The relationship between the circadian cycle of blood plasma analytes with feed efficiency in beef heifers

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ABSTRACT

The relationship between the circadian cycle of blood plasma analytes with feed efficiency in beef heifers

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Factors underlying biological differences in feed efficiency were investigated in order to determine if these factors could be utilized as indirect measures of feed efficiency. Residual feed intake (RFI) was determined in 36 beef heifers. Hourly blood samples were taken over 24 hours at 367 ± 15 days, 542 ± 23 days, and 704 ± 25 days of age corresponding to open, early pregnancy, and late pregnancy, respectively. Then hematological measures of basic metabolic processes that contribute significantly to energy expenditures were determined including albumin, urea, creatine kinase, glutamate dehydrogenase, aspartate aminotransferase and carbon dioxide. Creatine kinase, aspartate aminotransferase and carbon dioxide showed relationships with RFI in open heifers, urea and creatine kinase in late gestation and carbon dioxide overall. These associations tend to be reflective of differences in liver function, tissue turnover and whole body metabolism that may act as effective indirect measures of RFI.

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LIST OF ABBREVIATIONS

A Average
AA Amino acid
ADG Average daily gain
ADP Adenosine diphosphate
AST Aspartate aminotransferase
ATP Adenosine triphosphate
BIC Bayesian information criterion
BFT Back fat
BW Body weight
ºC Degrees Celsius
CK Creatine kinase
cm Centimeter
cwt Carcass weight
d Day
DNA Deoxyribonucleic acid
Δ Delta
DM Dry matter
DMI Dry matter intake
FI Feed intake
g Gram
G Gain
GLDH Glutamate dehydrogenase
h Hour
h^2 Heritability
IFT Intramuscular fat
kDa Kildalton
kg Kilogram
lbs Pounds
m Metre
mg Miligram
mm Millimetre
mRNA Messenger RNA
PT Pre-feed trial
QTL Quantitative trait loci
R^2 Coefficient of determination
REA Rib eye area
RFI Residual feed intake
Rpm Revolutions per minute
ROS Reactive oxygen species
SNP Single-nucleotide polymorphism
T Trial
T3 Triiodothyronine
T4 Tetraiodothyronine

Chapter 1

1. GENERAL INTRODUCTION

The Canadian beef industry provides a safe and nutritious food supply while also adding significantly to the national and provincial economies. Canada's beef industry is the largest single commodity source of farm cash receipts comprising about 20% of the total. In addition, Canadian beef production also contributes to the processing, retail, food service and transportation sectors. With these other sectors considered, beef production adds about $26 billion to the Canadian economy annually. The industry consists of 210, 400 bulls, 3, 956, 200 cows, 569, 800 breeding heifers, 996, 800 slaughter heifers and 1, 292, 500 steers, which comprises 1.1% of the world cattle inventory and provides 7.0% of the world exports (Statistics Canada, 2013). Therefore the Canadian beef industry is an integral part of the Canadian economy and great importance should be placed on its success allowing for its expansion. The trend for rising costs associated with raising cattle have been decreasing profit margins in the cattle industry, preventing expansion (CCA, 2011). Small improvements in feed efficiency can reduce the cost of gain considerably since the price of feed comprises 70 to 80% of the total gain cost (Field and Taylor, 2002). Feed efficiency is an economically important trait for the cattle feeder. It has been determined that a 5% improvement in feed efficiency is equivalent to any one of the following, reducing ration costs on a dry matter basis by $8 per ton, reducing purchase costs of the feeder animal by $1.75 cwt or increasing daily gain by 0.6 lbs per day (Field and Taylor, 2002). Investigations into factors related to feed efficiency is important for profitable beef production in Ontario and Canada.

Efficiency is defined as performance in the best possible manner with the least waste of time and effort. An efficient animal will attain the greatest amount of output, measured as meat or

milk for example, from the least amount of inputs, generally measured as feed consumption. Lessening inputs would maximize the profitability of beef cattle production through the minimization of input costs since feed provision is one of the greatest costs of production (Herd et al., 2003). Improvement in animal feed efficiency has the potential to not only increase profits for cattle producers, but also to decrease the environmental footprint of beef cattle production. Both are important in addressing the challenges of increasing feed costs and land pressure (Moore et al., 2009). There exists individual variation in animal feed efficiency which can be measured and utilized in replacement heifer selection (Herd et al., 2003).

The limitation to the application of this measurement is due to the time consuming and laborious need for the quantification of individual feed intake and body weight measurements over a relatively lengthy time period. The costs and equipment required to assess efficiency prohibit its wide scale application. In order to implement a measurement of animal feed efficiencies, there is the need to determine indirect assessments for feed efficiency that are more easily measured while still maintaining high accuracy. There have been studies into indirect assessments which give an indication of how there exists variation in efficiency between animals. Some of these factors are based directly off observations that affect animal energy expenditures while some are more complex underlying factors.

Animal behavioural responses can alter physical activity and thus influence total energy expenditure and feed efficiency (Susenbeth et al., 1998). A relationship has been shown between feeding behaviour and residual feed intake (Sowell et al., 1998; Pritchard and Bruns, 2003; Schwartzkopf-Genswein et al., 2003). Studies show that more feed efficient animals typically engage in less daily feeding events in addition to have shorter eating bouts (Robinson and Oddy, 2004; Kelly et al., 2010b). This may have evolved as an energy-sparing mechanism since low RFI animals may spend more time being sedentary, thereby utilizing less energy for activity. Another potential alternative to the direct assessment of feed efficiency is infrared thermography, which

measures the surface temperature of the animals' body. Montanholi et al. (2009 and 2010) have suggested that more efficient cattle have a lower body surface temperature than less efficient animals, potentially explained by their lower maintenance requirements resulting in less heat being dissipated through the body surface (Richardson et al., 2001; Nkrumah et al., 2006; Castro Bulle et al., 2007). Montanholi et al. (2008, 2009, and 2010) have found that more efficient steers had cooler body extremities which were better predictors of heat production than core body locations, since they play a large role in body heat dissipation (Whittow, 1962; van den Heuvel et al., 2004).

Mitochondria are the site of energy production in the cell and produce the majority of cellular ATP. They are also part of numerous functional, metabolic, and signalling networks (Poyton and McEwen, 1996; Hood and Saleem, 2007). Due to its importance in energy metabolism, variation in the function of this organelle is a candidate for the underlying control of maintenance energy requirements (McDonald et al., 2009). Data has shown that energetically efficient animals have higher mitochondrial respiration rate (Kolath et al., 2006), ADP control of oxidative phosphorylation (Lancaster et al., 2007), and mitochondrial complex protein concentration (Bottje and Carstens, 2009) while having lower mitochondrially derived reactive oxygen species production (Davis, 2009), uncoupling protein 3 mRNA (triggers mitochondrial proton leak in muscle; Ojano-Dirain et al., 2007), muscle protein carbonyl concentrations (indicative of oxidative stress; Sandelin et al., 2004).

Many quantitative trait loci (QTL) have been identified throughout the cattle genome (Nkrumah et al., 2007a; Sherman et al., 2009), and single nucleotide polymorphism (SNP) associated with RFI have been identified (Sherman et al., 2008). In addition, a whole genome association study by Barendse et al. (2007) identified many SNP throughout the bovine genome associated with RFI.

Glucocorticoids are increased under stressful situations (Mostl and Palme, 2002) and play a key role in energy metabolism, influencing the animals' performance (Sapolsky, 2002). Cortisol and its metabolites can be assessed in several manners, plasma cortisol represents the immediate response of the adrenal gland while fecal cortisol metabolites represents the cortisol that was released into the blood stream about 12 h before sampling (Palme et al., 1999 and 2005). How animals react to stressful situations may be impacting their efficiency. Koolhaas et al. (1999) found that shy animals have greater cortisol baseline and when exposed to stress situations have a predominantly parasympathetic response that is associated with energy conservation. Conversely, bolder animals have a lower cortisol baseline and when under stress exhibit a sympathetic response causing a fight and flight response that increases the energy expenditures. Montanholi et al. (2010) found that more efficient steers had greater baseline cortisol levels indicating a shy coping style.

Another factor that may provide an indirect measure of animal energy requirements is through hematological measurements. Concentrations of key metabolic hormones associated with feed intake, growth, fat accumulation, nutrient repartitioning, and nutrient utilization have been examined to identifying potential physiological markers of feed efficiency (Wood et al., 2004; Nkrumah et al., 2007b). Insulin-like growth factor-I, a hormone that has a number of effects on growth and metabolism, was determined to be genetically (Moore et al., 2005) and phenotypically (Brown et al., 2004) positively correlated with RFI in growing bulls and heifers and has also been shown to be heritable (Herd et al., 1995).

Leptin is another hormone that functions as a regulator of body weight, feed intake, and energy expenditure (Houseknecht et al., 1998). Richardson et al. (2004) observed a significant phenotypic correlation between serum leptin and residual feed intake. Studies have reported greater systemic insulin concentrations in high RFI steers at the end of a feedlot test (Richardson et al., 2004; Brown, 2005). This has been attributed to a decrease in leanness resulting from

increased fat deposition since insulin can reduce lipolysis and stimulate lipogenesis in adipose tissue (McCann and Reimers, 1986; Brown et al., 2004).

Plasma metabolites are useful biochemical indicators of energy metabolism and nutritional status of beef cows (Russel and Wright, 1983; Agenas et al., 2006). Previous reports in cattle by Richardson et al. (1996 and 2004) have found greater blood concentrations of urea in less efficient genotypes. This may be credited to a greater protein intake in high RFI animals, a greater rate of body protein degradation, or deviation in the supply of amino acids due in part to variation in the efficiency of microbial protein production in the rumen (Lush et al., 1991; Kahn, 2000). Creatinine is a breakdown product of creatine phosphate, an energy storage compound in the muscle and a proposed marker for muscle mass in cattle (Hansett and Michaux, 1985; Istasse et al., 1990). Studies by Richardson et al. (2004) and Lawrence et al. (2011) reported greater plasma creatinine concentrations in low RFI animals than in high RFI animals. Products of body fat mobilization such as nonesterified fatty acid and beta hydroxybutyrate increase in proportion to the degree of fat mobilization. Nonesterified fatty acid concentration has been found to be greater in the high efficiency animals (Richardson et al., 2004; Kelly et al., 2010a). Kelly et al. (2010a) found a positive relationship for beta hydroxybutyrate with residual feed intake.

In the current study, feed intake was recorded from puberty through to calving for the determination of feed efficiency measured by residual feed intake (RFI) defined by Koch et al. (1963). Residual feed intake is the difference between the actual feed intake and the expected intake for the maintenance of body weight and production calculated from measures of body weight. Animals classified as more efficient would be represented by a low RFI since they eat less than expected for the same growth while high RFI would be considered inefficient since they have intakes that are greater than expected. This feed efficiency trait represents variations in the requirements for basic metabolic processes rather than variations due to differences in level of production (Richardson et al., 2001; Nkrumah et al., 2006; Montanholi et al., 2010). These

requirements for background metabolic processes represent 60 to 70% of total energy expenditures (Ferrell, 1988) with a large proportion of individual variation due to differences in visceral organ metabolism (Kolath et al., 2006; Nkrumah et al., 2006).

Hematological measurements of visceral organ function were measured to assess their relationship to feed efficiency. They included metabolites such as urea and acetate, enzymes such as creatine kinase (CK), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH) and proteins such as albumin. The blood samples were taken over a 24 hour time period to determine their variability throughout the day. Any relationship between circadian analyte profiles and differences in residual feed intake could help to clarify the factors underlying individual observed differences in feed efficiency. If these profiles were found to significantly vary, they could be utilized as an indirect measure of efficiency, which could enable the classification of animals according to efficiency without the collection of feed intake and body weight measurements. Additionally measurements were taken at three ages corresponding to different physiological states to investigate how these influence animal energy requirements and overall animal metabolism potentially affecting hematological measurements. Results from this study could give some insight into factors underlying individual differences in animal feed efficiency. Utilizing this information to access and select for animals of improved efficiency could help to counteract the rising input costs that producers face from surging feed and land costs ensuring more profitable and sustainable beef production.

Chapter 2

2. LITERATURE REVIEW

2.1 Feed efficiency in livestock production

Historically, the majority of national genetic improvement programs for livestock have emphasized selection to improve outputs such as body weight and gains (Herd et al., 2003). More recently, selection has moved towards the focus on inputs in order to improve efficiency of production to increase profit (Herd et al., 2003). Providing feed to animals is responsible for a significant proportion of expenses with any animal production system. Therefore improving the efficiency with which animals use available feed is an important strategy to reduce feed costs resulting in an improvement in profitability. There is known variation in the efficiency in which nutrients supplied from feed are used for maintenance and growth (Herd et al., 2004). It is well known that differences in the amount and type of feed eaten, the sex and breed of the animal and the environmental conditions in which the animal is managed will influence feed efficiency (Herd et al., 2004). Still there is variation in efficiency of nutrient utilization between animals of the same breed, sex and age, raised under the same conditions and eating the same feed indicating that there is individual variation in the efficiency of utilization of feed (Herd et al., 2004).

2.1.1 Assessment of feed efficiency

Feed intake and its utilisation by the animal involve multiple biological processes and pathways, in addition to interactions with the environment and are highly correlated with live weight and level of production (Pitchford, 2004). Therefore to improve production system efficiency feed intake by itself cannot be used so other measures have been determined (Pitchford, 2004).

The most widely used index of efficiency in the literature is that of gross efficiency or its inverse, feed conversion ratio (Archer et al., 1999). Gross efficiency is defined as the ratio between production outputs and feed inputs. For meat production systems, outputs are often measured as weight gain of growing animals and feed conversion ratio becomes the ratio between feed intake and weight gain measured over a defined period of growth (Archer et al., 1999). Feed conversion ratio is defined as feed intake per unit gain in weight and has been the most common measure of efficiency used in the swine and poultry industries (Pitchford, 2004). However, despite widespread use, this measure is undesirable for genetic improvement purposes because they are often correlated with growth rate or other production traits, such as mature cow body weight which would result in higher feed requirements for the cow herd (Koots et al., 1994). The increase in feed requirements of the breeding herd would offset the gains in growth efficiency resulting in no change in production system feed efficiency. Also placing selective pressure on the components of a ratio trait is not predictable given that more intensity is usually placed on the component with greater variation (Gunsett, 1984).

Since maintenance requirements constuitue such a large proportion of total energy requirements measuring efficiency as maintenance efficiency may provide insight into differenes in efficiency. Maintenance requirements can be defined as the feed energy required for zero body weight change (Ferrell and Jenkins, 1985). Maintenance efficiency can then be defined as the ratio of body weight to feed intake at zero body weight change (Archer et al., 1999). Although maintenance efficiency is important there are difficulties associated with its measurement (Archer et al., 1999). Furthermore, maintenance efficiency cannot be measured in growing animals, as the requirement for weight stability is not satisfied (Archer et al., 1999). To obtain a true meaurement of maintenance requirements it is necessary to hold animals at a constant bodyweight, a process which can take up to 2 years in beef cattle (Taylor et al., 1981).

Another proposed measure of efficiency is the partial efficiency of growth which is defined as the ratio of weight gain to feed after the expected requirements for maintenance have been subtracted (Archer et al., 1999). Maintenance requirements can be estimated from feeding tables which are based on average body weight during the measurment period (Archer et al., 1999). However, problems are associated with their measurement since the estimation of maintenance requirements from feeding tables carries with it the assumption that no variation exists in the efficiency of feed use for maintenance.

Fifty years ago, Koch et al. (1963) suggested an alternative measure that avoids many of the problems listed above. He suggested that feed intake could be adjusted for live weight and weight gain and effectively partitioned into two components, the feed intake expected for a given level of production and a residual portion. The residual portion could then be used to identify those animals that deviate from their expected level of feed intake, which could then be classified as either high efficiency (negative residual feed intake) or low efficiency (positive residual feed intake). The concept of RFI adjusts an animal's intake for the variation in feed intake due to body mass and gain, while the remainder of its intake is considered representative of background energy requirements (Carstens and Tedeschi, 2006; Nkrumah et al., 2006). Improvements in maintenance energy requirements can decrease the amount of feed required in a cow herd, thereby reducing costs associated with feed and increasing profitability (Arthur and Herd, 2008; Swanson and Miller, 2008). Other advantages of the use of RFI evolve from it having less dependence on production and growth factors (Arthur and Herd, 2008), which allows for improved points of comparison between animals (Carstens and Tedeschi, 2006).

2.1.2 Biological differences in response to feed efficiency in other species

Utilizing work that has been done regarding feed efficiency in other species provides insight into indications of variation, which could be applied to the improvement of feed efficiency

in beef cattle. The concept of residual feed intake was originally proposed in the early 1940s as a direct approach to limit feed costs in laying poultry (Foster et al., 1983). Studies on heritabilities of RFI in poultry have shown heritabilities ranging from 0.21- 0.49 (Van Bebber and Mercer, 1994; Pakdel et al., 2005). The concept of RFI was then further developed for use in other species including pigs where several studies have indicated that RFI in growing pigs is heritable, with estimates ranging from 0.15 to 0.40 (Johnson et al., 1999; Gilbert et al., 2007; Cai et al., 2008; Hoque et al., 2009). Similar heritabilities have also been observed in sheep (Cammack et al., 2005), mice (Bunger et al., 1998), fish (Silverstein et al., 2005) and dairy cows (Williams et al., 2010).

Several studies have been done that evaluated the relationship of animal behaviour with RFI. In mice, it was found that high efficiency mice were 67% less active than the low efficiency line (Hastings et al., 1997; Bunger et al., 1998). As well laying hens from a low RFI line were less active than those from the high line (Braastad and Katle, 1989; Luiting et al., 1991). Feeding behaviour also contributes to animal feed efficiency. Finisher pigs that were selected for decreased RFI resulted in pigs that spent less time eating and ate faster (de Haer et al., 1993; Von Felde et al., 1996; Rauw et al., 2006a and 2006b; Young et al., 2011). There is also evidence that heat production factors into differences in RFI since it influences maintenance energy requirements. Barea et al. (2010) observed that less efficient pigs have a higher heat production relating to their greater physical activity and basal metabolic rate. At the same time, Bordas et al. (1992) reported that lower efficiency chickens also had lower rectal and comb temperature. Investigations into some genetic differences between animals selected for divergent efficiency provides insights into possible indirect measures of feed efficiency. SNPs have been identified in a Yorkshire pig experimental population, which were associated with differences in RFI (Fan et al., 2010). QTLs have also been found in chickens that affect feed efficiency (Van Kaam et al., 1999). Since the role of mitochondria is universal between species, any investigations into their

function would be easily applied to beef cattle. It has been determined that all 5 respiratory chain complex activities were greater in sheep exhibiting the low RFI phenotype compared with those exhibiting the high RFI phenotype (Rajaei Sharifabadi et al., 2012). The same trend was observed in broilers (Bottje et al., 2002) and pigs (Grubbs et al., 2013).

2.2 Evidences of variation in feed efficiency

2.2.1 Genetic evidences of variation in feed efficiency

Residual feed intake is a valuable tool for selection of more efficient animals since it is genetically independent of growth rate and body weight in growing cattle (Herd and Bishop, 2000; Arthur et al., 2001a) meaning that selection based on RFI could offer progeny that would eat less without compromising growth performance (Herd et al., 2004). However, the direct measurement of RFI for individual animals is an expensive process, and this has been a major limitation to the adoption of feed efficiency as an economically important trait in animal breeding. Therefore, DNA based marker-assisted selection would help beef breeders to accelerate genetic improvement for feed efficiency by reducing the generation interval and would avoid the high cost of measuring residual feed intake (Chen et al., 2011). The genetic assessment of feed efficiency would provide an accurate measurement that is not confounded by environmental or feed effects (Sherman et al., 2009). Sherman et al. (2009) identified associations between SNPs underlying five RFI QTL on five bovine chromosomes with measures of dry matter intake, RFI and feed conversion ratio in beef cattle. Six SNPs were found to have effects on RFI. Further studies by Sherman et al. (2010) found a total of 150 SNP were associated with RFI. This shows that these SNPs may be affecting the underlying biological mechanisms of feed efficiency beyond feed intake control and weight gain efficiency. Chen et al. (2011) used global gene expression profiling by microarray to identify genes that were differentially expressed in cattle of different efficiencies and to uncover candidate genes for residual feed intake. One hundred and sixty-one

unique genes were identified as being differentially expressed between animals with high and low residual feed intake. These genes were involved in seven gene networks affecting cellular growth and proliferation, cellular assembly and organization, cell signalling, drug metabolism, protein synthesis, lipid metabolism, and carbohydrate metabolism. This analysis allows for the identification of candidate genes for marker-assisted selection.

2.2.2 Behavioural evidences of variation in feed efficiency

Variation in energetic costs associated with feeding activities including eating time and eating frequency may contribute to variation in RFI because of their association with activities such as standing and walking (Lancaster et al., 2009). Energy expenditures are 19% greater during standing compared with lying (Susenbeth et al., 1998). For example more efficient bulls took 6% less steps than less efficient bulls, which accounted for 10% of RFI variation (Richardson et al., 2000). It has been found that more efficient animals eat smaller meals (Montanholi et al., 2010), eat slower (Kelly et al., 2010a; Montanholi et al., 2010), visit the feeder less often (Montanholi et al., 2010), eat fewer meals (Robinson and Oddy, 2004; Golden et al., 2008; Kelly et al., 2010a) and overall spend less time feeding (Lancaster et al., 2005; Nkrumah et al., 2006; Golden et al., 2008). This indicates that a decrease in feeding-associated activities of the more efficient animals may have evolved as an energy-sparing mechanism (Richardson and Herd, 2004; Nkrumah et al., 2006; Golden et al., 2008). Additionally, Hickman et al. (2002) and Golden et al. (2008) found greater variability of feed intake throughout the day for inefficient animals.

2.2.3 Metabolic evidences of variation in feed efficiency

As early as 1966, Hungate noted that animal variation in digestion, fermentation, and rumen turnover rate offered an opportunity to select ruminant livestock for increased productivity

(Hungate, 1966). Furthermore behavioural evidences may also affect animal digestion since eating rate is negatively related to total mean retention time (Forbes et al., 1972). Within the rumen, the retention time of feed will affect animal productivity by many means, including changes to the ratio of ruminal microbial species (bacteria, protozoa and methanogens), volatile fatty acid pattern, chemical composition of the microbes, alteration of the maintenance energy requirement of the microbes and also the energetic efficiency of the growth of microbes themselves (Meng et al., 1999). A large component of the individual variation is due to the microbial population since they are responsible for the breakdown of feed components, enabling ruminants to derive approximately 70% of their metabolic energy from the microbial fermentation of feedstuffs and act as a source of protein (Bergman, 1990). Recent studies have suggested that bacterial structure in the rumen is associated with cattle feed efficiency (Guan et al., 2008). Hernandez-Sanabria et al. (2012) identified associations between specific bacterial phylotypes and VFAs with feed efficiency traits. Three bacterial phylotypes (*Succinivibrio* sp., *Eubacterium* sp., and *Robinsoniella* sp.) were identified to be potentially associated with RFI based on their sequences. As well differences in rumen retention time are associated with starch digestion and absorption (Meissner et al., 1996; Orskov et al., 1988). It is possible that there is between animal variations in the capacity to digest starch in the small intestine (Channon et al., 2004). Inefficient digestion could result in an animal with an overall lower feed efficiency. Fermentation of starch in the hindgut follows poor starch digestion in the rumen and small intestine and it is the least desirable site of starch digestion in the gastrointestinal tract of ruminants (Orskov, 1986). Methane and heat losses make fermentation in the hindgut is a much less energetically efficient process than enzymatic starch digestion and absorption of glucose in the small intestine (Black, 1971; Owens et al., 1986). Any microbial protein produced in the hindgut cannot be absorbed and is lost in the faeces resulting in a loss of protein available to the animal (Channon et al., 2004). Channon et al. (2004) found that high efficiency parents generally

produced progeny that had higher faecal pH and faecal DM indicating that starch was more extensively utilised in the rumen and small intestine meaning less starch reached the hindgut to be fermented. This provided evidence for genetic differences between animals in their capacity to digest starch, which contributed to differences in efficiency of feed utilisation.

2.2.3.1 Visceral organ function

The maintenance of an optimum nutrient balance in ruminant animals is important for the most efficient growth and requires a wide range of responses to supply the necessary metabolites (Seal and Reynolds, 1993). The visceral organs play a pivotal role in the moderation of nutrients available for peripheral tissues since they are responsible for the digestion, absorption and intermediary metabolism of ingested nutrients (Huntington and Reynolds, 1987). Firstly, nutrients must cross the intestinal tissues since they form an interface between the diet and the animal and will influence the flux of nutrients from the intestinal lumen into the bloodstream. After passing the intestinal wall, nutrients must transverse the liver before reaching the vena cava. The liver is situated to integrate the flow of absorbed nutrients and their metabolites from the digestive tract to peripheral tissues. This will further moderate the supply of nutrients to peripheral tissues for maintenance or productive functions such as muscle deposition (Seal and Reynolds, 1993). This results in a pattern of nutrients in the bloodstream, which does not exactly reflect the quantity and form of those that were available from the diet, meaning that these organs have a profound impact on the supply of nutrients for production in ruminants. Removal of nutrients is a major determinant of the amount of nutrients reaching peripheral tissues, mainly responsible for whole-body protein retention (Lapierre et al., 2000). The degree of release of nutrients from the visceral organs has also been shown to be influenced by feed intake (Huntington and Prior, 1985; Huntington et al., 1988). Lapierre et al. (2000) observed an increase in hepatic removal of total amino acids with increasing feed intake.

The amount of nutrients available for gain in cattle is also influenced by the considerable amounts of energy requirements that are put towards visceral organ metabolism. Even though these organs only represent approximately 6-10 % of body-weight, estimates indicate that metabolically active visceral organs account for a large proportion of total energy requirements at 40-50 % (Webster, 1981). Liver accounts for about half of this amount (Baldwin, 1995) while only comprising 1.45% of the body weight in beef steers (Terry et al., 1995). Some of the metabolic events that contribute to the high energy expenditure of the visceral organs include diet digestion, nutrient absorption and metabolism, maintenance of gut epithelial structure and immune functions, and synthetic processes within the pancreas and spleen (Reynolds, 2002). On a biochemical level, these energy costs go towards Na+, K+-ATPase, protein synthesis, protein degradation, substrate cycling and urea synthesis (McBride et al., 1990). In particular, the maintenance of cell structure through protein turnover requires a high rate of energy expenditure and amino acid requirements (Reynolds, 2002). The high rate of protein synthesis in the gastrointestinal tract is associated with cellular turnover and sloughing, secretion and enzymatic action (McBride et al., 1990). While in the liver, protein synthesis is important in the mediation of hormonal induction, which influences regulation of body systems, synthesis of plasma proteins, enzymatic and cellular turnover and detoxification of blood (McBride et al., 1990).

The high metabolic capacity also requires these organs to have a high rate of blood flow to provide oxygen and remove carbon dioxide (Reynolds, 2002). The kidneys receive more blood flow per unit mass than any other tissue in the ruminant (Hales, 1973), due to their function in the excretion of waste products and the maintenance of fluid balance. Though their blood flow is relatively constant and their fractional extraction of oxygen is lower than for tissues such as the liver their metabolic functions represent a true maintenance cost (Reynolds et al., 1991). On the other hand, other visceral organs including the gastrointestinal tract, pancreas, spleen, and liver account for substantial portions of body oxygen consumption and show variability due to body

weight, physiological state or diet composition, indicating that their exists individual animal variation. Across a variety of experimental conditions, the total splanchnic tissues account for from 36 to 54% of body oxygen use (Reynolds, 1995). Evidence suggests that the proportions of maintenance energy requirements that visceral organs require is highly variable depending on genotype, ration, physiological state, nutritional status, maturity and physiological state (Ferrell, 1988). In particular, the level of nutrition evokes changes in visceral organs that results in changes to organ metabolic activity. This is both a function of organ size and tissue metabolic activity (Burrin et al., 1989). An increase in feed intake will influence blood flow and oxygen consumption (Burrin et al., 1989). Burrin et al. (1989) observed a 37% decrease in oxygen consumption of the portal drained viscera and a 63% decrease in oxygen consumption of liver in lambs fed at maintenance intake compared with lambs with ad libitum intake. It has been suggested that this regulation of blood flow ensures a constant rate of delivery of nutrients and removal of end products for a given unit of tissue.

2.2.3.2 Organ size and activity

A relationship has been shown between feeding level and organ size from changes of workload. In a series of experiments with pigs, rats and sheep, a decreased plane of nutrition consistently resulted in decreased sizes of visceral organs such as liver, kidney, stomach and intestines (Koong et al., 1982; Ferrell and Koong, 1986). Since metabolic activity of an organ is the product of organ size and metabolic activity per unit of tissue this greatly relates to animal energy requirements (Burrin et al., 1990). From the divergent selection for basal metabolic rate in mice, there was a resulting increase in liver size with increases in basal metabolic rate (Ksiazek et al., 2004). Some research has suggested that changes in visceral tissue mass are associated with differences in RFI (Archer et al., 1999; Basarab et al., 2003).

2.2.3.3 Organ function and cellular activity

A large proportion of dietary intake is directed towards "housekeeping" activities of the animal potentially decreasing the amount available for utilization for gain. There is evidence that these maintenance events are associated with genetic variation in RFI (Herd et al., 2004). Protein turnover in living animals is an energetically expensive process, with energy costs of protein turnover accounting for 15- 20% of basal metabolic rate across a range of species (Waterlow, 1988). Variation in protein metabolism has been shown to accompany genetic selection for growth and other traits in domestic animals (Oddy, 1999) and for cattle divergently selected for RFI (McDonagh et al., 2001). Transcriptomic analysis of liver biopsies from Angus bulls have identified 163 differentially expressed genes between animals with high and low RFI, that represent several cellular pathways, such as growth, proliferation, protein synthesis, lipid metabolism, and carbohydrate metabolism (Chen et al., 2008).

Inefficiency of energy production may also contribute to differences between animal feed efficiencies. There has been evidence of a link between inefficient mitochondrial respiration and decreased gain to feed ratio in poultry (Bottje et al., 2002) and rats (Lutz and Stahly, 2003). Mitochondria produce the majority of cellular ATP from oxidative phosphorylation through the electron transport chain from proton pumping across the inner mitochondrial membrane (Bottje et al., 2009). However, protons may also flow back into the mitochondria at sites other than the designated site in a process called proton leak (Brand, 1995). Therefore the inefficiency of mitochondria can be measured through proton leak. Proton leak represents up to 30% of oxygen consumption in isolated liver cells and up to 50% of oxygen use in a perfused muscle (Brand, 1990; Rolfe and Brand, 1996) meaning that proton leak could contribute as much as 25% of total basal metabolic rate of an animal, contributing to inefficiency (Rolfe and Brand, 1996 and 1997; Rolfe et al., 1999). Further adding to the trend of efficiency of mitochondrial complexes contributing to the efficiency of the animal has been shown by Bottje and Carstens (2009) that

found that reduced activities of respiratory chain complexes may be associated with reduced feed efficiency. Rajaei Sharifabadi et al. (2012) found that in sheep all 5 respiratory chain complex activities were greater in sheep exhibiting the low RFI phenotype compared with those exhibiting the high RFI phenotype. Moreover, in breast muscle, liver and duodenal mitochondria from higher efficiency broilers it was found that there was a more tightly coupled respiratory chain along with lower electron leak and reactive oxygen species production compared with lower efficiency broilers (Bottje et al., 2004).

2.2.4 Effect of selection for feed efficiency

The selection for RFI may only be viable if detrimental effects on output are not observed due to genetic correlations of RFI with other key production traits. These traits include carcass and meat quality traits at slaughter, and cow traits, such as age at puberty, mature size, milk production, and lifetime reproductive performance (Herd et al., 2003). Several studies have looked at the effects of selection for RFI on performance and carcass traits. Richardson et al. (1998) found no difference between the progeny of parents selected for low RFI or for high RFI in average daily gain over the test, and body weight at the end of the test. Carcass traits measured ultrasonically before slaughter showed that low RFI line steers had less subcutaneous fat depth at the 12/13th rib and rump, and a smaller cross-sectional area of the longissimus dorsi muscle. It was concluded that progeny of low RFI parents grew as fast, or faster, than steers of high RFI parents but ate less feed per unit gain and produced carcasses that were acceptable with no compromise in retail meat yield. Baker et al. (2006) also looked at meat quality and palatability after selection for RFI. No differences among RFI groups were found for hot carcass weight, longissimus dorsi muscle area, ultrasound fat thickness, USDA yield grade, marbling score, or quality grade. Shear force and sensory panel tenderness and flavour scores of steaks were similar across RFI groups.

The effects of selection of cows based on post weaning RFI measurements and later performance as a mature cow is important to understand. Arthur et al. (2005) found no significant selection line differences were observed in performance traits including fatness and weight. In addition, no significant differences in maternal productivity traits were observed between high and low RFI selection lines including differences in pregnancy, calving and weaning rates, milk yield and weight of calf weaned per cow exposed to bull. Basarab et al. (2007) observed that cows that produced more efficient calves were in better body fatness throughout their lifetime, had less calf death loss, had a similar calving interval and produced the same weight of calf weaned per 100 kg of cow exposed to breeding compared with cows that produced inefficient progeny. Crowley et al. (2011) also found that selection for improved feed efficiency had no deleterious effects on cow performance traits including fertility, calving difficulty, or perinatal mortality with the exception of delaying the age at first calving, which may be due to a delay in the onset of puberty, although it did not affect subsequent reproductive performance. Arthur et al., 1999 also reported that milk yield, did not differ between high and low efficiency lines. Overall the results indicate that females, which were more efficient as weanlings, required less feed as mature cows, with no compromise in performance. Therefore the addition of selection of RFI into breeding programs has been found to have very little negative effects on overall performance and value of end product while making significant improvements on animal feed efficiency.

The effects of selection for RFI on bull fertility are also very important factors influencing the profitability of beef production since bull fertility plays a key role in the success of calf production and identifying bulls with superior fertility and with superior feed efficiency could significantly impact cow-calf production efficiency (Awda et al., 2013). Studies have looked into selection for RFI and fertility traits (Arthur et al., 2001b; Fox et al., 2004; Schenkel et al., 2004). Wang et al. (2012) went further and looked into traits measured for breeding soundness evaluations including body weight at one year, scrotal circumference at one year, front

and hind feet score, temperament score, semen density score, normal sperm morphology and sperm motility. With the exception of sperm motility, none of the breeding soundness traits were associated with RFI (Wang et al., 2012). However the scoring procedure for motility is somewhat subjective and can be affected by many factors, such as temperature, time, concentration, contamination, and method of collection and should not be used as a sole measure of bull fertility (Barth, 2000; Agdex, 2002). Furthermore no effect on siring ability was found. Awda et al. (2013) also looked into measurements of scrotal circumference and semen quality, including sperm motility, viability and morphology to evaluate bull fertility. In this study they found that improved feed efficiency was negatively associated with sperm motility and viability. This may suggest the possibility of genetic associations between RFI and testicular tissue development, rate of maturation, spermatogenesis activity, sperm viability, and motility (Awda et al., 2013). Furthermore the more efficient bulls may be reaching puberty later and exhibiting less sexual activity while on the feed trial, which could benefit their RFI phenotype (Awda et al., 2013).

Benefits have been found to arise from the selection for improved efficiency, specifically differences in greenhouse gas emissions. Since livestock contribute 50% to Canada's agricultural greenhouse gas emissions (Environment Canada, 2004) this benefit would be important. Low RFI cattle have been found to have lesser methane emissions since the quantity of ration consumed is an important determinant of the daily methane emission of livestock (Nkrumah et al., 2005 and 2006; Hegarty et al., 2007). The reduction in dry matter intake of low RFI cattle would also be expected to reduce the amount of manure produced, decreasing the magnitude of nitrous oxide liberated from manure, and also decreasing overall nitrogen intake (Okine et al., 2001; Herd et al., 2002). Direct selection on RFI for multiple generations has proved to be effective in cattle (Arthur et al., 2005).

2.3 Blood plasma analytes as indicators of feed efficiency

2.3.1 Studies on blood plasma analytes as indicators of feed efficiency

Concentrations of metabolic hormones, enzymes and metabolites, mediators of nutrient uptake as well as inhibitors of tissue catabolism, have been examined with a view to identifying potential physiological biomarkers for feed efficiency in cattle (Richardson et al., 2004; Wood et al., 2004; Nkrumah et al., 2007b).

Hormones have been investigated to determine if their actions mediate any differences in efficiency of feed use. Leptin is a hormone synthesised primarily by adipose tissue whose role includes the participation in, and regulation of, multiple physiological systems, including reproduction, inflammation, and cell-mediated immunity, in addition to the coordination of whole body energy homeostasis (Houseknecht and Portocarrero 1998; Houseknecht et al. 1998; Friedman and Halaas 1998). Its concentration in plasma is related to the extent of body lipid depots (Ji et al., 1997; Chillard et al., 1998; Minton et al., 1998). Leptin has been shown to be positively correlated with steer RFI (Richardson and Herd, 2004; Richardson et al., 2004). Alternatively, Brown et al. (2004) and Kelly et al. (2010a) reported that systemic leptin concentrations were unrelated to RFI. Another hormone linked to RFI is insulin. Following divergent genetic selection, high RFI steers tended to have higher insulin concentration than low RFI steers at the end of a feedlot test (Brown, 2005; Richardson et al., 2004). Kelly et al. (2010b and 2011) also found a positive association between RFI and plasma insulin concentration. Higher blood insulin concentrations in the high RFI cattle may be associated with their greater fat composition, since insulin inhibits lipolysis and stimulates lipogenesis in adipose tissue (Hocquette et al. 1998; Orr et al. 1988). Furthermore insulin concentration increases with feeding frequency (Mineo et al. 1990) and since high RFI steers have a higher feed intake (Richardson et al. 1998, 2001) this may be contributing to observed differences.

There has been less research into the relationship of circulating enzymes on differences in RFI. Aspartate aminotransferase is a key enzyme in amino-acid metabolism (Stryer, 1988). It acts as a marker of liver function and indicates higher levels of protein catabolism in the liver of less efficient steers (Richardson et al., 2004). Richardson et al. (2004) demonstrated a positive correlation of -0.36 with feedlot RFI. Products of metabolism may give insight into underlying differences in animal metabolism which may reflect inherit differences in feed efficiency. Triglycerides are a reserve of energy that is stored in adipocyte cells (Vernon and Houseknecht, 2000). Plasma triglycerides have commonly been used as indicators of energy status (Cameron, 1992). Muscle tissue can use plasma triglycerides as an extra-muscular source of fuel following their transformation to non-esterified fatty acids (Hocquette et al., 1998). It has been suggested that lower plasma concentrations of triglycerides indicate higher use by muscle as an energy source for protein synthesis (Cameron, 1992). There has been evidence that less efficient animals had less plasma triglyceride concentrations than low RFI steers, indicating a greater energy requirement by their muscle, due to a higher protein turnover rate (Richardson et al., 2004). Beta-hydroxybutyrate (BHB) is a product of tissue fatty acid catabolism that is oxidized in muscle (Hocquette et al., 1998). Systemic concentrations increase in proportion to the degree of fat mobilization (Kelly et al., 2010b). Ruminants have a higher use of BHB as an energy substrate by muscles than monogastrics, with extraction rates by hindlimb muscle ranging from 10 to 45%, which are higher glucose utilization (Hocquette et al. 1998). In sheep, BHB is reported as positively correlated with carcass protein content (Cameron 1992; Clarke et al. 1996) and negatively correlated with subcutaneous fat depth (Clarke et al. 1996). Higher concentrations of BHB have been seen in high RFI cattle (Kelly et al., 2010a, 2010b; Lawrence et al., 2011). Richardson et al. (2004) observed the same trend, however, the direction of the association changed from positive to negative as the animals increased in age.

Urea is a product of protein degradation (Cameron, 1992) with increased plasma urea nitrogen indicating increased amino acid catabolism (Mortimore and Poso, 1987). Blood urea nitrogen was reported to be negatively related to protein content in bulls (Robinson et al., 1992), negatively related to lean growth (Cameron, 1992; Clarke et al., 1996) and positively related with back fat in sheep (Clarke et al., 1996). Blood urea concentrations have been reported as negatively correlated with high lean growth in sheep (Cameron, 1992; Clarke et al., 1996), with predicted carcass lean in sheep (Cameron et al. 1994), and positively correlated with back fat depth in sheep (Cameron et al., 1994; Clarke et al., 1996). Urea has also been shown to be positively related to genetic and phenotypic measures of RFI in steers (Richardson et al., 1996, 2004; Kelly et al., 2010b). There is evidence that high RFI steers have a higher rate of protein degradation than low RFI steers (Richardson et al., 1996; McDonagh et al., 2001). This may be credited to a greater protein intake in high RFI animals, a greater rate of body protein degradation, or deviation in the supply of amino acids due in part to variation in the efficiency of microbial protein production in the rumen (Lush et al., 1991; Kahn, 2000). Mixed results have been found regarding any relationship between RFI and concentration of glucose. Richardson et al. (2004) found a positive phenotypic correlation with feedlot RFI. Kelly et al. (2010a and 2011) did not find any relationship between circulating glucose and RFI. Plasma proteins have important functions as sources of amino acids for tissue protein synthesis and in the control of blood pressure and blood viscosity (Clarke et al., 1996). There have been observed trends for positive phenotypic relationships between RFI with total plasma protein. Richardson et al. (1996) observed this trend while Richardson et al. (2004) observed this relationship only from blood samples taken at weaning and Lawrence et al. (2011) found this on final sampling, indicating inconsistencies between day of testing. Increased plasma protein reported in the less efficient cattle may reflect differences in the rate of protein turnover (Richardson and Herd, 2004). Creatinine is a break-down product of creatine phosphate, an energy storage compound in the

muscle that has been shown to be positively associated with muscle mass in sheep (Cameron 1992; Clarke et al. 1996) and cattle (Hansett and Michaux, 1985; Istasse at al., 1990) and negatively associated with fat depth in sheep (Clarke et al., 1996). A study by Richardson et al. (2004) reported a negative correlation between RFI and plasma creatinine concentration which was in agreement with the findings of Lawrence et al. (2011) who found that plasma concentrations of circulating creatinine were higher for low RFI heifers than high RFI heifers relating to lower protein turnover.

Mediators of nutrient uptake may directly affect animal feed efficiency. Insulin-like growth factor I is a single-chain 70 amino acid, basic polypeptide that mediates the growth stimulating action and metabolic activities of growth hormone (Kelly et al., 2011). IGF-I has been determined to be positively correlated with RFI in growing animals (Moore et al., 2005; Brown et al., 2004). However no relationship between overall plasma IGF-I and RFI has been observed. Johnston et al. (2007) reported that as cattle became more physiologically mature, the genetic relationship between plasma IGF-I concentration and RFI became less positive.

2.3.2 Albumin

Albumin is one of the longest known and likely most studied of all proteins due to its abundance and ease of measurement. In mammals albumin is synthesized by the liver and is primarily regulated by the colloid osmotic pressure of the interstitial fluid surrounding hepatocytes (Peters and Anfinsen, 1950) and possesses a half-life in circulation of 19 days (Waldman, 1977). Thus albumin reflects the liver's synthetic ability (Lee, 2004). Albumin functions in the regulation and maintenance of the colloidal osmotic pressure in the blood and as a major transporter in the blood. The substances that albumin transports includes the bile acids, free fatty acids, bilirubin, cations, hormones, cholesterol, bilirubin, and heme in addition to trace elements including iron, copper, cobalt, manganese and zinc and many drugs such as penicillin (Reece, 2004). The transport of cortisol in the blood is through the attachment to carrier proteins

including corticosteroid binding globulin or by albumin (Reece, 2004). Albumin has a much greater capacity for binding cortisol since its levels are much higher in the plasma, but its binding is much weaker. Seventy five percent of albumin is bound to corticosteroid binding globulin, 15 percent is albumin bound, and 10 percent is unbound or free (Reece, 2004). The binding to albumin protects cortisol from kidney excretion and from liver inactivation, provides water stability, storage for the hormones and regulates availability to effector tissues (Reece, 2004). Albumin also maintains normal blood pressure by contributing to the viscosity of blood and regulating blood acid base balance (Reece, 2004). Albumin also possesses the ability to affect the solubility of carbohydrates, lipids, and other substances held in solution by the blood (Reece, 2004). There is evidence that albumin has antioxidant activity which may promote wound healing (Nicholson, 2000). Variation in serum albumin levels occur. Hyeralbuminemia is usually only observed with dehydration (Peters, 1996). Hypoalbuminemia can result from decreased synthesis due to malnutrition or liver disease, from increased degradation due to nephrosis or gastrointestinal tract losses or from increased loss from circulation from shock or edema (Peters, 1996). Even under optimal conditions variation may be observed (Peters, 1996). A higher than normal level of serum albumin is associated with body weight in humans (Micozzi et al., 1989) and with birth weight, neonatal survival, and growth rate in piglets (Stone and Leymaster, 1985; Wise et al., 1991).

2.3.3 Urea

Blood urea nitrogen is the concentration of nitrogen, as urea, in the serum. Its concentration in the blood depends on urea production in the liver as a result of protein metabolism and tubular reabsorption, in addition to glomerular filtration in the kidneys before excretion into the urine (Wilson, 2008). Elevated urea concentrations indicate malfunction of the kidneys due to acute or chronic kidney disease, damage, or failure. It may also be indicative of decreased blood flow to the kidneys, from problems such as congestive heart failure, shock,

stress, or from conditions that cause obstruction of urine flow, or from dehydration from inadequate fluid intake or from the administration of corticosteroids, tetracylines, or any drug with antianabolic effects (Lee, 2004). Lower than normal levels could indicate liver disease or damage, or malnutrition (Lee, 2004). Urea is often considered a waste product of protein metabolism, resulting from the need to detoxify ammonia arising from the oxidation of amino acids. However, urea has many other functions in different organisms. For example, in the kidney, urea is essential for concentrating urine (Sands et al., 1992), in marine animals it has an important osmotic role (Goldstein, 1970), in snails it is involved in shell formation (Campbell, 1970) and in ruminants urea is part of the "protein regeneration cycle" (Houpt, 1959). Ruminant animals are able to recycle substantial amounts of urea into the gastrointestinal tract rather than excreting it in the urine. After hydrolysis to ammonia, gut bacteria can use the nitrogen from urea to satisfy their metabolic need, ultimately providing the host ruminant animal with amino acids, nucleic acids and ammonia (Sejrsen et al., 2006). Urea enters the rumen in feeds, in saliva and by direct translocation from blood across the rumen wall (Reece, 2004).

2.3.4 Creatine kinase

Creatine Kinase (CK) is an enzyme found primarily in the heart and skeletal muscles and in smaller amounts in the brain (Wilson, 2008). Creatine kinase is a dimeric globular protein consisting of two subunits (Brancaccio et al., 2007). Its function is to buffer cellular ATP and ADP concentrations by catalyzing the reversible exchange of high energy phosphate bonds between phosphocreatine and ADP produced during contraction (Brancaccio et al., 2007). Total creatine kinase levels depend on age, gender, race, muscle mass, physical activity and climatic condition (Brancaccio et al., 2007). The serum level of skeletal muscle enzymes is a marker of the functional status of muscle tissue and varies widely in both pathological and physiological conditions (Brancaccio et al., 2007). An increase in the activity of creatine kinase in the plasma can be caused by fasting, exercise and the administration of adrenaline due to anaerobic

metabolism (Holmes et al., 1973). The production of lactic acid in muscle causes local and systematic acidosis resulting in a decrease in pH at the tissue level resulting in increased permeability of cell membranes and cell lysis, releasing intracellular enzymes including creatine kinase to the blood thus elevated concentrations of creatine kinase in serum or plasma reflect damage to skeletal and cardiac muscle (Bollinger et al., 1989). Elevation of serum creatine kinase concentration appears to be the most sensitive and specific index of muscle damage in mammals (Chalmers and Barrett, 1982). Elevated plasma creatine kinase also occurs in hypothyroidism (Scott et al., 2002) and from the administration of several drugs including statins, fibrates, anti-retrovirals, angiotensin-II receptor antagonists, immunosuppressants and hydroxychloroquine (Dugue et al., 2004).

2.3.5 Glutamate dehydrogenase

Glutamate dehydrogenase (GLDH) has the key role of acting as an interface between carbohydrates and amino acids in the vicinity of the citric acid cycle and the urea cycle (Lee et al., 1999; Thomas, 2005; Kravos and Malesic, 2008). It is a key enzyme in amino acid oxidation and urea production where it catalyzes the reversible deamination of glutamate to alpha-ketoglutarate and ammonium ions (Kravos and Malesic, 2008). It is a relatively liver specific enzyme (Lindena et al., 1986, Clampitt, 1978) and is located in the mitochondrial matrix (Zimmerman, 1974, Schmidt and Schmidt, 1988). GLDH activity is higher in the centrilobular region than in the periportal zone. Therefore GLDH can be considered a marker of liver function. It has several features, which makes it a viable biomarker of hepatocellular disease. It is highly conserved in structure, tissue-distribution, and function across a wide range of species (Schmidt and Schmidt, 1988). The remote intracellular concentration of GLDH in the mitochondrial matrix of hepatocytes and its large size (330 kDa) delays its release in cell damage and makes it a more specific marker of necrosis (Schmidt and Schmidt, 1988).

2.3.6 Aspartate aminotransferase

Aspartate aminotransferase (AST) is an enzyme involved in amino acid metabolism that catalyses the transfer of alpha keto group of ketoglutaric acid, to produce oxaloacetic acid and the transfer of an amino group from aspartate to produce glutamate (Kew, 2000). Oxaloacetate then acts as a substrate for the citric acid cycle and gluconeogenesis. It is widely distributed in the liver, cardiac muscle, skeletal muscle, red blood cells, kidneys, lungs, brain, pancreas, leukocytes, and erythrocytes (Lee, 2004). Greatest activity occurs in liver, heart, and skeletal muscle and in erythrocytes while minimal activity occurs in skin, kidneys and pancreas (Wu, 2006). Aspartate aminotransferase is found in both mitochondria (80% of total) and cytoplasm (20%), with a specific isoenzyme identifying each site (Rej, 1978). It may be released from hepatocytes into the circulation by necrosis or by changes in cell membrane permeability that allows the enzymes to leak out of cells resulting in elevated levels in the blood (Fregia and Jensen, 1994). It may also be elevated due to a variety of conditions including musculoskeletal diseases including muscular dystrophy, dermatomyostitis, trichinosis, gangrene, muscle damage, intestinal injury, renal infraction or failure (Fregia and Jensen, 1994).

2.3.7 Carbon dioxide

Carbon dioxide is produced by oxidative metabolism in the mitochondria (Reece, 2004). The amount produced depends on the rate of metabolism and the relative amounts of carbohydrate, fat and protein metabolized (Arthurs and Sudhaker, 2005). During the process of respiration, excess carbon dioxide is removed from the body and this is replaced by atmospheric oxygen; however there is still some level of carbon dioxide that remains in the blood (Lee, 2004). The major portion of carbon dioxide is present in the body either as bicarbonates or carbonic acid (Lee, 2004). A major determinant of serum bicarbonate levels is kidney function (Widmer et al., 1979). The kidneys reabsorb filtered bicarbonate to prevent depletion (Lee, 2004). Lower than normal levels may be due to ketoacidosis, kidney disease, lactic acidosis, and metabolic acidosis

while higher than normal levels may be due to breathing disorders, cushing syndrome, or hyperaldosteronism (Lee, 2004).

2.4 Sources of variation of blood plasma parameters

Blood metabolite concentrations can represent differences in energy, protein and mineral metabolism (Ndlovu et al., 2007). Blood metabolites that are commonly related to energy metabolism include blood glucose, betahydroxy butyrate and non-esterified fatty acids. While blood urea nitrogen, creatinine, and total protein, albumin, and creatinine levels can be related to protein metabolism. It is important to understand any underlying factors affecting these constituents in order to effectively use them as potential indictors of normal metabolic function. Many factors have been shown to influence blood metabolites including environmental factors such as season, management practices such as diet fed, as well as animal factors like sex and breed.

2.4.1 Circadian variation in blood plasma parameters

Circadian rhythm is a twenty-four hour period repetition of certain phenomena in living organisms. It may represent an important regulating mechanism for the physiological and behavioural responses of animals to their changing environment associated with the solar day (Aranas et al., 1987). There exists an intrinsic rhythm in mechanisms regulating metabolism that is evident by variations in rumination (Gordon and McAllister, 1970), body temperature (Bitman et al., 1984), and differences in blood constituents including cortisol (Lefcourt et al., 1993), prolactin (Evan et al., 1991), thyroid hormones (T3 and T4) (Bitman et al., 1994), aldosterone (Aranas et al., 1987) and insulin (Lefcourt et al., 1999). Blood metabolites including glucose (Shehab-El-Deen et al., 2010), cholesterol (Shehab-El-Deen et al., 2010), non-esterified fatty acids (Toharmat et al., 1999), urea nitrogen (Toharmat et al., 1999), and enzymes (Caldeira et al., 1999) have also been shown to be influenced by circadian rhythm indicating a potential circadian rhythm of animal metabolism.

More specifically, Caldeira et al. (1999) looked at the blood profiles of sheep over a twenty-four hour period. Glucose profiles were higher 9 to13 hours after time of feeding. The fluctuation of glycemia was a result of variations in the gluconeogenesis rate depending on availability of propionate (Katz and Bergman, 1969; Bergman, 1973). The production of beta hydroxybutyrate peaked 3 hours after feeding and decreased until the next meal. Triglyceride levels decreased after feeding. Albumin concentrations decreased 3 to 6 hours after the meal while globulin concentration increased after feeding along with total protein levels. Urea levels were characterised by a rise in concentration after feeding. Creatinine peaked after feeding and then decreased after the meal likely due to the switch to an anabolic state after feeding due to availability of amino acids for protein synthesis. Aspartate aminotransferase activities showed a decrease after the meal. A higher activity before feeding could be explained by an increase in the utilisation of amino acids as an energy source, or as glucose precursors to counterbalance a phase of energy negative balance in the daily cycle. Glutamate dehydrogenase activities also reflect the magnitude of protein metabolism and showed a decrease after feeding. In mammals, the circadian system is organized with the master pacemaker located in the suprachiasmatic nucleus that controls downstream oscillators in peripheral tissues (Ko and Takahashi, 2006). This system is entrained to the light/ dark cycle in addition to other inputs, such as temperature, social cues, or access to food (Stokkan et al., 2001). The peripheral tissues generate transcriptional rhythms thought to be important for the daily timing of physiological processes (Reppert and Weaver, 2001; Ripperger and Schibler, 2001). Some molecular studies on rodents identified the liver as the site of a food trainable oscillator (Stephan, 2001), which could be synchronized by feeding time (Damiola et al., 2000; Hara et al., 2001).

2.4.2 Age influence in the variation of blood plasma parameters

As animals age they undergo changes in growth and body composition due to differences in metabolism (Loyd et al., 2011). As cattle grow, composition of their gain shifts from protein accretion to fat deposition (Trenkle and Willham, 1977). Since the energetic expense associated with protein accrual is less than for fat deposition (Ferrell and Jenkins, 1985) the efficiency with which cattle convert feed into body weight gain is reduced as they mature. Metabolic rates tend to decline with age (Shaffer et al., 1981). The higher metabolic rates of younger animals can be attributed to the higher rates of cellular reactions and from the rapid synthesis of cellular materials and growth of the body (Guyton, 1976). The higher metabolic rate may affect the metabolite profile of blood.

Doornenbal et al. (1988) looked at blood component levels in shorthorn calves at birth, weaning and approximately one year of age then as heifers at 2 years of age and cows at 4 to 5 years of age and 6 to 10 years of age. Blood glucose levels were higher at birth and then decreased with age up to one year of age. In growing animals, glucose requirement is determined by growth rate (Reynolds et al., 2003). Growing animals have higher energy requirements for growth. Total protein and albumin levels were found to be lower in young animals and higher in mature animals agreeing with the findings of Shaffer et al. (1981). Blood urea nitrogen levels from birth to one year of age were slightly lower than those of older animals. Blood enzymes were also shown to be related to metabolism and therefore age of animal. Alkaline phosphatase, a group of enzymes, which catalyzes the liberation of Pi from many molecules, originates from the liver in mature animals and from bone tissue in growing animals (Doornenbal et al., 1988). Higher serum levels found in young animals would be indicative of rapid skeletal growth. Aspartate aminotransferase catalyzes the transfer of an a-amino group from an amino acid to an alpha keto acid and is widely distributed in animal tissues and generally increases with age (Doornenbal et al., 1988). Lactate dehydrogenase is a glycolytic enzyme, which is involved in the

reversible conversion of pyruvic acid to lactic acid and levels at birth are somewhat lower then with older animals. Calcium and phosphorus have a large function in skeletal growth in young animals. In older animals there is a decreased need for calcium and phosphorus for this purpose. This is reflective in decreased amounts in blood beyond one year of age. Shaffer et al., 1981 also observed age of animal effects on blood characteristics. Packed cell volume, a volume percentage of red blood cells in blood decreased with age. A decrease in red blood cells with age may be revealing a decreased capacity to produce red blood cells with age or an increased rate of destruction of red cells associated with old age or related to decreased activity with aging, thereby requiring less red cell capacity.

2.4.3 The effect of physiological status on blood parameters

Whether or not the animal is pregnant or non-pregnant or lactating will factor into differences in blood metabolite levels since pregnancy and lactation, especially in the early stages, are very demanding on the organism and are when nutritional requirements are highest (Goff and Horst, 1997; Antunovic et al., 2002; Roubies et al., 2006). The physiological status of an animal affects the glucose concentration (Otto et al., 2000). Glucose concentrations were lower in pregnant cows (Peterson and Waldern, 1981; Otto et al., 2000; Grunwaldt et al., 2005). Low glucose levels in high pregnancy are associated with fetal development and mobilization of maternal glucose to fetal blood circulation (Jacob and Vadodaria, 2001). This also occurs when comparing lactating cows to non-lactating cows (Peterson and Waldern, 1981; Otto et al., 2000; Antunovic et al., 2011) due to the high energy demand and constant energy loss with the milk (Pambu-Gollah et al., 2000). It has been found that in pregnant ewes and cows blood urea levels are higher than in non-pregnant counterparts (Peterson and Waldern, 1981; Doornenbal et al., 1988; El- Sherif et al., 2001; Grunwaldt et al., 2005). This has been explained by an increase in protein catabolism due to the high need for energy for pregnancy which would lead to an increase in urea levels to an extent above the ability of the kidneys to eliminate excess amounts (El- Sherif

et al., 2001). Creatinine levels rose during pregnancy (Peterson and Waldern, 1981; El- Sherif et al., 2001; Yokus and Cakir, 2006). Protein measured either as albumin, globulin or total protein during pregnancy have shown mixed results with Otto et al. (2000); El- Sherif et al. (2001); Yokus and Cakir (2006); Antunovic et al. (2011) finding increases with gestation while decreases have been found by Peterson and Waldern (1981) and Grunwaldt et al. (2005). During lactation, Peterson and Waldern (1981) and Antunovic et al. (2011) found a decrease of total protein and albumins over the lactation period which could be explained by a rapid extraction of immunoglobulin from the plasma during the last few months of pregnancy when colostrum is being formed in the mammary gland (Kaneko et al., 2008). Serum triglycerides and cholesterol in the blood of the pregnant ewes and cows were higher than non mated (Yokus and Cakir, 2006) this can be explained by increased energy requirements and negative energy balance (Antunovic et al., 2011). Increases in serum concentrations of hepatic enzymes (aspartate aminotransferase, alanine transaminase) during pregnancy have been observed by Otto et al. (2000); El- Sherif et al. (2001); Grunwaldt et al. (2005) which indicated an increase in hepatic metabolism. (Antunovic et al., 2011). Variation in levels of circulating hormones has been observed between lactating and non-lactating animals. Significantly lower concentrations of T3 were obtained in the blood of lactating ewes (Karapehlivan et al., 2007; Antunovic et al., 2011). Lower blood T3 concentration could reduce the rate of oxidation and the rate of continuous breakdown and formation of protein and fat in most, if not all mammary tissue (Riis and Madsen, 1985). Lower concentrations of serum leptin were obtained in the blood of lactating ewes if compared to pregnant ewes (Antunovic et al., 2011). At the same time, lower concentrations of serum insulin were obtained in the blood of lactating ewes if compared to pregnant ewes (Antunovic et al., 2011).

2.4.4 Other sources of variation in blood parameters

Differences in blood parameters with season are due to differences in environmental temperature, particularly from heat stress in the summer (Shaffer et al., 1981). Depression in

many blood parameters of animals under heat stress may be due to a hemodilution effect, where more water is transported in the circulatory system for evaporative cooling (Shaffer et al., 1981). Furthermore, there is a significant decline in feed consumption with rising temperature (Shaffer et al., 1981). An increased activity of some enzymes with rising temperature such as an increase in serum AST and CK activities observed by Georgie et al. (1973) and Shaffer et al. (1981) has been observed potentially due to the fact that their reactions may be simply accelerated at higher temperatures (Shaffer et al., 1981). Glucose levels decrease with an increase in body temperature and respiration rate of animals normally experienced in hot summer season (Ndlovu et al., 2007). This increase in respiration rate causes a rapid utilization of blood glucose by the respiratory muscles and thus results in a decrease in blood glucose content under heat stress (Hassan and Roussel, 1975). Heat stress also resulted in a depression of packed cell volume, red blood cells, hemoglobin, and oxyhemoglobin (Shaffer et al., 1981). This has been suggested to be due to a reduction in cellular oxygen requirements to reduce metabolic heat load to compensate for elevated environmental heat load (Lee et al., 1978). Total protein and albumin have also been shown to have a relation to changes in temperature showing an increase in hotter months (Shaffer et al., 1981), which may be indicative of a loss of extracellular fluid due to heat exposure (Rasooli et al., 2004). A higher concentration of blood urea is observed in summer likely as a result of a loss of extracellular fluid due to heat exposure (Rasooli et al., 2004).

Differences in diet fed would of course affect concentrations of some metabolites in blood including concentrations of blood glucose, albumin of serum, and nitrogen in urea due to differences in dietary intakes of energy and crude protein (Lee et al., 1978). Depending on when the animal had consumed its meal and when blood testing occurred, this could cause large variations in results.

It is well known that lean tissue growth is regulated largely by hormones and that differences in lean tissue growth may affect the blood profile of cattle (Doornenbal et al., 1987).

Concentrations of cortisol, urea nitrogen and albumin in serum were higher in steers than in intact males and those of glutamic oxaloacetic transaminase, lactate dehydrogenase, alkaline phosphatase and creatinine were lower in steers than in intact males (Doornenbal et al., 1987). Ban-Tokuda et al. (2007) looked at differences in the blood profile of males and females they found higher leptin, triglyceride and insulin concentrations in females than in males, possibly explained by the higher fat deposition of females than males.

Breed differences would likely reflect differences in body size and weight for the breed. For instance, larger size gives a greater consumptive capacity (Shaffer et al., 1981). Breed effects were found to be significant for hemoglobin, oxyhemoglobin, packed cell volume, alkaline phosphatase, creatine phosphokinase, total protein, albumin, globulin, urea, cholesterol, and calcium (Shaffer et al., 1981).

Differences in RFI reflect differences in metabolic processes (Koch et al., 1963). Blood measures can be utilized to look at differences in metabolic process of organs that account for large proportions of energy expenditures that may contributes to variations in animal feed efficiency. Therefore blood measures offer the potential to act as biological indicators of feed efficiency if an association are found. Associations have been observed between blood analytes and RFI (Richardson et al., 1996 and 2004; Richardson and Herd, 2004; Brown, 2005; Kelly et al., 2010a, 2010b and 2011; Lawrence et al., 2011). However these measures were limited in their sampling numbers therefore obtaining an understanding of the relation observed between blood analytes and RFI will require increased numbers of sampling.

Chapter 3

CIRCADIAN PROFILES OF BLOOD PLASMA ANALYTES IN BEEF HEIFERS AT DIFFERENT PHYSIOLOGICAL STATES WITH DISTINCT FEED EFFICIENCIES

ABSTRACT

Indirect methods for assessing feed efficiency would lower costs associated with individual feed intake determination making its use more accessible enabling more widespread improvement of feed efficiency in livestock. Therefore potential biological factors underlying differences in feed efficiency were assessed through hematological measures including albumin, urea, creatine kinase, glutamate dehydrogenase, aspartate aminotransferase and carbon dioxide. These measures of basic metabolic processes were determined in a population of 36 heifers from hourly blood samples collected over a 24 h period on three separate sampling occasions corresponding approximately to the yearling, early gestation, and late gestation stages in order to determine variation throughout the day, with physiological status as well as their associations with residual feed intake. It was found that there was variation in blood analytes with time of day with the most variation between 08:00 and 16:00. This variation was consistent between heifers of high and low residual feed intake and was generally consistent between physiological states. There were also significant differences in concentrations of analytes from open to pregnancy and throughout pregnancy. Albumin increased in pregnancy ($P<0.05$) with a lower concentration in late gestation compared to early. Plasma urea was higher in early gestation ($P<0.05$) then in open or late gestation. Activity of creatine kinase decreased in bred heifers ($P<0.05$) and through pregnancy ($P<0.05$). Plasma glutamate dehydrogenase activity was greatest in early pregnancy ($P<0.05$) and then decreased back to the same activity as open heifers. Aspartate aminotransferase decreased in bred heifers ($P<0.05$) and through pregnancy ($P<0.05$). A concentration of carbon dioxide

decreased from open to bred heifers ($P<0.05$) and was not effected by advancing gestation. In addition, associations between feed efficiency, measured as residual feed intake, on analyte concentrations and activities were observed. In open heifers that were more feed efficient there were higher activities of creatine kinase ($P<0.05$) and aspartate aminotransferase ($P<0.05$) and a lower concentration of carbon dioxide ($P<0.05$). While in late gestation more efficient heifers had lower urea concentrations ($P<0.05$) and lower creatine kinase levels ($P<0.05$). Over the whole experimental period carbon dioxide concentrations were also shown to be lower in more feed efficient heifers ($P<0.05$). This indicates that plasma analyte concentrations are related to animal feed efficiency through their reflection of differences in animal metabolism. These measured differences may be able to be utilized as indirect measures of feed efficiency.

INTRODUCTION

Improving feed efficiency in cattle is crucial for reducing feed costs and lessening the detrimental environmental impact of beef production, while allowing for industry sustainability and growth. Improvements in feed efficiency can reduce the cost of gain considerably because the price of feed comprises 70 to 80% of the total cost of body weight gain (Field and Taylor, 2002). However, the direct assessment of individual feed efficiency is not normally obtained until late in the life of cattle and it is a time-consuming, laborious, and expensive measurement (Arthur et al., 2004). The preferred method of measurement of feed efficiency currently used in beef cattle is residual feed intake (RFI), which is defined as the difference between the actual feed intake of an animal and expected feed intake based on body size, growth rate and body composition (Montanholi et al., 2009) over a specified test period. Residual feed intake is often a favoured measurement of efficiency as it represents variations in the requirements for basic metabolic processes rather than variations due to differences in level of production (Richardson et al., 2001;

Nkrumah et al., 2006; Montanholi et al., 2010) which allows for closer comparison between animals regardless of production level (Carstens and Tedeschi, 2006). It also allows for the investigation into biological processes and mechanisms that contribute to differences observed in feed intake (Arthur and Herd, 2008) which may represent an avenue for identifying new traits for predicting feed efficiency in beef cattle.

Hematological measures provide a readily accessible means of determining differences in efficiency between animals. Previous work has been done to look at relationships between RFI and metabolites such as urea (Richardson et al., 1996 and 2004; Kelly et al., 2010b), β-hydroxybutyrate (Kelly et al., 2010a and 2010b; Lawrence et al., 2011) and triglycerides (Richardson et al., 2004), total protein (Richardson et al., 1996), creatinine (Richardson et al., 2004; Lawrence et al., 2011), hormones such as insulin (Richardson et al., 2004; Brown, 2005; Kelly et al., 2010b and 2011), leptin (Richardson and Herd, 2004; Richardson et al., 2004) and enzymes such as aspartate aminotransferase (Richardson et al., 2004). These measures, however, were done at only one point in time during the day and typically at one particular physiological state. Since there are known sources of variation in blood variables including season (Shaffer et al., 1981), sex (Ban-Tokuda et al., 2007), circadian cycle (Caldeira et al., 1999), age (Doornenbal et al., 1988), and physiological status (Otto et al., 2000), it is important to determine how these factors cause variation in the concentrations of blood analytes. Here, the circadian cycle and physiological state were assessed, and additional metabolic and enzyme assays were performed that may better reflect differences in RFI then those previously determined.

There exists an inherent rhythm in the mechanisms regulating metabolism that is evident in variations in rumination patterns (Gordon and McAllister, 1970), body temperature (Bitman et al., 1984), and concentrations of blood constituents (Caldeira et al., 1999). This rhythm is controlled by the circadian system, which is largely controlled by the environmental light cycle in addition to other cyclic inputs, such as temperature, noise, social cues, or access to food (Stokkan

et al., 2001). These inputs will result in variations in measured blood analytes. Therefore it is important to investigate circadian variation in order to identify how time of measurement affects accuracy. The physiological status of the animal also influences blood metabolite levels since pregnancy is demanding on the organism and is when nutritional requirements rise (Goff and Horst, 1997; Antunovic et al., 2002; Roubies et al., 2006). There are large amounts of mobilization of body reserves to meet these increased energy demands associated with growth of the reproductive organs, fetus and growth of the heifer resulting in increases in organ metabolism to fulfill this mobilization (Bell and Bauman, 1997; Wallace, 2000; Herrera, 2002).

HYPOTHESIS AND OBJECTIVES

Since basal metabolic processes are responsible for a large proportion of energy requirements, blood variables that reflect the workload placed on them, measured over the circadian cycle as well as with growth and over the pregnancy period, may give indications of differences in animal efficiency. These measures of metabolic function include metabolites such as urea, enzymes such as creatine kinase (CK), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH), and proteins such as albumin. Differences in these levels may mirror differences in feed efficiency due to changes in organ function, protein turnover and whole body metabolism.

The objectives of this study were to define the circadian variation of blood analytes in addition to determining the differences in blood analyte concentrations and activities with physiological status and how they relate to feed efficiency. The study was intended to provide insight into the biological basis of observed differences in RFI and to assess the practicality of using hematological measures as an indirect measure of RFI and to define if a certain time of day and physiological status are most accurate for testing.

MATERIALS AND METHODS

Animals

Thirty-six cross-bred replacement heifers from the Elora Beef Research Centre, University of Guelph were used for this study. The study took place from March 2011 to April 2012. Average breed composition of the heifers was 63.4% Angus, 24.3% Simmental, 2.9% Gelbvieh, and 9.4% other European breeds. Characteristics of the heifers at each of the stages including age, body weight and day of gestation are found in Table 3.1. All experimental procedures followed the recommendations as outlined by the Canadian Council of Animal Care guidelines, 1993 and were approved by the University of Guelph animal care committee.

Housing, feeding and performance assessments

Animals were housed in pens of six, with each animal having individual feed access through the use of Calan gates (American Calan Inc., Northwood, NH, USA). Calan gates limit animal access by requiring the recognition of a specific electronic key in close proximity to the head gate, which disengages the locking mechanism. Unique electronic keys attached to a collar around the heifer's neck allowed entrance to only one feeder within the pen. Pens had a depth of 10.8 m, a width of 5.4 m, and a shelter over 29.2 m^2. The sheltered area contained straw bedding which was topped up weekly or when necessary.

A total mixed ration was offered once daily in the morning and feed refusals were removed and measured at least once weekly in order to maintain fresh bunks. Animals were offered feed for ad libitum intake at a rate of 105 to 110% of actual intake to ensure access to feed was unlimited, with adjustments in offerings calculated from the previous week's intake levels. Two total mixed ration diets, A and B, were fed over the duration of feed intake measurements (Table 3.2). Diet 1 was fed from weaning at an average age and body weight of 191 ± 17 days

and 242 ± 43 kg, respectively, until an average age and body weight of 584 ± 17 days and 575 ± 63 kg, respectively. Diet 2, of lower energy density, was fed from this point through to calving. Individual animal weights were recorded biweekly and ultrasound measures for body composition were performed every 28 days while animals were restrained within a hydraulic squeeze chute (Silencer® Hydraulic Squeeze Chute; Moly Manufacturing Inc., Lorraine, KS, USA). Ultrasound measurements were taken with the use of an Aloka SSD-500 ultrasound unit, 3.5 MHz long probe (Corometrics Medical Systems, Wallingford, CT, USA) and Auskey program (Animal Ultrasound Services, Ithaca, NY, USA) to measure back fat thickness (mm), rib eye area (cm^2), intramuscular fat score (score based on 2-10+%) and rump fat thickness (mm).

Residual feed intake determination

The trial started shortly after weaning at an average of 247 ± 17 and weight of 275 ± 47 kg, and concluded the week before calving for each animal. The trial was subdivided into a pre-feed trial period (PT) and a feed trial period (T). The pre-trial period covered the first 178 days of the trial and included the collection of biweekly body weights, and ultrasound measurements every 28 days in a similar manner to that described in details by Montanholi et al. (2009) but did not include the measurement of individual animal feed intake. The feed trial period consisted of the 286 to 342 days of the study remaining after the pretrial period included the assessment of individual feed intake assessment using Calan gates (American Calan Inc., Northwood, U.S.A.) and also included the collection of biweekly body weights, and ultrasound measurements every 28 days.

Several models were tested to calculate residual feed intake (RFI), similar to the approach used by Montanholi et al. (2009). The most appropriate model for explaining variation in feed intake had an R^2 of 0.43 and the lowest Bayesian information criteria (BIC) and was composed as follows: DMI = -6.102 + 0.013 × AGE + 1.354 × T_ADG + 0.002 × T_ABW + 0.584 × T_ABF -

60.509 × T_BFG + 1.600 × T_AIF + 4.675 × T_IFG + 0.011 × T_ARE + 3.430 × T_REG - 0.128 × T_ARF - 1.856 × T_RFG - 0.307 × PT_ADG - 1141.999 × PT_ABG + 14.561 × PT_IFG + 6.802 × PT_REG + 139.622 × PT_RFG + RFI where AGE is the Julian day of the year that the heifer was born; T_ADG is the test average daily gain; T_ABW is the test average body weight; T_ABF is the test average backfat; T_BFG is the test backfat average daily gain; T_AIF is the test average intramuscular fat; T_IFG is the test intramuscular fat average daily gain; T_ARE is the test average ribeye area; T_REG is the test ribeye area average daily gain; T_ARF is the test average rump fat; T_RFG is the test rump fat average daily gain; PT_ADG is the pre-test average daily gain; PT_ABG is the pre-test average daily backfat gain; PT_ IFG is the pre-test average daily intramuscular fat gain; PT_REG is the pre-test ribeye average daily gain and; PT_RFG is the pre-test average daily rump fat gain. Additionally, RFI is the residual proportion of the model that represents the deviation of the observed feed intake in relation to the expected feed intake.

Blood sampling and processing

Four stalls, each measuring 2.5 m × 1.1 m and bedded with wood shavings, were used to house the heifers during blood sample collection. Heifers were fitted with halters and tethered to a feed chamber located at the front of each stall to enable sample collection. Feed and water were provided for *ad libitum* consumption within the feed chamber throughout the duration of the sampling. Lighting in the room during sampling was controlled to mimic the lighting experienced by the animal in its regular pen setting. Stalls were kept clean and dry with the addition of shavings as required.

Groups of four heifers were moved into individual stalls on three different occasions for sample collection. Heifers were adapted to the sampling environment, stall, and feed chamber for 2 days prior to each day of blood sampling. Jugular catheters were inserted and blood samples

were taken and then processed as described by Montanholi et al. (2013). Blood collection started at 08:15 and concluded at 08:15 the following day.

Blood analyte determination

Measurements of plasma albumin, AST, CK, CO_2, GLDH and urea were performed at the Animal Health Laboratory at the University of Guelph using the cobas 4000 c311 biochemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The reagents used were ALB2: ACN 413 for albumin determination, UREA: ACN 418 for urea determination, CKL: ACN 057 for CK determination, GLDH3: ACN 588 for GLDH, ASTL: ACN 687 for plasma AST determination, and CO2: ACN 006 for CO_2 determination.

Statistical analysis

Homogeneities of variance were tested using residual plots as part of the MIXED procedure of SAS (SAS Inst. INC., Cary, NC). Three measures (AST, CK, and GLDH) showed significant skewness and, therefore, were transformed by taking their natural logarithm. Back-transformed data are presented in the results for clarity. Data were analyzed using the MIXED procedure of SAS (SAS Inst. INC., Cary, NC), fitting the following model, $Y_{ijklm} = \mu + startage_i + stage_j + rfigroup_{k(j)} + group_{l(j)} + \beta_{1(j)} \times hour + \beta_{2(j)} \times hour^2 + \beta_{3(j)} \times hour^3 + \beta_{4(j)} \times hour^4 + heifer_m + e_{ijklm}$ where Y_{ijklm} is the variable measured (albumin, urea, CK, GLDH, AST, CO2) in the m-th heifer, with i-th starting age, at j-th stage of pregnancy, belonging to the k-th RFI group, and in the l-th group of heifers; μ is the overall mean; $startage_i$ is the fixed effect of the i-th age at sampling; $stage_j$ is the fixed effect of the j-th stage of pregnancy (open, early pregnancy, late pregnancy); $rfigroup_{k(j)}$ is the fixed effect of the k-th RFI group (RFI groups were defined as group 1 having RFI < 0.10 and group 0 with RFI > 0.10) within the j-th stage of pregnancy; $group_{l(j)}$ is the fixed effect of the l-th group of measurement (group of heifers corresponded to the group of animals

sampled on the same day; there were 14 classes in Open Stage, 11 classes in Early Pregnancy Stage, and 11 classes in Late Pregnancy Stage) within the j-th stage of pregnancy; $\beta_{1(j)}\times$hour , $\beta_{2(j)}\times$hour^2 , $\beta_{3(j)}\times$hour^3 , and $\beta_{4(j)}\times$hour^4 are the fixed linear, quadratic, cubic and quartic regressions on the hour of measurement within the j-th stage; $heifer_m$ is the random effect of the m-th heifer; e_{ijklm} is the random residual effect. The Scheffe multiple comparison test was applied to evaluate all pairwise comparisons between the RFI group means and physiological stage group means. The correlation procedure of SAS was also used to determine the association between different analytes at each physiological stage. Differences were considered statistically significant at $P \leq 0.05$ and were considered trending toward significance at P values > 0.05 and ≤ 0.10. The means presented in the circadian curves are predicted means based on the model described above.

RESULTS

Mean values with their standard deviations, and minimum and maximum values of the assessments performed are shown in Table 3.3. The average DMI was 10.91 kg/day with a standard deviation of 0.93 kg/day. Residual feed intake had a standard deviation of 0.65 kg DM/ day, with the most feed efficient heifer eating 3.05 kg DM/d less than the least feed efficient heifer. Correlation coefficients between analyte values are shown in Table 3.4.

Circadian variation in analyte concentrations

The least squares means of analyte concentrations and activities over the circadian period are shown in Figure 3.1. This graphical representation displays the pattern of analytes throughout the day. The concentration of albumin increased to 12:00 then decreased to 21:00 and then increased again. Urea concentrations peaked at 20:00. Activity of CK in plasma peaked at 12:00. The profile of GLDH activity increased to 11:00 and then reached its lowest at 23:00 and then

increased. Plasma AST activity increased to 12:00. Concentration of CO_2 in plasma decreased to 01:00.

Effect of physiological status on analyte concentrations

Table 3.5 shows the mean analyte concentrations at each sampling period. Albumin concentrations showed a 3.89 g/L decrease between open and early gestation, a 1.35 g/L increase between early and late gestation, and a 2.54 g/L decrease between open and late gestation. Urea concentrations showed a 1.29 mmol/L decrease between open and early gestation, a 1.25 mmol/L increase between early and late gestation, and a 0.04 mmol/L decrease between open and late gestation. Creatine kinase concentrations showed a 12.51 U/L increase between open and early gestation, an 85.37 U/L increase between early and late gestation and a 97.88 U/L increase between open and late gestation. Glutamate dehydrogenase concentrations showed a 0.9 U/L decrease between open and early gestation, a 1.02 U/L increase between early and late gestation and a 0.12 U/L increase between open and late gestation. AST concentrations showed a 2.4 U/L increase between open and early gestation, a 1.8 U/L increase between early and late gestation and a 4.2 U/L decrease between open and late gestation. Carbon dioxide concentrations showed a 3.87 mmol/L increase between open and early gestation, no change between early and late gestation and a 3.87 mmol/L increase between open and late gestation. The least squares means of all measured analytes over the circadian period at each physiological state are presented in Figure 3.1.

Differences in circulating analyte concentrations with RFI

The means of each analyte separated by RFI group over each sampling period are shown in Table 3.6. Plasma albumin values differed between low and high RFI groups by -0.42 to +0.52 g/L. Plasma urea values differed by +0.02 to -0.46 mmol/L with low RFI heifers in late

pregnancy having significantly ($P < 0.05$) lower urea concentrations at late gestation. Plasma CK values differed by -42.7 to +113.62 U/L. Creatine kinase activity in low RFI open heifers was significantly higher ($P < 0.05$) than high RFI heifers while more feed efficient heifers in early and late pregnancy showed lower CK activities with a significant difference ($P < 0.05$) in late gestation. Plasma GLDH values differed by -0.63 to -1.68 U/L. Plasma AST values differed by -3.40 to 7.62 U/L with a significantly ($P < 0.05$) higher activity of AST in low RFI open heifers. Plasma carbon dioxide values differed by -0.57 to -1.34 mmol/L with all values lower in low RFI heifers with a significant difference in open heifers. The least square means over the circadian period of measured analytes that were found to have significant differences between low and high RFI animals are shown in Figure 3.2.

DISCUSSION

Values of DMI were similar to those measured by Bingham et al. (2009), Lancaster et al. (2009) and Kelly et al. (2010b) on growing heifers. Statistical difference was observed between the mean RFI value of -0.58 kg DM/ day in the low RFI group and the mean value of 0.41 kg DM/day in the high RFI group. The difference in feed intake of 0.99 kg DM/day is similar to the value of 0.87 kg DM/day found by Durunna et al. (2012), and the value of 1.15 kg/d found by Kelly et al. (2010a) in beef heifers. However, this difference is lower than the published value of 1.44 kg DM/day in young growing cattle reported by Basarab et al. (2003). The higher values of Basarab et al. (2003) are likely due to a larger population and the use of steers instead of heifers.

Values of albumin, urea and AST were in the range found by Doorenbal et al. (1988) and Grunwaldt et al. (2005) in beef heifers. Creatine kinase levels were higher than those observed in most studies but still within the range set by Latimer (2011) in cows. Observed GLDH levels

were lower than literature values (Latimer, 2011). Carbon dioxide levels were within the reference range of Kaneko et al. (2008) and Latimer (2011) in cows.

It is important to assess the variations that exist in blood analytes in order to determine how these differences are reflective of differences in animal feed efficiency. In animals, many physiological systems have some degree of circadian rhythm (Piccione and Caola, 2002). Circadian variation in blood metabolites may give insight into the circadian variation in feed intake of cattle. The major reason for studying eating and ruminating patterns is to understand both behavioral and physiological processes that control feeding since they may impact animal performance (Panksepp, 1978). Furthermore, pregnancy is one of the main physiological states during which large metabolic adaptations occur due to changes in the partitioning of nutrients to support growth of the conceptus, reproductive tissues and non-reproductive tissues (Bell and Bauman, 1997; Wallace, 2000; Herrera, 2002). Energy expenditures of pregnant cows have been determined to increase from 644 kcal/d in early gestation to 3,572 kcal/d in late gestation, but only approximately 50% of this increase can be accounted for by the uterus (Ferrell et al., 1976, Scheaffer, 1997). The remaining expenditures associated with gestation is hypothesized to be used by maternal tissues involved in service functions, including kidney and liver function (Ferrell, 1988 and 1991; Magness, 1998).

Circadian variation in analyte concentrations

Grazing cattle have a "crepuscular" feeding pattern, with a large proportion of intake consumed at dawn and dusk (Reviewed by Albright, 1993). A series of papers (Putnam and Davis, 1963; Putnam et al., 1964; Putnam et al., 1967) demonstrated that most of the eating activity of feedlot steers occurred between 06:00 and 18:00 hours. At the same time, when feed is first provided, over 16% of daily intake is consumed in the first 2 hours after feed is provided, which is nearly twice steady state consumption (Harvatine et al., 2012).

The greatest change in measured plasma analytes tended to occur between 8:00 and 16:00 which could be explained by the increase in eating activity that tends to occur between 6:00 and 18:00 (Putnam and Davis, 1963; Putnam et al., 1964; Putnam et al., 1967) and from the addition of feed to the bunk (Harvatine et al., 2012).

Plasma albumin tended to increase at 8:00, which lasted until 12:00 and began to decrease until 21:00 where another increase occurred. Albumin is synthesized in the liver and exported to the blood stream. The circadian pattern of albumin concentration after feeding agrees with Simon and Bergner (1983) who found that in rats, before food intake, the fractional rate of protein synthesis in liver was 55 to 67 %/d and it increased 1 to 4 hours after food ingestion to 80 to 120 %/d.

Plasma urea increased to a maximum at 20:00 before decreasing throughout the remainder of the day. This pattern of increase after a meal is relatively well known in ruminants (Manston et al. 1981; Gustafsson and Palmquist, 1993; Blum et al., 2000; Plaizier et al., 2005). However previous studies have found that the rhythm of urea vanishes when the animals are food deprived, indicating that it is driven by ingestion (Piccione et al., 2003).

Plasma CK showed an increase until 12:00 and then steadily declined. Haus and Touitou (1992) and Rivera-Coll et al. (1993) found that in humans, serum CK level peaked in the early afternoon. However, the daily changes were largely reduced under resting conditions (Gutenbrunner et al., 2000), indicating that they are largely due to physical activity and associated damage of cardiac and skeletal muscle cells.

Plasma GLDH showed the most variation in its circadian rhythm between physiological states. In open heifers plasma GLDH exhibited a small decrease until 16:00 and remained steady thereafter. In early and late gestation, plasma GLDH tended to increase until 11:00 and then decreased to 23:00, where an increase occurred. Caldeira et al. (1999) also found an increase in GLDH activity after feeding in sheep relating to the flux of amino acids.

Plasma AST tended to increase from 08:00 to 12:00 and remained relatively stable thereafter. Rivera-Coll et al. (1993) found that, in humans, an increase in AST occurred in the morning relating to feeding and flux of amino acids.

Plasma carbon dioxide tended to decrease until 01:00 when a steady state occurred. Piccione et al. (2004) also observed a decrease in plasma bicarbonate in the morning. The circadian oscillation of CO2 may be due to its production in tissues, since metabolic rate has been shown to increase 60 % during eating in sheep (Christopherson and Webster, 1972). It could also be interpreted in relation to pH stabilization to compensate for acid production associated with nutrient digestion and metabolism after feeding.

The circadian rhythm of all analytes measured showed patterns with acrophases occurring mainly between 08:00 and 16:00. This shows a distinct circadian rhythm of blood analytes associated with visceral organs.

Effect of physiological status on analyte concentrations

Albumin concentrations in plasma were higher in pregnant than in open heifers with higher levels in early than in late pregnancy. Decreases in plasma proteins throughout pregnancy have been observed (Brozostowski et al., 1996; El-Sherif and Assad, 2001; Antunovic et al., 2002 and are explained by fetal demands for albumin as a source of amino acids for development (Jainudeen and Hafez, 1994). The lowest level that was observed in open heifers may be a result of high protein utilization for growth.

Circulating urea concentrations increased from open to early pregnancy and then decreased again at late pregnancy. Greater urea concentration can be a result of catabolizing amino acids when provided in excess or increased degradation of protein in the rumen. Our findings are in agreement with those of Greenfield et al. (2000) and Antunovic et al. (2002), who

found a steady decline in plasma urea nitrogen in dairy cattle prior to calving, indicating a greater efficiency of amino acid use for protein synthesis.

Creatine kinase concentrations decreased from open to pregnant and late-pregnant heifers. Antunovic et al. (2011) also found a decrease in CK concentrations between non-pregnant and pregnant. The highest level observed in open heifers may have less to do with physiological status and more to do with the age of the heifers. Metabolic rate tends to slow down as an animal ages where adults usually show lower levels of skeletal muscle protein synthesis, degradation, and accretion (Castro Bulle et al., 2007) and CK is an indicator of myogenic growth (Hagiwara et al., 1989; Guglielmo et al., 2001). Higher plasma CK activity has been observed during rapid growth in turkeys (Wilson et al., 1990; Mills et al., 1998) and pigs (Mitchell and Heffron, 1975).

Circulating concentrations of GLDH were highest in early pregnancy and did not differ between open and late pregnancy. Glutamate dehydrogenase catalyzes the deamination of glutamate as part of ureagenesis (Kravos and Malesic, 2008). The pattern of levels in GLDH followed those of urea, possibly indicating that amino acid catabolism decreased throughout pregnancy while amino acids were partitioned into the fetus for growth. We are unaware of other studies on the effects of pregnancy on circulating GLDH.

Plasma concentrations of AST decreased throughout pregnancy. Studies in ewes have found increases in AST in pregnant ewes (Baranowski and Kmiec, 1997; El-Sherif and Assad, 2001; Antunovic et al., 2002) and in goats (Jana et al., 1991). Alternatively, Ramos et al. (1994) and Lebedevaa et al. (2012) found a drop towards the end of pregnancy, which may be explained by greater liver weight agreeing with our findings. As well, the highest level in open heifers may be associated with the muscle mass growth that can increase the serum AST activity (Soback et al., 1985).

Levels of carbon dioxide were lower in pregnant heifers then in open heifers and did not differ between open and late pregnancy. Production of CO_2 by portal drained viscera can be attributed to respiratory metabolism, ruminal fermentation, and salivary bicarbonate from the rapid transfer of CO2 across the rumen epithelium (Pell et al., 1986). The highest level observed in open heifers may be reflective of an increase in rate of metabolism (Arthurs and Sudhaker, 2005).

It was observed here that most of the measured analytes were at a level that indicated greater metabolism and organ function not in late gestation but instead in open heifers. Bauman and Currie (1980) proposed that maternal adjustments during pregnancy were both homeostatic and homeorhetic. Previous studies have indicated that pregnancy may increase the efficiency of visceral metabolism to act as an energy conservation tool designed to support pregnancy (Ferrell et al., 1976; Scheaffer et al., 2001; Scheaffer et al., 2003).

Differences in circulating analyte concentrations with RFI

In open heifers, significant differences between high and low RFI heifers were found in circulating concentrations of plasma CK, AST, and carbon dioxide. Plasma CK concentration were higher in low RFI heifers. This is the opposite trend of what was observed by Richardson et al. (2004) and Lawrence et al. (2011) who found that low RFI cattle had lower concentrations of CK, though neither were significant. Circulating concentrations of AST were higher in low RFI heifers (P= 0.0123). A study by Richardson et al. (2004) looked into the associations of efficiency, measured as RFI, on AST and found that blood plasma samples taken from the steers at weaning showed a positively phenotypic correlation of AST with steer RFI over the whole experiment. Following transport, this correlation was negative, and returned to a positive correlation in steers sampled at the start of feedlot RFI test. Lawrence et al. (2011) found the opposite pattern of what we observed with a lower concentration of AST in low RFI heifers.

Higher activity of plasma AST in low RFI heifers may indicate a greater workload placed on the organ for enzyme synthesis. The functional workload concept defined by Johnson et al. (1990), states that as metabolizable energy intake increases, there is a change in the mass, function and resulting energy consumption of tissues. Therefore a higher activity of AST may indicate an overall higher metabolic function of liver of low RFI yearling heifers. Plasma carbon dioxide was higher in high RFI heifers (P= 0.0137). This may indicate greater hepatic metabolism (Antunovic et al., 2011) due to rapid gluconeogenesis (Krebs, 1966). No previous studies have looked into the association of RFI on carbon dioxide concentrations. The amount produced depends on the rate of metabolism and the relative amounts of carbohydrate, fat and protein metabolized (Arthurs and Sudhaker, 2005). Therefore a lower amount of CO2 produced may be indicative of a lower workload placed on the visceral organs and consequently a lower energy expenditure of the visceral organs decreasing animal energy requirements. A lower carbon dioxide level in more feed efficient heifers may indicate a lesser amount of fat or protein metabolism from body reserves, which could translate into greater efficiency.

In early pregnancy, no significant differences were observed in blood analyte levels between RFI groups. When looking at differences between circulating analytes of high and low RFI in late pregnancy, urea and CK concentrations were significantly different. Circulating concentrations of urea were found to be lower in low RFI heifers (P= 0.0232). The same trend was observed in studies by Richardson et al. (2004) with a significant difference between groupings at the beginning of the trial and from Kelly et al. (2010a and b); Kelly et al. (2011). The lower concentration of urea in plasma of low RFI heifers means that the visceral organs are expending less energy for urea synthesis (McBride et al., 1990). The cost of urea synthesis in the ruminant liver is a major energy-consuming event and any process that alters ammonia production could alter energy use by the liver (McBride et al., 1990). It may also be possible that greater systemic concentrations of urea in high RFI animals is a function of their greater protein

intake and potentially greater ruminal passage rate (Hegarty et al., 2007), poorer protein digestibility, greater rate of body protein degradation, or deviation in supply of amino acids, in part because of variation in efficiency of microbial protein production in the rumen (Lush et al., 1991; Kahn et al., 2000). In addition, urea, the major mammalian end product of ammonia and amino acid metabolism, is produced by the liver in greater amounts than are eliminated in the urine (Sarrasecal et al., 1998). Ruminants recycle substantial amounts of nitrogen as urea by transfer from blood to the lumen of the gut, which is an important source of nitrogen for synthesis of microbial proteins (Egan et al. 1986; Sakata et al. 2003). In cattle, as much as 40 to 80% of urea produced by the liver can enter the gastrointestinal tract (Harmayer and Martens, 1980). Therefore less plasma urea may indicate a greater transfer efficiency of urea from blood to the rumen potentially translating to greater synthesis of microbial protein for body deposition. Against the trend observed in open heifers in our study, plasma CK concentrations were lower in low RFI heifers, which agree with the findings of Richardson et al. (2004) and Lawrence et al. (2011). The serum level of CK is a marker of the functional status of muscle tissue (Brancaccio et al., 2007). An increase in the activity of CK in the plasma can be caused by muscle turnover since protein degradation accounts for 7-10 % of muscle tissue energy use as well as a further 20% use for protein synthesis it is a very energy demanding process (Caton et al., 2000). Therefore, more efficient cattle put less energy expenditure to protein turnover.

Moreover, the differences in the circadian rhythms of the RFI groups can give insight into underlying causes of differences in efficiency. Circadian rhythms allow organisms to predict and prepare for changes in the environment before they occur allowing the animal to be more proactive instead of reactive (Davidson et al., 2004). For example, synthesizing and releasing enzymes prior to the ingestion of a meal can enable digestion to begin immediately, making the metabolic fuel available in a more efficient manner (Saito et al., 1976). This timing could also apply to the release of enzymes by the liver. The only enzyme that was found to be significant

between RFI groups was AST in open heifers. The higher activity of AST in low RFI heifers could indicate a better preparedness of the animal for the meal indicating greater efficiency of digestion after the meal.

The workload concept defined by Johnson et al., 1990 could be utilized to explain some of the variations observed between RFI groups. Since metabolic activity per unit of tissue greatly relates to animal energy requirements (Burrin et al., 1990), less metabolic workload placed on the organs may relate to less energy put towards them, allowing for greater efficiency of ingested feed, reflective on differences in RFI.

Several correlations between AST and analytes throughout stages. In open heifers, AST showed a positive correlation of 0.68 with CK. In heifers in early gestation, AST showed positive correlations CK (r= 0.57), GLDH (r= 0.55) and albumin (r= 0.56). Additionally, heifers in late gestation had a positive correlation of 0.50 between AST and CK and 0.54 with albumin. The overall positive correlations observed show consistencies between indicators of visceral organs function. The positive correlations of plasma AST activity with albumin concentrations through pregnancy could be due to the involvement of AST in amino acid metabolism. Baranowski (1995) also connected the concentration of the total protein in blood to AST activity. The associations observed between AST and GLDH indicate the consistency of liver enzymes with variations in organ workload.

CONCLUSION

Significant patterns of circadian variation with each analyte were found. The circadian rhythm indicated an increase of workload on organs and muscle in the morning relating to feed intake. There were differences in analyte levels between physiological statuses with significant differences in almost every analyte with the highest workload in yearling heifer to meet

requirements for growth. There were also associations between RFI and blood analytes which give indications into metabolic rates. More efficient yearlings appear to have greater metabolic rate in liver and muscle while more efficient pregnant heifers appear to have lower metabolic rate in liver and muscle. It was found that these hematological measures may have the potential for the application as indirect measures of RFI. Furthermore it was observed that the circadian variation was consistent between RFI groups throughout the day indicating that a specific time of day for sampling is not important. However physiological status must be factored into measurement. Based on these findings, carbon dioxide shows the most promise as an indicator of RFI since it held consistency between physiological states. Combinations of indicators may be more effective as an indirect screening method for feed efficiency in cattle. Further studies are required to see if these associations hold up under different housing conditions or with different animal types including cows, steers or bulls.

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Table 3.1. Average animal parameters and standard deviation for each sampling period

Traits (abbreviation; unit)	Open	Early Gestation	Late Gestation
Animal parameters			
Stage	open	early gestation	late gestation
Age (d)	367 ± 15	542 ± 23	704 ± 25
Body weight (BW; kg)	354 ± 49	501 ± 57	645 ± 62
Days in gestation (DIG; d)	0	82 ± 24	244 ± 4

Table 3.2. Ingredient composition of the experimental diet

	NE (Mcal/kg DM)	% as fed basis	% inclusion in diet on DM basis
Diet 1	1.60[m]; 0.99[g]		
Corn Silage		49.75	50.29
Haylage		49.75	48.17
Premix A[a]		0.50	1.53
Diet 2	1.41[m]; 0.82[g]		
Corn Silage		17.00	15.60
Haylage		74.97	65.90
Wheat Straw		7.30	16.70
Premix B[b]		0.73	1.80

DM = dry matter; NE = net energy.
[a] Contains 40% of calcium phosphate, 60% trace mineralized salt (96.5% NaCl, 7500 mg/kg Zn, 5000 mg/kg Mn, 2500 mg/kg Cu, 1600 mg/kg Fe, 70 mg/kg I, 40 mg/kg Co), 600, 000 IU/kg vitamin A, 200, 000 IU/kg vitamin D and 1, 000 IU/kg vitamin E
[b] Contains 43% of calcium phosphate, 57% trace mineralized salt (96.5% NaCl, 7500 mg/kg Zn, 5000 mg/kg Mn, 2500 mg/kg Cu, 1600 mg/kg Fe, 70 mg/kg I, 40 mg/kg Co), 600, 000 IU/kg vitamin A, 200, 000 IU/kg vitamin D and 1, 000 IU/kg vitamin E
[m] Net energy for maintenance calculated according to Weiss et al., (1992) and NRC (1996).
[g] Net energy for growth calculated according to Weiss et al., (1992) and NRC (1996).

Table 3.3. Descriptive statistics

Traits (abbreviation; unit)	Mean	S.D.	Minimum	Maximum
Performance traits[a]				
Dry matter intake (DMI; kg /day)	10.91	0.93	9.14	13.19
Residual Feed Intake (RFI; kg DM/day)	0.00	0.65	-1.31	1.74
Average body weight (kg)	553.00	58.00	420.00	667.00
Analyte Concentrations/ Activity				
Open				
Albumin (g/L)	32.60	3.66	17.0	41.00
Urea (mmol/L)	3.85	1.20	1.30	6.90
Creatine kinase (U/L)	261.00	1.80	90.00	2495.00
Glutamate dehydrogenase (U/L)	7.25	1.59	1.00	28.00
Aspartate aminotransferase (U/L)	61.50	1.27	27.00	140.0
Carbon Dioxide (mmol/L)	24.20	2.68	17.00	32.00
Early Gestation				
Albumin (g/L)	36.20	4.12	18.00	50.00
Urea (mmol/L)	5.03	1.11	2.40	8.20
Creatine kinase (U/L)	244.00	1.60	96.00	1101.00
Glutamate dehydrogenase (U/L)	8.12	1.52	2.00	19.00
Aspartate aminotransferase (U/L)	59.00	1.20	31.00	101.00
Carbon Dioxide (mmol/L)	20.20	2.49	12.00	29.00
Late Gestation				
Albumin (g/L)	35.20	4.00	19.00	49.00
Urea (mmol/L)	3.91	0.99	1.50	6.90
Creatine kinase (U/L)	160.00	1.54	49.00	774.00
Glutamate dehydrogenase (U/L)	6.96	1.54	2.00	18.00
Aspartate aminotransferase (U/L)	57.10	1.18	31.00	85.00
Carbon Dioxide (mmol/L)	20.14	2.30	12.00	28.00
Overall mean for all samples				
Albumin (g/L)	34.60	4.20	17.00	50.00
Urea (mmol/L)	4.25	1.23	1.30	8.20
Creatine kinase (U/L)	217.00	1.74	49.00	2495.00
Glutamate dehydrogenase (U/L)	7.42	1.56	1.00	28.00
Aspartate aminotransferase (U/L)	59.20	1.22	27.00	140.00
Carbon Dioxide (mmol/L)	21.54	3.14	12.00	32.00

[a] Over the duration of feed intake measurements

Table 3.4. Correlations between blood analytes

Traits (abbreviation; unit)	ALB (g/L)	UREA (mmol/L)	CK (U/L)	GLDH (U/L)	AST (U/L)	CO_2 (mmol/L)
Open						
ALB (g/L)		0.06	-0.07*	0.38**	0.09**	0.15**
UREA (mmol/L)			0.19**	0.23**	0.27**	0.25**
CK (U/L)				0.22**	0.68**	0.04
GLDH (U/L)					0.39**	0.06
AST (U/L)						0.19**
CO_2 (mmol/L)						
Early Gestation						
ALB (g/L)		0.04	0.11**	0.54**	0.56**	0.19**
UREA (mmol/L)			0.01	0.06	0.03	0.24**
CK (U/L)				0.43**	0.57**	-0.01
GLDH (U/L)					0.54**	0.08*
AST (U/L)						0.14**
CO_2 (mmol/L)						
Late Gestation						
ALB (g/L)		-0.01	0.001	0.42**	0.54**	0.18**
UREA (mmol/L)			0.14**	-0.09*	-0.06	0.03
CK (U/L)				0.26**	0.50**	-0.11**
GLDH (U/L)					0.40**	0.08*
AST (U/L)						0.11**
CO_2 (mmol/L)						
Overall mean for all samples						
ALB (g/L)		0.14**	-0.04*	0.44**	0.30**	-0.08**
UREA (mmol/L)			0.16**	0.13**	0.09**	-0.009
CK (U/L)				0.30**	0.60**	0.14**
GLDH (U/L)					0.43**	0.03
AST (U/L)						0.20**
CO_2 (mmol/L)						

ALB= albumin; UREA= urea; CK= creatine kinase; GLDH= glutamate dehydrogenase; AST= aspartate aminotransferase; CO_2 = carbon dioxide
* $P<0.05$; ** $P<0.01$

Table 3.5. Least square means by stage

Traits (abbreviation; unit)	Open	Early Gestation	Late Gestation
Analyte Concentrations/ Activity			
Albumin (g/L)	32.50 ± 0.55^{a}	36.40 ± 0.55^{b}	35.00 ± 0.55^{c}
Urea (mmol/L)	3.81 ± 0.10^{a}	5.10 ± 0.10^{b}	3.85 ± 0.10^{ac}
Creatine kinase (U/L)	254.00 ± 1.06^{a}	242.00 ± 1.06^{b}	157.00 ± 1.06^{c}
Glutamate dehydrogenase (U/L)	7.05 ± 1.08^{a}	7.95 ± 1.08^{b}	6.93 ± 1.08^{ac}
Aspartate aminotransferase (U/L)	61.30 ± 1.02^{a}	58.90 ± 1.02^{b}	57.10 ± 1.02^{c}
Carbon Dioxide (mmol/L)	24.00 ± 0.25^{a}	20.10 ± 0.25^{b}	20.10 ± 0.25^{bc}

Means within a row with different superscripts differ based on Scheffe multiple comparison test ($P<0.05$)

Table 3.6. Least square means by RFI group

Traits (abbreviation; unit)	Low RFI	High RFI	P Value
Residual feed intake (RFI; kg DM/d)	-0.47 ± 0.37	0.47 ± 0.52	<0.001
Analyte Concentrations/ Activity			
Open			
Albumin (g/L)	32.30 ± 0.78	32.70 ± 0.78	0.7082
Urea (mmol/L)	3.82 ± 0.14	3.80 ± 0.14	0.9104
Creatine kinase (U/L)	318.00 ± 1.09	204.00 ± 1.09	0.0007
Glutamate dehydrogenase (U/L)	6.74 ± 1.12	7.38 ± 1.12	0.5773
Aspartate aminotransferase (U/L)	65.20 ± 1.03	57.60 ± 1.03	0.0123
Carbon Dioxide (mmol/L)	23.40 ± 0.35	24.70 ± 0.35	0.0137
Early Gestation			
Albumin (g/L)	36.30 ± 0.78	36.40 ± 0.78	0.9781
Urea (mmol/L)	5.03 ± 0.14	5.18 ± 0.14	0.4264
Creatine kinase (U/L)	223.00 ± 1.09	262.00 ± 1.09	0.1871
Glutamate dehydrogenase (U/L)	7.00 ± 1.12	8.83 ± 1.12	0.1941
Aspartate aminotransferase (U/L)	57.24 ± 1.03	60.64 ± 1.03	0.2296
Carbon Dioxide (mmol/L)	19.70 ± 0.36	20.50 ± 0.36	0.1259
Late Gestation			
Albumin (g/L)	35.30 ± 0.78	34.80 ± 0.77	0.6448
Urea (mmol/L)	3.62 ± 0.14	4.08 ± 0.14	0.0232
Creatine kinase (U/L)	137.00 ± 1.09	179.00 ± 1.09	0.0288
Glutamate dehydrogenase (U/L)	6.37 ± 1.12	7.54 ± 1.12	0.2974
Aspartate aminotransferase (U/L)	56.60 ± 1.03	57.64 ± 1.03	0.7025
Carbon Dioxide (mmol/L)	19.80 ± 0.36	20.40 ± 0.36	0.2727
Overall mean for all samples			
Albumin (g/L)	34.60 ± 0.77	34.60 ± 0.77	0.9842
Urea (mmol/L)	4.00 ± 0.13	4.35 ± 0.13	0.3039
Creatine kinase (U/L)	213.00 ± 1.09	212.00 ± 1.09	0.9716
Glutamate dehydrogenase (U/L)	6.75 ± 1.12	7.89 ± 1.12	0.3300
Aspartate aminotransferase (U/L)	59.60 ± 1.03	58.60 ± 1.03	0.7283
Carbon Dioxide (mmol/L)	21.00 ± 0.36	21.90 ± 0.36	0.0795

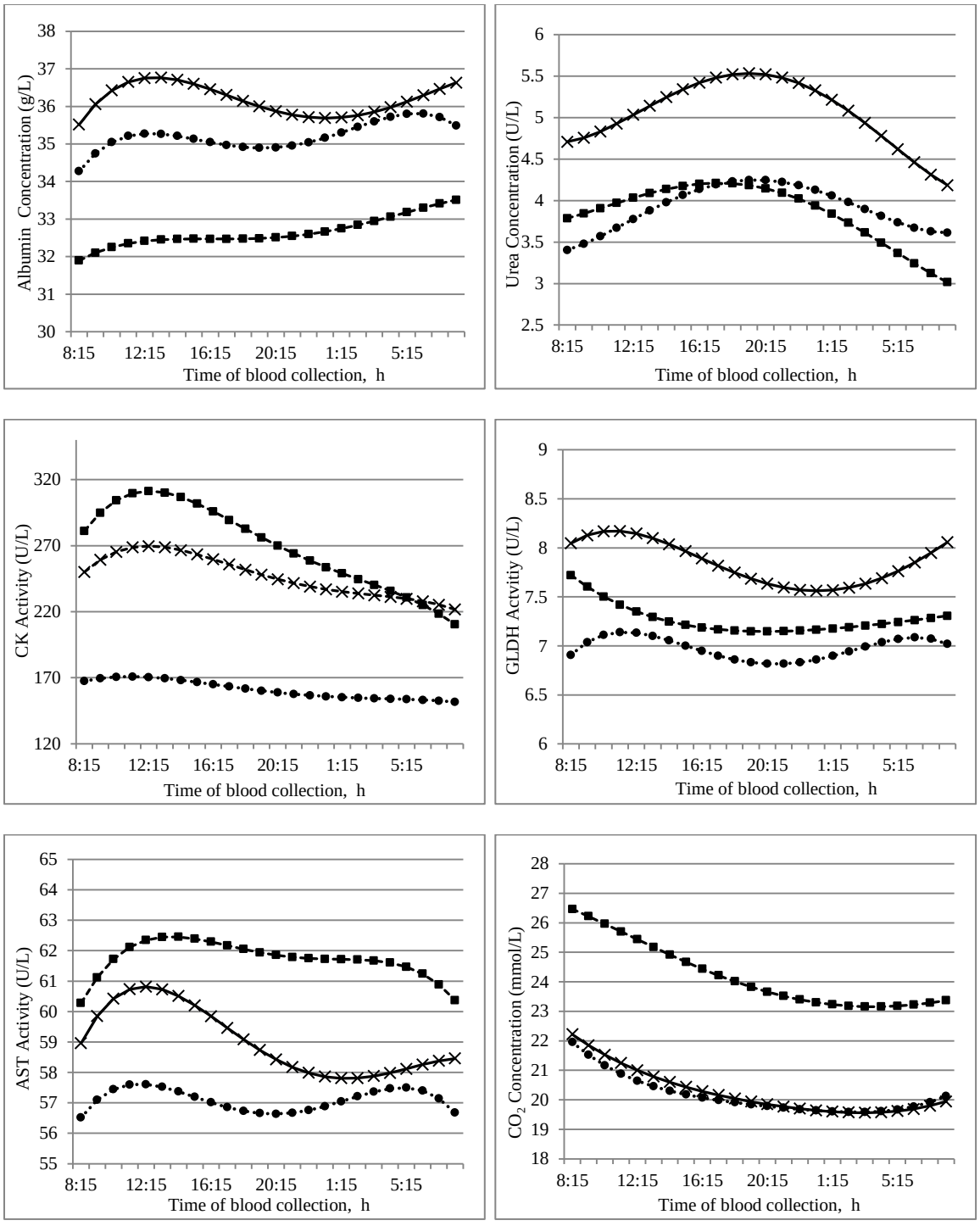

Figure 3.1. Circadian profiles of plasma albumin, urea, CK, GLDH, AST and CO_2 heifers at open (-■-), early gestation (—×—) and late gestation (···●···)

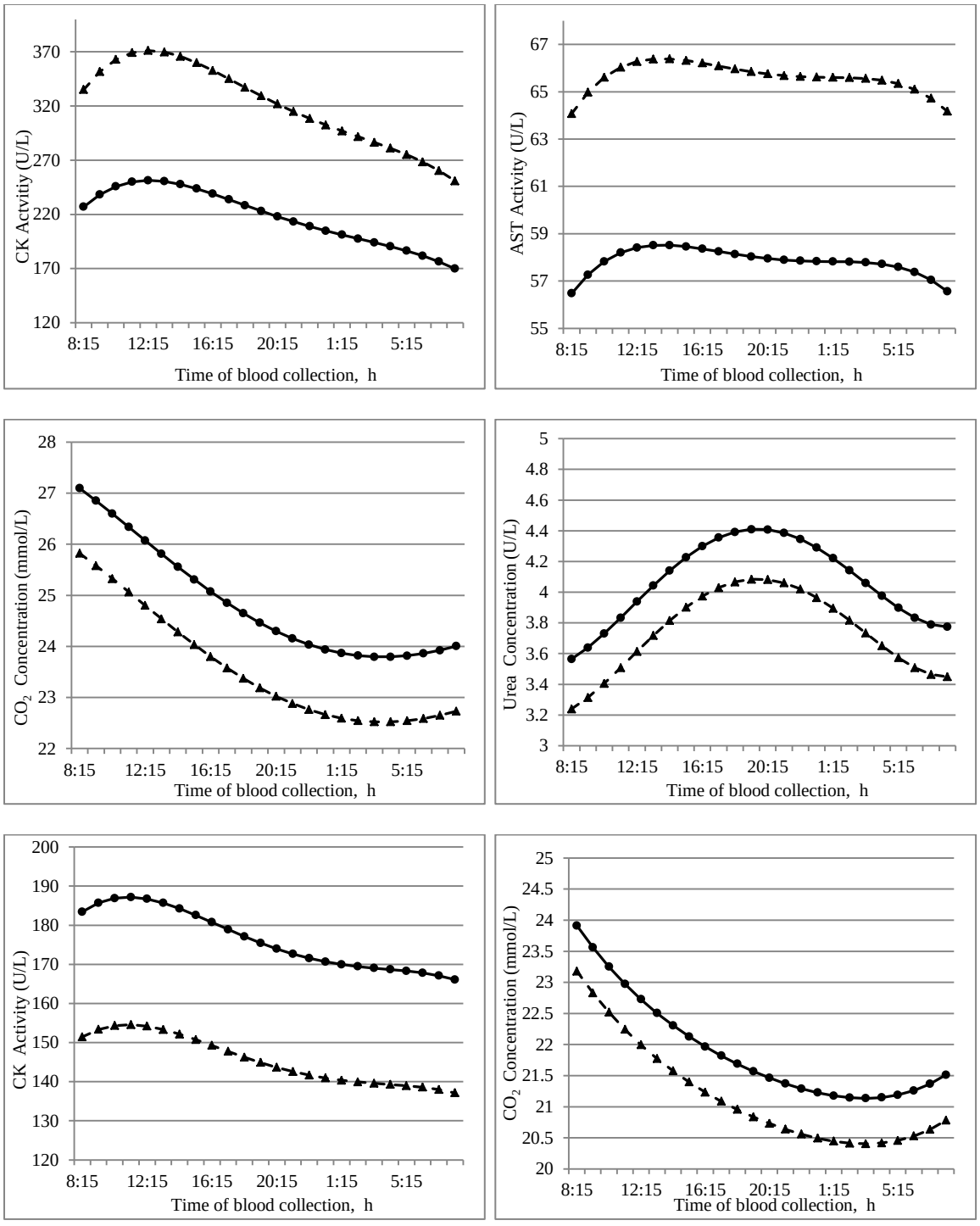

Figure 3.2. Circadian profiles of plasma CK, AST and CO_2 of open heifers, plasma urea and CK of heifers in late gestation and the average CO_2 separated by low RFI (▬▲▬) and high RFI (▬●▬)

Chapter 4

GENERAL DISCUSSION

In this research, the circadian variations of blood plasma analytes in beef heifers at three different physiological states with known feed efficiency were assessed. This was done to give insight into factors impacting variations in feed efficiency as well as to assess the effectiveness of these analytes as indirect measures of feed efficiency. Evaluating the efficiency of feed use is important so that progress can be done on its improvement to counteract the rising feed and land prices, which would allow for increased profitability and industry growth and to offset the environmental footprint of beef cattle production.

The limitation to the application of selection for improved feed efficiency is due to the laborious and costly measurements of individual feed intake and body weight measurements over a relatively lengthy time period. Biological indicators of feed efficiency represent alternative measurements that are more easily measured and provide insight into the individual variation in the efficiency of utilization of feed.

In this study, feed efficiency, measured as RFI, was determined in 36 heifers. Blood samples were collected on these heifers hourly over a 24 hour time period at three different times corresponding approximately to the yearling stage, early gestation, and late gestation. Plasma samples were analyzed for concentrations of albumin, urea, CK, GLDH, AST and CO_2. Measured values of analytes were assessed to determine their variation through the circadian cycle, their variability through physiological states and well as associations with RFI to provide insight into their effectiveness as indirect measures of feed efficiency. There was significant variation in analytes associated with feeding patterns. However the patterns of eating were consistent between RFI groups indicating that time of day for sampling may not need to be factored into sampling of blood analytes for RFI determination. On the other hand there was found to be significant

variation with physiological states on concentrations of analytes indicating that the physiological status at time of sampling should be factored into the assessment of RFI. RFI was found to be significantly associated with analytes in open and late pregnancy. Of the analytes that were found to be significant and show the most potential for its application as an indirect measure of RFI were plasma CO_2 and CK in open heifers, plasma urea or CK in late gestation or plasma CO_2 overall. Plasma carbon dioxide was lower in low RFI open heifers, which may indicate lesser hepatic metabolism (Antunovic et al., 2011). A lower amount of CO_2 produced may be indicative of a lower workload placed on the visceral organs and consequently a lower energy expenditure of the visceral organs decreasing animal energy requirements. Circulating concentrations of urea were found to be lower in low RFI heifers. The lower concentration of urea in plasma of low RFI heifers means that the visceral organs are expending less energy for urea synthesis (McBride et al., 1990). The cost of urea synthesis in the ruminant liver is a major energy-consuming event and any process that alters ammonia production could alter energy use by the liver (McBride et al., 1990). Urea is the major mammalian end product of amino acid metabolism. It produced by the liver in greater amounts than are then eliminated in the urine (Sarrasecal et al., 1998). Ruminants recycle substantial amounts of nitrogen as urea by transfer from blood to the lumen of the gut, which is an important source of nitrogen for synthesis of microbial proteins (Egan et al. 1986; Sakata et al. 2003). Therefore less plasma urea may indicate a greater transfer efficiency of urea from blood to the rumen potentially translating to greater synthesis of microbial protein for body deposition. Plasma CK concentrations were lower in low RFI heifers. The serum level of creatine kinase is a marker of the functional status of muscle tissue (Brancaccio et al., 2007). An increase in the activity of creatine kinase in the plasma can be caused by exercise (Holmes et al., 1973). Since it has been found that more efficient bulls are generally less active and were found to take 6% less steps than less efficient bulls (Richardson et al., 2000), this lower level of CK may be

indicative of less physical activity. Further studies are required to see if these associations hold up under different situation with bulls or feedlot cattle.

Overall these results suggest the potential for the alterative assessment in beef cattle for RFI and provides insight into observed differences in feed efficiency due differences in metabolism and function of the visceral organs altering animal energy requirements. A combination of these enzymes and metabolites may be effective indicators of feed efficiency.

Chapter 5

LITERATURE CITED

Agdex (2002) Breeding soundness evaluation of bulls [Online]. Alberta Agriculture and Rural Development, Agdex 423/15-1. Available at: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3545 (verified March 2002)

Agenas S, Heath MF, Nixon RM, Wilkinson JM, Philips CJC (2006) Indicators of under nutrition in cattle. Animal Welfare 15, 149- 160.

Albright JJ (1993) Feeding Behavior of Dairy Cattle. Journal of Dairy Science 76, 485-498.

Antunovic Z, Sencic D, Speranda M, Liker B (2002) Influence of the season and the reproductive status of ewes on blood parameters. Small Ruminant Research 45, 39-44.

Antunovic Z, Novoselec J, Sauerwein H, Speranda M, Vegara M, Pavic V (2011) Blood metabolic profile and some of hormones concentration in ewes during different physiological status. Bulgarian Journal of Agricultural Science 17, 687-695.

Aranas TJ, Roussel JD, Seybt SH (1987) Circadian rhythm of aldosterone in dairy cattle during the summer. International Journal of Biometeorology 31, 237-247.

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

Arthur PF, Archer JA, Herd RM, Richardson EC (1999) Relationship between postweaning growth, net feed intake and cow performance. In ´Proceedings of the 13th Conference on the Association for the Advancement of Animal Breeding and Genetics´. pp 484- 487 (Mandurah, Australia)

Arthur PF, Archer JA, Herd RM, Melville GJ (2001a) Response to selection for net feed intake in beef cattle. Proceedings of Advanced Animal Breeding and Genetics 14, 135- 138.

Arthur PF, Archer JA, Johnston DJ, Herd RM, Richardson EC, Parnell PF (2001b) Genetic and phenotypic variance and covariance components for feed intake, feed efficiency and other postweaning traits in Angus cattle. Journal of Animal Science 79, 2805- 2811.

Arthur PF, Archer JA, Herd RM (2004) Feed intake and efficiency in beef cattle, overview of recent Australian research and challenges for the future. Australian Journal of Experimental Agriculture 44, 361-369.

Arthur PF, Herd RM (2005) Efficiency of feed utilization by livestock - Implications and benefits of genetic improvement. Canadian Journal of Animal Science 85, 281- 290.

Arthur PF and Herd RM (2008) Residual feed intake in beef cattle. Revista Brasileira de Zootecnia 37, 269-279.

Arthur PF, Herd RM, Wilkins JF, Archer JA (2005) Maternal productivity of Angus cows divergently selected for post-weaning residual feed intake. Australian Journal of Experimental Agriculture 45, 985- 93.

Arthurs GJ, Sudhakar M (2005) Carbon dioxide transport. Continuing Education in Anaesthesia, Critical Care & Pain 5, 207-210.

Awda BJ, Miller SP, Montanholi YR, Vandervoort G, Caldwell T, Buhr MM, Swanson KC (2013) The relationship between feed efficiency traits and fertility in young beef bulls. Canadian Journal of Animal Science 93, 185- 192.

Baker SD, Szasz JI, Klein TA, Kuber PS, Hunt CW, Glaze JB Jr, Falk D, Richard R, Miller JC, Battaglia RA, Hill RA (2006) Residual feed intake of purebred Angus steers, effects on meat quality and palatability. Journal of Animal Science 84, 938-45.

Baldwin RL (1995) Modeling Ruminant Digestion and Metabolism. (Chapman and Hall, London)

Ban-Tokuda T, Orden EA, Barrio AN, Lapitan RM, Delavaud C, Chilliard Y, Fujihara T, Cruz LC, Homma A, Kanai Y (2007) Effects of species and sex on plasma hormone and metabolite concentrations in crossbred Brahman cattle and crossbred water buffalo. Livestock Science 107, 244-252.

Barea R, Dubois S, Gilbert H, Sellier P, van Milgen J, Noblet J (2010) Energy utilization in pigs selected for high and low residual feed intake. Journal of Animal Science 88, 2062-2072.

Barendse W, Reverter A, Bunch RJ, Harrison BE, Barris W, Thomas MB (2007) A validated whole-genome association study of efficient food conversion in cattle. Genetics 176, 1893-1905.

Baranowski P (1995) Certain Blood Haematological and Biochemical Indicators and Enzyme Activities in polish Merino and Polish Merino x Suffolk Ewes during Pregnancy and lactation. Animal Science Papers and Reports 13, 27-33.

Barth AD (2000) ´Bull Breeding Soundness Evaluation (2nd edn).´ (The Western Canadian Association of Bovine Practitioners, Saskatoon, Saskatchewan)

Basarab JA, Price MA, Aalhus JL, Okine EK, Snelling WM, Lyle KL (2003) Residual feed intake and body composition in young growing cattle. Canadian Journal of Agricultural Research 83, 189- 204.

Basarab JA, McCartney D, Okine EK, Baron VS (2007) Relationships between progeny residual feed intake and dam productivity traits. Canadian Journal of Animal Science 87, 489-502.

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

Bell AW, Bauman DE (1997) Adaptations of glucose metabolism during pregnancy and lactation. Journal of Mammary Gland Biology and Neoplasia 2, 265- 278.

Benlamlih S, de Pomyers H (1989) Changes in endogenous Urea recycling and the handling of renal urea in pregnant and lactating Sardi Jheep kept on a constant feeding level. Reproduction Nutrition Development 29, 129-137.

Bergman EN (1973) Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Veterinary 63, 341- 382.

Bergman EN (1990) Energy contribution of VFA from the gastrointestinal tract in various species. Physiological Reviews 70, 567-590.

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

Bitman J, Lefcourt A, Wood DL, Stroud B (1984) Circadian and ultradian temperature rhythms of lactating dairy cows. Journal of Dairy Science 67, 1014- 1023.

Bitman J, Kahl S, Wood DL, Lefcourt AM (1994) Circadian and ultradian rhythms of plasma thyroid hormone concentrations in lactating dairy cows. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 266, R1797-R1803.

Black JL (1971) A theoretical consideration of the effect of preventing rumen fermentation on the efficiency of utilisation of dietary energy and protein in lambs. The British Journal of Nutrition 25, 31- 55.

Blum JW, Bruckmaier RM, Vacher PY, Munger A, Jans F (2000) Twenty-four-hour patterns of hormones and metabolites in week 9 and 19 of lactation in high-yielding dairy cows fed triglycerides and free fatty acids. Journal of Veterinary Medicine.A, Physiology, Pathology, Clinical Medicine 47, 43- 60.

Bollinger T, Wobeser G, Clark RG, Nieman DJ, Smith JR (1989) Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by three methods for banding. Journal of Wildlife Diseases 25, 225-231.

Bottje W, Tang Z, Iqbal M, Cawthon D, Okimoto R, Wing T, Cooper M (2002) Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. Poultry Science 81, 546- 555.

Bottje WG, Iqbal M, Pumford NR, Ojano-Dirain C, Lassiter K (2004) Role of Mitochondria in the Phenotypic Expression of Feed Efficiency. Journal of Applied Poultry Research 13, 94- 105.

Bottje WG, Carstens GE (2009) Association of mitochondrial function and feed efficiency in poultry and livestock species. Journal of Animal Science 87, E48–E63.

Bottje W, Brand MD, Ojano-Dirain C, Lassiter K, Toyomizu M, Wing T (2009) Mitochondrial proton leak kinetics and relationship with feed efficiency within a single genetic line of male broilers. Poultry Science 88, 1683–1693.

Bordas A, Tixier-Boichard M, Merat P (1992) Direct and correlated responses to divergent selection for residual food intake in Rhode Island Red laying hens. British Poultry Science 33, 741–754.

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

Brand MD (1990) The proton leak across the mitochondrial inner membrane. Biochimica et Biophysica Acta 1018, 128- 133.

Brand MD (1995) Measurement of mitochondrial protonmotive force. In ´Bioenergetics. A Practical Approach.´ (Eds GC Brown, CE Cooper) pp 39-62. (IRL Press, Oxford, UK)

Braastad BO, Katle J (1989) Behavioural differences between laying hen populations selected for high and low efficiency of feed utilisation. British Poultry Science, 30, 533- 544.

Brown EG, Carstens GE, Fox JT, Curley KO Jr., Bryan TM, Slay LJ, Welsh TH Jr., Randel RD, Holloway JW, Keisler DH (2004) Physiological indicators of performance and feed efficiency traits in growing steers and bulls. In ´Beef Cattle Research in Texas´. pp. 163-166. (Texas A&M University, College Station, TX.)

Brown EG (2005) ´Sources of biological variation in residual feed intake in growing and finishing steers.´ PhD thesis, Texas A&M University, College Station.

Brozostowski H, Milewski S, Wasilewska A, Tanski Z (1996) The influence of the reproductive cycle on levels of some metabolism indices in ewes. Archivum veterinarium Polonicum 35, 53- 62.

Brun-Bellut J (1996) Urea recycling in the rumen of dairy goats, effects of physiological stage and composition of intake. Small Ruminant Research 23, 83-90.

Bunger L, MacLeod MG, Wallace CA, Hill WG (1998) Direct and correlated effects of selection for food intake corrected for liveweight in the adult mouse. In ´Proceedings of the 6th world congress on genetics applied to livestock production´. Vol. 26. (Ed L Piper) pp. 97-100. (University of New England, Armidale)

Burrin DG, Ferrell CL, Eisemann JH, Britton RA, Nienaber JA (1989) Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. British Journal of Nutrition, 62 23-34.

Burrin DG, Ferrell CL, Britton RA, Bauer M (1990) Level of nutrition and visceral organ size and metabolic activity in sheep. British Journal of Nutrition 64, 439- 448.

Cai W, Casey DS, Dekkers JCM (2008) Selection response and genetic parameters for residual feed intake in Yorkshire swine. Journal of Animal Science 86, 287- 298.

Caldeira RM, Almeida MA, Santos CC, Vasques MI, Vaz Portugal A (1999) Daily variation in blood enzymes and metabolites in ewes under three levels of feed intake. Canadian Journal of Animal Science, 79, 157-164.

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

Campbell JW (1970) Comparative biochemistry of arginine and urea metabolism in invertrebrates. In ´Urea and the kindey´. (Eds B Schmidt- Nielsen) (Excerpta Medical Foundation, Amsterdam)

Cammack KM, Leymaster KA, Jenkins TG, Nielsen MK (2005) Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. Journal of Animal Science 83, 777-785.

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

Castro Bulle FCP, Paulino PV, Sanches AC and Sainz RD (2007) Growth, carcass quality, and protein and energy metabolism in beef cattle with different growth potentials and residual feed intakes. Journal of Animal Science 85, 928- 936.

Caton JS, Bauer ML, Hidari H (2000) Metabolic components of energy expenditure in growing beef cattle. Asian Australasian Journal of Animal Science 13, 702-710.

CCAC—Canadian Council on Animal Care (1993) Guide to the use of experimental Animals. Canadian Council on Animal Care, Ottawa, Ontario, Canada.

Chalmers GA, Barrett MW (1982) Capture myopathy. In ´Non-infectious diseases of wildlife´. (Eds GL Hoff, JW Davis). pp. 84-94. (Iowa State University Press, Ames, Iowa) Channon AF, Rowe JB, Herd RM (2004) Genetic variation in starch digestion in feedlot cattle and its association with residual feed intake. Australian Journal of Experimental Agriculture 44, 469- 474.

Chen Y, Gondro K, Quinn K, Herd R (2008) Gene expression for cattle with high or low feed efficiency (Abstract). In ´Proceedings from the International Society of Animal Genetics´. (Wiley, Hoboken, NJ)

Chen Y, Gondro C, Quinn K, Herd RM, Parnell PF, Vanselow B (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal Genetics 42, 475-90.

Chillard Y, Ferlay A, Delavaud C, Bocquier F (1998) Plasma leptin in underfed or overfed adult Holstein and Charolais cows and its relationship with adipose tissue cellularity. International Journal of Obesity 22(Suppl. 3), S171.

Christopherson RJ, Webster AJF (1972) Changes during eating in oxygen consumption, cardiac function and body fluids of sheep. Journal of Physiology 221, 441-457.

Clampitt RB (1978) An investigation into the value of some clinical biochemical tests in the detection of minimal changes in liver morphology and function in the rat. Archives of Toxicology Supplement 1, 1- 13.

Clarke JN, Binnie DB, Jones KR, Mowat CM, Purchas RW, Uljee AE (1996) Repeatabilities of blood plasma metabolites and their associations with leanness in genotypes showing a wide divergence in carcass composition. Proceedings of the New Zealand Society of Animal Production 56, 180-183.

Crowley JJ, Evans RD, Mc Hugh N, Kenny DA, McGee M, Crews DH Jr., Berry DP (2011) Genetic relationships between feed efficiency in growing males and beef cow performance. Journal of Animal Science 89, 3372-3381.

Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes and Development 14, 2950-2961.

Davidson AJ, Castanon-Cervantes O, Stephan FK (2004) Daily oscillations in liver function, diurnal vs circadian rhythmicity. Liver International 24, 179- 186.

Davis M (2009) ´Influence of diet, production traits, blood hormones and metabolites and mitochondrial complex protein concentrations on residual feed intake in beef cattle.´ PhD thesis, University of Missouri, Columbia.

de Haer LCM, Luiting P, de Vries AG (1993) Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing. Livestock Production Science 36, 233- 253.

Doornenbal H, Tong AKW, Newman JA, Murray NL, Mears GJ (1987) Blood and Serum Components and Organ Weights in Steers, Bulls and Zeranol-Implanted Bulls. Journal of Animal Science 64, 489-496.

Doornenbal H, Tong AKW, Murray NL (1988) Reference Values of Blood Parameters in Beef Cattle of Different Ages and Stages of Lactation. Canadian Journal of Veterinary Research 52, 99-105.

Dugue A, Bagheri H, Lapeyre-Mestre M (2004) Detection and incidence of muscular adverse drug reactions, a prospective analysis from laboratory signals. European Journal of Clinical Pharmacology 60, 285- 292.

Durunna ON, Colazo MG, Ambrose DJ, McCartney D, Baron VS, Basarab JA (2012) Evidence of residual feed intake reranking in crossbred replacement heifers. Journal of Animal Science 90, 734-741.

Egan AR, Boda K, Varady J (1986) Regulation of nitrogen metabolism and recycling. In, ´Control of Digestion and Metabolism in Ruminants´. (Eds LP Milligan, WL Growum, A Dobson) pp. 386- 402. (Prentice-Hall, New Jersey)

El- Sherif MMA, Assad F (2001) Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi arid conditions. Small Ruminant Research 40, 269-277.

Fan B, Lkhagvadorj S, Cai W, Young J, Smith RM, Dekkers JCM, Huff-Lonergan E, Lonergan SM, Rothschild MF (2010) Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. Meat Science 84, 645- 650.

Ferrell CL, Garrett WN, Hinman N, Gritching G (1976) Energy utilization by pregnant and non-pregnant heifers. Journal of Animal Science 42, 937- 950.

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

Ferrell CL, Koong JL, Nienaber JA (1986) Effect of previous nutrition on body composition and maintenance energy costs of growing lambs. British Journal of Nutrition 56, 595-605.

Ferrell CL (1988) Contribution of visceral organs to animal energy expenditures. Journal of Animal Science 66, 23-34.

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

Field TG, Taylor RE (2003) ´Beef Production and Management Decisions. (4th edn).´ (Prentice Hall, Upper Saddle River, New Jersey)

Forbes JM, Wright JA, Bannister A (1972) A note on rate of eating in sheep. Animal Production 15, 211-214.

Foster WH, Kilpatrick DJ, Heaney IH (1983) Genetic variation in the efficiency of energy utilization by the fattening pig. Animal Production 37, 387- 393.

Fox JT, Carstens GE, Brown EG, White MB, Woods SA, Welsh TH Jr, Holloway JW, Warrington BG, Randel RD, Forrest DW, Lunt DK (2004) Net feed intake of growing bulls and relationships with performance, fertility and ultrasound composition traits. In ´Beef Cattle Research in Texas.´ pp 117- 120 (Texas A&M University, College Station, TX.)

Fregia A, Jensen D (1994) Evaluation of abnormal liver tests. Comprehensive Therapy 20, 50-54.

Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. Nature 395, 763- 770.

Georgie GC, Chand D, Razdan MN (1973) Seasonal changes in plasma cholesterol and serum alkaline phosphatase and transaminases activities in crossbred cattle. Indian Journal of Experimental Biology 11, 448- 450.

Gilbert H, Bidanel JP, Gruand J, Caritez JC, Billon Y, Guillouet P (2007) Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. Journal of Animal Science 85, 3182- 3188.

Goff JP, Horst RL (1997) Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science 80, 1260-1268.

Goldstein L (1970) Urea metabolism and osmoregulation in euryhaline elasomobranhes and amphibia. In ´Urea and the kidney´. (Eds B Schmidt- Nielse) (Excerpta Medical Foundation, Amsterdam)

Golden JW, Kerley MS, Kolath WH (2008) The relationship of feeding behavior to residual feed intake in crossbred Angus steers fed traditional and no-roughage diets. Journal of Animal Science 86, 180-186.

Gordon JG, McAllister IK (1970) The circadian rhythm of rumination. Journal of Agricultural Science Cambridge 74, 291-297.

Greenfield RB, Cecava MJ, Johnson TR, Donkin SS (2000) Impact of Dietary Protein Amount and Rumen Degradability on Intake, Peripartum Liver Triglyceride, Plasma Metabolites, and Milk Production in Transition Dairy Cattle. Journal of Dairy Science 83, 703-710.

Grubbs JK, Fritchen AN, Huff-Lonergan E, Gabler NK, Lonergan SM (2013) Selection for residual feed intake alters the mitochondria protein profile in pigs. Journal of Proteomics 80, 334-345.

Grunwaldt EG, Guevara JC, Estevez OR, Vicente A, Rousselle H, Alcuten N, Aguerregaray D, Stasi CR (2005) Biochemical and Haematological Measurements in Beef Cattle in Mendoza Plain Rangelands (Argentina). Tropical Animal Health and Production 37, 527-540.

Guan LL, Nkrumah JD, Basarab JA, Moore SS (2008) Linkage of microbial ecology to phenotype, correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiology Letters 288, 85- 91.

Guglielmo CG, Piersma T, Williams T (2001) A sport-physiological perspective on bird migration, evidence for flight-induced muscle damage. Journal of Experimental Biology 204, 2683- 2690.

Gunsett FC (1984) Linear index selection to improve traits defined as ratios. Journal of Animal Science, 59, 1185- 1193.

Gustafsson AH, Palmquist DL (1993) Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. Journal of Dairy Science 76, 475- 484.

Gutenbrunner C (2000) Circadian variations of the serum creatine kinase level- a masking effect? Chronobiology International 17, 583- 590.

Guyton, AC (1976) ´Textbook of medical physiology (5th edn)´. (WB Saunders Co., Philadelphia, PA)

Hagiwara Y, Shimo-Oka T, Okamura K. Ozawa E (1989) Basis for the assay of myogenic cell growth in vitro using creatine kinase activity as an index, with special reference to measurement of power ratio of transferrins in growth promotion. Japanese Journal of Pharmacology 49, 53- 58.

Hales JRS (1973) Radioactive microsphere measurements of cardiac output and regional tissue blood flow in sheep. Pflügers Archiv - European Journal of Physiology 344, 119-132.

Hansett R, Michaux C (1985) Characterization of biological types of cattle by the blood levels of creatine and creatinine. Journal of Animal Breeding and Genetics 103, 227–240.

Harmayer J, Martens H (1980) Aspects of urea metabolism in ruminants with reference to the goat. Journal of Dairy Science 63, 1707-1728.

Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, Shibata (2001) Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. Genes to Cells 6, 269- 278.

Harvatine KJ (2012) Circadian Patterns of Feed Intake and Milk Composition Variability. Tri-state Dairy Nutrition Conference 21st, Tri-state Dairy Nutrition Conference, 43-56.

Hassan A, Roussel JD (1975) Effect of protein concentration in the diet on blood composition and productivity of lactating Holstein cows under thermal stress. The Journal of Agricultural Science 85, 409- 415.

Hastings IM, Moruppa SM, Bunger L, Hill WG (1997) Effects of selection on food intake in the adult mouse. Journal of Animal Breeding and Genetics 114, 419- 434.

Haus E, Touitou Y (1992) ´Chronobiology in laboratory medicine. Biological rhythms in clinical and laboratory medicine´. (Springer-Verlag, Berlin)

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

Heitmann RN, Bergman EN (1981) Glutamate interconversions and glucogenicity in the sheep. American Journal of Physiology 241, E465-E472.

Herd RM, Arthur PF, Zirkler K, Quinn C, Oddy VH (1995) Heritability of IGF-1 in beef cattle. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 11, 694- 695.

Herd RM, Bishop SC (2000) Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livestock Production Science 63, 111-119.

Herd RM, Arthur PF, Hegarty RS (2002) Potential to reduce greenhouse gas emissions from beef production by selection to reduce residual feed intake. In ´Proceedings of the 7th World Congress on Genetics Animal Production´. pp. 10- 22. (Montpellier, France)

Herd RM, Archer JA, Arthur PF (2003) Reducing the cost of beef production through genetic improvement in residual feed intake, Opportunity and challenges to application. Journal of Animal Science 81, E9- E17.

Herd RM, Oddy VH, Richardson EC (2004) Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Australian Journal of Experimental Agriculture 44, 423- 430.

Hernandez-Sanabria E, Goonewardene LA, Wang Z, Durunna ON, Moore SS, Guan LL (2012) Impact of Feed Efficiency and Diet on Adaptive Variations in the Bacterial Community in the Rumen Fluid of Cattle. Applied Environmental Microbiology 78, 1203- 1214.

Herrera E (2002) Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine 19, 43- 55.

Hickman DD, Schwartzkopf-Genswein KS, Silasi R, Crews DH Jr., Krehbiel CR, McAllister TA (2002) Relationship between feeding behavior and performance of feedlot steers. Journal of Animal Science. 80(Suppl. 1), 15.

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

Hodgson JC, Mellor DJ, Field AC (1982) Foetal and maternal rates of urea production and disposal in well-nourished and undernourished sheep. British Journal of Nutrition 48, 49-58.

Holmes JHG, Ashmore CR, Robinson DW (1973) Hypertrophy Effects of Stress on Cattle with Hereditary Muscular. Journal of Animal Science 36, 684-694.

Hood DA, Saleem A (2007) Exercise-induced mitochondrial biogenesis in skeletal muscle. Nutrition, Metabolism and Cardiovascular Diseases 17, 332- 337.

Hoque MA, Kadowaki H, Shibata T, Oikawa T, Suzuki K (2009) Genetic parameters for measures of residual feed intake and growth traits in seven generations of Duroc pigs. Livestock Science 121, 45- 49.

Houpt TR (1959) Utilization of blood urea in ruminants. American Journal of Physiology 197, 115- 120.

Houseknecht KL, Baile CA, Matteri RL, Spurlock ME (1998) The biology of leptin, A review. Journal of Animal Science 76, 1405- 1420.

Houseknecht KL, Portocarrero CP (1998) Leptin and its receptors, regulators of whole-body energy homeostasis. Domestic Animal Endocrinology 15, 457- 475.

Hungate RE (1966) The rumen and its microbes. New York- London, Academic Press.

Huntington GB, Prior RL (1985) Net absorption of amino acids by portal-drained viscera and hind half of beef cattle fed a high concentrate diet. Journal of Animal Science 60, 1491-1499.

Huntington GB, Reynolds CK (1987) Oxygen consumption and metabolite flux of bovine portal-drained viscera and liver. Journal of Nutrition 117, 1167-1173.

Huntington G, Eisemann J, Whitt J (1988) Proportions of whole body blood flow and oxygen uptake attributable to gut and liver of beef steers. Journal of Animal Science 66 (suppl 1) 147.

Istasse L, Van Eenaeme C, Gabriel A, Clinquart A, Maghuin- Rogister G, Bienfait JM (1990) The relationship between carcass characteristics, plasma hormones and metabolites in young fattening bulls. Veterinary Research Communications 14, 19- 26.

Jana S, Bhattaacharyya B, Duttagupta R, Moitra DN (1991) A note of some biochemical constituents of blood in pregnant goats reared on extensive management system. Indian Veterinary Journal 68, 592-594.

Jacob N, Vadodaria VP (2001) Levels of glucose and cortisol in blood of Patanwadi ewes around parturition. Indian Veterinary Journal 78, 890-892.

Jainudeen MR, Hafez ESE (1994) Gestation, prenatal physiology and parturition. In ´Reproduction in farm animals´. pp. 247- 283. (Williams and Wilkins, Lippincott)

Ji SQ, Willis GM, Scott RR, Spurlock ME (1998) Partial cloning and expression of the bovine leptin gene. Animal Biotechnology 9, 1-14.

Johnson DE, Johnson KA, Baldwin RL (1990) Changes in Liver and Gastrointestinal Tract Energy Demands in Response to Physiological Workload in Ruminants. Journal of Nutrition 120, 649-655.

Johnson ZB, Chewning JJ, Nugent RA (1999) Genetic parameters for production traits and measures of residual feed intake in large white swine. Journal of Animal Science, 77, 1679-1685.

Johnston DJ (2007) Technical Update NFI & IGF-I. Beef Technical Note [Online]. Animal Breeding and Genetics. Unit, Univ. New England, Armidale, Australia. (Available at http,//agbu.une.edu.au/cattle/ beef9.pdf) (verified March 2007)

Kahn LP, Leng RA, Piper LR (2000) Rumen microbial yield from sheep genetically different for fleece weight. Asian-Australasian Journal of Animal Sciences 13, 137.

Kaneko JJ, Harvey JW, Bruss ML (2008) ´Clinical Biochemistry of Domestic Animals (6th edn).´ (Elsevier/Academic Press, Amsterdam)

Karapehlivan M, Atakisi A, Atakisi O, Yucayurt R, Pancarci SM (2007) Blood biochemical parameters during the lactation and dry period in Tuj ewes. Small Ruminant Research 73, 267-271.

Katz ML, Bergman EN (1969) Hepatic and portal metabolism of glucose, free fatty acids, and ketone bodies in the sheep. American Journal of Physiology 216, 953- 960.

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

Kelly AK, McGee M, Crews DH Jr., Sweeney T, Boland TM, Kenny DA (2010b) Repeatability of feed efficiency, carcass ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. Journal of Animal Science 88, 3214-3225.

Kelly AK, McGee M, Crews DH Jr., Lynch CO, Wylie AR, Evans RD, Kenny DA (2011) Relationship between body measurements, metabolic hormones, metabolites and residual feed intake in performance tested pedigree beef bulls. Livestock Science 135, 8-16.

Kew MC (2000) Serum aminotransferase concentration as evidence of hepatocellular damage. The Lancet 355, 591- 592.

Ko CH, Takahashi JS (2006) Molecular components of the mammalian circadian clock. Human Molecular Genetics 15, R271–R277.

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

Kolath WH, Kerley MS, Golden JW, Keisler DH (2006) The relationship between mitochondrial function and residual feed intake in Angus steers. Journal of Animal Science 84, 861-865.

Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen SG, Hopster H, De Jong IC, Ruis MAW and Blokhuis HJ (1999) Coping styles in animals, current status in behavior and stress physiology. Neuroscience and Biobehavioral Reviews, 23, 925- 935.

Koong LJ, Neinaber JA, Pekas JC, Yen JT (1982) Effects of plane of nutrition on organ size and fasting heat production in pigs. Journal of Nutrition 112, 1638-1642.

Koots KR, Gibson JP, Wilton JW (1994) Analyses of published genetic parameter estimates for beef production traits. 2. Phenotypic and genetic correlations. Animal Breeding Abstract 62, 825- 853.

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

Krebs HA (1966) Bovine Ketosis. Veterinary Research 78, 187- 192.

Ksiazek A, Konarzewski M, Lapo IB (2004) Anatomic and energetic correlates of divergent selection for basal metabolic rate in laboratory mice. Physiological and Biochemical Zoology 77, 890- 899.

Lapierre H, Bernier JF, Dubreuil P, Reynolds CK, Farmer C, Ouellet DR, Lobley GE (2000) The effect of feed intake level on splanchnic metabolism in growing beef steers. Journal of Animal Science 78, 1084-1099.

Lancaster PA, Cartens GE, Crews DH Jr and Woods SA (2005) Evaluation of feed efficiency traits in growing bulls and relationships with feeding behavior and ultrasound carcass estimates. In ´Proceedings, Western Section, American Society of Animal Science´ pp. 461- 464.

Lancaster PA, Carstens GE, Michal J, Brennan KM, Johnson KA, Slay LJ, Tedeschi LO, Davis ME (2007) Relationships between hepatic mitochondrial function and residual feed intake in growing beef calves. In ´International Symposium on Energy and Protein Metabolism and Nutrition´ pp. 57- 58.

Lancaster PA, Carstens GE, Ribeiro FRB, Tedeschi LO, Crews DH, Jr (2009) Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. Journal of Animal Science 87, 1528-1539.

Latimer KS (2011) ´Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology (5th Eds).´ (Wiley- Blackwell, Chichester, West Sussex)

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

Lebedevaa IY, Leibovab VB, Ernstc LK (2012) Activity of Protein and Carbohydrate Metabolism Enzymes in Black Pied Heifer Blood in Relation to Subsequent Reproductive Intensity. Russian Agricultural Sciences 38, 247- 250.

Lee AJ, Twardock AR, Bubar RH, Hall JE, Davis CL (1978) Blood Metabolic Profiles, Their Use and Relation to Nutritional Status of Dairy Cows. Journal of Dairy Science 61,1652-1670.

Lee M (2004) ´Interpreting Laboratory Data.´ (Bethesda, Maryland, American Society of Health-System Pharmacists)

Lee WK, Shin S, Cho SS, Park JS (1999) Purification and characterization of glutamate dehydrogenase as another isoprotein binding to the membrane of rough endoplasmatic reticulum. Journal of Cellular Biochemistry 76, 244- 253.

Lefcourt AM, Bitman J, Kahl S, Wood DL (1993) Circadian and ultradian rhythms of peripheral cortisol concentrations in lactating dairy cows. Journal of Dairy Science 76, 2607–2612.

Lefcourt AM, Huntington JB, Akers RM, Wood DL, Bitman J (1999) Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. Domestic Animal Endocrinology 16, 41- 55.

Lindena J, Friedel R, Rapp K, Sommerfeld U, Trautschold I, Deerberg F (1980) Long-term observation of plasma and tissue enzyme activities in the rat. Mechanisms of Ageing and Development 14, 379- 407.

Loyd AN, Long CR, Lewis AW, Randel RD (2011) Effects of physiological age on residual feed intake of growing heifers. Open Journal of Animal Sciences 1, 89-92.

Luiting P, Schrama JW, Van der Hel W, Urff EM (1991) Metabolic differences between White Leghorns selected for high and low residual feed consumption. British Poultry Science 32, 763- 782.

Lush JM, Gooden JM, Annison EF (1991) The uptake of nitrogenous compounds from the gut of sheep genetically different in wool production. Proceedings of the Nutrition Society of Australia 16, 144.

Lutz RT, Stahly TS (2003) Quantitative relationship between mitochondrial bioenergetics and efficiency of animal growth. Journal of Animal Science 81(Suppl. 1), 141.

Magness RR (1998) Maternal cardiovascular and other physiologic response to the endocrinology of pregnancy. In ´The Endocrinology of Pregnancy (8th edn).´ pp.507- 539. (Humana Press, Totowa, NJ)

Manston R, Rowlands GJ, Little W, Collis KA (1981) Variability of the blood composition of dairy cows in relation to time of day. Journal of Agricultural Science 96, 593- 598.

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

McCann JP, Reimers TJ (1986) Effects of obesity on insulin and glucose metabolism in cyclic heifers. Journal of Animal Science 62, 772- 782.

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

McDonald JM, Ramsey JJ, Miner JL, Nielsen MK (2009) Differences in mitochondrial efficiency between lines of mice divergently selected for heat loss. Journal of Animal Science 87, 3105- 3113.

Meissner HH, Paulsmeier DV, Leeuw K-J, Coetzer CM (1996) Ruminal and postruminal digestion of dietary protein and starch in steers. 2. Multivariate model prediction of non-ammonia nitrogen and starch passage and digestibility. South African Journal of Animal Science 26, 66-74.

Meng Q, Kerley MS, Ludden PA, Belyea RL (1999) Fermentation substrate and dilution rate interact to alter microbial growth and efficiency. Journal of Animal Science 77, 206–214.

Micozzi MS, Albanes D, Steves RG (1989) Relation of body size and composition to clinical biochemical and hematologic indices in US men and women. American Journal of Clinical Nutrition 50, 1276- 1281.

Mills LJ, Mitchell MA, Mahon M (1998) Incidence of skeletal muscle damage in selected and unselected strains of turkey. British Poultry Science 39, S27- S29.

Mineo H, Oyamada T, Yasuda T, Akiyama M, Kato S, Ushijima JI (1990) Effects of feeding frequency on plasma glucose, insulin and glucagon concentrations in sheep. Japanese Journal of Zootechnical Science 61, 411- 416.

Minton JE, Bindel DJ, Drouillard JS, Titgemeyer EC, Grieger DM, Hill CM (1998) Serum leptin is associated with carcass traits in finishing cattle. Journal of Animal Science 76(Suppl. 1), 231.

Mitchell G, Heffron JJ (1975) Factors affecting serum creatine phosphokinase activity in pigs. Journal of the South African Veterinary Association 46, 145- 148.

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

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

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

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

Moore KL, Johnston DJ, Graser HU, Herd R (2005) Genetic and phenotypic relationships between insulin-like growth factor-I (IGF-I) and net feed intake, fat, and growth traits in Angus beef cattle. Australian Journal Agricultural Research 56, 211-218.

Moore SS, Mujibi FD, Sherman EL (2009) Molecular basis for residual feed intake in beef cattle. Journal of Animal Science 87, E41-E47.

Moores RR Jr, Vaughn PR, Battaglia FC, Fennessey PV, Wilkening RB, Meschia G (1994) Glutamate metabolism in fetus and placenta of late-gestation sheep. American Journal of Physiology 267, R89-R96.

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

Mostl E and Palme R (2002) Hormones as indicators of stress. Domestic Animal Endocrinology 23, 67- 74.

Ndlovu T, Chimonyo M, Okoh AI, Muchenje V, Dzama K, Raats JG (2007) Assessing the nutritional status of beef cattle, current practices and future prospects. African Journal of Biotechnology 6(24), 2727-2734.

Nkrumah JD, Okine EK, Mathison GW, Schmid K, Li C, Basarab JA, Price MA, Wang Z, Moore SS (2005) Relationship of residual feed intake with metabolic rate, methane production and energy partitioning in beef cattle. Journal of Animal Science 83(Suppl. 1), 368.

Nkrumah JD, Okine EK, Mathison GW, Schmid K, Li C, Basarab JA, Price MA, Wang Z, Moore SS (2006) Relationships of feedlot feed efficiency, performance and feeding behaviour with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of Animal Science 84, 145- 153.

Nkrumah JD, Sherman EL, Li C, Marques E, Crews DH Jr., Bartusiak R, Murdoch B, Wang Z, Basarab JA, Moore SS (2007a) Primary genome scan to identify putative QTL for feedlot growth rate, feed intake, and feed efficiency of beef cattle. Journal of Animal Science, 85 3170- 3181.

Nkrumah JD, Keisler DH, Crews DH Jr., Basarab JA, Wang Z, Li C, Price MA, Okine EK, Moore SS (2007b) Genetic and phenotypic relationships of serum leptin concentration with performance, efficiency of gain, and carcass merit of feedlot cattle. Journal of Animal Science, 85, 2147- 2155.

Nicholson JP (2000) The role of albumin in critical illness. British Journal of Anaesthesia, 85 599-610.

NRC (1996) ´Nutrient requirements of beef cattle.´ (National Academy Press, Washington, D.C, United States)

NRC (2000) ´Nutrient requirements of beef cattle.´ (National Academy Press,Washington, D.C, United States)

Oddy VH (1999) Protein metabolism and nutrition in farm animals, an overview. Protein metabolism and nutrition. In ´EAAP Publication No. 96.´ (Eds GE Lobley, A White, JC Mackae) pp. 9-23. (European Association for Animal Production)

Ojano-Dirain C, Toyomizu M, Wing T, Cooper M, Bottje WG (2007) Gene expression in breast muscle and duodenum from low and high feed efficient broilers. Poultry Science 86, 372- 381.

Okine EK, Basarab JA, Baron V, Price MA (2001) Net feed efficiency in young growing cattle, III. Relationships to methane and manure production. Canadian Journal of Animal Science 81, 614.

Orr C, Hutcheson DP, Cummins JM, Thompson GB (1988) Nitrogen kinetics of infectious bovine rhinotraccheitis stressed calves. Journal of Animal Science 66, 1982-1989.

Orskov ER (1986) Starch digestion and utilisation in ruminants. Journal of Animal Science 63, 1624- 1633.

Orskov ER, Ojwang I, Reid GW (1988) A study on consistency of differences between cows in rumen outflow rate of fibrous particles and other substrates and consequences for digestibility and intake of roughages. Animal Production 47, 45- 51.

Otto F, Baggasse P, Bogin E, Harun M, Vilela F (2000) Biochemical blood profile of Angoni cattle in Mozambique. Israel Veterinary Medical Association 55(3), 1-9.

Owens FN, Zinn RA, Kim YK (1986) Limits to starch digestion in the ruminant small intestine. Journal of Animal Science 63 1634- 1648.

Pakdel A, van Arendonk JAM, Vereijken AL, Bovenhuis H (2005) Genetic parameters of ascites-related traits in broilers, correlations with feed efficiency and carcass traits. British Poultry Science 46, 43-53.

Palme R, Robia C, Messmann S, Hofer J, Mostl E (1999) Measurement of fecal cortisol metabolites in ruminants, a noninvasive parameter of adrenocortical function. Wiener Tierarztliche Monatsschrift 86, 237- 241.

Palme R, Rettenbacher S, Touma C, El-Bahr SM and Mostl E (2005) Stress hormones in mammals and birds, comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals New York Academy of Sciences 1040, 162- 171.

Pambu-Gollah R, Cronje PB, Casey NH (2000) An evolution of the use of blood metabolite concentrations as indicators of nutritional status in free-ranging indigenous goats. South African Journal of Animal Science 30, 115-120.

Panksepp J (1978) Analysis of feeding patterns, Data reduction and theoretical implications. In ´Hunger Models, Computable Theory of Feeding Control.´ (Ed. DA Booth) pp. 143. (Academic Press, New York.)

Pell JM, Caldarone EM, Bergman EN (1986) Leucine and a-ketoisocaproate metabolism and interconversions in fed and fasted sheep. Metabolism 35, 1005- 1016.

Peters T, Anfinsen CB (1950) Net production of serum albumin by liver slices. The Journal of Biological Chemistry 186, 805-813.

Peters T Jr (1996) ´All about Albumin, Biochemistry, Genetics, and Medical Applications.´ (Academic Press, Inc. San Diego, California)

Peterson RG, Walden DE (1981) Repeatabilities of serum constituents in Holstein- Friesians affected by feeding, age, lactation, and pregnancy. Journal of Dairy Science 64, 822-831.

Piccione G, Caola G (2002) Biological rhythm in livestock. Journal of Veterinary Science 3, 147-157.

Piccione G, Caola G, Refinetti R (2003) Circadian rhythms of body temperature and liver function in fed and food-deprived goats. Comparative Biochemistry and Physiology Part A 134, 563- 572.

Piccione G, Caola G, Mortola JP (2004) Day/night pattern of arterial blood gases in the cow. Respiratory Physiology & Neurobiology 140, 33-41.

Piccione G, Giannetto C, Casella S, Caola G (2009) Annual rhythms of some physiological parameters in Ovis aries and Capra hircus. Biological Rhythm Research 40, 455-464.

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

Plaizier JC, Fairfield AM, Azevedo PA, Nikkhah A, Duffield TF, Crow GH, Bagg R, Dick P, McBride BW (2005) Effects of monensin and stage of lactation on variation of blood metabolites within twenty-four hours in dairy cows. Journal of Dairy Science 88, 3595-3602.

Poyton RO, McEwen JE (1996) Crosstalk between nuclear and mitochondrial genomes. Annual Review of Biochemistry 65, 563- 607.

Pritchard RH, KW Bruns (2003) Controlling variation in feed intake through bunk management. Journal of Animal Science 81, E133- E138.

Putnam PA, Davis RE (1963) Ration effects on drylot steer feeding patterns. Journal of Animal Science 22, 437- 443.

Putnam PA, Lehmann R, Davis RE (1964) Rate of feed consumption and body weight of beef cattle. Journal of Animal Science 23, 425- 429.

Putnam PA, Lehmann R, Davis RE (1967) Ration selection and feeding patterns of steers fed in drylot. Journal of Animal Science 26, 647- 650.

Rajaei Sharifabadi H, Zamiri MJ, Rowghani E, Bottje WG (2012) Relationship between the activity of mitochondrial respiratory chain complexes and feed efficiency in fat-tailed Ghezel lambs. Journal of Animal Science 90, 1807-1815.

Ramos JJ, Verde MT, Marca MC, Fernandez A (1994) Clinical chemical values and variations in Rasa Argonesa ewes and lambs. Small Ruminant Research 13, 133-139.

Rasooli A, Nouri M, Khadjeh GH, Rasekh A (2004) The influences of seasonal variations on thyroid activity and some biochemical parameters of cattle. Iranian Journal of Veterinary Research 5(2), 1383.

Rauw WM, Soler J, Tibau J, Reixach J, Gomez Raya L (2006a) The relationship between residual feed intake and feed intake behavior in group-housed Duroc barrows. Journal of Animal Science 84, 956- 962.

Rauw WM, Soler J, Tibau J, Reixach J, Gomez Raya L (2006b) Feeding time and feeding rate and its relationship with feed intake, feed efficiency, growth rate, and rate of fat deposition in growing Duroc barrows. Journal of Animal Science 84, 3404- 3409.

Reece WO (2004) ´Duke's Physiology of Domestic Animals (12th edn).´ (Comstock Publishing Associates/ Cornell University Press. Ithaca, New York.)

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

Reppert SM, Weaver DR (2001) Molecular analysis of mammalian circadian rhythms. Annual Review of Physiology 63, 647- 676.

Reynolds CK and Huntington GB (1988) Partition of portal-drained visceral net flux in beef steers 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. British Journal of Nutrition 60, 539- 551.

Reynolds CK, Tyrrell HF, Reynolds PJ (1991) Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers, Whole body energy and nitrogen balance and visceral heat production. Journal of Nutrition 121, 994-1003.

Reynolds CK (1995) Quantitative aspects of liver metabolism in ruminants. In ´Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction Proceeding of the 8th International Symposium of Ruminant Physiology´. (Eds WV Engelhardt, S Leonhard-Marek, G Breves, D Giesecke) pp. 351. (Ferdinand Enke Verlag, Stuttgart, Germany)

Reynolds CK (2002) Economics of visceral energy metabolism in ruminants, Toll keeping or internal revenue service? Journal of Animal Science 80, E74-E84.

Reynolds CK, Aikman PC, Lupoli B, Humphries DJ, Beever DE (2003) Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. Journal of Dairy Science 86, 1201-1217.

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

Richardson EC, Herd RM, Archer JA, Woodgate RT, Arthur PF (1998) Steers bred for improved net feed efficiency eat less for the same feedlot performance. Animal Production in Australia, 22, 213- 216.

Richardson EC, Herd RM and Oddy VH (2000) Variation in body composition, activity and other physiological processes and their associations with feed efficiency. In ´Feed efficiency in beef cattle. Proceedings of the feed efficiency workshop´. (Eds JA Archer, RM Herd and PF Arthur) pp. 46- 50. (University of New England, Armidale, NSW, Australia)

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

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

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

Riis PM, Madsen A (1985) Thyroxine concentration and secretion rates in relation to pregnancy, lactation and energy balance in goats. Journal of Endocrinology, 107, 421-427.

Rivera-Coll A, Fuentes-Arderiu X, Diez-Noguera A (1993) Circadian rhythms of serum concentrations of 12 enzymes of clinical interest. Chronobiology International 10, 190-200.

Robinson DL, Hammond K, Graser HU, McDowell GH (1992) Relationships between breeding values and physiological responses to fasting and re-feeding in dairy bulls. Journal of Animal Breeding and Genetics 109, 26- 36.

Robinson DL, Oddy VH (2004) Genetic parameters for feed efficiency, fatness, muscle area and feeding behavior of feedlot finished beef cattle. Livestock Production Science 90, 255- 270.

Rodriguez MN, Tebot I, Le Bas A, Nievas C, Leng L, Cuio A (1996) Renal functions and urea handling in pregnant and lactating corriedale ewes. Canadian Journal of Animal Science, 76 469-472.

Rolfe DFS, Brand MS (1996) Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. American Journal of Physiology 271, C1380–C1389.

Rolfe DFS, Brand MD (1997) The physiological significance of mitochondrial proton leak in animal cells and tissues. Bioscience Reports 17, 9-16.

Rolfe DFS, Newman JM, Buckingham JA, Clark MG, Brand MD (1999) Contribution of mitochondria. Proton leak to respiration rate in working skeletal muscle and liver and to SMR. American Journal of Physiology 276, C692- C699.

Roubies N, Panousis N, Fytianou A, Katsoulos PD, Giadinis N, Karatzias H (2006) Effects of age and reproductive stage on certain serum biochemical parameters of chios sheep under greek rearing conditions. Journal of Veterinary Medicine Series A 53, 277-281.

Ripperger JA, Schibler U (2001) Circadian regulation of gene expression in animals. Current Opinion in Cell Biology 13, 357- 362.

Russel AJF, Wright IA (1983) The use of blood metabolites in the determination of energy status in beef cows. Animal Production 37, 335- 343.

Saito M, Murakami E, Suda M (1976) Circadian rhythms in disaccharidases of rat small intestine and its relation to food intake. Biochim Biophys Acta 421, 177-179.

Sakata T, Kojima T, Fujieda M, Takahashi M, Michibata T (2003) Influences of probiotic bacteria on organic acid production by pig caecal bacteria in vitro. Proceedings of the Nutrition Society 62, 73- 80.

Sandelin BA, Brown AH Jr, Ojano-Dirain C, Iqbal M, Bottje WG, Brown MA (2004) Herring WO, Johnson ZB, Baublits RT. Protein expression in muscle mitochondria in Angus steers with low and high feed efficiency. Journal of Animal Science Supplementary 1, 416.

Sands JM, Gargus JJ, Frohlich O, Gunn BB, Kokko JP (1992) Urinary concentration ability in patients with JK (a-b-) blood type who lack carrier- mediated urea transport. Journal of the American Society of Nephrology 2, 1689- 1696.

Sapolsky RM (2002) Endocrinology of the stress response. In ´Behavioral Endocrinology (2nd edn).´ (Eds JB Becker, M Breedlove, D Crews and MM McCarthy) pp. 409- 450. (The MIT Press, Cambridge, Massachusetts, USA.

Sarrasecal A, Milne E, Metcalf MJ, Lobley GE (1998) Urea recycling in sheep, effects of intake. British Journal of Nutrition 79, 79-88

Scheaffer AN (1997) ´Influence of pregnancy on body weight, ruminal characteristics, and visceral organ mass in beef heifers´. MSc thesis, North Dakota State University, Fargo.

Scheaffer AN, Caton JS, Bauer ML, Reynolds LP (2001) Influence of pregnancy on body weight, ruminal characteristics, and visceral organ mass in beef heifers. Journal of Animal Science 79, 2481-2490.

Scheaffer AN, Caton JS, Bauer ML, Redmer DA, Reynolds LP (2003) The effect of pregnancy on visceral growth and energy use in beef heifers. Journal of Animal Science 81, 1853-1861.

Schenkel FS, Miller SP, Wilton JW (2004) Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Canadian Journal of Animal Science 84, 177- 185.

Schmidt ES, Schmidt FW (1988) Glutamate dehydrogenase, biochemical and clinical aspects of an interesting enzyme. Clinica Chimica Acta 43, 43-56.

Schwartzkopf-Genswein KS, Beauchemin KA, Gibb DJ, Crews DH Jr., Hickman DD, Streeter M, McAllister TA (2003) Effect of bunk management on feeding behavior, ruminal acidosis and performance of feedlot cattle: A review. Journal of Animal Science 81, E149- E158.

Scott KR, Simmons Z, Boyer PJ (2002) Hypothyroid myopathy with strikingly elevated serum c reatine kinase level. Muscle Nerve 26, 141-144.

Seal CJ, Reynolds CK (1993) Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutrition Research Review 6, 185-208.

Sejrsen K, Hvelplund T, Nielsen MO (2006) In ´Ruminant Physiology, Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress.´ (Wageningen Academic Publishers. Wageningen, The Netherlands)

Shaffer L, Roussel JD, Koonce KL (1981) Effects of Age, Temperature-Season, and Breed on Blood Characteristics of Dairy Cattle. Journal of Dairy Science 64, 62-70.

Shehab-El-Deen MA, Fadel MS, Van Soom A, Saleh SY, Maes D, Leroy JL (2010) Circadian rhythm of metabolic changes associated with summer heat stress in high-producing dairy cattle. Tropical Animal Health and Production 42, 1119-1125.

Sherman EL, Nkrumah JD, Murdoch BM, Moore SS (2008) Identification of polymorphisms influencing feed intake and efficiency in beef cattle. Animal Genetics 39, 225- 231.

Sherman EL, Nkrumah JD, Li C, Bartusiak R, Murdoch B, Moore SS (2009) Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. Journal of Animal Science 87, 37-45.

Sherman EL, Nkrumah JD, Moore SS (2010) Whole genome single nucleotide polymorphism associations with feed intake and feed efficiency in beef cattle. Journal of Animal Science 88, 16-22.

Silverstein JT, Hostuttler M, Blemings KP (2005) Strain differences in feed efficiency measured as residual feed intake in individually reared rainbow trout, Oncorhynchus mykiss (Walbaum). Aquaculture Research 36, 704- 711.

Simon O, Bergner H (1983) Changes in protein synthesis in the liver and small intestine of rats after feeding. Biomedica Biochimica Acta 42, 1299-308.

Soback S, Bogin E, Weisman Y (1985) Biochemical changes in the blood, spleen and duodenum of turkeys experimentally and naturally exposed to hemorrhagic enteritis virus. Avian Pathology 14, 189-197.

Sowell BF, Bowman JGP, Branine ME, Hubbert ME (1998) Radio frequency technology to measure feeding behavior and health of feedlot steers. Applied Animal Behaviour Science 59, 277- 284.

Stephan FK (2001) Food-entrainable oscillators in mammals. In ´Handbook of Behavioral Neurobiology. Circadian Clocks Volume 12´. (Eds JS Takahashi, FW Turek, RY Moore) pp. 223- 246. (New York, Kluwer) Stokkan KA,Yamazaki S,Tei H, Sakaki Y, Menaker M (2001) Entrainment of the Circadian Clock in the Liver by Feeding. Science 291, 490-493.

Stone RT, Leymaster KA (1985) Relationship of birth weight and prenursing concentrations of serum albumin to survival and growth rate in swine. Growth 49, 263-270.

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

Susenbeth A, Mayer R, Kiehler B, Neumann O (1998) Energy requirements for eating in cattle. Journal of Animal Science 76, 2701- 2705.

Swanson K, Miller S (2008) Factors regulating feed efficiency and nutrient utilization in beef cattle. In ´Mathematical Modelling in Animal Nutrition´. (Eds J France, E Kebreab) pp. 419- 441 (CAB International, Cambridge, MA)

Taylor CS, Turner HG, Young GB (1981) Genetic control of equilibrium maintenance efficiency in cattle. Animal Production 33, 179- 194.

Terry CA, Knapp RH, Edwards JW, Mies WL, Savell JW, Cross HR (1990) Yields of by-products from different cattle types. Journal of Animal Science 68, 4200-4205.

Thomas L (2005) Glutamate-Dehydrogenase. In ´Labor und Diagnose 6th edn.´ (Eds L Thomas) pp. 103-106. (Frankfurt/Main, TH – Books Verlagsgesellschaft Gmbh)

Toharmat T, Nonaka I, Shimizu M, Kume S (1999) Changes of the blood composition of periparturient cows in relation to time of day. Asian-Australasian Journal of Animal Sciences 12, 1111-1115.

Trenkle A, Willham RL (1977) Beef production efficiency, The efficiency of beef production can be improved by applying knowledge of nutrition and breeding. Science 198, 1009-1015.

Van Bebber J, Mercer JT (1994) Selection for efficiency of broilers. A comparison of properties of residual feed intake and feed conversion ratio. In ´Proceedings of the 5th World Congress on Genetics Applied to Livestock Production´. pp. 53-56.

Van den Heuvel CJ, Ferguson SA, Gilbert SS and Dawson D (2004) Thermoregulation in normal sleep and insomnia, the role of peripheral heat loss and new applications for digital thermal infrared imaging (DITI). Journal of Thermal Biology 29, 457- 461.

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, Van Arendonk JAM (1999) Whole Genome Scan in Chickens for Quantitative Trait Loci Affecting Growth and Feed Efficiency. Poultry Science 78, 15- 23.

Vaughn PR, Lobo M, Battaglia FC, Fennessey P, Wilkening R, Meschia G (1995) Glutamine-glutamate exchange between placenta and fetal liver. American Journal of Physiology 268, E705- E711.

Vernon RG, Houseknecht KL (2000) Adipose tissue, beyond an energy reserve. In ´Ruminant Physiology, digestion, metabolism, growth and reproduction´. (Eds PB Cronje) pp.171-186. (CABI, Wallingford, UK)

Von Felde A, Roehe R, Looft H, Kalm E (1996) Genetic association between feed intake and feed intake behaviour at different stages of growth of group-housed boars. Livestock Production Science 47, 11- 22.

Waldmann, TA (1977) ´Albumin Structure, Function and Uses´. (Eds VM Rosenoer, M Oratz, MA Rothschild) pp. 255-273. (Pergamon, Oxford)

Wallace JM (2000) Nutrient partitioning during pregnancy, adverse gestational outcome in overnourished adolescent dams. Proceedings of the Nutrition Society 59, 107- 117.

Wang Z, Colazo MG, Basarab JA, Goonewardene LA, Ambrose DJ, Marques E, Plastow G, Miller SP, Moore SS (2012) Impact of selection for residual feed intake on breeding soundness and reproductive performance of bulls on pasture-based multisire mating. Journal of Animal Science 90, 2963- 2969.

Waterlow JC (1988) The variability of energy metabolism in man. In ´Comparative nutrition.´ (Eds K Blaxter, I McDonald) pp. 133-139. (John Libby and Co., London)

Webster AJF (1981) The energetic efficiency of metabolism. Proceedings of the Nutrition Society 40, 121-128.

Whittow GC (1962) The significance of the extremities of the ox (Bos taurus) in thermoregulation. Journal of Agricultural Science 58, 109-120.

Widmer B, Gerhardt RE, Harrington JT, Cohen JJ (1979) Serum electrolyte and acid base composition. The influence of graded degrees of chronic renal failure. Archives of Internal Medicine 139, 1099-1102.

Williams YJ, Pryce JE, Grainger C, Wales WJ, Linden N, Porker M, Hayes BJ (2010) Variation in residual feed intake in Holstein-Friesian dairy heifers in southern Australia. Journal of Dairy Science 94, 4715- 4725.

Wilson BW, Nieberg PS, Buhr FT, Kelly BJ, Shultz FT (1990) Turkey muscle growth and focal myopathy. Poultry Science 69, 1553-1562.

Wilson D (2008) ´McGraw- Hill's Manual of Laboratory & Diagnostic Tests.´ (McGraw- Hill Companies, Inc. Columbus, Ohio)

Wise T, Stone RT, Vernon MW (1991) Relationship of serum estriol, cortisol and albumin concentrations with pig weight at 110 days of gestation and at birth. Biology of the Neonate 59, 114-119.

Wood BJ, Archerb JA, van der Werf JHJ (2004) Response to selection in beef cattle using IGF-I as a selection criterion for residual feed intake under different Australian breeding objectives. Livestock Production Science 91, 69- 81.

Wu A (2006) Tietz Clinical Guide to Laboratory Tests. Saunders Elsevier. St. Louis, Missouri.

Yamamoto H, Aikawa T, Matsutaka H, Okuda T, Ishikawa E (1974) Interorganal relationships of amino acid metabolism in fed rats. American Journal of Physiology 226, 1428-1433.

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

Yang YT, Baldwin RL (1973) Lipolysis in isolated cow adipose-cells. Journal of Dairy Science 56, 366- 374.

Young JM, Cai W, Dekkers JCM (2011) Effect of selection for residual feed intake on feeding behavior and daily feed intake patterns in Yorkshire swine. Journal of Animal Science 89, 639-647.

Zimmerman HJ (1974) Serum enzyme measurement in experimental hepatotoxicity. Israel Journal of Medical Sciences 10, 328-33.