are different ($P<0.05$) based on student t-test.

Table 3.6 Blood parameter mean (confidence limit) comparison between younger and older bulls sampled during on-farm sampling period and at slaughter

Parameters (abbreviation; unit)	On-farm		Slaughter	
	Younger	Older	Younger	Older
Metabolic products				
Carbon dioxide (CO_2; mmol/L)	22.7 (22.2, 23.2)	22.6 (22.0, 23.2)	22.0 (21.3, 22.8)	23.1 (22.0, 24.3)
Glucose (mmol/L)	4.8^{a} (4.7, 4.9)	4.5^{b} (4.3, 4.6)	-	-
Urea (mmol/L)	3.8^{B} (3.6, 4.1)	4.2^{A} (3.8, 4.5)	4.2 (3.7, 4.8)	4.3 (3.5, 5.1)
Cholesterol (mmol/L)	2.3 (2.1, 2.5)	2.4 (2.2, 2.7)	2.67^{b} (2.3, 3.0)	3.26^{a} (2.8, 3.7)
Acetate (g/L)	31.2 (29.6, 32.9)	33.3 (31.1, 35.7)	33.4 (26.0, 40.8)	41.3 (30.7, 51.9)
Ions				
Sodium (mmol/L)	135.9 (134.2, 137.6)	136.4 (134.1, 138.7)	-	-
Potassium (mmol/L)	4.6^{a} (4.5, 4.7)	4.2^{b} (4.0, 4.3)	5.8 (5.1, 6.3)	5.42 (5.1, 6.2)
Chloride (mmol/L)	94.7 (93.5, 95.9)	95.5 (93.9, 97.0)	-	-
Osmolality (mmol/L)	269.9 (266.5, 273.3)	270.1 (265.6, 274.7)	271.5 (266.4, 276.6)	274.2 (266.8, 281.6)
Proteins				
Aspartate aminotransferase (AST; U/L)	57.3 (53.1, 62.3)	59.0 (53.2, 66.3)	73.3 (64.8, 82.8)	65.1 (54.5, 77.8)
Alkaline phosphatase (ALP; U/L)	126.9 (116.3, 137.5)	114.9 (100.9, 128.8)	106.5 (93.6, 119.4)	115.6 (96.9, 134.4)
Creatine kinase (CK; U/L)	-	-	363.3 (260.8, 506.2)	283.9 (175.4, 459.6)
Albumin (g/L)	31.7 (30.7, 32.6)	31.4 (30.1, 32.7)	33.6^{B} (31.6, 35.6)	36.6^{A} (33.7, 39.5)
Brain-derived neurotrophic factor (BDNF; ng/mL)	-	-	40.6 (22.9, 58.2)	62.9 (37.2, 88.5)
Sexual hormones				
Anti-Müllerian hormone (AMH; pg/mL)	460.5 (375.7, 564.3)	375.2 (286.9, 490.6)	468.7 (373.6, 563.9)	409.5 (266.5, 551.5)
Testosterone (ng/mL)	3.5^{b} (3.0, 4.0)	5.9^{a} (5.1, 6.8)	3.1 (2.3, 5.1)	4.7 (2.6, 5.5)
Follicle-stimulating hormone (FSH; ng/mL)	0.3^{B} (0.2, 0.3)	0.4^{A} (0.3, 0.4)	0.2^{b} (0.1, 0.4)	0.5^{a} (0.3, 0.7)
Luteinizing hormone (LH; ng/mL)	0.4^{B} (0.3, 0.5)	0.5^{A} (0.4, 0.7)	0.1 (0.1, 0.2)	0.2 (0.1, 0.2)
Prolactin (ng/mL)	59.2 (46.5, 73.5)	64.0 (46.7, 84.0)	114.0 (69.8, 158.2)	153.5 (90.1, 216.9)
Metabolic hormones				
Cortisol (ng/g)	35.0 (29.2, 41.4)	32.1 (24.8, 40.3)	53.4 (76.0, 137.7)	45.1 (46.2, 134.3)
Cholecystokinin (CCK; pg/mL)	-	-	43.4 (32.7, 55.6)	49.1 (33.0, 68.4)
Ghrelin (pg/mL)	-	-	29.4 (22.0, 36.8)	35.9 (25.2, 46.6)
Thyroxine (T_4; nmol/L)	84.3 (80.7, 87.9)	86.1 (81.3, 90.9)	97.4 (90.8, 103.9)	95.4 (85.9, 104.9)
Triiodothyronine (T_3; nmol/L)	2.7 (2.5, 2.8)	2.7 (2.5, 2.8)	2.8 (2.6, 3.0)	2.7 (2.4, 3.0)
Insulin-like growth factor 1 (IGF1; ng/mL)	545.3^{a} (517.9, 572.7)	474.7^{b} (438.4, 511.0)	403.2 (361.0, 445.4)	418.7 (357.5, 480.0)
Leptin (ng/mL)	2.6 (2.3, 3.0)	2.9 (2.4, 3.4)	2.7 (2.0, 3.4)	3.4 (2.4, 4.3)

Different subscript (a,b and A,B) in the same row and within the same AGE classification are different ($P<0.05$) and are a trend towards significance ($0.05<P\leq0.10$), respectively, based on student t-test

Table 3.7 Blood parameter mean (confidence limit) comparison between efficient and inefficient (RFI_{US}) bulls during on-farm sampling period and at slaughter

Parameters (abbreviation; unit)	On-farm-RFI_{US}		Slaughter-RFI_{US}	
	Efficient	Inefficient	Efficient	Inefficient
Metabolic products				
Carbon dioxide (CO_2; mmol/L)	22.9 (22.4, 23.4)	22.4 (21.9, 22.9)	22.3 (21.3, 23.3)	22.4 (21.6, 23.3)
Glucose (mmol/L)	4.6 (4.5, 4.8)	4.7 (4.6, 4.9)	-	-
Urea (mmol/L)	3.9 (3.6, 4.1)	4.0 (3.7, 4.3)	4.5 (3.9, 5.3)	4.1 (3.5, 4.6)
Cholesterol (mmol/L)	2.3 (2.1, 2.5)	2.4 (2.2, 2.6)	2.7 (2.3, 3.2)	2.9 (2.5, 3.3)
Acetate (g/L)	33.2^{a} (31.4, 35.1)	30.6^{b} (29.0, 32.4)	38.9 (30.0, 47.7)	33.4 (25.3, 41.5)
Ions				
Sodium (mmol/L)	136.3 (134.4,	135.9 (134.0,	-	-
Potassium (mmol/L)	4.4 (4.3, 4.6)	4.5 (4.3, 4.6)	5.7 (5.1, 6.3)	5.7 (5.1, 6.3)
Chloride (mmol/L)	94.9 (93.5, 96.2)	95.0 (93.7, 96.3)	-	-
Osmolality (mmol/L)	270.2 (266.5,	269.8 (266.1,	273.8 (267.6,	271.2 (265.7, 276.7)
Proteins				
Aspartate aminotransferase (AST; U/L)	59.8 (54.9, 65.8)	56.1 (51.7, 61.2)	70.6 (60.6, 82.2)	70.8 (61.8, 81.1)
Alkaline phosphatase (ALP; U/L)	118.0 (106.4,	127.6 (116.0,	100.0^{B} (85.2,	116.4^{A} (103.1,
Creatine Kinase (CK; U/L)	-	-	300.5 (201.0,	368.3 (257.1, 527.4)
Albumin (g/L)	31.6 (30.5, 32.6)	31.6 (30.5, 32.6)	34.1 (31.6, 36.8)	34.8 (32.5, 37.1)
Brain-derived neurotrophic factor (BDNF;	-	-	69.5^{a} (51.7, 87.4)	30.3^{b} (14.3, 46.2)
Sexual Hormones				
Anti-Müllerian Hormone (AMH; pg/mL)	395.6 (313.5,	458.7 (369.2,	445.7 (323.8,	454.3 (355.2, 553.4)
Testosterone (ng/mL)	4.8^{a} (4.0, 5.5)	3.8^{b} (3.1, 4.4)	2.8 (2.0, 4.6)	4.3 (2.8, 9.9)
Follicle-stimulating hormone (FSH; ng/mL)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.4 (0.2, 0.5)	0.3 (0.2, 0.4)
Luteinizing hormone (LH; ng/mL)	0.5^{A} (0.4, 0.6)	0.4^{B} (0.3, 0.5)	0.2 (0.1, 0.2)	0.1 (0.1, 0.2)
Prolactin (ng/mL)	59.7 (45.7, 75.6)	61.9 (47.6, 77.9)	140.7 (88.1, 193.4)	115.3 (67.6, 162.9)
Metabolic Hormones				
Cortisol (ng/g)	34.8 (28.4, 41.9)	33.2 (27.0, 40.1)	49.50 (31.2, 67.8)	51.91 (35.1, 68.7)
Cholecystokinin (CCK; pg/mL)	-	-	50.9 (37.4, 66.7)	40.8 (29.9, 53.4)
Ghrelin (pg/mL)	-	-	33.4 (24.4, 42.5)	29.8 (21.7, 37.9)
Thyroxine (T4; nmol/L)	85.0 (81.0, 89.0)	84.8 (80.8, 88.8)	93.8 (86.1, 101.6)	99.0 (92.1, 105.9)
Triiodothyronine (T3; nmol/L)	2.6^{b} (2.4, 2.7)	2.76^{a} (2.6, 2.9)	2.6^{b} (2.3, 2.8)	2.9^{a} (2.7, 3.1)
Insulin growth-like factor 1 (IGF1; ng/mL)	511.2 (476.9,	532.1 (497.8,	396.8 (346.0,	416.4 (371.1, 461.8)
Leptin (ng/mL)	2.9 (2.5, 3.3)	2.6 (2.2, 2.9)	3.5^{a} (2.8, 4.2)	2.5^{b} (1.8, 3.1)

Different subscript (a,b and A,B) in the same row and within the same RFI classification are different ($P<0.05$) and are a trend towards significance ($0.05<P\leq0.10$), respectively, based on student t-test.

Table 3.8 Blood parameter mean (confidence limit) comparison between efficient and inefficient (RFI_{AGE}) bulls sampled during on-farm sampling period and at slaughter

Parameters (abbreviation; unit)	On-farm-RFI_{AGE}		Slaughter-RFI_{AGE}	
	Efficient	Inefficient	Efficient	Inefficient
Metabolic products				
Carbon dioxide (CO_2; mmol/L)	23.0^{a} (22.5, 23.5)	22.3^{b} (21.8, 22.8)	22.2 (21.2, 23.2)	22.5 (21.6, 23.4)
Glucose (mmol/L)	4.7 (4.5, 4.8)	4.7 (4.5, 4.8)	-	-
Urea (mmol/L)	3.9 (3.6, 4.1)	4.0 (3.8, 4.3)	4.5 (3.9, 5.3)	4.1 (3.5, 4.7)
Cholesterol (mmol/L)	2.4 (2.2, 2.5)	2.3 (2.1, 2.5)	2.8 (2.3, 3.2)	2.9 (2.5, 3.3)
Acetate (g/L)	32.5 (30.7, 34.5)	31.3 (29.5, 33.1)	40.4 (31.8, 49.1)	32.1 (24.2, 40.0)
Ions				
Sodium (mmol/L)	136.3 (134.4,	135.8 (134.0, 137.8)	-	-
Potassium (mmol/L)	4.5 (4.4, 4,7)	4.4 (4.2, 4.5)	5.8 (5.2, 6.4)	5.6 (5.0, 6.1)
Chloride (mmol/L)	94.6 (93.3, 95.9)	95.3 (94.0, 96.6)	-	-
Osmolality (mmol/L)	270.4 (266.7, 274.1)	269.5 (265.8,	274.2 (268.1,	270.8 (265.4,
Proteins				
Aspartate aminotransferase (AST; U/L)	59.6 (54.7, 65.5)	56.24 (51.87,	72.2 (62.0, 84.0)	69.6 (60.7, 79.7)
Alkaline phosphatase (ALP; U/L)	122.1 (110.3, 134.1)	123.4 (111.5,	102.9 (87.5, 118.3)	114.1 (100.4,
Creatine Kinase (CK; U/L)	-	-	329.4 (219.0,	343.2 (238.4,
Albumin (g/L)	31.7 (30.6, 32.7)	31.5 (30.4, 32.5)	34.3 (31.7, 36.9)	34.8 (32.4, 37.1)
Brain-derived neurotrophic factor (BDNF;	-	-	59.8^{A} (38.6, 80.9)	37.8^{B} (18.9, 56.7)
Sexual Hormones				
Anti-Müllerian Hormone (AMH; pg/mL)	423.4 (333.5, 537.4)	433.2 (346.8,	419.5 (299.1,	470.3 (372.5,
Testosterone (ng/mL)	4.4 (3.7, 5.2)	4.1 (3.4, 4.8)	3.0 (2.1, 5.5)	4.0 (2.6, 8.4)
Follicle-stimulating hormone (FSH; ng/mL)	0.3 (0.2, 0.3)	0.3 (0.3, 0.4)	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)
Luteinizing hormone (LH; ng/mL)	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)
Prolactin (ng/mL)	53.9 (40.9, 68.6)	68.1 (53.5, 68.6)	145.8 (93.7, 197.8)	111.3 (64.2, 158.4)
Metabolic Hormones				
Cortisol (ng/g)	34.5 (28.2, 41.5)	33.5 (27.3, 40.4)	56.8 (38.8, 74.8)	46.0 (29.6, 62.5)
Cholecystokinin (CCK; pg/mL)	-	-	52.1 (38.5, 67.8)	40.0 (29.4, 52.3)
Ghrelin (pg/mL)	-	-	31.9 (22.7, 41.0)	31.0 (22.8, 39.2)
Thyroxine (T4; nmol/L)	86.3 (82.4, 90.2)	83.5 (79.6, 87.5)	97.1 (89.2, 105.0)	96.5 (89.5, 103.6)
Triiodothyronine (T3; nmol/L)	2.6^{b} (2.4, 2.7)	2.8^{a} (2.6, 2.9)	2.6^{B} (2.4, 2.9)	2.9^{A} (2.7, 3.1)
Insulin growth-like factor 1 (IGF1; ng/mL)	529.9 (495.2, 564.5)	513.4 (478.7,	419.5 (373.8,	470.3 (350.6,
Leptin (ng/mL)	2.8 (2.4, 3.2)	2.7 (2.3, 3.0)	3.1 (2.3, 3.9)	2.7 (2.0, 3.5)

Different subscript (a,b and A,B) in the same row and within the same RFI classification are different ($P<0.05$) and are a trend towards significance ($0.05<P\leq0.10$), respectively, based on student t-test.

Table 3.9 Blood parameter mean (confidence limit) comparison between on-farm sampling period and slaughter in bulls

Parameters (abbreviation; unit)	On-farm	Slaughter
Metabolic products		
Carbon dioxide (CO_2; mmol/L)	22.8 (22.3, 23.2)	22.9 (22.4, 23.5)
Glucose (mmol/L)	-	-
Urea (mmol/L)	4.0^b (3.7, 4.3)	4.4^a (4.1, 4.8)
Cholesterol (mmol/L)	2.4^b (2.2, 2.6)	2.9^a (2.7, 3.0)
Acetate (g/L)	33.2 (30.6, 36.3)	33.7 (30.7, 37.2)
Ions		
Sodium	136.3 (135.1, 137.6)	136.0 (134.6, 137.4)
Potassium (mmol/L)	4.3^b (4.2 to 4.5)	5.5^a (5.2, 5.7)
Chloride	95.1^a (94.3, 95.9)	92.0^b (91.1, 93.0)
Osmolality (mmol/L)	270.4 (267.9, 272.8)	273.4 (270.7, 276.1)
Proteins		
Aspartate aminotransferase (AST; U/L)	59.8^b (56.1, 64.0)	71.3^a (65.6, 78.2)
Alkaline phosphatase (ALP; U/L)	121.3^a (112.6, 129.9)	107.6^b (97.8, 117.3)
Creatine kinase (CK; U/L)	115.9^b (105.7, 128.2)	303.7^a (236.8, 423.4)
Albumin (g/L)	31.4^b (30.4, 32.3)	34.6^a (33.5, 35.6)
Brain-derived neurotrophic factor (BDNF; ng/mL)	-	-
Sexual Hormones		
Anti-Müllerian hormone (pg/mL)	449.4 (387.5, 511.3)	478.0 (408.8, 547.2)
Testosterone (ng/mL)	4.0 (3.2, 5.1)	4.5 (3.5, 5.8)
Follicle-stimulating hormone (FSH; ng/mL)	0.4 (0.3, 0.4)	0.3 (0.2, 0.4)
Luteinizing hormone (LH; ng/mL)	0.6^a (0.4, 0.7)	0.1^b (0.1, 0.2)
Prolactin (ng/mL)	57.3^b (45.8, 71.7)	104.1^a (80.6, 134.5)
Metabolic Hormones		
Cortisol (ng/g)	38.9^a (33.0, 47.4)	54.0^b (45.1, 63.8)
Cholecystokinin (CCK; pg/mL)	-	-
Thyroxine (T_4; nmol/L)	86.6^a (82.9, 90.2)	96.5^b (92.4, 100.6)
Triiodothyronine (T_3; nmol/L)	2.7 (2.6, 2.8)	2.8 (2.67, 2.9)
Insulin growth-like factor 1 (IGF1; ng/mL)	500.2^b (475.3, 525.0)	401.9^a (374.0, 429.8)
Leptin (ng/mL)	3.0 (2.7, 3.4)	2.9 (2.5, 3.3)

Subscripts (a,b) in the same row are different (P<0.05) based on T-test.

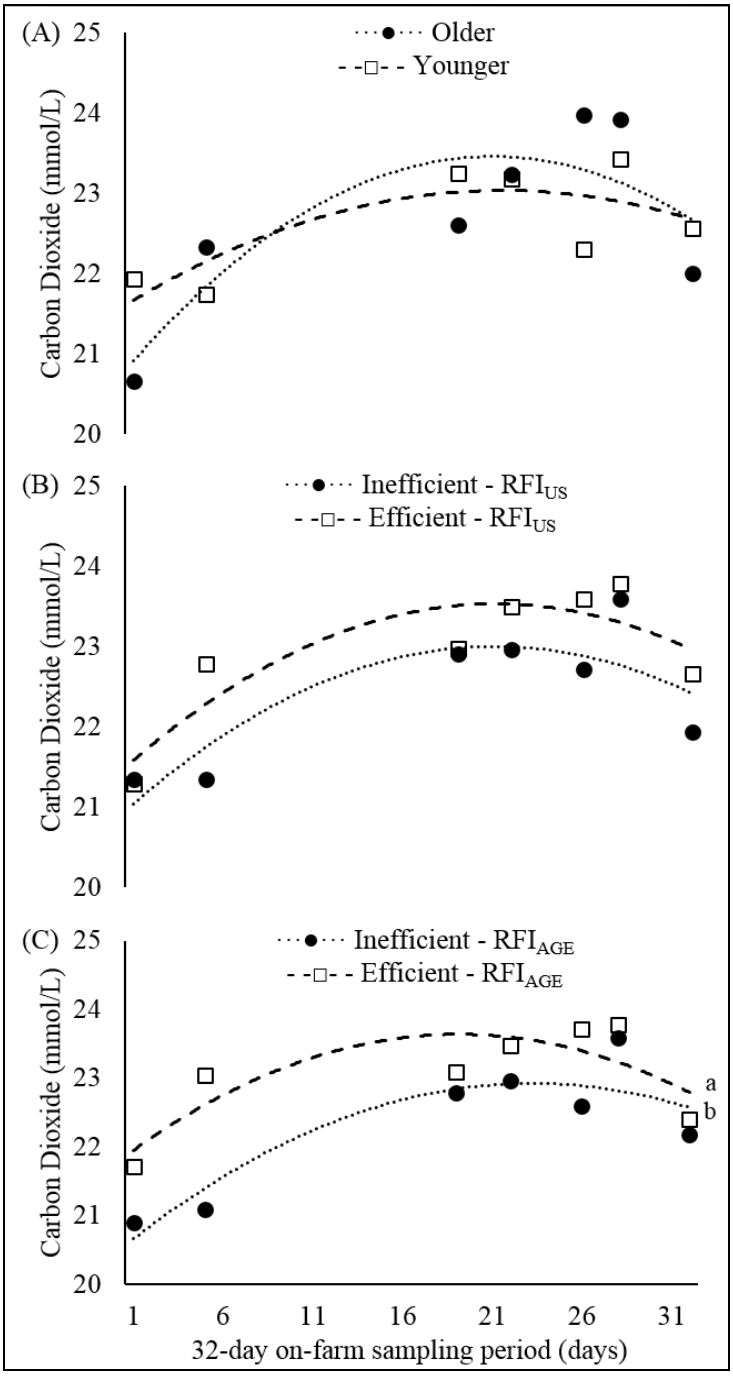

Figure 3.1 Plasma carbon dioxide concentration across age and feed efficiency groups of bulls sampled during on-farm sampling period. (A) younger (--□--) and older (···●···); (B) efficient (--□--) and inefficient (···●···) for $RFI_{US;}$ and (C) efficient (--□--) and inefficient (···●···) for RFI_{AGE}. Differing letters denote $P < 0.05$.

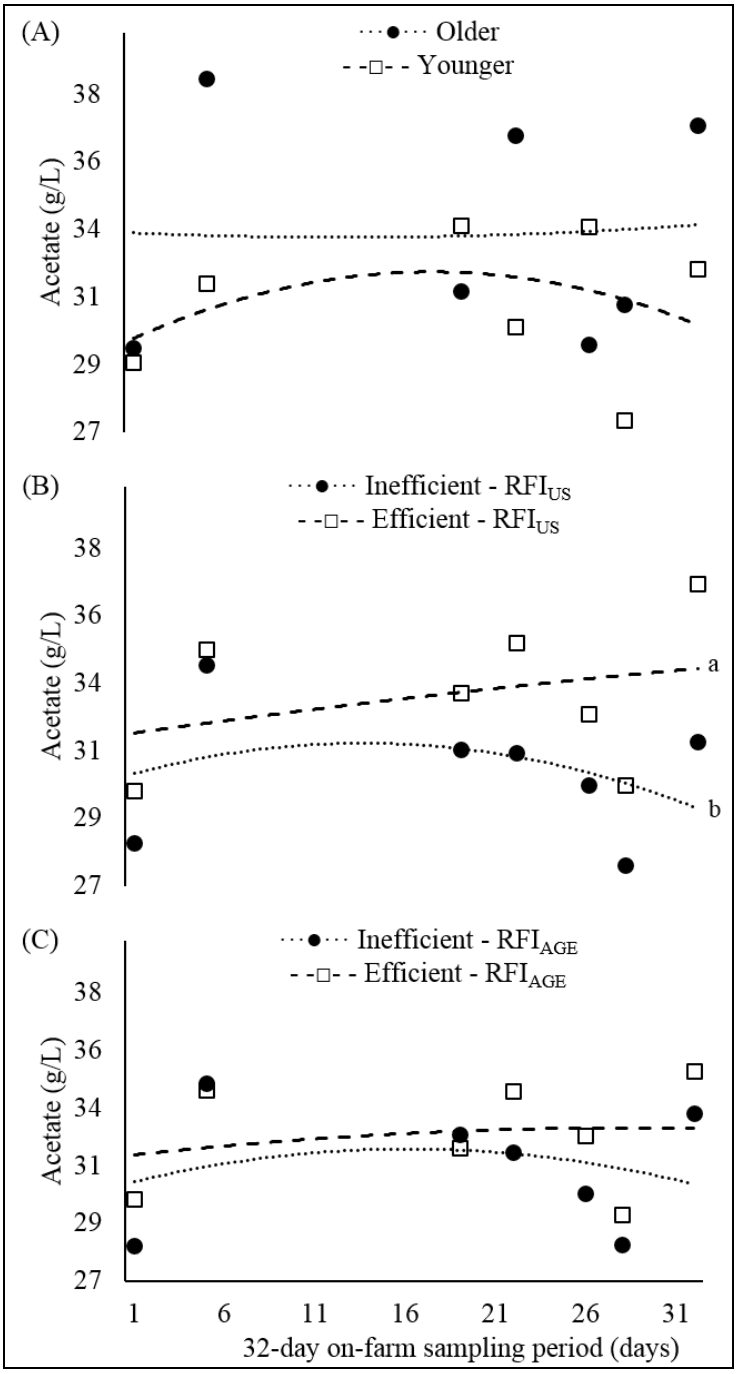

Figure 3.2 Plasma acetate levels across age and feed efficiency groups of bulls sampled during on-farm sampling period. (A) younger (--□--) and older (···●···); (B) efficient (--□--) and inefficient (···●···) for $RFI_{US;}$ and (C) efficient (--□--) and inefficient (···●···) for RFI_{AGE}. Differing letters denote $P < 0.05$.

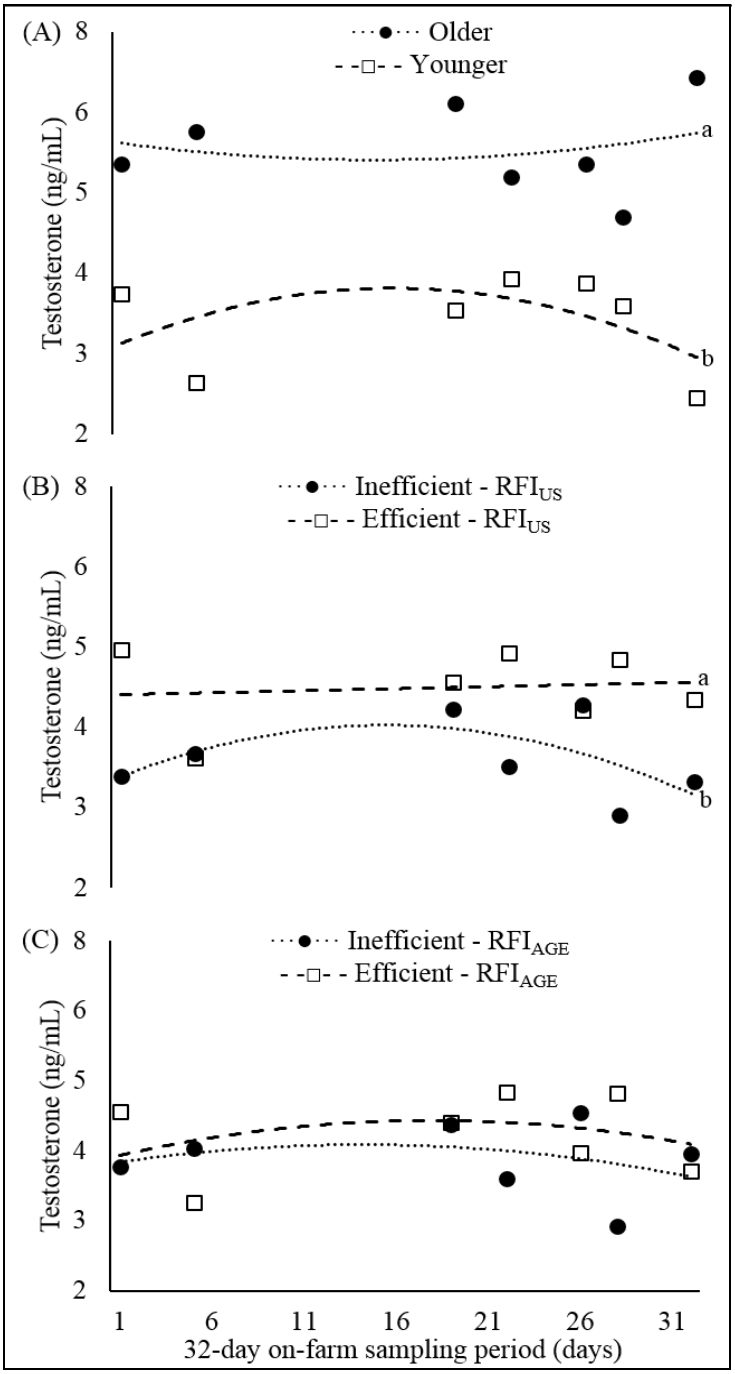

Figure 3.3 Plasma testosterone levels across age and feed efficiency groups of bulls sampled during on-farm sampling period. (A) younger (--□--) and older (···●···); (B) efficient (--□--) and inefficient (···●···) for $RFI_{US;}$ and (C) efficient (--□--) and inefficient (···●···) for RFI_{AGE}. Differing letters denote $P < 0.05$.

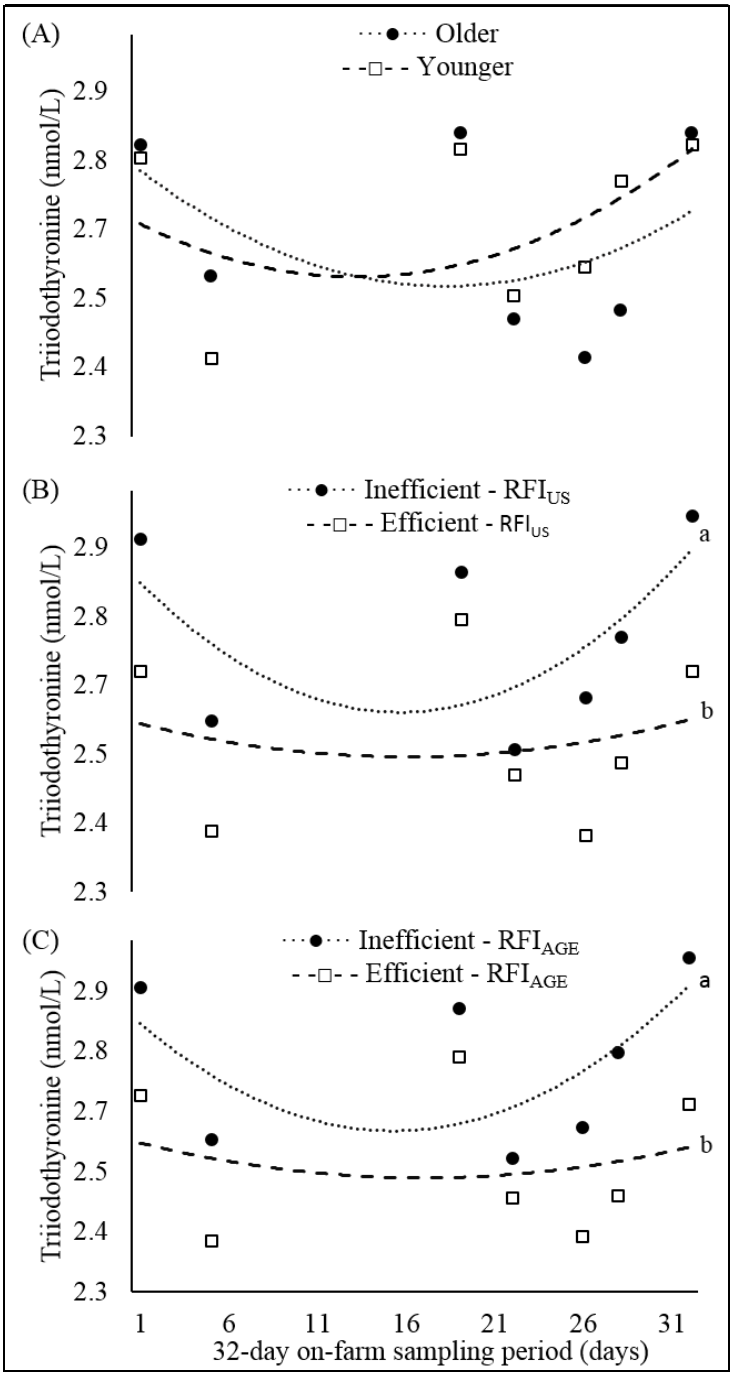

Figure 3.4 Plasma triiodothyronine levels across age and feed efficiency groups of bulls sampled during on-farm sampling period. (A) younger (--□--) and older (···●···); (B) efficient (--□--) and inefficient (···●···) for $RFI_{US;}$ and (C) efficient (--□--) and inefficient (···●···) for RFI_{AGE}. Differing letters denote $P < 0.05$.

CHAPTER 4

Relationships of age and feed efficiency with sexual development and fertility-related measures in young beef bulls

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Abstract

Variation in feed efficiency and age are associated with metabolic fluctuations, which may impact sexual development of young bulls. In order to characterize such variability, 158 bulls were evaluated in a performance test where scrotal circumference (SC), testis ultrasounds and blood hormone were determined at the start, middle and end of the test. Breeding soundness evaluation, scrotal thermographs and complete blood cell count (CBC) were obtained towards the end of the test. Bulls were characterized according to age (younger and older) and residual feed intake (efficient and inefficient) for least square means comparisons and partial regression analyses. The later, along with correlations, was also determined across the whole population. Younger bulls had smaller SC, higher scrotal radiant heat loss, reduced normal sperm morphology and suggested higher average testis pixel intensity (P=0.06). Efficient bulls had reduced SC and scrotal radiant heat loss, suggested greater sperm head abnormalities (P=0.08) and lower testis maximum pixel intensity (P=0.08). Triiodothyronine was positively correlated with SC, scrotal radiant heat loss and sperm tail defects. Variation in age was mainly explained by SC and CBC. Semen quality was the main factor contributing to the age variation in younger and older sub-groups. Scrotum thermography and semen quality were the two main factors explaining variation in RFI. In the efficient and inefficient sub-groups, the main factors contributing to the variation in RFI were semen quality and CBC. Metabolic differences associated with age and feed efficiency may impact reproductive function, leading to features of delayed sexual development in efficient bulls.

Keywords: scrotal circumference, infrared imaging, residual feed intake, semen quality, testes ultrasonography, trioodothyronine

Highlights

- Variability in scrotal circumference is related to age distribution of young bulls.
- Older growing bulls show greater oxygen requirements compared to younger bulls.
- Feed efficient bulls display features of delayed sexual development.
- Semen quality is a major source of variation in efficient and inefficient bulls.
- Resemblances in sexual development exist between feed efficient and younger bulls.

Introduction

Improvement in feed efficiency is important for economic and environmental sustainability of the beef industry. Residual feed intake (RFI) is a common measure of feed efficiency, defined as the difference between observed and expected feed intake, based on body weight, average daily gain and body composition (Montanholi et al. 2009). In addition to feed efficiency, desirable bull fertility influences the profitability of the industry, by contributing substantially to the reproductive outcome of the breeding herd (Wiltbank, 1994). Bulls that reach sexual maturity younger contribute to the overall genetic gain in fertility (Holroyd and McGowan, 2014). However, factors such as feed efficiency can influence sexual development in young bulls (Fontoura et al. 2016). There are concerns in the beef industry of undesirable associations between improved feed efficiency and sexual maturity in young bulls, such as delayed testicular development

(Awda et al. 2013; Fontoura et al. 2016), reduced semen quality (Awda et al. 2013; Fontoura et al. 2016; Wang et al. 2012) and indications of delayed sexual development based on the proteomic profile of the seminal plasma (Montanholi et al. 2016a).

Assessments of fertility-related measures and physiological parameters may provide important clues about the metabolic and reproductive profile of yearling bulls in relation to age and feed efficiency. During the breeding soundness evaluation (BSE), scrotal circumference (SC) and semen quality are assessed, along with other measurements (Hopper, 2015). Scrotal circumference is highly associated with age at puberty, daily sperm production and semen quality (Barth and Ominski, 2000), while sperm motility and sperm morphology may allow inferences about reproductive abnormalities and fertility potential (Hopper and King, 2015). Non-invasive imaging technologies can also be used in the reproductive assessment of young bulls. Testicular ultrasonography may be employed to assess testis echogenicity for testicular tissue development, which is related to sexual development in young bulls (Aravindakshan et al. 2000). In the same way, scrotal thermographs are useful to evaluate scrotum radiant heat loss, related to semen quality and bull fertility (Kastelic et al. 1995). Moreover, hormones associated with stage of sexual maturity and metabolic status are relevant assessments in young bulls (Rawlings et al. 1978). Complete blood cell count parameters, such as red blood cells (RBC) and white blood cells (WBC), are also associated with metabolic demands (Riedesel and Engen 2015) and thus, may be used as indirect indicators of feed efficiency, as suggested by others (Lawrence et al. 2011).

Variation in age is associated with changes in the reproductive development of bulls (Brito, 2015b). Feed efficiency relates to sexual development in young beef bulls, as

supported by discrepancies in fertility-related measures between bulls of distinct feed efficiency (Fontoura et al. 2016; Hafla et al. 2012; Montanholi et al. 2016a). These differences, which may be evident throughout the growth and development of the bulls, can be assessed via testis and scrotal biometrics, as well as through hormonal and blood cell profiles. Such characterization is important to portray the underlying biology, leading to practical assessments for commercial beef bulls. The objective of this study was to characterize the variability in sexual development and fertility-related measures in young bulls, in the context of age and feed efficiency, throughout the routine bull test performance evaluation and at occasion of the BSE.

Material and methods

Bull management and experimental design

Bulls were housed and tested for productive performance and reproductive traits at the Maritime Beef Testing Society (Nappan, Canada; Maritime bulls) and at the Elora Beef Research Center (Guelph, Canada; Elora bulls), from December to April (winter, Northern Hemisphere). In both stations, bulls were cared for according to the Canadian Council on Animal Care Guidelines (2009). Maritime bulls (initial age: 313 ± 20 days) consisted of 109 purebred and crossbred bulls classified, by breed type, as British or Continental predominance. Bulls were stratified by initial body weight (BW; kg) into 4 pens (Pen 1: n=27, BW=386 ± 63 kg; Pen 2: n=29, BW=413 ± 47 kg; Pen 3: n=26, BW=438 ± 36 kg and; Pen 4: n=27, BW=463 ± 66 kg). Each pen included an indoor feeding area (20.5 by 12.9 m), an outdoor area (15.5 by 9.1 m) and a sheltered bedding

area supplied with wheat straw (12.9 by 5.5 m). All bulls were fed *ad libitum* a mixed-ration diet (as fed, 70.00% grass silage, 15.42% corn silage, 12.75% barley grain, 1.08% soybean meal, 0.58% vitamin and mineral commercial premix (ATB brand beef premix med 125 (RUM)®) and 0.25% limestone), containing 21.15% starch and 73.12% total digestible energy. Elora bulls (initial age: 236 ± 10 days) consisted of 49 purebred and crossbred bulls classified, by breed type, as British or Continental predominance. Bulls were stratified by initial BW into 3 indoor pens (Pen 1: n=17, BW=300 ± 13 kg; Pen 2: n=17, BW=345 ± 25 kg and; Pen 3: n=15, BW=247 ± 17 kg). Each pen included a feeding area (12 by 29 m) and an area bedded with wood shavings (24 by 29 m). Elora bulls were fed *ad libitum* a high moisture corn-based diet (as fed, 52.20% high moisture corn, 42.40% alfalfa silage, and 5.04% soybean meal and 0.11% vitamin and a mineral premix, described by Montanholi et al. (2009)), containing 44.80% starch and 86.48% total digestible nutrients.

Bulls underwent a 112-day performance test, during which individual feed intake was recorded using automated feeding stations (Maritime: Growsafe®, Airdrie, Canada; Elora: Insentec®, B.V., Marknesse, The Netherlands). Ultrasounds for body composition traits (backfat thickness (BKFT; mm), rumpfat thickness (RUMP; mm) and ribeye area (RBEA; cm^2)), BW, scrotal circumference (SC; cm), testes ultrasounds and blood plasma samples were obtained at the start (days 5 ± 4), middle (days 59 ± 1) and end (days 108 ± 5) of the performance test, as described by Montanholi et al. (2009). Breeding soundness evaluation (Barth, 2002), scrotal thermography and complete blood cell count (CBC) analysis were performed around the end the performance test at 407 ± 22 days of age. Weather data was obtained from Climate Canada from the closest weather stations

(Maritimes; 45°45'34.400" North, 64°14'29.200" West; Elora: 43°39'00.000" North, 80°25'00.000" West). Ambient temperature and relative humidity were used to calculate hourly values of the temperature-humidity index (THI) at the start, middle and end, as described by Thom (1959).

Assessment of feed efficiency

Feed intake data was obtained from the automated feeding system, using only daily feed intake values with greater than a 98% probability of belonging to the normal distribution of daily feed intake were computed to determine the individual daily dry matter intake (DMI, kg/d). Body weight and ultrasound traits were regressed on time to calculate average BW, BKFT, RUMP and RBEA, as well as average daily gain (ADG; kg/d). These traits were used to calculate the predicted DMI accounting for the bull population effect, as described by Montanholi et al. (2009). Residual feed intake (kg/d) was used as a measure of feed efficiency and was calculated as the difference between the actual DMI and the predicted DMI, across the two bull populations ($R^2 = 0.58$), based on the following equation:

$$RFI = actual\ DMI - [3.87 + 0.02(average\ BW) + 0.03(ADG) - 0.01(average\ BKFT) - 0.03(average\ RBEA) - 0.30(average\ RUMP) + bull\ population]$$

where the effects of Maritime and Elora bull populations were 1.20 and 0.00, respectively.

Scrotal circumference and testes ultrasonography

Scrotal circumference was measured using a scrotal measuring tape (Lane Manufacturing Inc., Denver, USA) as described by Barth (2000). Ultrasonography of the testes was carried out, as described by Arteaga et al. (2005), using an ultrasound (Aloka SSD-500, Tokyo, Japan) connected to a 5 MHz linear array transducer. The ultrasound images were analyzed, using the imaging software ImageJ® (US National Institutes of Health, Bethesda, USA). A square was calculated proportional to the scrotal circumference obtained on each of assessment, and drawn laterally and medially to the mediastinum on the testicular parenchyma. Pixel intensity measures of the left and right testes were obtained and used to calculate average (AVG; pixels) and maximum (MAX; pixels) pixel intensity.

Scrotal thermography

Scrotal thermographs of the caudal aspect of the scrotum were obtained, as described by Fontoura et al. (2016). Thermographs were taken, prior to any handling of the scrotum and semen collection, while the bull was restrained in a squeeze chute. All images were interpreted using the ThermaCam™ Researcher Software version 2.10 (FLIR Systems AB, Danderyd, Sweden). Thermograph analysis of scrotal surface temperature was adapted from Lunstra and Coulter (1997). Scrotal surface temperature included the following traits: base average (STB; °C); apex average (STA; °C); total scrotal area average (STT; °C). Scrotal temperature gradient (STG; °C) was calculated as the difference between average temperature at the base and apex of the scrotum.

Semen quality assessment

Semen was collected, using an electro-ejaculator (Pulsator IV electro-ejaculator, Lane Manufacturing Inc., Denver, USA) and evaluated according to the guidelines of the Society for Theriogenology (Montgomery, USA; Chenoweth et al. 1992). Percentage of motile sperm was evaluated, as described by Arteaga et al. (2005), using phase-contrast microscopy (Nikon Eclipse 50i, Nikon, Tokyo, Japan). Sperm morphology and viability were determined from eosin and nigrosine-stained smears. A total of 100 sperm cells per bull were examined under 1000x magnification (Nikon Eclipse 50i, Nikon, Tokyo, Japan). Sperm morphology was classified, as described by Barth and Oko (1989) and results were grouped as percentages of normal sperm, detached sperm heads, head defects, midpiece defects and tail defects.

Blood sampling and analysis

Blood samples were collected between 8h00 and 16h00 via jugular venipuncture, using a 10 mL sodium heparin tube (Vacutainer ®, BD Inc., Franklin Lakes, USA) for blood hormone profile analysis and 7 mL EDTA blood collection tube (Monoject^{TM} Blood Collection Tube, Kendall Healthcare, Mansfield, USA) for CBC analysis. Samples for plasma hormone profile analysis were centrifuged (3000g for 25 minutes at 4 °C) and plasma was decanted and kept frozen until analysed. Samples for CBC analysis were stored at 4 °C and immediately submitted for analysis. Plasma testosterone (ng/mL) was analyzed, using a double-antibody radioimmunoassay (Coat-A-Count total testosterone, Siemens Healthcare Diagnostic Products, Los Angeles, USA) previously used in cattle

(Bagu et al. 2006); Total triiodothyronine (T_3; nmol/L) was analyzed using a solid-phase radioimmunoassay (Immulite® 1000 Total T3, Siemens Healthcare Diagnostic Products, Los Angeles, USA), previously used in cattle (Campos et al. 2004). Leptin (ng/mL) concentrations were determined using a liquid-phase radioimmunoassay (Multi-Species Leptin RIA Kit, Millipore, Missouri, USA) previously applied in the bovine (Astessiano et al. 2015). 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^{12} cells/L), hemoglobin (g/L), mean cell volume (MCV; fL), mean cell hemoglobin (MCH; pg) and platelets (10^9 cells/L). Total white blood cells (WBC; 10^9 cells/L) were also measured.

Statistical analysis

Data was analyzed using the Statistical Analysis System version 9.4 (SAS Institute Inc., Cary, USA). Data from both groups of bulls was combined in order to increase the statistical power of the analysis and is a common practice when assessing phenotypes in young bulls in the context of feed efficiency (Awda et al. 2013; Hafla et al. 2012; Wang et al. 2012). Normality was evaluated using the univariate procedure and data showing significant skewness were transformed for analysis. Results were back-transformed for presentation and reported with the standard error of the mean. Bulls were classified as either younger (n=80; 389 ± 14 days) and older (n=78; 427 ± 6 days) based on their age at the BSE. Bulls were also classified as either efficient (n=79; -0.56 ± 0.47 kg DM/day) and inefficient (n=79; 0.56 ± 0.40 kg DM/day), based on the RFI values. Repeated measures for SC, testes ultrasound traits and blood hormones were analyzed

through random regression, using the mixed procedure, according to the following model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_l + \eta_{ijklm} + \tau_{ijklm} + \kappa(THI_{ijkl} - THI_{..}) + \varepsilon_{ijklm}$$

where Y_{jtklm} is the dependent variable, μ is the overall mean; α_i is the fixed effect of age group (i = younger or older); β_j is the fixed effect of the RFI group (j = efficient or inefficient); $(\alpha\beta)_{ij}$ is the age and RFI groups interaction effect; γ_k is the fixed effect of the bull population (k = Maritime or Elora); δ_l is the fixed effect of the breed type (l = British or Continental); η_{ijklm} are fixed regression coefficients; τ_{ijklm} are the repeated regression coefficients; $\kappa(THI_{ijkl} - THI_{..})$ indicates the inclusion of THI as a covariate and; ε_{ijklm} is the residual random effect. The THI as a covariate was not included for analysis of blood hormones as preliminary analysis did not support association between blood hormones and THI. Additionally, a similar model, with inclusion of age as a covariate, was used to compare means between feed efficiency groups. Least square means of each trait for each day of assessment were obtained using a model similar to the above.

The general linear model procedure was used for means comparison of traits between age groups on the day of BSE according to the following model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_l + \kappa(THI_{ijkl} - THI_{..}) + \varepsilon_{ijklm}$$

where Y_{jtklm} is the dependent variable, μ is the overall mean; α_i is the fixed effect of age group (i = younger or older); β_j is the fixed effect of the RFI group (j = efficient or inefficient); $(\alpha\beta)_{ij}$ is the age and RFI groups interaction effect; γ_k is the fixed effect of the bull population (k = *Maritime or Elora)*; δ_l is the fixed effect of the breed type (l =

British, Continental and Other); $\kappa(THI_{ijkl} - THI_{\_})$ indicates the inclusion of THI as a covariate and; ε_{ijklm} is the residual random effect. The THI as a covariate was not included for analysis of blood hormones. Additionally, a similar model, with inclusion of age as a covariate, was used to compare means between feed efficiency groups. The interaction effect$(\alpha\beta)_{ij}$, was not significant in all analysis and, therefore, not reported.

Residuals for RFI analysis were determined by adjusting each trait for breed, bull population and age; while adjustment for age was removed for the residuals used in the age analysis. Additionally, residuals for testes ultrasound and scrotum thermography were also adjusted for THI. The relationships among age, RFI, fertility-related traits and CBC were measured through Pearson correlation using correlation procedure on the residuals. Partial least square procedure was applied on residuals to extract orthogonal vectors, to define single traits which represented each class of variables evaluated in this study (scrotal circumference, testes ultrasound traits, scrotum thermography traits, semen quality traits, blood hormones and CBC parameters). These indices were computed, using the regression procedure, to determine their partial contribution to the explained variation associated with age and feed efficiency. This analysis was repeated for bull population classified by age sub-groups (younger and older) and feed efficiency sub-groups (low-RFI and high-RFI). For all analyses, results were considered to be statistically significant when $P \leq 0.05$ and a trend towards significance when $0.10 \geq P > 0.05$.

Results

Table 4.1 lists the descriptive statistics of biometric measures at the end of the performance test, as well as fertility-related measures and complete blood count analysis

from pooled data obtained during repeated measures and during the BSE. Table 4.2 displays least square means of biometric measures between age groups and feed efficiency groups. The productive performance measures between age groups indicate lower DMI, BW, BKFT, RBEA and similar ADG in younger bulls when compared to older bulls. There was no difference in age on day of BSE between efficiency groups. As expected, the productive performance measures between efficiency groups indicate lower RFI and DMI in efficient bulls when compared to inefficient bulls and no difference in other productive performance traits.

Figure 4.1 represents the overall pattern of SC, AVG pixel intensity, testosterone, T_3 and leptin over the experimental period. Scrotal circumference increased between the start and middle assessment, but remained constant between middle and end of the test. Average pixel intensity had a marked positive slope, but the change was not significant. The concentration of testosterone increased between the start and middle of the experimental period, while both T_3 and leptin decreased between the middle and end of the experimental period.

Figures 4.2 illustrates AVG pixel intensity, SC, testosterone, T_3 and leptin measurements over repeated sampling days by age groups. Over time, younger bulls were observed to have lower SC, and tended to have higher AVG pixel intensity (P=0.066). No difference between age groups was observed for MAX pixel intensity (189.41 vs. 186.43; P=0.115), although numerically higher in younger bulls. Furthermore, no differences in levels of testosterone, T_3 and leptin were observed between age groups over repeated measures. Table 4.3 represents least square means comparison on day of BSE by age groups. Smaller SC in younger bulls and no difference in testes pixel

intensity between age groups were observed. In relation to thermographs, younger bull had increased STB and STT and no difference in STG was obtained between age groups. Lower percentage of sperm with normal morphology was detected in younger bulls, accompanied by increased sperm midpiece and tail defects, when compared to older bulls. There was no difference in sperm motility and viability between age categories. Lower MCH was observed in younger bulls compared to older bulls.

Figures 4.3 depicts AVG pixel intensity, SC, testosterone, T_3 and leptin measurements over repeated sampling days for each feed efficiency group. Efficient bulls had lower SC, and suggested lower MAX pixel intensity (131.89 vs. 134.86; $P = 0.065$) throughout the experiment. No difference between efficiency groups was observed for AVG pixel intensity. Similarly, no differences in levels of T3, testosterone and leptin were observed between efficiency groups over repeated measures. Table 4.4 represents means comparison on day of BSE by efficiency groups. Efficient bulls were shown to have smaller SC than inefficient bulls. No difference in testes pixel intensity traits was observed between efficiency groups. Lower STB and suggested higher STT were observed in efficient bulls, but no difference in STG was noted between efficiency groups. A higher percentage of sperm head defects was suggested ($P = 0.085$) in efficient bulls. Additionally, WBC count was greater in efficient bulls.

Evaluation of the relationship between age, RFI and fertility-related parameters and CBC traits revealed significant correlations between age and SC (r = 0.38; P =0.001) while RFI was correlated with MCH (r = 0.21; $P = 0.011$). Body weight and BKFT were both correlated with SC (BW: r = 0.53; P = 0.001, BKFT: r = 0.22; P = 0.006). Triiodothyronine was correlated with SC (r = 0.18; $P = 0.021$), STT (r = 0.24; 0.005)

while leptin was correlated with AVG pixel intensity (r = 0.20; *P* = 0.013). Occurrence of sperm tail defects was correlated with T_3 (r = 0.18; *P* = 0.024) and STT (r = 0.20; *P* = 0.046).

Figure 4.4 displays the partial regression analysis of age and RFI of scrotum circumference, testes ultrasound, scrotum thermography, semen quality, blood hormones and CBC. Overall, 32% of the age variation was explained by the measured traits. When considering only the younger and older bulls, 41% and 28%, respectively, of the variation associated with age was accounted for by the above traits. Similarly, 21% of the RFI variation was explained by those traits. When considering only the efficient and inefficient bulls, 12% and 33%, respectively, of the variation associated with RFI was accounted for by the traits under discussion.

Discussion

Cattle with superior feed efficiency are desirable to reduce production expenses while minimizing environmental impact of the beef industry. As reported elsewhere (Hafla et al. 2012; Kelly et al. 2011), lower feed intake of individual feed efficient bulls, compared to inefficient bulls, represents annual feed savings of over 400 kg DM, while ensuring the same level of productive performance and carcass composition. Feed efficiency has moderate to high heritability (Pitchford, 2004) and genetic improvement for this complex trait relies on extensive phenotyping of indirect indicators. The success of genomic selection depends on a large reference population associated with an accurate collection of phenotypic data (Gonzalez-Recio et al. 2014). However, selection for production efficiency can lead to undesirable effects on metabolism, health and reproduction of cattle and other livestock species (Rauw et al. 1998). Trade-offs between

productive and reproductive traits are commonly observed in livestock species. For example, maintenance of muscle mass is metabolically expensive and can lead to suppression of testosterone levels, reducing energy investment towards reproductive function (Bribiescas, 2001). Additionally, these trade-offs vary with age, with the greatest investment towards reproduction observed in younger growing animals and diminishing thereafter (Ellison, 2003). Thus, a characterization of sexual development and fertility-related measures in young bulls is needed to further understand the underlying biology linking age, feed efficiency and reproductive function in bulls, as well as to provide clues on complementary phenotypes and sampling protocols to facilitate decision-making in cattle production systems.

Over the years, SC has been used as an indicator of age at puberty in bulls (Pruitt et al. 1986) and widely utilized in genetic programs, due to its moderate to high heritability (Bourdon and Brinks, 1986). This trait is also commonly implemented as a measure of testicular development in bulls (Barth and Ominski, 2000). Testicular growth is initially rapid then plateaus (Brito, 2015b), which supports the pattern of SC presented in the present study. The smaller scrotal circumference observed in younger bulls, agrees with previous research (Abdel-Razek and Ali, 2005). Moreover, age (Lunstra et al. 1978), BW (Perumal, 2014) and BKFT (Hafla et al. 2012) have been shown to be positively associated with SC in young bulls; this is supported in the present study where positive correlations of age, BW and BKFT with SC have been observed. Thus, the relationship of SC with age and productive traits reinforces that these traits should be taken into account when assessing SC in bulls, as part of commercial bull test evaluations, as normally carried out in the industry (BIO®, 2015).

Association of improved feed efficiency and SC has been investigated in other studies (Awda et al, 2013; Fontoura et al. 2016; Hafla et al. 2012; Wang et al. 2012). Similar to Awda et al. (2013), the present study noted smaller SC in efficient bulls. These results disagreed with others (Fontoura et al. 2016; Hafla et al. 2012; Wang et al. 2012), where no difference in SC between efficiency groups was observed. Differences between studies may be due to disparities in sample size and RFI modelling. Comparably to this research, Awda et al. (2012) studied an intermediate size population, while Fontoura et al. (2016) worked with a smaller population, reducing the statistical power. Although both Hafla et al. (2012) and Wang et al. (2012) also evaluated intermediate size populations, observed differences may be due to variations in RFI modelling. Both studies compared SC between efficiency groups, using a model for RFI that did not account for body composition traits (Koch et al. 1963). Awda et al. (2012) compared two RFI models in relation to SC and only observed differences in SC when adjusting the RFI model for body composition traits. Lean and fat tissue deposition vary over time in cattle (Owens, 1995), which can have repercussions for reproductive development (Barth et al. 1995). This highlights the importance of adjusting for body composition when evaluating growing cattle, in order to accurately predict RFI ranking. Similar to the energetic trade-offs described in different types of dairy cows with distinct aptitudes for milk production and reproduction (Ollion et al. 2016), nutrient partitioning of bulls of similar body composition with diverging feed efficiency may vary; this suggests that feed efficient bulls may allocate energetic resources towards other physiological functions to the detriment of SC and sexual development.

Cellular content (Curtiss and Amann, 1981) and fluid content (Jegou et al. 1982) of the testis change throughout sexual development, as observed by an increase in testicular germ cell production and differentiation (Curtiss and Amann, 1981), a rise in testicular interstitial fluid (Sharpe and Cooper, 1983) and variation in testicular fluid protein composition (Sharpe and Bartlett, 1987). Thus, these changes in the micro-environment of the testis are closely related to testicular development and lead to changes in the relative density of the testicular tissue (Aravindakshan et al. 2000), resulting in variation in testicular echogenicity (Arteaga et al. 2005; Brito et al. 2012a). Increase in testicular growth, as observed by the increase in SC, explains the pattern of AVG pixel intensity observed over time in this study. Testicular echogenicity follows a constant increase 16 to 12 weeks prior to the onset of puberty and stabilizes around the time of puberty (Brito et al. 2012a). The trend towards higher pixel intensity over time in younger bulls is contrary to that observed elsewhere (Aravindakshan et al. 2000), where age was directly associated with pixel intensity. Although statistical models were adjusted for farm effect, a larger proportion of younger bulls were from the Elora farm compared to the Maritime farm (59% vs. 47%), where bulls received a diet higher in energetic substrates. Energy dense diets have been associated with increased testicular development (Barth et al. 2008). The sharp increase followed by a plateau in AVG pixel intensity in younger bulls, compared to the moderate increase in AVG pixel intensity in older bulls over time, confirms that younger bulls were at an earlier stage of sexual development, compared to older bulls (Figure 4.2). Moreover, the fact that both groups were post-pubertal when the BSE was performed explains the similarity in testicular echogenicity between age groups, noted at the end of the performance test.

The suggested lower MAX pixel intensity observed over time in efficient bulls in this study is similar to results reported by Fontoura et al. (2016). These authors observed lower testis echogenicity in efficient bulls when the BSE was performed, as well as greater seminiferous tubule diameter, accompanied by a lower prevalence of mature tubules in efficient bulls. The lack of difference in pixel intensity between efficiency groups on the day of BSE, in this study, was most likely due to the 34-day age difference between bulls, as described by Brito et al. (2012a). Interestingly, even though there was no age difference between efficiency groups, the pattern of AVG pixel intensity over time appeared to be consistently lower in efficient bulls. Difference in pixel intensity between efficiency groups may be associated with alterations in metabolic function of Sertoli cells, supporting the energetic demands of differentiating germ cells (Rato et al. 2012). Moreover, changes in testicular fluid composition (i.e. protein and cholesterol content) are associated with variation in testicular pixel intensity, such as elevated echogenicity (Dogra, 2003). In a recent study, Montanholi et al. (2016a), observed a positive association with seminal proteins related to sexual maturity and testicular pixel intensity of young bulls. Thus, it may be implied, that differences in energy allocation across bodily functions between efficiency groups may impact testicular growth and composition of testicular fluids, leading to changes in testicular development and function.

Bull testes must be maintained 2 to 6 °C cooler than core body temperature for optimal testicular function, as increased testicular temperature reduces semen quality (Kastelic et al. 1995). In beef bulls, average temperatures at the base and apex of the scrotum are around 30.4 and 28.8 °C, respectively (Cook et al. 1994). Ambient

temperature influences the temperature at the apex of the scrotum and has a smaller effect on the surface temperature at the base of the scrotum (Stelletta et al. 2013). In this study, bulls were evaluated during the Canadian winter months and scrotal surface temperature at the apex of the scrotum was lower than expected. However, Menegassi et al. (2015) also observed lower temperature at the apex of the scrotum during colder months. In general, younger bulls were observed to have lower scrotal radiant heat loss. Testicular development in young bulls is associated with vascular cone development, which is crucial for the increase in efficiency of counter-current mechanisms needed to regulate heat production associated with increased metabolic function (Cook et al. 1994). Thus, mature testicular tissue exhibit lower testicular metabolism, leading to reduced heat production by the testes and lower scrotal radiant heat loss as expected, due to the high correlation between scrotum and deep testicular temperature (Coulter et al. 1988).

Studies have investigated the association of feed efficiency with infrared thermography traits in cattle (Digiacomo et al. 2014; Montanholi et al. 2010), including its association with scrotum thermography traits (Fontoura et al. 2016; Montanholi et al. 2009). In general, the present research indicated lower radiant heat loss in efficient bulls, compared to inefficient bulls. These results are somewhat supported by other studies, where efficient cattle displayed lower radiant heat lost over different body locations such as the udder, shoulder, cheek and snout (DiGiacomo et al. 2014; Montanholi et al. 2010). Others (Fontoura et al. 2016), observed no difference in scrotal surface temperature but instead, noted differences in the temperature variability at the base of the scrotum in efficient bulls. Although the results between this study and others (Fontoura et al. 2016) have some differences, both support the variation in radiant heat loss between feed

efficiency phenotypes. Higher testicular metabolism results in increased testicular heat loss in response to oxygen utilization, leading to greater blood flow towards the testicles (Kastelic, 1995). Moreover, scrotal temperature is regulated independently from the whole body thermoregulatory control system via feedback on a local circuit (Maloney and Mitchell, 1996). Independence of scrotal temperature regulation from other body location temperatures was further demonstrated by Montanholi et al. (2009), where radiant heat loss by the scrotum had a small contribution to the explained variation in RFI, when compared to other body locations. Thus, these results suggest that the reduced heat loss observed in efficient bulls may be a result of lower energy expenditure towards testicular metabolism associated with a lower functional workload in this organ.

Spermatogenesis activity increases with age and reaches maximum adult levels at approximately 12 months of age in bulls (Killian and Amann, 1972). After puberty, semen quality improves over time as observed by increased sperm motility and decreased sperm abnormalities (Lunstra and Echternkamp, 1982). In this study, higher numbers of normal sperm were observed in older bulls, compared to younger bulls, accompanied by lower sperm tail and midpiece defects in older bulls. These results were in accordance with the literature (Arteaga et al. 2001; Lunstra and Echternkamp, 1982). Around 50% of *Bos taurus* beef bulls reached 70% normal sperm at 12 months of age (Arteaga et al. 2001). Although younger bulls had a higher number of sperm defects, these bulls still exceeded the minimum requirement for morphologically normal sperm at approximately 13 months of age.

The suggested increase in sperm head abnormalities in efficient bulls observed herein, agreed with the greater occurrence of sperm abnormalities in efficient bulls

presented by others (Fontoura et al. 2016; Hafla et al. 2012). Large numbers of sperm head abnormalities, such as pyriform heads, can result in lower fertilization rate (Saacke et al. 1998). Higher percentage of sperm head abnormalities were observed in bulls with smaller testicles and immature bulls, partly due to underdeveloped testes (Barth and Oko, 1989). Thus, suggested higher sperm head defects in the efficient bulls further support their delayed testicular development. There was no association between feed efficiency and sperm motility, in agreement with Hafla et al. (2012). However, contrary to these results, others (Awda et al. 2013; Fontoura et al. 2016; Wang et al. 2012) observed reduced sperm motility in efficient bulls, possibly due to dissimilarity in methodology for sperm motility assessment and age of bulls. Wang et al. (2012) used similar methodology to this study and observed lower sperm motility in efficient bulls compared to inefficient bulls, but bulls were 63 days younger than those in this study, which may explain their difference in sperm motility. When similar methodology and age of bulls were used, the authors also did not observe differences in sperm motility between efficiency groups (Hafla et al. 2012). The suggested number of sperm head abnormalities in efficient bulls supports the premise of delayed sexual maturity in efficient bulls.

Testosterone increases from 10 to 12 months of age to eventually reach a plateau in bulls (McCarthy et al. 1979), which was in accordance with the overall pattern observed for testosterone in this study. Levels of T3 decreased over the experimental period (Figure 4.1B), these results are in accordance with Todini (2007), who described variation in thyroid hormones in ruminants and noted lower T_3 in older growing animals due to reduced metabolic rate. A decrease in leptin levels was observed over time, which differed from other investigations (Brito et al. 2007), where an increase in leptin with age

in beef bulls was observed. The decrease in leptin levels observed over time here may have been caused by long exposure to cold, as bulls in this study were assessed during winter months. It has been shown that cold exposure reduces circulating levels of leptin in humans (Peinó et al. 2000). This suggests that in animals living in regions with large seasonal temperature variations, a decrease in serum leptin may represent an adaptive mechanism for maximizing the size of fat deposits, when environmental temperature is low (Margetic et al. 2002).

No difference in blood hormones was observed between age groups. These results disagreed with others who observed increased testosterone (Lunstra et al. 1978) and leptin levels (Brito et al. 2007), with advanced sexual development. The lack of difference between age groups may be due to greater age by the end of the experimental period, with both groups being post-pubertal, where the influence of age on blood hormones may not be as evident. In contrast, the lack of difference in T_3 may be explained by the inconsistency of blood T_3 levels during growth in young bulls (Pavlík et al. 2010). The positive correlation between T_3 and SC observed in this study was in agreement with other studies (Holsberger and Cook, 2005), where T_3 was observed to regulate the maturation and growth of the testis, via its influence on the Sertoli and Leydig cell proliferation and differentiation. It was also noted that T_3 was associated with STT in the present study. Both T_3 and STT were positively correlated with the occurrence of tail defects, while T_3 was also positively related to STT. Elevated thyroid hormones affect thermogenesis through stimulation of heat production (Hulbert, 2000), and may also influence scrotum temperature, through increased testicular metabolism (Wagner et al. 2009). In turn, elevated scrotum temperature leads to more sperm abnormalities,

including tail abnormalities (Kastelic et al. 1995). Moreover, leptin was positively related to AVG pixel intensity, agreeing with the association of leptin testicular function and growth (Ramos and Zamoner, 2014) and the onset of puberty (Gill et al. 1999). In fact, testicular echogenicity was found to be associated with timing of puberty (Brito et al. 2012a) supporting a relation between leptin and testicular growth.

Similarly, no difference in blood hormones was observed between feed efficiency groups. Contrary to this study, other studies reported that the anabolic property of testosterone influences performance traits, such as ADG and feed efficiency in bulls (Cook et al. 2000). Others (Walker et al. 2015), observed lower T_3 levels in efficient heifers, while lower leptin has been noted in efficient steers (Richardson et al. 2004) and heifers (Walker et al. 2015). The lack of association between these hormones and feed efficiency in this study, is likely due to the circadian pattern of these hormones. Varying levels of T_3 (Bitman et al. 1994; Montanholi et al. 2016b), testosterone (Thibier, 1976) and leptin (Kawakita et al. 2001) are observed during the day. Thus, variation of blood hormones between efficiency groups may be only apparent at specific time of the day and should be taken into consideration when sampling blood in large group of cattle over the day.

The CBC analysis is routinely used to assess health status in cattle. Mean values of CBC parameters reported here indicated that the bulls in the present study were healthy, according to recommended ranges in cattle (Jones et al. 2007; University of Guelph Animal Health Lab Guidelines, 2015). Younger bulls showed reduced MCH, compared to older bulls, agreeing with results by Mohri et al. (2007). Mean cell hemoglobin is an RBC parameter, indicative of animals' RBC oxygen-carrying capacity

(Jones et al. 2007) and thus, greater MCH in older growing animals serves to accommodate their relatively elevated oxygen requirements (Rawson et al. 1992). Mitochondria of circulating WBC react to oxidative stress by producing reactive oxygen species (Ijsselmuiden et al. 2008). Moderate production of reactive oxygen species is needed for normal functioning of cells. However, rise in reactive oxygen species, as a result of stress or illness, can cause damage in biological molecules (Kuznetsov et al. 2011). The greater WBC observed in efficient bulls agrees with findings by Richardson et al. (2002) and may suggest differences in cellular stress response between efficiency groups. Additionally, RFI was positively associated with MCH, agreeing with the results by Richardson et al. (2002), suggesting that animals with higher RFI values may have greater oxygen demands.

As shown in Figure 4.4, scrotal circumference contributed 46% of the variation in age, but only made a small contribution to age variation when bulls were sub-grouped as younger and older, revealing that evaluation of bulls of similar age result in reduced variation in SC. The contribution of testes ultrasound to the variation in age was minimal. However, this trait was of greater importance in older bulls, suggesting that younger bulls displayed similar stages of testicular development, while older bulls displayed greater variation in testicular tissue development. Bulls reach sexual maturity at varying ages (Lunstra et al. 1978), which may imply that bulls in the present study from the older population varied more in stage of sexual development. Semen quality was the most important trait to explain age variation in younger and older bulls, with a greater contribution explaining the age variation in younger bulls (89% higher than older bulls). Younger bulls have greater range in amount of sperm abnormalities compared to older

bulls (Coe, 1999), implying greater variation in semen quality traits. The greater variation in CBC in age of older bulls was most likely due to the greater oxygen demand of older bulls, which was further demonstrated by the positive correlation between age and MCH, agreeing with results by Mohri et al. (2007).

Variation in RFI was mainly attributed to scrotum thermography, reinforcing the relationship between radiant heat loss and testicular metabolic rate. Semen quality traits represented a major contribution in the explanation of RFI and also to variation in RFI for the sub-groups, suggesting that it is an important factor related to energy use. Olsson et al. (1997) studied male adders as a model to study energetic cost of spermatogenesis and noted that sperm production was a major energetic cost in males, supporting the importance of semen quality traits in animals with varying feed efficiency. Moreover, despite the fact that scrotal circumference is routinely used to assess the reproductive capacity of bulls (Barth and Ominski, 2000) and that testis echogenicity is associated with testicular development (Aravindakshan et al. 2000), these traits had limited inference to explain variation in RFI compared to scrotum thermography and semen quality. Complete blood count greatly contributed to the explained variation in RFI of inefficient bulls. Many CBC traits, including RBC, hemoglobin and WBC, have been shown to be related to oxygen consumption (Riedesel and Engen, 2015). Inefficient cattle appear to produce more heat (Montanholi et al. 2010) and are more active (Richardson et al. 2001) than efficient cattle. Additionally, increases in metabolic requirements for oxygen, stimulate RBC and hemoglobin production in cattle (Weiss and Wardrop, 2010), agreeing with other studies, which reported a rise in oxygen-carrying capacity of high RFI cattle (Richardson et al. 2002). Thus, the greater contribution of CBC traits to the

variation in RFI of inefficient bulls is likely due to the increased demand for oxygen transport by inefficient bulls, to support increased background energy requirements.

Conclusion

Biological characterization of yearling bulls revealed that younger bulls had lower scrotal circumference, higher scrotal radiant heat loss and lower hemoglobin content. Similarly, feed efficient bulls had smaller scrotal circumference, reduced pixel intensity, lower scrotal radiant heat loss, suggested higher sperm head abnormalities and increased white blood cell counts. In general, bulls with improved feed efficiency display features of delayed sexual development. Notably, variation in feed efficiency and age were similar in terms of the sources of variation considered. Trades-off in energy and nutrients across feed efficiency and age phenotypes, based on the fertility-related measures evaluated, support variability in the functional workload. This research indicates that scrotal circumference and scrotum thermography are primary traits to be considered during the BSE to maximize fertility in feed efficiency bulls. Further studies to investigate the microstructure and molecular aspects of reproductive organs, as well as, to develop strategies to identify bulls that combine superior feed efficiency and desirable sexual development are warranted.

Table 4.1 Least square means (± standard error of the mean) comparisons of biometric traits between age groups and efficiency groups at the end of performance test.

Trait (abbreviation; units)	Younger	Older	*P*
Age (days)	390.06 ± 1.60	426.33 ± 1.47	0.001
Body weight (kg)	440.33 ± 7.57	488.99 ± 6.94	0.001
Dry matter intake (DMI; kg/day)	9.33 ± 0.16	9.91 ± 0.14	0.001
Average daily gain (ADG; kg/day)	1.88 ± 0.06	1.76 ± 0.06	0.060
Residual feed intake (RFI; kg/day)	0.06 ± 0.11	-0.03 ± 0.10	0.414
Backfat thickness (BKFT; mm)	3.02 ± 0.17	3.44 ± 0.15	0.023
Rumpfat thickness (RUMP; mm)	3.58 ± 0.21	3.82 ± 0.15	0.281
Ribeye area (RBEA; cm^2)	68.77 ± 0.97	71.88 ± 0.89	0.004
Trait (abbreviation; units)	Efficient	Inefficient	*P*
Age (days)	411.37 ± 3.04	409.99 ± 2.94	0.678
Body weight (kg)	462.78 ± 7.23	466.19 ± 6.96	0.665
Dry matter intake (DMI; kg/day)	9.00 ± 0.12	10.16 ± 0.12	0.001
Average daily gain (ADG; kg/day)	1.77 ± 0.06	1.85 ± 0.06	0.212
Residual feed intake (RFI; kg/day)	-0.58 ± 0.06	0.53 ± 0.06	0.001
Backfat thickness (BKFT; mm)	3.27 ± 0.16	3.20 ± 0.16	0.675
Rumpfat thickness (RUMP; mm)	3.77 ± 0.20	3.64 ± 0.20	0.551
Ribeye area (RBEA; cm^2)	70.21 ± 0.94	70.41 ± 0.91	0.844

Table 4.2 Descriptive statistics of age, biometric traits, fertility-related measures, blood hormones and complete blood cell count over the experimental period.

Traits (abbreviation; unit)	Mean	Standard Deviation	Minimum	Maximum
Age at end of test (days)	407.90	21.83	358.00	443.00
Body weight (kg)	478.10	75.49	302.90	688.53
Dry matter intake (DMI; kg/day)	9.70	1.09	6.30	13.22
Average daily gain (ADG; kg/day)	1.67	0.35	0.77	2.52
Residual feed intake (RFI; kg/day) DM/day)	0.00	0.71	-2.56	1.49
Backfat thickness (BKFT; mm)	3.00	1.39	0.61	7.78
Rumpfat thickness (RUMP; mm)	3.80	1.46	1.09	9,71
Ribeye area (RBEA; cm^2)	69.40	6.79	56.09	85.68
Scrotal circumference (SC; cm)	32.50	3.81	21.00	42.60
Average pixel intensity (AVG; pixels)	132.31	18.77	70.48	185.11
Maximum pixel intensity (MAX; pixels)	186.16	19.91	115.00	238.50
Scrotum base average (STB; °C)	32.07	2.46	25.10	36.80
Scrotum apex average (STA; °C)	23.65	3.77	14.30	31.20
Scrotum total average (STT; °C)	28.45	2.70	21.00	33.20
Scrotum temperature gradient (STG; °C)	8.42	2.86	1.60	14.10
Motility (%)	70.36	18.97	10.00	90.00
Normal morphology (%)	68.60	18.30	4.00	93.00
Head defects (%)	15.39	10.26	2.00	71.00
Midpiece defects (%)	11.74	8.07	1.00	40.00
Tail defects (%)	3.60	3.59	0.00	20.00
Detached heads (%)	5.69	6.07	0.00	46.00
Alive sperm cells (%)	69.65	15.86	14.00	95.00
Dead sperm cells (%)	30.37	15.81	5.00	86.00
Testosterone (ng/mL)	2.05	1.43	0.01	8.16
Triiodothyronine (T_3; nmol/L)	3.38	0.72	0.86	6.11
Leptin (ng/mL)	5.14	2.78	0.42	25.63
Red blood cells (RBC; 10^{12} cells/L)	8.16	1.00	6.43	11.90
Hemoglobin (g/L)	126.32	15.15	98.00	173.00
Hematocrit (L/L)	0.34	0.04	0.26	0.49
Mean cell volume (MCV; fL)	42.17	3.31	34.00	51.00
Mean cell hemoglobin (MCH; pg)	15.53	1.01	13.00	19.00
Platelets (10^9 cells/L)	333.69	102.35	89.00	654.00
White blood cells (WBC; 10^9 cells/L)	9.68	1.99	4.20	18.10

Table 4.3 Least square means (± standard error of the mean) comparisons of reproductive-related measures, blood hormones and complete blood cell count between younger and older bulls at the end of performance test.

Traits (abbreviation; units)	Younger	Older	*P*
Scrotal circumference (SC; cm)	34.34 ± 0.37	35.36 ± 0.32	0.011
Testes ultrasonography			
Average pixel intensity (AVG; pixels)	139.03 ± 2.76	139.65 ± 2.42	0.831
Maximum pixel intensity (MAX; pixels)	189.99 ± 3.00	191.78 ± 2.64	0.570
Scrotum thermography			
Scrotum base average (STB; °C)	32.72 ± 0.34	31.94 ± 0.29	0.039
Scrotum apex average (STA; °C)	24.28 ± 0.40	23.90 ± 0.34	0.392
Scrotum total average (STT; °C)	29.28 ± 0.31	28.55 ± 0.27	0.039
Scrotum temperature gradient (STG; °C)	8.44 ± 0.38	8.04 ± 0.33	0.343
Semen quality			
Motility (%)	76.08 ± 17.89	79.32 ± 17.08	0.149
Normal morphology (%)	71.85 ± 16.80	76.73 ± 16.01	0.019
Head defects (%)	11.72 ± 1.09	10.81 ± 1.08	0.376
Midpiece defects (%)	9.77 ± 0.02	7.60 ± 0.02	0.033
Tail defects (%)	3.08 ± 1.09	2.24 ± 1.08	0.043
Detached heads (%)	4.42 ± 1.10	3.76 ± 1.09	0.271
Alive sperm cells (%)	74.34 ± 17.21	74.11 ± 16.40	0.916
Dead sperm cells (%)	25.31 ± 0.04	25.64 ± 0.04	0.886
Blood hormones			
Testosterone (ng/mL)	2.38 ± 0.23	2.44 ± 0.21	0.820
Triiodothyronine (T_3; nmol/L)	3.74 ± 0.10	3.64 ± 0.09	0.325
Leptin (ng/mL)	5.04 ± 1.08	5.43 ± 1.07	0.374
Complete blood count			
Red blood cells (RBC; 10^{12} cells/L)	8.43 ± 1.01	8.26 ± 1.01	0.159
Hemoglobin (g/L)	129.81 ± 1.77	130.97 ± 1.72	0.517
Hematocrit (%)	35.57 ± 0.50	35.75 ± 48	0.721
Mean cell volume (MCV; fL)	42.03 ± 0.53	42.89 ± 0.52	0.108
Mean cell hemoglobin (MCH; pg)	15.40 ± 0.16	15.80 ± 0.16	0.017
Platelets (10^9 cells/L)	322.91 ± 17.12	336.37 ± 16.61	0.437
White blood cells (WBC; 10^9 cells/L)	8.76 ± 1.03	9.13 ± 1.03	0.213

Table 4.4 Least square means (± standard error of the mean) comparisons of fertility-related measures, blood hormones and complete blood cell count between efficient and inefficient bulls at the end of performance test.

Traits (Abbreviation; Units)	Efficient	Inefficient	*P*
Scrotal circumference (SC; cm)	34.43 ± 0.34	35.29 ± 0.34	0.024
Testes ultrasonography			
Average pixel intensity (AVG; pixels)	137.74 ± 2.52	139.65 ± 2.65	0.507
Maximum pixel intensity (MAX; pixels)	190.21 ± 2.74	191.67 ± 2.88	0.640
Scrotum thermography			
Scrotum base average (STB; °C)	31.84 ± 0.31	32.68 ± 0.30	0.022
Scrotum apex average (STA; °C)	23.84 ± 0.37	24.30 ± 0.36	0.286
Scrotum total average (STT; °C)	28.54 ± 0.29	29.17 ± 0.28	0.060
Scrotum temperature gradient (STG; °C)	8.00 ± 0.36	8.38 ± 0.36	0.356
Semen quality			
Motility (%)	77.64 ± 17.56	77.77 ± 17.31	0.950
Normal morphology (%)	74.03 ± 16.59	74.91 ± 16.33	0.666
Head defects (%)	12.15 ± 1.08	10.43 ± 1.08	0.085
Midpiece defects (%)	8.96 ± 0.02	8.20 ± 0.02	0.444
Tail defects (%)	2.56 ± 1.08	2.65 ± 1.08	0.832
Detached heads (%)	3.98 ± 1.09	4.13 ± 1.09	0.796
Alive sperm cells (%)	74.07 ± 16.91	74.31 ± 16.64	0.913
Dead sperm cells (%)	25.65 ± 0.04	25.41 ± 0.04	0.916
Blood hormones			
Testosterone (ng/mL)	2.37 ± 0.23	2.45 ± 0.02	0.727
Triiodothyronine (T_3; nmol/L)	3.65 ± 0.10	3.73 ± 0.09	0.481
Leptin (ng/mL)	4.92 ± 1.08	5.50 ± 1.07	0.170
Complete blood count			
Red blood cells (RBC; 10^{12} cells/L)	8.32 ± 1.01	8.38 ± 1.01	0.618
Hemoglobin (g/L)	129.72 ± 1.69	131.21 ± 1.78	0.618
Hematocrit (%)	35.50 ± 0.48	35.85 ± 0.50	0.482
Mean cell volume (MCV; fL)	42.36 ± 0.51	42.60 ± 0.53	0.640
Mean cell hemoglobin (MCH; pg)	15.55 ± 0.16	15.67 ± 0.16	0.478
Platelets (10^9 cells/L)	332.72 ± 16.44	326.30 ± 17.28	0.707
White blood cells (WBC; 10^9 cells/L)	9.21 ± 1.03	8.63 ± 1.03	0.045

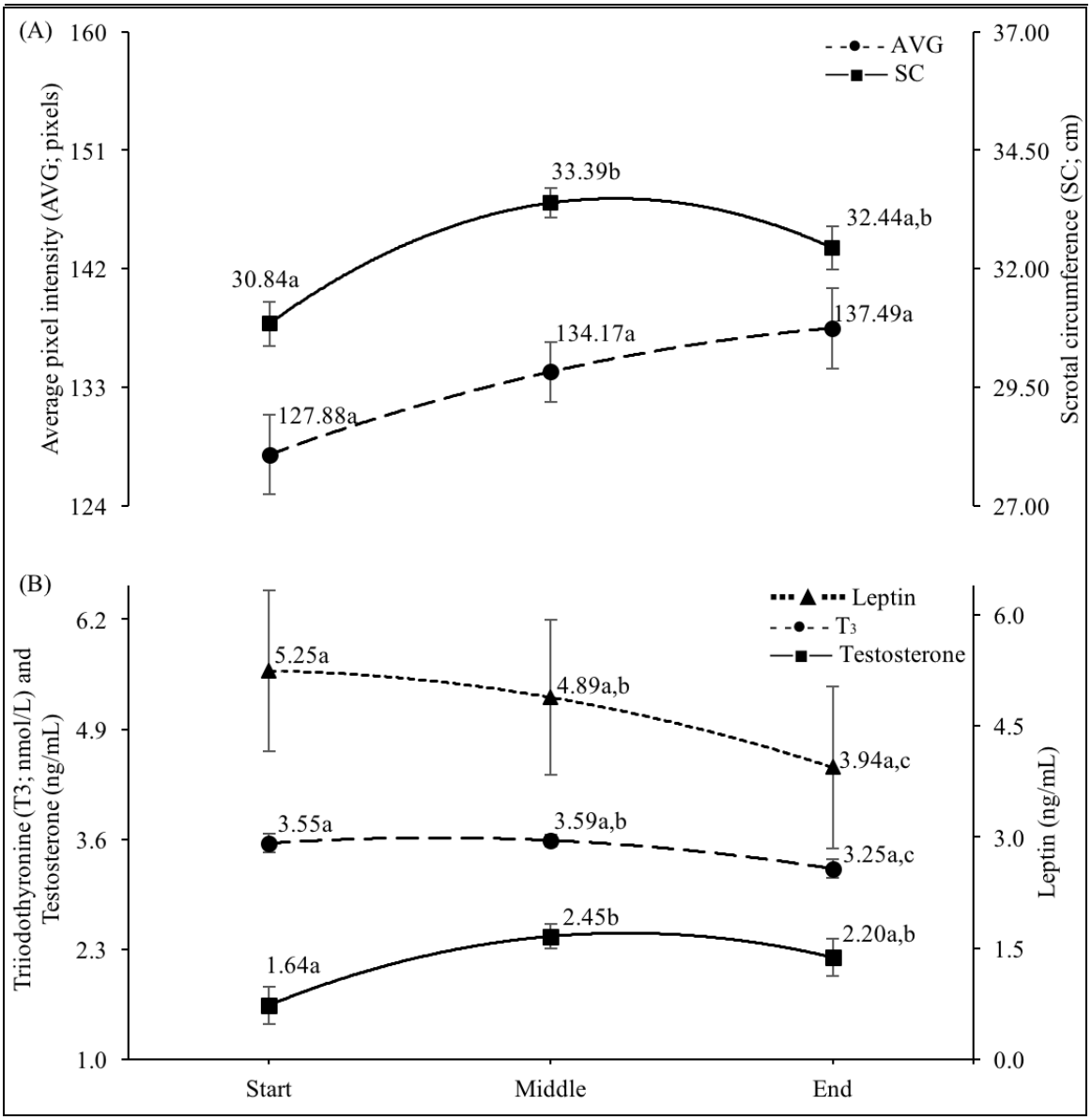

Figure 4.1 Least squares means and standard error for scrotal circumference, scrotum pixel intensity and hormones profile over the start, middle and end of the performance test. (A) Regression of scrotal circumference and pixel intensity (B) Regression of testosterone, triiodothyronine and leptin. Differing letters over time and for the same determination denote $P \leq 0.05$.

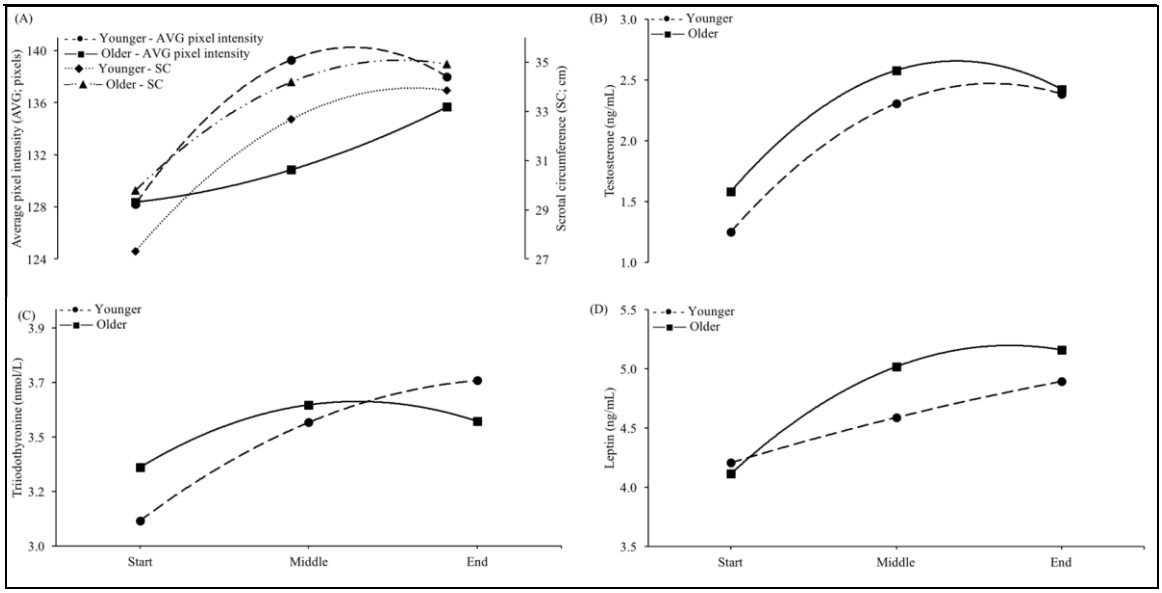

Figure 4.2 Least squares means and standard error for regression of scrotal circumference (SC), testis average (AVG) pixel intensity and hormones profile by age groups over the start, middle and end of the performance test. (A) Smaller SC (31.26 ± 0.34 vs. 33.00 ± 0.32 cm; *P*=0.001) and suggested higher AVG pixel intensity (135.27 ± 1.73 vs. 131.75 ± 1.57 pixels; *P*=0.059) in younger bulls. No differences in (B) testosterone (1.98 ± 0.15 vs. 2.19 ± 0.13 ng/mL; *P*=0.191), (C) triiodothyronine (3.44 ± 0.07 vs. 3.48 ± 0.07 nmol/L; *P*=0.636) and, (D) leptin (4.58 ± 1.06 vs. 4.71 ± 1.06 ng/mL; *P*=0.682) between younger and older bulls.

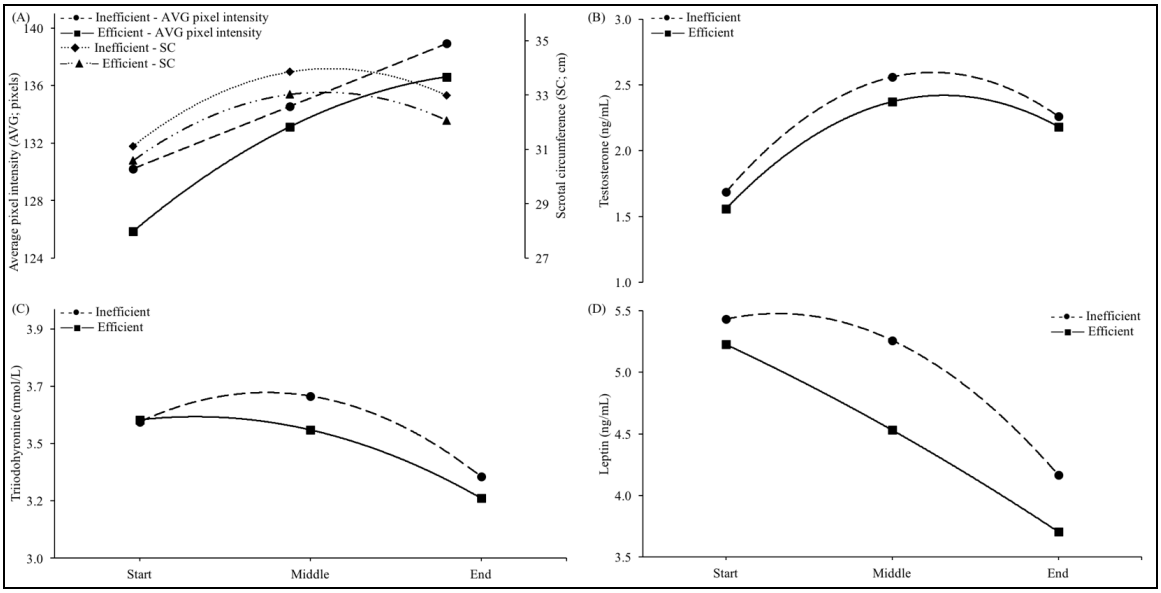

Figure 4.3 Least squares means and standard error for regression of scrotal circumference (SC), testis pixel intensity and hormones profile by feed efficiency groups over the start, middle and end of the performance test. (A) Smaller SC (31.91 ± 0.32 vs. 32.64 ± 0.31 cm; *P*=0.035) in efficient and no difference in AVG pixel intensity (131.84 ± 1.66 vs. 134.57 ± 1.64; *P*=0.137). No difference in (B) testosterone (2.04 ± 0.14 vs. 2.16 ± 0.14 ng/mL; *P*=0.425), (C) triiodothyronine (3.44 ± 0.07 vs. 3.50 ± 0.07 nmol/L; *P*=0.437) and, (D) leptin (4.47 ± 1.06 vs. 4.89 ± 1.06 ng/mL; *P*=0.173) between efficient and inefficient bulls.

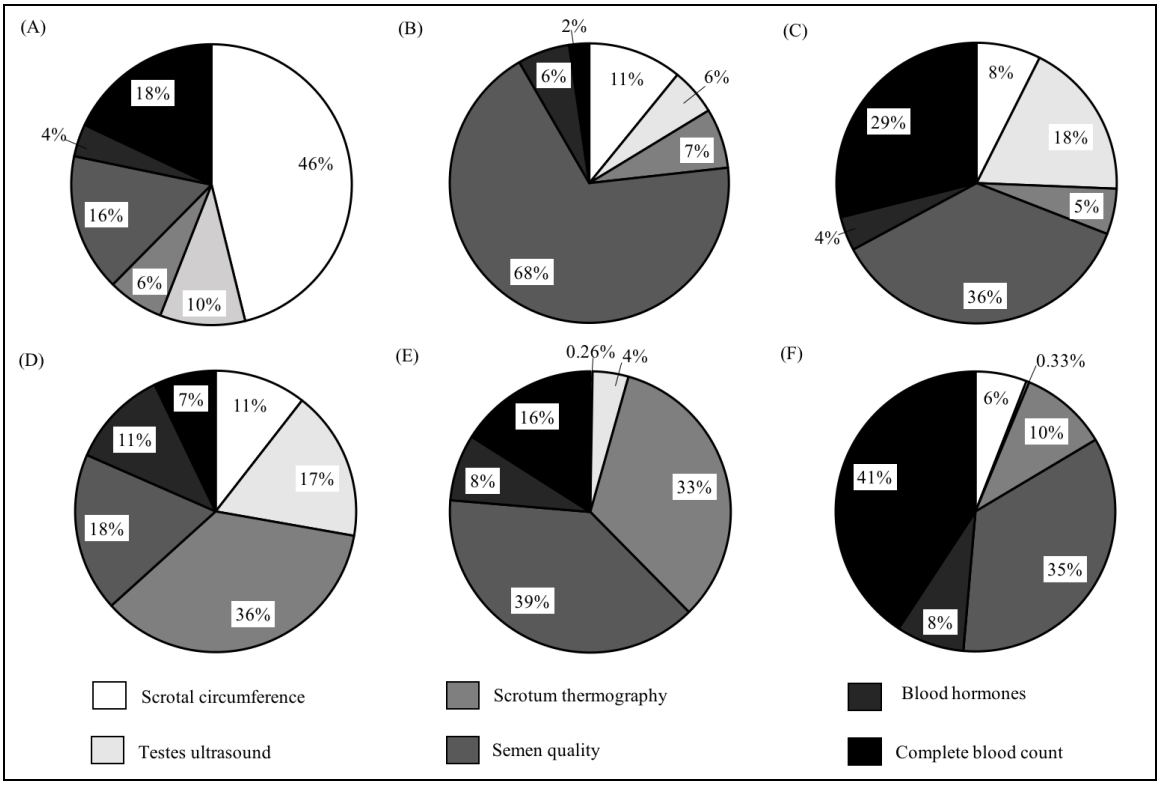

Figure 4.4 Relative contribution of scrotum thermography, testes ultrasonography, semen quality, blood hormones, scrotal circumference and complete blood cell count to the observed variation in age and feed efficiency. (A) Age, (B) Age variation of younger bulls, (C) Age variation of older bulls, (D) residual feed intake (RFI, feed efficiency), (E) RFI of efficient bulls and (F) RFI of inefficient bulls.

CHAPTER 5

GENERAL DISCUSSION

This research was conducted to advance application and knowledge regarding the relationship between feed efficiency and sexual development of young bulls, as well as to evaluate proxies for feed efficiency. Over the years, improvement of production traits has been important to improve profitability of livestock industries. However, the impacts of selection for production on the biology of the animals have not always been well understood, leading to unwanted collateral responses to selection. For example, 65 years ago genetic selection using inbreeding was a recommended practice in cattle with no concern about resulting phenotypes (Branston, 1951). Information about the undesirable side effects of selection for improved production has become available (Rauw et al. 1998). These authors reviewed the antagonism between productivity and fertility in livestock species. This antagonism is a current concern in the beef industry in relation to fertility related traits and feed efficiency in young bulls (Awda et al. 2013; Fontoura et al. 2016; Montanholi et al. 2016a). Thus, the characterization of underlying biology linking feed efficiency and reproductive function may serve to improve husbandry decisions to enhance feed efficiency without hampering reproduction.

In order to identify possible proxies for feed efficiency, blood parameters between age and feed efficiency groups were evaluated in Chapter 3. Characteristics of sexual immaturity were observed in younger bulls, such as smaller ribeye area (RBEA), decreased backfat thickness (BKFT), smaller SC, and lower testosterone concentration.

As bulls go through sexual development, testosterone production by the Leydig cells increases, leading to the development of secondary sex characteristics such as changes in body composition (Pietersen et al. 1992), as was observed in the current study. Greater concentrations of CO_2 and BDNF and lower T_3 concentrations were observed in efficient bulls, which are related to energy metabolism (Brody 1945; Hulbert, 2000; Rios et al. 2001). Moreover, T_3 has been associated with male testicular growth by acting directly on the Sertoli and Leydig cells, with lower T_3 concentration related to delayed sexual development (Holsberger and Cook, 2005). However, while no difference in SC was observed, variation in T_3 concentrations may suggest differences in deep testicular tissue maturation.

Following the assessment of blood parameters in the context of age and feed efficiency in Chapter 3, evaluation of fertility-related measures between age and feed efficiency groups was conducted in Chapter 4. Features of sexual immaturity were observed in younger bulls, such as smaller RBEA, lower BKFT, smaller SC and greater radiant heat loss by the scrotum. However, no difference in testosterone concentration between age groups was observed. Nonetheless, over the experimental period, testosterone levels in young bulls were consistently lower, supporting the characteristics of sexual immaturity. In general, features of delayed sexual maturity were observed in efficient bulls, as has been reported elsewhere (Awda et al. 2013; Hafla et al. 2012; Fontoura et al. 2016; Montanholi et al. 2016). In addition, lower radiant heat loss by the scrotum was also noted in efficient bulls, suggesting lower testicular metabolism. Similarly to Chapter 3, T_3 appeared lower in efficient bulls, however the difference was not significant. Moreover, the positive correlation observed between T_3 and SC, as well

as between leptin and average pixel intensity agrees with the function of these hormones during testicular development (Ramos and Zamoner, 2014). As each of these hormones is also associated with metabolism and sexual development in males (Ramos and Zamoner, 2014), this further supports the differences in metabolic rate and reproductive measures in bulls diverging in feed efficiency.

In Chapters 3 and 4, younger bulls had lower RBEA, BKFT and SC. Changes in body composition with sexual development in bulls have been demonstrated elsewhere (Perry and Arthur, 2000) and was further supported in Chapter 4 with the positive correlation between BKFT and SC. The lower testosterone concentration observed for younger bulls in Chapter 3 was not observed in Chapter 4. The difference in age between bulls of both studies was 49 days while the age-range between bulls was greater in Chapter 3 (106 vs 85 days). Greater variation in phenotypes related to sexual development is expected in a population with more animals in active sexual development and with a greater age-range (Brito, 2015a; Adolfsson et al. 2012). Additionally, blood parameters such as metabolic and sex hormones display circadian patterns (Montanholi et al. 2016b; Kawakita et al. 2001; Thibier, 1976) and time of collection can therefore impact results. This may explain the lack of difference in blood hormones between age groups in Chapter 4, due to a longer collection time. Nevertheless, similar numerical differences in blood hormones between age groups were observed between Chapter 3 and Chapter 4 suggesting resemblances in hormonal profiles of young bulls between studies.

While levels of T_3 in efficient bulls were not significantly lower in Chapter 4, numerical differences in T_3 over the experimental period and at end of test were similar to those observed in Chapter 3. Once again, difference in age and collection routine is

likely an important factor resulting in lower variation in T_3 concentration between studies. Bulls in Chapter 3 were kept on a higher plane of energy than bulls in Chapter 4, which may have been a contributing factor to the difference between studies. In cattle kept on a high plane of nutrition, observations of greater thyroid acinar cell heights and greater thyroid gland activity (Sorensen et al. 1959) have been reported. This suggests that variation in T_3 between bulls with diverging feed efficiency may be increased when fed diets with higher energy content. Additionally, changes in environmental temperature has important effects on thyroid function and secretion in cattle (Ekesbo, 2011; Saber et al. 2009). Thus, varying environmental temperature over the 112-day experimental period may have influenced T_3 levels in Chapter 4, as Maritimes bulls were housed in outdoor pens and were directly subjected to variation in environmental temperature the experimental period. The smaller SC in efficient bulls in Chapter 4 was not observed in Chapter 3. Differences between studies is likely due to the smaller population size studied in Chapter 3. When using a similar RFI model and diet composition as in Chapter 4, other studies also observed lower SC in efficient bulls when studying a population size of 110 bulls (Awda et al. 2013). In addition, higher CO_2 observed in Chapter 3 for the efficient bulls agrees with the higher white blood cell count of efficient bulls in Chapter 4. Both variables are associated with stress response (Grossman, 1983; Richardson et al. 2002) and therefore support the premise of a higher fight and flight response to a stressor in inefficient cattle.

This Thesis may have implications regarding the methodology used in the collection of feed efficiency and reproduction phenotypes and in the reproductive management of young bulls with improved feed efficiency. Further studies that involve

bulls at different physiological stages are necessary to further investigate this relationship. Circadian response of these parameters over the circadian pattern can be useful to identify the optimum time for data collection in large populations. Additionally, further studies to investigate the microstructure and molecular aspects of bull reproductive organs in the context of feed efficiency are warranted.

In essence, this Thesis contributes to the knowledge of the intricate association between reproductive function and feed efficiency in young bulls as evaluated by the measurements of T_3, SC and testes ultrasonography over time. The novel information generated may be useful to the development of protocols to identify bulls with superior feed efficiency and desirable sexual development. Such protocols are important to prevent an antagonistic relationship between production and reproductive traits previously reported in cattle and other livestock species (i.e. Hutchens et al. 1981; Nestor, 1977; Van Arendonk et al. 1989).

It is also important to highlight that the majority of feed efficient bulls were classified as satisfactory breeders following the BSE, suggesting good future breeding potential. This Thesis is a proactive call identifying the need to consider fertility-related measures when optimizing feed efficiency, as it may affect the reproduction capacity of the livestock specie with the largest generation interval (Wilton et al. 2013), which may be difficult to remediate, as was witnessed in other species where such caution was not considered.

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