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Faculty of Veterinary Medicine and Animal Sciences

Effects of altered body weight and body fat content on performance, recovery response and locomotion asymmetry in the Icelandic horse

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Abstract

The objective of this thesis was to investigate the effects of altered body weight and body condition score on physiological response to exercise in terms of performance, recovery and locomotion asymmetry in Icelandic horses. Obesity is a commonly rising problem in modern horse management. Related health disorders include laminitis and even impaired locomotion. The Icelandic horse is considered an easy keeper, with low energy requirements. It is therefore prone to obesity.

The study was in a change-over arrangement, where nine horses were submitted to two different forage-only feeding strategies, a high energy allowance (HA, 64 MJ ME/day) and a restricted energy allowance (RA, 32 MJ ME/day), for a 28-day adaptation period followed by a week of data collection. The horses were trained five times a week. For data collection, the horses performed a standardized exercise test (SET) and a simulated breed evaluation field test (BEFT) together with locomotion asymmetry analysis. Blood samples were collected together with measurements of rectal temperature, respiratory rate and heart rate.

The main results were that horses adapted to the high energy allowance had significantly higher body weight, body condition score and fat percentage. Altered body weight and body condition score affected the physiological response to exercise. Horses adapted to HA had lower judges´ scores in a BEFT for total score, gallop and for form under rider. Horses adapted to RA had higher plasma lactate. The RA horses moreover had higher mean speed and maximum speed during a BEFT, thus able to perform under higher exercise intensity. In the SET and BEFT, RA horses had higher haematocrit.

In both exercise test, the recovery pattern of respiratory rate and rectal temperature was altered, indicating a decreased capacity to cope with exercise for the HA horses. The horses adapted to HA had higher front limb asymmetry compared to RA horses. With all the results combined, it is concluded that horses with higher body weight and body condition score have a decreased performance capacity at high exercise intensities.

Keywords: Body weight, body condition score, breed evaluation field test, Icelandic horse, performance, recovery, locomotion asymmetry.

Ágrip

Markmið þessa verkefnis var að rannsaka áhrif mismunandi holdastigs á frammistöðu, endurheimt og hreyfifræðilegt jafnvægi í íslenskum hestum. Offita hrossa er stórt vandamál og er tengd háu holdastigi og hefur í för með sér heilsufarsvandamál, svo sem efnaskiptavandamál, hófsperru og jafnvel hamlaða hreyfigetu. Íslenski hesturinn er talinn vera “easy keeper”, með lágar orkuþarfir til viðhalds og hefur því tilhneigingu til offituvandamála.

Tilraun var framkvæmd á skipti formi, þar sem níu í tveimur hópum voru fóðruð á tvo mismuandi vegu, há-orku fóðrun (64 MJ ME/dag) og lág-orku fóðrun (32 MJ ME/dag), til að ná fram breytileika í holdastigi hópanna. Hrossin voru fóðruð í 28 dag í senn samhliða einni viku af gagnasöfnun. Hrossin voru þjálfuð fimm sinnum í viku. Fyrir söfnun gagna voru hrossin sett í tvenns konar frammistöðupróf, á hlaupabretti annars vegar og í eftirlíktri kynbótasýningu hins vegar. Einnig var hreyfifræðilegt jafnvægi mælt. Blóðsýni voru tekin, en einnig voru öndunartíðni og líkamshiti í endaþarmi mæld.

Hross á há-orku fóðrun voru þyngri og í hærra holdastigi samanborið við hross á lág-orku fóðrun. Hross í hærra holdastigi hlutu marktækt lægri einkunnir fyrir stökk, fegurð í reið og í aðaleinkunn hæfileika í kynbótadómi samanborið við hross í lægra holdastigi. Hrossin í lægri holdastigi höfðu hærri mjólkursýru í blóðui eftir kynbótasýningu, en höfðu hærri meðalhraða og hámarkshraða í sýningunni. Þau gátu því unnið undir meira álagi en hross í hærra holdastigi. Ennfremur höfðu hross á lág-orku fóðrun hærra hlutfall rauðra blóðkorna í blóði. Í báðum hlaupaprófum var mynstur endurheimtar breytt milli mismunandi meðferða. Hestar í hærra holdastigi höfðu hærri öndunartíðni, en hærri líkamshita í kynbótasýningu, vegna aukins álags. Þetta bendir til þess að hross í hærra holdastigi gætu verið lengur að ná fullri endurheimt eftir þjálfun.

Jafnframt höfðu hross í hærra holdastigi minna jafnvægi í hreyfingu, mælda í framfótum. Þegar niðurstöður eru dregnar saman er það ályktað að umfram líkamsþyngd og holdastig skerðir frammistöðu, breytir endurheimtarmynstri og minnkar jafnvægi í hreyfingum og þar sem getu til að framvæma vinnu undir hærra álagi, þ.e. skerðir möguleika hestsins að sýna sanna reiðhestshæfileika sína.

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Abbreviations

AST	Aspartate amino transferase
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
BEFT	Breed evaluation field test
BCS	Body condition score
B.p.m.	Beats per minute
BW	Body weight
BWR	Body weight ratio
CNS	Cresty neck score
CP	Crude protein
DM	Dry matter
GH	Growth hormone
HA	Horses adapted to high energy allowance
Hct	Haematocrit
HR	Heart rate
HR_{max}	Maximum heart rate
HR_{peak}	Peak heart rate
min	minutes
ME	Metabolizable energy
MJ	Mega joule
RA	Horses adapted to restricted energy allowance

RBC	Red blood cells
RT	Rectal temperature
RR	Respiratory rate
SD	Standard deviation
S.E.	Standard error
SET	Standardised exercise test
TPP	Total plasma protein
VOS	Digestibility of organic matter
VSF	Vector sum front
VSH	Vector sum hind
V_{mean}	Mean speed during BEFT
V_{max}	Maximum speed during BEFT
V_{La4}	Estimated speed at plasma lactate concentration of 4 mmol/L

1 Introduction

The Icelandic horse is a popular riding horse, in its native country as well as worldwide. Today roughly 75.000 horses populate Iceland. Over 260.000 Icelandic horses are registered worldwide in 21 countries (FEIF, 2017a).

The Icelandic horse breed is considered pure-bred, due to its isolation for centuries (Aðalsteinsson, 1981). The closest relatives are thought to be the Norwegian Fjord horse and the Shetland pony, originating from Scandinavia and Great Britain (Hreiðarsdóttir and Hallsson, 2010) and the Swedish Gotlandsruss (Vilá *et al*., 2001). The Icelandic horse is strictly speaking a pony, since the average height on withers is under 147 cm. The breed has long been considered strong, robust and healthy with a great working mentality (Björnsson and Sveinsson, 2006). Since the settlement of Iceland, the Icelandic horse was mainly used as a working animal and as a food source. After the industrialization of Icelandic agriculture, the horses' utility changed to a riding and leisure horse and the breed has been rapidly changing through selective breeding. The first regional breeding evaluation of the Icelandic horse was held in the year 1906 and its breeding has been progressing ever since (Björnsson and Sveinsson, 2006).

In contrast to the most common horse breeds of the world, the Icelandic horse is a five-gaited horse, able to perform in gaits of lateral locomotion pattern, namely tölt and pace in addition to walk, trot and canter. This is a result of a single base mutation on the DMRT3 gene (Andersson *et al*., 2012). The breeding goal of the Icelandic horse, first presented in 1950 is to breed the light body type of the Icelandic horse, with emphasis on strength, flexibility and muscular body (FEIF, 2015). The breeding goal is a multi-trait goal and therefore emphasis in training is more versatile compared to race horse breeds such as Standardbreds and Thoroughbreds where speed and endurance is a key component (Khadka, 2010; ASVT, 2015). The scientific knowledge on training effects and training response on the Icelandic horse is scarce. The study by Stefánsdóttir *et al*. (2014) is the first study documenting the physiological response to exercise in the Icelandic horse, clearing the path for future research regarding their exercise physiology and training response.

Training of the Icelandic horse can be divided into leisure riding and to improve gait qualities according to either BEFT- or competition standards. The traits measured in BEFT and the competitions have shown to be genetically correlated (Albertsdóttir *et al*., 2008) and contain similar attributes, thus training is expected to be of similar content, although physiological response between various disciplines is different (Stefánsdóttir, 2015).

In recent years, numerous related disorders have become more common in domesticated horses, such as obesity (Thatcher *et al*., 2008; Geor, 2008; Wyse *et al*., 2008; Robin *et al*., 2015). Obesity in horses can be a cause for several related symptoms, such as equine metabolic syndrome (EMS), laminitis and indications of impaired locomotion (Treiber *et al*., 2006; Frank *et al*., 2010). On the other hand, malnourishment has been connected to the prevalence of gastric ulcers in Standardbred trotters (Dionne *et al*., 2003).

The physical state of Icelandic horses has been recorded in recent studies (Ragnarsson and Jansson, 2011, Stefánsdóttir *et al*., 2014; Jensen *et al*., 2015). The Icelandic horse is considered to be an "easy keeper" relating to their low energy maintenance requirements, i.e. compared to Standardbred trotters (Ragnarsson and Jansson, 2011). A study by Jensen *et al*. (2015) showed that 24% of Icelandic horses in Denmark are overweight or obese (10.2%). Furthermore, they found that owners tend to underestimate the BCS of their horse compared to an experienced evaluator. The effects of body condition on metabolism and physiological response during and after exercise is yet to be determined in the Icelandic horse. In recent decades, the effects of body condition and body composition on performance have been shown in Arabian horses during endurance racing (Garlinghouse and Burrill, 1999), in Standardbred trotters (Kearns *et al*., 2002a; Kearns *et al*., 2002b: Leleu and Cotrel, 2006) and in Thoroughbred racehorses (Fonseca *et al*., 2013).

2 Literature review

2.1 Adipose tissue

When the amount of energy that enters the body exceeds the amount of energy expended, the excess energy is mainly stored in the form of triglyceride, or fat, forming adipose tissue, a subcutaneous fat layer and around internal organs. This process occurs when the body is in a positive energy balance as the animal builds up nutritional and energy reserves in the form of adipose tissue (Lafontan and Langin, 1995; Gray and Vidal-Puig, 2007). Distribution of body fat is inherited, an individual has no control over where the fat is allocated (Berry et *al*., 2013). When the energy expenditure exceeds the intake, the animal can use the reserve energy, due to exercise, poor feed availability or environmental conditions. Each fat molecule contains roughly twice as much energy compared to protein and carbohydrates, and is therefore a desirable energy storage unit (Sjaastad *et al*., 2016). Increased adiposity is obtained by adipocyte recruitment- and enlargement (Gray and Vidal-Puig, 2007). The triglycerides are hydrolysed into glycerol and free fatty acids, that are utilized for energy. There are two types of adipose tissue, namely white and brown, with white being predominant in larger mammals. Adipose tissue is distributed all over the body with prominent depots in the mesenteric, retroperitoneal, perigonadal, inguinal and scapular regions, described as either visceral or subcutaneous depots. Subcutaneous fat layer acts as an insulator, preventing rapid fluctuation in body core temperature.

The adipocyte distribution is genetically controlled through expression of peroxisome proliferator-activated receptor gamma (PPARγ), a hormone receptor in the nucleus of adipocytes. PPARγ plays a vital role in the genes controlling glucose sensing, lipid synthesis and lipid storage (Berry *et al*., 2013). In a positive energy balance, adipose tissue is deposited in intermuscular, intramuscular, visceral and subcutaneous regions (Zhou *et al*., 2014; Sjaastad *et al*., 2016). Dugdale *et al*. (2011b) showed equal distribution between visceral and subcutaneous fat deposition in ponies, meaning that only a proportion of the changes in fat mass are detected by palpation. A relationship between white adipose tissue deposition and BCS ($r^2 = 0.96$) has been shown in Welsh mountain ponies (Dugdale *et al*., 2011b).

2.2 Body condition scoring

Various methods have been applied to assess the subcutaneous fat reserves in horses, most often referred to as body condition score (BCS). The method can be used to evaluate health and as tool for feed ration calculations as an index of energy balance. Currently in Iceland, the body condition of the Icelandic horse is evaluated by a five-degree scale described by Stefánsdóttir and Björnsdóttir, (2001). This method involves palpation of the caudal rib area and scoring the fat deposition, together with subjectively evaluating the horse's general health, muscle mass, eye expression and coat condition.

Henneke *et al*. (1983) developed a nine-degree scale for subjective evaluation of subcutaneous fat deposition in horses. It involves physical palpation of six different body areas, namely neck, withers, shoulders, ribs, loins and tail head, along with visual assessment of overall picture. The scale was developed on mature Quarter Horse broodmares. It is the most widely spread BCS system for horses, but has also been validated for fat accretion description for other horse breeds such as Thoroughbred (Suagee *et al*., 2008) and Standardbred racehorses (Leleu and Cotrel, 2006). The Henneke scale positively correlates with body fat percentage, weight, height/weight ratio, girth circumference, but not to height (Henneke *et al*., 1983).

Westervelt *et al*. (1976) reported a method to evaluate actual body fat content of horses, using ultrasonic measurements on several sites of the body. The measurement of rump fat was most highly correlated to actual rump fat thickness and they showed a linear relationship between subcutaneous rump fat thickness and body extractable fat content. Ultrasonic measurements of the shoulders and ribs did not correlate to total body fat to the same extent and therefore the rump fat measurement has become the standardised measuring site. This method has been used widely (Henneke *et al*., 1983; Kearns *et al*., 2002a; 2002b; Dugdale *et al*., 2011a; Fonseca *et al*., 2013; Ringmark *et al.*, 2013) as an assessment for total body fat content, as for comparison in scientific research. Furthermore, the study showed that adipose deposition does not degenerate at the same rate, where shoulder fat and rump fat decreased after 30 days, but rib fat and BW remained constant, for ponies subjected to two different treatments, fed *ad libitum* and a restricted allowance. This implies the importance of evaluating multiple body parts when assessing equine body condition.

However, Dugdale *et al*. (2011a) showed an exponential relationship between percentage body fat (fat%) and BCS in ponies. Moreover, the sensitivity of the correlation between fat% and BCS decreases with increased fat deposition, implying that small changes in BCS in moderately obese to obese horses, (BCS over 6 on the Henneke scale) is unreliable.

Morphological measurements such as girth circumference and neck circumference have been used as indicators of BW (Matthíasdóttir, 2012) and BCS (Dugdale *et al*., 2010). Carter *et al*. 2009, developed a method for evaluating neck adiposity, that correlated highly to plasma insulin concentration. Frank *et al*., 2006 found neck circumference to correlate highly to metabolic profiles of glucose and insulin concentration, relating to risk of insulin resistance.

Excessive body condition, or obesity, has been linked to various metabolic complications such as plasma lipoprotein concentration, glucose intolerance, hyperinsulinemia possibly due to hormone production of leptin and adiponectin by the adipocytes, diabetes, hypertension and several cardiovascular diseases in horses (Després *et al.*, 1987), as well as cancer and heart diseases in humans (Friedman, 2009). Precise evaluation of body condition is beneficial when striving to maximize health and welfare of the horse.

2.2.1 BCS and performance

In humans, there has been various reports of correlation between lean body mass and aerobic exercise performance (Hetland *et al*., 1998; Cosgrove *et al*., 1999), as well as in horses.
BCS is affected by sex in Standardbreds, Thoroughbred and Icelandic horses (Kearns *et al*., 2002a; Christie *et al*., 2006; Fonseca *et al*., 2013; Stefánsdóttir *et al*., 2014). They all reported lower BCS in male horses and the lower BCS in stallions is contemplated as a possible explanation for their superior performance in a field exercise test compared to mares. Furthermore, several studies have showed the effects of fat mass and body composition on performance, connected to lower fat% (Leleu and Cotrel, 2006), increased fat-free mass (Kearns *et al*., 2002b; Fonseca *et al*., 2013) and power/weight ratio (McMiken, 1983). These studies included either Standardbred (Kearns *et al*., 2002b; Leleu and Cotrel, 2006) or Thoroughbred horses (Fonseca *et al*., 2013) with body fat content ranging from 5-14%. Garlinghouse and Burrill (1999) showed that BCS affected completion rate of Arabian horses in a 160-km race, where depleted BCS had detrimental effects on the completion rate.

The reasons for these effects are yet to be explained, whether they are due to altered metabolism, increased weight-bearing or both. Studies examining increased weight-bearing of horses have found increased load carried is proportionate to its oxygen consumption, implying higher exercise intensity (Taylor *et al*., 1980; Pagan and Hintz, 1986; Thornton *et al*., 1987). Compared with a heavier horse, a horse of less mass is expected to expend proportionally less energy to move the same distance at the same speed. From the available publications, it can be implemented that BCS affect the aerobic respiration and performance capacity, and a proper body condition is essential when striving for maximum health and performance in equine athletes.

2.3 Exercise energy metabolism

The athletic ability of an individual is determined by genetics, environmental factors and finally it´s training (Hodgson and Foreman, 2014). The environmental variables include aerobic respiration capacity, subcutaneous and intramuscular energy stores of fat and glycogen, respiratory capacity of the skeletal muscle, splenic contraction, gait efficiency and thermoregulation.

Locomotion is the result of muscle contractions. When muscle sarcomeres contract and alter the cross-bridge orientation, energy is needed (Valberg, 2014). This energy is derived from the cleavage of adenosine triphosphate (ATP), at the head of every myosin filament. The reaction is catalysed by the enzyme ATP-ase, and produces adenosine diphosphate (ADP), a free phosphate molecule and energy (Sjaastad *et al*., 2016). This energy drives the muscle contraction. This process is either performed by aerobic or anaerobic pathways, depending mainly on oxygen availability to the cell, and ATP/ADP ratio. The energy substrates used for muscle contraction are blood glucose, glycogen derived from the liver or skeletal muscle, fat and protein to a limited extent. Glycogen has proven to be the most important energy substrate for the athletic horse, especially at high exercise intensity, although fat can be a preferred energy substrate for low intensity exercise or endurance racing (Essén-Gustavsson *et al*., 1984), and a fat deposition to some extent can be beneficial and a preferred energy substrate for long distances (Garlinghouse and Burrill, 1999). The horse has a slower glycogen repletion post exercise compared to humans, around 72 hours in the horse (Lacombe *et al*., 2001) compared to 24 hours in humans (Snow *et al*., 1987).

2.3.1 Aerobic and anaerobic pathways

In the inner membrane of the mitochondria, β-oxidation of free fatty acids, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation via the electron transport chain are the aerobic production paths of ATP. Aerobic exercise of the body is determined by its capacity of uptake, transport and utilization of oxygen from the alveoli to the skeletal muscle cells. The process produces high amounts of energy but at a low rate (Hodgson, 2014; Sjaastad *et al*., 2016).

When there is insufficient oxygen available, the body performs anaerobic respiration, at the initial moment of exercise as well under intense exercise. The pathways occur in the muscle cell cytoplasm. The most important pathway for anaerobic energy metabolism is via the glycolytic pathway, producing ATP along with the by-products lactate and H^+, that causes a drop in cellular pH. This pathway produces fast available energy, but fewer ATP molecules per energy unit are produced, the residues resulting in the by-products and heat. Furthermore, to the aerobic capacity of the athletic horse, it has astonishing capabilities of respiratory compensation for metabolic acidosis due to lactate accumulation and a free hydrogen ion (H^+).

2.4 Exercise testing

To evaluate the physiological fitness of a horse it must be submitted to exercise. This can be done on a treadmill or in actual situations in the field. In this study, physiological responses were assessed in both a simulated breed evaluation field test and a treadmill test. Common protocols include standardised, incremental exercise test or a single step, high speed test. The only publication on Icelandic horses involving a field test is the study by Stefánsdóttir *et al*. (2014), which will be used as a reference comparison in this thesis.

2.4.1 Standardised exercise test

Standardised exercise tests (SET) are often performed on a treadmill, in controlled conditions. By performing a standardised treadmill test, numerous variables are closely controlled, such as exercise intensity with speed and incline. Environmental factors such as weather conditions are more carefully controlled and there is increased repeatability of the SET and it enhances the possibilities of parameters to be measured, most importantly blood sampling and measurements during exercise.

Several biological parameters have been linked to higher performance during a standardised exercise test on a treadmill, such as the lactate threshold (V_{La4}) and haematocrit (Hct) concentration (Persson, 1967; Persson and Ullberg, 1974; Seeherman and Morris, 1990). Heart rate and lactate response has been shown to be lower on an uninclined treadmill compared to a field test (Persson, 1983), possibly due to air resistance in field and the driving force of the treadmill. It has been estimated that roughly 25% of the horses' energy is expended at high speeds to overcome air resistance (Hodgson and Foreman, 2014). Nostell *et al*. (2006) found no differences in HR, plasma protein concentration, Hct in an inclined treadmill test simulating racing conditions compared to a simulated race on a field track, but difference in blood plasma lactate response.

The field test is most representative of actual competition- and training conditions, and are more easily interpreted for the industry and applicable to training and competition, but much more challenging to standardise, in terms of exercise intensity. To the author's knowledge, a study on the physiological response of the Icelandic horse to a standardised, incremental exercise test performed on a treadmill has not been done before. The treadmill exercise test designed and used in this study was a standardised, incremental exercise test, gradually increasing speed in four different steps.

2.4.2 Breed evaluation field test

A breed evaluation field test (BEFT) is a field exercise test, designed to determine which horses are best fitted for breeding. The breeding goal of the Icelandic horse (FEIF, 2015) describes the optimal riding horse. As previously described by Stefánsdóttir *et al*. (2014), the BEFT consists of three parts; objective body measurements, conformational evaluation and judging of riding abilities. Horses are evaluated and scored on a half-point scale from 5-10. For assessment of riding abilities, the horse is ridden on a straight track, 4-6 meters wide and 250-300 meters long, for six to ten rounds, where the horse is subjectively evaluated for gait quality on all gaits in addition to slow tölt and canter, along with spirit and form under rider. In an actual BEFT, horses are evaluated two times, the second assessment designed to highlight the horses´ quality or to improve faults from the initial assessment. In this study the horses were only evaluated in the first time. According to the evaluation scale for all gates except for walk, high speed is vital for reaching scores from 9 to 10. Speed has been correlated with higher heart rate, plasma lactate concentration and rectal temperature in the Icelandic horse (Stefánsdóttir *et al*., 2014) and therefore, both high aerobic as well as anaerobic metabolic capacity are important for reaching the highest scores. BEFT is a field exercise test, with no limits or goals regarding exact distance or speed. There is even variation in how many gaits are shown, depending on the capacity to pace and how often each gait is ridden. Therefore, it is difficult to standardise the test precisely between individual horses and experimental periods.

2.5 Physiological parameters of importance to exercise

2.5.1 Heart rate and oxygen uptake

The athletic horse has an extraordinary capacity to perform aerobic work. The equine cardiovascular system has a unique O_2 transport capacity from the lungs to the body tissues. The secret lies in the horse´s ability to release an extra amount of red blood cells into the bloodstream via splenic contraction, that is hormonally regulated through external stimuli and via the sympathetic nerve system. During maximal exercise, O_2 uptake can exceed resting values up to 50 times (McGowan and Hodgson, 2014). Oxygen uptake is commonly referred to as the limiting factor to a race horses' performance.

Oxygen is transported throughout the body via the bloodstream. During exercise, blood transport or cardiac output depends on the heart rate (HR) and the stroke volume of the heart. The heart´s stroke volume is an important contributor to blood transport capacity. The heart muscle has been shown to increase in size with training, increasing the volume of blood pumped out in each stroke (Evans and Polglaze, 1994; McGowan and Hodgson, 2014). Correlation between heart score and race performance has been documented (Young and Wood, 2001) in national hunt horses and its´ oxygen uptake capacity is affected by heart size in conditioned Thoroughbreds (Young *et al*., 2002). Heart rate has not been adapted as performance parameter, but it is widely used as an indication of exercise intensity. General heart rate measurements can however accurately control exercise intensity during exercise. Normal resting HR ranges between 20-30 b.p.m. in fit horses (McGowan and Hodgson, 2014). Maximum heart rate (Hr_{max}) has been measured at level up to 240-250 b.p.m. in Thoroughbred racehorses (Evans and Rose, 1988), compared to around 200 b.p.m. in athletic humans (Noakes, 1992). Lower HR during submaximal exercise is positively correlated to racing performance (Marsland, 1968). Maximum HR will not be altered by training, but the maximum speed under any given HR will be affected by training. The speed when the horse reaches their maximal oxygen uptake (VO_{2max}), has been shown multiple times to improve with training (Art and Lekeux, 1993; 1988; Tyler *et al*., 1996). This improvement is related to the cardiac output, O_2 extraction or both. Thoroughbred racehorses have values of VO_{2max} of around 140 mL O_2/kg/min, roughly twice the human capacity per kg bodyweight (Noakes, 1992; Rose *et al*., 1988). Fonseca *et al*. 2010 investigated the effects of body composition on physiological parameters in trained Thoroughbred race horses, ranging from 4.5-4.7 ± 0.2% in estimated total body fat content. No relationship was found between speed, heart rate or lactate concentration and body composition.

Icelandic horses performing at BEFT could be at their maximum heart rate level with a mean HR of 184 ± 13 b.p.m. during BEFT and HR_{peak} of 224 ± 9 b.p.m., indicating even supramaximal exercise (Stefánsdóttir *et al*., 2014). Oxygen uptake remains high post exercise due to the body's demand for O_2 for phosphocreatine resynthesis, catabolism and anabolism of lactate, high body temperature and hormonal restoration, referred altogether as oxygen dept. The rate of restoration to these parameters to resting state reflects upon the fitness of a horse to cope with exercise. This high demand is met by high HR and respiratory rate (Lekeux *et al.*, 2014). Recovery heart rate decreases in a bi-exponential manner (Bitschnau *et al*., 2010), and its rate can indicate aerobic capacity. Post exercise recovery heart rate is correlated with exercise intensity and plasma lactate accumulation in Icelandic horses following a BEFT (Stefánsdóttir *et al*., 2014).

2.5.2 Lactate and lactate threshold

Lactate is a by-product metabolite of anaerobic respiration in the process of anaerobic glycolysis (McMiken, 1983). Lactate is produced in the working muscle during all exercise intensities and increases exponentially with higher exercise intensity (Lindholm and Saltin, 1974; Judson *et al*., 1983; Harris *et al*., 1991). When there is insufficient O_2 to oxidize pyruvate in the mitochondria, lactate is produced.

Blood plasma lactate concentration is commonly used as a parameter to evaluate exercise intensity and the extent of anaerobic respiration. High levels of lactate (20 mmol/L) can indicate onset of fatigue (Schuback *et al*., 1999). Lactate accumulation increases exponentially with speed during an incremental SET (Davie *et al*., 2002) and can therefore accumulate at high rates during high intensity exercises. The production of lactate is although beneficial, as it regenerates NAD^+, which is used in oxidation of glyceraldehyde 3-phosphate during production of pyruvate from glucose in aerobic conditions. The lactate ensures continuous energy utilization via the glycolytic pathway, also being used directly as an energy source for liver and heart muscle during exercise (Pösö *et al*., 2008).

However, during high intensity exercise, the respiratory chain cannot keep up with the amount of hydrogen atoms that join to form NADH, and cannot regenerate NAD^+ at a sufficient rate. The lactate threshold represents the speed at which the athlete reaches lactate plasma concentration of 4 mmol/L (V_{La4}), referred to as the anaerobic threshold. This corresponds to the level of exercise when the production of lactate is greater than its efflux from muscle fibres to the bloodstream and it accumulates in the skeletal muscle and plasma (Valberg, 2014). Increased V_{La4} is reported as increased aerobic fitness (Hodgson and McGowan, 2014). V_{La4} has been correlated to performance in actual races for pacing Standardbreds (Davie *et al*., 2002). Resting values are generally around 0.3-1.0 mmol/L (Keenan, 1979; Judson *et al*, 1983; Snow *et al*., 1983; Bayly *et al*., 2006, Stefánsdóttir *et al*., 2014). Maximum values often rise to 20-40 mmol/L after high intensity exercise, such as Standardbred and Thoroughbred racing or breed evaluation field test in Icelandic horses (Keenan 1979; Snow *et al*., 1983; Evans *et al*., 2002; Stefánsdóttir *et al*., 2014). Numerous studies have shown that plasma lactate concentration after maximal exercise are higher in better performing horses (Persson and Ullberg, 1974; Räsänen *et al*., 1995; Stefánsdóttir e*t al*., 2014), underlining the importance of anaerobic metabolism at higher exercise intensities. The enzymatic activity related to lactate has been shown to alter by training and detraining, namely lactate dehydrogenase, the enzyme that catalyses the conversion to lactate to pyruvic acid and back. Thus, the cell controls the lactate production (Karlsson *et al*., 1974). Mykkänen *et al*. 2010 found breed differences in lactate transporters expression, being highest in Thoroughbred racehorses. The rate of removal of lactate from the bloodstream depends upon the metabolic state of the horse. Light exercise increases oxygen uptake and thus oxidation of lactic acid. The half-life of lactate can be decreased by 50% after a treadmill test compared to stationary horses (Marlin *et al*., 1987).

Blood lactate concentration is either measured by laboratory enzymatic methods or by portable devices in the field. Values derived from portable devices from blood are lower, 20-50% compared to plasma laboratory values, being inaccurate in high concentration of lactate (Räsänen *et al*., 1995; Stefánsdóttir *et al*., 2012).

2.5.3 Haematocrit

Red blood cells (RBC) are essential in the O_2 transporter units from the lungs alveoli to utilization in the skeletal muscle, and to transport CO_2 back out of the system (Alberts *et al*., 2008; Weibel *et al*., 1991). They are produced in the bone marrow of mature animals, containing hemopoietic stem cells (Alberts *et al*., 2008; Akers and Denbow, 2008). The possible oxygen concentration in arterial blood depends upon red blood cell ratio to the total blood volume, namely haematocrit (Hct) and red cell haemoglobin content. The haemoglobin subunit carries O_2 molecules using iron ions (Fe^{3+}) (Akers and Denbow, 2008; Kingston, 2008). Hct has been shown to increase with training and to be correlated with performance in racing- and riding horses (Persson 1968; 1983a; Stewart and Steel, 1975; Stefánsdóttir *et al*., 2014). The horse can release large amounts of red blood cells via splenic contraction into the bloodstream, from values of 32% to 46% and up to 60% to 70% (McGowan and Hodgson, 2014), regulated by hormonal release of mainly noradrenalin (McGowan and Hodgson, 2014). Persson *et al*. (1973) reported that a horse can withhold up to 50% of its red blood cells in the spleen, that can be released, greatly influencing blood volume and haematocrit during exercise and therefore the horse´s oxygen uptake capacity, thus withholding natural blood doping mechanism. Red blood cells also act as a repository for lactate during exercise (Bayly *et al*., 2006) and serve to keep intramuscular levels of lactate lower at bayan lowering pH, possibly delaying the onset of fatigue. Changes in Hct during exercise can also be a result of fluids shifts due to changes in heart rate and blood pressure (Snow *et al*., 1983). Resting values of Hct for Standardbreds and Thoroughbreds and Icelandic horses vary from 30 to 40 % (McGowan and Hogdson, 2014; Stefánsdóttir *et al*., 2014). Resting Hct has not been correlated to performance, but endurance horses have lower resting Hct indices than Thoroughbred race horses (McGowan and Hodgson, 2014). There may be a reduction in Hct with training in endurance horses because of an expansion of plasma volume (McGowan and Hodgson, 2014). A linear relationship between Hct and exercise intensity has been reported, reaching maximum concentration when approaching VO_{2max} (Rose and Allen, 1985). Maximum Hct in Standardbreds and Thoroughbreds racehorses reach 60-68% after high intensity exercise (Persson, 1983; Snow *et al*. 1983; Evans *et al*., 1993). The Icelandic horse has reported values of 45 ± 3 % sampled within 5 min post- BEFT exercise, ranging from 36-55% (Stefánsdóttir *et al*., 2014). This is a substantially lower concentration, implying lower aerobic capacity of the Icelandic horse, possibly due to breeding, training practices and other environmental factors (Stefánsdóttir, 2015).

2.5.4 Total plasma protein

Total plasma protein (TPP) is widely used as an indicator of hydration status in horses (McGowan and Hodgson, 2014). TPP increases during exercise due to decreased plasma volume as hydrostatic pressure pushes fluids out of vessels and arteries, increasing with duration and intensity of exercise. (Carlson, 1983; Judson *et al*., 1983; Seeherman and Morris, 1990; McKeever *et al*., 1993; Danielsen *et al*., 1995; Hargreaves *et al*., 1999). TPP has shown to be significantly elevated in dehydrated animals (Kingston, 2008; McGowan and Hodgson, 2014). In mature performing horses, normal resting levels are 55-75 g/L (McGowan and Hodgson, 2014). Due to the variation in resting values, it can be difficult to detect changes in fluid balance, especially in horses with lower resting values. The plasma concentration of albumin is affected by changes in plasma water content and intravascular water volume (Kingston, 2008). Dehydration can increase plasma fibrinogen concentration. Globulin changes indicate clinical signs such as inflammation. Increases in plasma protein associated with short-duration exercise return to pre-exercise values within 15–30 min of exercise, if not dehydrated (Hargreaves *et al*., 1999; Judson *et al*., 1983).

2.5.5 Respiratory rate

After exercise, physiological parameters return to resting values. The return rate depends upon the exercise intensity, duration, the state of fitness and the environmental conditions (Lekeux *et al.*, 2014). Oxygen uptake remains elevated post-exercise due to the body's demand for O_2 for phosphocreatine resynthesis, catabolism and anabolism of lactate, high body temperature and hormonal restoration, referred altogether as oxygen dept. The rate of restoration to these parameters to resting state reflects upon the horses' fitness to cope with exercise. This high demand is met by high respiratory rate (RR), while tidal volume remains constant (Lekeux *et al.*, 2014). The high respiratory rate is vital for post exercise thermoregulation, losing heat by evaporative cooling as moisture, taking part in returning the body in its normal, resting metabolic state. RR has been shown to be significantly higher in ponies after the same treadmill exercise in hot and humid conditions compared to dry and cold climate (Art and Lekeux, 1988).

RR is usually measured by counting by hand each breath manually or by listening. This can only be done accurately in a stationary position. RR can be measured during exercise with a capnograph analyser (Evans *et al*., 2011). In this study, RR was measured by feeling air flow by hand manually from the nostrils and by listening to lung activity with a stethoscope. During exercise, respiratory rate is in close correlation with stride frequency, increasing in breaths/stride with increased exercise intensity at trot. During gallop, the RR couples to the locomotion keeping a constant 1:1 stride/breath ratio (Ainsworth, 2008; Franklin *et al*., 2012). Normal equine respiratory rate varies from 8-19 breaths/min, and can exceed to levels of 120-130 breaths/min following a high intensity treadmill exercise (Butler *et al*., 1993; Ainsworth and Cheetham, 2010; Lekeux *et al*., 2014). Stefánsdóttir *et al*. (2014) reported resting RR of 30 ± 11 breaths/min and within 5 min post BEFT a RR of 101 ± 30 breaths/min. A decrease in RR with age was also seen.

RR was affected by speed, increasing by 5 breaths/min for every 1 km/hour in increase of speed. Values as high as 133 breaths/min have been reported in Standardbred horses running on a treadmill (Dahl *et al*., 1987; Franklin *et al*., 2012). RR increased by 3 breaths/min for every increase in body weight ratio (BWR) of the rider to the horse (Stefánsdóttir, 2015). Horses had longer recovery rate of RR after tölt compared to trot (Stefánsdóttir *et al*., 2015). Horses did not reach resting values of RR after 100m pace race 30 min post-exercise.

2.5.6 Rectal temperature

Exercise produces high heat loads in an exercising horse (Hodgson *et al*., 1993). Low surface area to mass ratio puts great demands on thermoregulation through respiration. It has been suggested that a combination of elevated body temperature, lowered pH and oxygen transport all contribute to limiting exercise capacity in horses (Hodgson *et al*., 1990). Rectal temperature differs from core temperature due to the time it takes the circulatory system to dissipate throughout the whole body. In a study by Hodgson *et al*. (1993), horses were exercised on a treadmill at different intensities to the onset of fatigue. RT continued to rise 3 min post high intensity exercise, and had similar maximum values after various exercise intensities, as well as showing lower temperature values in the rectum compared to venous blood- and muscle temperature, illustrating that RT could deviate from actual core temperature due to the time it takes the circulatory system to dissipate throughout the whole body. In this study, rectal temperature was measured.

Normal rectal temperature ranges from 37.5-38.5 °C (Hodgson *et al*., 1993; Stefánsdóttir *et al*., 2014; Hodgson, 2014). The greatest challenge to the healthy equine athlete regarding thermoregulation is generally exercise and its intensity is closely related to post-exercise body temperature. RT can rise from resting values of 37.2 °C to 40.3 °C post high intensity treadmill exercise of around 5 min (Hodgson *et al*., 1993), and from 37.8 ± 0.3 °C up to 39.5 ± 0.5 °C within 5 min post BEFT (Stefánsdóttir *et al*., 2014). Peak values were recorded up to 40.4 °C in BEFT and are similar for Standardbred trotters following high intensity treadmill exercise (Lindholm and Saltin, 1974).

2.5.7 Aspartate amino transferase

Aspartate amino transferase (AST), is a muscle enzyme commonly used to indicate muscle damage. AST is a cytoplasmic and mitochondrial enzyme that catalyses the deamination of aspartate to form oxaloacetate, which can enter the citric acid cycle. In humans, elevations in AST has been connected to decreased locomotion coordination and fatigue associated to overtraining, increasing the risk of injury (Fry *et al*., 1991). Elevations in AST concentration are found in horses with rhabdomyolysis, and have been connected to hepatocyte damage, muscle damage, or in vitro haemolysis (McGowan and Hodgson, 2014). The enzyme enters the bloodstream due to changes in membrane permeability changes after strenuous exercise (Snow *et al*., 1983; Stefánsdóttir *et al*., 2014; Tyler-McGowan *et al*., 1999) and in over trained horses (Hamlin *et al*., 2002). Normal values have been reported from 150-400 Units/L (McGowan and Hodgson, 2014). After severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days in humans, and has a reported half-life of 7-8 days in horses (Kingston, 2008). Stefánsdóttir *et al*. 2014 reported a significant elevation in AST after performing a BEFT, ranging from 353 ± 98 U/L up to 382 ± 106 U/L. In this thesis, AST concentration will be presented in two units, U/L but also ukat/L, the replacing SI-unit.

2.6 Locomotion asymmetry

When a horse trots, the head moves up and down twice during one complete stride, following the diagonal stride pattern. In a sound horse, there is high symmetry between left and right limb load distribution. In horses with unilateral forelimb lameness, this movement becomes asymmetric (Merkens and Schamhardt, 1988; Keegan *et al*., 2001). Furthermore, the movement of the pelvis during each step can be asymmetric. Abnormalities in the muscoskeletal system such as difference in strength or flexibility between the left and right side of the horse, stiffness or fatigue can emerge as locomotion asymmetry. The quantity of this asymmetry has been used to evaluate the degree of lameness. However, various degrees of asymmetry can occur without clinical signs of lameness nevertheless affecting performance. When a horse becomes tired, the locomotion pattern and joint load are altered (Johnston *et al*., 1999) and the risk of mistakes such as mistepping and cross firing may increase substantially, along with cumulative and acute overloading of limbs. These loads are a known lameness cause.

When the locomotion of a horse is evaluated, parameters and methods of kinetics and kinematics are used. Abnormalities in movements along with symmetry between left and right side can be measured. A subjective, visual evaluation by trained personnel, especially veterinarians has been the standard, most frequent diagnostic method (McCracken *et al*., 2012). However, it has been shown unreliable and even biased, especially in mild lameness cases (Arkell *et al*., 2006, Fuller *et al*., 2006, Keegan *et al*., 2010). Force plate measuring the kinetics of the running horse, stance and swing phases of every stride and the ground reaction forces hitting the ground is highly accurate and gives a clear description of load distribution (Merkens and Schamhargt, 1988). Poor mobility of the heavy equipment and the fact that only one stride at a time can be recorded. Along with high costs of the equipment limits it use to a research facility (Weishaupt *et al*., 2004b; Keegan, 2007; Keegan *et al*., 2012).

In the present study, a wireless, accelerometer sensor system was used to evaluate locomotion symmetry (Lameness Locator, Equinosis LLC). The software uses three accelerometers, placed on the top of the head between the ears, on top of the pelvis and one on the distal right front pastern to measure symmetry between the left and the right side in trot. The head- and pelvic sensors are a uni-axial accelerometer. The right front sensor is a uni-axial gyroscope. The difference in movement of both the head and the pelvis are described as minimum difference (min diff) and maximum difference (max diff). By calculating a vector sum, cumulative quantity of vectors from each stride, the degree of asymmetry or even lameness can be detected (Figure 1). A sensor placed on the distal right front pastern tells where in in stride cycle each vector originates and thus indicates the source of each vector, thus pointing out the possible origin of the asymmetry. That can although be challenging to detect, due to compensatory load distribution (Weishaupt *et al*., 2004a), especially during multi-limb lameness. However, the software assumes the possibility of these effects. The system has been validated in comparison to video-based evaluations (Keegan *et al*., 2004), to force plate analysis (Keegan *et al*., 2012) and to subjective, visual evaluations (McCracken *et al*., 2012).

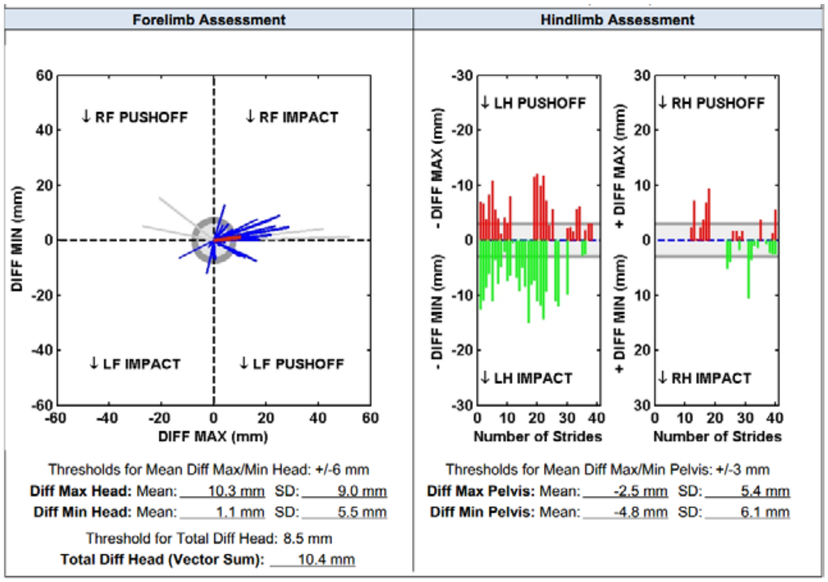

Figure 1. Locomotion analysis produced by the Lameness Locator software. The figure to the left illustrates vectors that represent head movements in terms of direction and magnitude of each stride (mm). The red line marks the vector sum that is used for interpretation of the analysis. The figure to the right illustrates the vertical locomotion of the pelvis and the difference between the left and right side (mm). Red lines represent max-diff and the green vectors represent min-diff of each stride that are used to calculate the vector sum.

2.6.1 Locomotion symmetry and performance

The effects of locomotion asymmetry on health and performance has been evaluated in several studies, though with different methods. Clinical, subjective examinations have been used as scientific as well as practical evaluations of health status in Swedish Warmblood horses (Jönsson *et al.*, 2013). Locomotion was evaluated on a straight line on walk and trot by hand, and in trot after a 60 second full limb flexion test on all limbs. Health scores in the 4-year-old Warmblood horses tested affected overall lifetime performance as well as longevity (Jönsson *et al.*, 2014). It has been shown by Ringmark (2014) that locomotion asymmetry increases after introduction of new speed training. Furthermore, lower asymmetry was associated with early qualification for races, indicating greater athletic abilities. The type of training to which racehorses are submitted is associated with alterations in locomotive tissues (Firth, 2006). Mild induced lameness can reduce VO_{2max} and increase lactate accumulation rate during exercise (Parente *et al.*, 2002). Hindquarter asymmetry has had negative effect on lifetime earnings, race number, racing records (min/km) and difficulties performing at higher speeds (Dalin *et al.*, 1985).

3 Aim of the thesis

The aim of the study in whole was to submit the horses to two kinds of exercise tests, namely a standardised incremental exercise test and a simulated breed evaluation field test and evaluate the effects of altered body fat content on the physiological response and performance and recovery response. Furthermore, the aim was to evaluate effects of altered body weight and body fat content on locomotion asymmetry in Icelandic horses.

Specific aims were to:

- Determine if altered body condition affects the physiological response to exercise in the Icelandic horse.
- Study the recovery response of Icelandic horses in different body condition, following a standardised incremental exercise test and a simulated breed evaluation field test.
- Evaluate the effects of two different body condition states on locomotion asymmetry, before and after a breed evaluation field test.

The hypothesis tested were:

- Higher body fat content impairs performance in a breed evaluation field test.
- Horses with higher body fat content have a lower recovery rate following exercise compared to horses in lower body fat content and thus decreased ability to cope with exercise.
- Horses with higher body fat content have higher locomotion asymmetry and are therefore less balanced compared to horses with lower body fat content.
- The performance of a breed evaluation field tests increases locomotion asymmetry in Icelandic horses.

4 Materials & methods

4.1 Experimental design

The study was approved by the National Animal Research Committee of Iceland. It was conducted at Hólar University College in Iceland. The study was initiated at 20th of March and ended on the second of June 2016. The study was a change-over design, where horses were fed a high energy allowance (HA) and restricted energy allowance (RA) for 36 days to create a desired body condition. Horses were subjected to both treatments, thus eliminating the effect of individual. Each period consisted of a 28-day adaptation- and training period, followed by a week involving the two exercise tests and locomotion asymmetry evaluation. Prior to the study, horses were assigned to groups so they would have similar means of body weight, body condition and age.

4.2 Horses

Ten geldings from Hólar University College were recruited for the study. They were six to eight years old, all in similar training stage. All horses have had the same type of training that winter up until the study. At initiation of the study horses weighed 401 ± 16 kg (mean ± SD). The horses were divided into two groups that were aimed for the closest mean of BCS, BW, girth circumference, V_{la4} and peak Hct. Variation between groups before initiation of the study can be

Figure 2. The coat of the horses was clipped in the presented manner, as an attempt to eliminate the effects of variations in coat loss during the experiment (experiment performed in the spring).

seen in Table 1. At the onset of the study, the horses were clipped where the winter coat was partly removed to eliminate the possible effect of different coat coverage on thermoregulation (Figure 2).

Table 1. *Mean body condition score (Henneke et al. 1983, BCS), score for overall picture, body weight (BW), girth circumference (cm), Lactate threshold (V_{La4}) and peak haematocrit (Hct) (LSM ± SE) prior to the study in the two groups of horses.*

	BCS_{mean}	Overall picture	Body weight (kg)	Girth circ.(cm)	V_{La4}	Peak Hct (%)
Group 1	6.4 ± 0.2	6.9 ± 0.7	398 ± 22	176.2 ± 4.5	5.3 ± 0.7	47.4 ± 2.4
Group 2	6.4 ± 0.2	6.7 ± 0.7	401 ± 17	175.6 ± 4.2	5.4 ± 0.5	46.8 ± 1.9

4.3. Body condition scoring

The horses were weighed and scored for body condition every week according to the nine-degree scale by Henneke *et al*. (1983). Two mean scores were calculated, one including back score and one without the score for back. Icelandic horses tend to accumulate fat in that area to a less extent than other horse breeds. The challenges of scoring the back for fat accumulation has also been reported for Thoroughbred geldings (Suagee *et al*., 2008). By including the score for back there is a risk of underestimating the actual BCS of the horse. Therefore, the mean score excluding the back score was used and will be referred to as BCS_{mean}. Cresty neck score was evaluated according to the scoring system presented by Carter *et al*. (2009).

4.3.1 Rump fat

Estimation of body fat content was done using ultrasonic measurements as described by Westervelt *et al*. (1976), using the same ultrasound machine (VET E Magic 2200, 5.0 MHz, with maximum depth set on 6.47) and probe (5.0 MHz linear rectal probe, 80 elements, 4-step multi-frequency 3.5/5.0/6.0/7.0 MHz, Eickemeyer, Tuttlingen, Germany), measuring subcutaneous fat thickness over the rump, 5 cm lateral from the midline at the centre of the pelvic bone (Figure 3). The measurement site was shaved prior to every recording. Measurements were performed by the same personnel every time. Initial rump fat measurements indicated that the horses had roughly 15 % body fat, around 1.5 cm, corresponding to approximately 60 kg body fat (Westervelt *et al*., 1976). Athletic horses in body condition around 5 have roughly 7-10 % body fat (0.9 cm) corresponding to 28-40 kg body fat (Ringmark *et al*., 2013), both using the following equation:

$$Y = 8.64 + 4.70x$$

with Y as fat percentage and x the rump fat measurement in cm. Therefore, 30 kg were deducted from the BW and a target body weight was calculated and used for feed ration calculations.

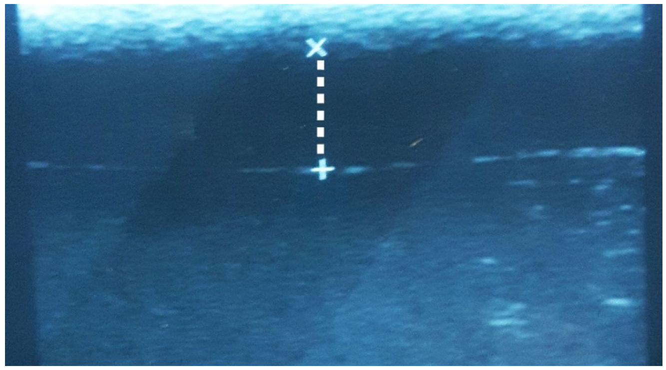

Figure 3. Measurement of rump fat thickness using ultrasound. The distance measured ranges from the dermis above, to the fascia of the underlying muscle below.

4.4 Feeding & management

The horses were housed separately from other horses in individual 2.4x3 m (7.2 m^2) boxes. Outside the horses were kept in two separate groups, for 1-5 hours every day, depending on training and weather situations. Water was available *ad libitum* from water bowls (flow rate 7 L/min) in the boxes. The horses were fed three times per day, at 07:00-08:00, 13:00-14:00 and 21:00-22:00. The horses were fed on a forage only diet complemented with a commercial mineral and vitamin supplement to fulfil nutrient requirements in accordance to NRC (2007) (Racing mineral, Trouw nutrition, Netherlands), together with 40 g of NaCl/day. 50 g/day of commercial muesli (Besterly Herbic, Besterly Horse Feed, Netherlands) was added and mixed with the minerals to increase palatability and ensure full uptake of the minerals. Mineral allowance was adjusted according to NRC (2007), using preliminary feed analysis and the PC-horse software (PC-horse, Hove Software LTD, Norway). Feed samples were gathered every other day and at opening of every new bale, pooled together weekly and stored at -18°C. The haylage samples were analysed for metabolizable energy, digestibility, crude protein and ash, all at the Department of animal nutrition and management at SLU, Sweden.

Big bale haylage batches used in the study, grown at Hólar were chosen with emphasis on energy and crude protein content. The haylage had a mean content of 10.7 ± 0.3 MJ ME (range 9.9-11.2 MJ ME) and a mean CP content of 163 ± 9 g/kg (range 14.0-17.8 g/kg). Mean nutrient values for each period can be seen in Table 3. Diets were designed in cooperation with researchers and trainers at Hólar University College. The allowance was 0.8 kg DM/100kg BW as restricted allowance, corresponding to 8.5 MJ ME/100 kg BW or 32 MJ ME/day and 1.6 kg DM/100 kg BW as high allowance, corresponding 17 MJ ME/100kg BW or 64 MJ ME/day, respectively (Table 2). The Icelandic horse is considered an easy keeper, with high capability to maintain body mass (Ragnarsson and Jansson 2011) and therefore this amount of restricted allowance was decided.

Table 2. *Daily nutritional allowance in terms of energy (MJ ME), crude protein (CP), ash and minerals (Ca, P, Mg, Na and K) for horses adapted to a high energy allowance and a restricted energy allowance, respectively.*

	High allowance	Restricted allowance
Kg DM	5.9 ± 0.3	3.0 ± 0.1
MJ ME/100 kg BW/day	17 ± 0	8.5 ± 0.2
MJ ME/day	64 ± 0.3	32 ± 0.3
Ash g/day	390.0 ± 19.0	194.0 ± 9.0
CP g/day	970.0 ± 45.0	483.0 ± 22.0
Ca g/day	34.0 ± 2.0	27.0 ± 1.0
P g/day	26.0 ± 14.0	13.0 ± 1.0
Mg g/day	18.0 ± 1.0	10.0 ± 0.4
Na g/day	27.0 ± 1.0	24.0 ± 0.4
K g/day	61.0 ± 3.0	30.0 ± 1.0

Table 3. *Mean nutritional values of haylages from period one and two, presented as means ± SD.*

	DM (g/kg DM)	Ash (g/kg DM)	CP (g/kg DM)	VOS (%)	ME (MJ/kg DM)
Period 1	641 ± 36	64 ± 5	160 ± 8	81.3 ± 0.6	10.5 ± 0.1
Period 2	616 ± 25	67 ± 4	166 ± 12	83.9 ± 1.3	10.9 ± 0.3

4.5 Training and training response

The horses were trained five times per week, on weekdays. The training program was designed together with a professional trainer and riding instructors employed at Hólar University College. Three types of training intensities were developed, namely training I, II and III. The mean HR, duration over 180 b.p.m., duration under 180 b.p.m., velocity and distance of the training types can be seen in Table 4. Variation of training intensities between training periods is presented in Table 5. Training was either conducted inside a riding hall or outside on a packed dirt road. To be representative of Icelandic horse population, the horses were trained as is considered a conventional preparation for a breeding evaluation field test. All horses were shod according to regulations of the International Federation of Icelandic Horse Associations (FEIF, 2015) for breeding evaluations. The horses were trained by students together with two professional trainers employed at Hólar University College.

Training I was regarded as light training, where HR did not exceed 180 b.p.m. Training II was regarded as a normal training day, where horses would reach 180 b.p.m. for a short duration. Training III was conducted one time per week which involved gallop intervals, designed to reach peak HR, involving 3x200 m gallop sprints. The variation in HR over and under 180 b.p.m. as well as training duration for each intensity between treatments is presented in Table 4, and between period 1 and 2 in Table 5. every training session was logged by the rider. In the second period, an attempt was made to repeat every individual training session in order. Average training HR was significantly different between training intensities (P<.0001) and there was a significant difference of duration over-and under 180 b.p.m. between training intensities (P<.0001). Training HR or training duration did not differ between treatments (P>0.05, Table 4). Training HR did not differ between periods (Table 5). However, duration of training in training intensity 2 was significantly higher in period 2 compared to period 1.

Table 4. *Mean heart rate, duration under and above HR 180 b.p.m. and duration in training between different training intensity steps. The same training intensity steps did not show a significant difference between treatments (values are presented as LSM ± SE)*

	Training intensity 1		Training intensity 2		Training intensity 3	
	Restricted	High	Restricted	High	Restricted	High
Mean HR b.p.m.	101 ± 2	104 ± 2	125 ± 1	127 ± 1	133 ± 2	133 ± 2
< 180 b.p.m. (min)	23.1 ± 1.0	23.3 ± 1.0	26.2 ± 0.5	26.9 ± 0.5	23.5 ± 1.0	24.9 ± 1.0
> 180 b.p.m. (min)	0.1 ± 0.3	0.0 ± 0.3	1.8 ± 0.2	2.0 ± 0.2	3.4 ± 0.3	2.9 ± 0.3
Duration (min)	23.2 ± 0.8	23.3 ± 0.8	27.7 ± 0.5	28.5 ± 0.5	27.0 ± 1.0	27.1 ± 1.0

Table 5. *Differences in mean heart rate, duration under and over 180 b.p.m. and duration in training of each training intensity 1, 2 and 3 between Period 1 and Period 2 (values presented as LSM ± SE). * marks significant difference between periods (P<0.05).*

	Period 1			Period 2		
	Training 1	Training 2	Training 3	Training 1	Training 2	Training 3
Mean HR b.p.m.	100 ± 2	125 ± 1	135 ± 2	105 ± 2	127 ± 1	131 ± 2
< 180 b.p.m. (min)	23.0 ± 1.0	25.4 ± 0.5	24.3 ± 1.0	23.4 ± 1.0	27.7 ± 0.5	24.2 ± 1.0
> 180 b.p.m. (min)	0.1 ± 0.3	2.1 ± 0.2	3.5 ± 0.3	0.0 ± 0.3	1.7 ± 0.2	2.8 ± 0.3
Duration (min)	23.1 ± 0.8	27.3 ± 0.5*	27.3 ± 1.0	23.4 ± 0.8	29.0 ± 0.5*	26.7 ± 1.0

4.6 Exercise testing

Two types of standardised tests were performed in each period, namely a standardised V_{La4} treadmill test and a Breed evaluation field test (BEFT).

4.6.1 Standardised exercise tests

Before the study, the peak velocity for every individual horse was determined by a preliminary V_{La4} test, using a portable lactate blood analyser (Lactate Pro™ 2 LT-1730, Arkray Inc., Japan). The standardised exercise test (SET) consisted of a 4-minute warm-up walk at 1.2 m/s with 0% incline followed by four, two-minute-long incremental steps with 6.25% incline, ending with the peak velocity determined beforehand for every individual, ending with two-minute walk uninclined cool down. Mean speed at each step is presented in Table 6. Blood samples were drawn on the last seconds of every incremental step, as well as 15 and 30 min after the test. HR, RR and BF were also recorded as recovery parameters right after the exercise and after 15 and 30 min post-exercise.

Table 5. *Mean speed (LS means ± SE) for each step during the SET.*

Step	Speed (m/s)
1	3.5 ± 0.05
2	4.3 ± 0.05
3	5.0 ± 0.05
4	6.0 ± 0.05

4.6.2 Breed evaluation filed test

A simulated breed evaluation field test (BEFT) was performed at Hólar, Iceland, according to FIZO regulations (FEIF, 2015) and as previously described by Stefánsdóttir *et al*. (2014). In an actual BEFT, horses are evaluated two times. In this study the horses were only evaluated the first time. The horses are scored for riding abilities on a half-point scale from 5-10. The horses were ridden by the studies´ head trainer that is an experienced BEFT rider. The structure of the BEFT for every horse was fixed between treatments. The rider chose the order and the speed in which the gaits were ridden in the first BEFT. That structure was then repeated in the second BEFT. Same two certified BEFT judges evaluated the horses both times and were blinded to the treatment of the horses. A blood sample was drawn before the BEFT, two min after exiting the track, 15 and 30 min post-exercise. HR, RR and BF were also recorded as recovery parameters after 15 and 30 min.

4.7 Locomotion asymmetry

Locomotion asymmetry of each individual was assessed in the horses using the wireless sensor system Lameness locator (EQUINOSIS®, Columbia and St. Louis, MO; www.equinosis.com). The horses were equipped with the sensors according to McCracken *et al.* (2012). Recordings were conducted three times in each period, one day before BEFT (BF), one day after BEFT (DA) and two days after BEFT (2-DA), in the same order of horses from 08:00-12:00. Two types of surface were used, a packed dirt road outside and loose sand inside a 20x60m riding hall. The horses were run for at least 50 m in each direction. Records were considered for analysis if a minimum of 25 strides were included.

Recordings were done on a straight line and on the diagonal in the riding hall. Horses that had trouble trotting easily were equipped with 240 g heel weight riding boots to assist the keeping the gait for more accurate measurements. Two handlers ran the horses by hand with a halter. One outside and the other handled the horses inside. By calculating vector sum, if a horse is lame it can indicate on which leg it is lame. The mean vector sum for front and hind limbs respectively gives an indication of locomotion asymmetry. It is derived from maximum and minimum height difference off the head and pelvic sensors (max diff and min diff) separately using the following equation:

$$VS = \sqrt{(maximum\ difference)^2 + (minimum\ difference)^2}$$

Vector sum for both front limbs (VSF) and hind limbs (VSH) were derived from the equation. The effects of locomotion asymmetry on the physiological response to exercise was studied using the simulated BEFT. Multiple recordings were performed until a desirable, consistent measurement was obtained. From those recordings, data was selected by two different selection criteria. Firstly, data was selected by the lowest standard deviation. Secondly, data was selected by the number of strides in each measurement.

4.8 Data collection

4.8.1 Heart rate, respiratory recording and rectal temperature recording

Heart rate, velocity and distance during all training sessions and exercise tests were recorded using heart rate monitor (Polar Pro Trainer 5 Equine Edition, Polar Electro, Kempele, Finland) and equine H1 heart rate sensor electrode based set used with Polar equine T56H transmitter W.I.N.D. (Kempele, Finland), synchronized with a GPS (Polar G3, GPS sensor, Polar Electro, Kempele, Finland). From HR recordings, warm up HR, duration of HR>180 b.p.m., duration of HR<180 b.p.m., total distance with HR >180 b.p.m. and average velocity when HR was >180 b.p.m. was calculated. Recovery HR and peak HR, namely the highest recorded HR for each horse and period were also determined from the HR curves. Resting HR was recorded using a stethoscope. Respiratory rate was counted by placing the hand in front of the nostrils for 15 seconds. Breaths per minute was evaluated by multiplying the value by four. Rectal temperature was recorded at rest, at the offset of exercise and 15- and 30 min post-exercise, using a digital thermometer (Disney, Hartman, Heidenheim, Germany).

4.8.2 Blood sampling and analysis

Blood samples were collected for the two exercise tests. All blood drawing was performed by certified personnel. For the SET, blood samples were drawn from *vena jugularis*, using a jugular vein catheter inserted under local anaesthesia (5ml Xylocain 20 mg/mL (Astra Zenecaa)).

Blood samples were drawn before the V_{La4} test, on the last seconds of every incremental step as well as 15 and 30 min after the exercise, seven samples in total. Resting samples were taken at the same time for all horses, in the stall box, after morning feeding of 1 kg DM.

For the BEFT, blood samples were drawn by jugular venipuncture. A blood sample was drawn before the BEFT, two min after exiting the track, 15 and 30 min post-exercise, four samples in total. Samples were collected in chilled lithium heparin tubes (9 ml, Vacuette ®; Greine-Bio-One, Kremsmuenster, Austria) and stored on ice until analysed.

From each blood sample, blood was collected into non-heparinised capillary glass tubes and centrifuged for 8 minutes, using (Cellocrit 2, AB Lars Ljungberg and Co, Stockholm, Sweden) for haematocrit analysis. Each sample was run in triplicate and the mean value used for statistical analysis. Plasma was then separated as all samples were centrifuged in the heparin tubes for 15 minutes (520 x G, EPA 12, Hettich zentrifugen, Tuttlingen, Germany). The separated plasma was frozen and stored at -18°C.

The muscle enzyme AST was analysed by enzymatic method on a fully automated, open-system clinical chemistry/immunoassay analyser (spectrophotometer, Architect c4000, Abbott Park, IL, USA). Total plasma protein was analysed using a refractometer (Atago, Tokyo, Japan). Blood plasma lactate concentration was analysed by an enzymatic method (Boehringer&Mannheims, lactat/r-biopharmkit, kit no 10139084035, Skandinavien Diagnostiska, Göteborg, Sweden).

4.9 Statistical analysis and calculations

Statistical analysis was performed using SAS (Statistical Analysis System package, Inst. Inc. Cary, NC, USA, version 9.4). ANOVA was performed using a general linear model (procedure GLM). The effect of treatment was analysed using the model $Y=\mu+ a_i+b_l+ c_k + d_j + e_{ilkj}$, where Y_{ijkr} is the observation, μ is the mean value, a_i is the fixed effect of treatment, b_l is the fixed effect of period, c_k the fixed effect of sample, d_j is the random effect of horse and e_{iljkl} is the residuals. Results are expressed as least squares means ± standard error, and as mean ± standard deviation, where stated. Tukey test was used for comparison. The significance level was set at $P<0.05$. Correlations calculations were performed using Pearson correlations test.

5 Results

5.1 General

Five weeks into the study, one horse was withdrawn due to abnormal behaviour and loss of appetite, and therefore excluded from all analysis. Two other horses registered days lost in training, 3 days due to a lost shoe resulting in mild hoof injury, and one day for the other horse due to mud fever. Prior to the study, all horses passed a thorough health examination performed by a certified veterinarian. The horses that participated in the study, finished completed without clinical signs of injury, fatigue or metabolic syndromes, examined by a veterinarian.

5.2 Body condition and body weight

Horses adapted to high energy allowance were significantly heavier compared to horses adapted to restricted energy allowance (P<.0001, Figure 4). The weight difference was significant already at second week of treatment. The horses weighed on average 405.6 ± 1.2 kg for HA compared to 388.8 ± 1.2 kg for RA respectively, varying by 16.8 kg on average.

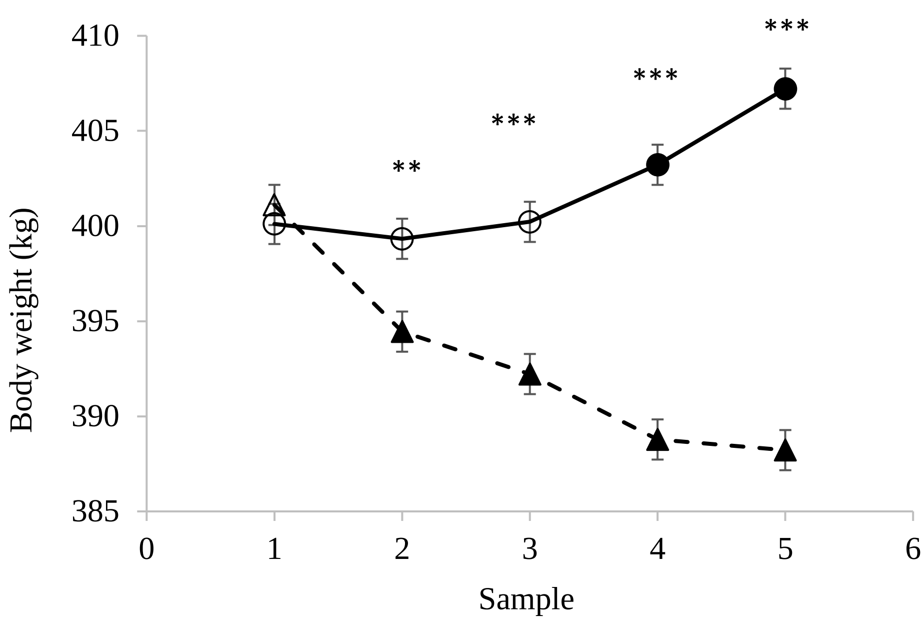

Figure 4. Weekly changes in total body weight between treatments. Δ and dotted line represent RA, ○ and filled line represent HA. Filled labels are significantly different from the initial value. ** marks significance of P<0.05. *** Marks significance between treatments of P<.0001.

All parameters of the body condition scoring showed significant difference between treatments with increased body weight and BCS on high allowance, apart from the score for back (Table 7). Fat% differed between treatments ($P<0.05$) being on average 0.55% higher for HA compared to RA. Mean BCS was 6.5 ± 0.02 in HA and 6.2 ± 0.02 in RA ($P<.0001$) varying by 0.3 points on average. The morphological measurement of girth circumference was significantly different between treatments, higher for HA compared to RA. Neck circumference did not differ between treatments. Cresty neck score tended to be higher for RA compared to HA ($P<0.1$).

Table 6. *Mean values of body weight and individual parameters of the BCS between treatment groups (LSM ± SE), and the level of significance between treatment groups (n=9).*

Variable	High allowance	Restricted allowance	Effect of treatment
Body weight (kg)	405.6 ± 1.2	388.8 ± 1.2	P<.0001
Fat (%)	14.6 ± 0.13	14.05 ± 0.13	P<0.05
Neck	6 ± 0.05	5.7 ± 0.05	P<0.005
Back	4.7 ± 0.02	4.6 ± 0.02	0.06
Rump	5.2 ± 0.02	5.1 ± 0.02	P<0.05
Tail head	5.7 ± 0.04	5.4 ± 0.04	P<0.005
Ribs	6.9 ± 0.06	6.5 ± 0.06	P<0.005
Shoulder blade	7.5 ± 0.06	7.1 ± 0.06	P<0.005
BCS_{mean} + back	6 ± 0.02	5.7 ± 0.02	P<.0001
BCS_{mean} - back	6.5 ± 0.03	6.2 ± 0.03	P<.0001
Overall picture	7.1 ± 0.1	6.5 ± 0.1	P<0.05
Cresty neck score	2.25 ± 0.03	2.34 ± 0.03	P= 0.07
Girth circ. (cm)	174.1 ± 0.1	171.7 ± 0.1	P<.0001
Neck circ. (cm)	103.4 ± 0.8	102.2 ± 0.8	0.35

All evaluated parameters correlated highly with BW and BCS (Table 8), apart from CNS and neck circumference. Girth circumference had the highest correlation to BW. Rib score correlated the highest to BCS_{mean}.

Table 7. *Correlations between weekly changes in body weight, body condition score and changes in individual parameters of the total BCS.*

	Body Weight	*P*-value	BCS_{mean}	*P*-value
Body weight	.	.	0.904	<.0001
Neck	0.758	0.0003	0.846	0.0003
Back	0.632	0.0049	0.576	0.0124
Tail head	0.822	<.0001	0.831	<.0001
Rump	0.598	0.0088	0.521	0.0265
Ribs	0.801	<.0001	0.917	<.0001
Elbow area	0.801	<.0001	0.875	<.0001
Girth circ. (cm)	0.851	<.0001	0.758	0.0003
Overall picture	0.898	<.0001	0.778	0.0001

5.3 Performance

5.3.1 A breed evaluation field test

All horses completed the BEFT without injury and considered healthy by a veterinarian. Duration of warm up was 9.0 ± 1.0 min. Mean duration of BEFT was 10.9 ± 1.3 min. Distance of warm up was 2,010 ± 180 m. Distance ridden in the BEFT was 2617 ± 302 m. Mean velocity during BEFT was 4.2 ± 0.2 m/s and maximum velocity was 10.9 ± 0.6 m/s. Judges scores for total score for riding abilities, form under rider and gallop were significantly lower for HA compared to RA ($P<0.05$, Table 9). All scores for riding abilities were numerically lower for horses on HA compared to RA except for walk and the same mean score for canter.

Weather conditions

Mean wind speed at BEFT was 3.2 ± 0.2 m/s. Wind speed was significantly lower for the HA compared to RA (3.0 ± 0.1 m/s vs. 3.4 ±0.1 m/s respectively) ($P<0.05$). Mean ambient temperature during BEFT was 6.8 ± 0.2 °C. No difference in ambient temperature between treatment groups during the BEFT.

Table 8. Scores from an experimental breed evaluation field test (LSM ± SE) on horses subjected to a high allowance (HA) and restricted allowance (RA) treatment. The judges were blinded to treatments.

	HA	RA	SE	P-value
Tölt	7.09	7.41	0.139	0.146
Slow tölt	6.92	7.14	0.2	0.456
Trot	7.11	7.28	0.108	0.328
Pace	5.1	5.29	0.074	0.118
Gallop	7.62	7.83	0.062	0.047
Canter	7.44	7.44	0.109	1
Spirit	7.43	7.62	0.132	0.351
Form under rider	7.44	7.67	0.056	0.018
Walk	7.65	7.57	0.126	0.639
Total	6.95	7.16	0.0618	0.048

5.4 Physiological response

5.4.1 Standardised incremental exercise test

Exercise intensity

Mean HR tended to be higher for HA (118 ± 2 b.p.m.) compared to RA (116 ± 2 b.p.m.), respectively, (P<0.1). HR increased linearly with speed during SET. Furthermore, lactate response differed between treatments (Figure 5).

Rectal temperature and respiratory rate

Rectal temperature was higher in HA compared to RA (P<0.05) in the SET. There was a significant difference between groups in resting rectal temperature (37.6 ± 0.1 °C for HA vs. 37.3 ± 0.1 °C for RA, respectively), (P<0.05). In both treatment states, rectal temperature had not lowered to resting values 30 min post-exercise. RT 15 min post BEFT was higher in HA (38.5 ± 0.1 °C) compared to RA (38.2 ± 0.1 °C) (P<0.05). The indoor temperature was significantly higher for HA compared to RA (12.4 ± 0.08 °C for HA, vs. 11.8 ± 0.08 °C for RA, respectively). However, the ambient temperature did not have significant on the rectal temperature.

Mean respiratory rate was significantly higher for HA compared to RA (P<0.05). Clearest variation was detected at 2 min post-exercise (97 ± 5 breaths/min vs. 71 ± 5 breaths/min respectively, P<.0001). Horses had reached resting RR 15 min post-exercise in both treatments.

Haematocrit and plasma protein concentration

There was a significant difference in haematocrit between treatments (39.9 ± 0.2 % vs. 40.9 ± 0.2 %, $P<0.01$) (Figure 5). There was a significant difference already in resting Hct, 35.4 ± 0.5 % for HA vs. 37.0 ± 0.5 % for RA, respectively ($P<0.05$). Peak Hct did not differ between treatments (45.8 ± 0.5% for HA vs. 46.7 ± 0.5% for RA, respectively, $P>0.05$). Horses in both treatments had reached resting values for Hct 15 min post-exercise. There was no effect of treatment on TPP during the SET (Figure 5). TPP elevated in relation to speed during the SET. Horses had reached their resting values already 15 min post-exercise.

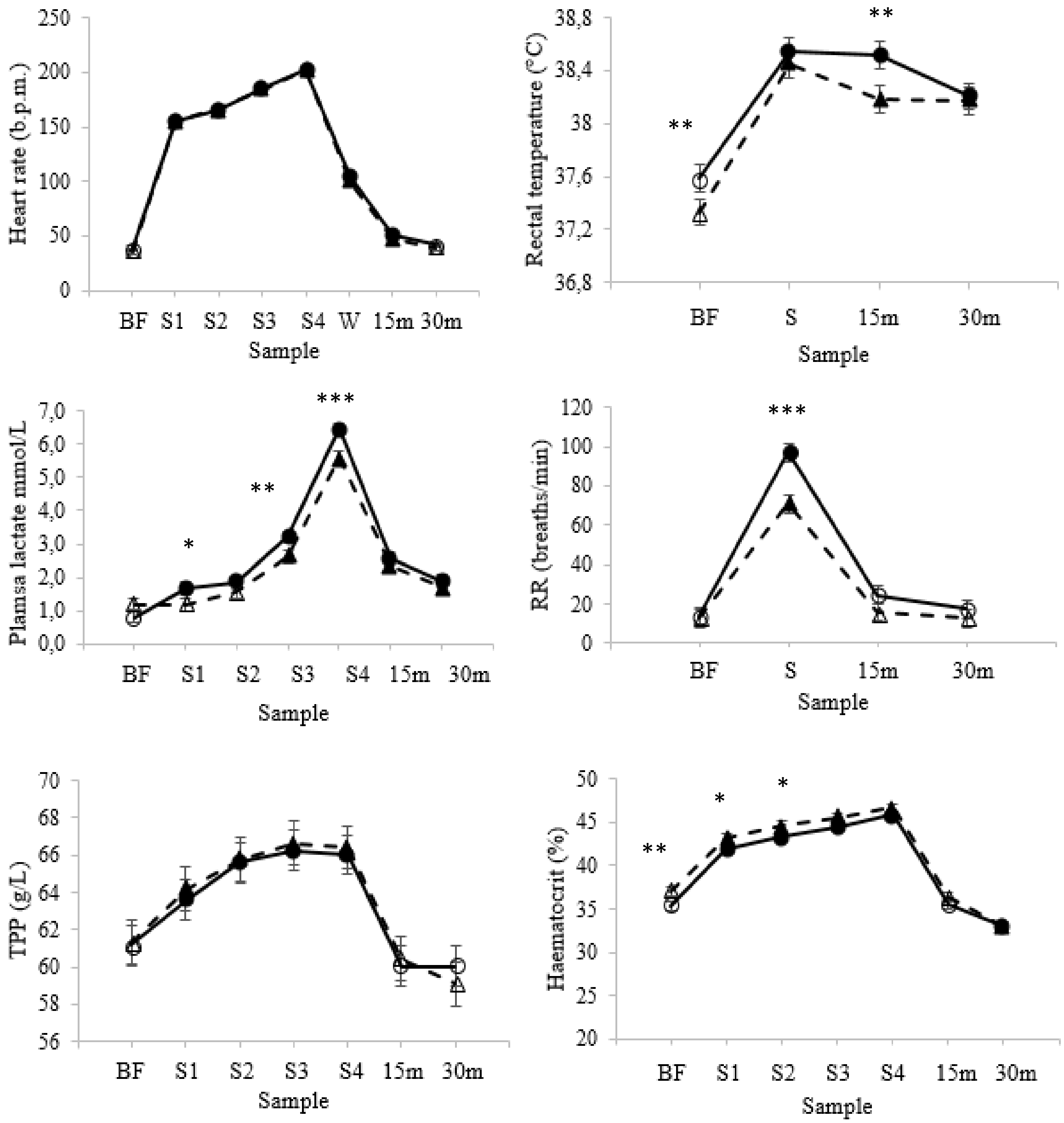

Figure 5. Physiological response to a standardised, incremental exercise test in terms of heart rate, rectal temperature, respiratory rate, plasma lactate concentration, total plasma protein concentration and haematocrit (LSM ± SE. Samples were taken Before (BF), at the end of each step (S1), (S2), (S3) and (S4) of the exercise test, at the end of two-minute cooldown in walk (W), right after stop (S), and 15 and 30 min post exercise (15m) and (30m). Δ and dotted line represent RA, ○ and filled line represent HA. Filled labels are significantly different from before value. * marks a statistical tendency between treatments in each sample (P<0.1). ** marks significant difference of individual samples between treatments (P<0.05). *** marks significance level of (P<.0001) between treatments of individual samples

5.4.2 BEFT

Exercise intensity

Mean HR of warm up was 149 ± 6 b.p.m. Mean HR of BEFT was 168 ± 6 b.p.m. Mean HR_{peak} was 217 ± 7 b.p.m. There was a significant effect of treatment on mean HR during BEFT, with a mean of 167 ± 1 b.p.m. in HAs vs. 170 ± 1 b.p.m. in RA. Peak HR did not differ between treatments. Plasma lactate concentration was significantly higher for RA (Figure 6). Peak plasma lactate concentration, 2 min post-exercise was significantly lower in HA, 3.4 ± 0.8 mmol/L vs. 5.4 ± 0.8 mmol/L in RA, respectively (P<0.05, Figure 6).

There was no difference of sampling days on aspartate amino transferase (AST) concentration, before BEFT and two days after BEFT (p>0.05), nor between treatments, 5.4 ± 0.2 ukat/L in HA vs. 5.2 ± 0.2 ukat/L in RA, or 325.3 ± 12 U/L vs. 313.3 ± 12 U/L, respectively.

Rectal temperature and respirator rate

Rectal temperature (RT) differed between treatments (P<0.05), with higher mean RT in RA compared to HA (38.5 ± 0.05 °C vs. 38.3 ± 0.05 °C. respectively, P<0.05). Peak RT did not differ between treatments (P>0.05). The horses had not reached resting RT 30 min post-exercise in both treatments (Figure 6) .

There was significant difference in mean respiratory rate (RR) between treatments (P<0.05) being higher in HA compared to RA. The respiratory rate 2 min-post BEFT was higher in HA compared to RA (107 ± 6 breaths/min vs. 88 ± 6 breaths/min, respectively) and 15 min post-BEFT (47 ± 6 breaths/min vs. 29 ± 6 breaths/min, respectively), P<0.05. Horses in both treatments had reached resting respiratory rate 30 min post-exercise. Following the BEFT, the HA had reached resting HR values 30 min' post-exercise, while the RA had not (Figure 6).

Haematocrit and plasma protein

There was significant difference in mean Hct concentration between treatments (P<0.05) (Figure 6). Hct had diminished to resting values 30 min post exercise (Figure 6). There was a tendency of effect of treatment on mean total plasma protein concentration (TPP) (P<0.1), being higher in RA.

compared to HA with mean values of (60.12 ± 0.6 g/L in RA and 58.5 ± 0.6 g/L respectively). TPP levels did not significantly exceed resting values (Figure 6).

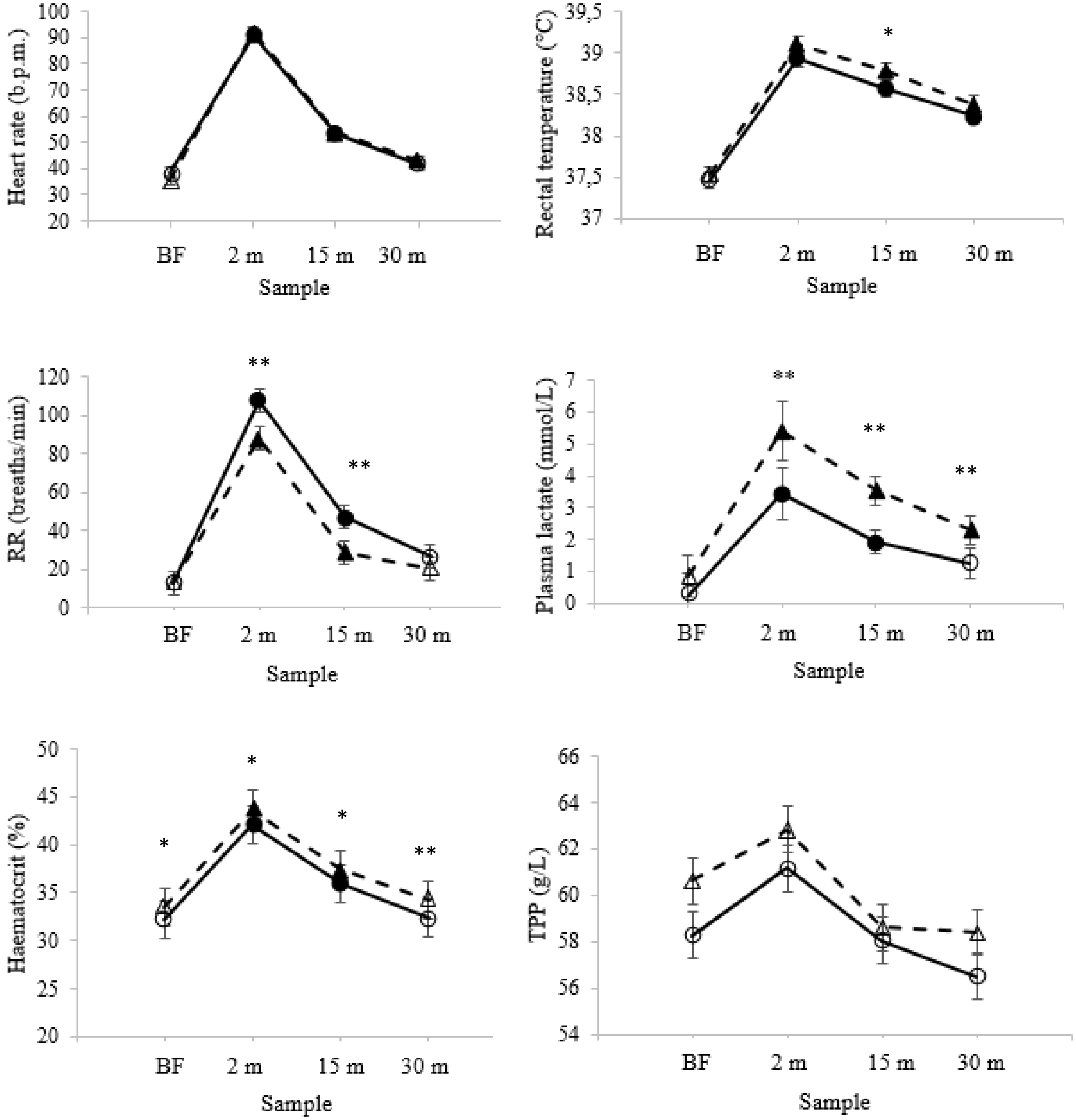

Figure 6. Physiological response to a breed evaluation field test in terms of heart rate, rectal temperature, respiratory rate, plasma lactate concentration, haematocrit (%) and total plasma protein concentration (LSM ± SE). Before sample (BF), 2 minutes post-exercise (2 m), 15 minutes post-exercise (15 m) and 30 minutes- post-exercise (30 m). Δ and dotted line represent RA, ○ and filled line represent HA. Filled labels are significantly different from before value. * marks a statistical tendency between treatments in each sample (P<0.1). ** marks significant difference of individual samples between treatments (P<0.05). *** marks significance level of (P<.0001)

5.5 Locomotion asymmetry

For analysis of locomotion asymmetry, the effects of stride rate, stride length and surface were not significant and were therefore excluded from the final statistical model. In general, there was a significant difference between treatments, with higher front limb asymmetry, reported as vector sum front (VSF) for horses adapted to high energy allowance (HA) compared to horses adapted to restricted energy allowance (Figure 7). Hind limb asymmetry did generally not differ between treatments, though variation could be detected prior to the BEFT. Participation in BEFT had lowering effects on locomotion asymmetry after the field test.

With selection criteria of lower standard deviation there was significant effect of treatment for VSF ($P<0.05$). Mean VSF for HA was 11.0 ± 0.6 mm vs. 8.7 ± 0.6 mm in RA, respectively. Horses tended to have greater front limb asymmetry the day before BEFT compared to two days after BEFT ($P<0.1$), regardless of treatment. Horses in HA had greater hind limb asymmetry day before BEFT ($P<0.05$). Different treatments did not affect overall VSH (Figure 7).

When data was selected from stride quantity of the measurement, there was a tendency of treatment effect for VSF ($P<0.1$), 11.2 ± 0.7 mm in HA vs. 9.5 ± 0.6 mm in RA, respectively. VSH tended to be higher for HA, 5.8 ± 0.4 mm in HA vs. 4.8 ± 0.4 mm in RA ($P<0.1$). No differences between groups on individual sampling days. For horses adapted to restricted energy allowance, VSF was significantly lower day after BEFT and as well as 2 days after BEFT (Figure 7). Regardless of treatment, VSF day before BEFT tended to be higher than the day after ($P<0.1$), and were significantly higher than 2 days after BEFT ($P<0.05$).

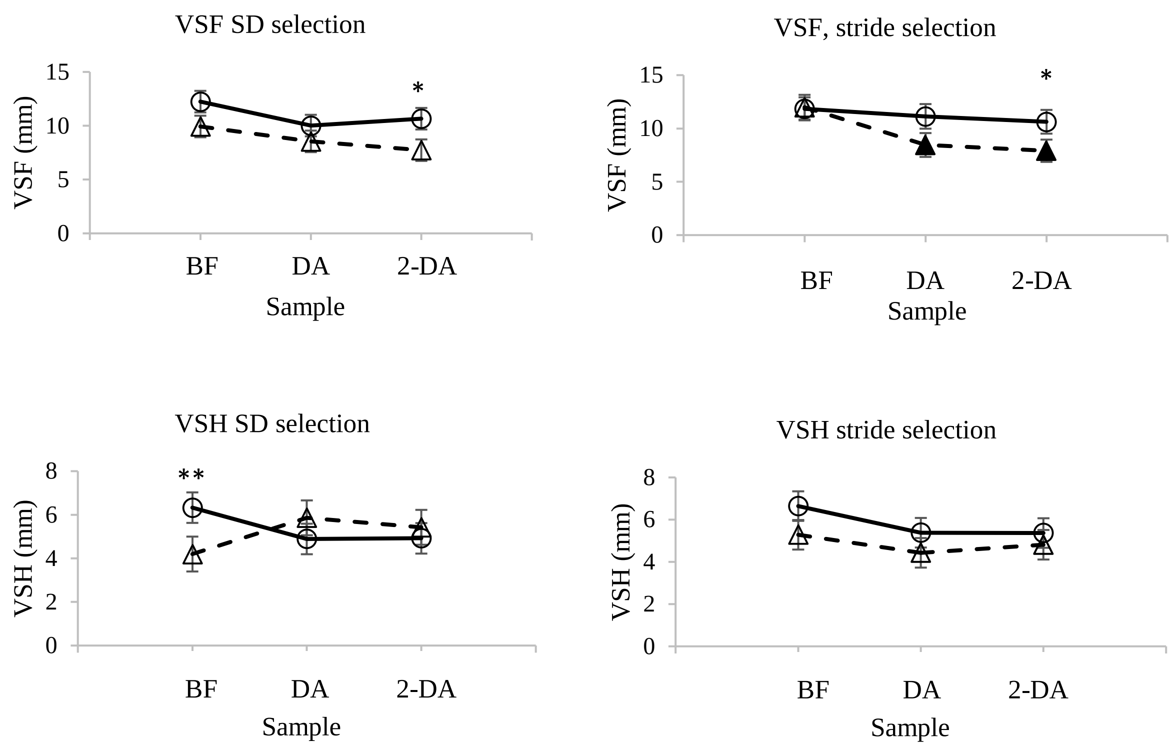

Figure 7. Locomotion asymmetry (vector sum front and hind, VSF and VSH) of front legs (top) and hind legs (bottom) in nine horses subjected to two treatments: high energy allowance (○) and restricted energy allowance (∆). Left figures respresent a data selection based on lowest standard deviation and right figures a data selection based on a minimum of 25 strides (LSM ± SE). Measurements were performed a day before a breed evaluation filed test (BEFT) (BF), a day after BEFT (DA) and two days after BEFT (2-DA). Filled labels are significantly different from BF sample. * marks statistical tendency of effects of treatment ($P<0.1$). ** marks significant difference between treatments ($P<0.05$).

5.5.1 Locomotion asymmetry post-exercise

For a better demonstration of the effect of treatments on the symmetry response to BEFT, a statistical analysis was performed, excluding the measurements from the day before the BEFT (Figure 8). With selection criteria of lower standard deviation, front limb asymmetry was higher for HA compared to RA with VSF = 10.4 ± 0.7 mm in HA vs. 8.2 ± 0.7 mm for RA, respectively ($P<0.05$). VSF tended to be lower for RA compared to HA two days after BEFT ($P<0.1$). No difference in VSH was found between sampling days or between treatments.

When selected for stride quantity, front limb asymmetry (VSF) was also higher for HA compared to RA, 11.1 ± 0.7 mm in HA vs. 8.1 ± 0.7 mm in RA ($P<0.05$). Moreover, VSF tended to be higher for HA compared to RA the day after BEFT as well as two days after BEFT ($P<0.1$). VSH did not differ between treatments or sampling days.

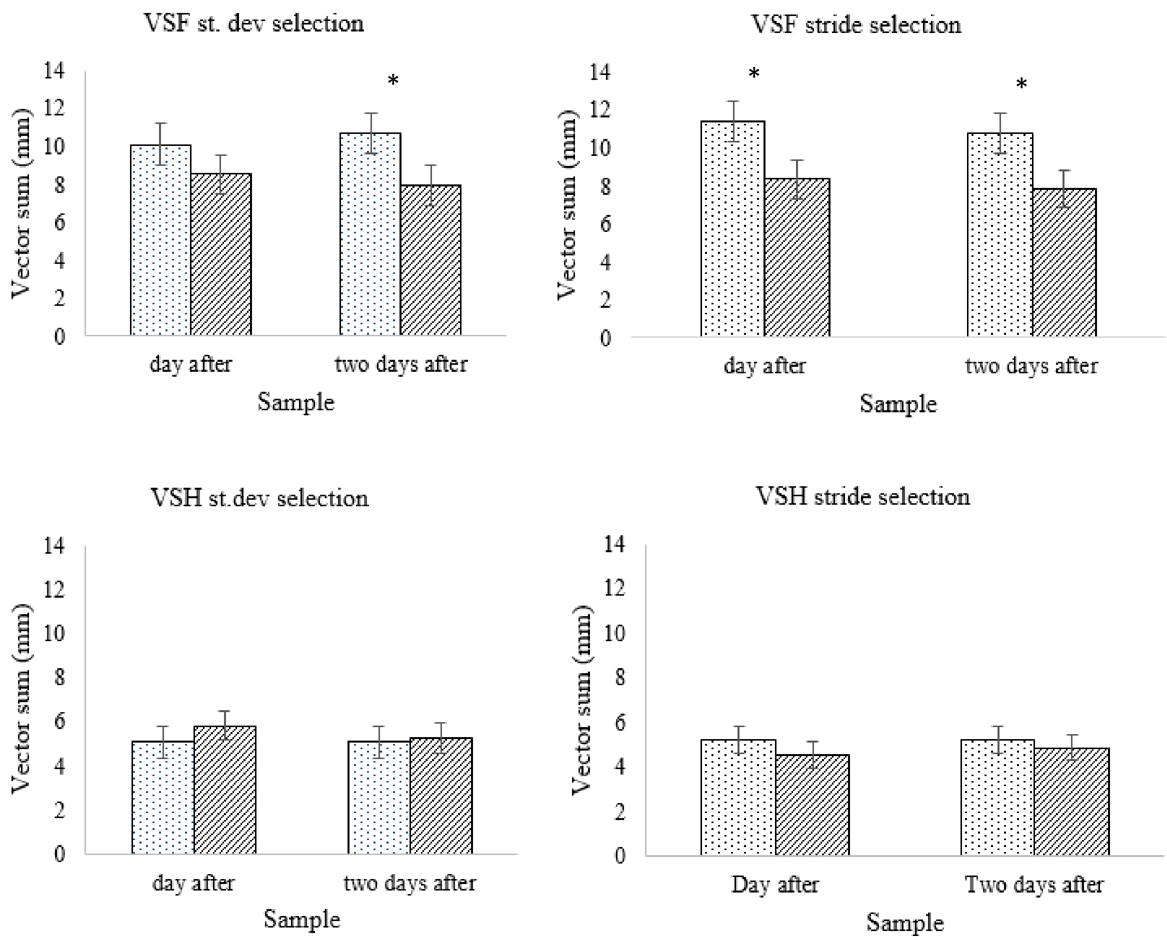

Figure 8. Locomotion asymmetry of front legs and hind legs between treatments in two different strategies of data selection (LSM ± SE). Measurements from a day after BEFT (DA) and two days after BEFT (2-DA) are included in ths analysis. First for lowest standard deviation and secondly for stride count of the measurement. Asymmetry is reported as vector sum front, VSF (mm) and vector sum hind,VSH(mm). Striped columns represent RA; dotted columns represent HA. Filled labels are significantly different from before value. * marks statistical tendency between treatment (P<0.1).

5.5.2 Locomotion asymmetry and performance

Front limb asymmetry (VSF) was strongly correlated between sampling days, VSF day after BEFT (r=0.62. P<0.05) and two days after BEFT (r=0.74. P<0.05), meaning that horses with high asymmetry before the BEFT also had high asymmetry the days after it as well. In general, the horses had lower locomotion asymmetry in the days after the BEFT compared to the day before. VSF the day after BEFT (DA) correlated to scores for gallop (r=0.50. p=0.0328) and canter (r=0.47. P<0.05) from BEFT.

Hind limb asymmetry (VSH) day before BEFT tended to be negatively correlated with scores for slow tölt (r = -0.41. P=0.08), meaning that higher hind limb asymmetry may result in lower scores for slow tölt. Mean VSH tended to be negatively correlated to canter (r=-0.44. p=0.07). No direct correlations were found between locomotion asymmetry and BCS or BW.

6 Discussion

6.1 General

The most clear and present findings in this thesis are that altered body condition does affect physiological response to exercise and performance in Icelandic horses (Figures 5 and 6). Specifically, horses adapted to high allowance diet and therefore had higher BCS had lower judges´ scores from a breed evaluation field test, lower aerobic capacity and altered recovery pattern. Moreover, horses in higher body condition had greater locomotion asymmetry in front limbs (Figures 7 and 8). Participation in a BEFT had lowering effects on locomotion asymmetry to some extent.

6.2 Body condition

The Henneke scale has proven to be a successful tool in evaluating fat accretion in Icelandic horses. In general, the study was successful in altering the BCS, BW and fat content of the horses with the given feeding, management and training practice to a statistically significant extent, with merely a 28-day adaptation period. Quite interestingly, there was a significant difference between treatments even though the mean variation of BCS_{mean} was 0.3 points, with a mean variation in BW of 16.8 kg (Figure 4, Table 7). As seen in Figure 4, the horses lost weight more rapidly than they gained weight. All horses were subjected to the same training program, resulting in that alterations in BW and BCS were due to the treatment and not training.

Since 2001, the body condition of Icelandic horses has been evaluated according to Stefánsdóttir and Björnsdóttir, (2001), in their native country. This is a half-point scale ranging from 0-5. Apart from visually assessing the horses' general health status, the only palpation required to give score is the area covering the caudal ribs. From the results of this study, we can see that rib score correlates the highest to mean BCS ($r = 0.92$, Table 8). Therefore, if only to palpate one body part of the horse, the rib area is the appropriate approach. However, our results correlate to Westervelt *et al*. (1976), that fat deposition between areas of the body variates within every horse, as between individuals. To gain an accurate assessment of a horse's body condition, detecting slight changes and reducing risk of underestimating body fat deposition, the Henneke scale is a more precise method, clearly being beneficial for the horse.

At the beginning of each adaptation period, it was observed that the horses in the HA were close to maximum levels of their feed intake capabilities. After a few days, they ate at a higher rate and the horses´ feed intake could have been increased. With a longer adaptation period and higher feed allowance for the HA, the result would probably have been even more pronounced, with higher exercise intensity in relation to increased body weight and body fat content.

6.2.1 Morphology and cresty neck score

Morphological measurements of girth circumference correlated highly to BW due to possibly changes in fat deposition, but also in gut fill. These findings correlate with previous findings on Icelandic horses (Matthíasdóttir, 2012) as well as in other breeds (Carroll and Huntington, 1988; Dugdale *et al*., 2011b). No differences were seen in neck circumference. That can be because of the high measurement error of the parameter or higher heterogeneity of the measured horses. Even though placements sites of measurement tape were marked in the horses´ coat, only a slight posture change of the horse altered the measurement of several cm, and is therefore highly challenging to standardise the procedure of measuring this parameter. Frank *et al*. 2006 concluded that neck circumference is a good indicator of the horse´s physical state. That study compared obese horses (BCS≥7/9) to normal horses ranging from 4-6 out of 9, thus a much more heterogenous group of horses, the obese group possibly containing much more prominent fat deposition in the neck. The horses were also restrained during every measurement, which is likely to be necessary for standardization of the measurement.

Cresty neck score (CNS), applied at the end of each period was not different between treatments. No correlations between CNS and BCS, nor to neck circumference were found. To detect a significant change in the fat deposition of the neck, it is likely that more time is needed for the horse to build up the fat reserve to a higher extent. Our findings concur with Dugdale *et al*. 2011b, that girth measurements have a higher correlation to BCS than neck circumference. The relatively low change in CNS between treatments compared to other parameters of the total BCS, it is possible to speculate that fat deposits sooner in other areas, and longer adaptation time is needed to see a substantial fat deposition in the neck.

6.2.2 Fat deposition and weight gain

No correlations between fat percentage and BCS were found in this study, in contrast to older findings (Henneke *et al*., 1983; Leleu and Cotrel, 2006; Ragnarsson and Jansson, 2011). The number of measurements (n=18) and the relatively high homogeneity compared to the range in BCS of 2-4 (Leleu and Cotrel, 2006) and 4.5 – 7.5 (Ragnarsson and Jansson, 2011) in both mean BCS and the fat% could be the explanation. Ringmark *et al*. (2013) assessed horses in BCS around 5 with 7-10% fat%, carrying 28-40 kg of fat. The calculated fat content of the horses in this study according to Westervelt *et al*. (1976), the horses were carrying roughly 57 kg of total fat reserves, compared to roughly 67 kg using the exponential relationship presented by Dugdale *et al*. (2011). It is though vital to address the conclusion of Dugdale *et al*. (2011b) that when evaluating BCS of 6 and higher, the correlation becomes much more insensitive and therefore not a useful indicator of actual body fat content. By increase of one or two points in BCS, actual body fat can double and thus, small errors of measurement in BCS can result in larger error in body fat content. BCS systems are based on detecting changes in subcutaneous fat deposition, leaving blanks in visceral adiposity (Dugdale *et al*., 2010; Dugdale *et al*., 2012).

By calculating the estimated body fat, according Westervelt *et al*. (1976), we can see that 1% of body fat corresponds to 4 kg of actual body fat. The average difference between treatment groups of 0.55% corresponds to 2.2 kg of extractable body fat, or 80 grams of weight gain per day. The equation used was derived from a study using eight horses of unknown breed, ranging in BW from 336-559 kg. Out of mean difference in BW of 16.8 kg, the value is low and unrealistic. However, assuming an exponential relationship between fat% and BCS that Dugdale *et al*. (2011a) described using obese Welsh mountain ponies in BW = 219 ± 21 kg of BCS 6.8-9 out of 9, average fat accumulation between treatments is 7.5 kg or 270 grams/day, and the residues are expected to be gut fill and possible increased balance retention, although Dugdale *et al*. (2011a) states that weight gain is generally attributed through accumulation of body fat. Many horses in this study fell within the lower limits of obesity used by Dugdale *et al*. (2011b). Average difference between treatments on forage allowance was 4.7 kg/day. From organic matter digestibility of the haylage, we can see that roughly 0.5 kg/day of indigestible material was eaten. Whether it is all released with faeces or is resided for some time is not known.

If all excessive energy intake would be stored in the form of adipose tissue, the horses adapted to high energy allowance would have deposited roughly 20 kg of fat, which is clearly unrealistic, given that the mean BW difference was less than 17 kg. Every kg of fat withholds roughly 38 MJ ME (Alberts *et al*., 2008; Sjaastad *et al*., 2016) By calculating the difference in total energy intake between treatments, it is estimated that around 35% of the energy is stored in adipose tissue, assuming 74% energy efficiency of lipid synthesis (MSU, 2017). The residual energy is most likely utilized for increased workload due to greater weight-bearing, more heat production due to increased microbial activity and chewing activity and due to maintenance cost of the increased adipose tissue.

Water uptake has been shown to correlate with forage intake (Lewis, 1995), and that water intake increases with forage only diet vs. concentrate diet (Jansson and Lindberg, 2012). As more water can be bound to the increased fibre content in the hind gut, it could contribute partly to the weight gain for the horses adapted to high energy allowance.

6.3 Physiological response

6.3.1 Standardised exercise test

The exercise intensity for the horses adapted to high energy allowance was clearly higher compared to horses adapted to restricted energy allowance. They tended to have higher heart rate, had higher lactate accumulation, higher rectal temperature and higher respiratory rate. Furthermore, unpublished results from this study show that horse in HA had higher plasma lactate concentration and lower V_{La4} compared to horses in RA, implying lower aerobic capacity for horses with higher body condition and body fat content. This corresponds to the findings of Kearns *et al*. 2002a, where horses with higher fat% had lower VO_{2max}. Other publications referred to in this thesis included field exercise tests.

6.3.2 BEFT

In summary, judges´ scores for gallop, form under rider and total score for riding abilities were significantly higher for RA compared to HA. Furthermore, mean heart rate during BEFT, rectal temperature and haematocrit were as well higher for RA compared to HA. Respiratory rate and plasma lactate concentration were though significantly higher for HA compared to RA.

In comparison to Stefánsdóttir *et al*. (2014), this study had lower mean HR and peak HR, Hct and distance covered. The rider weight including tack was 72 kg in this study compared to average of 83 ± 11 kg (Stefánsdóttir *et al*., 2014). When only observations are included with riders of comparable weight to this study, the horses still had lower plasma lactate concentration. Moreover, horses in this study had limited abilities to show pace. By that it can be concluded that the exercise intensity of this field test was somewhat lower than an actual breed evaluation field test. The quality of the horses used in the study could be lower than in Stefánsdóttir *et al*. (2014) (7.69 vs. 7.06). For the horses with a rider of comparable weight, the mean total score was 7.50 compared to 7.06 points in this study. For an actual BEFT, there is a high selection intensity. From 1990-2001, 12% of horses born attended at a BEFT (Albertsdóttir *et al*., 2011). The horses used in the study were all geldings, that already have been through a certain selection of potential performance quality. Although no correlations were found in this study between the body weight ratio between the rider and the horse (BWR) and physiological response, as only one rider performed the BEFT, we can conclude that the riders weight did not have incremental effects on lactate accumulation.

Nevertheless, increased weight bearing as adipose tissue or as gut fill could contribute to increased exercise intensity.

The body weight of the horses was also different is this study compared to Stefánsdóttir *et al.* (2014), that reported mean BW of 339 ± 14 kg. This study had mean BW of 397 ± 1.2 kg, relating to the additional weight carried as fat or gut fill. Training state of the horses between studies could vary. The horses used in the study were all property of Hólar University College, possibly lacking the pressure and demands of the owners and trainers of horses preparing for an actual BEFT. In addition, the mean Hct of 42.8 ± 0.6% vs. 45.0 ± 3% indicates lower oxygen transport capacity of the horses in this study. This difference can partly be explained by higher Hct in stallions, which this study did not include. In real situations, most of the horses in this study would most likely not been considered fit to perform in a BEFT.

Stefánsdóttir *et al.* (2017) investigated the effects of added rider weight on the physiological response of the horse, running at 5.3 m/s. In that study (BWR) > 25% resulted in lactate accumulation. In this study, the mean BWR was 18% and the mean velocity was 4.2 m/s. Based on this information, it is not surprising that lactate could be lower in the present study.

6.3.3 Effects of treatment

There was a significant difference in exercise intensity between treatment groups during BEFT. There was higher wind speed for RA compared to HA during the BEFT, with a variation of 0.4 m/s on average which could have increased work load and rectal temperature 2 min post-BEFT. This difference is probably coincidental, as the horses were ridden in a random order, in addition to a variable wind direction, making it difficult to speculate on the true effects of wind speed on exercise intensity.

However, there was a significant effect between treatments on maximum speed (V_{max}) (10.8 ± 0.1m/s vs. 11.1 ± 0.1 m/s), mean speed (V_{mean}) (4.1 ± 0.03 m/s in HA vs. 4.2 ± 0.1 m/s in RA), and HR_{mean} during BEFT, confirming the higher exercise intensity in the RA, in accordance to the lactate response between treatment groups. Exercise intensity and speed have been closely correlated (Evans and Rose, 1988). It is therefore difficult to conclude on the effects of wind on the physiological response, and is more logical to point out the speed, gaits and movements as the reason for difference in exercise intensity of the RA during the BEFT. The RA horses worked at higher intensity and to a greater extent, utilized anaerobic pathways.

Unpublished data from this study also show that horses on RA had greater suppleness, speed and self-carriage according to the rider. Moreover, there was a tendency of difference in willingness, gaits and movements. Therefore, the results indicate strongly that increased body condition score negatively affect ridden abilities of the Icelandic horse.

6.3.4 Correlation to performance

Higher BCS had direct, detrimental effects on riding abilities during BEFT, seen by the difference between treatment on scores for total core, gallop and form under rider, but also by the negative correlations between mean BCS and score for tölt ($r=-0.61$, $P<0,05$) and mean speed during BEFT ($r=-0.51$, $P<0,05$). This indicates decreased performance with increased fat deposition. Kearns (2002c) explains the inverse relationship to the relative energy expenditure required to perform submaximal or even maximal exercise. Adipose tissue is a non- working tissue and a low-fat mass improve the power to-weight ratio of the whole body. This handicap of excessive weight bearing may be a substantial factor during high intensity exercises. There was a tendency of correlation between V_{La4} and total score in BEFT ($r=0.43$, $P=0.07$). Strong correlation between V_{La4} and gallop was found ($r=0.61$, $P=0.007$), as well as moderate correlation to form under rider ($r=0.56$, $P=0.015$).

Therefore, as reported for other breeds (McMiken *et al*., 1983; Kearns *et al*., 2002a; Kearns *et al*., 2002b; Leleu and Cotrel, 2006), there is a relationship between aerobic capacity and performance in the Icelandic horse and most importantly, a relationship between BCS and aerobic capacity and performance. Whether the reason for these effects are solely due to altered metabolic pattern, increased added weight or both, remains to be known. However, as Stefánsdóttir *et al*. 2014 reported positive correlation between scores and plasma lactate response as well as velocity, marking the importance of anaerobic capacity at the highest intensities during a BEFT. This is also seen in this study by the treatment effect on lactate and total score for riding abilities. Interestingly, V_{La4} correlated to peak Hct (2 min post BEFT, $r=0.58$, $P<0.05$), pointing out the relationship between oxygen transport and aerobic respiration capacity, is in accordance to previous report on aerobic capacity (Persson 1968; 1983a; Stewart and Steel, 1975; Stefánsdóttir *et al*., 2014).

In addition, plasma lactate concentration correlated significantly both to total score for riding abilities and to score for pace (Stefánsdóttir, 2015). Neither relationship was found in this study. The horses in this study, in only three out of 18 observations, a score for pace was given, never higher than 6.5. As 7.5 is an average score (FEIF, 2015), the horses were not considered as decent pacers. This is a possible reason for the variation between studies in correlations to lactate, and in total score average. In addition, there is a slight variation in sampling time of the first blood sample post-exercise. In this study, blood was sampled precisely 2 min post BEFT. Stefánsdóttir *et al*. 2014 sampled within 5 min post-exercise. Although these correlations were not observed in this study, there was a significant overall effect of treatment on both total score for riding abilities and plasma lactate concentration.

6.3.5 Muscle enzyme

The lack of variation on AST concentration before and after the BEFT contrasts with Stefánsdóttir *et al*. (2014), Snow *et al*. (1983), Tyler-McGowan *et al*. (1999) and Hamlin *et al*. (2002), raising AST following submaximal or even maximal exercise. This could be explained by lower exercise intensity of the field test, in terms of lower speed and a lighter rider than in previous studies.

6.4 Recovery

A different pattern of recovery parameters was detected between different treatments, indicating a possible lower capacity to cope with strenuous exercise in higher body condition. Furthermore, an altered pattern of recovery was seen between the two exercise tests.

6.4.1 Standardised incremental exercise test

Under controlled situations of the SET, recovery parameters showed slightly altered pattern compared to following a BEFT. Despite the tendency of higher HR for HA, both groups reached their resting HR 30 min post exercise, showing the same pattern of recovery HR. There was a tendency of higher mean HR in HA and significant difference between treatments regarding RR and RT in the SET. There was a significant difference in resting RT between treatments, being lower in RA, nevertheless both ranging within normal values (Hodgson, 2014). RT differed between treatments 15 min' post-exercise. The groups had reached similar levels of RT after 30 min post exercise.

The peak RR in this study (71 – 97 ± 5) corresponds to RR of Standardbred trotters working at 7-10 m/s during an incremental exercise test. Thoroughbreds performing a SET, galloping at 10 m/s at 2-4° incline reach RR of 120 breaths/min and up to 133 breaths/min (Franklin *et al*., 2012). Moreover, levels remain at that level 10 m. post-exercise (Ainsworth, 2008; Lekeux *et al*., 2014). Butler *et al*. (1993) reported Thoroughbred horses not reaching resting levels of oxygen consumption 30 m. post-exercise. These studies present similar resting RR (16-19 breaths/min). The reason for this variance is clearly explained by higher exercise intensities for the race horse breeds.

The results indicate that excessive body condition could affect body temperature, possibly by limiting heat dissipation to some extent. Heat production could also be greater in the HA, due to increased microbial activity from increased forage intake and chewing activity, as seen by Pagan and Hintz (1986). There was a difference in RR at the end of SET, corresponding to higher loads, increasing oxygen demand.

There was a higher measured lactate accumulation in the SET compared to the BEFT. That is likely the result of sampling at maximum speed during the exercise test, while sampling was done 2 min post exercise in the BEFT and maximum speed could be reached anywhere in the ten rounds ridden, not necessarily in the end. Resting RR was reached by both groups 15 min post-exercise following a SET, but 30 min post-exercise after the BEFT.

Adaptation to the treadmill can vary. The gait adaptations occur fast, after about three times exercising in a treadmill (Buchner *et al*., 1994), but it might take much longer for horses to adapt mentally. Until complete adaptation occurs, nervousness is likely to cause a higher workload compared to fully adapted horses (Scheffer and van Oldruitenborgh-Oosterbaan, 1996).

There was a difference in RR and RT between exercise tests. As in the study by Nostell *et al*. (2006), RT and RR were higher after a treadmill test compared to field test was hypothesized to be because of diminished heat loss due to reduced heat convection. In the present study, higher RR and RT were documented after BEFT than after the treadmill test, corresponding to the extent of exercising intensity, anaerobic metabolism, and distance covered.

6.4.2 BEFT

To this date, this is the first study that measures recovery parameters of Icelandic horses following a simulated or real breed evaluation field test, exceeding observations 5 min post-exercise. There was a difference between treatments in recovery heart rate. HA had reached resting HR values 30 min post exercise, while the RA did not reach resting values 30 min post-exercise, relating to the greater exercise intensity of the treatment group followed by the lactate accumulation, lowered pH and oxygen dept of the skeletal muscle.

Respiratory rate differed between the two treatments, in total as well as significant difference between treatments in peak RR at 2 min post BEFT as well as 15 min post-exercise, with lower

RR in the RA compared to HA. Both treatments had reached resting values 30 min post exercise. The peak RR falls within boundaries of the peak RR in Stefánsdóttir *et al*. (2014), where RR within 5 min post-exercise was 101 ± 30 breaths/min. Higher resting RR in Stefánsdóttir *et al*. (2014) than in this study (30 ± 11 breaths/min vs. 13 ± 6 breaths/min) could be explained by location effects is that this study was performed at home, while Stefánsdóttir *et al*. (2014) was performed at a regional breeding evaluation, where horses needed to be transported over variable distances. With traffic, people and unfamiliar sightings, that could induce stress related behaviour, such as increased breathing frequency (Leadon and Hodgson, 2014).

On the contrary, rectal temperature was higher in RA compared to HA after the BEFT, being the opposite in the SET, most likely because they performed at higher intensity. Both groups did not reach their resting levels 30 min post exercise following BEFT and SET. After BEFT, plasma lactate concentration had reached insignificance from resting value 30 min post-exercise in the HA, not implying a faster lactate metabolism, but merely lower lactate values and therefore lower extent of anaerobic metabolism during BEFT. Interestingly, even though plasma lactate and RT were lower in HA, the HA seemed to need longer recovery post breed evaluation field test. The results indicate the significant effect of treatment on the pattern of recovery parameters, possibly lowering the horses´ capacity to cope with exercise.

6.5 Haematological responses

6.5.1 Haematocrit

The significant rise in Hct in relation to exercise intensity, both in BEFT and SET is in accordance to previous findings (Seeherman and Morris, 1990; McKeever *et al.*, 1993; Danielsen *et al*., 1995; Stefánsdóttir *et al*., 2014), as there was a strong correlation between velocity and Hct during SET. Variation in Hct between treatment groups is significant though being numerically low, varying on average by 1.7% after BEFT and 0.9% after SET. Interestingly, the difference in Hct can be seen already at resting state.

A possible reason for the increased Hct on the restricted allowance is that they have had a period of increased growth hormone (GH) levels to maintain plasma glucose concentration. GH secretion increases with lowered blood glucose, but unpublished results from this study indicate that blood glucose concentration is maintained or even slightly elevated. However, glucose measurement was only during a 90-minute feeding period and during SET, leaving a gap of undetected fluctuations in blood glucose. Growth hormone (GH), also called somatotropin, or recombinant equine somatotropin in horses (eST), stimulates growth of body mass and elongation of bones. GH-producing cells are dominant in the anterior pituitary gland (Sjaastad *et al*., 2016). GH also stimulates the production of red blood cells, through the stimulation of IGF-1 release that plays a vital role in the process of erythropoiesis in the bone marrow (Akers and Denbow, 2008; Alberts *et al*., 2008). Furthermore, temporary feed-deprivation and overnight fasting (18-20 hours) has shown to elevate eST levels (Sticker *et al*., 1995; Christiensen *et al*., 1997). Horses differ from ruminants and do not respond with decreased GH after feeding. Due to the episodic nature of GH secretion, increases can be of various reasons. eST infusion has not been found to affect the exercise capacity of horses, their indices or fitness of young Standardbreds in training (Gerard *et al*., 2002), or in unfit, geriatric mares (McKeever *et al*., 1998). However, there are several ways GH could affect exercise capacity, one being an increase in Hct (Christ *et al*., 1997).

Hct had reached resting values after 15 min of recovery time, both after BEFT and SET, showing a rather quick return of red blood cells to the spleen. The relative low concentration of Hct found in this study and by Stefánsdóttir *et al*. 2014 compared to other breeds (McGowan and Hodgson, 2014) seems to be consistent. The reason for this breed effects remains to be explained. It could be due to the versatile breeding goal of the Icelandic horse (FEIF, 2015), being much broader than to breed solely for speed, as is Thoroughbred and Standardbred racehorses (ASVT, 2015; Khadka, 2010). Moreover, it could be due to the prolonged conditioning to exercise compared to racing breeds, as it is tradition to start training of Icelandic horses at the age of three or older.

6.5.2 Total plasma protein

There was a tendency of treatment effect, with higher mean TPP for horses adapted to restricted energy allowance following a BEFT, but not in the SET ($P<0.1$). Therefore, the possible loss of fluids from the vascular compartment does not explain Hct variation between treatments entirely. The rise in TPP following BEFT and SET is in accordance to previous findings (Carlson, 1983; Judson *et al*., 1983 Seeherman and Morris, 1990; McKeever *et al.*, 1993; Danielsen *et al*., 1995; Hargreaves *et al*., 1999), that plasma protein increases with exercise intensity. However, these publications report peak TPP level at maximum exercise intensity, where the peak value is reached at step three out of four during the SET.

No significant different difference was found between sampling times during BEFT. Horses had recovered to resting values 15 min post-exercise after SET. The significant difference between resting value and during exercise-values in SET for all horses ($P<0.05$) is therefore likely to be explained by the fact that elevated exercise intensity results in temporary increase in heart rate and blood pressure, forcing a portion the plasma fluid through the capillary walls, that returns to a normal state as blood pressure decreases, followed by increased seating due to elevated body temperature (Hodgson and McGowan, 2014; Kingston, 2008). The horses had reached the resting values after 15 min after BEFT and SET.

Another possible contributor could be that horses adapted to a forage-only diet have higher values of extracellular fluids, possibly explained by greater fluid reservoir in the hindgut. Higher forage allowance increases water intake (Pagan and Harris, 1999; Jansson and Lindberg, 2012). Jansson and Lindberg, (2012) also found lower TPP in forage vs. concentrates diets. The results show the same pattern as Danielsen *et al*. (1995), with higher TPP during exercise and lower TPP in horses in high allowance diet compared to low allowance diet in Thoroughbred horses during an endurance type treadmill exercise test. The absence of variance between treatments in this study could be due to that the exercise intensity of the SET was not great enough to express responses in TPP. Changes in TPP values post-exercise reflect redistribution of circulating fluid volume, as previously found by Seeherman and Morris (1990), loosing fluid from the vascular compartment.

6.6 Locomotion asymmetry

To the authors knowledge, this is the first time that an objective evaluation of locomotion asymmetry on gaited horses is performed for scientific purposes. Therefore, to keep an open door for future research, two kinds of data selection were performed.

In general, results from statistical analysis of both datasets shown similar trends. Locomotion front limb symmetry was affected by treatment for both selection criteria, with higher asymmetry for horses adapted to high energy allowance. For hind limb symmetry, a tendency for treatment effect with higher asymmetry for HA compared to RA was found when selecting for strides, not for standard deviation. Ringmark *et al*. (2013) reported higher asymmetry for early race-qualifying horses, only in front limbs. A possible explanation is that horses carry naturally more weight on their front legs (Dutto *et al*., 2004) and are thus prone to show effects of added weight in front. The effects of compensatory weight shifting cannot be excluded, as locomotion asymmetry originating in the hind limbs can show as front limb asymmetry due to weight shifting with the head and trunk (Weishaupt *et al*., 2004a).

Interestingly, locomotion asymmetry was generally lower the days after the BEFT, more specifically for front limbs in the stride selection dataset. During the week before the BEFT, the only exercise the horses were submitted to was the SET. Therefore, the horses could have been stiff in the measurement the day before. Examining the analysis for the two days after BEFT (Figure 8), the same pattern can be seen, with higher front limb asymmetry in horses adapted to high energy allowance and no difference between treatments for hind limb asymmetry.

VSF day after BEFT correlated to judges scores for gallop in the BEFT. Gallop is shown at maximum speed capacity of the horse, with high loads on the dominant front limb in every stride. During gallop, high amounts of strain and stress have been recorded in horses during the weight-bearing phase on speeds up to 7.4 m/s (Biewner, 1998; Wilson *et al*., 2001). Several studies have shown the relationship between speed and ground reaction forces and strain, increasing risk of injury (Rubin and Lanyon, 1982; Nunamaker *et al*., 1990; Dutto *et al*., 2004). Horses in this study performed on $V_{max} \approx 11$ m/s. Icelandic horses have been recorded in up to 14 m/s during a 100m pace race (FEIF, 2017b) and can therefore be subjected to these high loads during BEFT and other disciplines, resulting in possible soreness, swelling or microdamage in front limbs following the BEFT, after showing gaits at higher performance levels.

VSH had negative correlations to canter and slow tölt. Both are shown at slow speeds, but require the most amount of collection, balance and suppleness during a BEFT. These results indicate that high asymmetry of hind legs could negatively affect balance and collection capacity of the Icelandic speed at slower speeds.

Prior to the study, the horses were adapted to run in hand on both surfaces. Icelandic horses are five-gaited horses, varying in the capacity to trot in a precise and secure manner, often changing from the diagonal locomotion pattern of trot to the lateral locomotion pattern of either tölt or pace. Therefore, some horses had substantial challenges running in even trot needed for a consistent recording and therefore we used boots for assistance. For these horses, it took a higher number of evaluations to get an acceptable measurement. It would have interesting to count those incidences and correlate to the extent of locomotion asymmetry. For future researchers, it is essential to be fully assured that the gaited horse is capable of trotting in hand without assistance and without additional footwear, that possibly needs some level of training and adaptation to be adequate.

There is no clinical equipment available for locomotion- or lameness evaluation available in Iceland, merely subjective evaluation by trained personnel and veterinarians. The portable device used in this study makes it possible to perform studies on site producing objective measurements and for veterinarians and other evaluators to compare results and data due to greatly increased standardization.

6.7 Strengths, challenges and limitations

The results from this thesis, are an important addition of information to the physiological response to exercise in the Icelandic horse. This study is the first attempt to implement a standardised training protocol where all individuals are introduced to precisely the same training in two separated treatments. Thus, we gain knowledge on the metabolic and physiological response towards feeding and exercise in the Icelandic horse, when its body composition is altered and how it affects its health, performance capacity and durability as an athlete. As this was the first attempt to do so, the training protocol was an experimental procedure. Although the two training periods were performed in a very similar manner, they were not identical.

There were in total five riders that participated, the three students performing the study along with two trainers employed by Hólar University College. To ensure the highest level of training standardization between individual horses, it is preferable that fewer riders handle the training, decreasing the chance of differentiation in duration and intensity.

The second assessment of an actual BEFT was not included in the study. That is ridden from one to three days after the first round, to attempt to raise marks for something that went wrong in the first assessment or simply to emphasize and advertise the horse´s quality. For an evaluation of full scale effects of a BEFT, a study with the both assessments are needed.

At the onset of the study, the horses were all in the target BCS following the high allowance treatment. The change between treatments was significant but not dramatic, and thus the horses did not reach moderate BCS of around 5, and the study only involved moderately obese horses (BCS > 6).

Moreover, this is the first time that locomotion asymmetry is evaluated in the Icelandic horse, clearing the path for further research in the field. Some of the horses had troubles trotting on a straight line in hand, but got better as the study progressed. Therefore, it is essential that the horses would have been fully accustomed to the procedure, to be able to exclude the effects of gait changes, though the software already accounts for those effects.

7 Conclusions

The overall conclusion from the results presented in the thesis is that higher body condition score and fat content impairs performance, increases locomotion asymmetry and alters recovery pattern in the Icelandic horse. Detailed conclusions were that:

- Altered body condition score and fat content affects the physiological response to exercise in the Icelandic horse.
- Horses with lower body condition score and fat content had higher judges scores for riding abilities in a simulated breed evaluation field test, namely for total score, gallop and form under rider.
- Horses with lower body condition score and fat content were able to perform at higher intensity in a simulated breed evaluation field test, utilizing anaerobic pathways to a greater extent.
- Altered body condition and fat content affects the pattern of recovery parameters following a BEFT and a SET, implying decreased capacity to cope with exercise in higher body condition score.
- Locomotion symmetry of front limbs was negatively affected by higher body condition score and body fat content.
- The participation in a breed evaluation field test did not increase locomotion asymmetry.

8 Future research

This study involved horses with only slight variation in BCS. Effects of BCS with greater variation in BCS and weight, obtained by longer adaptation periods and more excessive energy allowance would show us the physiological response of obese horses (BCS≥7.5) and horse in moderate BCS (4-5), being reported the optimum in other breeds. To study the true effects of altered BCS, a study on the physiological response to different weight-bearing, during both a field test but most importantly a standardised exercise test under controlled conditions, and hopefully answer the questions if the effects from this study are solely due to increased weight bearing and not due to altered metabolic profile. Long term effects of excessive BCS needs to be evaluated. Effects of chronic overweight on health, metabolic profile, joints and muscoskeletal system will be beneficial to the breed, hopefully helpful in increasing knowledge for increased health and performance capacity even more.

To study the full effects of the exercise intensity of a BEFT, a measurement of the energy expenditure during the test is optimal. Muscle glycogen measurements can be done to reach further understanding of the intensity and moreover, recovery following a BEFT, assessing when the horses have fully recovered and if they are fit to perform at the second assessment, performed one to three days later. Furthermore, it would be very interesting to gain knowledge on the responses on altered BCS on 100-250 m pace races and sport competitions, closing the circle on official competition disciplines for the Icelandic horse.

Furthermore, this is the first time the physiological response to a standardised, incremental exercise test performed on a treadmill is studied in the Icelandic horse. This makes way for future research on the Icelandic horse, able to use these results as comparison for methodology and physiological response. There are numerous effects that are yet to be evaluated in the Icelandic horse using a standardised exercise test, such as training state, age, sex, gait capacity and more.

The mystery of the low Hct concentration in Icelandic horses needs to be explained. Therefore, a long-term training study, conditioning young horses from 18 months of age, including a control group that is subjected to the traditional training methods, and then compare the groups using standardised exercise test and a breed evaluation field test.

A study on locomotion asymmetry study in top class competitions and BEFT at the highest levels in needed, as the horse quality and exercise intensity is most likely at a higher level, possibly with a different pattern of locomotion asymmetry. For a greater understanding of recovery pattern in the Icelandic horse, a study including exercise to the onset of fatigue, and measure at shorter intervals RR and RT.

This study included forage-only feeding. The effects of feeding forage vs. concentrate and high quality forage vs. lower quality on performance will be beneficial, as those are all possible feed allowances that Icelandic horses are subjected to in the real world.

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