DEVELOPMENT OF A MATHEMATICAL MODEL FOR PREDICTING DIGESTIBLE ENERGY INTAKE TO MEET DESIRED BODY CONDITION PARAMETERS IN EXERCISING HORSES

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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December 2016

Major Subject: Animal Science

ABSTRACT

Information regarding dietary requirements to maintain or alter body condition in the horse is scarce; however, a recently developed nutritional model was acceptably accurate in prediction of the energy required to alter BCS in sedentary mares. The objective of this study was to expand the scope of this model to include exercising horses. Previously published estimates of energy expenditure in the exercising horse were incorporated into the model. Stock type horses (n=24) were assigned to treatments of light exercise, heavy exercise or control and fed according to the model to gain or lose 2 BCS within 60 d. The energy expenditure for exercise was quantified for each horse via indirect calorimetry using the K4b2 (Cosmed) adapted for use in horses. Body parameters were also measured including, BCS, %BF estimated from RFT, BW, body length, heart girth circumference and neck circumference at 2 wk intervals throughout the study.

Model evaluation revealed acceptable precision when predicting BCS and BW in control horses (r^2 = 0.91 and r^2 = 0.98 respectively) but was less precise when predicting %BF (r^2 = 0.51). Model precision for BCS, BW and %BF in lightly (r^2 = 0.29, 0.85, 0.57) and heavily (r^2 = 0.04, 0.84, 0.13) exercised horses was low. Statistical analysis of indirect calorimetry data revealed that the observed and model predicted DE (Mcal/d) expenditure for lightly exercised horses were similar (0.71 vs 0.81, P = 0.46); however, the observed energy expenditure in heavily exercised horses was lower than the model predicted value (3.63 vs 6.79, P = 0.04). Also, observed energy expenditures were lower than the NRC recommendations for both light and heavy exercise groups ($P < 0.05$). Regression analysis revealed that rider BW, environmental humidity and horse age are significant contributors to VO_2. Further investigation into the relationship between these factors, VO_2 and body composition could yield a more precise predictive equation which would increase the precision of both the model and NRC recommendations for exercising horses.

DEDICATION

This manuscript is dedicated to my husband Patrick and my son Carson for their patience, love and support.

ACKNOWLEDGEMENTS

I would like to first thank my committee chairs, Dr. Clay Cavinder and Dr. Dennis Sigler, for the opportunities they have afforded me throughout the years and for their guidance and support in school, research and life in general. I would also like to thank my committee members, Dr. Luis O. Tedeschi for his knowledge and most especially his willingness to share a portion of that knowledge with me, and Dr. Julie Harlin for her support both in my research and in my career path. Thanks also to Dr. Martha Vogelsang for her guidance and friendship throughout my graduate career and to my fellow graduate students, Courtney Phillips, Raul Valdez, and Chelsie Huseman for their help during this research project and beyond.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis (or) dissertation committee consisting of Professor Clay Cavinder (advisor), Professor Dennis Sigler (co-advisor) and Professor Luis Tedeschi of the Department of Animal Science and Professor Julie Harlin of Agriculture Leadership Education and Communication.

All work for the thesis (or) dissertation was completed independently by the student.

Funding Sources

There are no outside funding contributions to acknowledge related to the research and compilation of this document.

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CHAPTER I

INTRODUCTION

Maintaining optimal nutritional status is an important managerial concern for horse owners and managers. Previous research indicates that over or under nutrition can be detrimental to reproductive efficiency (Henneke et al., 1984; Cavinder et al., 2009), athletic ability (Kearns et al., 2002) and overall health (Hoffman et al., 2003; Adams et al., 2009) of the horse. The body condition score system was developed to help researchers and professionals better quantify the energy status or fatness of horses (Henneke et al., 1983); however, current NRC (2007) nutritional recommendations for horses are based on body weight (BW) and information regarding dietary requirements to maintain or alter body condition in horses is scarce.

In the cattle industry, nutrition models have been created to estimate energy requirements based on body composition (Fox et al., 2004; Tylutki et al., 2008), and these practices have recently been applied to horses (Cordero et al., 2013). Cordero et al. (2013) developed a computer model that can adequately predict the digestible energy required to alter body condition in broodmares within a set period of time. Application of this model has the potential to create more efficient feeding practices both nutritionally and economically; however, this model was evaluated on sedentary mares at maintenance. Many horses in the United States are used for athletic activities such as racing, showing, and recreation (American Horse Council, 2005). Expansion of the model developed by Cordero et al. (2013) to include exercising horses would make the model more applicable to the larger population of equine athletes.

In order to accomplish this expansion, the energy expended during exercise must be quantified. Energy metabolism during exercise has been measured by indirect calorimetry in several studies (Eaton et al., 1995b; Tyler et al., 1996; McCutcheon et al., 1999); but, due to equipment limitations these measurements could only be taken while the subjects were being worked on a treadmill. Field conditions present many more factors such as ground resistance, elevation changes, and rider and tack weight that will alter the intensity of the exercise bout (NRC, 2007). In an effort to better estimate energy expended under these conditions, researchers have developed correlations between HR and oxygen consumption (Eaton et al., 1995b; Courouce et al., 2010). More recent technological advances have made it possible to analyze oxygen consumption during equestrian events (Art et al., 2006; Votion et al., 2006; Lepretre et al., 2009) and application of this technology may allow for more accurate quantification of energy expenditure, leading to more precise predictions when applied to the aforementioned nutrition model.

The largest and most variable storage form of energy in the body is fat (Lohman, 1971). In humans, several technologies have emerged to more precisely measure body fat (Haarbo et al., 1991); however, due to practical constraints, such as body size, these options are not available for evaluating body composition in the horse. The most widely accepted form of measuring body fat in the horse is through ultrasonic evaluation of rump fat thickness (Westervelt et al., 1976); although, more recent work indicates that administration of deuterium oxide (D_2O) as a tracer may provide more accurate whole body assessment (Dugdale et al., 2011b). Still, producers in the equine industry do not routinely have access to ultrasound equipment, nor is application of the tracer dilution technique a practical way for body fat estimation in the field. Correlation of body fat, as determined by ultrasound and D_2O, with body parameter measurements, which can be easily measured in the field, could provide a useful tool for industry professionals to quantify body fat in horses. Additionally, %BF is one of the inputs required in the model created by Cordero et al. (2013), and providing producers with a tool to quantify body fat would make the model more applicable in the industry.

Therefore, the objectives of this study are to:

1. Alter the current nutrition model by Cordero et al. (2013) to include exercising horses, based on exercise energy expenditure estimates from previous research,
2. Quantify the energy expenditure of horses being ridden in field conditions in order to evaluate and enhance the model,
3. Evaluate the effectiveness of the model in predicting changes in BCS for exercising horses, and
4. Determine what relationships exist, if any, between estimation of BF via ultrasonic evaluation, and measurement of body parameters.

CHAPTER II

LITERATURE REVIEW

Dietary Energy

The conversion of chemical energy, present in feedstuffs and animal tissue, to mechanical energy gives off heat and fuels maintenance and athletic activities in the horse. Energy can be quantified in terms of calories, the nomenclature most often used in the United States; or it can be presented as joules. A calorie is defined as the amount of heat required to increase 1g of water by 1°C, and equine nutrition energy requirements are most commonly expressed in terms of kilocalories (kcal) or megacalories (Mcal).

There is a hierarchy of systems in place to partition and define different types of energy. Gross energy is the chemical energy contained in feed and is defined as the heat produced when a feedstuff is combusted via bomb calorimetry. Combustion involves reducing a feed to its most oxidized form with oxygen and heat. Using this technique the amount of energy being provided to the animal can be quantified, and is termed intake energy (IE). However, during digestion, not all of the IE provided can be absorbed and utilized by the animal due to the type of digestive system or the composition of the feed provided. The IE that is available to the animal after digestion is termed DE and is calculated by subtracting the GE contained in the fecal matter from the intake energy. Digestible energy is the unit most commonly used in conjunction with equine nutrition because fecal energy represents the largest source of energy lost during digestion (NRC, 2007). However, ruminant nutritionists often describe energy in terms of metabolizable energy or net energy. Metabolizable energy accounts for urinary and gaseous energy losses in addition to fecal losses. In the horse, energy lost as gas and urine is minimal and up to 90% of DE is converted to ME (NRC, 2007). In ruminants, the gaseous losses tend to be larger due to the greater extent of microbial fermentation during digestion. Metabolizable energy can further be partitioned into energy lost as heat (HE) and recovered energy (RE), which comprises the NE system. Recovered energy includes energy stored in the body tissues, and energy used to generate a product, such as during pregnancy, lactation and growth. Heat losses can further be attributed to separate components as follows: heat associated with basal metabolism, voluntary activity, thermal regulation, product formation, digestion and absorption, waste formation and excretion, and the heat of fermentation. Because the NE system can partition HE losses for separate activities it may be more useful at predicting how well a diet will meet the requirements of the animal (NRC, 2007); however, it also requires more information and is more complicated to use.

Although DE is the most widely accepted system in the equine industry, it is not without its limitations. First, during digestion internal cells may be sloughed which can attribute to fecal GE that was not derived from IE. The difference between IE and fecal GE is termed apparent DE and may be lower than the actual or true DE of the feedstuff (Pagan, 1998). True DE can only be determined if endogenous losses are known, however, most studies do not account for endogenous losses and so report apparent DE (NRC, 2007). Secondly, a feeding trial is the most accurate method for determining DE content of a feed; however, feeding trial data in horses is limited in comparison to other livestock animals. In earlier versions of the NRC (1978), DE values for horse feeds were designated based on information from other species, but more recent insight shows that DE is variable between species due to differences in digestive processes (NRC, 2007). Recognition of the need to develop more equine specific methodologies led the NRC (1989) to adopt an equation developed by Fonnesbeck et al. (1981) which estimates DE based on the chemical composition of the feed.

For dry forages and roughages, pasture, range plants and forages fed fresh:

$$\text{DE (Mcal/kg)} = 4.22 - 0.11 \times (\%\text{ADF}) + 0.0332 \times (\%\text{CP}) + 0.00112 \times (\%\text{ADF}^2);\ R^2 = 0.80$$

Energy feeds and protein supplements:

$$\text{DE (Mcal/kg)} = 4.07 - 0.055 \times (\%\text{ADF})$$

where ADF = acid detergent fiber and CP = crude protein (Fonnesbeck et al., 1981). However, researchers failed to report the composition of the diets that were evaluated, leading to doubts about the versatility and adequacy of the formula. In 1998, a meta-analysis of 30 different studies resulted in the following equation for estimating DE (Pagan, 1998):

$$\text{DE (kcal/kg DM)} = 2{,}118 + 12.18 \times (\%\text{CP}) - 9.37 \times (\%\text{ADF}) - 3.83 \times (\%\text{hemicellulose}) + 47.18 \times (\%\text{fat}) + 20.35 \times (\%\text{non-structural carbohydrate}) - 26.3 \times (\%\text{ash});\ R^2 = 0.88$$

Where hemicellulose = ADF – neutral detergent fiber (NDF) and nonstructural carbohydrate = 100 - %NDF - %Fat - %Ash - %CP. Pagan et al. (1998) reported that the predictions were similar between the equations of Fonnesbeck (1981) and Pagan (1998) for many feeds, but neither was adequate when predicting the DE content of feeds high in fiber or fat. Evaluation of 287 digestion trials led to yet another formula to estimate DE (Zeyner and Kienzle, 2002):

$$\text{DE (MJ/kg DM)} = -3.60 + 0.211 \times \text{CP} + 0.421 \times \text{AEE} + 0.015 \times \text{CF} + 0.189 \times \text{NFE};$$

$$R = 0.626$$

where AEE = acid ether extract; CF = crude fiber; and NFE = nitrogen-free extract. The authors designated a limit of validity for this equation at less than 8% crude fat in DM and less than 35% CF in DM. The Zeyner and Kienzle (2002) equation may be more adequate at predicting DE content of high fat rations, however, the chemical components required by this equation are not as readily available in databases when compared to the NRC (1989) and Pagan (1998) equations (NRC, 2007). The NRC (2007) retained the equations used by the NRC (1989) for concentrates and utilized the Pagan (1998) equation when evaluating forages. The formulas utilized by the current NRC (2007) will be applied to estimate the DE of concentrate and forage fed in the current study.

There are many factors that can influence the DE requirements of horses, such as environmental temperature, body composition, diet composition (Potter, 2004) and exercise (Pagan et al., 1998). Potter et al. (1990) determined that horses exercised in an environment outside of their thermal neutral zone required greater DE to maintain BW and those animals in a fleshy body condition required an even greater increase in DE. Exercising horses were also shown to have a decreased DM digestibility and mean retention time, both resulting in an increase in DE requirements. Further research into the degree to which each of these factors affects DE requirements in the horse is needed and could potentially add precision to prediction equations for energy requirements. According to Potter (2004), accounting for these factors would "move to more of a NE description of energy requirements" for horses.

The NE system has been developed and widely used in the United States for cattle; however, these values were determined through studies of comparative slaughter and calorimetric methods, neither of which are widely acceptable or applicable in equine research (NRC, 2000). Still, a NE system for horses is currently utilized in France which was developed on the basis of long term feeding trials and extrapolations from ruminant data (Martin-Rosset et al., 1994; Cuddeford, 2004). This system reports NE in terms of Unite Fourragere Cheval (UFC) or horse feed unit, which relates the NE value of feeds and NE requirements to the NE value of 1 kg of barley (Martin-Rosset et al., 1994; NRC, 2007). While barley is a widely used and therefore appropriate reference feed in France, it may not be a commonly used feed in other parts of the world, making the French NE system more difficult to apply. Also, the value of the NE system lies in the partitioning of HE losses into activity or production classes, but the French NE system is deficient in this regard. Incomplete information on the efficiency of feeds to meet the requirements for different purposes, such as reproduction or exercise, leaves room for improvement (Cuddeford, 2004; NRC, 2007). The UFC method, with further research, has the potential to improve our understanding of energy partitioning in the horse; however, the DE system continues to be utilized by equine nutritionists in the US due to its simplicity of use, availability of information and wide spread acceptability.

Nutrition Models

Models are numerical representations of complex systems that can be used to help understand how systems function and aid in decision making processes (Tedeschi, 2006). The digestive system is indeed a complex system that has been described by nutritional models in several species (Baldwin et al., 1977; Fox et al., 2004; Tylutki et al., 2008; Cordero et al., 2013). Such nutrition models integrate information from the cellular, tissue, organ, and whole system level into useful predictions about the needs of the whole animal and even the herd, which can aid producers in selecting the most efficient and economic management practices. Two such models have been created, among others, to describe cattle nutritional requirements, the Cornell Net Carbohydrate and Protein System (CNCPS) and the UC Davis Baldwin Molly Model (Molly) (Baldwin et al., 1977; Tylutki et al., 2008). The objective of developing Molly was, "to develop a model of ruminant digestion for use in evaluating the biochemical, microbial, physiological and chemical factors which determine the nutritive value of feeds" (Baldwin et al., 1977). The goal of the authors was to integrate information in a way that would help researchers to identify areas where further investigation is needed, and due to the detailed focus of the model, the inputs required are often unavailable in the field (Tedeschi et al., 2005). Conversely, the CNCPS was developed as a tool for producers, to help balance minimizing cost and nutrient excretion with maximal animal gain (Fox et al., 2004). While these systems have different end goals, they both aim to better explain the digestive processes in cattle; and when research and modeling work in concert it can only lead to improvement of our knowledge of nutrition from the cellular to the whole animal level (Baldwin et al., 1977; Tedeschi et al., 2005).

Unfortunately, modeling techniques have not been extensively employed to understand and quantify nutrition in the horse. Recently, researchers have utilized equations from the Nutrient Requirements of Horses (sixth revised edition; NRC 2007) to develop an equine nutrition model, Fancy (Tylutki, 2011). However, Fancy has not been evaluated for accuracy through implementation. Also, the recommendations made in the NRC (2007), and thus Fancy, are all based on the current BW of the animal; very little information is included on how to alter composition of an animal. Cordero et al. (2013) developed an equine nutrition model, with the goal of quantifying the amount of DE required to alter body condition within a specified time period (Cordero et al., 2013). This model relies on determining the total energy (TE) of the body and then adjusting the TE to achieve BCS gain or loss. Total energy is determined by quantifying BF and body protein (BP) of the animal and then multiplying each by their heat of combustion:

$$TE = 9.367\ BF + 5.554\ BP$$

Cordero et al. (2013) determined %BF by ultrasonic measurement of RF thickness and %BP was estimated to be 21.37% of fat free mass (FFM) based on data from previous studies (Cordero et al., 2013). Data from an earlier study (Cavinder et al., 2009) was utilized to develop a weight adjustment factor (WAF) to predict final BW when the desired change in BCS is achieved:

$$WAF = 1 - 0.038827 * (5 - BCS)$$

$fEBW = (iEBW / iWAF) * fWAF$

where fEBW = final empty body weight (BW*0.96*0.851); iEBW = initial empty body weight; iWAF = initial weight adjustment factor and fWAF = final weight adjustment factor. A predictive equation for final BF was also developed based on the same data set:

$fBF = 1.0656 * fBCS + 6.9844$

where fBCS = final BCS. The DE required to meet these goals was determined by subtracting initial TE from final TE. Energy conversions were utilized as follows:

$ME = TE * 0.6$

$DE = ME * 0.85$

The authors determined that the model was reliable at predicting changes in BW ($r^2 = 0.94$, $p < 0.001$) and BCS ($r^2 = 0.907$, $p < 0.001$) in nonlactating Quarter Horse mares over a 30-d feeding trial. However, the model was not as accurate in predicting changes in %BF ($r^2 = 0.607$) and further research is necessary to identify the relationships between BCS, BF and RF (Cordero et al., 2013). Additionally, the Cordero et al. (2013) model is predictive for sedentary horses while the majority of the horse population is subjected to some form of work, thus limiting the usefulness of the model for producers (American Horse Council, 2005). Still, the model developed by Cordero et al. (2013) provides an important starting point and highlights areas where further research can add to the efficiency and practicality of the model.

Body Composition

The body consists of several distinct components, including muscle, bone and fat. The most variable component of the body is fat while fat free mass remains relatively constant once maturity is reached (Lohman, 1971). Fat is the most abundant storage form of energy in the body and the fatness of an animal has long been considered a reflection of nutritional status and well-being. Correspondingly, research has indicated the importance of body condition in the maintenance of reproductive function (Henneke et al., 1984; Cavinder et al., 2009), athletic ability (Kearns et al., 2002), and overall health (Hoffman et al., 2003; Adams et al., 2009) of the horse. With the knowledge that body composition affects several maintenance and production parameters, comes the need to reliably and consistently measure body condition in the horse. Several methods have been utilized in other species to measure BF including cadaver evaluation, bioelectrical impedance, dual energy X-ray absorption (DEXA), underwater (hydrostatic) weighing, air-displacement, assessment of total body water, body condition scoring and ultrasound. Unfortunately the sheer size and nature of the horse restrict the use of DEXA, hydrostatic weighing, or air-displacement methodologies.

Cadaver dissection is the “gold standard” for assessment of actual body composition. While application of this method is widely utilized for evaluating meat producing animals who are destined for slaughter, it is more limited with regard to horses. First, in most societies horses are not traditionally cultivated as meat producing animals, and so slaughter is not a common practice. Also, many cultures claim a certain emotional attachment between people and horses, making it difficult in some situations to justify sacrificing the animal for research purposes. Also, this is a terminal assessment, so changes in body composition over time cannot be evaluated. Still, valuable information has been derived about the relative composition of the horse from the few studies evaluating equine cadavers. Robb et al. (1972) reported that the %BF of ponies (n=11) ranged from 6.6 – 18.9 in the empty body, while the fat-free empty body was comprised of 70.7% body water (BWa), 22.6% BP and 6.0% body ash (BA). Similarly, gross chemical evaluation of mature pony mare carcasses (n=7) yielded 60.4 ± 3.2% BWa; 18.4 ± 0.9% BP; 15.3 ± 4.1% BF; and 4.6 ± 0.4% BA (Dugdale et al., 2011a). This trend was continued when Gee et al. (2003) assessed young horses and reported that the empty BF ranged from 5.5 to 13%, and the fat-free empty body was made up of 73.2 ± 0.6 BWa; 22.7 ± 0.9 BP and 4.1 ± 0.4 BA. Each of these studies indicate that the most variable component of BW is BF, which is consistent with other species (Lohman, 1971). This variation has spurred researchers to study how differences or changes in BF affect metabolic functions such as reproduction, and exercise; however, in order to make theses assessments a less invasive method for determining body composition must be employed.

One such method is the BCS system. Body condition scoring involves visual appraisal of the “fleshiness” of an animal and application of a numerical score based on that subjective assessment. Henneke developed the most frequently applied BCS method for horses by evaluating Quarter Horse mares (n = 20) of varying condition to determine where fat deposits could most easily be seen and palpated as an estimation of whole BF storage (Henneke et al., 1983). The areas identified were the ribs, behind the shoulder, along the neck, along the withers, crease down the back and the tailhead. A score ranging from 1 being emaciated to 9 being obese is then applied based on the visual and tactile assessment of these 6 body areas. While the Henneke et al. (1983) BCS system is the most widely utilized in the equine industry, it has been criticized for being too subjective in the assignment of scores. This resulted in the modification proposed by Kohnke (1992), who states that appraisers should assign a score from 1 to 9 for each of the 6 body areas evaluated and then those scores should be averaged to get the final BCS. Also, the French National Institute for Agricultural Research (INRA) has developed a BCS scheme with scores ranging from 1 being emaciated to 5 being obese (Martin-Rosset et al., 2008). The Henneke BCS system will be used in the current study due to ease of application and wide spread adoption.

The BCS system is a powerful tool for qualitative assessment of an animal's nutritional status which does not require technology and so it can be utilized in the field. Also, it is simple to understand and apply making it easily implemented in the industry. However, it is limited with respect to quantitative measurements of actual BF. While evidence supports that there is a relationship between BCS and actual BF, it is unclear what that relationship is and how applicable it may be. Several studies have indicated a positive linear correlation between BCS and BF extrapolated from RF thickness measurements (Henneke et al., 1983; Gentry et al., 2004) and from chemical composition analysis (Gee et al., 2003). However, none of these authors provided the regression equation describing the relationship. More recent work suggests that the relationship between BCS and BF is exponential (Martin-Rosset et al., 2008; Dugdale et al., 2011a; Dugdale et al., 2012). Comparison of BCS to actual BF measured via dissection in adult French sport horses (n=20) expressed the following relationship ($r^2 = 0.990$, $P = 0.001$)

$$TFT=5.868e^{0.563*BCS_{1-5}}$$

where TFT = total fat tissues weight in kg (Martin-Rosset et al., 2008). However, it is important to note that these investigators utilized the 1 to 5 BCS scale created by INRA. Dugdale et al. (2012) also reported an exponential relationship between BCS and BF ($r^2 = 0.79$)

$$e^{TBF} = 0.006 + e^{1.56*BCS_{1-9}}$$

where TBF = total body fat and BCS was measured using the 1 to 9 Kohnke BCS system. In this study actual BF was not quantified but instead predicted using the tracer dilution technique. Also, the authors reported that the equation became less accurate at BCS > 6.83, and 2 ponies with low BCS (1.25 and 2.67) were removed from the study based on improbable BF predictions. Taken together, these studies consistently confirm a positive relationship between BCS and BF; however, efforts to pinpoint a predictive equation have yielded contrasting results leaving room for further investigation. Also, BCS is a visual appraisal which excludes evaluation of changes in visceral fat mass. Visceral fat mass has been reported to be the most altered during periods of weight change (Macfarlane et al., 2008); and information again calling into question the appropriateness of quantifying BF using BCS alone.

The most common method for quantitative assessment of BF in the horse is ultrasonic measurement of fat cover. Westervelt et al. (1976) took ultrasonic measurements of fat cover at the rib, shoulder and rump of mature horses (n=8) and compared those measurements to actual body fat determined through chemical carcass analysis (Westervelt et al., 1976). Rump fat was found to be the most highly correlated to whole BF as expressed by the following equation ($r^2 = 0.86$)

$$Y = 8.64 + 4.70X$$

where Y = percent of ether extractable fat and X = cm of rump fat measured 5 cm lateral from the midline at the center of the pelvic bone. This relationship was further evaluated when Kane et al. (1987) reported the following relationship between empty BF and RF measured via ultrasound ($r^2 = 0.90$)

$$Y = 2.47 + 5.47X$$

Where Y = empty body fat and X = cm of rump fat. Similarly, ultrasonically measured retroperitoneal fat depth was correlated to chemically-extracted BF in pony mare cadavers ($n=7$, $r^2 = 0.88$); however, the regression equation was not provided (Dugdale et al., 2011a). Ultrasonic measurements are noninvasive and relatively easy to conduct, leading to widespread application across research disciplines, ages and breeds of horses (Henneke et al., 1984; Kearns et al., 2002; Gee et al., 2003; Fonseca et al., 2013). However, it should be noted that the available studies validating this procedure were restricted to mature horses of undisclosed sex and breed type and mature pony mares. In humans, the sites of fat deposition are significantly affected by gender and genetics of the individual (Leibel et al., 1989). The limited variation in subjects coupled with small sample sizes leads to the question of whether or not this method is accurate for all varieties of horses (Dugdale et al., 2011a).

Another point of variation between these studies is placement of the ultrasound probe. Westervelt et al. (1976) measured 5 cm lateral from the midline at the center of the pelvic bone, while Dugdale et al. (2011) measured "equidistant between the left point of hip and the center of the tail-head root". Furthermore, Kane et al. (1987) measured rump fat at 5 different sites starting 6 cm anterior to the tailhead and continuing anteriorly at 5 cm increments for each of the 5 sites of measurement. Rump fat measured at sites 1 through 4 were reportedly significantly correlated to BF; however regression equations varied greatly for each site (Kane et al., 1987). Also, other researchers reported a difference between rump fat measured on the left versus the right side of the body (Gee et al., 2003). These disparities again call into question the validity of measuring 1 anatomical point to make assumptions about the whole body composition.

The isotope dilution technique offers an alternate quantitative method for measuring BF which takes into consideration the entire body, as opposed to estimation from 1 anatomic measurement point as with ultrasound. There is a strong linear correlation between BWa and BP ($r^2 = 0.996$) such that lean body mass contains 73.2 % of BWa (Pace and Rathburn, 1945). This relationship allows the prediction of BF when BWa is known:

$$\% \text{ fat} = 100 - \% \text{ water} / 0.732$$

Body water can be estimated using the tracer dilution technique. Administration of a biological tracer is followed by an interval of time to allow for dissemination of the tracer throughout the body, typically 3 to 4 hours. A subsequent body fluid sample is collected and evaluated for the concentration of the tracer, and BWa is estimated based on the ratio of tracer to water in the sample (Powers and Howley, 2009).

A number of different tracers have been utilized, including tritium oxide (T_2O), deuterium oxide (D_2O), and urea. Tritium oxide and D_2O are forms of water that contain larger than normal amounts of the hydrogen isotope tritium or deuterium. These isotopes are heavier than the protium isotope commonly found in water, and so D_2O is sometimes referred to as heavy water and T_2O as super-heavy water. Tritiated water methodology holds an advantage in that it is very easily measured via assay; however, its radioactive nature restricts access and limits subjects (Schoeller et al., 1980). Deuterium is much more stable but is not without disadvantage. First, D_2O is an expensive tracer and also measurement requires very sensitive mass spectrometry, making this technique cost prohibitive. A more economical option is urea, which is also relatively easy to quantify; however, the accuracy of using urea to estimate BWa has been called into question. Comparison of estimated BWa using D_2O versus urea to actual BWa determined by cadaver examination in dairy cattle revealed inconsistencies between the methodologies. Estimation of empty BWa using urea space ($r^2 = 0.31$) was less accurate than estimation using D_2O ($r^2 = 0.73$) (Andrew et al., 1995). This discrepancy has also been observed in the horse (Geerken et al., 1988) and has been attributed to endogenous urea content, or metabolism and excretion of the tracer (Schoeller et al., 1980; Andrew et al., 1995). These results indicate that D_2O is the most stable and accurate tracer when measuring BWa, and recent advancements in mass spectrometry have made it possible to administer smaller amounts of the tracer which reduces the cost of the procedure.

The isotope dilution method has been used to estimate BWa and BF in several species including humans, horses and cattle (Sheng and Huggins, 1979; Andrew et al., 1995; Andrews et al., 1997; Dugdale et al., 2011b). The, D_2O administration allowed for sufficient estimation of BWa in horses (n = 6), with estimated values falling within the normal range as determined by whole body evaluation in previous studies (Andrews et al., 1997). The D_2O estimation of BWa and BF was also used as the standard of comparison to support the use bioelectrical impedance analysis in horses (Forro et al., 2000). However, the validation of this method in the horse, by comparison to actual BWa measured postmortem in the same subjects, was conducted relatively recently (Dugdale et al., 2011b). Dugdale et al. (2011) administered D_2O (0.11 – 0.13 g/kg BW) to mature pony mares (n = 7) ranging in BCS from 1.25 to 7 assigned according to the Kohnke BCS procedure. Following a 4 hour equilibration period, a blood sample was taken and plasma was analyzed via gas isotope ratio mass spectrometry to determine D_2O content. The D_2O space was determined by the following equation.

$$D_2O \text{ space (g)} = \frac{[\text{Dose (g)x } P_b \text{ amu}]}{\text{Dose amu}} \text{ x } \frac{(\text{Dose ppm} - P_b \text{ ppm})}{P_e \text{ ppm} - P_b \text{ ppm}}$$

where P_b is baseline plasma, and P_e is equilibrium plasma. Body fat was then determined using the aforementioned equation developed by Pace and Rathburn (1945). Total body water as determined by D_2O dilution was significantly correlated to actual BWa ($r^2 = 0.98$, $p < 0.0001$) and the same association was observed when comparing estimated to actual BF ($r^2 = 0.995$, $p < 0.0001$). The authors concluded that administration of D_2O to estimate BWa and BF was a viable, non-terminal, option to quantify body composition in the horse; however, they also acknowledged that the number of subjects utilized in this study was small. Further studies would be useful to help solidify the validity of this method as the "gold standard" for body composition evaluation in the horse.

In the field, estimation of BF by isotope dilution techniques is unrealistic, also many horse owners or managers do not have access to ultrasound technology for the extrapolation of BF from rump fat thickness. Body condition scoring is an applicable tool; however, the sensitivity and reliability of using BCS to estimate BF has been called into question. The use of morphometric data, perhaps in conjunction with BCS, has been suggested as an alternative, easily applied, estimator. Body measurements have been used successfully to estimate BW of horses. Length and heart girth circumference (n = 372) were positively correlated to BW ($r^2 = 0.90$) resulting in the following predictive equation,

$$\text{BW (kg)} = \frac{\text{girth}^2 \text{ x length (cm)}}{\text{Y}}$$

where Y is a weight adjustment factor specific to age and type of horse (Carroll and Huntington, 1988). Several authors have attempted to quantify a similar relationship between body measurements and BF. Henneke et al. (1983) measured weight, height at the withers, heart girth circumference and estimated BF based on ultrasonically measured rump fat of 32 quarter horse mares. A significant, though weak, correlation was reported between BF and the ratio of weight:height ($r^2 = 0.43$, $p < 0.05$) and heart girth:height ($r^2 = 0.44$, $p < 0.05$) (Henneke et al., 1983). Evaluation of 34 horses of Thoroughbred and Arabian breeding, based on BW, height, length, girth circumference, neck length, neck circumference and BCS yielded a positive relationship between girth:height ratio and BCS ($r2 = 0.64$, $p < 0.001$); however BF was not measured (Carter et al., 2009). Similarly, the following predictive equation was reported when 77 horses and ponies were measured and BF was quantified by administration of D_2O ($r2 = 0.86$),

$$BF = 0.118 + e^{1.22*BCS} + 0.006 * RFT(mm) + 0.007 * height + 0.007 * girth$$

where RFT = rump fat thickness measured by ultrasound (Dugdale et al., 2012). This multivariable model explained more of the variation than did BCS alone ($r^2 = 0.79$); however, it did include RFT which limits the applicability across all field situations. It has also been suggested that a body condition index (BCI) be developed similarly to the body mass index (BMI) used in humans. To this end researchers collected physical measurements on 22 horses ranging in BCS from 4 to 8.5 (Kohnke, 1992) and reported the following relationship of these measurements to BF as estimated by D_2O ($r^2 = 0.745$)

$$BCI = [(HG^{0.5} + BG + NC^{1.2}) / H^{1.05}]^{2.2}$$

where HG = heart girth, BG = belly girth, NC = neck circumference and H = height to the withers (Potter et al., 2015). It is unclear if body length was measured in this study, which may have improved the model. Also, inclusion of BCS in the equation may have explained more of the variability as it was included in the equation proposed by Dugdale et al. (2012). None of the available models have been compared to actual BF but instead have relied on different predictors, RFT and BWa; however, the development of a method to predict BF that is accurate and does not require technology, expense or invasive techniques would hold great utility in the equine industry thus warranting further investigation.

Exercise

The horse is unique among livestock species in that many are athletes, and the energy expended during exercise significantly affects their nutritive requirements. Work requires the movement of limbs carried out by the contraction of muscles which requires energy in the form of ATP. A large proportion of this energy is supplied by the conversion of DE to chemical energy, and so as work requirements increase so do DE requirements. Attempts have been made to develop predictive equations for DE requirements for exercising horses; however there are many factors which influence the intensity of a work bout including speed, weight, environment, gait and BCS (Hoyt and Taylor, 1981; Anderson et al., 1983; Eaton et al., 1995b; Harris, 1997; Jones and Carlson, 2010)**.** The exact relationship between many of these factors to each other and energy requirements is still unclear. In order to investigate these relationships, information about energy expenditure has been gathered in several different ways including feeding trial, assessment of oxygen consumption, and evaluation of HR (Anderson et al., 1983; Pagan and Hintz, 1985; Eaton et al., 1995).

Hintz et al. (1971) evaluated polo horses (n = 9) and horses used in equitation events (n = 7) to determine the amount of DE necessary to maintain BW for each activity. From these data a curve was developed and energy requirements for varying levels of work were estimated and are presented in Table 1.

Table 1. Digestible energy requirements above that of maintenance for various activities (Hintz et al., 1971)

Activity	Kcal of DE/hr/kg mass
Walking	0.5
Light (slow trot, some canter)	5.1
Medium (fast trot, canter, some jumping)	12.5
Heavy (canter, gallop, jump)	24
Strenuous effort	39

These data were presented as feeding recommendations for working horses in the NRC (1978) and the partitioning of activity into light, medium and heavy categories continues to be used in the NRC (2007). However, the authors recognized that the method of data collection, although practical, may not be completely precise or applicable to a wide range of situations (Hintz et al., 1971). Anderson quantified DE required for exercise by feeding horses (n = 4) to maintain BW and BF while being subjected to varying levels of exercise (Anderson et al., 1983). Work was defined by weight over distance (kg-m) and horses were exercised at work intensities of 0.89×10^3, 1.80×10^3 and 3.56×10^3 kg-km on a treadmill at a constant speed of 155m/min. Digestible energy consumed to maintain BW was best represented by a quadratic equation, suggesting a curvilinear relationship between work and DE ($r^2 = 0.926$)

$$DE_{Mcal/d} = 5.97 + 0.021 * Wt_{kg} + 5.036\ X - 0.48\ X^2$$

where X = work in kg/m (Anderson et al., 1983). Work is more objectively defined in this equation in comparison to the subjective assignment of light, medium or heavy utilized by Hintz et al. (1971). However, exercise in this study was achieved on a treadmill and may not be reflective of work performed in natural conditions where terrain and rider can impact energy expenditure (Courouce et al., 2010). Also, speed of travel was held constant between trials while distance was altered to change work intensity. Speed variation is an important attribute in equine competitive events, and significantly contributes to the intensity of an exercise bout thus affecting DE requirements (Eaton et al., 1995b). Feeding trials have provided important information about how the animal is utilizing DE for work; however, a more precise estimation of energy expended during exercise can be achieved by employing calorimetry.

Direct calorimetry involves the quantification of energy released by the body as heat. All cellular processes in the body produce heat and so by quantifying heat released metabolism can be measured (Brooks et al., 1996). While this presents the most precise measure of metabolism, the technology required is advanced, cost prohibitive and unrealistic for obtaining energy expenditure values for the exercising horse. Indirect calorimetry is a viable alternative that is more easily measured by evaluating oxygen consumption (VO_2) (Brooks et al., 1996). The aerobic conversion of dietary energy to chemical energy requires oxygen to act as the terminal electron acceptor at the conclusion of the electron transport chain. For each ml of O_2 consumed 4.8 kcal of energy is produced by the body and so when steady state VO_2 is measured energy expenditure can be calculated (Harris, 1997). Indirect calorimetry has been utilized in several species including humans, cattle and horses (Pagan and Hintz, 1985; Eaton et al., 1995; Graf et al., 2013; Hales et al., 2014). Pagan and Hintz (1986) developed a novel mobile calorimeter that was pulled on a wagon behind a tractor and powered by a diesel generator. Geldings (n = 4) were fitted with a facemask that held connective tubing to the calorimeter and were ponied by another rider around an oval track at various speeds ranging from 40 m/min to 390 m/min (Pagan and Hintz, 1986a). The authors reported that total energy expenditure could best be described by the following equation (r^2 = 0.92)

$$Y = e^{3.02 + 0.0065X}$$

where Y = energy expended (cal/kg/min) and X = speed (m/min). Based on an assumed efficiency of utilization the following equation was suggested for DE requirements above maintenance.

$$\text{DE (kcal/kg/h)} = \frac{e^{3.02+0.0065X} - 13.92}{0.57} \times 0.06$$

Recommendations based on this equation when applied to observed gaits at different speeds are listed in Table 2.

Table 2. Digestible energy requirements above maintenance at various speeds (Pagan and Hintz, 1986a)

Gait	Speed (m/min)	DE/h/kg BW (kcal)
Slow walk	59	1.7
Fast walk	95	2.5
Slow trot	200	6.5
Medium trot	250	9.5
Fast trot/slow canter	300	13.7
Medium canter	350	19.5

These values are similar to DE recommendations proposed by Hintz et al. (1971) with the exception of the slow walk, but Pagan and Hintz expand on previous research by defining the speed and gait at which energy expenditure was observed. Unfortunately this data was not applied in a feeding trial to test DE recommendations and the cumbersome nature of the calorimeter utilized by was restrictive to future studies. Treadmill exercise allows a horse to be stationary while exercising which eliminates the need to move large metabolic measurement systems and several studies have utilized treadmill exercise to evaluate VO_2 in the horse (Knight et al., 1991; Eaton et al., 1995b; Hiraga et al., 1995; Tyler et al., 1996; McCutcheon et al., 1999). Treadmills also provide the researcher with more precise regulation over incline, speed and duration of exercise. While this makes for a more controlled research setting it may not parallel more practical exercise environments. Horses performing a standardized exercise test on the treadmill had a significantly lower HR when compared to the same test performed on the track (Courouce et al., 2010). This discrepancy coupled with the restrictive nature of metabolic measurement devices leads to the quest for a predictor of energy expenditure that can be more easily measured in the field.

Heart rate has been suggested as a predictor of VO_2 (Eaton et al., 1995b; Coenen, 2005). Evaluation of thoroughbred horses (n = 5) exercised on a treadmill at various speeds and inclines revealed a linear relationship between VO_2 and HR which is expressed by the equation below ($r^2 = 0.865$) (Eaton et al., 1995).

$$VO_2 \text{ (ml/kg/min)} = 0.833 * HR - 54.7$$

Similarly Coenen (2005) reported a correlation between VO_2 and HR after evaluation of 87 independent studies ($r^2 = 0.9$) (Coenen, 2005; NRC, 2007).

$$VO_2 \text{ (ml/kg/min)} = 0.0019 * HR^{2.0653}$$

It has been suggested that the equation developed by Eaton et al. (1995) is more accurate at high HR and the Coenen (2005) equation is more precise at lower HR; however the distinction between what is considered high versus low HR was not defined (NRC, 2007). In humans, energy expenditure can also be estimated from HR; however, the development of an individual HR-VO_2 curve gives more precision to the estimation (Ainslie et al., 2003). Development of this curve is achieved by determining the relationship of HR to VO_2 for each individual, which requires the use of indirect calorimetry equipment, thus negating the ease of using HR alone. In the absence of this curve, estimations of energy expenditure from HR can vary up to 30% in individuals (Ainslie et al., 2003). Heart rate measurements on their own may not be a precise measurement of energy expenditure; however, when taken in conjunction with other parameters such as BW, BCS or speed a more precise estimation could possibly be reached. In order to examine these possible correlations a more accurate, in-field indirect calorimetry method must be employed.

Portable metabolic units have been used to measure energy expenditure in humans for several years (Pinnington et al., 2001; Eisenmann et al., 2003; Duffield et al., 2004), and more recently this technology has become available for use in horses (Art et al., 2006; Votion et al., 2006; Lepretre et al., 2009). The Cosmed K4b2 consists of a small light weight box that analyzes VO_2 and VCO_2 on a breath by breath basis and transmits that data via telemetry to a computer for analysis. This system also measures HR, via an on board HR monitor, and speed of travel through an integrated GPS system, as well as tidal volume (VT), respiration rate (RR) and more. The adaptation of this system for use in horses required the development of an equine specific mask, which has a built in hackamore to allow the horse to be ridden during testing. The mask is an open-flow system with 2 turbines on the front of the mask. With each breath the turbines rotate and a sensor reads the rate of rotation to determine respiration rates. Votion et al. (2006) first tested the system using military horses (n=4). Each horse was subjected to 3 treadmill tests and 1 field test while wearing the K4b2 system and results were compared to determine the validity of field testing. The authors reported that values obtained were similar between treadmill and field tests, and within expected normal ranges based on previous research with the exception of VCO_2 which was lower than expected at high RR. The authors speculated that this discrepancy could be due to a slow response time of the analyzer that measures $FETCO_2$ when respiration was elevated (Votion et al., 2006). Art et al. (2006) sought to validate the K4b2 system through measures of repeatability and comparison to a reference method (Art et al., 2006). Saddle horses (n=5) were subjected to incremental treadmill tests to exhaustion while metabolic measurements were obtained alternately between the K4b2 and a reference method consisting of mass spectrometer to analyze expired gasses and ultrasonic pneumotachometers for flow measurements, with 1 week rest periods between methods. The authors reported that the K4b2 is a valid measurement tool for VO_2 as the measurements were not statistically different between the 2 systems. However, like Votion et al. (2006), the current study reported that VCO_2 values were significantly lower when obtained with the K4b2 at higher work intensities. A third study sought to compare the K4b2 with yet another reference method, the Quark laboratory analyzer which is a stationary metabolic measurement system (Lepretre et al., 2009). Seven mature warmblood horses were subjected to standardized incremental treadmill tests and measurements were obtained with both systems alternately. The authors reported no statistically significant differences between the 2 methods for measurements of VO_2, VCO_2 or VT. However, they did state that the respiratory exchange ratio (RER) was underestimated in comparison to previous studies using different measurement systems, which was also reported by Art et al. (2006). This discrepancy was again hypothesized to be linked to the delayed response of the CO_2 electrode. Although there are some questions about the validity of using the K4b2 system for measurement of VCO_2, it has consistently proven reliable for the measurement of VO_2. Also, this is the only portable metabolic unit available for horses which allows for measurement of energy expenditure while being ridden. Based on these merits, the Cosmed K4b2 system will be utilized in the current study to measure energy expenditure of horses during exercise.

CHAPTER III

MATERIALS AND METHODS

Model Modifications

The model developed by Cordero et al. (2013) was altered to include energy expenditure of work equations developed by Hintz et al. (1971) as follows:

$DE_{exlight}$ (Mcal/hr) = $0.0051*BW$

$DE_{exmoderate}$ (Mcal/hr) = $0.0125*BW$

$DE_{exheavy}$ (Mcal/hr) = $0.024*BW$

$DE_{exveryheavy}$ (Mcal/hr) = $0.039*BW$

Additionally, the Cordero model was only equipped to calculate the change in DE required to alter BCS, it did not take into account whether or not the horse in question was already on an increasing or decreasing plane of nutrition. In order to address this issue, the model was modified to include maintenance DE requirement (NRC, 1978).

DE_m (kcal) = $155*BW^{0.75}$

The final recommendation of the model was then calculated as follows,

DE_{adj} (Mcal/d) = $DE_m + DE_{ex} – DE_i + \Delta DE$

where DE_{adj} is the adjustment required to meet the desired BCS, DE_m is the maintenance DE requirement, DE_{ex} is the DE required to meet the demands of exercise, DE_i is the initial DE intake and ΔDE is the change in DE required to alter BCS as calculated by the original Cordero model (Cordero et al., 2013).

Model Application

Mature stock type horses (n = 24; 3 to 16 yr of age; mean = 8), with initial BW ranging from 400 kg to 569 kg (mean = 488 kg) and initial BCS of 3 to 6.5 (scale of 1 – 9; 1 = emaciated; 9 = obese; 5 = moderate) were used in this study. Horses belonged to Texas A&M University or were leased from local owners and were individually housed in 3.6 x 4.3 m stalls at the Texas A&M Equestrian Center. Individual housing was necessary to insure precise management of dietary intake; however, horses were rotated through individual turn out for 2 d each wk. Horses were acclimated to diet and exercise regimens over a 3 wk period. All animals were treated with a broad-spectrum dewormer during the first wk of the acclimation period (450 kg dose; Equimectrin Paste, 1.87% Ivermectin equine dewormer in oral syringe). Use of animals was approved by the Texas A&M University Institutional Agricultural Animal Care and Use Committee using guidelines set forth by the Federation of Animal Science Societies (2014).

Physical Measurements

Pre-trial measurements of BCS, BW and RF thickness were obtained to assign animals to treatment groups, and were reevaluated every two wk throughout the 60 d trial. Body condition scores were evaluated by 3 independent, experienced individuals and these scores were averaged to determine the final BCS. Scores were assigned based on the 1 to 9 BCS system developed by Henneke et al. (1983) where 1 is emaciated, 5 is moderate, and 9 is obese. Assessment included visual and tactile appraisal of the neck, withers, shoulders, ribs, loin, and tailhead to determine the amount of fat present. Appendix Table 1 outlines the criteria used for score assignment.

Body weight was obtained by leading each horse individually to a livestock weighbridge scale (Paul Livestock Scale, Adrian J. Paul Co., Inc., Duncan, OK). Rump fat thickness was assessed via ultrasonic scanning equipment with a 5MHz transducer (MicroMaxx Ultrasound System, SonoSite, Inc., Bothell, WA). An approximately 5cm x 7cm section of the horses hip, measured 5cm from the midline at the center of the pelvic bone, was shaved to allow better conduction for the transducer and also to insure consistent sampling location across all dates. Body fat percentage was then calculated using the equation developed by Westervelt et al. (1976). The following physical measurements were taken at d 0 and d 60 to compare to measurements of BF; height measured from the ground to the tallest point of the withers, heart girth circumference, length from the point of the shoulder to the middle of the buttock, length from middle of the chest to the middle of the buttock, and neck circumference at the base of the neck, middle of the neck and at the throatlatch.

Treatments

Pre-treatment BCS were used to assign animals to 1 of 6 treatment groups as follows:

Table 3. Initial BCS groups for horses fed to achieve a targeted body condition

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
No Exercise	No Exercise	Light Exercise	Light Exercise	Heavy Exercise	Heavy Exercise
Fed to increase by 2 BCS	Fed to decrease by 2 BCS	Fed to increase by 2 BCS	Fed to decrease by 2 BCS	Fed to increase by 2 BCS	Fed to decrease by 2 BCS
N = 4	N = 4	N = 4	N = 4	N = 4	N = 4

Exercise was conducted under saddle at the Texas A&M Equestrian Center. Protocols were designed to mimic the descriptions of workloads outlined in the NRC (2007).

Table 4. Exercise protocol utilized in the current study

	Light	Heavy
Walk (min)	18	12
Sitting trot (min)	11	14
Extended trot (min)	12	16
Lope (min)	4	9
Extended Lope (min)	0	9
Total Duration (min)	45	60
No. of rides per wk	3	4

Horses for each protocol were ridden simultaneously to keep environmental factors the same across all horses in the group. During each exercise bout, 1 horse in the group was fitted with a portable metabolic measurement device (Cosmed K4b2) to record VO_2, VCO_2, HR, temperature, distance traveled, and speed of travel. This system consists of a mask fitted with a mechanical hackamore to allow simultaneous sampling of respiratory gasses and riding. Horses were acclimated to the mask prior to the start of the trial and each horse wore the mask for metabolic assessment during 4 different exercise bouts throughout the 60 d trial. The K4b2 was calibrated according to the manufacturer's instructions prior to each measurement period. The O_2 and CO_2 sensors were calibrated with ambient air containing 20.9% O_2 and 0.03%CO_2, and also with calibration gas containing 16%O_2 and 5%CO_2. The flow meter was calibrated with a 3L syringe. Humidity was recorded from a portable weather station (AcuRite®). Rider and tack weights were recorded.

Diet

Diet consisted of forage and pelleted concentrate. Forage offered was Coastal Bermuda grass hay (89.9% DM). The concentrate fed was a 13% crude protein pelleted feed (Brazos County Producer's Co-Operative Association, Bryan, Texas). Samples were obtained by random core sampling of forage, and random grab samples of concentrate. All samples were submitted to a commercial laboratory for analysis which is outlined in Appendix Tables 2 and 3 (Dairy One, Ithaca, New York).

Initial BCS, BW and %BF data for each horse was put into the proposed model along with the desired BCS after a 60 d period. The diet for each horse was manipulated to meet the model recommendations. Animals were fed 1% BW in forage per day, pelleted feed was fed to meet the gap between the DE available in forage and the DE recommended by the model. There were 5 horses, all being exercised and expected to increase in BCS, for whom the model recommended DE could not safely be met with forage and pelleted feed alone. These horses were fed corn oil in addition to forage and grain to meet the DE requirement. Horses were fed individually in stalls equipped with hay and grain combo stall feeders. Feedings were twice/d spaced 12 h apart. Clean water was offered *ad libitum*. Refusals were collected, weighed and recorded after each feeding. At the conclusion of the 60 d period, final BCS, BW and %BF were recorded.

Statistical Analysis

The model was evaluated for precision and accuracy using the Model Evaluation System (MES) developed by Tedeschi (2006). Accuracy can be defined as how closely the model predicted values are to the true observed values, while precision evaluates how closely individual model predicted values are within each other (Tedeschi, 2006). The coefficient of determination (r^2) was evaluated as a good indicator of the precision of the model. Mean bias (MB) represents the mean difference between observed and predicted values, and is an indicator of model accuracy (Tedeschi, 2006). This statistic also provides information about model over or under prediction, with a positive MB indicating model under prediction and a negative MB signifying model over prediction. Modeling efficiency (MEF) offers a measure of goodness of fit by quantifying the proportion of variation between the observed values and model-predicted values explained by the linear regression. The closer the MEF is to 1, the better the fit (Loague and Green, 1991; Mayer and Butler, 1993). The coefficient of model determination (CD) is another measure of model predictability, where the closer to 1 the greater the model predictability. This statistic evaluates the proportion of the total variance of the observed values explained by the predicted values (Loague and Green, 1991). The mean square error of prediction (MSEP) is a reliable and common estimate of the accuracy of a model, with a low MSEP denoting greater accuracy (Bibby and Toutenburg, 1977). Mean absolute error (MAE) is the deviance between observed and predicted values, and the lower the MAE the more accurate the model is (Mayer and Butler, 1993). Regression analysis was conducted using SAS to evaluate the validity of using body parameters to estimate BF and also to evaluate estimators of VO_2 (SAS, INC., 2014). SAS was also utilized to conduct analysis of variance (ANOVA) in order to compare methods of determining %BF.

CHAPTER IV

RESULTS

Table 5 below illustrates how animals were assigned to the 6 treatment groups based on their initial BCS upon arrival at the research housing facility. Condition scores within each group were not uniform, with the exception of group 2; however, each animal was individually fed according to the model in order to achieve a change in 2 BCS units over a 60 d period.

Table 5. Assignment of horses to treatment groups based on initial body condition scores per animal.

Treatment Groups											
Group 1 No Exercise		Group 2 No Exercise		Group 3 Light Exercise		Group 4 Light Exercise		Group 5 Heavy Exercise		Group 6 Heavy Exercise	
Horse ID	$BCS_{initial-desired}$	Horse ID	$BCS_{initial-desired}$	Horse ID	$BCS_{initial-desired}$	Horse ID	$BCS_{initial-desired}$	Horse ID	$BCS_{initial-desired}$	Horse ID	$BCS_{initial-desired}$
407	5 → 7	6	5.5 → 3.5	128	4.5 → 6.5	11	5 → 3	36	4 → 6	5	6 → 4
410	4 → 6	116	5.5 → 3.5	137	3 → 5	106	6 → 4	107	4 → 6	104	6.5 → 4.5
412	4 → 6	409	5.5 → 3.5	503	4 → 6	138	6.5 → 4.5	108	4 → 6	120	5.5 → 3.5
420	3.5 → 5.5	508	5.5 → 3.5	504	3.5 → 5.5	418	5.5 → 3.5	112	4.5 → 6.5	405	6 → 4

Table 6 lists the model predicted change in DE required for each horse along with the initial and final DE intake. Initial values were calculated to maintain current BW according to the NRC recommendations and animals were fed initial DE for 3 wk prior to the start of the study (NRC, 1978). Horses were very lightly exercised under saddle on 3 different days prior to the start of the trial in order to familiarize them to the mask used for analysis of respiratory gasses.

Table 6. Digestible energy intake changes per horse as predicted by model

Horse ID	BCS Initial - desired	Initial DE Intake (Mcal/d)	Proposed DE Change (Mcal/d)	Proposed Total DE Intake (Mcal/d)
Group 1				
407	5-7	15.20	6.23	21.43
410	4-6	15.59	4.91	20.49
412	4-6	15.97	4.96	20.93
420	3.5-5.5	17.03	7.60	24.63
Group 2				
6	5.5-3.5	16.45	-3.15	13.30
116	5.5-3.5	14.90	-5.14	9.76
409	5.5-3.5	15.70	-3.30	12.40
508	5.5-3.5	16.05	-3.23	12.82
Group 3				
128	4.5-6.5	14.67	6.85	21.52
137	3-5	13.87	6.18	20.05
503	4-6	16.32	8.33	24.65
504	3.5-5.5	17.36	8.75	26.11
Group 4				
11	5-3	16.35	-2.36	13.99
106	6-4	15.66	-4.01	11.64
138	6.5-4.5	18.06	-4.24	13.82
418	5.5-3.5	17.87	-4.60	13.27
Group 5				
36	4-6	15.69	13.39	29.08
107	4-6	14.96	11.67	26.63
108	4-6	16.44	14.03	30.48
112	4.5-6.5	15.54	14.22	29.76
Group 6				
5	6-4	14.49	2.28	16.77
104	6.5-4.5	17.58	2.94	20.52
120	5.5-3.5	17.95	2.71	20.66
405	6-4	16.45	2.89	19.35

Model Evaluations

Evaluating BCS

Body condition scores were assigned by 3 independent assessors and then averaged for a mean BCS. Values were rounded to the nearest 0.5 BCS. Final observed BCS for each horse was compared to the model predicted final BCS value using the Model Evaluation System. The coefficient of determination (r^2) is a measure of how well the observed values fit the line represented by the predicted values, or the proportion of variation in observed values that can be explained by the model. All animals ($n = 24$) were included in the evaluation of BCS predictability by the model, resulting in an r^2 value of 0.37316 ($P = 0.00152$) with a maximum error (ME) of 2 BCS units. Meaning that, when all animals are included, the model accounted for 37.3% of the variation in observed BCS values and at least 1 horse did not change in BCS for the duration of the study. The mean bias (MB) was -0.08333 BCS units indicating that the model over predicted DE requirements. Mean absolute error (MAE) was 0.83333 BCS units, modeling efficiency (MEF) was -0.73633, coefficient of model determination (CD) was 0.36205 and the mean square error of prediction (MSEP) was 0.9375 BCS units (Table 7).

Table 7. Model Evaluation System (MES) statistical results for BCS prediction when all horses are included in the model evaluation

Coefficient of determination (r^2)	0.37
Maximum error (ME)	2 BCS units
Mean bias (MB)	-0.08 BCS units (model over-prediction)
Mean absolute error (MAE)	0.83 BCS units
Modeling efficiency (MEF)	-0.74
Coefficient of model determination (CD)	0.36
Mean square error of prediction (MSEP)	0.94 BCS units

Figure 1 below illustrates the observed and predicted BCS values in a scatterplot. Values above the Y = X line represent model under-predictions while values below the Y = X line represent model over-predictions.

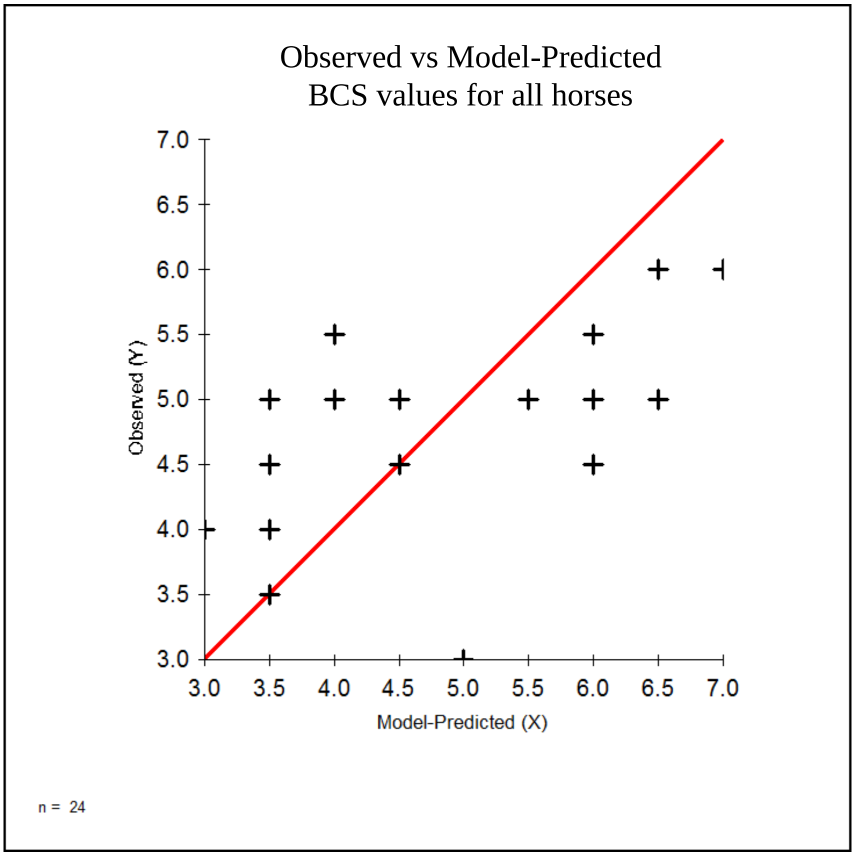

Figure 1. Scatterplot of observed vs model-predicted BCS values. Values above the line represent model under predictions, while values below the line represent model over predictions.

Evaluation of the data revealed that 1 horse did not change BCS at all during the 60d period. This horse was a 3 yr old gelding, the youngest horse included, and was most likely using energy for growth, which is not accounted for in the current model. Removal of this horse from analysis revealed higher model precision (r2 = 0.52 vs 0.37) and greater model accuracy (MSEP = 0.80 vs 0.94), this horse was subsequently removed from all further analysis, results listed in Table 8.

Table 8. Model Evaluation System (MES) statistical results for BCS prediction when Horse 137 is removed from analysis

Coefficient of determination (r^2)	0.52
Maximum error (ME)	1.5 BCS units
Mean bias (MB)	-0.00 BCS units (model over-prediction)
Mean absolute error (MAE)	0.78 BCS units
Modeling efficiency (MEF)	-0.92
Coefficient of model determination (CD)	0.27
Mean square error of prediction (MSEP)	0.80 BCS units

In order to evaluate the predictability of the model for exercised versus non exercised horses the observed and predicted BCS values were organized into 3 distinct data sets. Groups 1 and 2 were combined into one data set (n = 8) which represents the non-exercised control horses. Statistical analysis resulted in an r^2 value of 0.9084 (P = 0.00025) with a ME of 1 BCS unit. Groups 3 and 4 were combined into a data set (n = 8) of lightly exercised horses, resulting in an r^2 value of 0.29313 (P = 0.16578) with a ME of 2 BCS units. Groups 5 and 6 were combined (n = 8) to represent heavily exercised horses, resulting in an r^2 value of 0.03623 (P = 0.65165) with a ME of 1.5. Complete statistical results for non-exercise, light exercise and heavy exercise data sets are outlined below in Table 9.

Table 9. Model Evaluation System (MES) statistical results for BCS prediction for groups 1 and 2 (no exercise), groups 3 and 4 (light exercise) and groups 5 and 6 (heavy exercise)

	Groups 1 and 2 No Exercise	Groups 3 and 4 Light Exercise	Groups 5 and 6 Heavy Exercise
Coefficient of determination (r^2)	0.91	0.57	0.04
Maximum error (ME)	1 BCS units	1.5 BCS units	1.5 BCS units
Mean bias (MB)	-0.06 BCS units (model over-prediction)	-0.07 BCS units (model over-prediction)	0.13 BCS units (model under-prediction)
Mean absolute error (MAE)	0.69 BCS units	0.64 BCS units	1 BCS units
Modeling efficiency (MEF)	-0.06	-0.23	-19.27
Coefficient of model determination (CD)	0.27	0.37	0.048
Mean square error of prediction (MSEP)	0.53 BCS units	0.68 BCS units	1.19 BCS units

Horses were also divided into two distinct data sets to analyze the predictability of the model for increasing BCS versus decreasing BCS. Groups 2, 4 and 6 were combined (n = 12) to examine decreasing BCS resulting in an r^2 value of 0.34028 (P = 0.04648) and a ME of 1.5 BCS units (Table 9). Groups 3, 5 and 7 were combined (n =

12) to evaluate increasing BCS resulting in an r^2 of 0.59036 (P = 0.00351) and a ME of 2 BCS units (Table 10).

Table 10. Model Evaluation System (MES) statistical results for BCS prediction for groups 2, 4 and 6 (decreasing BCS) and groups 1, 3, and 5 (increasing BCS)

	Groups 2, 4 and 6 Decreasing BCS	Groups 1, 3 and 5 Increasing BCS
Coefficient of determination (r^2)	0.34	0.59
Maximum error (ME)	1.5 BCS units	2 BCS units
Mean bias (MB)	0.75 BCS units (model under-prediction)	-0.92 BCS units (model over-prediction)
Mean absolute error (MAE)	0.75 BCS units	0.92 BCS units
Modeling efficiency (MEF)	-1.38	-0.88
Coefficient of model determination (CD)	0.44	0.53
Mean square error of prediction (MSEP)	0.79 BCS units	1.08 BCS units

Evaluating BF

Final observed and model predicted values were compared to test the certainty of the model with regards to BF. When all values (n = 23) were included the r^2 = 0.36572

(P = 0.00380). Data were also evaluated for groups subjected to no exercise (n = 8; r^2 = 0.50548; P = 0.04803), light exercise (n = 7; r^2 = 0.74472; P = 0.03015) and heavy exercise (n = 8; r^2 = 0.12638; P = 0.38746). Further statistics are outlined below in Tables 11 and 12. Figure 2 illustrates the final observed BF values versus the model predicted values represented by the Y = X line for all values.

Table 11. Model Evaluation System (MES) statistical results for BF prediction when all horses are included in the model evaluation

Coefficient of determination (r^2)	0.37
Maximum error (ME)	3.39
Mean bias (MB)	-0.90
Mean absolute error (MAE)	1.16
Modeling efficiency (MEF)	-0.79
Coefficient of model determination (CD)	0.42
Mean square error of prediction (MSEP)	2.25

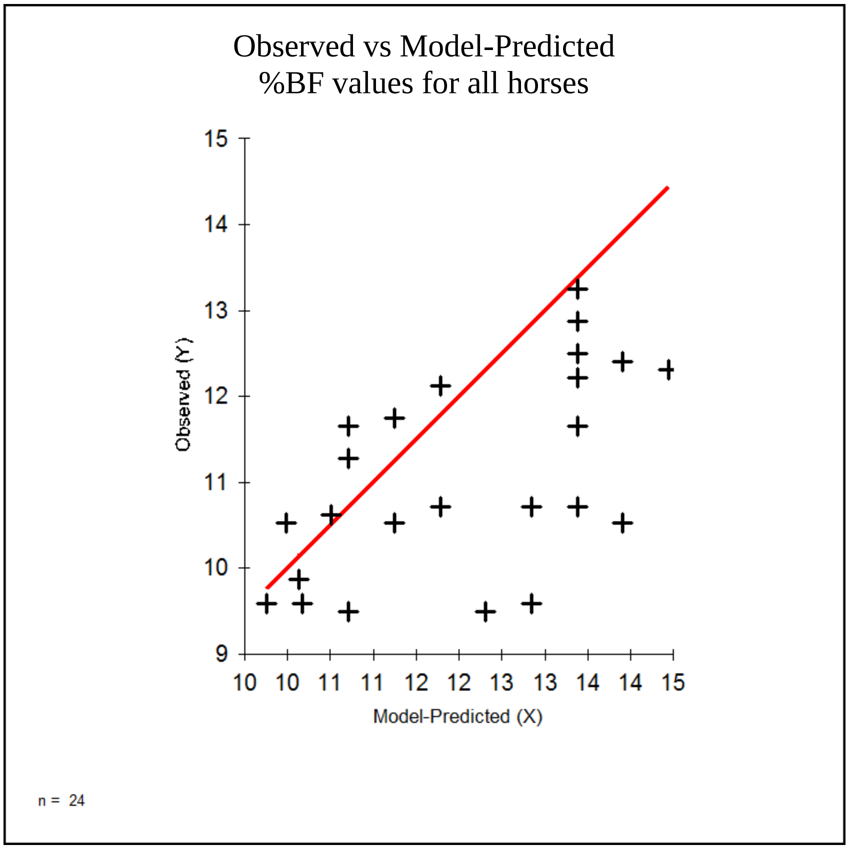

Figure 2. Scatterplot of observed vs model-predicted %BF values. Values above the line represent model under predictions, while values below the line represent model over predictions.

Table 12. Model Evaluation System (MES) statistical results for %BF prediction for groups 1 and 2 (no exercise), groups 3 and 4 (light exercise) and groups 5 and 6 (heavy exercise)

	Groups 1 and 2 No Exercise	Groups 3 and 4 Light Exercise	Groups 5 and 6 Heavy Exercise
Coefficient of determination (r^2)	0.51	0.74	0.13
Maximum error (ME)	3.27	2.67	3.39
Mean bias (MB)	-0.91	-1.42	-0.43
Mean absolute error (MAE)	1.07	1.42	1.01
Modeling efficiency (MEF)	-0.63	-2.16	-1.45
Coefficient of model determination (CD)	0.37	0.21	0.40
Mean square error of prediction (MSEP)	2.23	2.51	2.04

Evaluating BW

Observed final and model predicted values were compared in order to test the certainty of the model with regards to BW. When all values (n = 23) were included the r^2 = 0.90604 (P = 0.00001) (Table 13). Data were also evaluated for groups subjected to no exercise (n = 8; r^2 = 0.98466; P = 0.00001), light exercise (n = 7; r^2 = 0.87895; P = 0.00107) and heavy exercise (n = 8; r^2 = 0.83772; P = 0.00143) (Table 14). Figure 3 illustrates the final observed BW values versus the model predicted values represented by the Y = X line for all values.

Table 13. Model Evaluation System (MES) statistical results for BW prediction when all horses are included in the model evaluation.

Coefficient of determination (r^2)	0.91
Maximum error (ME)	31.91
Mean bias (MB)	4.52
Mean absolute error (MAE)	14.13
Modeling efficiency (MEF)	0.81
Coefficient of model determination (CD)	0.65
Mean square error of prediction (MSEP)	281.99

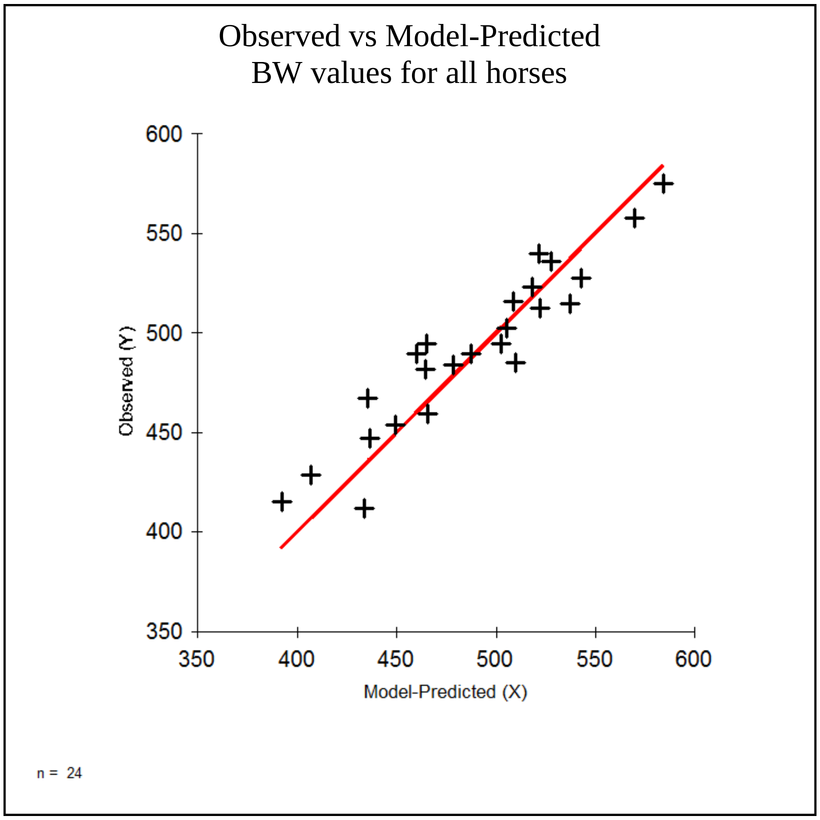

Figure 3. Scatterplot of observed vs model-predicted BW values. Values above the line represent model under predictions, while values below the line represent model over predictions.

Table 14. Model Evaluation System (MES) statistical results for BW prediction for groups 1 and 2 (no exercise) groups 3 and 4 (light exercise) and groups 5 and 6 (heavy exercise.

	Groups 1 and 2 No Exercise	Groups 3 and 4 Light Exercise	Groups 5 and 6 Heavy Exercise
Coefficient of determination (r^2)	0.98	0.88	0.84
Maximum error (ME)	21.68	31.91	29.32
Mean bias (MB)	4.05	5.24	4.37
Mean absolute error (MAE)	10.13	16.07	16.43
Modeling efficiency (MEF)	0.90	0.74	0.73
Coefficient of model determination (CD)	0.63	0.60	0.66
Mean square error of prediction (MSEP)	144.22	373.88	339.35

Evaluation of Energy Expenditure

Exercise Effect

Descriptive statistics for exercise parameters are listed below in Table 15. Oxygen consumption was determined to be significantly different between heavy and light exercise groups by mixed model analysis ($P < 0.0001$).

Table 15. Exercise parameter descriptive statistics for light and heavy exercise groups.

	Heavy Exercise			Light Exercise		
Descriptive Statistic	HR, bpm	VO_2, L/min	VCO_2, L/min	HR, bpm	VO_2, L/min	VCO_2, L/min
Mean	124.09	8.83	5.45	109.14	3.05	1.98
Std Dev	23.32	2.81	1.93	18.55	1.66	1.11
Minimum	91.76	3.85	2.46	88.15	1.34	0.92
Maximum	185.83	15.22	10.16	157.70	8.75	6.40
n	32	32	32	30	30	30

Mixed model analysis, assigning horse as the random variable, was applied to determine what relationships exist between VO_2 and measured variables. BW, age*exercise and heat index (HI)*exercise were determined to be significant contributors to oxygen consumption ($P \leq 0.05$) (Table 16).

Table 16. Statistical results of mixed model analysis of VO_2 correlation to exercise variables

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
exercise	1	34	0.07	0.7942
BW	1	34	6.68	0.0142
BW*exercise	1	34	0.01	0.9437
HI	1	34	1.29	0.2637
HI*exercise	1	34	3.79	0.0599
age	1	34	2.91	0.0972
age*exercise	1	34	3.94	0.0553
FFM	1	34	2.75	0.1062
FFM*exercise	1	34	0.16	0.6886
%BF	1	34	0.29	0.5924
%BF*exercise	1	34	0.02	0.8977
HR	1	34	2.01	0.1655
HR*exercise	1	34	0.26	0.6124
BCS	1	34	0.16	0.6881
BCS*exercise	1	34	0.18	0.6737

Several tests of mixed model analysis were conducted based on these significant variables and the following predictive equations were developed from the fixed effects of each model. Each equation is outlined in Table 17 and Figures 4-7 depict the relationship between observed and predicted values for each equation. Previous predictive equations for energy expenditure in horses relied heavily on HR and BW as predictors (Eaton et al., 1995b; Coenen, 2005). Models 2, 3 and 4 were developed to test the relationship between these variables and VO_2 based on the current data.

Table 17. Proposed VO_2 predictive equations based on statistical analysis of the current data.

Model No		τ^2		Fit Statistics			
	Equation	Residual	Horse	AIC	-2 log	R^2	Pr > F
1	VO_2 (L/min) = $(-5.3664 + a_1) + (-0.00606 + b_1)*HI + (-0.1358 + c_1)*age + 0.01739*BW$	1.21	2.00	240.2	236.2	0.77	<0.0001
2	VO_2 (ml/min/kgBW) = $(4.8590 + a_2) + (0.01146 + b_2)*HR$	8.47	8.76	340.3	336.3	0.72	<0.0001
3	VO_2 (ml/min/kgBW) = $3.4969 + 0.07279*HR$	9.14	39.23	364.1	360.1	0.26	<0.0001
4	VO_2 (ml/min/kgBW$^{0.75}$) = $(22.9190 + a_4) + (0.05348 + b_4)*HR$	185.86	199.61	519.9	515.9	0.71	<0.0001

For light exercise parameters are zero, otherwise
$a_1 = -5.0799$; $b_1 = 0.05972$; $c_1 = 0.7046$
$a_2 = -0.9556$; $b_2 = 0.1009$
$a_4 = -3.8202$; $b_4 = 0.4706$

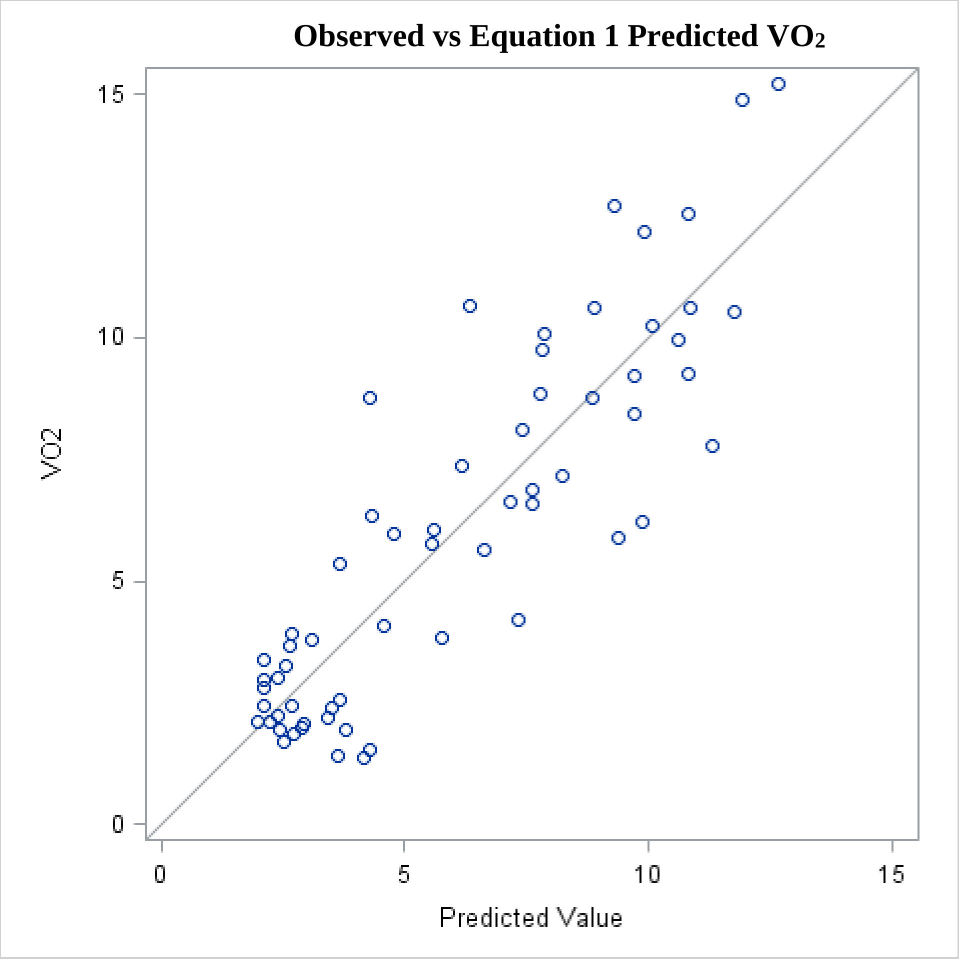

Figure 4. Scatterplot of observed vs Equation 1-predicted VO_2 (L/min) values. Values above the line represent model under predictions, while values below the line represent model over predictions.

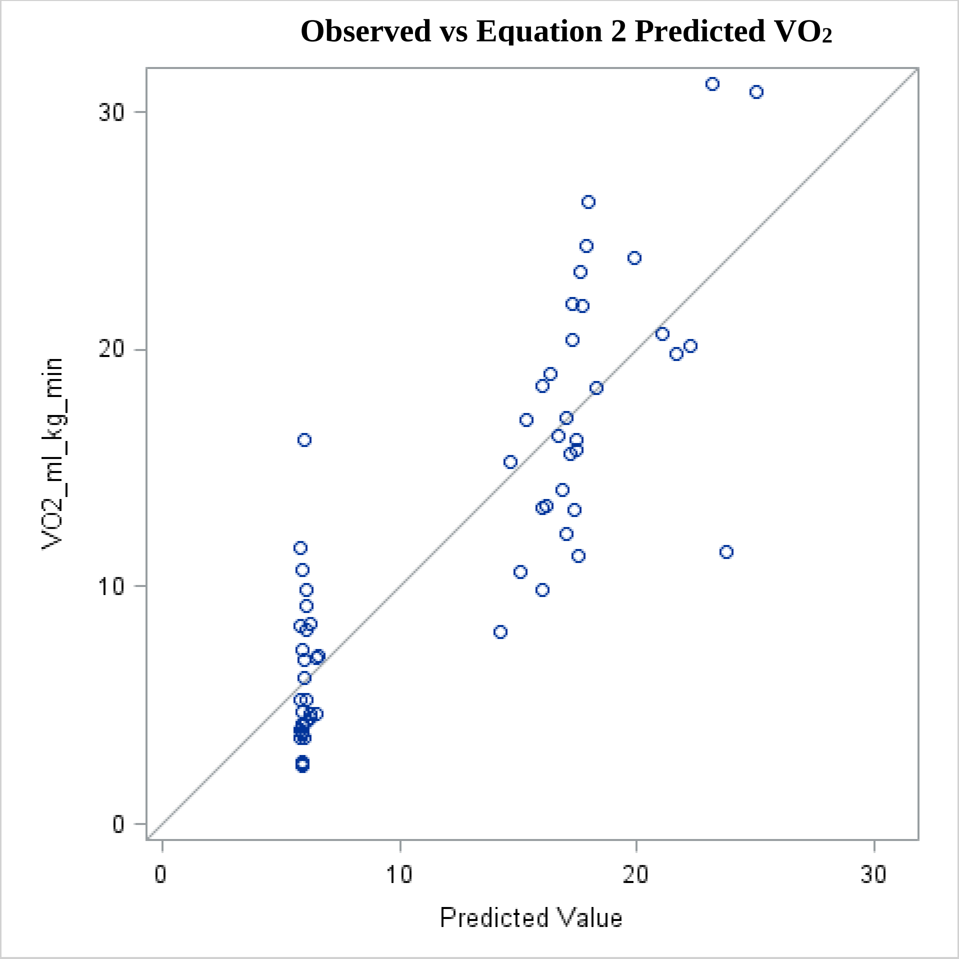

Figure 5. Scatterplot of observed vs Equation 2-predicted VO_2 (ml/min/kgBW) values. Values above the line represent model under predictions, while values below the line represent model over predictions.

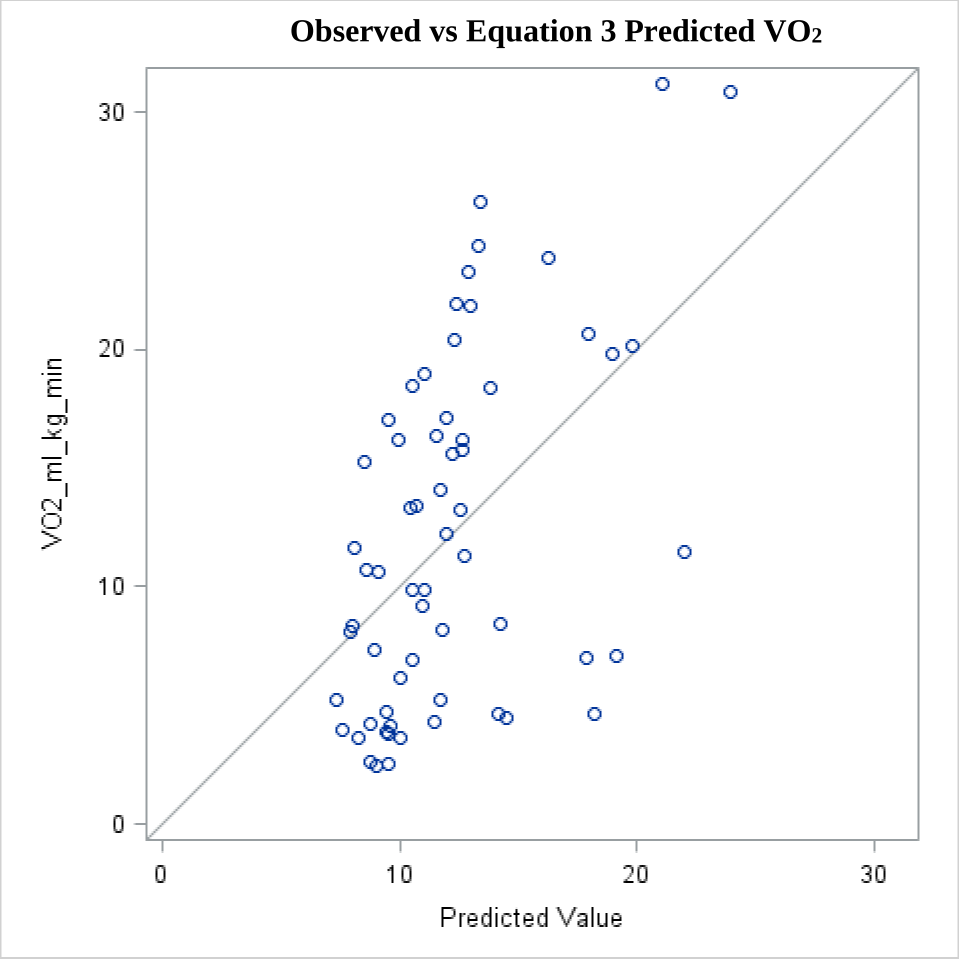

Figure 6. Scatterplot of observed vs Equation 3-predicted VO_2 (ml/min/kgBW) values. Values above the line represent model under predictions, while values below the line represent model over predictions.

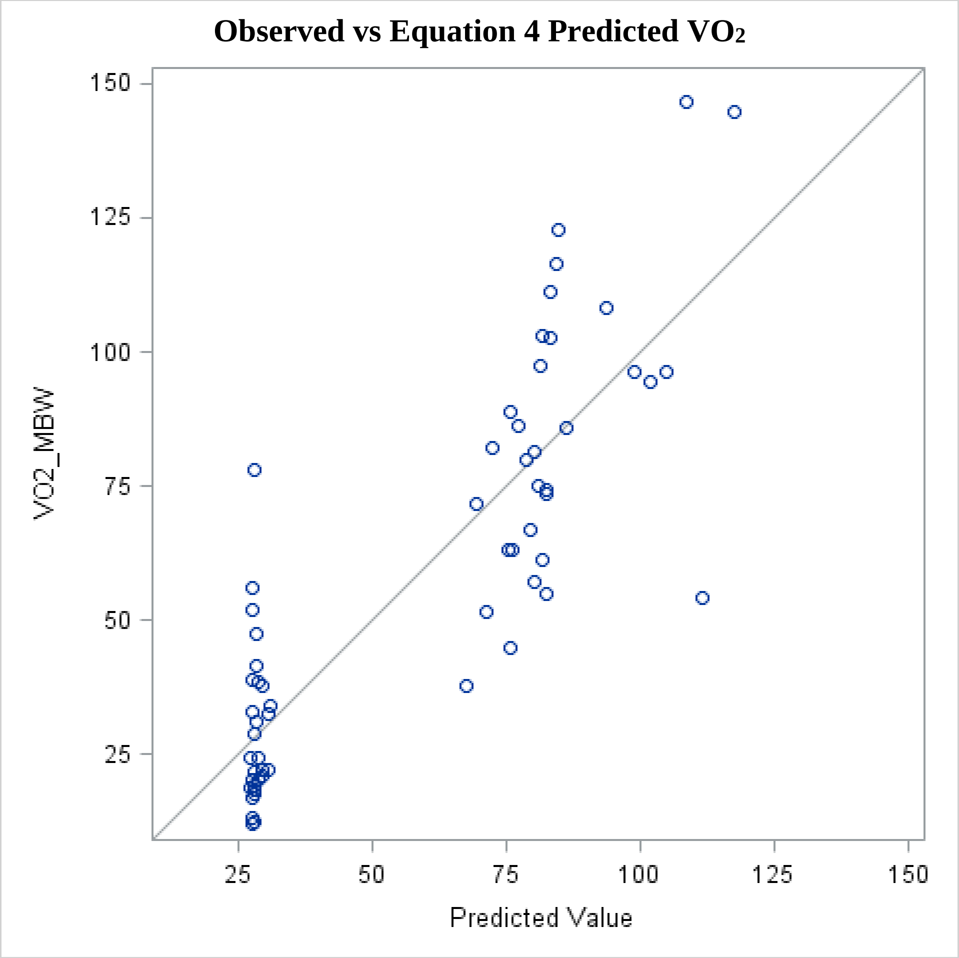

Figure 7. Scatterplot of observed vs Equation 4-predicted VO_2 (ml/min/kgBW$^{0.75}$) values. Values above the line represent model under predictions, while values below the line represent model over predictions.

Comparison of Equations

The observed VO_2 values were converted to energy using the following equation (Harris, 1997):

NE (Mcal/hr) = VO_2 (L/min) * 4.8 (kcal/L) * 60 (min/hr) / 1000

The efficiency of conversion of DE to NE for exercise based on previous research can range from 20 to 50% (NRC, 2007). The NRC (2007) estimated that the efficiency of conversion for strenuous activity is 30% versus 40% for animals undergoing a mild or moderate exercise regimen. Based on the NRC estimation, observed NE was converted to DE as follows:

DE (Mcal/hr) = NE (Mcal/hr) / 0.4

Values were converted to DE requirement per day and compared to the predicted requirement based on equations 2 & 3 developed from the current data, the original equation as applied to the model which was based on data from Hintz et al. (1971), and the NRC (2007) recommendation. Analysis was conducted using one way ANOVA followed by Fishers LSD. The results are listed in Table 18.

Table 18. Comparison of observed energy expenditure to equations predicting DE requirement for lightly and heavily exercised horses

	Mean DE (Mcal/d) Light Exercise	Mean DE (Mcal/d) Heavy Exercise
Observed[1]	0.71^a	3.63^a
Hintz[2]	0.81^a	6.79^b
NRC[3]	3.29^b	9.90^c
Equation 1[4]	0.76^a	4.07^a
Equation 2[4]	0.70^a	3.63^a

^{abc}Values with differing superscripts are significantly different ($P < 0.05$)

[1] Observed VO_2 values converted to estimated DE

[2] Hintz (1971) equations for energy expenditure

[3] NRC (2007) equations for energy expenditure

[4] Equations developed from current data

Body Composition

Mixed model analysis with horse assigned as the random variable was conducted to determine what relationships exist between %BF estimated from RFT and body parameter measurements. The measures of BW, body length from point of shoulder to point of buttock, average neck circumference and BCS were significant contributors to %BF ($P \leq 0.1$). There was also a significant effect of exercise on the relationship of body length and BCS to %BF ($P \leq 0.05$) (Table 19).

Table 19. Mixed model analysis of %BF correlation to body parameters

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Exercise	2	16	10.62	0.0012
BW	1	16	3.08	0.0981
Length	1	16	2.43	0.1386
Length*exercise	2	16	11.54	0.0008
Neck average	1	16	9.05	0.0083
BCS	1	16	71.85	<0.0001
BCS*exercise	2	16	9.23	0.0022

Several tests of mixed model analysis were conducted based on these significant variables and the following predictive equations were developed from the fixed effects of each model. Each equation is outlined in Table 20 and Figures 20 - 22 depict the relationship between observed and predicted values for each equation.

Table 20. Proposed predictive equations for %BF based on the current data

Model No	Equation	τ^2		Fit Statistics			
		Residual	Horse	AIC	-2 log	R^2	Pr > F
1	$\%BF = (5.7910 + a_1) + -0.00827*BW + (-0.01478 + b_1)*length + 0.09428*neck + (0.6980 + c_1)*BCS$	0.11	0.57	123.4	119.4	0.6	<0.0001
2	$\%BF = (8.3759 + a_2) + (0.5578 + b_2)*BCS$	0.24	0.55	113.5	109.5	0.5	<0.0001
3	$\%BF = 6.7010 + 0.9008*BCS$	0.31	0.46	119.4	115.4	0.46	<0.0001

For no exercise parameters are zero, otherwise for heavy exercise
$a_1 = -18.0108$; $b_1 = 0.1005$; $c_1 = -0.1749$
$a_2 = -0.8405$; $b_2 = 0.1977$
or for light exercise
$a_1 = -1.8174$; $b_1 = -0.0066$; $c_1 = 0.6724$
$a_2 = -3.4009$; $b_2 = 0.6915$
BW = kg
Length = point of shoulder to point of buttock (cm)
Neck = average circumference of the neck measured at the throatlatch, mid neck and base of the neck (cm)

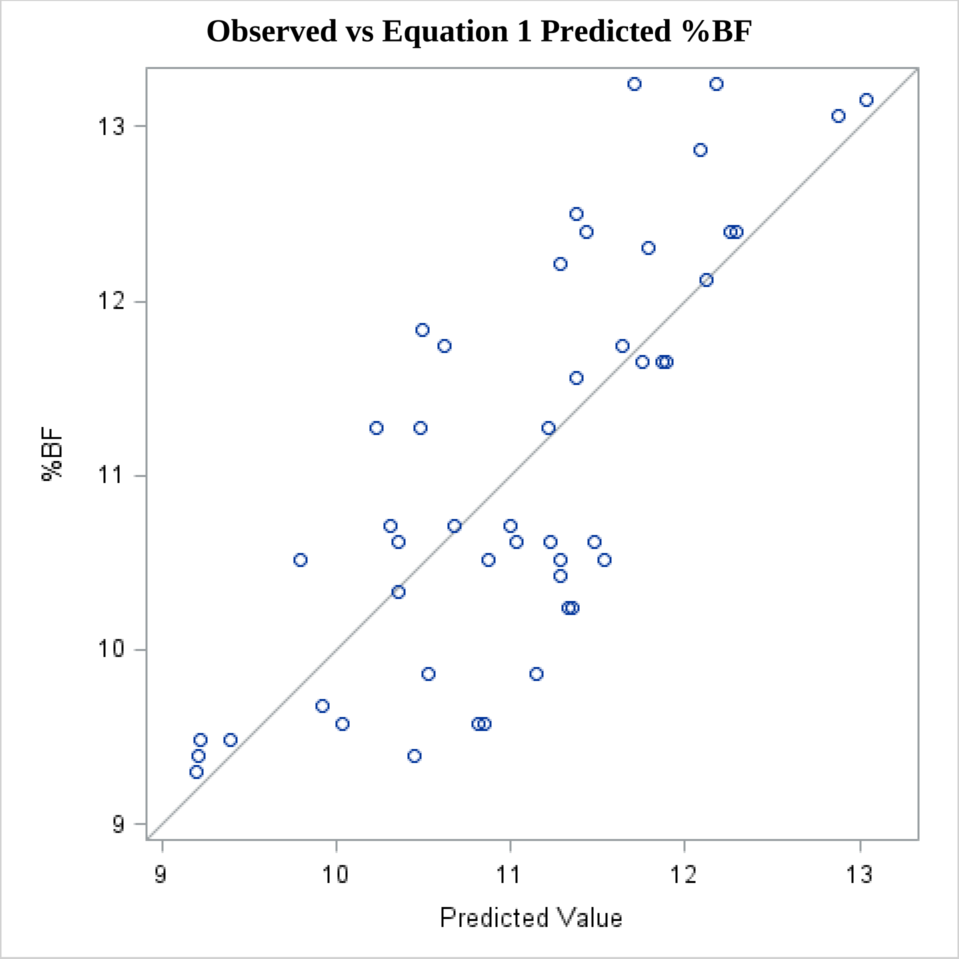

Figure 8. Scatterplot of observed vs Equation 1-predicted %BF values. Values above the line represent model under predictions, while values below the line represent model over predictions.

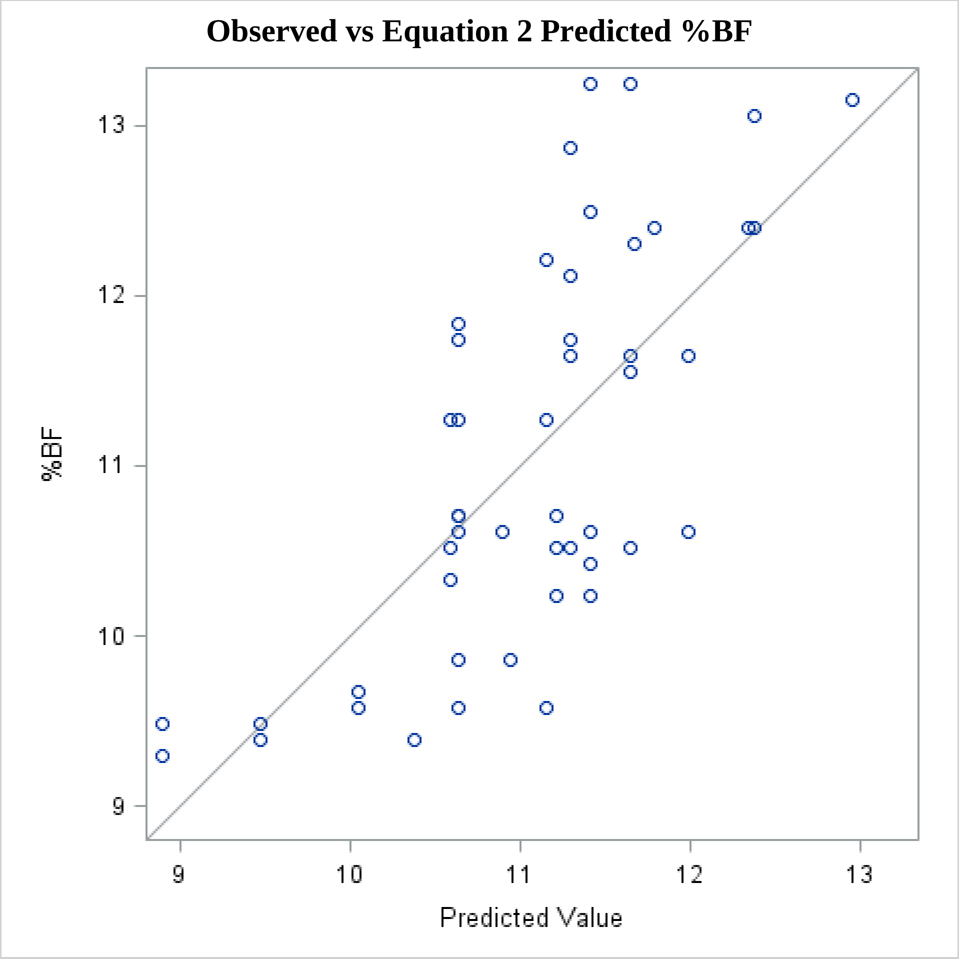

Figure 9. Scatterplot of observed vs Equation 2-predicted %BF values. Values above the line represent model under predictions, while values below the line represent model over predictions.

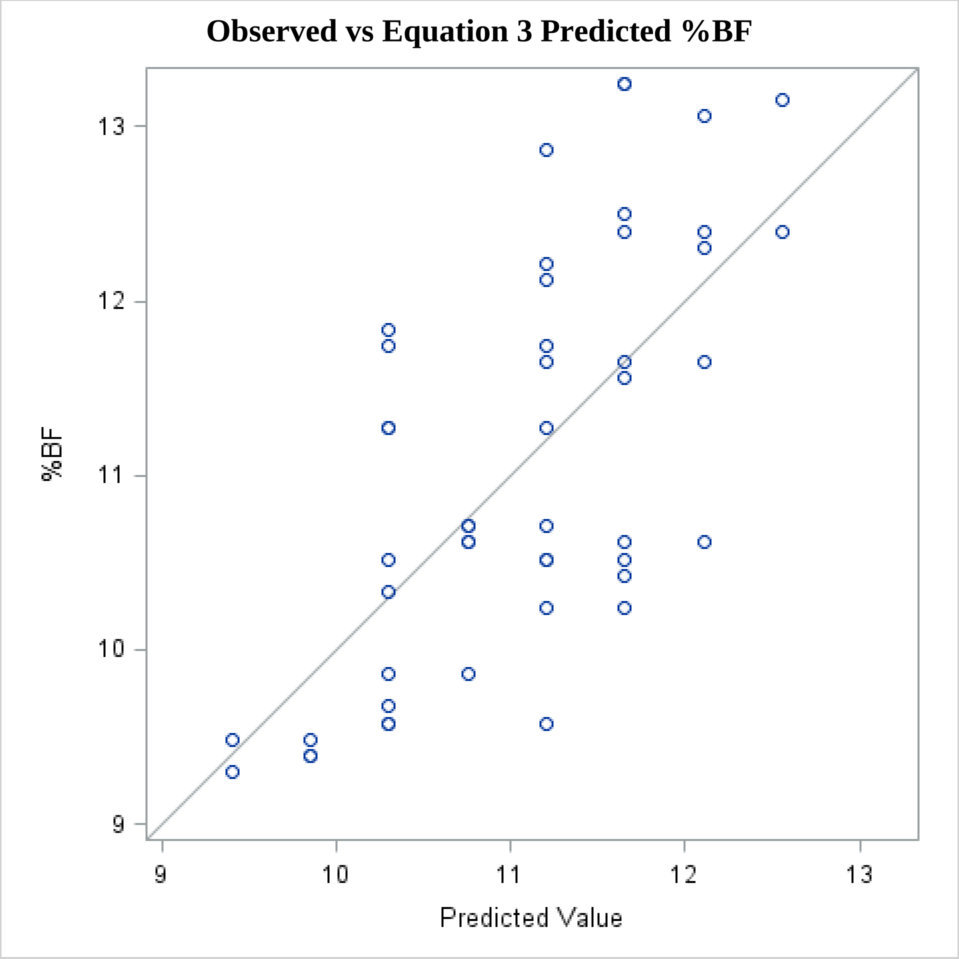

Figure 10. Scatterplot of observed vs Equation 3-predicted %BF values. Values above the line represent model under predictions, while values below the line represent model over predictions.

CHAPTER V

DISCUSSION

Model Evaluation

Mathematical models are useful tools to further our understanding of complex biological systems and aid in the decision making process (Tedeschi, 2006). Statistical evaluation of the model utilized in the current study yielded results very similar to those reported by Cordero et al. (2013) when only sedentary horses were included in the analysis. Cordero reported r^2 values of 0.907, 0.607 and 0.944 when comparing observed to model predicted values for BCS, BF and BW respectively in non-exercising mares. Correspondingly, evaluation of only control horses in the current study revealed r^2 values of 0.908, 0.505 and 0.985 for BCS, BF and BW respectively. These results indicate that the model offers an acceptable level of precision for predicting BCS and BW in non-exercising horses; however, the model in its current state is less precise when predicting BF. This could be due to inconsistencies related to estimating whole BF from RFT. The reliability of using RFT as an estimator of whole BF has been called into question due to the limited number and variety of subjects utilized when validating the method, leading researchers to search for a non-invasive whole body assessment (Dugdale et al., 2011b). Also, it may be prudent to reevaluate the BF predictive equations utilized in the model which rely on a previously quantified relationship between BCS and BF. The data used to generate this equation was gathered from sedentary mares with BCS ranging from 5-8 (Cavinder et al., 2009). The results of this study indicate that the relationship between BCS and BF changes with exercise; however even when exercise is accounted for only 50% of the variation in BF could be explained by BCS (Table 20). Further information about the relationship between BF, RFT and BCS could help to make the model more precise with regards to predicting BF. Still, the repeatability of results between the research conducted by Cordero et al. (2013) and the current study lends further credence to the reliability of the model for sedentary animals.

Further statistical evaluation revealed that the model is less precise with regard to exercising horses. Comparison of observed to model predicted values for BCS, BF and BW in light exercise groups yielded r^2 values of 0.57, 0.74 and 0.88 respectively; and 0.036, 0.126, and 0.838 for heavy exercise groups. Each of these representations of model precision are lower than the sedentary group alone, with the exception of BF for the light exercise group which is higher than the control group. The r^2 for BCS in the heavy exercise group is exceptionally low, with only 3% of the variation in BCS explained by the model. The difference in model precision between the control and exercised groups indicates that the equations utilized to predict energy expenditure of exercise in the current model require revision.

Energy Expenditure of Exercise

The data gathered in this study is the first to illustrate the energy expenditure of the stock type horse under saddle in normal riding conditions. This was made possible through the implementation of new technology, the K4b2 (Cosmed, Inc.), which allows onboard monitoring of metabolic parameters. The most important parameter to this study being VO_2 which allows for the estimation of energy expenditure. Previous studies were conducted to validate this equipment utilizing subjects of saddlebred and warmblood breeding (Art et al., 2006; Votion et al., 2006; Lepretre et al., 2009). These researchers reported that the K4b2 did accurately measure metabolic parameters in field conditions; however, the breed differences between studies make it difficult to compare actual values collected for oxygen consumption to the findings of the current study. Animals of Thoroughbred decent have been found to have higher VO_2max values when compared to Arabians (Prince et al., 2002). Similarly, Thoroughbreds are also reported to have greater aerobic capacity than Standardbreds (Rose et al., 2010). Therefore, it is not surprising that due to breed differences and differences in exercise protocols, the VO_2 and HR values observed in the current study (walk VO_2 = 4.6 HR = 86.12, sitting trot VO_2 = 7.86 HR = 112.91, extended trot VO_2 = 9.84 HR = 129.45, lope VO_2 = 16.04 HR = 148.49, extended lope VO_2 = 30.93 HR = 167.13) are much lower than those reported by Art et al. (2006) (walk VO_2 = 18.22 HR = 103, extended trot VO_2 = 37.99 HR = 162.4, Lope VO_2 = 76.33, HR = 193.2) and Votion et al. (2006) (extended trot VO_2 = 40.2 HR = 141.3) (Art et al., 2006; Votion et al., 2006).

The exercise protocols for the current study were developed based on the descriptions of exercise in the NRC (2007), which indicate that light and heavy exercise would have mean HR of 80 and 110bpm respectively. The NRC authors emphasize that these HR are consistent with the work descriptions for each category but should not be used to define that category. This is likely because many factors can affect HR during a given exercise bout, including size of the animal, the environment, or the terrain. It is probable that due to these factors the observed HR for both light and heavy exercise groups were higher than the NRC suggested HR at 109±18bpm for light exercise and 124±23bpm for heavy exercise. Statistical tests for ANOVA did reveal that the HR ($P = 0.007$) and VO_2 ($P < 0.0001$) were significantly different between light and heavy exercise groups. This statistical difference coupled with the fact that the exercise protocols were specifically designed to match the descriptions published by the NRC (2007) lead to the conclusion that these protocols adequately reflect industry standards of light and heavy exercise.

The objective of collecting the exercise data was to develop equations to predict the energy expenditure of horses ridden in field conditions. To that end, mixed model statistical analysis was conducted to examine what relationships exist, if any, between VO_2 and measured variables including: humidity, rider BW, BCS, age, environmental temperature, %BF, FFM, BW and HR. Heat index was calculated from measures of humidity and temperature of the environment. Also, previous research indicates that rider BW does not alter VO2 per unit of mass (Pagan and Hintz, 1986; Thornton et al., 1986), so horse BW and rider BW were combined prior to statistical analysis. Age, HI and BW were determined to be significant contributors to VO_2 and from these variables VO2 equation 1 was developed, which has the highest r^2 value of 0.77 and the lowest AIC at 240.2.

It is widely accepted that VO_2 corresponds linearly with BW in human subjects participating in weight bearing exercise (Malhotra et al., 1962; van der Walt and Wyndham, 1973), and this relationship has also been demonstrated in the horse (Pagan and Hintz, 1986). Due to this linear relationship, it is common for VO_2 to be expressed as a function of BW (mL/kg/min) which allows for the comparison of energy expenditures over a range of BW and exercise protocols. There is also some evidence to suggest that FFM may have an even greater effect on oxygen consumption in the horse than BW alone. Kearns et al. (2002) reported a significant correlation between BW and VO_{2max} ($r = 0.541$; $P<0.01$) but an even stronger relationship was observed between FFM and VO_{2max} independent of BW ($r = 0.857$; $P<0.001$) in mature Standardbred mares. Although FFM was not a significant predictor of VO_2 in the current study, a trend towards a positive relationship between FFM and VO_2 was observed. Thus, further investigation into the effects body composition on energy expenditure in the exercising horse is warranted.

In humans, age effects VO_2max along with VO_2 kinetics. Evaluation of VO_2max in volunteers aged 22 to 87 (n = 184) revealed an inverse relationship between VO_2max and age. The authors also measured muscle mass and concluded that the age related decrease in VO_2max was likely due to a decrease in muscle mass with advancing age (Fleg and Lakatta, 1988). To date this relationship has not been examined in the horse; however, if muscle mass does decrease with age then this could explain the greater oxygen consumption during heavy exercise observed with increased age in the current study. Also, with advancing age researchers found that VO_2 is slower to respond to an increase in exercise intensity in humans (Babcock et al., 1994). Again, this has not been examined in the horse, but a slower VO_2 response could lead to an oxygen deficit that must be overcome over time leading to an increase in average oxygen consumption. Further investigation into the relationship of muscle mass and VO_2 kinetics with regards to age in the horse could lead to better understanding of the relationship between age and VO_2.

The final significant parameter in equation 2 is HI, which had a positive correlation to oxygen consumption during heavy exercise. This relationship is most likely due to the decrease in heat dissipation ability in high temperature, high humidity conditions (Mostert et al., 2010) coupled with increased heat production during heavy exercise. Humans exercising in hot, humid environments exhibit a decrease in work performance due to factors including “decrease in VO_{2max}, disproportionate rise in rectal temperature, narrowing of the difference between the core and the skin temperature and attainment of maximal sweating rate” (Gupta et al., 1981). Potter et al. (1990) demonstrated that equine athletes maintained in hot environments require greater DE to maintain BW than do horses maintained in temperate environments. Although humidity was not a contributing factor in the study conducted by Potter et al. (1990), the data does reinforce that environment has a significant effect on the energy requirements of exercising horses.

Identification of these significant contributors to energy expenditure during exercise allow for a greater understanding of exercise dynamics in the horse and further investigation could lead the industry to a nutrition system based on NE as opposed to the current DE system. However, practical constraints of applying equation 1 in the field, led to the development of equations 2, 3 and 4 in which HR is the sole variable for estimation which is similar to previous equine studies (Eaton et al., 1995; Coenen, 2005). Because it is widely accepted that VO_2 increases linearly with BW, equation 2 (r^2

= 0.72, $P < 0.0001$) estimates VO_2 relative to BW (ml/kg/min) using HR. Mixed model analysis revealed that exercise was a significant factor in this estimation, indicating that the relationship between HR and VO_2 increases as exercise increases from light to heavy. While the r^2 value of 0.72 is less than that attributed to equation 1, this is still an acceptable level of variation explained by the equation. Also, HR can be easily measured with onboard HR monitors or manually upon the completion of exercise and averaged, leading to more straightforward application of the equation. In order to examine the importance of the exercise effect, equation 3 was developed using only HR in absence of the exercise variables. This resulted in an r^2 value of 0.26, meaning that the equation only explained 26% of the variation in VO_2 between subjects. This is an unacceptable level of predictability; however, it does illustrate the importance of the exercise variable and highlights the difference in the HR/VO_2 relationship with changing intensity of exercise.

In other livestock species energy requirements are most often expressed as a function of metabolic BW in order to account for differences in surface area; however, the current equine NRC (NRC, 2007) does not scale BW when listing nutritional requirements. This is largely due to a study which found no significant advantage to scaling BW when expressing energy requirements in the horse (Pagan and Hintz, 1986). However, the NRC does recognize that this study was conducted on very few horses leaving doubt as to the validity of dismissing scaled BW. In order to examine the effect of using metabolic BW with regards to energy expenditure, equation 4 was developed, in which VO2 is estimated per metabolic BW ($ml/min/kgBW^{0.75}$). Equation 4 accounted for a similar amount of variation as equation 2 (r^2=0.71 vs r^2=0.72), leading to the conclusion that, in this study, scaling BW does not offer any advantage over BW when estimating energy expenditure in exercising horses. However, subjects utilized in the current study were of similar breeding, and size, so it is not surprising that scaling BW offers no advantage. Further examination using horses of more varied size and type could reveal a benefit for utilizing metabolic BW when estimating energy expenditure as is observed in other species.

Comparison of Equations

The goal of measuring and estimating VO_2 is to calculate the energy expended and thus the energy required by the horse. To this end VO_2 was converted to energy expenditure using a previously validated equation (Harris, 1997). Energy values were then translated from NE to DE by applying the estimation that 40% of DE is utilized for NE of exercise in mild to moderate exercise conditions in the horse (NRC, 2007). It should be noted that according to previous research DE efficiency of use for exercise in the horse can vary from approximately 20 to 50%, and the NRC recognizes that further research is needed to evaluate the effects of type of work, feed intake and diet composition on this efficiency.

After completing these calculations, values for DE required based on observed VO_2 were compared to DE required based on estimated VO_2 from several different equations. These comparisons are outlined in Table 18. For light exercise, observed DE was not statistically significantly different ($P > 0.05$) from values predicted from the Hintz equation which was used in the current model or from equation 1 or 2. For heavy exercise observed DE was not significantly different ($P > 0.05$) from predicted DE based on equations 1 or 2. Notably, for both light and heavy exercise groups there was a significant difference between DE based on observed energy expenditure in the current study and the NRC recommended DE above maintenance for exercise. There are many factors that can affect the daily energy requirements of an equine athlete, this coupled with a need for simple equations applicable in field conditions led the NRC to adopt the recommendations of 20, 40, 60 and 90% above maintenance for lightly, moderately, heavily and very heavily exercised horses respectively. While these recommendations offer a starting point for developing equine nutrition programs, they are vague and may not be accurate in all situations, thus the difference in observed and NRC predicted requirements in this study and the need for more focused research into the factors that affect energy requirements in equine athletes.

As previously discussed, the current model was less accurate in predicting energy requirements for lightly and to a greater extent heavily exercised horses, than for sedentary horses. However, according to Table 18 the observed and model predicted values for energy expenditure of light exercise were not significantly different. The similarity between the model predicted and observed energy expenditure in the light exercise group coupled with the overall lack of precision of the model to predict BCS changes in that same group, leads to the conclusion that either the efficiency of conversion calculation between NE and DE is inaccurate, or perhaps continued exercise effects basal metabolism causing a gap in energy requirements outside of what is expended during exercise bouts. This is further supported by the fact that predicted energy expenditure of heavy exercise was much higher than the observed values, yet animals expected to increase in BCS did not. Again, leading to the hypothesis that continued exercise has an effect on DE energy requirements beyond what is expended during exercise. Also, heavily exercised animals fed to increase BCS were fed larger amounts of concentrate, and some topped with vegetable oil, to meet the high energy demand predicted by the model. It could be that horses consuming this type of diet had a decreased digestibility or lost more energy as heat, and so there could be an effect of diet on the NE available for exercise. Several studies have conducted feeding trials to test the energy required to maintain BW in equine athletes, which would encompass both exercise energy needs and any increase in BMR (Pagan and Hintz, 1985; Potter et al., 1990); however, partitioning the energy requirement between exercise and BMR using this method is difficult and unprecise. More information on the specific effect of exercise on BMR and the effect of diet composition on energy retention in the horse would be beneficial not only to increase the precision of the current model but more importantly to further our understanding of the energy requirements of equine athletes.

Body Composition

Body Fat has a large impact on the energy requirements of animals which is why BF is one of the required variable inputs in the current model. The most widely accepted tool for estimating BF in the horse is through ultrasonic measurement of RFT; however as previously discussed, this application is limited in the field leading to the need for a more simplistic estimation procedure. To that end physical measurements were taken in the current study and statistical analysis was conducted to examine the relationship of these measurements to BF. Of the equations developed, model 1 explained the most variability in BF with an $r^2 = 0.6$, which is greater than those models suggested by Henneke et al. (1983) ($r^2 = 0.43$ & 0.44) (Henneke et al., 1983), but less than the model proposed by Potter et al. (2015) ($r^2 = 0.745$) (Potter et al., 2015). Potter et al. (2015) utilized the isotope dilution technique for measuring BF which may account for the greater variability explained by their model, as the accuracy of measuring RFT via ultrasound to estimate BF has been called into question. Although model 1 does reflect an adequate amount of the variability in BF, further analysis using the more precise isotope dilution technique could explain even more of the variability leading to an even more accurate method of estimation using body parameters.

CHAPTER IV

CONCLUSIONS

This body of data further supports and expands upon the model created by Cordero et al. (2013). The modification of the model in the current study to include maintenance energy requirements and input of current energy intake helps to determine the energy balance of the horse prior to BCS modification. This allows the model to predict the DE that the horse requires to alter BCS rather than a change in DE from maintenance requirements, which was the end product of the Cordero model. Packaging this model in a format that facilitates industry application could lead to more efficient feeding practices of sedentary horses, which would be of health and economic benefit. Still, as mentioned previously the majority of horses are subjected to some form of athletic activity and the model in its current state is not reliable when applied to the exercising horse. Further investigation into the relationships between exercise, diet, basal metabolism and BCS could yield a more dynamic model applicable to a wider variety of horses.

The exercise data obtained in this study is the first to quantify energy expenditure of the exercising stock type horse under saddle in field conditions. Averages of VO_2 at the walk, sitting trot, extended trot, lope and extended lope across all horses were obtained, along with overall VO_2 averages for lightly and heavily exercised horses. While this is valuable insight into overall energy expenditure under field conditions, the goal of collecting this information was to develop predictive equations for each exercise protocol that could then be incorporated into the model. Equations 1 and 2 offer an acceptable level of precision for predicting energy expenditure of the stock type horse during exercise.

This study also revealed a significant difference between observed exercise energy expenditure and the NRC recommendation of energy requirement above maintenance for lightly and heavily exercised horses (Table 18). Admittedly, the NRC recommendations are most likely attempting to compensate for possible changes in BMR or DE efficiency of use attributed to continued exercise; however, these relationships have not been clearly quantified in the horse. Further investigation into the effects of type of work, feed intake and diet composition on DE efficiency of use for exercise and BMR would enhance both the model and NRC recommendations for exercising horses.

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APPENDIX

Appendix Table 1. Body Condition Score descriptions.

Score	Description	
1	Poor	The horse is emaciated. The spinous processes (backbone), ribs, tailhead and hooks and pins all project prominently. The bone structures of the withers, shoulders and neck are easily noticeable, and no fat can be felt anywhere.
2	Very Thin	The spinous processes are prominent. The ribs, tailhead and pelvic bones stand out, and bone structures of the withers, neck and shoulders are faintly discernable.
3	Thin	The spinous processes stand out, but fat covers them to midpoint. Very slight fat cover can be felt over the ribs, but the spinous processes and ribs are easily discernable. The tailhead is prominent, but individual vertebrae cannot be seen. Hook bones are visible but appear rounded. Pin bones cannot be seen. The withers, shoulders and neck are accentuated.
4	Moderately Thin	The horse has a negative crease along its back and the outline of the ribs can just be seen. Fat can be felt around the trailhead. The hook bones cannot be seen and the withers, neck and shoulders do not look obviously thin.
5	Moderate	The back is level. Ribs cannot be seen but can be easily felt. Fat around the tailhead feels slightly spongy. The withers look rounded and the shoulder and neck blend smoothly into the body.
6	Moderate to Fleshy	There may be a slight crease down the back. Fat around the tailhead feels soft and fat over the ribs feels spongy. There are small deposits along the sides of the withers, behind the shoulders and along the sides of the neck.
7	Fleshy	There may be a crease down the back. Individual ribs can be felt, but there is noticeable fat between the ribs. Fat around the tailhead is soft. Fat is noticeable in the withers, the neck and behind the shoulders.
8	Fat	The horse has a crease down the back. Spaces between ribs are so filled with fat that the ribs are difficult to feel. The area along the withers is filled with fat, and fat around the tailhead feels very soft. The space behind the shoulders is filled in flush and some fat is deposited along the inner buttocks.
9	Extremely Fat	The crease down the back is very obvious. Fat appears in patches over the ribs and there is bulging fat around the tailhead, withers, shoulders and neck. Fat along the inner buttocks may cause buttocks to rub together, and the flank is filled in flush.

Appendix Table 2. Forage Analysis

	As Sampled	Dry Matter
Estimated Digestible Energy, Mcal/lb	0.72	0.81
Crude Protein (%)	11.5	12.8
Estimated Lysine (%)	0.40	0.45
Lignin (%)	6.0	6.6
ADF (%)	34	37.8
NDF (%)	61.1	68
Starch (%)	1.9	2.1
NFC (%)	6.3	7.0
Crude Fat (%)	1.9	2.1
Ash (%)	9.1	10.1
Calcium (%)	0.57	0.64
Phosphorous (%)	0.23	0.26
Magnesium (%)	0.15	0.17
Potassium (%)	1.50	1.67
Sodium (%)	0.047	0.053
Iron (ppm)	419	466
Zinc (ppm)	28	31
Copper (ppm)	9	10
Manganese (ppm)	47	52
Molybdenum (ppm)	1.0	1.1

Appendix Table 3. Concentrate Analysis

	As Sampled	Dry Matter
Estimated Digestible Energy, Mcal/lb	1.30	1.45
Crude Protein (%)	15.9	17.8
ADF (%)	12.2	13.7
NDF (%)	24.2	27.1
Calcium (%)	0.93	1.04
Phosphorous (%)	0.64	0.72
Magnesium (%)	0.26	0.29
Potassium (%)	0.98	1.09
Sodium (%)	0.360	0.403
Iron (ppm)	186	208
Zinc (ppm)	101	113
Copper (ppm)	38	43
Manganese (ppm)	124	138
Molybdenum (ppm)	0.9	1.0

Appendix Table 4. Body Condition Score (BCS) results per animal

Animal ID	**Day**	**BCS**			**Mean BCS**	**BCS$_a$**
		Judge1	*Judge 2*	*Judge 3*		
Group 1						
407	**initial**	**5**	**5**	**4.5**	**4.83**	**5**
	9-Jul	5	4.5	4.5	4.67	5
	30-Jul	5	4.5	5.5	5.00	5
	13-Aug	N/A	6	6	6.00	6
	Final	6	5.5	6	5.83	6
	Targeted Final BCS					**7**
410	**initial**	**4**	**4**	**4**	**4.00**	**4**
	9-Jul	4	4.5	4.5	4.33	4.5
	30-Jul	4.5	4.5	4.5	4.50	4.5
	13-Aug	N/A	5	5	5.00	5
	Final	5.5	5	5.5	5.33	5.5
	Targeted Final BCS					**6**
412	**initial**	**4**	**4.5**	**4**	**4.17**	**4**
	9-Jul	4.5	5	5	4.83	5
	30-Jul	5	5	5	5.00	5
	13-Aug	N/A	5	5	5.00	5
	Final	5	5	5.5	5.17	5
	Targeted Final BCS					**6**
420	**initial**	**3**	**3.5**	**4**	**3.50**	**3.5**
	9-Jul	4	4	4.5	4.17	4
	30-Jul	4.5	4	4.5	4.33	4.5
	13-Aug	N/A	5	5	5.00	5
	Final	5	4.5	5	4.83	5
	Targeted Final BCS					**5.5**
Group 2						
6	**initial**	**5**	**5.5**	**5.5**	**5.33**	**5.5**
	9-Jul	5	5.5	5	5.17	5
	30-Jul	4.5	5	5	4.83	5
	13-Aug	N/A	5	4.5	4.75	5

	Final	4	5	4.5	4.50	4.5
	Targeted Final BCS					**3.5**
116	**initial**	**5**	**5.5**	**6**	**5.50**	**5.5**
	9-Jul	5	5.5	5.5	5.33	5.5
	30-Jul	5	5	4.5	4.83	5
	13-Aug	N/A	5	4.5	4.75	5
	Final	4	4	4	4.00	4
	Targeted Final BCS					**3.5**
409	**initial**	**5**	**5.5**	**5.5**	**5.33**	**5.5**
	9-Jul	5	5	5.5	5.17	5
	30-Jul	4	4	4.5	4.17	4
	13-Aug	N/A	4	4	4.00	4
	Final	4	4	3.5	3.83	4
	Targeted Final BCS					**3.5**
508	**initial**	**5**	**5.5**	**5.5**	**5.33**	**5.5**
	9-Jul	5	4.5	5	4.83	5
	30-Jul	4.5	4.5	5	4.67	4.5
	13-Aug	N/A	4	4	4.00	4
	Final	4.5	4	3.5	4.00	4
	Targeted Final BCS					**3.5**
Group 3						
128	**initial**	**4.5**	**4.5**	**4**	**4.33**	**4.5**
	9-Jul	4.5	4.5	4.5	4.50	4.5
	30-Jul	5	4.5	5	4.83	5
	13-Aug	N/A	5.5	5.5	5.50	5.5
	Final	6	6	6	6.00	6
	Targeted Final BCS					**6.5**
137	**initial**	**3**	**3**	**3**	**3.00**	**3**
	9-Jul	3	3	3	3.00	3
	30-Jul	3	3	3	3.00	3
	13-Aug	N/A	3.5	3.5	3.50	3.5
	Final	3	3	3.5	3.17	3
	Targeted Final BCS					**5**

503	**initial**	**4**	**3.5**	**4**	**3.83**	**4**
	9-Jul	4	3.5	4	3.83	4
	30-Jul	4	4	4.5	4.17	4
	13-Aug	N/A	4.5	4.5	4.50	4.5
	Final	4.5	4.5	5	4.67	4.5
	Targeted Final BCS					**6**
504	**initial**	**3.5**	**3.5**	**3.5**	**3.50**	**3.5**
	9-Jul	4	4	4.5	4.17	4
	30-Jul	4.5	4.5	4.5	4.50	4.5
	13-Aug	N/A	5	5	5.00	5
	Final	5	5	5	5.00	5
	Targeted Final BCS					**5.5**
Group 4						
11	**initial**	**5**	**5**	**5**	**5.00**	**5**
	9-Jul	5	5	5	5.00	5
	30-Jul	4.5	4.5	4.5	4.50	4.5
	13-Aug	N/A	5.5	5	5.25	5
	Final	4	4	3.5	3.83	4
	Targeted Final BCS					**3**
106	**initial**	**5**	**6.5**	**6**	**5.83**	**6**
	9-Jul	5.5	6	5.5	5.67	5.5
	30-Jul	5	5.5	5	5.17	5
	13-Aug	N/A	5.5	5	5.25	5.5
	Final	5	5.5	5	5.17	5
	Targeted Final BCS					**4**
138	**initial**	**6**	**7**	**6.5**	**6.50**	**6.5**
	9-Jul	5.5	5.5	5.5	5.50	5.5
	30-Jul	5.5	5.5	5.5	5.50	5.5
	13-Aug	N/A	5.5	5	5.25	5.5
	Final	4.5	5	4.5	4.67	4.5
	Targeted Final BCS					**4.5**
418	**initial**	**5**	**6**	**5.5**	**5.50**	**5.5**
	9-Jul	5	5.5	5.5	5.33	5.5
	30-Jul	5	4.5	4.5	4.67	4.5

	13-Aug	N/A	4	4.5	4.25	4
	Final	3.5	4	3.5	3.67	3.5
	Targeted Final BCS					**3.5**
Group 5						
36	**initial**	**3.5**	**4**	**4**	**3.83**	**4**
	9-Jul	4.5	4.5	4.5	4.50	4.5
	30-Jul	4.5	5	5	4.83	5
	13-Aug	N/A	5	5.5	5.25	5
	Final	5	5.5	5.5	5.33	5.5
	Targeted Final BCS					**6**
107	**initial**	**4**	**4**	**4**	**4.00**	**4**
	9-Jul	4.5	5	4.5	4.67	4.5
	30-Jul	5.5	5	5	5.17	5
	13-Aug	N/A	6	6	6.00	6
	Final	5	5	6	5.33	5.5
	Targeted Final BCS					**6**
108	**initial**	**4**	**4**	**4**	**4.00**	**4**
	9-Jul	4.5	4.5	4.5	4.50	4.5
	30-Jul	5	5	5	5.00	5
	13-Aug	N/A	5.5	5.5	5.50	5.5
	Final	5	5	5.5	5.17	5
	Targeted Final BCS					**6**
112	**initial**	**4.5**	**4.5**	**4**	**4.33**	**4.5**
	9-Jul	5	4.5	4.5	4.67	4.5
	30-Jul	5.5	5	5.5	5.33	5.5
	13-Aug	N/A	5	5	5.00	5
	Final	5	5	5.5	5.17	5
	Targeted Final BCS					**6.5**
Group 6						
5	**initial**	**5.5**	**6**	**6**	**5.83**	**6**
	9-Jul	5.5	6	5.5	5.67	5.5
	30-Jul	5	5.5	5	5.17	5
	13-Aug	N/A	6	5.5	5.75	5.5
	Final	6	6	5	5.67	5.5

	Targeted Final BCS					**4**
104	**initial**	**6**	**6.5**	**6.5**	**6.33**	**6.5**
	9-Jul	5	5.5	5.5	5.33	5.5
	30-Jul	5	5.5	5.5	5.33	5.5
	13-Aug	N/A	6	5.5	5.75	6
	Final	5	5.5	5	5.17	5
	Targeted Final BCS					**4.5**
120	**initial**	**5**	**5.5**	**5.5**	**5.33**	**5.5**
	9-Jul	5.5	5.5	6	5.67	5.5
	30-Jul	5	5.5	5	5.17	5
	13-Aug	N/A	5.5	5	5.25	5.5
	Final	5	5	5	5.00	5
	Targeted Final BCS					**3.5**
405	**initial**	**5.5**	**6**	**6**	**5.83**	**6**
	9-Jul	6	6	5.5	5.83	6
	30-Jul	6	6	5	5.67	6
	13-Aug	N/A	6	6	6.00	6
	Final	5	5.5	5	5.17	5
	Targeted Final BCS					**4**

[a] Mean BCS rounded to nearest 0.5 BCS

Appendix Table 5. Body fat (%BF) results per animal

Animal ID	Day	Rump Fat (cm)	Extractable Fat (%)
Group 1			
407	**initial**	**0.56**	**11.272**
	9-Jul	0.58	11.366
	30-Jul	0.62	11.554
	13-Aug	0.8	12.4
	Final	0.78	12.306
	Targeted Final %BF		**14.44**
410	**initial**	**0.66**	**11.742**
	9-Jul	0.56	11.272
	30-Jul	0.7	11.93
	13-Aug	0.7	11.93
	Final	0.82	12.494
	Targeted Final %BF		**13.38**
412	**initial**	**0.68**	**11.836**
	9-Jul	0.52	11.084
	30-Jul	0.68	11.836
	13-Aug	0.28	9.956
	Final	0.76	12.212
	Targeted Final %BF		**13.38**
420	**initial**	**0.16**	**9.392**
	9-Jul	0.14	9.298
	30-Jul	0.12	9.204
	13-Aug	0.16	9.392
	Final	0.2	9.58
	Targeted Final %BF		**12.85**
Group 2			
6	**initial**	**0.42**	**10.614**
	9-Jul	0.54	11.178
	30-Jul	0.38	10.426
	13-Aug	0.36	10.332
	Final	0.42	10.614
	Targeted Final %BF		**10.51**

116	**initial**	**0.98**	**13.246**
	9-Jul	0.88	12.776
	30-Jul	0.74	12.118
	13-Aug	0.54	11.178
	Final	0.56	11.272
	Targeted Final %BF		**10.71**
409	**initial**	**0.34**	**10.238**
	9-Jul	0.36	10.332
	30-Jul	0.18	9.486
	13-Aug	0.14	9.298
	Final	0.2	9.58
	Targeted Final %BF		**9.76**
508	**initial**	**0.38**	**10.426**
	9-Jul	0.3	10.05
	30-Jul	0.22	9.674
	13-Aug	0.22	9.674
	Final	0.26	9.862
	Targeted Final %BF		**10.14**
Group 3			
128	**initial**	**0.42**	**10.614**
	9-Jul	0.38	10.426
	30-Jul	0.46	10.802
	13-Aug	0.66	11.742
	Final	0.8	12.4
	Targeted Final %BF		**13.91**
137	**initial**	**0.14**	**9.298**
	9-Jul	0.16	9.392
	30-Jul	0.12	9.204
	13-Aug	0.16	9.392
	Final	0.18	9.486
	Targeted Final %BF		**12.31**
503	**initial**	**0.22**	**9.674**
	9-Jul	0.18	9.486

	30-Jul	0.22	9.674
	13-Aug	0.32	10.144
	Final	0.44	10.708
	Targeted Final %BF		**13.38**
504	**initial**	**0.16**	**9.392**
	9-Jul	0.18	9.486
	30-Jul	0.2	9.58
	13-Aug	0.32	10.144
	Final	0.44	10.708
	Targeted Final %BF		**12.85**
Group 4			
11	**initial**	**0.34**	**10.238**
	9-Jul	0.4	10.52
	30-Jul	0.36	10.332
	13-Aug	0.24	9.768
	Final	0.2	9.58
	Targeted Final %BF		**10.18**
106	**initial**	**0.94**	**13.058**
	9-Jul	0.76	12.212
	30-Jul	0.6	11.46
	13-Aug	0.54	11.178
	Final	0.4	10.52
	Targeted Final %BF		**11.25**
138	**initial**	**0.96**	**13.152**
	9-Jul	0.78	12.306
	30-Jul	0.64	11.648
	13-Aug	0.58	11.366
	Final	0.44	10.708
	Targeted Final %BF		**11.78**
418	**initial**	**0.8**	**12.4**
	9-Jul	0.64	11.648
	30-Jul	0.5	10.99
	13-Aug	0.38	10.426
	Final	0.18	9.486

	Targeted Final %BF		**10.71**
Group 5			
36	**initial**	**0.36**	**10.332**
	9-Jul	0.42	10.614
	30-Jul	0.56	11.272
	13-Aug	0.68	11.836
	Final	0.64	11.648
	Targeted Final %BF		**13.38**
107	**initial**	**0.56**	**11.272**
	9-Jul	0.58	11.366
	30-Jul	0.7	11.93
	13-Aug	1.04	13.528
	Final	0.98	13.246
	Targeted Final %BF		**13.38**
108	**initial**	**0.4**	**10.52**
	9-Jul	0.42	10.614
	30-Jul	0.64	11.648
	13-Aug	0.82	12.494
	Final	0.9	12.87
	Targeted Final %BF		**13.38**
112	**initial**	**0.26**	**9.862**
	9-Jul	0.2	9.58
	30-Jul	0.32	10.144
	13-Aug	0.4	10.52
	Final	0.4	10.52
	Targeted Final %BF		**13.91**
Group 6			
5	**initial**	**0.42**	**10.614**
	9-Jul	0.44	10.708
	30-Jul	0.48	10.896
	13-Aug	0.36	10.332
	Final	0.4	10.52
	Targeted Final %BF		**9.98**

104	**initial**	**0.8**	**12.4**
	9-Jul	0.82	12.494
	30-Jul	0.78	12.306
	13-Aug	0.76	12.212
	Final	0.74	12.118
	Targeted Final %BF		**11.78**
120	**initial**	**0.62**	**11.554**
	9-Jul	0.74	12.118
	30-Jul	0.74	12.118
	13-Aug	0.66	11.742
	Final	0.64	11.648
	Targeted Final %BF		**10.71**
405	**initial**	**0.64**	**11.648**
	9-Jul	0.64	11.648
	30-Jul	0.62	11.554
	13-Aug	0.7	11.93
	Final	0.66	11.742
	Targeted Final %BF		**11.25**

Appendix Table 6. Body weight (BW) results per animal

Animal ID	**Day**	**Full BW**		**Empty BW**	
Group 1		lbs	kg	lbs	kg
407	**initial**	**997.00**	**452.23**	**814.51**	**369.46**
	9-Jul	1020.00	452.23	833.30	377.98
	30-Jul	1053.00	452.23	860.26	390.21
	13-Aug	1042.00	452.23	851.27	386.13
	Final	1080.00	452.23	882.32	400.21
	Targeted Final BW	**1074.42**	**487.35**	**877.76**	**398.15**
410	**initial**	**1031.00**	**467.65**	**842.29**	**382.05**
	9-Jul	1048.00	467.65	856.17	388.35
	30-Jul	1060.00	467.65	865.98	392.80
	13-Aug	1058.00	467.65	864.34	392.06
	Final	1108.00	467.65	905.19	410.59
	Targeted Final BW	**1114.30**	**505.44**	**910.34**	**412.92**
412	**initial**	**1065.00**	**483.08**	**870.06**	**394.65**
	9-Jul	1120.00	483.08	915.00	415.03
	30-Jul	1112.00	483.08	908.46	412.07
	13-Aug	1120.00	483.08	915.00	415.03
	Final	1130.00	483.08	923.16	418.74
	Targeted Final BW	**1151.03**	**522.10**	**940.35**	**426.53**
420	**initial**	**1160.00**	**526.17**	**947.67**	**429.86**
	9-Jul	1214.00	526.17	991.79	449.87
	30-Jul	1215.00	526.17	992.61	450.24
	13-Aug	1227.00	526.17	1002.41	454.69
	Final	1230.00	526.17	1004.86	455.80
	Targeted Final BW	**1255.64**	**569.55**	**1025.81**	**465.30**
Group 2					
6	**initial**	**1108.00**	**502.58**	**905.19**	**410.59**
	9-Jul	1098.00	502.58	897.02	406.88
	30-Jul	1055.00	502.58	861.89	390.95
	13-Aug	1040.00	502.58	849.64	385.39
	Final	1062.00	502.58	867.61	393.54
	Targeted Final BW	**1023.61**	**464.30**	**836.25**	**379.31**

116	**initial**	**971.00**	**440.44**	**793.27**	**359.82**
	9-Jul	965.00	440.44	788.37	357.60
	30-Jul	947.00	440.44	773.66	350.93
	13-Aug	940.00	440.44	767.94	348.33
	Final	945.00	440.44	772.03	350.19
	Targeted Final BW	**897.04**	**406.89**	**732.84**	**332.41**
409	**initial**	**1041.00**	**472.19**	**850.46**	**385.76**
	9-Jul	1005.00	472.19	821.04	372.42
	30-Jul	985.00	472.19	804.71	365.01
	13-Aug	976.00	472.19	797.35	361.67
	Final	986.00	472.19	805.52	365.38
	Targeted Final BW	**961.70**	**436.22**	**785.67**	**356.37**
508	**initial**	**1072.00**	**486.25**	**875.78**	**397.25**
	9-Jul	1046.00	486.25	854.54	387.61
	30-Jul	1025.00	486.25	837.38	379.83
	13-Aug	990.00	486.25	808.79	366.86
	Final	1000.00	486.25	816.96	370.57
	Targeted Final BW	**990.34**	**449.21**	**809.07**	**366.99**
Group 3					
128	**initial**	**951.00**	**431.37**	**776.93**	**352.41**
	9-Jul	975.00	431.37	796.54	361.30
	30-Jul	991.00	431.37	809.61	367.23
	13-Aug	1007.00	431.37	822.68	373.16
	Final	1013.00	431.37	827.58	375.38
	Targeted Final BW	**1026.32**	**465.53**	**838.46**	**380.32**
137	**initial**	**882.00**	**400.07**	**720.56**	**326.84**
	9-Jul	893.00	400.07	729.55	330.92
	30-Jul	885.00	400.07	723.01	327.95
	13-Aug	902.00	400.07	736.90	334.25
	Final	908.00	400.07	741.80	336.47
	Targeted Final BW	**956.26**	**433.75**	**781.22**	**354.36**
503	**initial**	**1096.00**	**497.14**	**895.39**	**406.14**
	9-Jul	1098.00	497.14	897.02	406.88

	30-Jul	1083.00	497.14	884.77	401.32
	13-Aug	1116.00	497.14	911.73	413.55
	Final	1135.00	497.14	927.25	420.59
	Targeted Final BW	**1184.54**	**537.30**	**967.72**	**438.95**
504	**initial**	**1190.00**	**539.77**	**972.18**	**440.97**
	9-Jul	1210.00	539.77	988.52	448.39
	30-Jul	1240.00	539.77	1013.03	459.50
	13-Aug	1253.00	539.77	1023.65	464.32
	Final	1268.00	539.77	1035.91	469.88
	Targeted Final BW	**1288.12**	**584.28**	**1052.34**	**477.33**
Group 4					
11	**initial**	**1099.00**	**498.50**	**897.84**	**407.25**
	9-Jul	1115.00	498.50	910.91	413.18
	30-Jul	1035.00	498.50	845.55	383.54
	13-Aug	1150.00	498.50	939.50	426.15
	Final	1079.00	498.50	881.50	399.84
	Targeted Final BW	**1013.66**	**459.79**	**828.12**	**375.63**
106	**initial**	**1037.00**	**470.38**	**847.19**	**384.28**
	9-Jul	1028.00	470.38	839.83	380.94
	30-Jul	1025.00	470.38	837.38	379.83
	13-Aug	1011.00	470.38	825.95	374.64
	Final	1030.00	470.38	841.47	381.68
	Targeted Final BW	**959.47**	**435.21**	**783.85**	**355.55**
138	**initial**	**1255.00**	**569.26**	**1025.28**	**465.06**
	9-Jul	1233.00	569.26	1007.31	456.91
	30-Jul	1191.00	569.26	973.00	441.35
	13-Aug	1207.00	569.26	986.07	447.27
	Final	1182.00	569.26	965.65	438.01
	Targeted Final BW	**1162.92**	**527.49**	**950.06**	**430.94**
418	**initial**	**1237.00**	**561.09**	**1010.58**	**458.39**
	9-Jul	1215.00	561.09	992.61	450.24
	30-Jul	1194.00	561.09	975.45	442.46
	13-Aug	1150.00	561.09	939.50	426.15
	Final	1153.00	561.09	941.95	427.26

	Targeted Final BW	**1142.77**	**518.35**	**933.59**	**423.47**
Group 5					
36	**initial**	**1040.00**	**471.74**	**849.64**	**385.39**
	9-Jul	1058.00	471.74	864.34	392.06
	30-Jul	1075.00	471.74	878.23	398.36
	13-Aug	1088.00	471.74	888.85	403.18
	Final	1070.00	471.74	874.15	396.51
	Targeted Final BW	**1124.03**	**509.85**	**918.28**	**416.53**
107	**initial**	**976.00**	**442.71**	**797.35**	**361.67**
	9-Jul	1033.00	442.71	843.92	382.80
	30-Jul	1032.00	442.71	843.10	382.42
	13-Aug	1061.00	442.71	866.79	393.17
	Final	1067.00	442.71	871.70	395.39
	Targeted Final BW	**1054.85**	**478.47**	**861.77**	**390.89**
108	**initial**	**1107.00**	**502.13**	**904.37**	**410.22**
	9-Jul	1130.00	502.13	923.16	418.74
	30-Jul	1151.00	502.13	940.32	426.52
	13-Aug	1146.00	502.13	936.24	424.67
	Final	1163.00	502.13	950.12	430.97
	Targeted Final BW	**1196.43**	**542.69**	**977.43**	**443.36**
112	**initial**	**1027.00**	**465.84**	**839.02**	**380.57**
	9-Jul	1051.00	465.84	858.62	389.47
	30-Jul	1070.00	465.84	874.15	396.51
	13-Aug	1063.00	465.84	868.43	393.91
	Final	1090.00	465.84	890.49	403.92
	Targeted Final BW	**1108.33**	**502.73**	**905.46**	**410.71**
Group 6					
5	**initial**	**935.00**	**424.11**	**763.86**	**346.48**
	9-Jul	945.00	424.11	772.03	350.19
	30-Jul	938.00	424.11	766.31	347.59
	13-Aug	938.00	424.11	766.31	347.59
	Final	916.00	424.11	748.34	339.44
	Targeted Final BW	**865.12**	**392.41**	**706.77**	**320.58**

104	**initial**	**1210.00**	**548.85**	**988.52**	**448.39**
	9-Jul	1208.00	548.85	986.89	447.64
	30-Jul	1181.00	548.85	964.83	437.64
	13-Aug	1150.00	548.85	939.50	426.15
	Final	1137.00	548.85	928.88	421.33
	Targeted Final BW	**1121.20**	**508.57**	**915.98**	**415.48**
120	**initial**	**1244.00**	**564.27**	**1016.30**	**460.99**
	9-Jul	1248.00	564.27	1019.57	462.47
	30-Jul	1213.00	564.27	990.97	449.50
	13-Aug	1193.00	564.27	974.63	442.09
	Final	1190.00	564.27	972.18	440.97
	Targeted Final BW	**1149.25**	**521.29**	**938.89**	**425.87**
405	**initial**	**1108.00**	**502.58**	**905.19**	**410.59**
	9-Jul	1120.00	502.58	915.00	415.03
	30-Jul	1095.00	502.58	894.57	405.77
	13-Aug	1090.00	502.58	890.49	403.92
	Final	1090.00	502.58	890.49	403.92
	Targeted Final BW	**1025.17**	**465.01**	**837.52**	**379.89**