ASSOCIATIONS OF CARDIOVASCULAR FUNCTION AND STRUCTURE WITH FEED EFFICIENCY IN BEEF CATTLE

by

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ABSTRACT

Heart rate as a feed efficiency proxy could aid in the reduction of beef cattle feed costs. Applicable heart rate assessments remain to be developed and the basis of the association between heart rate and feed efficiency evaluated, before heart rate proxies are implemented. Study 1 assessed associations of overnight and acute stress heart rate with feed efficiency in heifer calves and yearling heifers. Study 2 evaluated relationships of cardiovascular function and structure measures with feed efficiency in bulls, steers and heifers. Efficient heifer calves displayed lower overnight heart rates and higher heart rate responses to acute stress, no difference in heart rate was observed in yearling heifers. Cardiovascular function and structure measures were associated with feed efficiency; with structure measures (i.e. ventricle thickness) indicating reduced cardiovascular workload. Heart rate through associations with other cardiovascular measures and metabolism has potential as an applicable proxy for beef cattle feed efficiency improvement.

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percent
% HCW	Grams per kilogram of hot carcass weight multiplied by 100
% DM	Percent dry matter
°C	Degrees Celsius
©	Copyright
®	Registered trademark
1/HR*1000	Inverse of heart rate x 1000
50K	50,000
ACTH	Adrenocorticotropic hormone
ADF	Acid detergent fibre
ADG	Average daily gain
$Adj.\ R^2$	Adjusted coefficient of determination
ADMI	Average dry matter intake
AT	Atria
AT_{WT}	Atria weight
AUDC	Area under dilution curve
AUHC	Area under heart rate curve
B	Bulls
BIC	Bayesian information criteria
BPM	Beats per minute
BV	Blood volume
BW	Body weight
c_0	Concentration of Indocyanine green in plasma at administration
C_1	First knot
C_2	Second knot
Ca	Calcium
CI	Confidence interval
Cl	Chlorine
cm^2	Centimeter squared
CO	Cardiac output
Co	Cobalt
Co.	Corporation
COI	Cardiac output indicator
CP	Crude protein
CR	Carcass
CRH	Corticotropin releasing hormone
Cu	Copper
D	Day

DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
e	Random residual effect
ECG	Electrocardiogram
$\mathrm{Fat}_{\mathrm{YLD}}$	Fat yield
FBW	Final body weight
FCR	Feed conversion ratio
Fe	Iron
FFT	Fast Fourier Transformation
fL	Femtoliters per cell
g/ kg HCW	Grams per kilogram or hot carcass weight
g/L	Grams per liter
GLM	General linear model
GPS	Global positioning system
h	Hour
H	Heifers
Hb	Hemoglobin
Hct	Hematocrit
HCW	Hot carcass weight
HF	High frequency
High-RFI	Feed inefficient
HPA	Hypothalamic–pituitary–adrenal
HR	Heart rate
$\mathrm{HR}_{\mathrm{ABA}}$	Average transport heart rate
$\mathrm{HR}_{\mathrm{ABH}}$	Average of highest twenty percent of abattoir heart rates
$\mathrm{HR}_{\mathrm{ABL}}$	Average of lowest twenty percent of abattoir heart rates
$\mathrm{HR}_{\mathrm{AFT}}$	Average heart rate over 20 seconds after umbrella exposure
$\mathrm{HR}_{\mathrm{BEF}}$	Average heart rate over 20 seconds prior umbrella exposure
$\mathrm{HR}_{\mathrm{CHG}}$	$\mathrm{HR}_{\mathrm{AFT}} - \mathrm{HR}_{\mathrm{BEF}}$
$\mathrm{HR}_{\mathrm{MAX}}$	Maximum heart rate after umbrella exposure
$\mathrm{HR}_{\mathrm{OVA}}$	Average overnight heart rate
$\mathrm{HR}_{\mathrm{OVH}}$	Average of highest twenty percent of overnight heart rates
$\mathrm{HR}_{\mathrm{OVL}}$	Average of lowest twenty percent of overnight heart rates
$\mathrm{HR}_{\mathrm{TRA}}$	Average transport heart rate
$\mathrm{HR}_{\mathrm{TRH}}$	Average of highest twenty percent of transport heart rates
$\mathrm{HR}_{\mathrm{TRL}}$	Average of lowest twenty percent of transport heart rates
HRV	Heart rate variability
HS	Heart structure
$\mathrm{HT}_{\mathrm{WT}}$	Heart weight
Hz	Hertz
IBI	Interbeat interval
ICG	Indocyanine green

ID	Indicator dilution
Inc.	Incorporated
IU/kg	International units per kilogram
kg	Kilogram
kg DM/d	Kilograms of dry matter per day
kg/d	Kilograms per day
L/L	Liters per liter
Lean_{YLD}	Lean yield
LF	Low frequency
LF:HF	Low frequency to high frequency ratio
LF_{AB}	Area under abattoir amplitude/frequency curve from 0.01-0.1 Hz
LF_{OV}	Area under overnight amplitude/frequency curve from 0.01-0.1 Hz
LF_{TR}	Area under transport amplitude/frequency curve from 0.01-0.1 Hz
LLC	Limited liability company
Low-RFI	Feed efficient
Ltd.	Limited
LV	Left ventricle
LV_{TH}	Left ventricle wall thickness
LV_{WT}	Left ventricle weight
LV_{WT}:RV_{WT}	Left ventricle weight to right ventricle weight ratio
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
Mg	Milligram
Mg	Magnesium
mg/kg	Milligram per kilogram
mg/kg BW	Milligrams per kilogram of body weight
mg/mL	Milligrams per milliliter
MHz	Megahertz
m_{ICG}	Mass of Indocyanine green administered
mL	Milliliter
mL/kg	Milliliters per kilogram
mm	Millimeter
Mn	Manganese
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MY_{WD}	Myocyte width
N	Number of observations
Na	Sodium
NaCl	Sodium chloride
NDF	Neutral detergent fibre
NIRS	Near-infrared spectroscopy
nm	Nanometer

OC	Oxygen-carrying capacity
P	P-value
P	Phosphorus
PF	Performance
PIT	Physiological indicator traits
PNS	Parasympathetic nervous system
R	Correlation coefficient
R^2	Coefficient of determination
RAAS	Renin-angiotensin-aldosterone system
RBC	Red blood cell count
Rd.	Road
RFI	Residual feed intake
RV	Right ventricle
RV_{TH}	Right ventricle thickness
RV_{WT}	Right ventricle weight
S	Steers
SA	Sino atrial
SAS	Statistical Analysis Software
SD	Standard deviation
SE	Standard error
SM_{LG}	Sarcomere length
SNP	Single nucleotide polymorphism
SNS	Sympathetic nervous system
SV	Stroke volume
SVI	Stroke volume indicator
T	Time value
T_{AFT}	Time from HR_{MAX} to start of HR_{AFT}
T_{BEF}	Time from umbrella exposure to HR_{MAX}
TDN	Total digestible nutrients
UCP-3	Uncoupling protein-3
USA	United States of America
VLF	Very low frequency
X 10^12/L	Cells per liter
Z	Polynomial regression covariate
Zn	Zinc
β	Regression coefficient
µm	Micrometers

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CHAPTER 1

INTRODUCTION

Beef cattle are a marvellous spectacle, producing a high-quality protein source from high-fibre feed sources not suitable for human consumption. In Canada the roots of cattle production date back to the futile attempt to manage imported Portuguese cattle on Sable Island, Nova Scotia in 1518 (MacLachlan, 2006). Various other attempts at establishing cattle herds across Canada have been noted from the 16th to 18th centuries (Jordan, 1993; Rasmussen, 1995) however, Canadian weather, feed resources, markets and competing industries stagnated growth (MacLachlan, 2006). Substantial growth in the Canadian cattle population did not occur until between 1840 and 1874, when mechanization allowed for winter-feeding and export markets to the United Kingdom were developed (MacLachlan, 2006).

The dynamics of pressure on beef cattle production continue to change as perceptions of livestock production's environmental impact and husbandry practises develop and land and market competition diversifies (Thornton, 2010; Capper and Bauman, 2013). Feed costs continue to represent a major proportion of production costs (Lancaster *et al.,* 2014) and when coupled with increased market volatility (Garrido *et al.,* 2016), have hindered the profitability of beef production in Canada. Furthermore, beef production is attributed with 40% of livestock greenhouse gas emissions (methane and nitrous oxide) while requiring one-third of the global cropped area (both as pasture and feed crops) for its production (Gerber *et al.,* 2013). Improvement of feed efficiency serves as a potential method to optimize the feed and land area required for beef production (Capper and Bauman, 2013). Recent feed efficiency initiatives such as the American National Program for Improvement of Feed Efficiency have recognized the importance of feed efficiency improvement (Spangler, 2015). However, the expense of individual feed intake recording technologies and time required to obtain an accurate determination of feed efficiency continue to limit feed efficiency improvement.

Feed efficiency is a complex trait influenced by a plethora of physiological processes. Physiological indicator traits (PIT) have been recognized as a complementary method, to direct trait selection, for the improvement of economically relevant traits including feed efficiency (Pollak *et al.,* 2012). Nielsen *et al.* (2013) recognized the benefits of correlated traits (physiological indicators) and recommended their use along with direct measures of feed intake in the improvement of feed efficiency. In the biological network of feed efficiency, PIT are located closer to the genome in comparison to the direct trait, therefore: a higher heritability and greater success in identifying associated genomic regions should be achieved (Thallman *et al.,* 2008). Due to their association with other traits of interest (i.e., reproduction, meat quality and temperament), PIT have the ability to identify the antagonistic indirect effects of selection and reduce selection bias (MacNeil *et al.,* 2011). Furthermore, a PIT is easier to record than the direct trait and its associations with additional traits could allow for the improvement of animal husbandry and nutrition in addition to direct trait improvement. A potential PIT will therefore not only have a strong genetic correlation with the trait of interest (feed efficiency) but also be associated with other relevant traits.

Heart rate (HR, beats per minute; BPM) is associated with feed efficiency (Hafla *et al.,* 2013; Montanholi *et al.,* 2014) along with other traits of importance including activity (Frondelius *et al.,* 2015), stress response (Stewart *et al.,* 2010), health status (Sheldon *et al.,* 2006) and welfare (Rushen *et al.,* 1999). Therefore, recording of HR on-farm could aid in the improvement of animal husbandry in addition to feed efficiency. Past associations between HR and feed efficiency were identified over long-term resting assessments (Hafla *et al.,* 2013; Montanholi *et al.,* 2014) and during transport (Montanholi *et al.,* 2014). While these associations serve as a basis for understanding the association between HR and feed efficiency during both rest and stress, additional HR assessments need to be developed that could be implemented on-farm. Associations of HR with handling (Waynert *et al.,* 1999), restraint (Lay *et al.,* 1992b), freeze branding (Lay *et al.,* 1992a), disbudding and ear-tagging (Stewart *et al.,* 2013) indicate that HR can be recorded during routine husbandry practises. Differences in HR during routine husbandry practises could be associated with feed efficiency and perform as PIT for feed efficiency.

An intricate network of physiological mechanisms and relationships could influence the association of HR with feed efficiency. Cardiac output (CO) is the product of HR and stroke volume (SV) and is influenced by body size, oxygen carrying capacity and metabolic demand (Walley, 2011), through neural, endocrine and renal mechanisms of regulation (Hall, 2015) all of which could be associated with feed efficiency during both rest and stress. Sustained changes in HR, CO and SV and differences in energy metabolism are associated with changes in heart structure (Maillet *et al.,* 2013), heart histomorphometry (Ahmet *et al.,* 2011) and blood volume (BV) (Messerli *et al.,* 1978). Evaluation of the underlying cardiovascular parameters contributing to, and affected by, the association of HR and feed efficiency, will aid in identifying antagonistic effects of selection on HR and could identify additional PIT for feed efficiency. Evaluation of this association must precede large-scale phenotyping of HR and its use in beef cattle breeding programmes.

This thesis commenced in September 2014 and sought 1) to assess the associations between two different HR assessments and feed efficiency in heifer calves and yearling heifers, to determine their ability as on-farm feed efficiency indicators and 2) to evaluate the associations among measures of cardiovascular function, structure, histomorphometry and their coinciding associations with feed efficiency, performance and body composition in yearling bulls, steers and heifer calves, to increase our understanding of the associations between the cardiovascular system and feed efficiency.

CHAPTER 2

LITERATURE REVIEW

2.1 Feed efficiency

2.1.1 Determination

Recognition of feed efficiency and its importance can be traced to 1896, when Manly Miles controversially stated: "*Our domestic animals may then be looked upon as machines for doing work in the repairs and other vital activities of the animal machine itself, including muscular exercise, and the manufacture of animal products used as food by man. The importance of these animal machines as factors in domestic economy leads us to inquire as to their relative efficiency in utilizing the potential energy of foods in the special work they are fitted to perform*." Miles (1896) measured relative efficiency as the amount of energy intake (input) required to give a 100 lbs increase in live weight (output) across various livestock species. Although multiple feed efficiency measures have since been developed, such as feed conversion ratio, partial efficiency of growth (Kellner and Goodwin, 1910), maintenance efficiency (Garrett *et al.,* 1959) and residual feed intake (RFI) (Koch *et al.,* 1963), the concept of assessing inputs and outputs (component traits) has remained constant (Johnson *et al.,* 2003). In multiple feed efficiency measures, selection pressure on component traits is uncontrolled, limiting the prediction of genetic change (Gunsett, 1984). For instance, use of feed conversion ratio has resulted in undesirable selection responses such as increased mature weight and age at first calving (Crowley *et al.,* 2011). Selection pressure on the component traits of RFI can be controlled when using genetic regression, resulting in a measure that is independent of production (Kennedy *et al.,* 1993).

The concept of RFI in its classical determination is based on the partitioning of feed intake into maintenance (body weight) and production (average daily gain) requirements, a method that was first postulated in poultry. Byerly (1941) developed a series of partitioning equations for laying Leghorn chickens predicting feed intake based on body weight, body weight change and egg production, where feed efficient chickens consumed less feed. Koch *et al.* (1963) developed a similar method by regressing feed intake on mid-test body weight and gain where RFI represented the residual error of this regression. Although predicted dry matter intake (DMI) is independent of body weight and average daily gain, the configuration of these parameters can differ if body composition is not considered. Richardson *et al.* (1998) observed decreased rib fat and rump fat in cattle classified as low-RFI when body composition was not adjusted. Additional experiments (Herd and Bishop, 2000; Arthur *et al.,* 2001) observed similar relationships and suggested that low-RFI cattle have increased lean tissue and decreased fat accretion when using the predicted equation developed by Koch *et al.* (1963). On a wet-tissue basis the energetic efficiency of protein relative to fat accretion is greater (Owens *et al.,* 1995). Increased lean tissue and decreased fat accretion suggests that cattle identified as low-RFI by the Koch *et al.* (1963) method, could be leaner, later maturing animals potentially affecting the overall efficiency of beef production and carcass value. Inclusion of ultrasound measures of body composition in the determination of RFI has become a common and recommended practise to prevent this undesirable selection (Arthur *et al.,* 2001; Schenkel *et al.,* 2004; Lancaster *et al.,* 2009; Montanholi *et al.,* 2009). Inclusion of compositional measures for component traits in the determination of any residual measure of feed efficiency (i.e. milk composition in residual milk production; Berry and Pryce, 2014) is recommended to increase the control of selection pressure.

2.1.2 Genetic parameters

Residual feed intake is moderately heritable with recent values ranging from 0.21 to 0.49 (Bolormaa *et al.,* 2013; Khansefid *et al.,* 2014; Saatchi *et al.,* 2014). Durunna *et al.* (2012) determined RFI in crossbred heifers over two subsequent feeding periods to have a rank correlation of 0.50 to 0.52. A similar study design in yearling steers calculated a ranking correlation of 0.40 (Gomes *et al.,* 2012). Changing feed from a growing to finishing ration reduced rank correlations to 0.33 in feedlot steers (Durunna *et al.,* 2011). Correlations between post-wean heifer RFI and finished heifer RFI of 0.62 (Kelly *et al.,* 2010) and between post-wean heifer RFI and near mature cow RFI of 0.39 (Herd *et al.,* 2006) have also been reported. Adjustment for sources of non-genetic component trait variation (i.e. breed, age, environmental conditions and diet composition) could have increased the repeatability values reported by these studies. Breed, age and environmental conditions are often recorded in industry; therefore, their adjustment is possible and would concentrate selection on mainly differences in background energy expenditure. Despite the potential for improvement, repeatability estimates for RFI are higher than values reported for other feed efficiency measures (Kelly *et al.,* 2010; Durunna *et al.,* 2011 Gomes *et al.,* 2012).

2.2 Physiological indicators

2.2.1 Use in breeding programmes

Although the advantages of RFI over alternative feed efficiency measures are recognized, the requirement of an accurate, impartial average DMI measurement continues to limit improvement. Currently, Beef Improvement Federation guidelines recommend that a 21 day (d) adjustment plus 70 d feed recording period is installed for the determination of average DMI (Beef Improvement Federation, 2010). Culbertson *et al*. (2015) determined that for the calculation of average DMI for inclusion in feed intake selection indices, feed recording could be reduced to 42 d, while recording for determination of RFI could be reduced to 56 d. Industry wide adoption of this shortened feed recording period would aid feed efficiency improvement; however, phenotype throughput would still be limited by the requirement and expense of feed intake recording technologies (Neilsen *et al.,* 2013). Physiological indicator traits of feed efficiency, with continued development, could be determined along with or independent of feed intake, increasing the potential for on-farm use and phenotype throughput (MacNeil *et al.,* 2011). As direct measures of physiological processes, PIT are less influenced by non-genetic variation supporting a higher heritability (Thallman *et al.,* 2008); therefore, their use could also accelerate feed efficiency improvement (Nielsen *et al.,* 2013). Assuming identical selection pressure for PIT and a moderate genetic association with the trait of interest, greater genetic progress can be achieved through PIT than through direct trait selection (Falconer and Mackay, 1996). Furthermore, the close association of PIT with physiological processes suggests the presence of associations with other relevant traits and an ability to monitor correlated responses to selection.

Multi-trait selection indices for feed intake and other relevant traits with appropriate weights on component and physiological indicator traits have been developed (MacNeil *et al.,* 2011). These indices could serve as the evaluation tool in future selection decisions for improved feed efficiency, removing the need for phenotypic feed efficiency measures. However, prior to inclusion in selection indices, PIT of feed efficiency must be determined and understood at the physiological level, independent of genetic and environmental influences. After adjusting for non-energetic and compositional sources of variation, RFI is independent of production (Schenkel *et al.,* 2004; Berry and Crowley, 2013), mainly reflecting variation in biological processes such as digestion, heat increment of feeding and basal energy expenditure (Carstens and Kerley, 2009). Recognition of RFI as a potential measure for the evaluation of PIT has led to the completion of numerous studies evaluating relationships between physiological processes and RFI.

2.2.2 Potential physiological indicators

In a summarization of RFI studies on steer progeny, Richardson and Herd (2004) determined that biological variation in RFI could be explained by differences in digestion (10%), heat increment of feeding (9%), composition of gain (5%), activity (5%), protein turnover, stress and tissue metabolism (37%) and cellular energy expenditure (27%). Differences in microbial populations (Guan *et al.,* 2008; Carberry *et al.,* 2012), methane production (Freetly and Brown-Brandl, 2013; McDonnell *et al.,* 2016) and heat production (Montanholi *et al.,* 2016), a lower ruminal acetate to propionate ratio (Lawrence *et al.,* 2011; Fitzsimons *et al.,* 2013), increased ruminal butyrate and valerate (Guan *et al.,* 2008), increased intestinal mucosal densities (Meyer *et al.,* 2014), increased cellularity in the intestinal epithelium (Montanholi *et al.,* 2013) and decreased intestinal vascularization (Meyer *et al.,* 2014) in low-RFI cattle suggest differences in ruminal fermentation, digestive efficiency and heat increment of feeding. Use of ultrasound body composition measures in the determination of RFI removes the variation in skeletal muscle composition, thereby maintaining carcass value (Baker *et al.,* 2006); however, variation in visceral and adipose tissue composition can still exist (Richardson *et al.,* 2001) and could contribute to variation in RFI. Similar activity patterns (Lawrence *et al.,* 2011; Hafla *et al.,* 2013) and discrepancies in feeding behaviour measures (Nkrumah *et al.,* 2006; Montanholi *et al.,* 2010; Hafla *et al.,* 2013; Chen *et al.,* 2014) suggest that the association between activity and RFI is marginal, as noted by Richardson and Herd (2004). Decreased skeletal muscle uncoupling protein concentration (Kelly *et al.,* 2011), increased skeletal and liver mitochondrial respiratory rates (Kolath *et al.,* 2006a; Lancaster *et al.,* 2014), decreased HR (Hafla *et al.,* 2013) and increased liver oxygen consumption (Montanholi *et al.,* 2016) indicate an increased tissue metabolic efficiency and/or increased cellular energy expenditure in low-RFI cattle.

Although a plethora of physiological measures have been identified, each serving as a potential PIT for RFI, of greater interest is the network of associations among contributing physiological processes. A physiological measure that is associated with multiple physiological processes across the general mechanisms suggested by Richardson and Herd (2004) could explain a greater proportion of the variation in RFI and could have greater potential as a PIT for RFI.

2.3 Heart rate assessments

2.3.1 Feed efficiency assessments

Among the measures discussed, HR - a direct physiological measure of the cardiovascular system - has also been associated with heat production (Brosh *et al.,* 1998), activity patterns (Frondelius *et al.,* 2015) and stress (Stewart *et al.,* 2013; Van Reenen *et al.,* 2013) in cattle and is reflective of differences in metabolic demand (Walley, 2011). Johnson *et al.* (2003) first suggested the collection of HR phenotypes as a method to improve the energetic efficiency of beef cattle. Hafla *et al.* (2013) observed a lower HR in low-RFI pregnant Bonsmara beef cows, with RFI determined as heifers, over a 7-day resting assessment. A lower HR was also observed in low-RFI yearling beef bulls overnight and during transport (Montanholi *et al.,* 2014). However, no difference in HR between low-RFI and high-RFI yearling Nellore steers was observed during a 4-day resting assessment (Chaves *et al.,* 2015). Confounding results could be explained by a combination of genetic and non-genetic differences (i.e. environment, recording technologies, contemporary group), reinforcing the complexity of the association between HR and RFI that remains to be explored.

2.3.2 Routine husbandry and stress assessments

Although past associations between HR and RFI (Hafla *et al.,* 2013; Montanholi *et al.,* 2014) have been identified over long-term resting and transport assessments, the simplicity and non-invasiveness of HR technologies could allow phenotypes to be collected across a broad range of conditions. Associations of HR during transport with feed efficiency observed by Montanholi *et al.* (2014) suggest that differences in HR in response to stress could be associated with feed efficiency. Waynert *et al.* (1999) observed increased

HR in beef heifers exposed to novel handling equipment noise and handler vocalisation compared to heifers exposed to silence, a result also observed in steers and heifers during routine handling and restraint (Lay *et al.,* 1992b). Lay *et al.* (1992a) observed that cattle displayed increased HR responses to freeze branding relative to sham branding. An increased HR has also been observed in dairy heifer calves upon novel exposure to either routine ear-tagging or disbudding (Stewart *et al.,* 2013). Both sudden and novel exposure to a scarf increased HR in lambs, an increase attributed to sympathetic nervous system (SNS) activity (Désiré *et al.,* 2004). Assessments that evaluate HR in response to stress either during routine husbandry practise or during short and novel exposure could be implemented on-farm; however, associations between HR in response to stress and feed efficiency remain to be evaluated.

2.4 Cardiovascular measurements

A.D. Galen in his second century treatise *On the Usefulness of the Parts of the Body* described the heart as "*...the hearthstone and source of the innate heat by which the animal is governed*" (Talladge, 1968). Although not the source of heat, the cardiovascular system is integral in energy metabolism. Continuous contraction and relaxation of the heart and an intricate network of blood vessels allow the cardiovascular system to transport metabolites required for the function of cells, antibodies and inflammatory cells as part of the immune system and hormones that regulate cellular function (Wagner, 2007). Cardiovascular system function is assessed through CO, a measure of blood flow calculated as the product of HR and SV.

2.4.1 Cardiac output and stroke volume

Early determinations of CO were completed using the indicator dilution technique where a known mass of an indicator was suddenly administered into the central venous circulation, blood samples were continuously collected downstream and indicator concentration in blood was determined (Stewart, 1897). Concentration of this indicator in blood was initially presumed to be constant and indicator mass was divided by the product of indicator concentration in blood and assessment length to determine CO. This concept, the law of conservation of mass, was adapted from the Fick principle, the initial method developed for estimating CO (Fick, 1870). Hamilton *et al.* (1928) recognized that indicator concentration was not constant but that it initially increased and then decreased in an exponential matter. The generation of a concentration-time curve allowed this change in concentration over time to be quantified and the division of indicator mass by area under the concentration curve yielded an estimate of CO (Hamilton *et al.,* 1928). Stroke volume was then be calculated by dividing CO by mean HR over the assessment interval (Zierler, 1962). The indicator dilution technique, is dependent on five assumptions: 1) recirculation of the indicator does not occur or is accounted for, 2) the circulatory system displays stationarity, 3) volume of the system is constant, 4) indicator mixing in blood is complete and uniform and 5) blood flow is laminar (Zierler, 1962). Indocyanine green (ICG), a fluorescent dye, has become the predominant indicator used as it rapidly and tightly binds to plasma protein and is metabolized solely and rapidly by the liver (Cherrick *et al.,* 1960). Thermodilution (Ganz *et al.,* 1971) and lithium dilution (Linton *et al.,* 1993) techniques adapted from the conventional indicator dilution technique, are more feasible, can be completed quicker and are influenced less by recirculation (Reuter *et al.,* 2011).

With advances in technology various minimally invasive methods of determining CO and SV have been developed including Doppler echocardiography, magnetic resonance imaging (MRI), pulse contour analysis, thoracic bioimpedance and bioreactance methods. Doppler echocardiography measures mean blood flow velocity which is multiplied by blood vessel cross sectional area to determine SV with further multiplication by HR to determine CO (Colocousis *et al.,* 1977). Although Doppler echocardiography is simple, accurate values are dependent on laminar blood flow and measurement of cross sectional area at the exact location velocity is measured (Quiñones *et al.,* 2002), requirements that could be difficult to meet outside a clinical setting. Magnetic resonance imaging uses images of the two long axis planes of the heart and the assumption that left ventricle volume is represented by an ellipsoid to predict end diastolic and end systolic volumes with the difference equalling SV (Bellenger *et al.,* 2000). Minimal access to a MRI scanner in a livestock research facility and equipment cost could explain the absence of use in beef cattle. Pulse contour analysis is based on the relationship between pulse pressure and SV (Marik, 2013). If arterial compliance and vascular resistance are known the arterial pressure waveform can be used to determine SV and CO using algorithms (Slagt *et al.,* 2014). However, as pulse contour analysis is dependent on blood pressure, discrepancies in values have been observed as the algorithm is challenged to differentiate the direction of the relationship between blood pressure and SV (Camporota and Beale, 2010).

Thoracic bioimpedance measures changes in voltage when a high frequency electric current is passed across the thorax. The change in current relative to amplitude (transthoracic resistance or impedance), is directly related to the volume of fluid. The instantaneous change in impedance is proportional to CO; therefore, the division of this change by ventricular ejection time is equivalent to SV (Bernstein, 2010). Movement, electrode placement, temperature, humidity and other factors that influence conductivity have limited the accuracy of SV values determined by bioimpedance (Marik, 2013). Bioreactance using a similar concept, overcomes the limitations of bioimpedance by using changes in frequency rather than amplitude to determine SV, limiting the influence of background variation (Vergnaud *et al.,* 2015).

Of the described techniques, CO and SV values have been calculated in cattle using indicator dilution, (Holt *et al.,* 1962; Sato, 1984; Ono *et al.,* 2011), thermodilution (Davis *et al.,* 1988; Lin *et al.,* 1991; Amory *et al.,* 1992) and Doppler echocardiography (Starke *et al.,* 2011; Zarifi *et al.,* 2012; Kutter *et al.,* 2015). Indicator dilution techniques are also used in the estimation of BV, using the concentration-time graph and hematocrit through the back-extrapolation technique (Kisch *et al.,* 1995), an additional capability not held by other techniques. However, recirculation, stationarity, volume, mixing and laminar blood flow again must be considered (Zierler, 1962).

2.4.2 Heart rate

A complex network of modified cardiac muscle cells innervates the heart forming the cardiac conduction system (Anderson *et al.,* 2009). Following spontaneous depolarization of the sino atrial (SA) node a coordinated electrical impulse travels through the cardiac conduction system causing and controlling the timing of atrial and ventricular contraction (Laske *et al.,* 2009). Each firing of the SA node and consequential series of contractions represents a heartbeat. Electrodes placed on the skin record the voltage of the electrical field generated by this coordinated contraction (Dupre *et al.,* 2009). An electrocardiogram (ECG) machine processes the signal received from the electrodes to produce a voltage versus time graph, an electrocardiogram. The pattern of each series of contractions (heart beats) is comprised of three major waves (P, QRS and T) that represent atrial depolarization, ventricular depolarization and ventricular repolarization (Dupre *et al.,* 2009). Heart rate is then determined based on the distance between successive QRS complexes (Ashley and Niebauer, 2004).

Cost and complexity of ECGs has led to the development of applicable telemetric HR recording technologies (Laukkanen and Virtanen, 1998). Polar (Polar Electro Oy ©, Kempele, Finland) telemetric based HR systems consist of two surface electrodes that detect the electrical impulses of the heart that are recorded and processed by a pulse-to-pulse time-averaging algorithm using a moving average process to determine HR (Karvonen *et al.,* 1984). In cattle, Polar systems have been validated during rest, activity and stress (Hopster and Blokhuis, 1994) and used to record HR during rest (Hafla *et al.,* 2013), handling (Waiblinger *et al.,* 2004), ear-tagging and disbudding (Stewart *et al.,* 2013). Signer *et al.,* (2010) developed an accelerometer based telemetric HR system where a bolus located in the reticulum records the mechanical vibrations of the heart against the reticulum wall, an algorithm then differentiates heart from ruminal vibrations to determine HR. This system has been validated with an ECG during rest in sheep (Signer *et al.,* 2010) and tested in beef cattle (Munro *et al.,* 2015). Accelerometer systems have potential for applications in extensive production systems such as grazing, due to their ability to record HR non-invasively over extended periods. However, the validity of this accelerometer during periods of activity, stress or extensive rumination must first be assessed. Foulkes *et* *al.,* (2013) determined HR in cattle by equipping a modified ear tag with a pulse oximeter; however, the system was unable to calculate accurate HR values. Using a similar pulse oximetry system logical HR values were observed but power consumption and physical size limited further testing and commercial production (Nagl *et al.,* 2003). Despite advances in accelerometery and pulse oximetry, Polar Electro systems remain as the leading HR technology used in livestock research due to their simplicity and commercial availability.

2.5 Long-term changes in cardiovascular function

As part of a service system (Baldwin *et al.,* 1980) supplying body systems and tissues with the metabolites demanded, CO, SV and HR are influenced by multiple mechanisms. Differences in body size, oxygen-carrying capacity and metabolic demand alter CO in the long-term (Walley, 2011) through neural, endocrine and renal modes of regulation (Hall, 2015). Lower long-term resting HR in low-RFI cattle (Hafla *et al.,* 2013; Montanholi *et al.,* 2014) indicates that the mechanisms and/or modes of regulation influencing HR could be associated with RFI, mechanisms that in addition could cause variation in SV and/or CO.

2.5.1 Body size and oxygen-carrying capacity

The inclusion of body weight in the predicted DMI equation for RFI negates the influence of body size. Oxygen delivery to the periphery is the product of CO and blood oxygen-carrying capacity (Walley, 2011); therefore, changes in hemoglobin (Hb) concentration, red blood cell (RBC) count, or hematocrit (Hct) alter oxygen-carrying capacity and could cause a subsequent CO change in order to meet oxygen delivery requirements. No differences in Hb concentration or RBC have been observed across RFI groups of yearling non-pregnant heifers (Kelly *et al.,* 2016), primiparous heifers (Lawrence *et al.,* 2011), yearling bulls (Gomes *et al.,* 2011; Santana *et al.,* 2013) or yearling steers (Gomes *et al.,* 2011). Richardson *et al.* (2002) observed no correlation between sire estimated breeding value for RFI and steer progeny Hb or RBC count either at pasture or in the feedlot. However, a positive relationship was observed between sire estimated breeding value for RFI and steer progeny Hb concentration measured during a feed intake test (Richardson *et al.,* 2002). Additional generations of selection on RFI could further decrease Hb in low-RFI cattle without changing RBC count, suggesting a lower oxygen-carrying capacity. Furthermore, lower RBC counts have been observed in low-RFI ewes and rams (Rincon-Delgado *et al.,* 2011), suggesting that relationships between oxygen-carrying capacity and RFI differ across species and require further investigation.

2.5.2 Metabolic demand

Metabolic demand is dependent on metabolic rate, which has long been estimated in cattle through the measurement of oxygen consumption or heat production (Kleiber, 1932; Kleiber, 1947). Associations between HR and oxygen consumption in cattle can be traced to the experiments of Benedict (1924) where a lower HR was observed in feed restricted (50 % of basal energy requirements) cattle compared to non-restricted cattle. Eisemann and Nienaber (1990) observed that oxygen consumption and CO decreased on a tissue-specific (portal drained viscera, liver and hindquarter) and whole-animal basis in fasted versus fed beef steers. This decrease in CO was explained in part by a decrease in

HR between the fed and fasted state (Eisemann and Nienaber, 1990). Similar differences have been observed in liver and portal blood flow between fed and fasted lactating and non-lactating dairy cows (Lomax and Baird, 1983). Decreased whole-animal oxygen consumption (Burrin *et al.,* 1990) and decreased portal drained viscera and liver oxygen consumption and blood flow (Burrin *et al.,* 1989) have also been observed in maintenance versus *ad libitum* fed sheep. Lower heat production, measured by a calorimeter, was observed in feed restricted low-RFI cattle (Nkrumah *et al.,* 2006; Chaves *et al.,* 2015). A positive relationship between feed intake and both oxygen consumption and heat production suggests a lower metabolic demand in low-RFI cattle. However, the feed intake restriction applied in the aforementioned studies could confound this relationship (Blaxter, 1967). Montanholi *et al.* (2009; 2010) observed decreased radiant heat production in *ad libitum* fed low-RFI beef steers and bulls, measured using thermography. In a follow up study, Montanholi *et al.* (2016) measured oxygen consumption through indirect calorimtery and radiant heat production through repeated thermographic measurements over the circadian period and observed increased whole-animal and liver oxygen consumption and decreased radiant heat production in *ad libitum* fed low-RFI beef heifers. Indirect calorimeter measurements are made on trained animals at rest, likely representing tissue, cellular and digestive energy expenditures. Consequently, a higher oxygen consumption and a lower radiant heat production suggest that a greater proportion of metabolic demand is associated with tissue, cellular and digestive mechanisms but that total metabolic demand is lower in low-RFI cattle, supporting the lower CO observed in feed restricted cattle and sheep (Burrin *et al.,* 1989; Burrin *et al.,* 1990; Eisemann and Nienaber, 1990). Furthermore, higher liver oxygen consumption in low-RFI cattle

(Montanholi *et al.,* 2016) suggests that tissue differences in metabolic demand could be associated with total metabolic demand and CO.

Increased liver mitochondrial function and reduced liver size were attributed with increasing feed efficiency during compensatory growth in limit-fed beef steers (Connor *et al.,* 2010) which in conjunction with increased liver oxygen consumption (Montanholi *et al.,* 2016) suggests increased liver metabolic activity in low-RFI cattle. Hepatic mitochondria in low-RFI beef heifers fed a grain-based diet had increased state 3 respiration rates, acceptor control ratios and respiratory control ratios (Lancaster *et al.,* 2014). However, when heifers were fed a forage-based diet, no difference in state 3 respiration rate, acceptor control ratio and respiratory control ratio was observed (Lancaster *et al.,* 2014). Higher mitochondrial state 2 and 3 respiration rates and respiratory control ratios were observed in the *longissimus dorsi* muscle of low-RFI beef steers fed a grain-based diet (Kolath *et al.,* 2006a). State 3 respiration rate displays a positive, exponential relationship with mitochondrial efficiency (Mogensen and Sahlin, 2005). A higher respiratory control ratio (state 3/state 4) results from reduced proton leak from respiration to oxidative phosphorylation. Kelly *et al.* (2011) evaluated the messenger ribonucleic acid (mRNA) expression of genes involved in the respiratory chain complex and mitochondrial biogenesis in *longissimus dorsi* muscle and observed decreased uncoupling protein 3 (UCP3; induces proton leak) expression in low-RFI beef heifers across forage- and grain-based diets, supporting the theory of decreased proton leak in low-RFI cattle. However, in blood or masseter muscle mitochondria no difference in mRNA expression of UCP3 was observed between RFI groups of Angus steers (Kolath *et al.,* 2006b) or Nellore cattle (Fonseca *et al.,* 2015). Expression of UCP3 is high in skeletal muscle relative to other locations (Ledesma *et al.,* 2002), explaining the absence of an association with RFI in non-skeletal tissue. Increased oxygen consumption, cell size, respiration rates, respiratory control ratio, acceptor control ratio and decreased UCP3 expression indicate increased metabolic activity (Brand and Nicholls, 2011) and efficiency (Kolath *et al.,* 2006a) in the liver and skeletal muscle of low-RFI cattle fed grain-based diets.

Increased metabolic efficiency can decrease vascularity (less vascular tissue) and CO as metabolic demand is reduced (Hall, 2015). Meyer *et al.* (2014) observed a positive correlation between small intestine vascularity and DMI and a trend towards a positive correlation between small intestine vascularity and RFI in steers and heifers. While Montanholi *et al.* (2013) observed an increased cell number in the crypts of the small intestine of low-RFI steers. Decreased vascularity and increased cell number in the small intestine of low-RFI cattle supports the theory of increased metabolic activity and efficiency in productive tissues and could support a decreased CO in low-RFI cattle. Differences in metabolic activity and efficiency associated with RFI appear to be: 1) tissue specific, 2) reflective of differences in proton leakage and vascularity and 3) influenced by diet.

Overall, higher calorimetric and lower thermographic heat production suggest that low-RFI cattle have an increased metabolic demand for tissue, cellular and digestive mechanisms, partitioning less energy to non-productive mechanisms such as activity and stress, resulting in a lower total metabolic demand. In addition, increased liver, skeletal and small intestine metabolic activities and efficiencies in low-RFI cattle suggest a more efficient use of this metabolite supply for productive processes, which could aid in explaining how low-RFI cattle achieve a parallel production at a lower feed intake.

Associations of metabolic demand, energy partitioning, metabolic activity and metabolic efficiency with RFI and the absence of any defined associations between oxygen-carrying capacity and RFI indicate that low-RFI cattle could have a decreased CO. A suggested lower CO and a lower HR observed during rest in low-RFI cattle (Hafla *et al.,* 2013; Montanholi *et al.,* 2014) suggest that SV has either a negative or no association with RFI. However, studies directly assessing CO and SV in the context of feed efficiency in cattle are warranted.

2.5.3 Neural, endocrine and renal regulation

Decreased CO is sustained by decreasing blood pressure equilibrium or by increasing total peripheral resistance - complementary mechanisms that in the long-term are based on blood pressure regulation by renal, neural and endocrine (renin-angiotensin-aldosterone system (RAAS)) mechanisms. Reduced oxygen consumption or increased metabolic efficiency decrease CO, reducing blood pressure below the equilibrium point of the renal output curve, stimulating the increased retention of water and salt by the kidneys. This retention acts to increase BV and the amount of blood returning to the heart, increasing HR and contractility, returning CO and blood pressure to original levels (Hall, 2015). Renin release is stimulated by reduced blood flow to the kidneys and the SNS, which precipitates the conversion of angiotensinogen to angiotensin I in the liver and subsequent conversion to angiotensin II, a vasoconstrictor that induces the release of aldosterone, further stimulating the reabsorption of water and salt (Laragh, 1985). However, based on sustained HR reductions in low-RFI cattle (Hafla *et al.,* 2013; Montanholi *et al.,* 2014), CO could remain at a lower level, suggesting a reduced blood pressure value at the equilibrium point on the renal input/output curve or reduced activity of the RAAS.

Messerli *et al.* (1978) compared the CO, arterial blood pressure, renal blood flow and plasma renin activity of humans with low, normal and high cardiac indices (CO scaled by body weight) and observed that patients with low CO had both lower SV and HR than high CO patients. Furthermore, CO was positively correlated with BV, renal blood flow and hepatic blood flow, while total BV was negatively correlated with total peripheral resistance. The results of Messerli *et al.* (1978) suggest that decreased resting HR in low-RFI cattle could be: 1) sustained through neural, endocrine (RAAS) and renal mechanisms of regulation and 2) associated with a decreased SV and total BV. Overall, renal function and RAAS activity appear to be positively associated with RFI, suggesting that decreased metabolic demand and increased metabolic efficiency in low-RFI cattle reduce blood pressure, CO, HR and SV in the long-term through neural, endocrine and renal mechanisms.

2.5.4 Heart structure

Differences in metabolic demand alter the workload on the heart causing changes in heart structure, described as either concentric or eccentric physiological hypertrophy (Maillet *et al.,* 2013). Concentric hypertrophy is characterized by an increase in wall thickness and cardiac mass with or without a decrease in ventricular capacity through the parallel addition of sarcomeres increasing cell width (Wikman-Coffelt *et al.,* 1979; Bernardo *et al.,* 2010). Eccentric hypertrophy is characterized as an increase in cardiac mass and ventricular capacity through the addition of sarcomeres in series increasing cell length (Bernardo *et al.,* 2010). Seymour *et al.,* (2006) induced 15% caloric restriction in rats for 18 weeks and observed lower end diastolic left ventricular volume, relative wall thickness, and left ventricle mass scaled for body weight and increased cardiac index (CO per unit of body weight) in calorie restricted versus non-restricted rats. Decreased left ventricle mass, posterior wall thickness expansion, end diastolic volume, SV and CO was observed in 40% calorie restricted rats after adjustment for body weight (Ahmet *et al.,* 2011). Furthermore, when assessing left ventricle microstructure in transverse tissue sections, decreased myocyte width and myocardial fibrosis and increased myocyte density were observed in calorie-restricted rats (Ahmet *et al.,* 2011). The results of past studies in rats when adjusting for body weight indicate that variation in feed efficiency could be associated with changes in heart structure, where high-RFI cattle could display characteristics of concentric hypertrophy when compared to low-RFI cattle. Although feed intake is reduced in low-RFI cattle, it remains *ad libitum* which could alter the impact on heart structure in comparison to induced caloric restriction; therefore, studies in cattle under conditions of *ad libitum* feed intake reduction are warranted.

2.6 Short-term changes in cardiovascular function

Lower HR observed during transport in low-RFI cattle (Montanholi *et al.,* 2014) represent changes in HR during short-term, stressful conditions. Furthermore, changes in HR in response to acute stress challenges such as routine husbandry practises (Lay *et al.,* 1992a and 1992b; Waynert *et al.,* 1999; Stewart *et al.,* 2013) are also regulated by short-term mechanisms; therefore, differences in the mechanisms regulating short-term cardiovascular function could be associated with differences in RFI. Heart rate, SV and CO regulation in the short-term differs from long-term regulation whereby the influences of body size, oxygen-carrying capacity and metabolic demand are minimal and changes are largely regulated by neural and endocrine mechanisms (Ulrich-Lai and Herman, 2009).

2.6.1 Mechanisms of stress response

When a stimulus is perceived as a stressor neural impulses travel to the posterior hypothalamus (sympathetic activation) and the anterior hypothalamus (parasympathetic activation) stimulating the SNS and inhibiting the parasympathetic nervous system (PNS, vagal) (Everly and Lating, 2013). Stimulation of the SNS causes the release of noepinephrine from postganglionic neurons that acts on specific target organs, including the heart, causing an increase in HR (Purves, 2001). Stimulation of the SNS increases contractility (Obrist *et al.,* 1974) resulting in an increased SV and CO (Hall, 2015). Longer perceived stressors (minutes versus seconds) in addition are fought by the activation of the hypothalamic-pituitary-adrenal (HPA) axis whereby neurons in the paraventricular nucleus are stimulated, causing the secretion of corticotropin releasing hormone (CRH) (Ulrich-Lai and Herman, 2009). Corticotropin releasing hormone stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, acting on the adrenal cortex, stimulating the production and release of cortisol, causing an increase in HR (Tsigos and Chrousus, 2002; Ulrich-Lai and Herman, 2009). The adrenal cortex is also innervated by the SNS (Purves, 2001) allowing for a rapid response, as the two-step hormonal activation process of HPA axis is bypassed.

2.6.2 Cortisol and behavioural reactivity

Knott *et al.* (2008) challenged rams with an exogenous administration of ACTH and observed decreased plasma cortisol responses in low-RFI rams. Plasma cortisol responses were also lower in feed efficient pigs after exogenous administration of ACTH (Hennessy *et al.,* 1988; Jenkins *et al.,* 2013). It is unclear if these increased cortisol responses are associated with increased sympathetic reactivity, increased HPA axis reactivity or both, as blood samples were collected 45 (Knott *et al.,* 2008) and 60 (Hennessey *et al.,* 1988) minutes after administration or were the average of 30, 45, 60 and 90 minute collections (Jenkins *et al.,* 2013).

After nine generations of divergent selection on RFI, chickens were challenged with an exogenous ACTH source and lower cortisol responses were observed in low-RFI chickens (Luiting *et al.,* 1994). The cortisol response was maintained longer in low-RFI chickens, which could indicate differences in parasympathetic reactivity. After eight generations of selection on RFI, castrated male pigs were exposed to both novel object and human approach tests to evaluate behaviour responses to stressful stimuli (Colpoys *et al.,* 2014). Low-RFI pigs had less head movements and froze and defecated less frequently in response to the novel object test and had fewer escape attempts during the human approach test, indicating decreased behavioural reactivity (Colpoys *et al.,* 2014). However, it is unknown if physiological reactivity (cortisol or HR response) was also associated with RFI. Although previous studies have indicated associations between stress response and RFI, sampling frequencies have limited the ability to differentiate the influences of the SNS and HPA axis.

2.6.3 Heart rate variability

Although inferences regarding autonomic activity can be made using HR the influences of the SNS and PNS are difficult to differentiate (Kindlon *et al*., 1995). An increase in HR in response to stress is typically generated through an increase in sympathetic activity (Purves, 2001); however, decreases in parasympathetic activity may or may not occur. Heart rate variability (HRV) has developed into a non-invasive indicator of autonomic activity and stress in cattle (von Borell *et al.,* 2007), allowing for differentiation of sympathetic and parasympathetic influences. The interval between heartbeats varies constantly from beat to beat reflecting the oscillation of largely the SNS and PNS; this irregularity of interbeat intervals (IBI) is termed HRV (Pumprla *et al.,* 2002). A graph of interbeat intervals displays oscillations on a time scale, where SNS oscillations are at a lower, and PNS at a higher frequency; therefore, analysis of IBI and instantaneous HR can provide information regarding the inputs of the two branches of the autonomic system (von Borell *et al.,* 2007).

Heart rate variability analysis can be divided into four categories: 1) time domain, 2) geometric 3) non-linear and 4) spectral. Time domain analysis involves the statistical analysis of IBI or instantaneous HR, including standard deviations and the square root of the sum of the squares of differences between consecutive IBI (Malik, 1996). Geometric analyses convert IBI into geometric forms, such as histograms that allow for interpretation of density distributions (von Borell *et al.,* 2007). Non-linear analysis is based on chaos theory and uses recurrence, dimension analysis, Lyapunov exponents and entropy estimates to identify non-linear properties in time series data that are related to physiological processes (Mansier *et al.,* 1996, von Borell *et al.,* 2007). In spectral analysis the harmonic waves that comprise each signal are divided into their sine and cosine components using parametric or non-parametric methods (Pichon *et al.,* 2006). In nonparametric methods the Fast Fourier Transformation (FFT) folds the time series data before calculating the power of the harmonics; therefore, the sampling frequency must be consistent (von Borell *et al.,* 2007). Sampling frequency will also determine the highest frequency that can be assessed; where according to the Nyquist limit the highest frequency is equal to 1/sampling interval (von Borell *et al.,* 2007). A power spectrum will be generated by FFT, allowing the powers within each frequency range to be assessed (Mansier *et al.,* 1996). Low frequency (LF; 0.04-0.15 Hz) and very low frequency bands (VLF; <0.04 Hz) are associated with sympathetic activity and the high frequency band (HF; 0.15-0.4 Hz) with parasympathetic activity (McCraty *et al.,* 1995). Parametric methods such as the autoregressive method, utilize models to generate smooth spectral components, a more complex method requiring verification of the selected model (Sztajzel, 2004).

In calves, Mohr *et al.* (2002) evaluated the associations of HRV parameters during no stress, external stress (increased temperature and insect presence) and internal stress (diarrhea) and observed a higher low frequency to high frequency ratio (LF:HF) during external and internal stress compared to no stress. A higher LF:HF was observed when comparing IBI 5 minutes after to 5 minutes before disbudding without anaesthetic in calves, which was accompanied by an increase in HR (Stewart *et al.,* 2008). Stewart *et al.* (2010) castrated calves with or without anaesthetic analyzing IBI 5 minutes before and 5 minutes after castration and observed a decreased LF:HF and increased HR and cortisol levels in response to castration without anaesthesia (Stewart *et al.,* 2010). A decrease in LF:HF indicates a shift in the sympathovagal balance towards parasympathetic activity, an opposite response to that observed by Mohr *et al.* (2002) and Stewart *et al.* (2008). Heart rate increased before castration with maximal HR response occurring at castration, followed by a rapid decrease within the first two minutes after castration. Therefore, the decreased LF:HF following castration represents the return to homeostasis (increased parasympathetic reactivity) and the higher LF:HF prior to castration represents an increase in sympathetic reactivity. Although calves were habituated to the handling facility and restraint prior to the experiment, handling prior to castration could have caused this increase in HR and sympathetic activity, confounding the HR and HRV results.

Spectral measures of HRV appear to be accurate and sensitive indicators of autonomic reactivity in cattle in response to stressful stimuli typically encountered on-farm. Although the associations between HR, acute stress and RFI indicate that differences in HRV in response to stress will be associated with RFI, this association has not been evaluated in cattle. Studies measuring cortisol and behaviour reactivity to stress (Hennessy *et al.,* 1988; Luiting *et al.,* 1994; Knott *et al.,* 2008; Jenkins *et al.,* 2013; Colpoys *et al.,* 2014) suggests that low-RFI cattle have either lower sympathetic reactivity, lower HPA axis reactivity, or both. Evaluation of HRV parameters in response to acute stress and their association with HR and RFI will enable the influence of the autonomic nervous system to be differentiated from the HPA axis, potentially clarifying the mechanisms contributing to the association between acute stress HR and RFI (Montanholi *et al.,* 2014). This evaluation of HRV could also provide information on the associations of RFI with SV and CO under conditions of acute stress, due to their positive relationships with sympathetic stimulation (Obrist *et al.,* 1974; Hall, 2015). Although HRV analysis is recommended as a measure of autonomic responsiveness rather then autonomic tone (Saul, 1990), analysis of HRV during rest could further our understanding of the associations between resting HR and RFI (Hafla *et al.,* 2013; Montanholi *et al.,* 2014).

2.7 Opportunities for heart rate in beef cattle production

Heart rate as a potential proxy for feed efficiency could have the ability to improve beef cattle husbandry, environmental impact and social perception by reducing feed and land use (Capper and Bauman, 2013). The ability of HR to indicate stress level on-farm has also been reviewed throughout this document, associations that could lead to improvements in animal welfare. Nagel *et al.* (2016) observed an increase in HR from the expulsive phase of labour, just prior to behavioural signs, until calving in Simmental cows. Heart rate increased with behavioural signs of calving onset in Holstein-Friesian cows, peaking at calving and increasing with calf body weight and cow body condition during calf delivery (Kovács *et al.,* 2015). Increases in HR are associated with clinical signs of puerperal metritis, a severe infection of the uterus following parturition in cows (Sheldon *et al.,* 2006). Heart rate displayed a positive association with horn fly density in steers (Schwinghammer *et al.,* 1987) and insect harassment in calves (Mohr *et al,* 2002). Associations of HR with calving onset, calf weight and cow body condition indicate that HR could be used as an indicator of calving, calf weight, calving ease and cow body condition, measures that are of importance to both commercial and seedstock cow-calf producers (BIF, 2010). Pending the continued development of non-invasive telemetric HR monitors, such as the system designed by Signer *et al.* (2010), HR thresholds in relation to infection or insect level could be set and used to determine when to treat cattle, potentially reducing labour and cost while increasing productivity. However, repeatability of these assessments and the feasibility of HR technologies in commercial settings will have to be determined prior to commercial use.

Heart rate has the potential to be a multipurpose management tool allowing for the indirect assessment of traits that are of economic, social and environmental importance to both producers and consumers. Therefore this thesis, assessing HR as a potential proxy for feed efficiency and evaluating the role of underlying mechanisms, could serve as a model for future studies in the development and assessment of HR based proxies for the beef industry.

CHAPTER 3

Associations of acute stress and overnight heart rate with feed efficiency in beef heifers

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Abstract

Proxies have the potential to accelerate feed efficiency (residual feed intake; RFI; kg DM/d) improvement, assisting with the reduction of beef cattle feed costs and environmental impact. Heart rate (HR; BPM) is associated with feed efficiency and influenced by autonomic activity and peripheral metabolism, suggesting HR could be used as a proxy for feed efficiency. Objectives were to assess associations between overnight HR, lying patterns and RFI, and between acute stress HR and RFI. Heifer calves (n = 107; 408 ± 28 days of age, 341 ± 42.2 kg) and yearling heifers (n = 36; 604 ± 92 days of age, 539 ± 52.2 kg) were exposed to a performance test to determine productive performance. Overnight HR (electrode based) and lying patterns (accelerometer based) were monitored on a subgroup of heifer calves (n = 40; 20 lowest-RFI; 20 highest-RFI). In the 10-minute acute stress assessment, all heifers were individually exposed to the opening and closing of an umbrella and HR before (HR_{BEF}), in response to (HR_{MAX}), after (HR_{AFT}) and change (HR_{CHG}; HR_{AFT}-HR_{BEF}) as a result of exposure were determined. Using polynomial regression, rate of HR decrease pre-exposure (β_1) and rates of HR increase (β_2) and decrease (β_3, β_4) post-exposure were determined. Heifer calves in the overnight assessment were classified into equal RFI groups (low-RFI; high-RFI) and HR means were treated as repeated measures and compared using multiple regression. In the acute stress assessment, heifers were classified within cattle category into equal RFI groups (low-RFI; high-RFI) and means and polynomial regression parameters were compared using multiple regression. Low-RFI heifer calves had a lower overnight HR (69.2 vs. 72.6 BPM), similar HR change from lying to standing intervals (8.9 vs. 9.2 BPM) and similar time lying (61.1 vs. 64.5 %) compared to high-RFI heifer calves. Low-RFI heifer calves had a higher absolute HR_{MAX} (162.9 vs. 145.7 BPM) and β_2 (-0.34 vs. -0.20) than high-RFI heifer calves. Low-RFI yearling heifers had similar acute stress HR means and a lower β_1 (0.003 vs. 0.006) than high-RFI yearling heifers. Overnight HR and acute stress HR are potential indicators of RFI in heifer calves. However, acute stress HR results varied in yearling heifers, suggesting previous handling experience and/or age influence stress response. Pending further development (predictive ability, repeatability), the acute stress assessment could have potential for on-farm application as a feed efficiency proxy in young heifers with minimal handling experience.

Keywords: biomarker, bovine behaviour, cardiovascular function, coping style, residual feed intake

Implications

Heart rate as a proxy for feed efficiency can aid in improving beef cattle husbandry, reducing feed costs and environmental impact. Feed efficient heifer calves have a lower overnight heart rate and increased heart rate upon acute stress. Contrasting acute stress results in yearling heifers suggest that coping styles vary across cattle categories. Overall, results indicate that overnight and acute stress heart rates are potential proxies for feed efficiency in heifer calves. Due to its short duration, with further development the acute stress assessment could have potential for on-farm use as a feed efficiency proxy in minimally handled young heifers.

Introduction

The beef industry is challenged with reducing feed costs that represent the largest production expense and with minimizing environmental impact from resource use and greenhouse gas emissions (Gerber *et al., 2013*; Lancaster *et al.,* 2014). Improving the efficiency of feed utilization could mitigate these challenges. Residual feed intake (**RFI**; kg DM/d) is a feed efficiency measurement that has experienced increased use due to its phenotypic independence from production traits (Montanholi *et al.,* 2009), demonstrated repeatability (Kelly *et al.,* 2010) and moderate heritability (Schenkel *et al.,* 2004). Direct determination of RFI cannot be conducted without substantial input and labour costs, limiting trait improvement. Proxies of feed efficiency have been recognized as alternatives for determination and improvement (Pollak *et al.,* 2012) and upon development could be implemented on-farm as screening tools.

Heart rate (**HR**; beats per minute, **BPM**) is a non-invasive measure that has been associated with RFI during rest, transport and long-term assessments (Hafla *et al.,* 2013; Montanholi *et al.,* 2014), suggesting HR could be developed as a feed efficiency proxy. Cardiovascular function is regulated by the autonomic nervous system and responds to the metabolic activity of peripheral tissues (Purves *et al.,* 2001). Variation in peripheral tissue metabolic activity has been associated with variation in RFI (Kolath *et al.,* 2006a; Lancaster *et al.,* 2014). Variation in peripheral tissue metabolic activity is associated with a change in peripheral resistance and blood pressure. Baroreceptors sense blood pressure changes and send sensory information via afferent nerves to the cardiac control center. Sensory information is integrated and an autonomic response is generated, altering cardiovascular function (heart rate, stroke volume and arterial dilation) in order to restore blood pressure (Purves *et al.,* 2001; Hall*,* 2015). Variation in HR could be associated with RFI through a combination of metabolic and autonomic mechanisms depending on the conditions under which HR is measured (rest, long-term stress or acute stress).

Hafla *et al.* (2013) identified associations between 7-day resting HR and RFI in pregnant beef cows and pregnant yearling heifers. Evaluation of the association between resting HR and RFI in young female cattle will determine the ability of resting HR assessments as screening tools for breeding stock on-farm. However, during this extended resting period the influence of lying patterns on the association between HR and RFI should be evaluated as, lying patterns are associated with HR (Frondelius *et al.,* 2015). Associations between transport HR and RFI in yearling bulls (Montanholi *et al.,* 2014) suggest that HR upon exposure to a stressful stimulus is also associated with RFI. Furthermore, Désiré *et al.* (2004) observed that both sudden exposure and novel stimuli increased HR and that response to the stimulus was associated with changes in autonomic nervous system activity, which could be associated with RFI. Associations among sudden and novel exposure, HR and RFI suggest that short assessments that suddenly expose cattle to a novel stimulus, such as an umbrella (Sandem *et al.,* 2004), could be developed as feed efficiency screening tools for inclusion in routine husbandry practices, potentially increasing on-farm use. Therefore, the objectives of this study were (1) to assess associations of HR and lying patterns recorded overnight during rest with RFI in beef heifer calves and (2) to assess associations of HR recorded during exposure to an acute stressor (umbrella) with RFI in beef heifer calves and yearling heifers.

Material and methods

Animals, husbandry and facilities

Experimental procedures were determined in accordance with the recommendations of the Canadian Council on Animal Care (2009). Beef heifer calves (n = 107; initial age: 285 ± 28 days) and yearling heifers (n = 36; initial age: 480 ± 92 days) were obtained from 20 commercial producers from The Maritimes of Canada (7.2 ± 5.4 cattle per producer). Genomic breed composition of both cattle categories (heifer calves and yearling heifers; Table 3.1) was determined on hair follicle DNA submitted for 50K SNP sequencing (ADMIXTURE® software, University of California, Los Angeles, USA) using pairwise comparison by estimating individual and population breed allele frequencies (Connolly *et al.,* 2014). Heifers were housed in a straw-bedded group pen facility (Maritime Beef Test Station, Nappan, Canada) from June to October 2014. Heifer calves were housed in three pens and yearling heifers in one pen at a stocking rate of 15.1 ± 0.3 m^2 per animal. Within each pen 50% of space was a concrete outdoor yard, 20% was a straw pack covered by a roof and 30% was a barn containing feeding stations and water bowls. Each pen contained five automated feeding stations (GrowSafe Systems Ltd., Airdrie, Canada) that recorded the individual daily feed intakes of all animals, for the determination of average daily feed intake. Animals were fed twice daily a grass-based ensilage ration of the composition listed in Table 3.2. Ration samples were collected weekly, homogenized on a monthly basis for chemical analysis and monthly results were averaged (Table 3.2).

Upon arrival at the research station, an acclimation period of three weeks was given before feed intake recording began. Daily feed intake and productive performance were then evaluated for heifer calves and yearling heifers over a 124-day test (Lawrence *et al.,* 2011), determining body weight and ultrasound body composition on 30.6 ± 3.0-day intervals.

The acute stress assessment was completed on all heifer calves and yearling heifers over days 121 to 124 within the handling facility at the research station. The overnight assessment was completed on a subgroup of heifer calves selected divergently on RFI (20 lowest-RFI; 20 highest-RFI) from the 107 heifer calves, over days 126 to 130 in a separate building within the research facility after a one-day acclimation period. A separate building was used as it was indoors, increasing the ease of monitoring and reducing the daily variation in relative humidity and air temperature while allowing stocking rate to be maintained. At the end of the productive performance test heifer calves were 408 ± 28 days of age and weighed 341 ± 42.2 kg while yearling heifers were 604 ± 92 days of age, weighed 539 ± 52.2 kg and were 109 ± 85.8 days in gestation.

Productive performance assessment

On an individual basis, daily feed intakes were assessed and recording errors were removed. Daily feed intakes that had less than a 2% probability of belonging to the normal distribution of daily feed intake were removed and average feed intake was calculated and standardized to dry matter intake (kg DM/d). Bodyweights were collected using a calibrated weigh scale (CattleMaster, Linwood, Canada). Ultrasound measurements were completed using an Aloka SSD-500 long probe, ultrasound unit (model 5044, 172 mm, 3.5 MHz; Corometrics Medical Systems, Wallingford, USA). Ultrasound measures including back fat thickness (mm), rib eye area (cm^2), rump fat thickness (mm) and marbling (1 (devoid) – 11 (abundant)) were collected. Average daily gain (kg/day) was calculated as the linear regression of BW on day of BW measurement (d0, d31, d62, d93, d124). Feed conversion ratio was the result of dry matter intake divided by average daily gain. Predicted feed intake (kg/d) was calculated using multiple linear regression in the GLM procedure of SAS software® (University Edition, Copyright©, SAS Institute Inc., Cary, USA). In both heifer calves and yearling heifers a predicted feed intake model was selected with the highest R^2 and the lowest Bayesian information criteria value, resulting in the following models:

$$\textit{Predicted feed intake}_{\textit{Heifer calves}} = -3.66 + 0.02(\textit{BW}) + 2.95(\textit{average daily gain}) + 0.07(\textit{back fat thickness}) - 0.04(\textit{rib eye area}) - 0.002(\textit{rump fat thickness}) - 0.02(\textit{marbling}) + 0.02(\textit{days of age}) + \textit{RFI},\ R^2=0.53$$

$$\textit{Predicted feed intake}_{\textit{Yearling heifers}} = -2.88 + 0.02(\textit{BW}) + 2.66(\textit{average daily gain}) + 0.08(\textit{back fat thickness}) - 0.02(\textit{rib eye area}) - 0.15(\textit{rump fat thickness}) - 0.11(\textit{marbling}) - 0.01(\textit{days in gestation}) + 0.01(\textit{days of age}) + \textit{RFI},\ R^2=0.49$$

Heart rate, lying pattern, humidity and temperature assessments

Heart rate was recorded continuously and averaged on 5-second intervals using an electrode-based system (Polar RC3 GPS or RS 800CX Science, Polar Electro, Kempele, Finland) adapted for heifers (Figure 3.1). Cattle were equipped with the Polar system and a custom leather harness that consisted of three parts: (1) a chest strap containing the Polar electrodes, transmitter and logger, (2) a neck strap and (3) four horizontal straps. The chest strap was fastened around the girth with one electrode located behind the scapula and the second behind the elbow. The Polar logger was attached to the chest strap at the midpoint between the shoulders, recording and displaying HR using data sent via wireless transmission from the transmitter. The additional neck and horizontal straps ensured system security during movement. Relative humidity (%), air temperature (°C), lying patterns (%) and body surface temperature (°C) were recorded during the overnight assessment. Relative humidity and air temperature were recorded using a portable weather station (HOBO Micro Station, Onset Computer Corporation, Bourne, USA) located centrally within the separate building over 10-second intervals and then averaged over 30-minute intervals. Lying patterns were determined using a tri-axial accelerometer (HOBO Pendant®, Onset Computer Corporation, Bourne, USA) that recorded the degree of vertical tilt (x-axis) over each 1-minute interval. Heifers were deemed to be standing when <60° of tilt and lying when ≥60° of tilt (Ito, 2009). Heifers were then classified as lying or standing by assessing tilt readings over 30-minute intervals, as follows: (1) standing if <50% of the interval was deemed lying and (2) lying if ≥50% of the interval was deemed lying. Using a technique adapted from Ito (2009), the acceleration logger was attached to the lateral side of the rear right leg at the midpoint of the long pastern, perpendicular to the ground to ensure proper recording of tilt. Body surface temperature was recorded at 2-second intervals using a temperature sensor (iButtonLink LLC, Whitewater, USA) and then averaged over 30-minute intervals. The temperature sensor was placed at the midpoint of the long pastern between the pedometer and the lateral side of the leg. The acceleration logger and temperature sensor were secured to the leg with Vetrap™ (3M, Saint Paul, USA).

Overnight assessment

In the subgroup of heifer calves HR, lying patterns and body surface temperature were recorded overnight. Monitoring occurred from 1600 h to 0530 h the following morning in the separate building. Heart rate and body surface temperature were averaged and lying patterns determined over 30-minute intervals. Difference in HR during standing relative to lying classifications (mean HR during standing classifications – mean HR during lying classifications; BPM) and time lying (%) were calculated for each animal.

Acute stress assessment

Heifer calves and yearling heifers were individually brought through a corral system, restrained in a squeeze chute and equipped with the Polar system. No interaction with the animal occurred for the first 4 minutes of HR monitoring, allowing HR to approach a resting value for the current animal state. At 4 minutes of monitoring, the animal was exposed to the opening and closing of an umbrella in front of their snout, 3 times, within 5 seconds. The animal was not interacted with for up to another 6 minutes when the Polar system was then removed and the animal was released from the squeeze chute and returned to the pen. Average HR over 20 seconds immediately prior to umbrella exposure ($\mathbf{HR_{BEF}}$; BPM), maximum HR after umbrella exposure ($\mathbf{HR_{MAX}}$; BPM), time from umbrella exposure to HR_{MAX} ($\mathbf{T_{BEF}}$; seconds), average HR over 20 seconds after umbrella exposure with ≤10 BPM increase in HR ($\mathbf{HR_{AFT}}$; BPM), time from HR_{MAX} to start of HR_{AFT} ($\mathbf{T_{AFT}}$: seconds) and $HR_{AFT} – HR_{BEF}$ ($\mathbf{HR_{CHG}}$; BPM) were calculated.

A linear-linear-quadratic segmented polynomial was used to determine and compare predicted HR curves across RFI groups. Heart rate was averaged over five-second intervals, transformed to the inverse of HR to obtain normality and multiplied by 1000 to increase the scale of the values. Adjusted heart rate means for each RFI group (high-RFI; heifer calves 0.9 ± 0.6 kg DM/d n = 54, yearling heifers 1.0 ± 1.0 kg DM/d n = 18, low-RFI; heifer calves -0.8 ± 0.7 kg DM/d n = 53, yearling heifers -1.0 ± 0.4 kg DM/d n = 18) were generated for each time (**T**; 0 to 355 on 5 second intervals) point using the MIXED procedure fitting the following models:

Heifer calves:

$$Y_{ijkl} = \mu + RFIgroup_i + time_j + RFIgroup_i \times time_j + \sum_{k=1}^{8} \beta_{1k}\, breed_k + e_{ijkl}$$

Yearling heifers:

$$Y_{ijkl} = \mu + RFIgroup_i + time_j + RFIgroup_i \times time_j + \sum_{k=1}^{8} \beta_{1k}\, breed_k + \beta_2 days\ in\ gestation + e_{ijkl}$$

where Y_{ijkl} is the HR measured on the l-th animal, from the i-th RFI group, at the j-th time. Where μ is overall mean; $RFIgroup_i$ is the fixed effect of the i-th RFI group (i = high- or low-RFI); $time_j$ is the fixed effect of the j-th time (0 - 355 seconds); $RFIgroup_i$ x $time_j$ is the interaction effect of the i-th RFI group and j-th time; β_1 is the contribution of the k-th breed to the breed composition of the animal; β_2 is the days in gestation of the animal; and e_{ijkl} is the random residual effect. The least squares mean value was determined for each time point using the lsmeans option.

Two knots were selected within each RFI group at inflection points (Figure 3.2). The first knot (**C_1**) represented the time value (T) when HR started to increase due to umbrella exposure. The second knot (**C_2**) represented the T when HR started to decline after reaching a maximum value. Knot selection resulted in the generation of three subintervals: (1) $0 \leq T \leq C_1$, (2) $C_1 < T \leq C_2$, and (3) $C_2 < T \leq 355$. Each interval was defined by equations and criteria used to determine covariates for each time value as indicated in Figure 3.2. Using these covariates and the GLM procedure, linear-linear-quadratic segmented polynomials were estimated and the polynomial that showed a competitively high R^2 for both cattle categories was used to predict HR separately for heifer calves and yearling heifers. Described below is the selected linear-linear-quadratic segmented polynomial used to predict heart rate separately for heifer calves and yearling heifers across all three subintervals ((1) $0 \leq T \leq C_1$, (2) $C_1 < T \leq C_2$, and (3) $C_2 < T \leq 355$):

$$HR_{ij} = \mu + RFIgroup_i + \beta_1 T_{(i)} + \beta_2 Z_{1(i)} + \beta_3 Z_{2(i)} + \beta_4 Z_{3(i)} + e_{ij}$$

where HR_{ij} is the *j*-th mean HR, recorded at time T, within the *i*-th RFI group; μ is the overall HR mean; RFI*group*$_i$ is the fixed effect of *i*-th RFI group (*i*= high- and low-RFI) and $\beta_1, \beta_2, \beta_3,$ *and* β_4 are fixed regression coefficients on T, $Z_{1(i)}$, $Z_{2(i)}$, and $Z_{3(i)}$ within the *i*-th RFI group, where: $Z_{1(i)} = 0$ if $T \leq C_{1(i)}$; $Z_{1(i)} = (T - C_{1(i)})$ if $T > C_{1(i)}$; $Z_{2(i)} = 0$ if $T \leq C_{2(i)}$; $Z_{2(i)} = (T - C_{2(i)})$ if $T > C_{2(i)}$; $Z_{3(i)} = 0$ if $T \leq C_{2(i)}$; $Z_{3(i)} = (T - C_{2(i)})^2$ if $T > C_{2(i)}$ and; e_{ij} is the random residual effect. Fixed regression coefficients in the segmented polynomial represented the linear rate of HR decrease pre-exposure ($\boldsymbol{\beta_1}$), linear rate of HR increase post-exposure ($\boldsymbol{\beta_2}$) and linear ($\boldsymbol{\beta_3}$) and quadratic ($\boldsymbol{\beta_4}$) rates of HR decrease post-exposure. Using an on-line integral calculator (Integral Calculator, David Scherfgen, Bonn, Germany) the area under the HR curve over the 2nd and 3rd subintervals was calculated and the difference between RFI groups was determined.

Statistical analysis

Heifer calves and yearling heifers were treated as separate datasets for all analyses. Descriptive statistics were determined and normality was assessed using the UNIVARIATE procedure of SAS software®. Normality was assessed using the histogram statement, normal option and Anderson-Darling test. Transformations for heifer calves were: natural logarithm (feed conversion ratio, overnight HR, T_{MAX}), inverse (back fat thickness), inverse x 1000 (predicted HR) and cubed (body surface temperature) and for yearling heifers were: natural logarithm (HR_{AFT}), inverse (HR_{MAX}) and inverse x 1000 (predicted HR). Values are presented on their original scale unless otherwise noted.

The repeated measure of overnight HR was analyzed and compared between RFI subgroups of heifer calves (high-RFI; 1.5 ± 0.6 kg DM/d n = 20, low-RFI; -1.5 ± 0.6 kg DM/d n = 20) through random regression using the MIXED procedure according to the following model:

$$Y_{ijklm} = \mu + RFIgroup_i + time_j + lying_k + RFIgroup_i \times time_j + RFIgroup_i \times lying_k + \sum_{l=1}^{8} \beta_{1l}\, breed_l + \beta_2 age + e_{ijklm}$$

where Y_{ijklm} is the HR measured on the *m*-th animal, from the *i*-th RFI group, at the *j*-th time, during a *k*-th lying classification. Where μ is overall mean; $RFIgroup_i$ is the fixed effect of the *i*-th RFI group (*i* = high- or low-RFI); $time_j$ is the fixed effect of the *j*-th time (1600 h to 0530 h; 30-minute intervals); $lying_k$ is the fixed effect of the *k*-th lying classification (lying or standing); $RFIgroup_i$ x $time_j$ is the interaction effect of the *i*-th RFI group and *j*-th time; $RFIgroup_i$ x $lying_k$ is the interaction effect of the *i*-th RFI group and *k*-th lying classification; β_1 is the contribution of the *l*-th breed to the breed composition of the animal; β_2 is the age of the animal; and e_{ijklm} is the random residual effect. The autoregressive covariance structure was selected on the basis of Bayesian information criteria. The type III test for fixed effects indicated that body surface temperature, relative humidity, air temperature and the interaction effect of lying x time were non- significant ($P > 0.05$), permitting their removal from the model. Least square means were compared across RFI group and RFI group x lying classification using the Scheffé multiple comparison test within the MIXED procedure.

Significant fixed effects for single measures of overnight, productive performance and acute stress HR traits were determined using the GLM SELECT procedure with the backward and BIC options. Using these options all effects are included in the model and in a stepwise manner effects that produce the least significant decrease in BIC are removed until removal increases BIC. The GLM procedure was then used to adjust trait means for overnight, productive performance and acute stress HR traits for each RFI group (high-RFI; heifer calf subgroup 1.5 ± 0.6 kg DM/d n = 20, heifer calves 0.9 ± 0.6 kg DM/d n = 54, yearling heifers 1.0 ± 1.0 kg DM/d n = 18, low-RFI; heifer calf subgroup -1.5 ± 0.6 kg DM/d n = 20, heifer calves -0.8 ± 0.7 kg DM/d n = 53, yearling heifers -1.0 ± 0.4 kg DM/d n = 18) using the following models:

Productive performance (yearling heifers) and acute stress HR traits (heifer calves and yearling heifers):

$$Y_{ijk} = \mu + RFIgroup_i + \sum_{j=1}^{8} \beta_{1j}\, breed_j + e_{ijk}$$

Productive performance (heifer calves) and overnight traits (heifer calf subgroup):

$$Y_{ijk} = \mu + RFIgroup_i + \sum_{j=1}^{8} \beta_{1j}\, breed_j + \beta_2 age + e_{ijk}$$

where Y_{ijk} is the trait of interest measured on the *k*-th animal, from the *i*-th RFI group (*i* = high- and low-RFI groups). Where μ is the unadjusted mean for the trait of interest; $RFIgroup_i$ is the fixed effect of the *i*-th RFI group; β_1 is the contribution of the *j*-th breed to the breed composition of the animal; β_2 is the age of the animal; and e_{ijk} is the random residual effect. Means of overnight, productive performance and acute stress HR traits were tested across RFI groups using the Scheffé multiple comparison test within the GLM procedure. Intercept and fixed regression coefficients of the segmented polynomials (β_1, β_2, β_3, β_4) for predicted acute stress HR, presented as inverse x 1000, were tested across RFI groups using a two-tailed *t*-test and interpreted on their original scale. For all analyses results were termed significant when $P \leq 0.05$.

Results

Productive performance

Group means and descriptive statistics for productive performance traits are shown in Table 3.3. In both heifer calves and yearling heifers, low-RFI cattle consumed less feed (dry matter intake) and had a lower feed conversion ratio while obtaining the same average daily gain and body composition (back fat thickness, rib eye area, rump fat thickness and marbling) as high-RFI cattle.

Overnight assessment

Means and confidence intervals of overnight HR, time lying, and body surface temperature were 70.7 ± 9.7 BPM, 61.2 ± 41.3 % and 32.7 ± 1.8 °C. Heart rate decreased over the recording (Figure 3.3) and was lower in low-RFI heifer calves than in high-RFI heifer calves ($P < 0.01$). Low-RFI heifer calves had a lower HR during lying and standing classifications than high-RFI heifer calves ($P < 0.05$) (Figure 3.4). No difference in HR change between standing and lying classifications (Figure 3.4) and time lying (low-RFI vs.

high-RFI; 61.1 ± 5.9 vs. 64.5 ± 5.9 %, $P = 0.44$) was observed between RFI groups. Means comparisons quantified the significant fixed effects of RFI group (Figure 3.3 and 3.4), time (Figure 3.3) and lying classification (Figure 3.4) ($P < 0.05$) and confirmed the absence of an interaction effect of RFI group x time (Figure 3.3) and RFI group x lying classification (Figure 3.4).

Acute stress assessment

Group means and descriptive statistics for acute stress HR traits are shown in Table 3.4. In heifer calves, there was no difference in HR_{BEF}, T_{BEF}, or T_{AFT} between RFI groups. Low-RFI heifer calves had a greater HR_{MAX}, HR_{AFT} and HR_{CHG} than high-RFI heifer calves. In yearling heifers, no difference in acute stress means was observed between RFI groups. Segmented polynomials were effective in clarifying the observed variation in HR as displayed through the R^2 in both cattle categories (Figure 3.5). Low-RFI heifer calves had a higher Intercept (RFI group effect), and greater absolute values for β_2, β_3 and β_4 than high-RFI heifer calves. No difference in β_1 was found between RFI groups of heifer calves (Table 3.5). Low-RFI yearling heifers had a lower Intercept and absolute β_1 value and higher absolute β_4 value than high-RFI yearling heifers however, no differences in β_2 and β_3 were observed (Table 3.5). Area under the HR curve over the 2nd and 3rd subintervals was 13.8 beats higher in low-RFI heifer calves compared to high-RFI heifer calves and 5.7 beats lower in low-RFI yearling heifers compared to high-RFI yearling heifers.

Discussion

The reduced feed intake and similar productivity of low-RFI commercial cattle in this study indicate the potential benefits of improved feed efficiency. Furthermore, the absence of a difference in performance and ultrasound traits between RFI groups is in agreement with past studies (Lawrence *et al.,* 2011; Gonano *et al.,* 2014), reinforcing the ability of RFI to identify cattle that have equal performance at a lower feed intake. This study sought to promote the use of feed efficiency and associated feed savings on-farm by developing non-invasive, applicable HR proxies for feed efficiency.

Heifer calf dry matter intake, BW and average daily gain values were comparable to values obtained in beef heifers (Abdelhadi *et al.,* 2005). In yearling heifers, values for RFI, dry matter intake, BW and average daily gain were similar to values obtained in pregnant beef yearlings (Gonano *et al.,* 2014). Overnight HR values recorded in the heifer calf subgroup in this study were in agreement with values observed in beef cows using a similar Polar recording system (Hafla *et al.,* 2013). Percentage of time lying was comparable to the range observed in beef steers during the overnight period (Robért *et al.,* 2011). Heifer calf values for HR_{BEF}, and HR_{AFT} were lower and values for HR_{MAX} were higher than coinciding values observed in beef heifers, where HR was recorded for one minute prior, during and after exposure to handling facility and handler noise (Waynert *et al.,* 1999). Longer pre- and post-exposure periods could increase the ability to cope with previous handling and the stressor, resulting in a lower HR_{BEF} and HR_{AFT}. Furthermore, exposure to a novel acute stressor as occurred in this study could result in an increased HR_{MAX} compared to exposure to a previously experienced stressor as occurred in Waynert *et al.* (1999).

Overnight assessment

The lower overnight HR in the low-RFI heifer calf subgroup is in agreement with Hafla *et al.* (2013) and Montanholi *et al.* (2014). Changes in cardiovascular function (i.e. HR) are generated by autonomic responses to changes in peripheral resistance that vary based on multiple factors including changes in peripheral tissue metabolic activity (Hall, 2015). Increased state 2 and 3 mitochondrial respiration in skeletal muscle (Kolath *et al.,* 2006a) and a trend towards increased state 3 mitochondrial respiration in liver (Lancaster *et al.,* 2014) have been observed in beef cattle with low-RFI. These results suggest that differences in peripheral tissue metabolic activity associated with RFI could contribute to the lower overnight HR in low-RFI heifer calves. It is unknown if differences in peripheral metabolism associated with RFI are sustained as only one assessment was made (Kolath *et al.,* 2006a; Lancaster *et al.,* 2014). Changes in HR and overall cardiac output restore blood pressure on a moment-to-moment basis (Purves *et al.*, 2001). However, if peripheral metabolic differences are sustained, additional cardiovascular parameters (i.e. myocardial contractility, chamber size and blood volume) could also be altered, proposing areas of future research.

The differences in HR during lying and standing across RFI groups (Figure 3.4) suggest that energy expended during lying and standing was lower in the subgroup of low-RFI heifer calves. The absence of a difference in percentage of time lying and in heart rate change from standing to lying classifications between RFI groups in this study suggest that RFI is not related to lying patterns. A study by Lawrence *et al.* (2011) also observed no difference in standing and lying percentages between low and high-RFI beef heifers. Therefore, the lower HR across lying patterns appears to be a result of underlying physiology rather than differences in energy expended for lying or standing. These results suggest that overnight HR has potential to be a feed efficiency proxy without knowledge of lying patterns, decreasing cost and increasing ease of implementation. Furthermore, the absence of a difference in percentage of time lying between RFI groups suggests that further evaluation is needed before the ability of lying patterns as RFI proxies can be determined.

Acute stress assessment

The increased HR_{MAX}, HR_{CHG}, β_2, β_3 and β_4 observed in low-RFI heifer calves may represent differences in autonomic nervous system regulation. Koolhaas *et al.* (1999) described animals with greater sympathetic reactivity (i.e. increased HR response), lower parasympathetic reactivity and lower humoral reactivity as proactive and the inverse as reactive. Greater HR_{MAX} and greater rate of HR change (β_2) suggest that low-RFI heifer calves had higher sympathetic reactivity in response to the stressor. Whereas greater HR_{AFT}, despite greater rate of HR decrease (β_3,β_4), suggests that low-RFI heifer calves had lower parasympathetic reactivity after HR_{MAX} was reached. While this study did not assess humoral reactivity, Knott *et al.* (2008) observed that low-RFI rams had a decreased humoral response to an ACTH challenge. The acute stress results in low-RFI heifer calves in conjunction with the results of Knott *et al.* (2008) suggest that feed efficient heifer calves have a proactive coping style (Koolhaas *et al.*, 1999). Careau *et al.* (2008) theorized that proactive animals evacuate a stressor more effectively resulting in a lower resting metabolic rate, a potential explanation for the associations between acute stress HR and RFI observed in this study.

Although low-RFI heifer calves had a greater rate of HR decrease (β_3, β_4), the higher HR_{AFT} and greater area under the HR curve of low-RFI heifer calves suggest that low-RFI heifer calves did not come to rest faster. Potentially, heifer calves were not in a state of rest when the assessment was ceased, as values for β_3 and β_4 for both RFI groups were not zero. Considering the lower overnight HR observed in the subgroup of low-RFI heifer calves, the higher intercept value in low-RFI heifers and absence of a difference in HR_{BEF} suggest that a state of rest was also not obtained pre-exposure. Handling prior to entry and the associated stress of restraint in a squeeze chute (Grandin, 1997) may explain why a state of rest was not obtained, suggesting that the acute stress assessment should not focus on periods pre- and post-exposure unless a resting state is verified. Instead, the agreement of the relationship of HR_{MAX} and β_2 with RFI proposes that the acute stress assessment may only need to include the time interval from exposure until HR_{MAX}. This reduction of assessment length would increase the likelihood of on-farm use in heifer calves. However, evaluation of acute stress assessment predictive ability and repeatability across cattle categories must precede this on-farm implementation.

The higher intercept of high-RFI yearling heifers may reflect differences in HR response to the stress of handling prior to entering the chute, an association observed in beef bulls during transport (Montanholi *et al.,* 2014). The higher β_1 in high-RFI yearling heifers suggests that habituation to restraint occurred at a faster rate, potentially compensating for this greater response to previous handling. Habituation to non-aversive handling has been reported (Grandin, 1997) and may explain the absence of a difference in the remaining acute stress HR parameters in yearling heifers. Some producers were seedstock operators where yearling cattle were handled frequently and may have habituated. This habituation to handling in yearling heifers may have resulted in a decreased HR response. In contrast, depending on producer selection programs, heifer calves may not have been exposed to frequent handling before entering the research facility. Further evaluation of the influence of handling experience on the relationship between acute stress HR and RFI is warranted. If acute stress assessment results are consistent across gender the assessment could be focused on younger heifers and bulls with minimal handling experience, a requirement that complements the selection programs of beef operations.

Influence of stress on the association between heart rate and feed efficiency

Lower overnight HR in the subgroup of low-RFI heifer calves and the absence of lying pattern differences suggest low-RFI cattle function at a lower HR during periods of rest due in part to differences in peripheral tissue metabolic activity (Kolath *et al.,* 2006a; Lancaster *et al.,* 2014; Hall, 2015). Increased parasympathetic or decreased sympathetic stimulation during periods of rest (Purves *et al.,* 2001) may be associated with this variation in peripheral tissue metabolism, potentially causing this lower HR, among other potential changes in cardiovascular function. Increased HR_{MAX}, β_2 and area under the HR curve (increased sympathetic reactivity) and increased HR_{AFT} (decreased parasympathetic reactivity) in low-RFI heifer calves in response to acute stress reflect a proactive coping style (Koolhaas *et al.,* 1999). A proactive coping style may be associated with long-term energy conservation (Careau *et al.*, 2008), potentially explaining the influence of stress on the association between HR and RFI in heifer calves. Overall, the overnight and acute stress HR results in heifer calves indicate that stress level influences the association between HR and RFI through differences in metabolic function and coping style. Evaluation of associations between bovine energetics (at animal and tissue-specific levels), autonomic regulation and feed efficiency is warranted. Assessment of these associations during both rest and acute stress exposure and across different categories of cattle could help determine the validity of these interpretations.

Conclusions

According to our study, overnight heart rate and acute stress heart rate are potential proxies for feed efficiency in heifer calves. Overnight assessment results suggest that overnight heart rate is associated with feed efficiency without the requirement of lying pattern information. Acute stress assessment results suggest that the assessment may only need to include the time interval from exposure until maximum heart rate, which may simplify its further application in commercial settings. Acute stress heart rate dynamics differ in yearling heifers, suggesting that the assessment should be focused on younger cattle with minimal handling experience, a requirement that complements the majority of beef operations. Further studies evaluating predictive ability and repeatability of heart rate across cattle categories and production systems could result in the development of on-farm heart rate assessments that increase the efficiency of the beef industry.

Acknowledgements

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Beef Improvement Opportunities is highly acknowledged. The first author acknowledges the graduate scholarship received from the Nova Scotia Department of Agriculture through the Growing Forward 2 initiative.

Table 3.1 *Genomic based breed composition of heifer calves and yearling heifers*

Breed (%)	Heifer calves	SD	Yearling heifers	SD
Black Angus	26.2	27.5	14.1	24.7
Charolais	10.7	19.5	7.1	17.8
Hereford	10.5	21.4	25.2	41.9
Limousin	16.8	25.5	1.6	8.5
Simmental	17.6	23.0	31.6	40.3
Shorthorn	3.2	14.2	13.0	31.3
Others[1]	14.9	14.3	7.3	5.2

[1] Includes; Gelbvieh, Maine Anjou, Piedmontese and Red Angus.

Table 3.2 *Ingredient and chemical composition of heifer calf and yearling heifer ration*

	Percentage	SD
Ingredient composition (% as fed)		
Mixed grass ensilage	99.5	
Vitamin and mineral premix[1]	0.5	
Chemical composition[2] (% DM)		
Dry matter	36.1	4.9
Crude protein	12.6	1.1
Neutral detergent fibre	53.7	3.3
Acid detergent fibre	34.2	1.8
Relative feed value	108.5	9.5

[1] Contains 7.8 % Na, 27 % Ca, 0.02 % P, 2.5 % Mg, 2 400 mg/kg Fe, 900 mg/kg Cu, 75 mg/kg I, 2 300 mg/kg Mn, 2 400 mg/kg Zn, 13 mg/kg Co, 3 000 mg/kg Cl, 200 000 IU/kg Vitamin A, 27 000 IU/kg Vitamin D-3, 4 000 IU/kg Vitamin E.

[2] Analyzed by NIRS (Agri-Food Laboratories Inc., Guelph, Canada).

Table 3.3 *Descriptive statistics and residual feed intake group means for productive performance and ultrasound traits of heifer calves and yearling heifers*

			High-RFI	Low-RFI	High-RFI	Low-RFI
Trait	Mean	SD	Mean	Mean	CI[1]	CI
Heifer calves						
RFI (kg DM/d)	0.0	1.1	0.8^{A}	-0.8^{B}	0.6, 1.0	-0.6, -1.0
Dry matter intake (kg/d)	7.5	1.6	8.4^{A}	6.6^{B}	8.1, 8.6	6.4, 6.9
Average daily gain (kg/d)	0.7	0.2	0.7	0.7	0.7, 0.8	0.7, 0.8
Feed to gain (ratio)[2]	10.3	2.6	11.5^{A}	9.2^{B}	10.8, 12.2	8.7, 9.8
Body weight (kg)	304.0	39.5	306.7	301.3	297.5, 316.0	292.1, 310.4
Rib eye area (cm^2)	40.7	5.1	41.2	40.2	39.8, 42.6	38.8, 41.6
Back fat thickness (mm)	1.2	0.8	1.3	1.2	1.2, 1.4	1.1, 1.3
Rump fat thickness (mm)	1.6	1.1	1.8	1.5	1.5, 2.0	1.3, 1.7
Marbling (score)[3]	7.0	0.4	7.0	7.0	6.9, 7.1	6.9, 7.1
Yearling heifers						
RFI (kg DM/d)	0.0	1.3	1.0^{A}	-0.9^{B}	0.6, 1.4	-1.3, -0.5
Dry matter intake (kg/d)	9.7	1.7	10.6^{a}	8.8^{b}	9.9, 11.4	8.1, 9.6
Average daily gain (kg/d)	1.0	0.2	1.0	1.0	0.9, 1.0	0.9, 1.1
Feed to gain (ratio)	10.0	2.1	11.2^{a}	8.8^{b}	10.4, 12.0	8.0, 9.7
Body weight (kg)	480.3	48.6	475.3	485.2	450.9, 499.8	460.7, 509.6
Rib eye area (cm^2)	51.1	6.9	51.6	50.6	48.6, 54.7	47.6, 53.7
Back fat thickness (mm)	2.0	0.9	2.0	1.9	1.6, 2.5	1.5, 2.4
Rump fat thickness (mm)	3.2	1.9	3.3	3.1	2.5, 4.2	2.2, 3.9
Marbling (score)	6.9	0.4	6.9	7.0	6.7, 7.0	6.8, 7.2

RFI = residual feed intake

[1] Confidence interval = lower limit, upper limit.

[2] Ratio of dry matter intake to average daily gain.

[3] 1 = devoid to 11 = abundant.

Table 3.4 *Descriptive statistics and residual feed intake group means for acute stress heart rate traits of heifer calves and yearling heifers*

			High-RFI	Low-RFI	High-RFI	Low-RFI
Trait	Mean	SD	Mean	Mean	CI[1]	CI
Heifer calves						
HR_{BEF} (BPM)	84.8	15.9	83.2	86.5	78.8, 87.6	82.1, 90.8
HR_{MAX} (BPM)	154.3	33.5	145.7^{a}	162.9^{b}	136.5, 154.9	153.7, 172.1
T_{BEF} (seconds)	24.5	10.9	23.9	25.0	21.6, 26.4	22.6, 27.6
HR_{AFT} (BPM)	88.8	16.9	84.6^{a}	92.9^{b}	80.1, 89.1	88.4, 97.4
T_{AFT} (seconds)	131.4	70.5	120.5	142.4	100.6, 140.3	122.6, 62.2
HR_{CHG} (BPM)	4.3	11.0	1.9^{a}	6.9^{b}	-1.2, 4.9	3.8, 10.0
Yearling heifers						
HR_{BEF} (BPM)	83.0	17.2	83.7	82.3	74.9, 92.5	73.5, 91.1
HR_{MAX} (BPM)	108.8	25.1	108.5	109.2	99.9, 118.6	100.5, 119.6
T_{BEF} (seconds)	23.1	8.8	21.7	24.4	17.2, 26.2	19.9, 28.9
HR_{AFT} (BPM)	83.5	22.1	85.9	81.1	76.3, 96.7	72.0, 91.3
T_{AFT} (seconds)	71.7	42.7	71.5	71.8	50.0, 93.1	50.3, 93.4
HR_{CHG} (BPM)	2.9	11.5	4.4	1.3	-1.3, 10.1	-4.7, 7.2

HR_{BEF} = average heart rate over 20 seconds immediately prior to umbrella exposure; HR_{MAX} = maximum heart rate after umbrella exposure; T_{BEF} = time from umbrella exposure to HR_{MAX}; HR_{AFT} = average heart rate over 20 seconds after umbrella with ≤10 BPM increase in heart rate; T_{AFT} = time from HR_{MAX} to start of HR_{AFT}; $\text{HR}_{\text{CHG}} = \text{HR}_{\text{AFT}} - \text{HR}_{\text{BEF}}$; BPM = beats per minute

[1] Confidence interval = lower limit, upper limit.

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 3.5 *Estimated intercept and regression coefficients of predicted heart rate curves (inverse x 1000) for residual feed intake groups of heifer calves and yearling heifers during the acute stress assessment*

	Heifer calves		Yearling heifers	
Parameter	High-RFI	Low-RFI	High-RFI	Low-RFI
Intercept[1] (1/HR*1000)[2]	0.345^{A}	0.000^{B}	-0.543^{A}	0.000^{B}
β_1	0.005	0.005	0.006^{A}	0.003^{B}
β_2	-0.198^{A}	-0.337^{B}	-0.118	-0.120
β_3	0.223^{A}	0.387^{B}	0.000	0.000
β_4	-0.0001^{A}	-0.0002^{B}	0.1570^{A}	0.1620^{B}

β_1 = linear rate of heart rate decrease pre-exposure; β_2 = linear rate of heart rate increase post-exposure; β_3 = linear rate of heart rate decrease post-exposure; β_4 = quadratic rate of heart rate decrease post-exposure

[1] RFI group effect.

[2] Inverse of heart rate x 1000.

^{A,B} Values within a row and cattle category with different superscripts differ significantly at $P < 0.01$.

Figure 3.1 Polar system and custom leather harness equipped on steers using identical equipment as used in this research trial. The leather harness consisted of a chest strap containing the Polar electrodes and transmitter, a neck strap and four horizontal straps to secure the device.

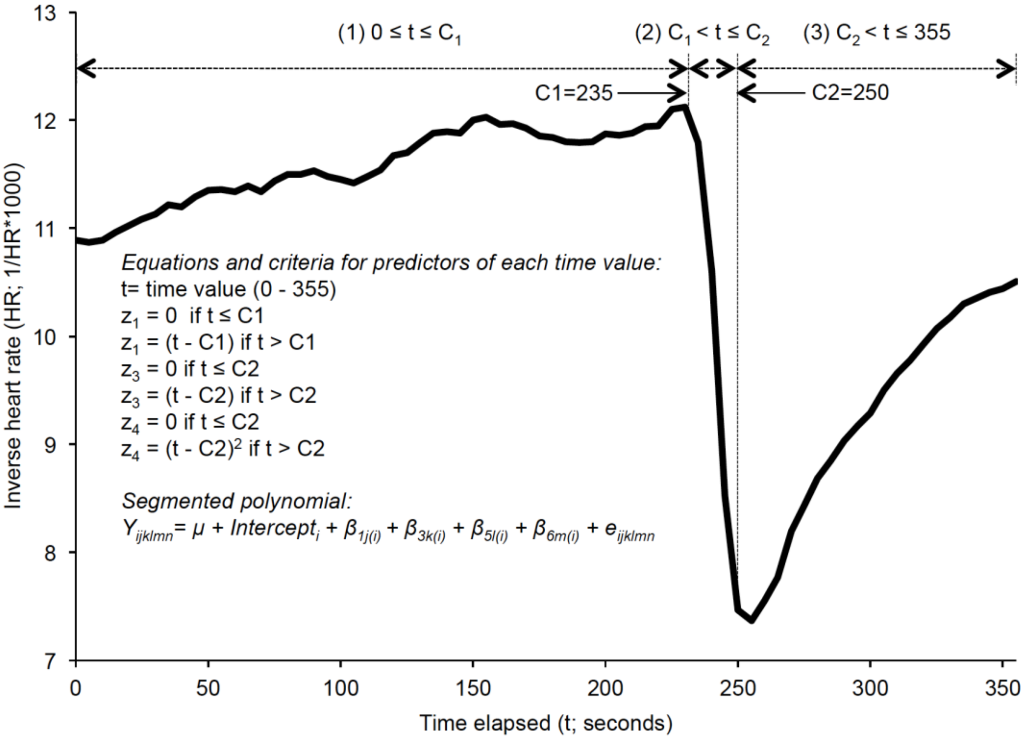

Figure 3.2 Heart rate (HR; beats per minute) profile of low-RFI heifer calves during the acute stress HR assessment, used in polynomial regression for prediction of HR curves. Two knots (C1, C2) were selected for each RFI group within each cattle category to generate three subintervals ((1), (2), (3)). The selected knots were: heifer calves; C1, low-RFI = 235 high-RFI = 230 C2, low-RFI = 250 high-RFI = 250, yearling heifers; C1, low-RFI = 235 high-RFI = 230 C2, low-RFI = 255 high-RFI = 255. Equations and criteria based on subintervals were followed for each RFI group within each cattle category to determine the covariates (T, Z_1, Z_2, Z_3) for each time (T; seconds) point within each subinterval that were used with the GLM procedure to estimate a linear-linear-quadratic segmented polynomial. The segmented polynomial predicted HR over all three subintervals for each RFI group within each cattle category.

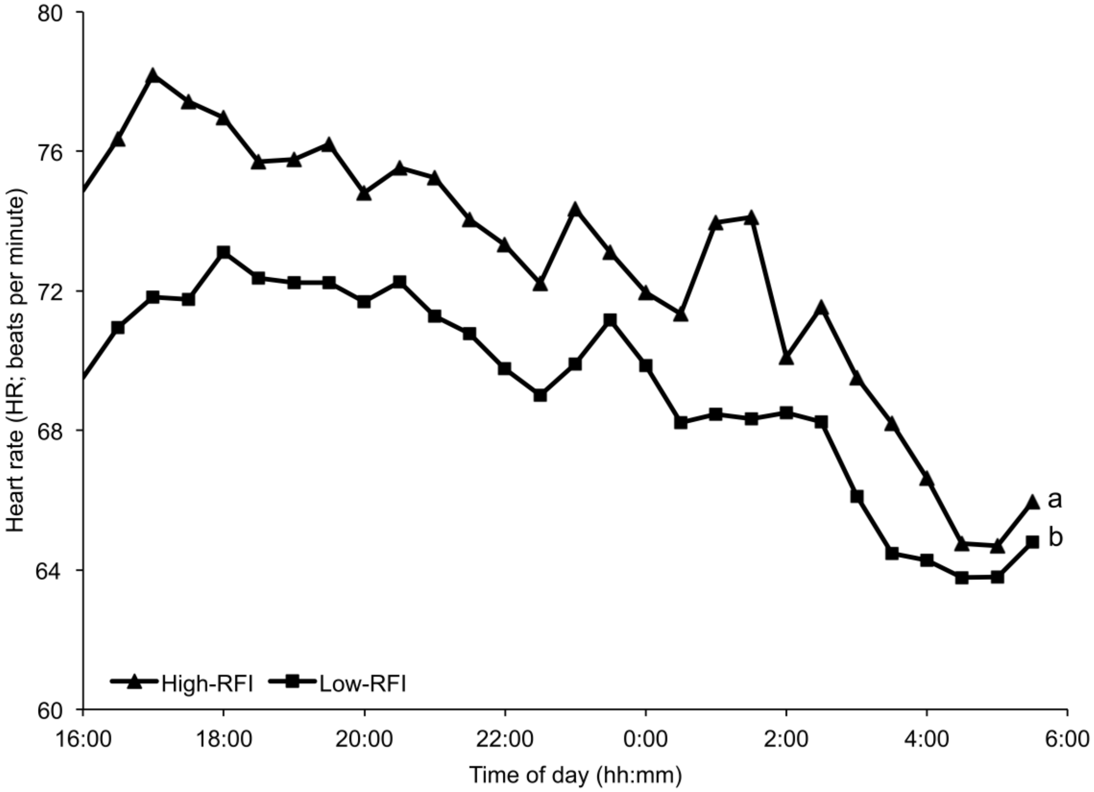

Figure 3.3 Overnight heart rate (HR; BPM) profiles of high-RFI (▲) and low-RFI (■) subgroups of heifer calves. Limits of 95% confidence intervals were 71.3 – 74.0 BPM for high-RFI and 67.9 – 70.5 BPM for low-RFI heifer calves. Interaction effect of RFI group x time was non-significant ($P > 0.05$). Differing superscripts denote $P < 0.05$.

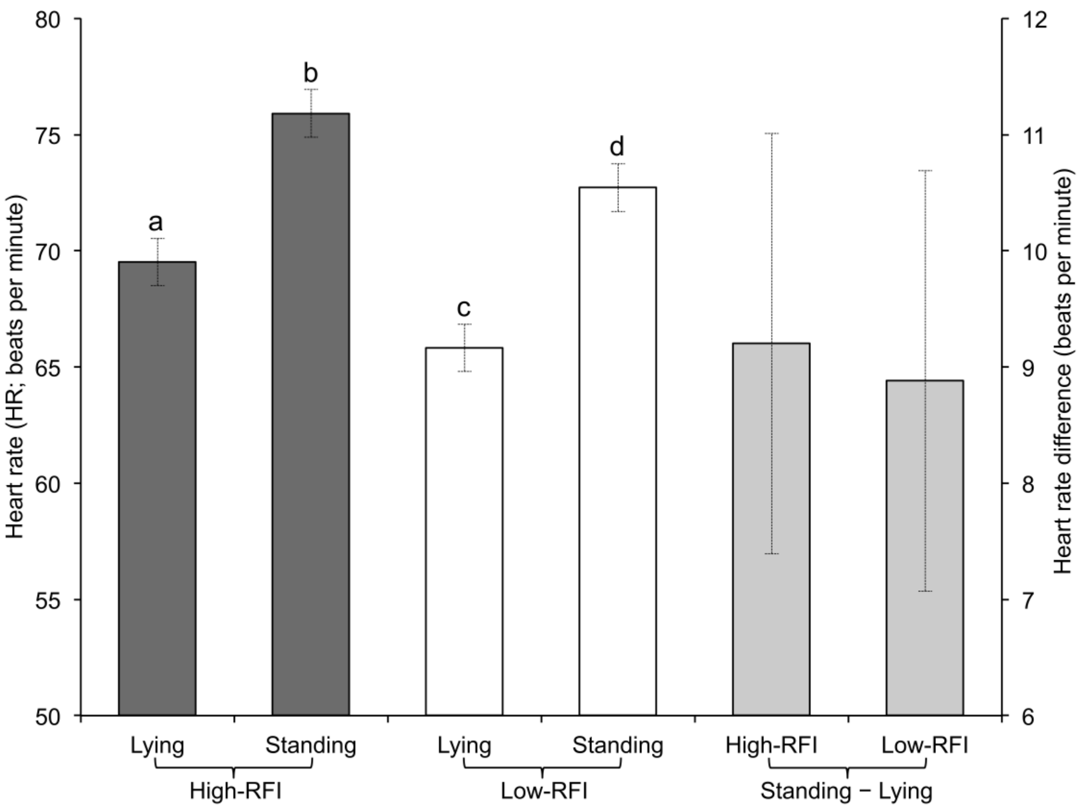

Figure 3.4 Overnight heart rate (HR; beats per minute) means of high-RFI (■) and low-RFI (□) heifer calf subgroups during lying and standing classifications and mean difference in heart rate between standing and lying classifications (■) for each RFI group. Vertical bars represent limits of a 95% confidence interval. Interaction effect of RFI group x lying classification was non-significant ($P > 0.05$). Differing superscripts denote $P < 0.05$.

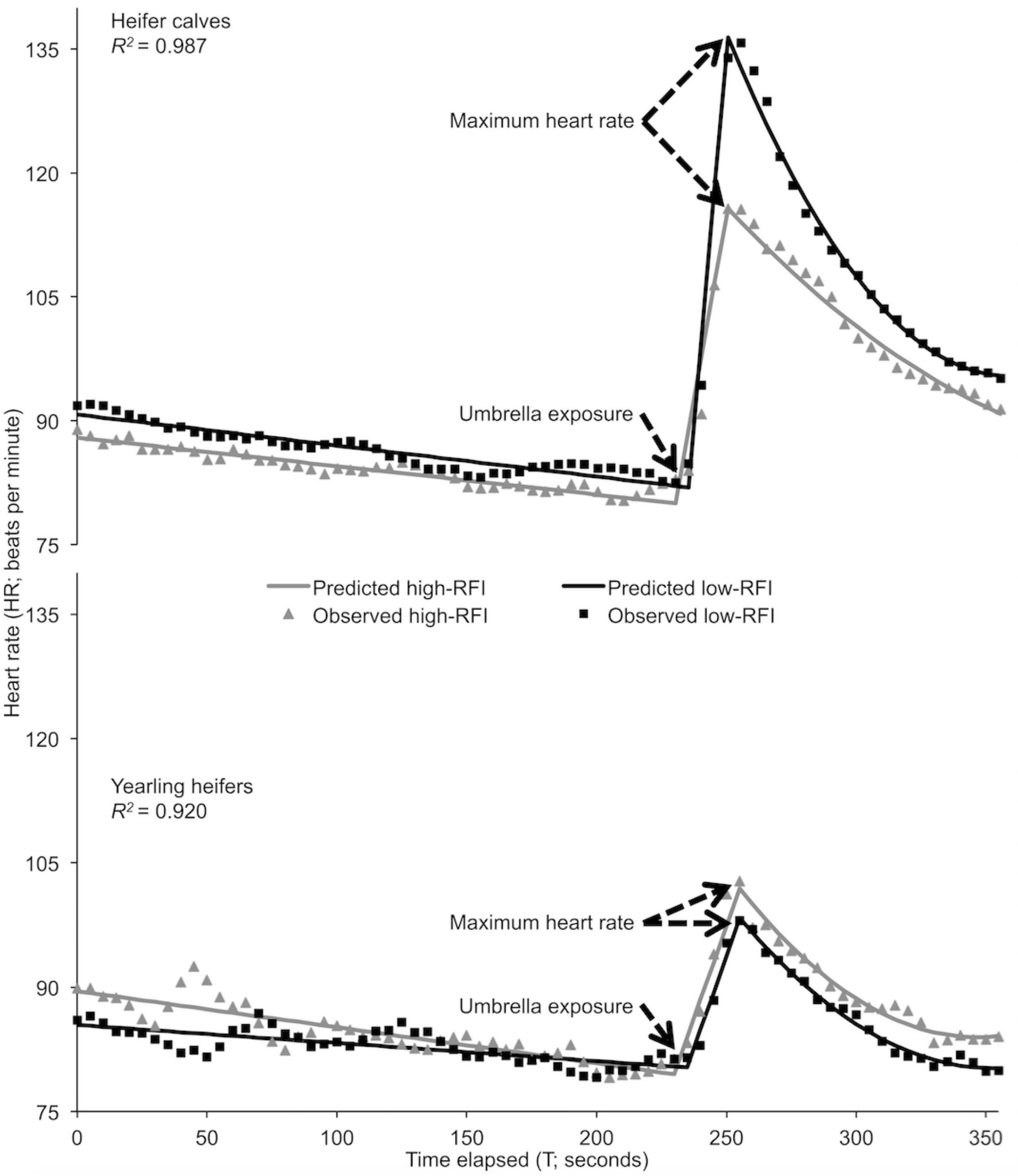

Figure 3.5 Heart rate (HR; beats per minute) curves (observed; ■/▲ predicted; −/−) of high-RFI (grey) and low-RFI (black) heifer calves and yearling heifers during the acute stress assessment.

CHAPTER 4

Associations of cardiovascular function and structure with productive performance and carcass composition in beef cattle

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Running Head

Cardiac function, structure, composition and feed efficiency in beef cattle

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Abstract

Heart rate (HR) during rest and stress as a bovine feed efficiency proxy (residual feed intake; RFI) has potential to reduce environmental impact and feed costs. Further understanding of this association and its physiological basis will aid in HR proxy development. Objectives were to assess the relationships between cardiovascular function and structure traits, their relationships with RFI and carcass composition, and the variation in RFI they explained. Residual feed intake, HR, low-frequency power (LF), stroke volume indicator (SVI), cardiac output indicator (COI), blood volume (BV), oxygen-carrying capacity, heart structure, histomorphometry and carcass composition were determined in heifers, bulls and steers. Correlations amongst traits and with RFI were determined and RFI-dependent multiple regression models containing linear and quadratic effects were selected. Positive and negative effects of HR and LF within and across overnight, transport and abattoir recordings were observed. Of the oxygen-carrying capacity traits only mean corpuscular volume had a suggested positive effect on RFI ($P \leq 0.10$). While carcass composition traits were only correlated with heart structure traits. Positive effects of overnight HR ($P \leq 0.10$), BV ($P \leq 0.10$), right ventricle thickness ($P \leq 0.01$) and myocyte width ($P \leq 0.01$) and negative effects of SVI ($P \leq 0.05$) on RFI were observed. Furthermore, heart structure measures explained the largest amount of RFI variation ($P \leq 0.01$). Relationships of HR with RFI are associated with changes in heart workload and HR regulation, while potentially reflecting differences in metabolic efficiency not oxygen-carrying capacity and carcass composition, suggesting potential for their development into RFI proxies for the improvement of bovine feed efficiency and husbandry.

New & Noteworthy

This novel study investigates the associations of cardiovascular function and structure with feed efficiency in the bovine. Relationships between heart rate and feed efficiency are associated with additional changes in cardiovascular function and structure but are not influenced by oxygen-carrying capacity or carcass composition, supporting heart rate proxy development.

Keywords

beef cattle, blood volume, heart rate, residual feed intake, stroke volume

Introduction

Improvement of beef cattle feed efficiency can aid in meeting increasing global meat demand (Davis *et al*., 2015) while reducing the environmental impact (Capper and Bauman, 2013; Davis *et al*., 2015; Gerber *et al*., 2013) and feed costs of beef production (Herd *et al*., 2003). Residual feed intake (RFI; kg DM/day), a feed efficiency measure determined as the difference between average feed intake and predicted feed intake (Koch *et al*., 1963), is independent from production traits (Schenkel *et al*., 2004), moderately heritable (Saatchi *et al*., 2014) and repeatable (Kelly *et al*., 2010), suggesting potential as method of feed efficiency improvement. However, improvement has been limited due to the expense and labour of feed intake recording equipment (Nielsen *et al*., 2013) and the time required for determination (Culbertson *et al*., 2015). Physiological indicator traits of RFI have been recognized as alternative methods of feed efficiency improvement (Pollak *et al*., 2012) by decreasing the need for feed intake recording.

Among physiological indicator traits identified (Carstens and Kerley, 2009; Richardson and Herd, 2004), those that are associated with multiple physiological processes could achieve greater improvement due to a higher heritability and associations with multiple specific genomic regions (Thallman *et al*., 2008). Lower heart rates (HR; beats/min; BPM) have been observed in low-RFI (feed efficient) cattle during rest (Hafla *et al*., 2013) and transport (Montanholi *et al*., 2014) with higher heart rates observed in low-RFI cattle in response to acute stress (Munro *et al*., 2016). Heart rate is also an indicator of multiple physiological processes that are associated with RFI (Richardson and Herd, 2004) including: heat production (Brosh *et al*., 1998), activity (Frondelius *et al*., 2015) and stress response (Stewart *et al*., 2013; Van Reenen *et al*., 2013), suggesting potential as a physiological indicator trait that could improve overall beef cattle husbandry. However, the relationship of resting and stress HR with RFI across stressors of different severity and duration has not been assessed in beef cattle. Furthermore, associations of cortisol response with RFI (Luiting *et al*., 1994; Knott *et al*., 2008; Jenkins *et al*., 2013) and behavioural reactivity with RFI (Colpoys *et al*., 2014) suggest that neural, endocrine and renal mechanisms regulating HR could also be influenced by stress severity and duration.

As a component trait, changes in HR could be accompanied by changes in cardiac output (CO) and/or stroke volume (SV). Oxygen-carrying capacity and metabolic demand influence HR, CO and SV by altering the demands on the cardiovascular system (Walley, 2011). Decreased cardiac output and HR has been associated with lower oxygen consumption in cattle (Eisemann and Nienaber, 1990). Furthermore, decreased CO and SV were observed in calorie-restricted rats after adjustment for body weight (Ahmet *et al*., 2011), suggesting that a lower HR in low-RFI cattle could be associated with a change in CO and/or SV. Increased calorimetric oxygen consumption (Montanholi *et al*., 2016), decreased thermographic heat production (Montanholi *et al*., 2009 and 2010), and increased liver (Lancaster *et al*., 2014), skeletal muscle (Kelly *et al.,* 2011; Kolath *et al.,* 2006a) and intestinal (Meyer *et al*., 2014) metabolic efficiencies and activities in low-RFI cattle indicate a decreased total oxygen consumption, suggesting a lower metabolic demand (Kleiber, 1932, 1947). Positive associations of RFI with hemoglobin (Hb) (Richardson *et al*., 2002) and mean corpuscular volume (MCV) (Crane *et al*., 2016) and equal red blood cell counts (RBC) across RFI groups (Kelly *et al.,* 2016; Lawrence *et al.,* 2011) suggest a lower oxygen-carrying capacity in low-RFI cattle. Given the aforementioned studies, decreases in HR in low-RFI cattle could be associated with changes in CO and SV and influenced by metabolic demand and oxygen-carrying capacity. Furthermore, carcass composition has been associated with CO and SV in humans (Collis *et al*., 2001) and was identified by Richardson and Herd (2004) as one of five major mechanisms influencing RFI, suggesting it could influence the associations of HR, SV and CO with RFI.

Sustained changes in CO and its components are largely mediated by changes in blood volume (BV; mL/kg) through neural, endocrine and renal mechanisms of regulation (Hall, 2015) that have been associated with RFI (Santana *et al*., 2014). Sustained changes in cardiovascular workload (i.e. decreased cardiac output or BV) are also associated with changes in heart structure (Ahmet *et al*., 2011; D'Andrea *et al*., 2013; Maillet *et al*., 2013; Xiao *et al*., 2014) and heart histomorphometry (Ahmet *et al*., 2011), suggesting potential for an association with RFI.

Although HR could serve as a potential proxy for RFI the basis of this association and its regulation requires further evaluation across stress severity and duration. Lower HR in low-RFI cattle could also be associated with differences in CO, SV, BV, heart structure and heart histomorphometry potentially due to differences in metabolic demand and/or oxygen-carrying capacity. The further assessment of the associations between resting and stress HR with RFI and the evaluation of potential correlated changes in cardiovascular function and structure could aid in explaining the biological basis of the association between HR and RFI. Therefore, the objectives of this study were to: 1) assess the relationships of HR and HR regulation with RFI across stress severity and duration 2) assess the relationships between HR, SV, CO, BV, heart structure, heart histomorphometry and oxygen-carrying capacity and their relationships with RFI and carcass composition and 3) evaluate the variation in RFI explained by these different measures of cardiovascular function and structure in the bovine.

Methods

Animal husbandry and experimental timeline

Experimental procedures involving the use of animals were conducted in agreement with the recommendations of the Canadian Council on Animal Care (Canadian Council on Animal Care, 2009). Four cohorts of beef cattle were used in this study (Cohorts A to D). Trait classes assessed, cohort intervals, cattle descriptions and diet compositions are detailed in Table 4.1. Cohorts were housed in group pen facilities (Cohorts A, B and D; Elora Beef Research Centre, Elora, Canada, Cohort C; Maritime Beef Test Station, Nappan, Canada) equipped with individual feed intake recording systems (Cohorts A, B and D; Insentec BV, Hokofarm Group BV, Marknesse, The Netherlands and, Cohort C; GrowSafe Systems Ltd., Airdrie, Canada). Animals were given ad libitum access to a diet (Table 4.1) offered twice daily. Cohorts were allocated a minimum 21-day period to acclimate to the experimental environment. Daily feed intake was recorded over 115 ± 23 days with productive performance (body weight, ultrasound body composition) evaluated on 28 ± 1-day intervals. Indicator dilution assessments occurred after productive performance evaluations over one day in a handling facility located within each research station. Heart rate assessments occurred after productive performance and indication dilution evaluations. Blood collection for determination of oxygen-carrying capacity occurred during the indicator dilution assessment (Cohort C) or at evisceration (Cohort B and Cohort D). During harvest (Cohorts A, B and D) hearts were excised for later structural assessments and carcass assessments were completed.

Productive performance traits

On an individual basis, daily feed intakes with less than a 2% probability of belonging to the normal distribution of daily feed intake were removed (two-sided test, student's *t*-distribution, critical value = 2.326) and average dry matter intake (ADMI; kg DM/ day) was calculated. Body weights (BW; kg) were recorded using calibrated cattle weigh scales (Moly Manufacturing Inc., Lorraine, Kansas, USA; CattleMaster, Linwood, Canada). Body composition was determined using a common livestock ultrasound technique adapted from Bergen *et al.* (2005). Briefly, ultrasound images were collected using an Aloka SSD-500 long probe, ultrasound unit (model 5044, 172 mm, 3.5 MHz; Corometrics Medical Systems, Wallingford, USA). Measurements of back fat thickness (mm), rib eye area (cm^2), rump fat thickness (mm) and marbling (1 (devoid) – 11 (abundant)) were completed using ImageJ® (U.S. National Institutes of Health, Bethesda, USA). Average daily gain (ADG; kg/d) was the slope of the regression of BW on time of assessment. Average dry matter intake was modelled across all cohorts using BW (body size measure), ADG (growth measure), ultrasound traits (body composition measures) and cohort in the GLM procedure of SAS software® (University Edition, Copyright©, SAS Institute Inc., Cary, USA). The ADMI model represented by the highest R^2 ($R^2 = 0.64$) and lowest Bayesian information criteria (BIC) value was selected and predicted ADMI was calculated using intercept, regression coefficients (body weight, average daily gain, back fat, rib eye area, marbling) and fixed effects (cohort). Residual feed intake was the residual of the ADMI model, where a negative RFI (low-RFI) denotes a feed efficient animal and a positive RFI (high-RFI) a feed inefficient animal.

Heart rate traits

Heart rate was recorded continuously and averaged on 5-second intervals using an external-electrode, telemetry HR system (Polar RC3 GPS or RS 800CX Science, Polar Electro, Kempele, Finland). The Polar system was modified for use in cattle and equipped as described by Munro *et al.* (2016). Cattle were equipped while physically restrained in the handling facility and returned to their respective pen. In Cohorts A and B heart rate recording began at 2200 h and stopped the following day at stunning, after cattle had been transported to, and harvested at, a federally inspected abattoir. In Cohort D recording occurred from 1600 h to 0530 h the following morning as animals were not transported or harvested.

Recordings were offloaded to a HR software package (Polar ProTrainer 5, Polar Electro, Kempele, Finland) and artefacts were corrected using the error correction tool under default settings. Corrected HR values were compared with original and surrounding HR values to ensure algorithmic corrections were logical. Heart rate recordings were divided into overnight (2200 h and 0500 h), transport, (initial 20 minutes of transport; Cohort A and B only) and abattoir (initial 10 minutes of abattoir arrival; Cohort A and B only) segments. Within each segment, animals missing more than 30% of observations were removed. Average HR over the entire segment (HR_{OVA}, HR_{TRA}, HR_{ABA}; BPM) and average of the highest (HR_{OVH}, HR_{TRH}, HR_{ABH}; BPM) and lowest (HR_{OVL}, HR_{TRL}, HR_{ABL}; BPM) twenty percent of HR were calculated for each animal within each segment.

A frequency-domain analysis was then completed separately on each HR segment and recording. Each HR recording was plotted, missing values were removed and first differences were calculated to ensure stationarity. A Fast Fourier Transformation (FFT) was completed on first differences using a Hanning Window (OriginPro; OriginLab Co., Northamption, USA) to calculate the power spectrum. Based on the Nyquist limit (Kuo and Chen, 1998) and the equidistant sampling frequency of 0.2 Hz, area under the generated amplitude/frequency curve from 0.01 to 0.1 Hz was calculated as an indicator of low-frequency (LF) power and sympathovagal balance (Ori *et al.*, 1992) (overnight, LF_{OV}; transport, LF_{TR}; abattoir, LF_{AB}).

Indicator dilution traits - blood volume, cardiac output indicator and stroke volume indicator

A modification of the indocyanine green (ICG) dilution technique was used to determine BV and indicators of CO and SV. Cattle were restrained, equipped with the Polar system and the right jugular vein was catheterized with a 2.1 x 133 mm internal jugular catheter (Beckon, Dickinson and Company, Franklin Lakes, New Jersey, USA) and secured with a skin suture and adhesive wrapping according to the method of Montanholi *et al.* (2013). Heart rate was recorded continuously over the assessment from the time of ICG administration until final sample collection. Aliquots of ICG (AdooQ BioScience, Irvine, California, USA) for injection were prepared fresh at six-hour intervals at a concentration of 2.5 mg ICG/ mL distilled water, according to manufacture recommendations. Before dye injection two 10 mL blank blood samples were collected into heparinized vacuum blood collection tubes and stored at 4 °C. A single intravenous injection of ICG (0.5 mg/ kg BW) was administered through the jugular catheter. Blood samples were collected 3, 6, 9, 12, 15 and 18 minutes after injection through the jugular catheter into heparinized vacuum blood collection tubes. Catheter and catheter tubing was flushed with 12 mL of isotonic saline (0.9 % NaCl) after ICG administration and each sample collection to prevent clogging (Montanholi *et al.,* 2013). One blank blood sample was submitted for a red blood cell count analysis as detailed below. Remaining blood samples were centrifuged at 2500 *g* for 25 minutes at 4 °C and plasma from each blood collection tube was separated into a microtube using a new transfer pipette and stored at -80 °C. At each sample collection a sub-sample of blood was collected into a heparinized micro-hematocrit capillary tube, centrifuged in a micro-centrifuge for five minutes and hematocrit (Hct) was determined using a micro-hematocrit tube reader, as the average of seven collections.

Blood volume determination. Spectral absorbance of plasma samples was determined in triplicate using spectrophotometry at 800 nm (Powerwave XS2, BioTek, Winooski, Vermont, USA) at a 10% coefficient of variation threshold for each plasma sample. Four dilutions of ICG in blank plasma were completed, spectral absorbance was read at 800 nm and a four-point standard absorbance versus concentration curve was generated and fitted with a linear function (Prism 6, GraphPad Software Inc., La Jolla, USA). Using the standard curve linear function, plasma sample concentration was determined and a dilution curve was generated and fitted with a monoexponential function. Blood volume was calculated as follows: $BV = m_{ICG}/(c_0 \text{ X } (1 - Hct) \text{ X } BW)$. Where m_{ICG} was the mass (mg) of ICG administered; c_0 was the hypothetical concentration of ICG in plasma (mg/mL) at time of administration, calculated using the monoexponential function; Hct was the hematocrit determined using a micro-hematocrit tube reader; and BW was the body weight recorded on the day of the ICG dilution assessment.

Cardiac output and stroke volume indicator determination. Area under the monoexponential dilution curve (AUDC) and heart rate curve (AUHC) from three minutes after administration until last blood sample collection was calculated for each animal using Prism 6 (GraphPad Software Inc., La Jolla, USA). Area under the dilution curve represented the total quantity of ICG pumped past the catheter over the sampling interval, a parameter proportional to total blood flow. The division of this parameter by total heartbeats over the sampling interval resulted in a parameter proportional to SV. Using these relationships an indicator of CO indexed by BW (cardiac output indicator index; COI) was calculated as: $COI = [(m_{ICG}/AUDC)/BW]$ and an indicator of SV indexed by BW (stroke volume indicator index; SVI) was calculated as: $SVI = [(COI/AUHC)/BW]$. As proportional equations were used in calculations, COI and SVI were unitless parameters.

Carcass traits

Cattle were harvested (Meat Science Laboratory, University of Guelph, Guelph, Canada) using accepted agricultural methods (Canadian Food Inspection Agency, 2013) . Cattle were stunned via captive bolt, vertically exsanguinated, skinned and eviscerated. Blood was collected at evisceration into heparinized blood collection tubes and stored at 4 °C. Carcasses were split, washed and dressed, hot carcass weight (HCW; kg) was recorded prior to overnight chilling at 1 °C. A 21 cm section spanning from the 10th to 12th ribs was dissected into lean, subcutaneous fat, intermuscular fat, body cavity fat and bone, each fraction was weighed (kg) and yields of lean ($Lean_{YLD}$; %) and fat (Fat_{YLD}; %) were determined according to Bergen *et al*. (2006).

Oxygen-carrying capacity traits

Heparinized blood samples were stored at 4 °C until submission to an accredited laboratory (Animal Health Laboratory, University of Guelph, Guelph, Canada; Diagnostic Services, University of Prince Edward Island, Charlottetown, Canada) for red blood cell analysis, determining: red blood cell count (RBC; X 10^12/L), hemoglobin (Hb; g/L), Hct (L/L), mean corpuscular volume (MCV; fL) and mean corpuscular hemoglobin concentration (MCHC; g/L).

Heart structure and histomorphometry traits

Myocardial tissue collection, heart dissection and structural measures. After evisceration the heart was excised and an incision was made from base to apex through the posterior wall of the right ventricle and septum. A myocardium sample was collected from the midpoint of the septum, weighed and fixed in 10% neutral phosphate buffered formalin for later histomorphometric analysis. The heart was then stored at -20 °C until dissection.

Hearts were removed from -20 °C storage and thawed over 24 h at a controlled temperature. Visceral fat, pericardial fat, blood vessels and connective tissue were removed from the heart and heart weight (HT_W; g) was recorded. Atria (AT) were separated from the ventricles along the coronary sulcus and weighed (AT_{WT}; g). Ventricles were separated into left ventricle plus septum (LV) and right ventricle (RV) along the anterior and posterior longitudinal sulci and weighed (LV_{WT}, RV_{WT}; g) and the ratio of LV to RV determined (LV_{WT}:RV_{WT}). An incision was made from the tip of the apex to the level of the coronary sulcus at the junction of the pericardial sheath and left atrium dividing the LV into two parts and LV wall thickness (LV_{TH}; g) was measured anteromedial to the junction of the chordae tendineae and posterior papillary muscle at the crest of the trabeculation. In the RV, an incision was made in an anterior/posterior direction from the tricuspid valve along the medial edge of the posterior papillary muscle and RV wall thickness (RV_{TH}; g) was measured anteromedial to the junction of the chordae tendineae and medial papillary muscle at the crest of the trabeculation. All thickness measurements were determined using a Vernier caliper. All measurements were scaled by HCW.

Myocardium histomorphometry. Myocardial samples were fixed in 10% neutral phosphate buffered formalin and longitudinal myocardial slices were prepared for embedding. Embedded myocardial slices were sectioned at a thickness of 5 µm using a microtome (Shandon Finesse®, Model 325: Thermo Electron Co., Waltham, U.S.A.) and stained with hematoxylin and eosin according to the method described by Carson and Hladik (2009). Myocyte images were collected under oil immersion at 1000X magnification through a light microscope (Carton CN-A©: Carton Optical Co. Ltd., Khlong Luang, Thailand) equipped with a video camera (Tucsen CMO Discovery Series: Tucsen Photonics Co. Ltd., Fuzhou, China) using imaging software (ISCapture: Tucsen Photonics Co. Ltd., Fuzhou, China). Images were then uploaded to ImageJ® (U.S. National Institutes of Health, Bethesda, USA) for histomorphometric measurements. Only myocytes with a longitudinal orientation, a central nucleus (Obayashi *et al*., 1997), defined Z-lines, defined myocyte edges and that were free of branching or cutting damage (Gerdes, 2015) were measured (Fig. 4.1). Based on previous studies (Obayashi *et al.,* 1997; Vliegen *et al.,* 1987) a threshold of 30 acceptable myocytes per animal was set for inclusion in further analysis. Myocyte width (MY_{WD}; µm) was measured as the distance between the two edges of the myocyte at the midpoint of the nucleus. Sarcomere length (SM_{LG}; µm) was determined and corrected for shrinkage using an equation developed by Tracy and Sander (2011): SM_{LG} = [(number of Z-bands within a 30 µm segment/30) X 1.21]. Myocyte width and sarcomere length were scaled by HCW. Myocyte imaging and measurements were completed by different single observers that were unaware of the treatment group.

Statistical analysis

All statistical analyses were completed using SAS software® (University Edition, Copyright©, SAS Institute Inc., Cary, USA). Data from all cohorts were combined in order to increase the statistical power of the analyses. Using the GLM procedure, residuals for productive performance, heart rate, carcass, oxygen-carrying capacity, heart structure and histomorphometry traits were generated by fitting the fixed effects of cohort and breed and for indicator dilution traits by fitting the fixed effects of cohort, breed and previous feed intake. Significant fixed effects and covariates for the above traits were identified using the GLMSELECT procedure using the backward and BIC options. Pearson's correlation coefficients amongst the adjusted productive performance, heart rate, indicator dilution, carcass, oxygen-carrying capacity, heart structure and histomorphometry traits (residuals) were determined using the CORR procedure. To control for the occurrence of type I errors in multiple comparisons, false discovery rate (FDR) (Benjamini and Hochberg, 1995) was controlled, where FDR ≤ 0.05 denoted significance and 0.05 < FDR ≤ 0.10 a trend towards significance.

The REG procedure, using the backward option and an inclusion level of *P* = 0.20, was used to determine the amount of variation in RFI explained by each class of cardiovascular trait (heart rate, indicator dilution, oxygen-carrying capacity, heart structure and histomorphometry) and the linear and quadratic effects of each trait on RFI. Using this procedure, within each trait class, all linear and quadratic trait effects are included in a RFI-dependent model, then in a stepwise manner the trait effect (linear and quadratic) with the highest *P* is removed until all remaining effects are significant at *P* ≤ 0.20, while manually maintaining the hierarchy within each effect. An inclusion level of *P* = 0.20 was used to increase predictive ability and reduce the likelihood of over parameterization. Coefficients were assessed to determine the direction and magnitude of each effect on RFI. To determine the portion of explained variation in RFI attributed to each trait, partial R^2 was summed across linear and quadratic effects. Predicted RFI calculated using each selected multiple regression model was plotted against observed RFI to visually assess the predictive ability of each model. All models and effects were termed as significant when $P \leq 0.05$ and as a trend approaching significance when $0.05 < P \leq 0.10$.

Results

Cardiovascular function and structure, productive performance and carcass traits

Correlations of heart rate, indicator dilution, oxygen-carrying capacity, heart structure and histomorphometry traits with productive performance and carcass traits and effects of heart rate, indicator dilution, oxygen-carrying capacity, heart structure and histomorphometry traits on RFI are shown in Table 4.2. Overnight and transport HR and LF traits were not correlated with productive performance or carcass traits. Positive correlations were observed between HR_{ABH} and DMI and LF_{AB} and DMI, but abattoir HR traits were not correlated with RFI. All three overnight HR traits had quadratic effects on RFI, where HR_{OVL} and HR_{OVH} had suggested positive quadratic effects ($P \leq 0.10$) and HR_{OVA} a negative quadratic effect on RFI. Of the transport HR traits, only HR_{TRA} had an effect on RFI, displaying a trend for a positive quadratic relationship ($P \leq 0.10$). Abattoir HR traits had either a positive (HR_{ABL}, HR_{ABH}, LF_{AB}) or negative (HR_{ABA}) linear effect on RFI. With the exception of the suggested positive correlation between BV and DMI ($P \leq 0.10$), no correlations between indicator dilution traits and productive performance traits were observed. Blood volume also had a positive quadratic effect on RFI, while SVI had a negative quadratic effect and AUHC and COI had no effect.

Oxygen-carrying capacity traits were not correlated with RFI, ADG, Lean_{YLD} or Fat_{YLD} but negative correlations of RBC and Hb ($P \leq 0.10$) with DMI and MCHC with FBW ($P \leq 0.10$) were observed. Furthermore, MCV had positive correlations with DMI ($P \leq 0.10$), and FBW and had a suggested positive linear effect on RFI ($P \leq 0.10$). Right ventricle thickness had a positive correlation with RFI and negative correlations with FBW, ADG and Fat_{YLD} ($P \leq 0.10$). Left ventricle thickness was not correlated with RFI but was negatively correlated with DMI, FBW, ADG and Fat_{YLD}. No other heart structure traits had correlations with productive performance or carcass traits. Positive linear effects on RFI were observed for AT_{WT} ($P \leq 0.10$) and RV_{TH}, while RV_{WT} had a negative quadratic association with RFI. In histomorphometry traits, a positive association was observed between MY_{WD} and RFI ($P \leq 0.10$) but not SR_{LG} and RFI and both MY_{WD} and SR_{LG} had negative correlations with FBW and ADG. While, MY_{WD}, but not SR_{LG}, had a positive linear effect on RFI.

Correlations amongst cardiovascular function and structure traits

Correlations of heart rate traits with indicator dilution and oxygen-carrying capacity traits are shown in Table 4.3. Transport HR traits (HR_{TRH}, HR_{TRL}, HR_{TRA}) had higher positive correlations with AUHC ($P \leq 0.10$) than the suggested correlations of HR_{OVL} and HR_{ABL} with AUHC ($P \leq 0.10$). As well, HR_{TRA} had a suggested negative correlation with SVI ($P \leq 0.10$) and HR_{TRL} a suggested positive correlation with BV ($P \leq 0.10$), while no overnight or abattoir HR traits were correlated with SVI or BV. No correlations between heart rate traits and oxygen-carrying capacity traits were observed. However, RBC did have a positive correlation with AUHC and a negative correlation with SVI (Table 4.4). Furthermore, suggested positive correlations were observed between AUHC and Hb and Hct ($P \leq 0.10$) and between MCV and BV ($P \leq 0.10$). Of the heart structure traits and indicator dilution traits only LV_{WT} and SVI were correlated, displaying a trend towards a positive correlation ($P \leq 0.10$) (Table 4.5). Left ventricle weight had positive correlations with Hb and Hct, positive correlations that were also observed with HT_{WT} (Table 4.5). Histomorphometry traits had no correlations with indicator dilution or oxygen-carrying capacity traits (Table 4.5).

Explained variation in residual feed intake

Transport HR traits explained the largest portion (39 %) of the variation in RFI accounted for by the heart rate model, followed by abattoir (34 %) and overnight HR (27 %) traits (Fig. 4.2, A1). The heart rate model was able to explain a significant amount of the variation in RFI as displayed in Fig. 4.2, A2. Variation in BV contributed most to the explained variation in RFI by the indicator dilution model, followed by SVI and AUHC (Fig. 4.2, B1) and the indicator dilution model showed a low ability to explain and discriminate the variation in RFI (Fig. 4.2, B2; $P \leq 0.10$). Fig. 4.3, A1 shows the relative contributions of each oxygen-carrying capacity trait to the variation in RFI explained by the oxygen-carrying capacity model, which did not explain a significant amount of the variation in RFI (Fig. 4.3, A2). Of the selected heart structure traits, RV_{WT} and RV_{TH} explained 92 % of the variation in RFI accounted for by the heart structure model (Fig. 4.3, B1). Furthermore, the heart structure model was successful in explaining and predicting the variation in RFI as seen in Fig. 4.3, B2. The selected histomorphometry model contained only MY_{WD}, which alone explained a substantial amount of the variation in RFI ($R^2 = 0.136$; $Adj.\ R^2 = 0.104$; $P \leq 0.05$).

Discussion

To our knowledge, this study is the first to 1) evaluate the relationships of resting and stress heart rate with residual feed intake on a continuous scale in beef cattle and 2) to evaluate the associations between functional and structural measures of the cardiovascular system and their relationships with productive performance in beef cattle. Our study determined that quadratic relationships exist between heart rate and residual feed intake, relationships that change with stress severity and duration. Multiple associations were identified between functional and structural measures of the cardiovascular system, and residual feed intake. The results of this study increase our understanding of the association between heart rate and residual feed intake and will aid in the development of heart rate based feed efficiency proxies for the beef industry.

Relationships of heart rate and heart rate regulation with stress and residual feed intake

The negative quadratic effect of HR_{OVA} in the RFI model indicates that the relationship between RFI and HR_{OVA} is not linear, and that for animals with a very low RFI or a very high RFI, HR changes only slightly with changing RFI, whereas in the mid-range changes in HR are rapid. For animals with the highest RFI, HR appears to increase again, suggesting these animals may be exhibiting a pathological relationship between RFI and cardiovascular function. These varying relationships illustrate the dangers involved in treating populations as a group rather than exploring the details of biological relationships.

Trends for positive quadratic effects of HR_{OVL} and HR_{OVH} indicate that during periods of low and high HR, while at rest, HR increases with RFI at an increasing rate. The differential quadratic effects of HR_{OVA}, HR_{OVL} and HR_{OVH} on RFI indicate a lower resting HR in low-RFI cattle if the plateauing effect of HR_{OVA} on RFI is minimal and only occurs at high levels of HR_{OVA}. A lower resting HR in low-RFI beef cows was observed during a long-term resting assessment (Hafla *et al*., 2013), supporting the overall positive relationship of overnight HR and RFI observed in this study when controlling for transport and abattoir HR.

Positive linear effects and trends for negative and positive quadratic effects of HR_{ABL} and HR_{ABH} on RFI (Table 4.2) indicate that during periods of low stress RFI increases at a decreasing rate with HR, while during periods of high stress RFI increases an increasing rate with HR. A negative linear effect of HR_{ABA} on RFI (Table 4.2) is in agreement with Munro *et al*. (2016), where low-RFI cattle displayed a greater HR response when suddenly exposed to the sudden, repeated opening and closing of a novel umbrella. Opposite effects of HR_{ABL} and HR_{ABH} on RFI compared to the effect of HR_{ABA} on RFI and the suggested positive quadratic effect of HR_{TRA} on RFI indicate that both stress severity and duration could influence the relationship between HR and RFI. In agreement, Colpoys *et al*. (2014) following eight generations of divergent RFI selection exposed pigs to both human approach and novel object tests, observing differences in behavioural reactivity to each stressor within RFI group. Residual feed intake, represents an energy reserve that has various uses including stress response (Rauw, 2012), where differences in stress severity and duration influence the energetic requirements of the response (Moberg and Mench,

2000) and could explain the changing relationships between stress response and RFI observed across stress severity and duration by Colpoys *et al*. (2014) and our study.

The absence of an association between LF_{OV} and RFI (Table 4.2) indicate that the associations of HR_{OVA}, $HR_{OVL,}$ HR_{OVH} with RFI are not influenced by sympathetic tone. The positive linear effect of LF_{AB} on RFI (Table 4.2) suggests that high-RFI cattle have an increased sympathetic reactivity in response to a high level of stress (von Borell *et al*., 2007), explaining the positive linear and quadratic effects of HR_{ABH} on RFI. Low-RFI rams (Knott *et al*., 2008), pigs (Hennessy *et al*., 1988; Jenkins *et al*., 2013) and chickens (Luiting *et al*., 1994) displayed a reduced cortisol response to an ACTH challenge which could support a decrease in sympathetic reactivity. Results that support the suggested positive quadratic effect of HR_{TRA} on RFI observed in this study. However, greater HR responses to acute stress in low-RFI beef cattle (Munro *et al*., 2016) indicate increased sympathetic reactivity to acute stress (Koolhaas *et al*., 1999). Maximum HR responses were observed within 30 seconds in Munro *et al*. (2016) primarily representing sympathetic regulation (Ulrich-Lai and Herman, 2009). Delayed sample collection (Hennessy *et al*., 1988; Knott *et al*., 2008) after the ACTH challenge and a low sampling frequency (Jenkins *et al*., 2013) limited the ability to determine if increased cortisol response observed in high-RFI animals were caused by increased HPA reactivity, increased sympathetic reactivity or both. The absence of a significant linear or quadratic effect of LF_{TR} on RFI (Table 4.2) indicates that the suggested positive quadratic effect of HR_{TRA} on RFI was not a result of decreased sympathetic reactivity but potentially decreased HPA axis reactivity. Although the positive linear effect of LF_{AB} on RFI suggests an increase in sympathetic reactivity, previous results (Munro *et al*., 2016) indicate that the relationships between sympathetic reactivity and RFI also depend on stress severity and duration. Furthermore, the suggested positive quadratic effect of HR_{TRA} on RFI and the results of previous studies in sheep (Knott *et al*., 2008), pigs (Hennessy *et al*., 1988; Jenkins *et al*., 2013) and chickens (Luiting *et al*., 1994) suggest that low-RFI cattle could have decreased HPA axis reactivity, independent of stress severity and duration, a topic that requires further evaluation.

Associations between overnight heart rate, stroke volume, cardiac output, blood volume and residual feed intake

Absence of a significant correlation between AUHC, SVI, COI, BV and RFI (Table 4.2) indicates that the effects of overnight HR traits on RFI were not associated with a change in SVI, COI or BV. However, cattle were restrained in a standing position during the indicator dilution assessment; therefore, a higher HR in comparison to HR_{OVA} is expected (Purwanto *et al*., 1993) and supports the correlations of HR_{TRL} and HR_{TRA} with AUHC, HR_{TRL} with BV and the negative correlation of HR_{TRA} with SVI (Table 4.3). After adjusting for the effects of AUHC and BV, SVI had a negative linear effect on RFI (Table 4.2), indicating that the decrease in HR_{OVA} with RFI (Table 4.2) could be associated with an increase in SVI. Potentially the adjustment for AUHC removed the variation in SVI caused by the non-resting conditions of the indicator assessment, resulting in a SVI that was representative of rest and associated with RFI. The suggested opposite relationships of HR_{OVA} and SVI with RFI appear to offset, as COI was not associated with RFI.

Decreases in BV with RFI (positive linear effect) in the absence of a decreased COI (Table 4.2) could indicate that right atrial pressure decreases, resulting in an unchanged venous return (CO) at a lower BV. A suggested decrease in right atrial pressure without an increase in COI, indicates that the decrease in BV and/or an increase in vascular compliance offset the increase in the pressure gradient associated with the reduction in right atrial pressure (Berlin and Bakker, 2014). Positive relationships between right atrial pressure and pulmonary hypertension (Gaynor *et al*., 2005), pulmonary hypertension and mitochondrial proton leak (Iqbal *et al*., 2001) and mitochondrial proton leak and RFI (Carstens and Kerley, 2009) support that right atrial pressure could have a positive relationship with RFI. No correlation of LF_{OV} with RFI and the absence of a linear or quadratic effect of LF_{OV} on RFI (Table 4.2) suggest that changes in BV were not regulated by the SNS. A decreased activity of the renin-angiotensin-aldosterone system (RAAS) could sustain the decreases in BV and suggested decreases in right atrial pressure by reducing/preventing the increased retention of water and salt (Hall, 2015). Associations between the RAAS and RFI observed in a genome-wide association study in cattle (Santana *et al*., 2014) indicated that fluctuations in salt reabsorption could decrease with RFI. This negative relationship between RAAS activity and RFI (Santana *et al*., 2014) supports the positive effect of BV on RFI, suggesting that neural and renal mechanisms could regulate the relationship between RFI and BV.

Influence of oxygen-carrying capacity and metabolic demand

The suggested positive linear effect of MCV on RFI supports the suggested lower MCV observed in low-RFI heifer calves and yearling heifers (Crane *et al*., 2016). Furthermore, the absence of an association between RBC, Hb, Hct and RFI observed in this study (Table 4.2) is in agreement with previous studies in heifers (Crane *et al*., 2016; Kelly *et al*., 2016), steers (Gomes *et al*., 2011) and bulls (Santana *et al*., 2013). The suggested positive relationship between MCV and RFI, in the absence of an association of RBC, Hb and Hct with RFI, indicates a decreased oxygen-carrying capacity in low-RFI cattle. Mean corpuscular volume is calculated as the quotient of Hct and RBC, traits that displayed similar relationships with multiple cardiovascular traits (Table 4.3, 4.4 and 4.5), reducing the likelihood of an association of the given trait with MCV. The absence of a correlation between MCV and traits associated with RFI (HR traits, SVI and BV) supports this observation and indicates that although MCV has potential for use as a RFI proxy (Richardson *et al*., 2002; Crane *et al*., 2016) it does not appear to influence the associations of other cardiovascular measures with RFI.

The positive relationship of HR_{OVA}, negative relationship of SVI and absence of relationship of COI with RFI (Table 4.2) are in agreement with changes in functional capacity that occur in humans following exercise training (Rerych *et al*., 1980). Increased state three respiration rates and reduced proton leakage in skeletal muscle have been observed both in humans following exercise training (Iaia *et al*., 2008; Phielix *et al*., 2010; Zoll *et al*., 2002) and in low-RFI cattle (Kelly *et al*., 2011; Kolath *et al*., 2006a). Increases in mitochondrial efficiency (Kelly *et al*., 2011; Kolath *et al*., 2006a) could, in part, explain the decreases in heat production in low-RFI cattle (Montanholi *et al*., 2009 and 2010), which indicate reduced metabolic demand, potentially explaining the relationships of HR_{OVA}, SVI and COI with RFI (Walley, 2011). The positive linear effect of BV on RFI (Table 4.2) indicates that BV increases with RFI, an opposite relationship to that observed in response to exercise training (Rerych *et al*., 1980). Blood volume but not HR_{OVA}, AUHC, SVI and COI was correlated with DMI (Table 4.2), suggesting that BV decreases with RFI due to reductions in both feed intake and metabolic demand.

Associations of residual feed intake and cardiovascular function with heart structure and histomorphometry

The suggested relationships of BV and right atrial blood pressure with RFI could explain the positive correlation of RV_{TH} and MY_{WD} with RFI and the positive linear effects of RV_{TH} and MY_{WD} on RFI (Table 4.2). Increased BV and right atrial pressure can cause RV pressure overload (Gaynor *et al*., 2005; Gonçalves *et al*., 2003; Mirsalimi *et al*., 1993). The RV adapts to pressure overload through RV hypertrophy increasing cell width (Werchan *et al*., 1989; Zierhut *et al*., 1990) and wall thickness (Voelkel *et al*., 2006), supporting the positive correlations of RV_{TH} and MY_{WD} with RFI. Increases in RV weight have also been observed as an adaptation to RV pressure overload (Werchan *et al*., 1989), as observed in this study through the negative quadratic effect of RV_{WT} on RFI (Table 4.2) and the positive correlation between RV_{WT} and RV_{TH} ($R = 0.47$; $P < 0.001$). Boostani *et al*. (2010) observed that non-restricted chickens had a decreased feed efficiency and increased RV_{WT} (adjusted for BW), supporting the negative quadratic relationship between RV_{WT} and RFI. Furthermore, cattle are known to display RV hypertrophy without pulmonary disease (Han *et al*., 2008), suggesting RV hypertrophy could be present in high-RFI cattle without noticeable effects on animal health or performance. The absence of a correlation of RV_{TH}, MY_{WD} and RV_{WT} with BV (Table 4.5) despite their relationships with RFI suggests that the relationships amongst these traits were not linear or were influenced by additional sources of variation that were not associated with RFI. Effects of an increase in BV and suggested increase in right atrial pressure on the LV as RFI increases are less clear, as LV_{WT}, LV_{TH}, LV_{WT}:RV_{WT} and HT_{WT} were not correlated with RFI and did not have an effect on RFI (Table 4.2). The trend for a positive correlation of LV_{WT} with SVI (Table 4.5) and the negative linear effect of SVI on RFI (Table 4.2), suggest that LV capacity should display a negative relationship with RFI. Decreases in RV_{WT} to total ventricle weight ratio without an increase in total heart weight were observed in feed efficient chickens (Boostani *et al.*, 2010), supporting the HT_{WT} results of our study and indicating an increased LV_{WT} with improved feed efficiency. Disparity in the associations of contractility, preload and afterload with measures of LV function and structure have been observed (Mahler *et al.*, 1975) and could explain why a relationship of LV_{WT} and LV_{TH} with RFI was not observed. The absence of an association between HT_{WT} and RFI has also been observed in pigs following divergent selection on RFI (Lefaucheur *et al.*, 2011) supporting that despite the disparities in the relationships between RV_{WT}, LV_{WT}, LV_{WT}:RV_{WT} and HT_{WT} , changes in HT_{WT} are not associated with the variation in RFI.

Of the cardiovascular parameters measured, only RV_{TH} and MY_{WD} were correlated with RFI, in the presence of additional variation from other related traits (Table 4.2). As structural measures, associations of RV_{TH} and MY_{WD} with RFI are the result of additive effects from multiple parameters. Linear and quadratic relationships of HR_{OVA}, SVI and BV with RFI indicate that these parameters additively decrease the workload on the right side of the heart in part, causing the positive linear relationships of RV_{TH} and MY_{WD} with RFI. Furthermore, RV_{TH} and MY_{WD} were correlated with multiple productive performance traits (Table 4.2), traits that are adjusted for during the calculation of RFI, supporting that these relationships are a result of differences in metabolic demand, not body size, carcass composition or oxygen-carrying capacity.

Explained variation in residual feed intake

The heart rate multiple regression model for RFI explained the largest amount of variation in RFI (Fig. 4.2); however, the selected model was over parameterized resulting in a model that was unable to explain or predict the variation in RFI. Heart rate in response to stress is influenced by neural and endocrine regulation (Ulrich-Lai and Herman, 2009), coping ability (Koolhaas *et al*., 1999), underlying metabolism (Careau *et al*., 2008) and energy reserves (Rauw, 2012) which are associated with RFI (Colpoys *et al*., 2014; Knott *et al*., 2008; Kolath *et al*., 2006a; Montanholi *et al*., 2010). Whereas the association between resting HR and RFI is influenced by neural, endocrine and renal regulation and underlying metabolism (Hall, 2015). Differences in mechanisms that influence the association of HR and RFI with stress could explain the over parameterization of the heart rate model. The additional relevant mechanisms during stress could explain why a larger amount of explained variation in RFI was attributed to transport and abattoir HR traits (Fig 4.2, A1).

Blood volume appeared to be influenced by both differences in DMI and metabolic demand in this study whereas the negative effect of SVI on RFI was attributed to differences in only metabolic demand, suggesting why BV explained the largest amount of variation in RFI attributed to indicator dilution traits (Fig. 4.2, B1). Furthermore, the significant effects of both SVI and BV on RFI explain why the indicator dilution model showed a trend towards explaining a significant amount of variation in RFI (Fig. 4.2, B2), despite the non-significant effect of AUHC (Table 4.2). Furthermore, as RFI decreased the ability of the indicator dilution model to differentiate RFI increased, as indicated by the slight reduction in RFI dispersion when moving from right to left in Fig. 4.2, B2. As oxygen-carrying capacity traits were not correlated with RFI and did not affect RFI, the low R^2 and adjusted R^2 of the non-significant oxygen-carrying capacity model (Fig. 4.3) was anticipated and explains the inability of the model to differentiate RFI.

Structure traits were influenced by HR_{OVA}, SVI and BV, traits that were associated with RFI; the additive effects of these measures could explain why the heart structure model and MY_W explained the largest amount of variation in RFI (Fig. 4.3, B2). The greatest portion of explained variation by the heart structure model was attributed to RV_{WT} and RV_{TH} (Fig. 4.3, B1), supporting the theory of reduced workload on the right side of the heart in low-RFI cattle, as indicated by the reduced BV and suggested reduction in metabolic demand. Predictive ability also appeared to be highest when using the heart structure model based on the reduced dispersion of low RFI values (Fig. 4.3, B2), in comparison to the indicator dilution model (Fig. 4.2, A2). However, an increased number of observations could have contributed to this reduced dispersion.

Summary

Our study has indicated that resting heart rate has a changing curvilinear relationship with residual feed intake that ultimately results in a positive relationship that appears to be explained by increases in metabolic efficiency, not sympathetic regulation. The changing direction of the relationship of transport and abattoir heart rate and low frequency power measurements with residual feed intake, as stress severity and duration change, suggest that increases in energy reserves and metabolic efficiency associated with decreases in residual feed intake could allow for a more specific and energy conserving stress response in feed efficient cattle. Reductions in overnight heart rate associated with decreases in residual feed intake appears to be offset by an increase in stroke volume indicator, resulting in an unchanged cardiac output indicator, a mechanism reflective of increases in metabolic efficiency, not oxygen-carrying capacity. Improvements in metabolic efficiency and/or changes in endocrine modes of regulation associated with increased feed efficiency appear to also explain the positive relationship observed between blood volume and residual feed intake. A positive relationship that indicates decreased right heart workload in feed efficient cattle, which is associated with the positive relationships of right ventricle weight, wall thickness and myocyte width with residual feed intake. Structural measures also explained the greatest variation in residual feed intake and were the most accurate predictors of feed efficiency supporting that they are the result of the additive influence of heart rate, stroke volume and blood volume.

In conclusion, a reduced heart rate, increased stroke volume indicator, reduced blood volume and suggested reduced right heart workload in feed efficient cattle, at an equivalent performance, could indicate a more efficient cardiovascular system that appears to be due to improvements in metabolic efficiency, decreasing metabolic demand and the demand on the cardiovascular system. Results that indicate that associations of both resting and stress heart rate with residual feed intake largely reflect differences in metabolic efficiency, that do not appear to impede performance or health, suggesting the potential for their use in the development of robust, applicable feed efficiency proxies for bovine.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors

Author Contributions

Author contributions: J.C.M., Y.R.M., P.W.P. and S.P.M. designed experiments; J.C.M., S.L., A.M., T.C. and Y.R.M. performed experiments; J.C.M., F.S.S., and Y.R.M. analyzed data; J.C.M. interpreted results, prepared figures and drafted manuscript; J.C.M., S.L., A.M., T.C., P.W.P., F.S.S., S.P.M. and Y.R.M. edited and revised manuscript; J.C.M., S.L., A.M., T.C., P.W.P., F.S.S., S.P.M. and Y.R.M. approved final version of manuscript.

Table 4.1 *Animal and diet information within cohort.*

	Cohort A	Cohort B			Cohort C	Cohort D
		B1	B2	B3		
n	34	16	16	16	40	43
Type	B	S	B	S	H	B
Time Period	Nov12-May13	Jan14-Aug14	Apr14-Sep14	May14-Sep14	Jul14-Oct14	Dec14-May15
Traits	PF, HR, CR	PF, HR, ID‡, CR, OC, HS	PF, HR, ID, CR, OC, HS	PF, HR, ID, CR, OC, HS	PF, HR, ID, OC	PF, CR, OC, HS
Age, d*	366 ± 30.9	379 ± 33.3	410 ± 14.4	432 ± 6.3	408 ± 28.0	374 ± 24.8
Weight, kg*	583 ± 85.5	550 ± 46.2	641 ± 73.0	607 ± 89.1	341 ± 42.2	527 ± 62.3
Breed, %						
Angus	52.8 ± 17.5	36.2 ± 17.5	46.7 ± 12.9	36.9 ± 13.4	27.5 ± 26.6	61.4 ± 22.4
Simmental	33.0 ± 20.3	53.0 ± 26.8	32.6 ± 14.5	46.5 ± 18.4	17.1 ± 20.4	15.6 ± 15.8
Crossbred	7.3 ± 5.3	4.6 ± 6.7	20.1 ± 6.3	15.2 ± 7.1	0	14.6 ± 11.1
Other	6.9 ± 5.4	6.3 ± 14.1	0.6 ± 1.7	1.4 ± 3.4	55.4 ± 35.1	8.4 ± 20.4
Diet†						
DM, %	53.9 ± 1.1	55.9 ± 5.2	53.8 ± 0.9	55.9 ± 5.2	36.1 ± 4.9	53.9 ± 1.1
CP, %DM	16.3 ± 2.2	12.5 ± 0.2	13.9 ± 0.9	12.5 ± 0.2	12.6 ± 1.1	16.3 ± 2.2
NDF, %DM	19.2 ± 1.4	20.3 ± 0.9	22.2 ± 0.9	20.3 ± 0.9	53.7 ± 3.3	19.2 ± 1.4
ADF, %DM	11.3 ± 1.6	8.2 ± 1.8	10.9 ± 1.6	8.2 ± 1.8	34.2 ± 1.8	11.3 ± 1.6
TDN, %DM	85.1 ± 1.4	88.4 ± 1.6	86.0 ± 1.5	88.4 ± 1.6	61.5 ± 1.6	85.1 ± 1.4

Values are means ± SD. *n*, number; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; TDN, total digestible nutrients; B, bulls; S, steers; H, heifers; PF, performance; HR, heart rate; ID, indicator dilution; CR, carcass; OC, oxygen-carrying capacity; HS, heart structure. *value at end of performance evaluation; †analyzed by near-infrared spectroscopy (Agri-Food Laboratories Inc., Guelph, Canada). ‡used for determination of blood volume and indicators of cardiac output and stroke volume.

Table 4.2 *Correlations of heart with performance and carcass traits and regression coefficients of heart traits on residual feed intake.*

Traits	RFI$^{\#}$	DMI$^{\#}$	FBW$^{\#}$	ADG$^{\#}$	Lean$_{YLD}^{\#}$	Fat$_{YLD}^{\#}$	Linear§	Quadratic§
Heart rate								
HR$_{OVL}$	0.05	0.01	-0.09	0.01	0.05	-0.01	-0.10 ± 0.17	0.03 ± 0.01‡
HR$_{OVH}$	0.20	0.18	0.01	0.10	0.06	-0.04	-0.10 ± 0.13	0.01 ± 0.01†
HR$_{OVA}$	0.15	0.11	-0.04	0.06	0.04	-0.01	0.18 ± 0.28	-0.04 ± 0.02‡
LF$_{OV}$	0.07	0.12	0.13	0.08	0.06	-0.04		
HR$_{TRL}$	0.10	0.15	0.09	0.11	-0.03	0.10	-0.07 ± 0.09	-0.007 ± 0.004
HR$_{TRH}$	0.10	0.24	0.23	0.23	-0.05	0.09		
HR$_{TRA}$	0.13	0.23	0.18	0.19	-0.02	0.08	0.04 ± 0.07	0.001 ± 0.003†
LF$_{TR}$	0.03	0.10	0.11	0.03	-0.01	0.01	9.3 ± 15.2	-575 ± 345
HR$_{ABL}$	0.15	0.05	-0.14	0.00	0.16	-0.07	0.33 ± 0.11‡	-0.004 ± 0.002†
HR$_{ABH}$	0.27	0.34**	0.17	0.20	-0.12	0.14	0.12 ± 0.06‡	0.001 ± 0.001†
HR$_{ABA}$	0.22	0.20	0.01	0.12	0.06	0.00	-0.36 ± 0.15‡	
LF$_{AB}$	0.19	0.29**	0.16	0.12	-0.05	-0.02	29.3 ± 10.8‡	
Indicator dilution								
AUHC	0.09	0.17	0.15	0.20	0.19	-0.04	-0.002 ± 0.001	
SVI	-0.13	-0.16	-0.11	-0.04	-0.09	0.04	-1157 ± 557‡	8.1e5 ± 5.4e5
COI	-0.05	-0.04	-0.03	0.05	-0.02	0.05		
BV	0.23	0.29*	0.11	0.17	-0.12	0.11	0.27 ± 0.10‡	
Oxygen-carrying								
RBC	-0.15	-0.24**	-0.15	-0.09	0.13	-0.13	1.4 ± 0.9	
Hb	-0.15	-0.17*	-0.03	0.00	0.02	0.04	-0.06 ± 0.09	
Hct	-0.15	-0.14	0.03	0.03	0.05	0.02	-14.1 ± 29.1	-84.6 ± 60.1
MCHC	-0.01	-0.10	-0.19*	-0.15	-0.11	0.14	0.02 ± 0.03	
MCV	0.06	0.20*	0.26**	0.20	-0.13	0.19	0.32 ± 0.19†	
Heart structure								
AT$_{WT}$	0.16	0.05	-0.03	-0.07	0.21	-0.27**	25.4 ± 14.0†	
LV$_{WT}$	0.08	0.02	0.00	0.02	-0.03	-0.03		
LV$_{TH}$	0.13	-0.26*	-0.52**	-0.35**	0.20	-0.28**		
RV$_{WT}$	-0.01	-0.05	0.01	0.03	0.02	0.02	-15.6 ± 15.1	-1150 ± 459‡
RV$_{TH}$	0.29**	-0.10	-0.40**	-0.38**	0.19	-0.23*	0.46 ± 0.15‡	
LV$_{WT}$:RV$_{WT}$	0.08	0.06	-0.04	-0.03	0.02	0.02	0.23 ± 0.71	
HT$_{WT}$	0.09	0.01	0.00	0.01	0.03	0.03		
Histomorphometry								
MY$_{WD}$	0.37*	0.08	-0.43**	-0.49**	-0.07	-0.11	0.93 ± 0.45‡	
SM$_{LG}$	0.05	-0.37*	-0.78**	-0.77**	0.26	-0.31		

[#]Columns are Pearson's correlation coefficients; §Columns are regression coefficients ± SE of significant effects ($P \leq 0.20$) according to the backward selection procedure. **Significant correlations at a 5% false discovery rate (Benjamini and Hochberg, 1995). *Significant correlations at a 10% false discovery rate. Significant effects: †$0.05 < P \leq 0.10$, ‡$P \leq 0.05$. RFI, residual feed intake; DMI, average dry matter intake; FBW, final body weight; ADG, average daily gain; Lean_{YLD}, lean yield; Fat_{YLD}, fat yield; HR_{OVA}, average overnight heart rate; HR_{OVL}, average of lowest twenty percent of overnight heart rates; HR_{OVH}, average of highest twenty percent of overnight heart rates, LF_{OV}, area under overnight amplitude/frequency curve from 0.01-0.1 Hz; HR_{TRA}, average transport heart rate; HR_{TRL}, average of lowest twenty percent of transport heart rates; HR_{TRH}, average of highest twenty percent of transport heart rates; LF_{TR}, area under transport amplitude/frequency curve from 0.01-0.1 Hz; HR_{ABA}, average transport heart rate; HR_{ABL}, average of lowest twenty percent of abattoir heart rates; HR_{ABH}, average of highest twenty percent of abattoir heart rates; LF_{AB}, area under abattoir amplitude/frequency curve from 0.01-0.1 Hz; AUHC, area under heart rate curve; SVI, stroke volume indicator; COI, cardiac output indicator; BV, blood volume; RBC, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; AT_{WT}, atria weight; LV_{WT}, left ventricle weight; LV_{TH}, left ventricle wall thickness; RV_{WT}, right ventricle weight; RV_{TH}, right ventricle thickness; LV_{WT}:RV_{WT}, left ventricle weight to right ventricle weight ratio; HT_{WT}, heart weight; MY_{WD}, myocyte width; SM_{LG}, sarcomere length.

Table 4.3 C*orrelations of heart rate with indicator dilution and oxygen-carrying capacity traits.*

Traits	AUHC	SVI	COI	BV	RBC	Hb	Hct	MCV	MCHC
HR_{OVA}	0.31	0.00	0.20	0.26	0.02	0.02	-0.09	-0.10	-0.05
HR_{OVL}	0.40*	-0.03	0.27	0.29	0.03	0.03	-0.08	-0.11	0.02
HR_{OVH}	0.16	0.02	0.06	0.15	0.03	0.03	-0.05	-0.09	-0.13
LF_{OV}	-0.12	0.11	-0.04	-0.07	0.00	0.00	-0.05	-0.06	-0.28
HR_{TRA}	0.78**	-0.38*	-0.01	0.38	0.29	0.29	0.20	0.01	-0.32
HR_{TRL}	0.68**	-0.27	0.13	0.43*	0.18	0.18	0.07	-0.04	-0.28
HR_{TRH}	0.74**	-0.38	-0.09	0.28	0.33	0.33	0.26	0.05	-0.34
LF_{TR}	0.28	-0.25	-0.24	0.04	0.12	0.12	0.07	-0.08	-0.50
HR_{ABA}	0.36	-0.16	0.04	0.00	0.04	0.04	-0.06	-0.15	0.08
HR_{ABL}	0.49*	-0.23	0.05	0.16	0.21	0.21	0.06	-0.19	0.01
HR_{ABH}	0.16	-0.09	-0.01	-0.09	-0.13	-0.13	-0.14	-0.06	0.15
LF_{AB}	0.01	-0.13	-0.15	-0.04	-0.01	-0.01	-0.24	-0.20	-0.10

Data are Pearson's correlation coefficients; **Significant correlations at a 5% false discovery rate (Benjamini and Hochberg, 1995). *Significant correlations at a 10% false discovery rate. HR_{OVA}, average overnight heart rate; HR_{OVL}, average of lowest twenty percent of overnight heart rates; HR_{OVH}, average of highest twenty percent of overnight heart rates, LF_{OV}, area under overnight amplitude/frequency curve from 0.01-0.1 Hz; HR_{TRA}, average transport heart rate; HR_{TRL}, average of lowest twenty percent of transport heart rates; HR_{TRH}, average of highest twenty percent of transport heart rates; LF_{TR}, area under transport amplitude/frequency curve from 0.01-0.1 Hz; HR_{ABA}, average abattoir heart rate; HR_{ABL}, average of lowest twenty percent of abattoir heart rates; HR_{ABH}, average of highest twenty percent of abattoir heart rates; LF_{AB}, area under abattoir amplitude/frequency curve from 0.01-0.1 Hz; AUHC, area under heart rate curve; SVI, stroke volume indicator; COI, cardiac output indicator; BV, blood volume; RBC, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

Table 4.4 *Correlations of oxygen-carrying capacity with indicator dilution traits.*

Traits	AUHC	SVI	COI	BV
RBC	0.40**	-0.42**	-0.30	0.10
Hb	0.29*	-0.24	-0.13	0.04
Hct	0.28*	-0.19	-0.08	0.06
MCHC	-0.19	-0.05	-0.10	-0.30*
MCV	0.02	0.25	0.34*	0.12

Data are Pearson's correlation coefficients; **Significant correlations at a 5% false discovery rate (Benjamini and Hochberg, 1995). *Significant correlations at a 10% false discovery rate. AUHC, area under heart rate curve; SVI, stroke volume indicator; COI, cardiac output indicator; BV, blood volume; RBC, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

Table 4.5 *Correlations of indicator dilution and oxygen-carrying capacity with heart structure and histomorphometry traits.*

Traits	AUHC	SVI	COI	BV	RBC	Hb	Hct	MCV	MCHC
Heart structure									
AT_{WT}	-0.11	0.18	0.12	-0.08	0.11	0.07	0.10	-0.03	-0.12
LV_{WT}	-0.20	0.26*	0.17	0.08	0.16	0.37**	0.33**	0.19	0.17
LV_{TH}	-0.11	0.21	0.13	-0.05	0.04	-0.05	-0.05	-0.16	-0.04
RV_{WT}	-0.26	0.03	-0.11	-0.08	0.07	0.21	0.18	0.12	0.04
RV_{TH}	-0.18	0.25	0.16	-0.09	0.15	0.13	0.13	-0.09	0.05
LV_{WT}:RV_{WT}	0.16	0.20	0.32	0.20	0.07	0.04	0.06	-0.01	0.10
HT_{WT}	-0.24	0.21	0.10	0.03	0.14	0.30**	0.28*	0.14	0.09
Histomorphometry									
MY_{WD}	0.16	-0.26	-0.19	-0.11	0.27	0.30	0.28	-0.01	0.08
SM_{LG}	0.11	-0.04	0.00	-0.17	0.34	0.19	0.22	-0.27	-0.16

Data are Pearson's correlation coefficients; **Significant correlations at a 5% false discovery rate (Benjamini and Hochberg, 1995). *Significant correlations at a 10% false discovery rate. AUHC, area under heart rate curve; SVI, stroke volume indicator; COI, cardiac output indicator; BV, blood volume; RBC, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; AT_{WT}, atria weight; LV_{WT}, left ventricle weight; LV_{TH}, left ventricle wall thickness; RV_{WT}, right ventricle weight; RV_{TH}, right ventricle thickness; LV_{WT}:RV_{WT}, left ventricle weight to right ventricle weight ratio; HT_{WT}, heart weight; MY_{WD}, myocyte width; SM_{LG}, sarcomere length.

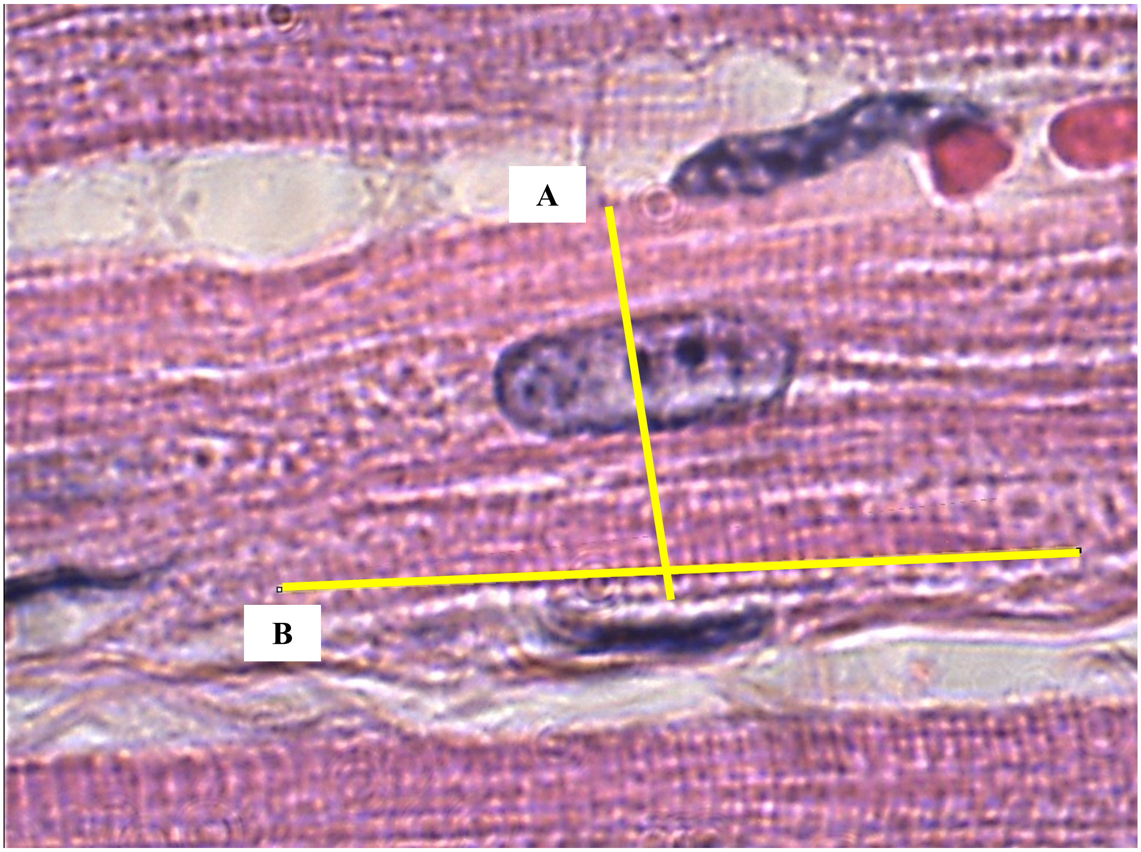

Fig. 4.1 Longitudinal myocyte image collected under oil immersion using light microscopy. A, myocyte width (MY_{WD}) measurement; B, sarcomere length (SR_{LG}) measurement.

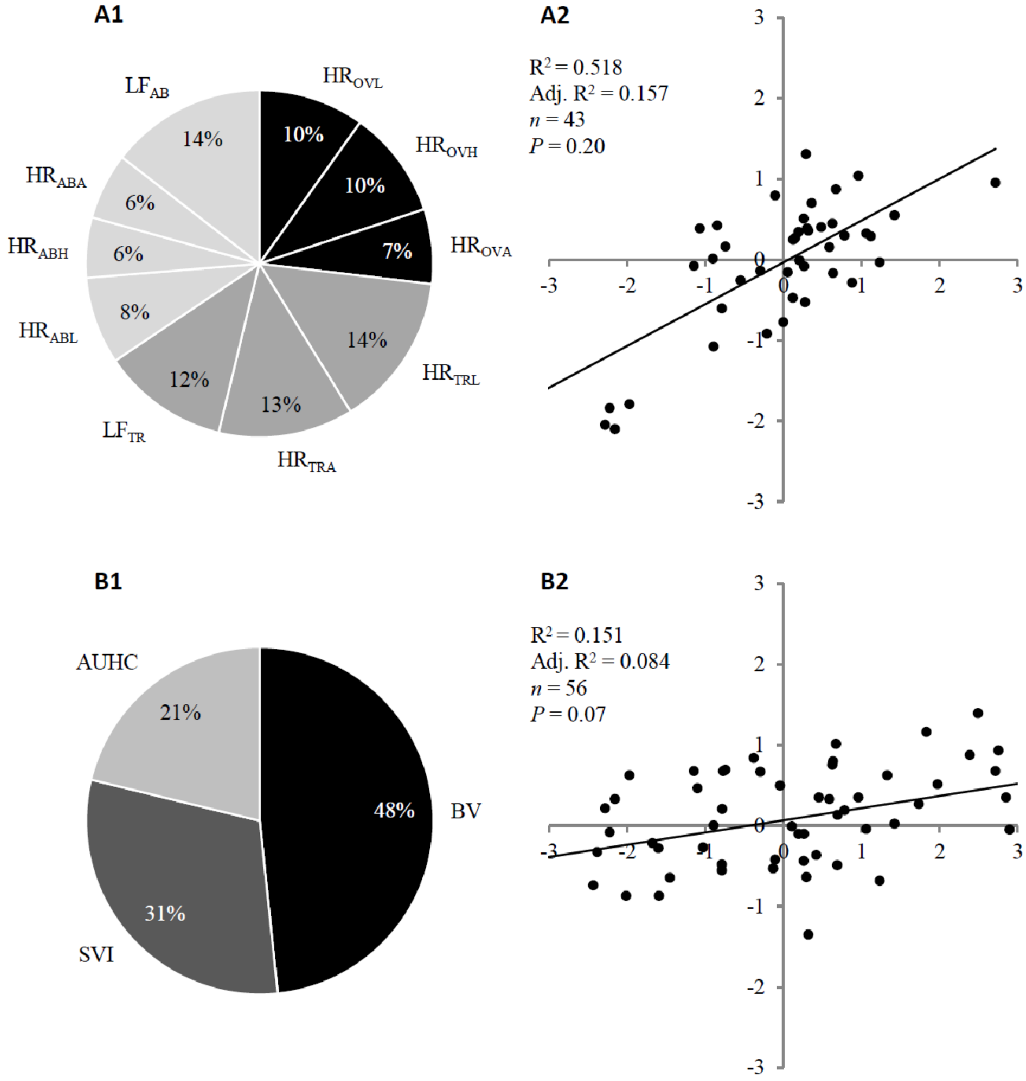

Fig. 4.2 Partial contribution of each trait towards variation in residual feed intake explained by selected heart rate (A1) and indicator dilution (B1) multiple regression models and plot of observed versus predicted residual feed intake determined using selected multiple regression model (A2, B2). $\mathrm{HR_{OVA}}$, mean heart rate during overnight segment; $\mathrm{HR_{OVL}}$, mean of lowest twenty percent of heart rates during overnight segment; $\mathrm{HR_{OVH}}$, mean of highest twenty percent of heart rates during overnight segment; $\mathrm{HR_{TRL}}$, mean of lowest twenty percent of heart rates during transport segment; $\mathrm{HR_{TRH}}$, mean of

highest twenty percent of heart rates during transport segment; LF_{TR}, area under transport segment amplitude/frequency graph from 0.01-0.1 Hz; HR_{ABA}, mean heart rate during abattoir segment; HR_{ABL}, mean of lowest twenty percent of heart rates during abattoir segment; HR_{ABH}, mean of highest twenty percent of heart rates during abattoir segment; LF_{AB}, area under abattoir segment amplitude/frequency graph from 0.01-0.1 Hz; AUHC, area under heart rate curve; SVI, stroke volume indicator; BV, blood volume; n, number of observations.

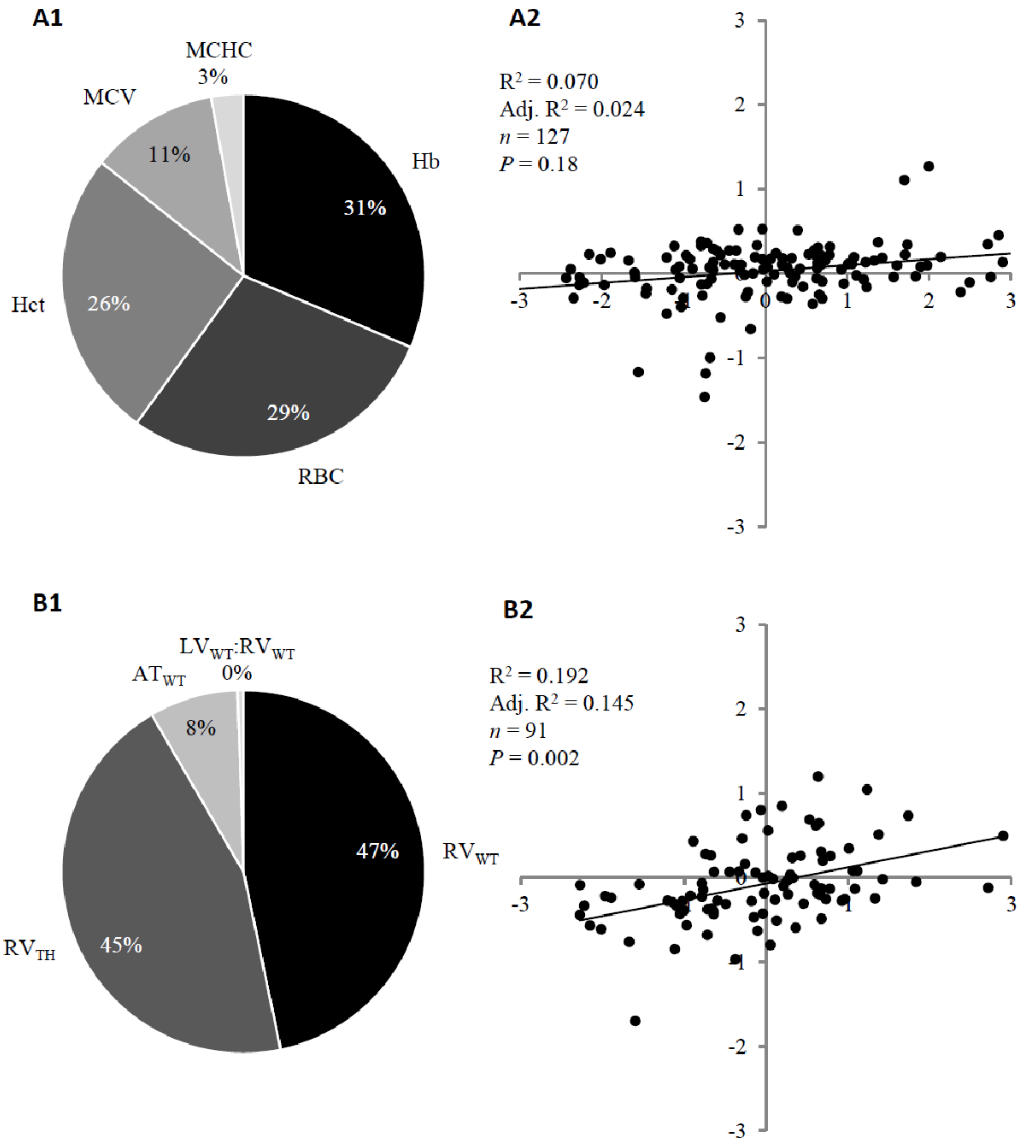

Fig. 4.3 Partial contribution of each trait towards variation in residual feed intake explained by selected oxygen-carrying capacity (A1) and heart structure (B1) multiple regression models and plot of observed versus predicted residual feed intake determined using selected multiple regression model (A2, B2). RBC, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; AT_{WT}, atria weight; LV_{WT}, left ventricle weight; RV_{TH}, right ventricle thickness; LV_{WT}:RV_{WT}, left ventricle weight to right ventricle weight ratio; HT_{WT}, heart weight; n, number of observations.

CHAPTER 5

CONCLUSION

The dynamics of pressure on the Canadian beef industry continue to change, where today, and into the foreseeable future, beef cattle production is challenged with reducing feed and land resource use and environmental impact (Gerber *et al.*, 2013). Improvement of feed efficiency through the use of RFI can optimize the feed and land required for beef cattle production (Capper and Bauman, 2013). However, expense and time required for the determination of RFI, despite continued efforts (Culbertson *et al.*, 2015), continue to limit improvement and industry adoption, leading to the development of RFI proxies, traits that are associated with RFI and applicable on-farm. Heart rate is associated with both RFI (Hafla *et al.*, 2013) and other traits of economic importance (Stewart *et al.*, 2013; Frondelius *et al.*, 2015), suggesting it could serve as an ideal on-farm feed efficiency indicator while improving overall beef cattle husbandry. However, HR assessments that are applicable in a production setting have not been developed and the basis of the relationship of HR with RFI has not been explored. An exploration that could identify antagonistic associations and/or novel HR proxies. This thesis set forth to develop and assess the applicability of on-farm HR assessments and to explore the cardiovascular, regulatory and metabolic mechanisms that underlie the association between HR and RFI.

Previous associations of HR and RFI during rest (Hafla *et al.*, 2013) and the reactivity of HR to novel, sudden stress (Désiré *et al.*, 2004) proposed that both resting and acute stress HR assessments could be evaluated for associations with RFI and their on-farm applicability as was investigated in Chapter 3. Lower overnight HR in low-RFI heifer calves appears to reflect differences in metabolism rather than energy expended for the act of lying or standing (Lawrence *et al.*, 2011). Increased HR responses to sudden, novel umbrella exposure in low-RFI calves could reflect differences in coping ability (Koolhaas *et al.*, 1999) and HR regulation (Désiré *et al.*, 2004) that in conjunction with other RFI studies (Knott *et al.*, 2008) indicate that low-RFI heifer calves have a proactive coping style that could represent a form of energy conservation (Careau *et al.*, 2008) and/or differences in the utilization of energy reserves (Rauw, 2012). The disparity in HR response to umbrella exposure between heifer calves and yearling heifers could be a result of differences previous handling experience (Grandin, 1997). Short duration (10 minutes), ability for inclusion during routine husbandry practices (handling) and potential for further shortening indicate that with further development the umbrella exposure assessment has potential for on-farm use as a RFI proxy (Figure 5.1).

While identifying potential, applicable HR proxies, Chapter 3 also suggested potential mechanisms influencing the relationships of resting and stress HR with RFI. Chapter 4 addressed these potential mechanisms by beginning to evaluate the underlying cardiovascular, regulatory and metabolic mechanisms defining the associations between HR and RFI during rest and stress. Changes in the direction of the relationship of HR and LF with RFI within and across overnight, transport and abattoir HR recordings, suggest that stress severity and duration influence both HR (Moberg and Mench, 2000) and HR regulation. The negative relationships of SVI and overall suggested positive relationship of overnight HR with RFI appear to reflect reductions in metabolic demand with RFI (Zoll *et al.*, 2002; Kolath *et al.*, 2006a; Montanholi *et al.*, 2010) rather than differences in oxygen-carrying capacity or sympathetic regulation (Figure 5.1). While the positive effect of BV on RFI could be explained by metabolic demand and feed intake. Additive associations of resting HR, SVI and BV with RFI could explain the overall positive relationships of RV_{TH}, RV_{WT} and MY_{WD} with RFI, which are secondary to and indicate a reduced right heart workload (Maillet *et al.*, 2013) (Figure 5.1). This additive influence from multiple functional traits could also explain why heart structure and histomorphometry models explained the largest amount of variation in RFI.

When considered together the relationships of HR_{OVL}, $HR_{OVH,}$ and HR_{OVA} from Chapter 4 indicate a lower overnight HR in low-RFI cattle as observed in Chapter 3 (Figure 5.1). However, the changing direction of these relationships as HR increases in Chapter 4 indicates that other mechanisms, in addition to metabolic demand, could affect the relationship between resting HR and RFI. Overnight during a resting period cattle could still be exposed to stressors of varying severity and duration, which could explain these changing relationships with increases in HR (Moberg and Mench, 2000). An influence that would not be detected when assessing overall HR means (Chapter 3). The higher HR response to umbrella exposure in low-RFI heifer calves in Chapter 3 are in agreement with the positive relationship between HR_{ABA} and RFI observed in Chapter 4, an agreement when considering the results of LF_{AB} (Chapter 4) that indicates low-RFI cattle could have a higher sympathetic reactivity to that particular stress severity. Previous handling experience before transport to the abattoir could have influenced the changing relationships of transport and abattoir HR with RFI in Chapter 4, similar to the suggested effect of previous handling experience (Grandin, 1997) on umbrella HR response observed in Chapter 3.

Chapters 3 and 4 identified various physiological mechanisms that aided in developing a network of physiological relationships that contribute to the association between resting HR and RFI (Figure 5.1). The absence of a lying pattern effect on the relationship between overnight HR and RFI (Chapter 3) and the absence of a relationship of LF_{OV} and oxygen-carrying capacity with RFI (Chapter 4), support that associations between resting HR and RFI are largely influenced by metabolic demand. Reduced metabolic demand in low-RFI cattle (Montanholi *et al.*, 2009; Montanholi *et al.*, 2010) appears to increase SVI, offsetting a decreased resting HR resulting in an unchanged COI (Zoll *et al.*, 2002; Iaia *et al.*, 2008; Phielix *et al.*, 2010) and in conjunction with a reduced feed intake could explain the reduced BV (Figure 5.1). Overall changes in cardiovascular function support a reduced right heart workload in low-RFI cattle

Throughout the experiments of this Thesis avenues for new research that could further advance the understanding and improvement of feed efficiency were identified. Chapter 3 and 4 indicate that previous handling experience, stress severity and stress duration influence the predictive ability of HR assessments. As we move forward in the development of applicable HR proxies, accounting for these three factors, along with ensuring repeatability and accuracy of the assessments will become a major challenge. Routine husbandry practices could become ideal HR assessments (Figure 5.1) that address all three factors while still ensuring applicability. Practices such as tagging and disbudding if consistent are associated with a known level of stress and are similar in length between animals (Stewart *et al.*, 2013). These husbandry practices occur at young age reducing the influence of previous experience while meeting the selection programs of beef producers.

Chapter 4 also identified gaps in research that could have implications at the production level. While the SNS was proposed not to influence the association of resting HR with RFI the associations of endocrine and renal mechanisms with RFI remain uncertain (Knott *et al.*, 2008; Santana *et al.*, 2014; Kelly *et al.*, 2016). Furthermore, it is suggested that low-RFI cattle could have a lower blood pressure that is associated with additional changes in cardiovascular function and structure. This suggestion although founded in relevant research (Iqbal *et al.*, 2001; Gaynor *et al.*, 2005; Carstens and Kerley, 2009) could be validated by future studies in beef cattle. Blood pressure measurements could be collected at a low-cost and, pending the development of technology, could be practical assessments on-farm, suggesting potential as proxies for feed efficiency.

This Thesis effectively developed and assessed heart rate proxies for residual feed intake while building the physiological framework of this association and, as such, could serve as a model for future studies focused on applicable feed efficiency improvement. Furthermore, associations of heart rate with estrous detection (Lewis and Newman, 1984), calving onset (Kovács *et al.*, 2015), disease onset (Sheldon *et al.* 2016) and pest pressure (Schwinghammer *et al.*, 1987) suggest that this Thesis could become the precedent to future studies focused on developing heart rate into an applicable, diverse management tool for the improvement of beef cattle husbandry.

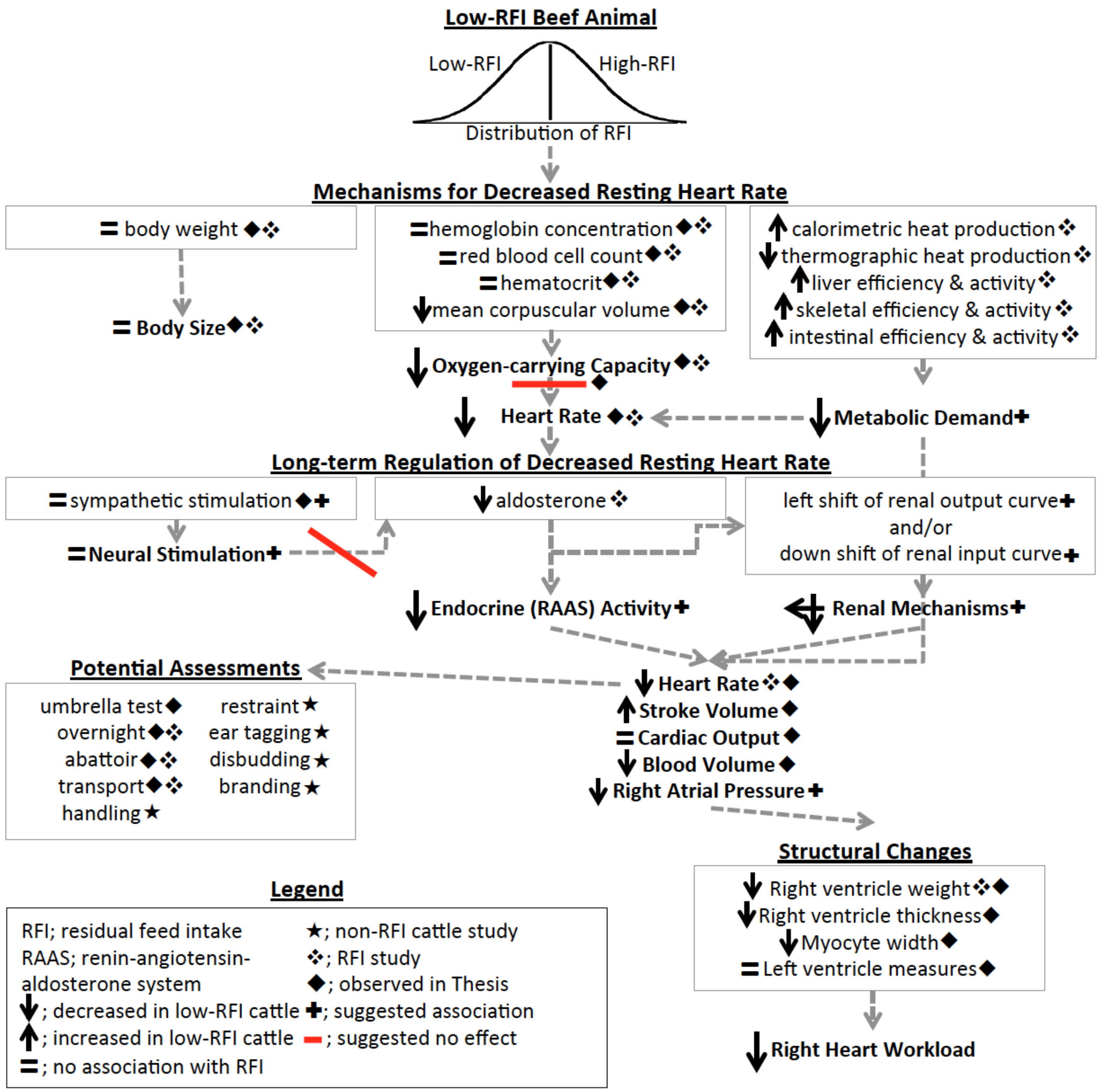

Figure 5.1. Proposed physiological basis of decreased resting heart rate in low residual feed intake cattle and identified and potential heart rate assessments for residual feed intake proxies.

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