Sveriges lantbruksuniversitet
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Metabolic effects of altered body fat content in the Icelandic horse

- responses to feeding and exercise

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Abstract

Obesity is one of the most common welfare problems affecting domesticated horses and is believed to stimulate the development of other metabolic syndromes, i.e. insulin resistance and laminitis. The Icelandic horse is considered to be an „easy keeper“, indicating that it requires lower amount of feed compared to many other horse breeds to maintain its body condition. However, obesity and its effect is an unexplored subject in the breed.

The aim of the thesis was to evaluate how altered body fat content affects metabolic- and physiological responses to feeding and exercise, respectively, in the Icelandic horse. The experiment was designed as a change-over study comprising of two experiments and two experimental periods. Ten Icelandic geldings, at the age of 6-8 years were used and equally divided into two groups and fed on different energy allowance over each period, restricted energy allowance diet vs. high energy allowance diet. The metabolic plasma profile was compared at the end of each treatment period by using a meal feeding trial and a standardised exercise treadmill test (SET). In the feeding trial collected blood samples were analysed for plasma insulin, glucose and non-esterified fatty acids (NEFA) and from the SET, blood samples were analysed for plasma glucose, NEFA and lactate.

The results from the study showed that body condition (BCS) and body weight were positively correlated to each other as well as other assessed body parameters, indicating that greater number of assessed body parameters increases the accuracy of the estimated BCS. In the meal feeding trial, all horses irrespective of treatment showed normal metabolic responses. However, horses adapted to the high energy allowance diet had significantly higher plasma insulin concentration, but significantly lower plasma glucose and NEFA concentrations. In the standardised exercise treadmill test horses adapted to restricted energy allowance showed significantly higher plasma glucose and NEFA concentration and significantly lower plasma lactate concentration. They also reached significantly higher V_{La2} and V_{La4} implying a greater aerobic capacity.

It was concluded that altered body condition and fat content affects the metabolic response to feeding and metabolic- and physiological responses to exercise. Keeping horses in a moderate body condition and physically active is of great importance. Feeding strategy and physical activity should be adapted to each other and maintained at a fine balance to positively affect the horse athletic abilities, enabling it to reach and maintain higher performance capacity in a healthy and sound state.

Ágrip

Offita er talið eitt af algengustu heilsufarsvandamálum sem herja á nútímahestinn, burtséð frá því af hvaða hestategund hesturinn er. Offita er talin ýta undir þróun annarra efnaskiptasjúkdóma, líkt og aukið insulín viðnám og hófsperru. Samanborið við mörg önnur hestakyn þá er íslenski hesturinn flokkaður sem „easy keeper“, sem gefur til kynna að hann getur viðhaldið holdastigi sínu á minna fóðurmagni eða orkuminna fóðri. Offita og áhrif hennar eru hins vegar, enn sem komið er, órannsakað viðfangsefni meðal íslenska hestsins.

Markmið þessarar rannsóknar var að meta hvaða áhrif breytt holdafar, það er aukin líkamsfita, hefur á efnaskipta- og lífeðlisfræðilega svörun jafnt við fóðurát sem og við þjálfun meðal íslenska hestsins. Rannsóknin samanstóð af tveimur aðskildum meðferðum og tveimur tímabilum og voru allir hestar prófaðir í báðum meðferðum. Tíu íslenskir geldingar, 6-8 vetra gamlir voru notaðir og skipt jafnt í tvo hópa sem fóðraðir voru annars vegar á gjöf þar sem orkuinnihald var takmarkað frá viðhaldsþörfum og hins vegar á gjöf þar sem orkuinnihald var umfram viðhaldsþarfir. Efnaskipta svörun var mæld í lok hvors tímabils með notkun sérstakrar fóðurrannsóknar og staðlaðs æfingaprófs sem framkvæmt var á hlaupabretti. Blóðsýni sem tekin voru á meðan fóðurrannsókn stóð voru greind fyrir insúlíni, blóðsykri og frjálsum fitusýrum og blóðsýni sem tekin voru á meðan staðlaða æfingaprófið fór fram voru greind fyrir blóðsykri, frjálsum fitusýrum og mjólkursýru.

Niðurstöður rannsóknarinnar sýndu fram á jákvæða fylgni milli holdastigs og líkamsþunga sem og við aðra mælda líkamsparta. Þetta gefur til kynna að nákvæmni holdastigunar eykst þegar fleiri heldur en færri líkamspartar eru teknir með við gerð matsins. Niðurstöðurnar fóðurranssóknarinnar gáfu til kynna að allir hestarnir, óháð meðferð, sýndu heilbrigða efnaskipta svörun við fóður inntöku, sem dregur fram mikilvægi þjálfunar. Hins vegar, hestar sem fóðraðir voru umfram orkuþarfir höfðu marktækt hærra magn af insúlíni í blóðinu en marktækt lægra magn af blóðsykri og frjálsum fitusýrum. Niðurstöður frá staðlaða æfingaprófinu sýndu að hestar sem fóðraðir voru á takmörkuðu orkuinnihaldi höfðu marktækt hærri blóðsykur og magn frjálsra fitusýra en um leið marktækt lægra magn mjólkusýru í blóði. Þessir hestar náðu einnig meiri hraða áður en magn mjólkursýru fór yfir 2 mmol/L og 4 mmol/L en hið síðarnefnda er hinn eiginlegi „mjólkursýruþröskuldur“ og bentu niðurstöður til aukinnar loftháðrar getu eða þols.

Holdastig hefur áhrif á efnaskipta svörun við fóður inntöku sem og efnaskipta- og lífeðlisfræðilega svörun við þjálfun. Mikilvægt er að stilla fóðrun og þjálfun saman og viðhalda í jafnvægi og þannig hafa jákvæð áhrif á heilbrigði og afkastagetu hestsins.

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Abbreviations

ATP	Adenosine-triphosphate
BCS	Body condition score
BCSmb	Mean body condition score with back score included
BCSub	Mean body condition score without back score
BW	Body weight
CP	Crude protein
CV	Coefficient variation
DM	Dry matter
F	Forage-only diet
FO	Forage-oats diet
GIP	Gastric inhibitory peptide
GLP-1	Glucagon-like polypeptide
GLUT-4	Glucose transporters found in the cell membrane
GPS	Global positioning sensor
Hct	Haematocrit
HR	Heart rate
HR_{max}	Maximal heart rate
IR	Insulin resistance
LSM	Least mean squares
MJ ME	Megajoule metabolizable energy
NEFA	Non-esterified fatty acid
SE	Standard error
SD	Standard deviation
SE	Standard error
SET	Standardised incremental exercise treadmill test
TBFP	Total body fat percentage
V_{La2}	Velocity (m/s) when reaching lactate concentration of 2 mmol/L
V_{La4}	Velocity (m/s) when reaching lactate concentration of 4 mmol/L
VOS	Organic matter digestibility

WAT	White adipose tissue
WSC	Water soluble carbohydrate

1 Introduction

1.1 The Icelandic horse

The popularity of the Icelandic horse has increased substantially over the past decades. Breeding organisations are found in 21 countries (FEIF, 2017) and total number of horses found in the world is approximately 265.000 of which near 75.000 individuals are registered in Iceland (FEIF, 2017) its certified country of origin (Ministry of Fisheries & Agriculture, 2011). Because of Iceland’s geographical location and historical low import, the Icelandic horse is considered to be one of the world´s most pure horse breed (Adalsteinsson, 1981; Björnsson and Sveinsson, 2006; Hreiðarsdóttir and Hallson, 2007).

1.1.1 The history of the Icelandic horse

The history of the Icelandic horse reaches all the way back to the beginning of 10th century from the period 874-930 (Adalsteinsson, 1981; Árnason *et al.*, 1994; Björnsson and Sveinsson, 2006) during the settlement of Iceland (Adalsteinsson, 1981; Björnsson and Sveinsson, 2006). In the first centuries until the beginning of the 20th century the horse was essential to humans where it was primarily used for transportation as well as work, mainly in agriculture and referred to as “þarfasti þjónninn” or “the most useful help” (Björnsson and Sveinsson, 2006).

After mechanisation the horse became a popular riding horse used for leisure and/or sport competitions (Björnsson and Sveinsson, 2006). In the year of 1950 an official breeding goal for the Icelandic horse was presented (Árnason *et al.*, 1994; Björnsson and Sveinsson, 2006) with high emphasis on improving conformation and riding abilities (Árnason *et al.*, 1994; Björnsson and Sveinsson, 2006; RML, 2017) and at the same time breed healthy, fertile and durable individuals (RML, 2017).

1.1.2 Influenced by the environment

The Icelandic horse is considered to be a pony (Björnsson and Sveinsson, 2006) with mean height at withers around 140 cm and body weight (BW) 330-370 kg (Stefánsdóttir *et al.*, 2014). Since settlement the horse has adapted to harsh and strenuous environment, both in landscape and climate conditions with limited food source (Árnason *et al.*, 1994; Björnsson and Sveinsson, 2006).

In Iceland, it has been a common management system throughout the years to keep horses out in the field, especially during the first years after birth and also during adulthood. Horses are often provided a break from training during autumn and first months of winter. Because of this management system the horse natural condition of increasing its BW, mainly through increased adipose tissue content, in the late summer or early autumn to use as an energy source during winter when feed availability is limited, has been vital for its survival (Gudmundsson and Dyrmundsson, 1994). The Icelandic horse does not require a substantial amount of feed to maintain its body condition and therefore it is known as an “easy keeper” (Ragnarsson and Jansson, 2011).

2 Literature Review

1.2 Adipose tissue

Adipose tissue, a highly specialized connective tissue, is the main energy storage site in mammalian species. Energy is stored in the form of triglycerides in adipocytes, a high density droplets found in a cell vacuole (Lafontan and Langin, 1995; Sjaastad *et al.,* 2016). In comparison to carbohydrates and proteins, triglycerides store approximately twice as much chemical energy (Akers and Denbow, 2008; Sjaastad *et al.,* 2016). If levels of absorbed carbohydrates and proteins from the diet exceed the body´s need, they are transported to the liver and can be used for triglyceride synthesis which are stored in the liver or transported in the form of lipoproteins to adipose tissue (Ferrier, 2014; Sjaastad *et al.*, 2016).

Two distinct types of adipose tissue can be found, white and brown where white is the prevalent (Akers and Denbow, 2008; Berry *et al.,* 2013; Sjaastad *et al.,* 2016) and possesses two major functions, storage and release of triglycerides and non-esterified fatty acids (NEFA), respectively (Lafontan and Langin, 1995). The adipose tissue is distributed all over the body, but is particularly abundant subcutaneously or visceral (Berry *et al.*, 2013; Sjaastad *et al.,* 2016) and is its allocation largely controlled by genes (Berry *et al.,* 2013; Gesta *et al.*, 2007). Within mammalian species, brown adipose tissue is dominant at neonatal stage, among young as well as small animals (Akers and Denbow, 2008; Sjaastad *et al.,* 2016) and its energy expenditure is of great importance for heat production (Gesta *et al.,* 2007; Akers and Denbow, 2008; Sjaastad *et al.,* 2016).

The white adipose tissue (WAT) is considered to be the largest endocrine organ that is involved in lipid synthesis and secretion of hormones and proteins, which affect body´s cells and tissues function (Gesta *et al.,* 2007). Regardless of horses body condition score (BCS) it is equally distributed between internal and external sites (Dugdale *et al.,* 2011b) however, when horses are overweight or obese the sensitivity of BCS as an indicator for total WAT content decreases (Dugdale *et al.*, 2011a). WAT plays an important role in plasma lipid metabolism and is the primary location for lipolysis, when triglyceride is hydrolysed and broken down to three energy-rich NEFAs and one glycerol molecule by stimulation from hormone-sensitive lipase (Lafontan and Langin, 1995). The hormone sensitive lipases are stimulated by catecholamines such as epinephrine (Pösö *et al.,* 1989; Ferrier, 2014; Sjaastad *et al.*, 2016).

Concentration of NEFA in the blood plasma is largely controlled by the adipose tissue. Vital organs, i.e. kidneys and heart, rely almost solely on NEFAs as an energy substrate, therefore, there is a constant demand of fatty acids supply to the blood circulation (Lafontan and Langin, 1995; Akers and Denbow, 2008). During starvation, stressful situations (Lafontan and Langin, 1995) and prolonged exercise (Sjaastad *et al.,* 2016), plasma NEFAs are believed to be the body's main energy source.

1.3 Body condition score and fat content effects

When keeping horses it is essential to have basic knowledge, both on optimum physical – and physiological conditions, to be able to ensure the horse welfare. Today, excessive adipose tissue accumulation or obesity in horses is one of the most common welfare problems known among domesticated horses (Owers & Chubbock, 2013) as majority of horse owners and trainers often underestimate their horse physical condition (Jensen *et al.,* 2016). It is known that obesity can be associated with various metabolic syndromes, i.e. insulin resistance (IR) (Hoffman *et al.*, 2003; Frank *et al.,* 2006; Geor, 2008; Ferrier, 2014; Treiber *et al.*, 2006b) and laminitis (Geor, 2008; Treiber *et al.,* 2006b).

Throughout the years, various methods of estimating BCS have been applied on humans (Hetland *et al.,* 1998) and different farm animals such as, dairy cows (Markusfeld *et al.*, 1997), beef cattle (Selk *et al.*, 1988) and sheep (Sejian *et al.,* 2010) for estimation of stored internal fat and its effects. These subjective methods are implemented by palpating different body parts and with the use of eye assessment (Henneke *et al.*, 1983). It is considered to be a relevant indicator for total energy balance and for the creation of feeding plans and management (Suagee *et al.,* 2008).

Westervelt *et al.* (1976) established that with the use of ultrasonic measurements it is possible to estimate the horse subcutaneous body fat. From these findings, a method was developed by Henneke *et al.* (1983) on mature Quarter Horse mares, known as the Henneke-scale. Mean BCS is derived from six palpated and visually assessed body parts, neck, shoulders, withers, ribs, loin and tailhead with scoring system extending from 1 (emaciated) to 9 (obese) (Henneke *et al.,* 1983). However, Suagee *et al.* (2008) established that mean BCS for

Thoroughbreds, derived from four body areas, neck, shoulders, ribs and tail head, with back area excluded from the scale, corresponds to Henneke´s mean BCS.

Carter *et al.* (2009) developed a scoring system (ranging from 0-5) for regional adiposity occurring in the crest of the neck, since increased neck adiposity is believed to alter metabolic reaction in the horse (Frank *et al.,* 2006; Treiber *et al.,* 2006b). Other methods have been developed to assess BW and BCS that can give indication on overweight or obesity in horses. These methods can involve weight tapes (Hoffmann *et al.,* 2013), weight formulas (Carroll and Huntington, 1988; Hoffmann *et al.,* 2013) and morphometric measurements, mainly girth circumference:height at withers ratio, neck circumference:height at withers ratio (Carter *et al.,* 2009; Jensen *et al.,* 2016), neck circumference (Frank *et al.,* 2006) and girth circumference in relation to body length (Carroll and Huntington, 1988; Hoffmann *et al.,* 2013).

A special system has been developed for the Icelandic horse where BCS is scored from 1 (extremely emaciated) to 5 (obese) (Stefánsdóttir and Björnsdóttir, 2001). A great proportion of the Icelandic horse population in Denmark is overweight or obese (Jensen *et al.,* 2016). From measurements performed by Ragnarsson and Jansson (2011) rump fat thickness is positively correlated to the BCS in Icelandic horses.

Body composition can be used as an indicator when health (Dionne *et al.,* 2003; Treiber *et al.,* 2006b), reproductive performance (Henneke *et al.,* 1984) and physiological responses in horses (Garlinghouse and Burrill, 1999; Kearns *et al.,* 2002a; Kearns *et al.,* 2002b; Leleu & Cotrel, 2006; Jansson *et al.,* 2015) are up for an evaluation. Increased body fat content among human athletes has been shown to reduce aerobic capacity and hence, performance (Hetland *et al.,* 1998). Increased energy expenditure needed for locomotion is undisputable in horses with increased body mass (McMiken, 1983; Pagan and Hintz, 1986) and weight loss can be stimulated with restricted energy diets (Sticker *et al.,* 1995a).

1.3.1 Body condition and effects of management on the domesticated horse

The horse is developed as an all year around grazing animal that relies on stored fat within the adipose tissue as an energy source during winter when feed is of limited source. The horse then enters a negative energy balance state where it starts to loose BW and body mass (Dawson *et al.,* 1945; Gudmundsson and Dyrmundsson, 1994), due to slower metabolic rate that results from reduced intake (Arnold *et al.,* 2006) as feed availability is limited (Gudmundsson and Dyrmundsson, 1994). By this, the horse reduces its energy demands to be able to thrive in the harsh environment (Dawson *et al.,* 1945; Arnold *et al.,* 2006). However, in the modern society horses are provided with harvested forage often fed in a mixture with concentrates and limited physical activity (Scheibe and Streich, 2003). This alters their natural seasonal respond where they compensate for reduced BW and body mass during winter with summer grazing and increased BW and body fat content (Dawson *et al.,* 1945; Gudmundsson and Dyrmundsson, 1994), and influences development of obesity (Hoffman *et al.,* 2003; Scheibe and Streich, 2003) and other metabolic syndromes (Treiber *et al.,* 2006b; Geor, 2008; Hoffman *et al.,* 2003) that can cause the animal pain and reduced quality of life (Treiber *et al.,* 2006b; Geor, 2008).

1.4 Metabolic responses to feed intake

1.4.1 Plasma insulin

Insulin, a small peptide hormone, is produced within the endocrine pancreas by β-cells found in the islets of Langerhans (Ferrier, 2014; Sjaastad *et al.,* 2016). Insulin plays an essential role in providing the body tissues with fuels used for energy utilisation. With feed intake, epithelial cells in the gastrointestinal tract, produce and release glucagon-like polypeptide (GLP-1) and gastric inhibitory peptide (GIP) hormones. These hormones stimulate the β-cells for insulin production and secretion where increased plasma insulin levels are rapidly seen after feed consumption (Sjaastad *et al.,* 2016). In addition, feed intake increases plasma glucose concentration that also stimulates β-cells for insulin production and secretion (Ferrier, 2014).

Insulin metabolism is considered to be anabolic, as it stimulates uptake on plasma glucose and NEFA and the synthesis of energy stores, i.e. glycogen within the liver and skeletal muscles and triglycerides synthesis within adipocytes (Ferrier, 2014; Sjaastad *et al.,* 2016). Insulin increases the number of glucose transporters (GLUT-4) in the cell-membrane which expand the cell’s efficiency on glucose uptake (Ferrier, 2014).

It is believed that increased BW and BCS affect plasma insulin by increasing its concentrations (Hoffman *et al.,* 2003; Ragnarsson and Jansson, 2011) during sedentary state (Carter *et al.,* 2009) and within active horses in training (Pagan *et al.,* 2009). During feed deprivation (DePew *et al.,* 1994; Connysson *et al.,* 2010) and when feeding frequency is altered/lowered (Jansson *et al.,* 2006) plasma insulin concentrations decreases significantly. However, following feed intake (Stull and Rodiek, 1988; DePew *et al.,* 1994; Sticker *et al.,* 1995a), increased feeding frequency (Jansson *et al.,* 2006) and ingestion on diets rich in carbohydrates there is a gradual increase in plasma insulin levels (Connysson *et al.,* 2010).

1.4.2 Plasma glucose

Glucose is derived from the diet as a carbohydrate, classified to simple sugars (monosaccharides) and is a hexose (Ferrier, 2014). It is essential for cells metabolism and considered to be the body´s primary energy source. Hepatocytes, cells located in the liver, can convert glucose into glycogen for storage in the liver or to triglycerides for storage in liver or adipose tissue. Plasma glucose can also be absorbed by muscles cells and stored within the muscle in the form of glycogen (Ferrier, 2014; Sjaastad *et al.,* 2016).

With feed intake, plasma glucose concentration increases substantially and hence, tissues and liver uptake of glucose. The extensive process of glucose uptake, utilisation and energy storage synthesis restrains high plasma concentrations of glucose within a healthy individual (Sjaastad *et al.,* 2016). Body condition is believed to affect insulin sensitivity and plasma glucose concentrations where obese horses rely primarily on glucose-mediated glucose disposal (Hoffman *et al.,* 2003). Daily plasma glucose level is not significantly different when horses are fed energy restricted diets (Sticker *et al.,* 1995a) but following feed intake, plasma glucose concentration rises within 2 hours (Stull and Rodiek, 1988; DePew *et al.,* 1994; Sticker *et al.,* 1995a).

1.4.3 Plasma NEFA

Absorbed triglycerides are mainly broken down in adipose tissues or muscle capillaries. There they are broken down to three NEFA molecules and one glycerol molecule (Ferrier, 2014; Sjaastad *et al.,* 2016). NEFAs can be absorbed by muscle cells where they are oxidised for energy utilisation or by adipocytes where they are either utilised as a fuel substrate or re-esterified to triglyceride. Plasma NEFA can also bind to plasma albumin and be transported to various tissues for energy utilisation. Increased levels of plasma insulin decreases the rate of lipolysis and promotes adipocytes uptake of NEFA resulting in low plasma NEFA concentrations (Ferrier, 2014; Sjaastad *et al.,* 2016). Increased levels of plasma glucose also promotes triglyceride storage (Ferrier, 2014).

Daily plasma NEFA concentrations are higher in horses in negative energy balance than horses in positive energy balance (Sticker *et al.,* 1995a). During feed deprivation, plasma NEFA levels increase (Sticker *et al.,* 1995b; Connysson *et al.,* 2010) and are also higher when horses are fed forage-only diets compared to carbohydrate rich diets (Connysson *et al.,* 2010). However, plasma NEFA concentration decreases with feed intake (DePew *et al.,* 1994; Sticker *et al.,* 1995a) and its reduction is more rapid on energy-restricted diets (Sticker *et al.,* 1995a). Following a day of exercise, plasma NEFA levels are higher in horses adapted to forage-only diet than to forage-concentrate diet (Jansson and Lindberg, 2012) and differences in NEFA concentration are believed to be breed independent and influenced of BW and BCS (Ragnarsson and Jansson, 2011).

1.5 Standardised exercise treadmill tests

With the use of standardised exercise test (SET) performed on a treadmill, it is possible to evaluate the horse metabolic- and physiological responses to training and how fitness is affected with prolonged training. Exercise tests can be implemented both in the field and on a treadmill. In 1967 the first SET was performed on horses (Persson, 1967) and since then it has been widely used in equine studies. With the use of treadmills, data can be collected while the horse performs the exercise without a rider and unnecessary equipment. Exercises are easily standardised, enabling repeated measurements to estimate parameters stability (Couroucé, 1999; Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999; Evans, 2008; Bitschnau *et al.*, 2010; Hodgson and McGowan, 2014).

SETs are optimal for measurements on heart rate (HR) and plasma variables (Persson, 1967; Valberg *et al.*, 1985; Harris and Snow, 1988; Rose *et al.*, 1988; Sexton and Erickson, 1990; Scheffer and Sloet van Oldruitenborgh-Oosterbaan, 1996). Locomotion patterns have also been determined with the use of treadmills (Frederickson *et al.*, 1983; Buchner *et al.*, 1994). Field exercise tests are not as easily standardised due to environmental factors, weather conditions (Hargreaves *et al.*, 1999) and exercise intensities which can affect performance and make it difficult to repeat previously performed exercise (Evans, 2008; Hodgson and McGowan, 2014) However, field tests are performed in the horse training and competition surroundings, where it is effected by its rider or driver, and prolonged adaption periods to the exercise equipment are most often unnecessary (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999; Evans, 2008; Hodgson and McGowan, 2014).

Studies have shown that the two form of exercise tests are not equivalent, as field tests are thought to be more demanding than treadmill tests. Trotters performing an uninclined SET have lower maximal HR (HR_{max}) and plasma lactate concentrations compared to horses exercising on the field (Couroucé *et al.*, 1999). To increase SET level of difficulty and push the horse to reach its maximal output without increased risk of injury, treadmill inclination by 2° to 10° is believed to be an optimal solution (Persson, 1967; Sexton and Erickson, 1990; Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999). Adaption time to the treadmill can vary between horses, ranging from few minutes (Frederickson *et al.*, 1983) up to prolonged time and training bouts (Buchner *et al.*, 1994; Scheffer and Sloet van Oldruitenborgh-Oosterbaan, 1996).

1.6 Energy utilisation pathways

The horse is considered to be an outstanding athlete that can perform various and strenuous work. From its domestication, humans have interfered with its development and nowadays nearly all horse breeds are bred to meet different roles (Gerard *et al.,* 2014). However, regardless of breed, all horses rely on the same pathways for energy utilisation. The horse relies on the body's metabolic ability to transform stored chemical energy to mechanical energy that initiates muscle contraction, enables locomotion and promotes performance (Gunn *et al.*, 1979; McMiken, 1983; Gerard *et al.*, 2014).

The term "fitness" is a synonymous that describes the individual ability to perform physical work for prolonged time (McMiken, 1983). Carbohydrates, fats and proteins are considered to be the body main fuel substrates as they provide the internal metabolism the energy substrate adenosine-triphosphate (ATP). Intramuscular ATP stores are limited (2.5 to 6 mmol/kg muscle) and are rapidly depleted after the exercise onset (Lindholm and Piehl, 1974; McMiken, 1983).

Within the body, aerobic phosphorylation and anaerobic phosphorylation are the two distinct pathways that take place for ATP synthesis (McMiken, 1983; Gerald *et al.*, 2014). Aerobic phosphorylation is oxygen dependent and relies on plasma glucose and NEFA oxidation where carbon dioxide (CO_2) is the end product. Through this energy production pathway ~36 ATPs can be produced from one glucose molecule (Gerard *et al.*, 2014). In contrast, anaerobic phosphorylation is independent of O_2 and can maintain the muscles rapid energy requirement during high-intensity exercise (Hodgson and McGowan, 2014; Gerard *et al.*, 2014). Muscle glycogen is the main energy substrate that undergoes anaerobic glycolysis with lactate as end product. Two to three ATPs are produced from one glucose molecule through anaerobic glycolysis and is this energy utilisation pathway substrate limited (Lindholm and Piehl, 1974; Gerard *et al.*, 2014).

During most exercises, both aerobic and anaerobic metabolisms can occur, however, aerobic metabolism is most often dominant (McMiken, 1983; Gerard *et al.*, 2014; Hodgson and McGowan, 2014). Muscles energy requirement is positively correlated to total body mass, as more energy is needed to mobilise a horse with increased body mass (McMiken, 1983; Pagan and Hintz, 1986; Kearns *et al.*, 2002b) and at slower speed the demand for energy is extended (Pagan and Hintz, 1986). There is undisputable rise in energy requirements when the body muscle:fat ratio increases (Blaxter, 1989; Kearns *et al.*, 2002b).

1.7 Physiological responses to standardised exercise treadmill test

1.7.1 Heart rate

The horse total heart mass is believed to be in relation to its body weight and in highly trained horses it can reach up to 1.1% of total body mass (Webb and Weaver, 1979). At rest the horse average HR is around 27-40 beats/min (Marsland, 1968; Krzywanek *et al*, 1970; Lindholm and Saltin, 1974) and during strenuous exercise HR_{max} is in the range of 225 beats/min (Krzywanek *et al.*, 1970; Stefánsdóttir *et al.,* 2014) up to 240 beats/min (Lindholm and Saltin, 1974).

Total heart mass or the heart score, relates to the heart size and is believed to influence athletic performance (Stewart, 1981) as higher heart score is in relation to greater performance capacity (Steel and Stewart, 1974; Rose *et al.*, 1979). Total heart size is in relation to body mass, body fat content and exercise intensity (Kline and Foreman, 1991). Heart size increases with elevated training intensity (Kline and Foreman, 1991) resulting in a lower resting rate, lower peak HR, longer time before reaching HR_{max} during exercise and rapid recovery rate (Marsland, 1968) following increased stroke volume (Persson, 1968).

HR during exercise increases linearly with extended training intensity (Persson and Ullberg, 1974; McKeever *et al.,* 1993; Persson, 1997; Eaton *et al.*, 1995). Training intensity is greatly affected by velocity which is significantly correlated to energy expenditure needed during exercise (Pagan and Hintz, 1986). After a strenuous exercise, rapid recovery has been reported during active cool-down (Kang *et al.,* 2012) whereas, speed of HR recovery is believed to imply greater fitness (Marsland, 1968). Today, modern HR meters, i.e. Polar-electro (Finland), have been developed for the use in equine studies that provide reliable results (Vermeulen *et al.*, 2006; Bitschnau *et al.,* 2010; Parker *et al.,* 2010; Stefánsdóttir *et al.,* 2014; Stefánsdóttir *et al.,* 2015).

1.7.2 Plasma glucose in response to exercise

During exercise, glucose is an essential energy source for the working muscle both in the form of absorbed plasma glucose and glucose in its storage form, glycogen (McMiken, 1983; Ferrier, 2014). However, glycogen stores are limited within the liver and skeletal muscles (McMiken, 1983; Ferrier, 2014) and when depleted the body starts producing energy through gluconeogenesis where lactate and triglycerides are hydrolysed for ATP synthesis (Ferrier, 2014).

Glucose uptake from the plasma during exercise is to a very low degree controlled by insulin (Treiber *et al.*, 2006a) and regardless of insulin sensitivity, absorption of plasma glucose is termed glucose effectiveness (Bergman *et al.,* 1979). Muscle contraction during exercise is believed to increase number of GLUT-4 transporters irrespective of plasma insulin concentration and stimulate glucose absorption (Henriksen *et al.,* 1990; Stewart-Hunt *et al.,* 2006). Along with muscle contraction that is stimulated through exercise, feeding strategies can also affect plasma glucose concentration during exercise (Pagan and Harris, 1999; Jansson and Lindberg, 2012) and following exercise (Sticker *et al.,* 1995b). Horses fed forage-only diet have higher plasma glucose levels (Jansson and Lindberg, 2012) which are believed to increase endurance during exercise (Lacombe *et al.,* 2001; Jansson and Lindberg, 2012).

Regardless of feeding regimes, exercise increases insulin sensitivity (Powell *et al.,* 2002; Pratt *et al.,* 2006; Stewart-Hunt *et al.,* 2006; Treiber *et al.,* 2006a) and glucose effectiveness (Treiber *et al.,* 2006a). Elevated concentration of plasma glucose in healthy individuals is believed to have positive influence on performance capacity (Lacombe *et al.,* 2001; Jansson and Lindberg, 2012). However, when horses are IR they can become hyperglycaemic which can promote obesity (Ferrier, 2014) resulting in altered performance (Treiber *et al.,* 2006a; 2006b). Walking has positive effects on horses during exercise recovery as they reach pre-exercise glucose values more rapidly (Kang *et al.,* 2012).

1.7.3 Plasma NEFA in response to exercise

In the post-absorptive state, NEFAs are the body main fuel substrate to use for maintenance and often termed as glucose sparing substrate (Sjaastad *et al.,* 2016). Exercise increases the activity of hormone sensitive lipases and hence, promotes lipolysis (Sjaastad *et al.,* 2016) and parallel, exercise increases insulin sensitivity (Powell *et al.,* 2002). Glycerol, produced from lipolysis, is transported to the liver where it can take part in triglyceride synthesis, glycolysis or gluconeogenesis. NEFAs can either be re-esterified within the adipocyte and promote triglyceride synthesis or diffuse through the adipocyte cell membrane to be transported to various tissues where they are oxidised within the cells mitochondria for energy utilisation (Ferrier, 2014; Sjaastad *et al.,* 2016).

It is believed that lipolysis, NEFA oxidation and triglyceride synthesis occur concurrently during exercise (Pösö *et al.,* 1989). Exercise at medium or high intensity level increases plasma NEFA production through adipose tissue lipolysis (Snow and MacKenzie, 1977; Pösö *et al.,* 1989; Lawrence *et al.,* 1993) and plasma NEFA oxidation (Pösö *et al.,* 1989), resulting in decreased plasma NEFA concentration during short but intense exercise bout (Jansson and Lindberg, 2012). In contrast, plasma NEFA concentration increases significantly during endurance exercise (Lindholm *et al.,* 1974; Essén-Gustavsson and Jensen-Waern, 2002) and during recovery (Snow and MacKenzie, 1977; Sticker *et al.,* 1995b).

Overweight and obese men show greater plasma insulin concentration and low concentration of plasma NEFA during exercise when compared to lean men (Mittendorfer *et al.,* 2004). When feed is withheld from horses prior to exercise it results in a greater plasma NEFA concentration and increased responsiveness compared to horses fed grains couple of hours before exercise (Lawrence *et al.,* 1993). Horses fed forage-only diet have higher plasma NEFA availability during exercise (Pagan and Harris, 1999) and higher NEFA concentration after exercise (Jansson and Lindberg, 2012) than horses adapted to forage-concentrate diet.

1.7.4 Plasma lactate in response to exercise and V_{La4}

Measurements of plasma lactate concentration, in samples collected during or after high intensity exercise, are frequently performed to evaluate physiological responses and fitness (Evans *et al.,* 1995; Hodgson and McGowan, 2014). When oxygen is deficient, anaerobic glycolysis occurs within the active muscles where lactate is the end-product (Gerard *et al.,* 2014). It diffuses into the cardiovascular system and may increase plasma lactate concentration (McMiken, 1983; McGowan and Hodgson, 2014).

When lactate concentration increases substantially, it is often referred to as the lactate threshold or anaerobic threshold, when the muscles convert from aerobic to anaerobic metabolism (Wesserman *et al.,* 1973; Hodgson and McGowan, 2014) and rate of lactate accumulation is greater than rate of lactate removal (Valberg, 2014). With increased plasma lactate concentration there is a gradual decrease in blood and muscle pH (Evans and Rose, 1988; Rose *et al.,* 1988). The anaerobic threshold is believed to occur when plasma lactate concentration reaches 4 mmol/L, and the speed when it occurs (V_{La4}) has been of great interest (Von Wittke *et al.,* 1994; Eaton *et al.,* 1995; Hodgson and McGowan, 2014; Ringmark *et al.,* 2015). Velocity, training intensity and repetition are the most important factors when horses are trained with the aim to increase V_{La4} (Von Wittke *et al.,* 1994; Ringmark *et al.,* 2015).

At resting state, lactate values are in the range of 1 mmol/L (Keenan, 1979; Lucke and Hall, 1980). Mean plasma lactate values measured post-exercise in Icelandic horses attending a breeding evaluation test was between 13.1 mmol/L to 18.6 mmol/L with individual values reaching 34.4 mmol/L (Stefánsdóttir *et al.,* 2014). Similar values have been seen among Thoroughbred racehorses performing a SET (Harris and Snow, 1988; Rose *et al.,* 1988; Seeherman and Morris, 1990; Evans *et al.,* 1995).

Plasma lactate concentration is positively correlated to velocity (Persson and Ullberg, 1974; Seeherman and Morris, 1990; Eaton *et al.*, 1995) and different workload, i.e. treadmill inclination (Eaton *et al.*, 1995). It is believed that horses with high aerobic capacity, showing high maximal performance have low lactate accumulation during exercise (Persson and Ullberg, 1974; Evans *et al.*, 1993) or faster lactate removal from the blood after exercise (Evans *et al.*, 1993) and increased V_{La4} (Bitschnau *et al.*, 2010). Body composition and BW have indirect effect on blood lactate concentrations during exercise as increased total fat percent (F%) is negatively correlated to V_{La4} (Leleu and Cotrel, 2006) and to aerobic capacity and performance level (Kearns *et al.*, 2002a; 2002b).

During the first steps in a standardised exercise, lactate concentration is stable but increases exponentially in relation to exercise intensity and reaches peak value during the first minutes of recovery (Rose *et al.*, 1988; Seeherman and Morris, 1990). Not all blood lactate is oxidised to CO_2 or H_2O (Rose *et al.*, 1988) as some proportion can be converted to glucose and re-used as an energy substrate, both during exercise (Karlsson *et al.*, 1972) and afterwards (Rose *et al.*, 1988). The rate for blood lactate removal is more rapid during active cooldown (Kang *et al.*, 2012).

2 Aim of the thesis

The aim of the thesis was to evaluate how altered body fat content affect metabolic and physiological responses to feeding and exercise, respectively, in the Icelandic horse.

Specific objectives were to:

Evaluate metabolic responses to feed intake in horses with different body condition and fat content.

Measure metabolic- and physiological responses to a standardised incremental exercise test performed on a treadmill in horses with different body condition and fat content.

The general hypotheses were:

1) The horse metabolic response to feed intake is altered with increased body weight, body condition and fat content.
2) The horse metabolic- and physiological responses to exercise are altered with increased body weight, body condition and fat content, resulting in lower performance level.

and specific hypotheses were:

- Plasma insulin concentrations is higher when horses have increased body weight and fat content
- Plasma glucose concentration is higher when horses have increased body weight and fat content
- Plasma NEFA concentrations are lower when horses have increased body weight and fat content
- Plasma glucose concentrations are higher during exercise when horses have lower body weight and fat content
- When horses have lower body weight and fat content they rely to a greater extent on plasma NEFA for energy utilisation
- Plasma lactate accumulation during exercise is lower when horses have lower body weight and fat content
- Performance capacity is greater in horses when they have lower body weight and fat content

3 Material and methods

3.1 Experimental setup

The experiment was designed as a change-over study comprising of two treatments and two experimental periods, each covering 5 weeks. The horses were divided into two groups and fed on different energy allowance over each period. For the first experimental period one group was fed on a high energy allowance and the other group was fed on a restricted energy allowance diet and for the next experimental period the energy allowances were shifted. The metabolic plasma profile was compared by the end of each treatment period by using a standardised incremental exercise test (SET) performed on a treadmill and a meal feeding trial. The study was conducted at Hólar University College in Iceland from 21st of March until 2nd of June and was approved by the National Animal Research Committee of Iceland.

3.2 The horses

Ten Icelandic geldings at the age of 6-8 years were used in the study. Their initial BW ranged from 401 ± 16 kg (mean ± SD) and were all evaluated healthy and sound by a veterinarian prior to the study. Before the study began the horses coat was clipped (Figure 1). This was done since the experiment was performed in the spring and an individual variation in the rate of change to summer coat was expected which would cause an unwanted variation in thermoregulatory responses.

Figure 1. A horse coat after being clipped.

3.2.1 Management

During the experiment the horses were kept in a stable where each individual had its own box (7 m^2) with saw dust bedding. Inside the box, the horses had access to water *ad libitum* from a water bowl (flow 7 L/min) and they were fed three times during the day, in the morning between 07:00-08:00 a.m., at lunch time between 14:00-15:00 p.m. and between 21:00-22:00 p.m. in the evening. The horses were divided to two groups (4 and 6 horses, respectively) when kept outside in a gravel paddock for approximately one up to five hours every day.

3.2.2 Body condition scoring

The horses were weighed and their body condition scored by using the Henneke scale (Henneke *et al.*, 1983) once a week during both periods. Accuracy of the weighing scale used for BW determination was ±100 g. With the use of BCS images (Henneke *et al.*, 1983) the horses were scored for overall picture. Two different mean values were calculated, as one included back score (BCSmb) and the other did not (BCSub). The main reason for the two different mean values is that the Icelandic horse is prone to increase body fat content in other places than on the back and seldom reaches the mean score of 5 for that area. This has also been observed among Thoroughbred horses (Suagee *et al.*, 2008). By using BCSmb it is possible that body fat content would be underestimated and therefore using BCSub is believed to give more accurate estimation. Along with BCS using the Henneke scale (1983), the horse´s neck line and fat content was evaluated by using a cresty neck scale created by Carter *et al.* (2009) with scoring system ranging from 0 to 5. Girth circumference was measured at the same time once every week but neck circumference was only measured at the end of first period but in the second period it was measured at the same time as other measurements once every week.

3.2.3 Rump fat

The horses' total body fat percentage (TBFP) was estimated by using ultrasonic equipment to measure total rump fat according to Westervelt *et al.* (1976). The desired spot for rump fat thickness measurements was found by measuring 5 cm from the horses' midline towards the centre of their pelvic bone. The horses' fur was clipped and shaved at the exact measurement place on their rump (Figure 2) and ultra-sound transmission gel (Sonavelle, Elefant-Cherrie, Hannover, Germany) was used to increase reflectivity. The measurement was performed by the same person and 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) were used in both periods to limit measurements deviations. TBFP was calculated by using the following equation, *TBFP = 8.64 + 4.70 * X* where X is the total cm of rump fat (Westervelt *et al.*, 1976).

Figure 2. Measurement place on a horse rump for calculation on total TBFP.

3.2.4 Creation of groups

Horses were divided into two groups. The aim was to have high similarities between groups based on initial information regarding BW, BCSub, girth circumference, V_{La4} with the use of instant lactate analysis (Lactate Pro analyser, Akray Factory Inc., Koji Konan-cho, Koka, Shiga, Japan) and peak haematocrit (Hct) (Table 1).

Table 1. *Parameters used for creation of groups prior to the study and mean values for the groups*

	BCSub	Overall picture	Body weight	Girth circumf. (cm)	VLa4	peak Hct
Group 1	6.4 ± 0.2	6.9 ± 0.7	398.2 ± 21.5	176.2 ± 4.5	5.3 ± 0.7	47.4 ± 2.4
Group 2	6.4 ± 0.2	6.7 ± 0.7	401.4 ± 16.8	175.6 ± 4.2	5.4 ± 0.5	46.8 ± 1.9

3.3 Feeding management

The method used to cause variation in body fat content and BCS was a high or low daily feed allowance (energy intake) during 36 days (each period). Prior to the study, the BCS of the experimental horses ranged from 6 - 6.6 and with the "low allowance" treatment the aim was to get closer to a BCS observed in other sport horses (Garlinghouse and Burrill, 1999; Suagee *et al.,* 2008; Ringmark *et al.,* 2013b). In the "high allowance" treatment, BCS was maintained or slightly increased compared to pre-study levels. The target BW on the low allowance treatment was possible to evaluate from the initial BW and BCS with the use of results from previously conducted studies. Results from Ringmark *et al.* (2013b) indicate that athletic horses in BCS 5 have approximately 7-10% of body fat, corresponding to 28-40 kg of body fat. The rump fat measurement performed prior to our study indicated that the horses had approximately 15% body fat (1.5 cm) corresponding to 60 kg of body fat (Westervelt *et al*., 1976). The horses used in our study therefore needed to lose nearly 30 kg to reach the "athletic horse" BCS, corresponding to approximately 1 kg per day before the last experimental week within each period. Therefore, the horses' daily energy requirements were calculated from the target BW (30 kg retracted from the initial BW). Since the Icelandic horse is known as an "easy keeper" it was also decided to reduce this daily restricted energy allowance with 20% compared to the recommendation for non-easy keepers, to increase the possibility of the horses reaching their desired BW and BCS before the experimental weeks.

All horses were fed on forage only diet, a haylage where high emphasis was put on energy and crude protein (CP) content. Daily energy and protein intakes in treatment high and low are shown in Table 2. For the restricted energy allowance treatment, daily allowance of haylage was calculated to 0.8 kg DM/100kg BW, corresponding to 8.5 MJ ME/100kg BW or 32 ± 0.3 MJ ME/day. For high energy allowance treatment, daily intake was calculated to 1.6 kg DM/100kg, corresponding to 17 MJ ME/100kg BW or 64 ± 0.3 MJ ME/day. A suitable feeding plan was calculated for each individual with a help from an equine nutritional program (PC-Horse software, Hove software LTD, Norway). To be able to fulfil the horses nutritional requirements (NRC, 2007) they were fed, along with the haylage, minerals and salt. With each evening meal, the horses received 90-160 g of minerals (Racing Minerals, Trouw nutrition, Netherlands) were the ratio was dependent on daily haylage intake and 40 g of salt. To ensure that all horses ate the minerals, 50 g of müsli (Besterly Herbic Müsli, Energys, Netherlands) was mixed with both minerals and salt.

Feed samples were collected every other day and when a new bale was opened. Samples that were collected during weeks 1 and 2 were mixed together in one big plastic bag and samples from weeks 3 and 4 were mixed together in another separated bag. Samples that were collected during the experimental weeks were kept in separated bags. Over both periods, six bags were collected in total and stored in -18°C until analysed. The haylage used in the study had a mean content of 10.7 ± 0.3 MJ ME (9.9-11.2 MJ ME) and mean CP content of 163 ± 9 g/kg. Energy and nutrient values in the two experimental periods are shown in Table 3.

Table 2. *Daily energy, protein and mineral intake within treatments (values presented as LSM ± SE)*

	High	Restricted
DM kg	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

DM = dry matter; MJ ME = metabolisable energy; CP = crude protein;
Ca = calsium; P = phosphor; Mg = magnesium; Na = natrium; K = kalium

Table 3. *Energy and nutrients values (Mean ± SD) from the haylages used in each period of the study*

	DM (g/kg)	Ash (g/kg)	CP (g/kg)	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

DM = dry matter; CP = crude protein
VOS = Organic matter of the haylage. Found with IVOMD (in vitro organic matter digestibility) analysing method
ME = metabolisable energy

3.4 **Training**

3.4.1 Training setup

During the experiment, the horses were trained five days each week and rested for two days. In total, 19 training sessions were within each period. Over both periods there were five different riders training the horses. To make sure the training was as standardised as possible, every rider wrote down every training session in period 1, date, duration and what kind of exercise/gait that was performed. In period 2, the training was performed in exactly the same way and the horses were ridden and trained by the same riders as in period 1.

3.4.2 Training intensity

Training sessions were classified according to three different levels of difficulty, training intensity 1, 2 and 3. Training intensity 1 was categorised as very low, heart rate <180 beats/min, and performed once every week. The training involved riding a horse that was irrelevant to the experiment and having the experimental horses running along in the riding tour as hand horses, one at a time. Training intensity 2 was defined as medium intense training, heart rate >180 beats/min for couple of short periods during the exercise. These training sessions included exercise under a rider (both indoor and outdoor) on average 3 times per week. The horses were never pushed up to their highest limits in speed as they were mostly ridden in walk and slow up to medium speed tölt, trot or canter. Training intensity 3 was defined as highly intense training, heart rate > 180 beats/min for couple of longer periods during exercise, and was performed once per week. The horses were exercised under a rider, outdoors and gallop interval training was performed. The riders warmed the horses up on walk as well as riding them on slow up to medium speed on either tölt or trot. The horses were then pushed to their limits and ridden at maximum speed on gallop, 3 times covering approximately 200 m distance, respectively, during a training session. For further information on different training intensity see Table 4 and Table 5.

A statistical analysis was performed to confirm that the training performed was not different between treatments. There was not a difference (P>0.05) in training between treatments. For mean training HR there was a significant difference between HR and different training intensities (P<.0001). There was a significant difference between total time spent <180 beats/min and training intensity (P<.0001) as well as between total time spent >180 beats/min and training intensity (P<.0001). Total time spent at each training intensity was significantly different between training intensities (P<.0001, Table 4).

Table 4. *A gradual increase in HR, time spent under and above HR 180 beats/min and duration 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.m.p.	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

b.p.m. = beats/min

Table 5. *Similarities in training intensity steps 1, 2 and 3 between Period 1 and Period 2 (values are presented as LSM ± SE)*

	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

* Significant difference (*P*<0.05)
b.p.m = beats/min

3.4.3 Heart rate recording equipment

During all training sessions and the SET, horse's heart rates were recorded using an equipment called, Polar HR Monitor (RS800CX, Polar Electro Oy, Kempele, Finland) and equine H1 heart rate sensor electrode base set used with Polar Equine T56H transmitter W.I.N.D. (Kempele, Finland). During outdoor training sessions, Polar G3 global positioning sensor (GPS; Polar Electro Oy, Kempele, Finland) was used to monitor and register speed and total distance. All recorded data was transferred into the software Polar Pro Trainer 5 Equine Edition (Polar Electro, Kempele, Finland), enabling evaluation on training intensity due to heart rate frequency per minute over each training session and during both exercise tests. In the morning of the experimental days, before the tests, resting heart rate was recorded when the horses stood relaxed inside their own box, with the use of stethoscope.

3.5 Data collection

3.5.1 Adjustments before experiment initiation

The week before the initiation of the study all horses were adjusted to the treadmill to gain necessary information regarding each horse and to reduce the risk of stress affecting the real experiment. During the first day, horses were only led up on to the treadmill for couple of times and stopped to stand still for few seconds on the board. The second day, the treadmill was turned on to get the horses used to its sudden start and for approximately 5 minutes the horses walked and trotted at slow speed on the non-inclined treadmill. At the third day the horses walked and trotted at a slow speed, first on a non-inclined treadmill and then the incline was increased from 0% up to 6.25% (Persson, 1967). The fourth day the horses trotted at two different speeds on the inclined treadmill (6.25%). During days five and six, the horses performed SET with four incremental speeds ranging for 2 minutes each. The heart rate was monitored and when reaching 190-200 beats/min the speed was registered. Blood samples were collected at the end of each step for lactate analyses that provided information on plasma lactate concentration and at what speed the horse was on when reaching 4 mmol/L plasma lactate concentration, or at what speed the horse reached its lactate threshold. From both heart rate and lactate information it was possible to decide all horses' individual speeds on the treadmill during the experiment.

3.5.2 Data sampling

A veterinarian inserted an I.V. catheter to the horses' *vena jugularis* when they were under local anaesthesia (using 5 ml of Xylocain 20 mg/ml (Astra Zeneca)) in the morning, prior to the SET and the following feeding trial to enable blood sampling. The blood was collected using vacutainer-technique into chilled lithium heparainzed tubes (9 ml, Vacuette®; Greine-Bio-One, Kremsmuenster, Austria). The respiratory rate (RR) was recorded by counting the number of breaths/min and data collection took place prior to the tests, at the end of the exercise, and during recovery, 15-, and 30 minutes post exercise. The same person measured the RR during the experimental period to avoid unnecessary inconsistency. Rectal temperature (RT) was measured in the morning using a digital thermometer, (Disney, Hartmann, Heidenheim, Germany), prior to the treadmill test, when the horses were relaxed inside their boxes. The RT was again measured at the end of the exercise and 15-, and 30 minutes post exercise. Indoor temperature was measured before each individual started the SET.

3.5.3 Treadmill test

In periods 1 and 2, on day 29 during the experiment a SET was performed. The test started with a 4 minute warm-up on walk (1.6 m/s) on a non-inclined treadmill. During the last 20 second of the walk the treadmill was inclined for 6.25% (Persson, 1967). Each incremental step was 2 minutes and speed was constantly increased throughout each step (Table 6). After the final step, speed was reduced to 1.6 m/s for 2 minutes and the inclined treadmill was lowered back to horizontal position.

Table 6. *Average speed (LSM ± 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

Blood samples were collected prior to the exercise, during steps one to four and 15-, and 30 minutes after step four. Heart rate was recorded prior to the exercise, during steps one to four, warm-down and, 15- and 30 minutes after step four. The same person handled the treadmill (speed, duration and inclination) in both experimental periods.

3.5.4 Feeding trial

On the same day as the SET was performed, blood samples were collected before, during and after feeding 1 kg DM (Table 7). The feed was offered 1-9.5 h after exercise (variation between individual horses) but at the same time post exercise for every individual in both periods.

Table 7. *Analysis of the haylage used during the feeding trial*

	Haylage
DM (g/kg)	678
Ash (g/kg)	63.0
CP (g/kg)	140
WSC (g/kg)	136
VOS (%)	77.5
ME (MJ/kg DM)	9.9

WSC= Water soluble carbohydrates
VOS = Organic matter (OM) of the haylage
ME = Metabolisable energy

Sufficient amount of haylage was stored in a freezer from period 1 to period 2, enabling usage of haylage from the same bale in period 1 and 2. Blood samples were collected 20-, 60-, and 90 minutes after the horses had been fed, through the I.V. catheter before its removal.

3.6 Data analysis

3.6.1 Preparation of blood samples

Collected blood samples were placed on ice before being prepared for storage until later analysed. For plasma separation the blood samples were centrifuged for 15 minutes (520 x g, EPA 12, Hettich zentrifugen, Tuttlingen, Germany) and stored in -18 °C until analysed.

3.6.2 Blood analysis

Enzymatic methods were used for quantitative analysis on plasma glucose (D-Glucose, kit no: E0716251, R-Biopharm, Skandinavien Diagnostiska, Göteborg, Sweden) and plasma NEFA (Waco NEFA-HR (1), (2), kits no. 434-91795(R1) and 436-91995(R2), Nordic Biolabs, Täby, Sweden). Plasma insulin concentration was analysed by ELISA (Mercodia equine insulin kit, Uppsala, Sweden) and plasma lactate concentration by using an enzymatic method (Boehringer&Mannheims, lactat/r-biopharmkit, kit no 10139084035, Skandinavien Diagnostiska, Göteborg, bSweden.). If samples coefficient variation (CV) exceeded 15%, analysis on particular samples were repeated. Mean CV for plasma glucose, plasma insulin and plasma NEFA feeding samples was 4.0%, 3.9% and 2.9%, respectively. The mean CV for plasma glucose, plasma NEFA and plasma lactate samples collected prior to, during and after the SET within both treatments was 4.8%, 2.8% and 3.3%, respectively.

3.6.3 Statistical analyses

Descriptive data calculations for means and standard deviations and Pearson's correlations coefficients were performed in SAS (Statistical Analyses Systems package, 9.4, Gary, NC, USA). ANOVA and proc GLM was implemented. All data is presented as least square means (LSM) with standard error (SE) and statistical significance set to $P< 0.05$.

When data was calculated for LSM ± SE and level of significance the following model was used; $y = \mu + a_i + b_l + c_k + d_j + e_{ilkj}$, where y variable stands for the observation, μ the mean value, a_i is treatment fixed effects, b_l is period fixed effects, c_k is samples fixed effects, d_j is horse as random effects and e_{ilkj} the residuals. Tukey test was used in comparison where level of statistical significance was set to $P<0.05$.

When raw data was prepared for Pearson's correlation coefficients, values from all BCS parameters and BW measured during the first week within each treatment were subtracted from the last measurements made during the final week. This method provided an accurate value that showed overall change in BCS parameters and BW during the treatment.

Pearson's coefficient was used to estimate correlation between plasma insulin concentration during feeding and BCS parameters and BW. Two runs were performed, where the first insulin sample, taken before the horses were fed, was run in relation to BCS parameters and BW. The latter run was to estimate the correlation between BCS parameters and BW and mean insulin value obtained from samples collected 20-. 60-, and 90 minutes post feeding.

Speed at plasma lactate concentration of 2 mmol/L (V_{La2}) and 4 mmol/L (V_{La4}) was calculated on an individual basis from SET. The exponential regressions of V_{La2} and V_{La4} were calculated with the use of Microsoft Excel, 2013 (Microsoft, Redmond, Washington, USA) where following equation was implemented; $y = A_1 \exp(A_2 x)$, where y is the y-offset, A_1 is the magnitude and A_2 is the exponent factor. The equation was adjusted from Bitschnau *et al.* (2010) equation for V_{La4} determination.

4 Results

4.1 Errors

Three horses experienced some kind of different errors that affected their overall participation in the trial or training.

During first period one horse in the high energy allowance treatment showed strange behaviour with a loss of appetite. In an agreement with veterinarian the horse was excluded from the trial. In the first period, another horse showed symptoms of mild mud fewer. Before a veterinarian analysed the severity of the infection the horse missed out one training day. At the beginning of period two, one horse lost one of its hind limb shoe outside in the gravel paddock. The shoe clip became stuck under the sole and in agreement with veterinarian the horse was not trained for 3 days afterwards due to risk of inflammation and infection.

At the end of the experiment, all horses that participated in both treatments finished in a healthy and sound state after thorough examination performed by a veterinarian.

4.2 Body condition and body weight

Horses adapted to high energy allowance were significantly heavier (*P*<.0001) than horses on restricted energy allowance (405.6 ± 1.2 kg vs. 388.8 ± 1.2 kg; respectively) and had higher TBFP and greater girth circumference (*P*<0.05, Table 8). All body condition scoring variables were higher in horses adapted to high energy allowance treatments except for cresty neck score (Table 8).

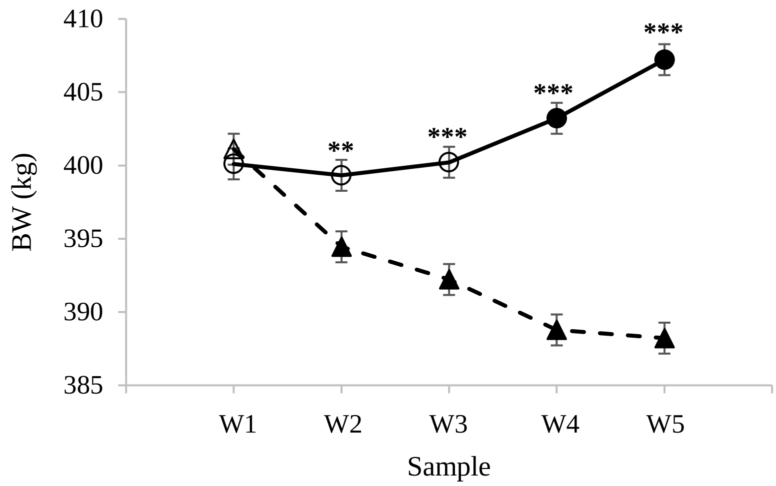

Figure 3. Weekly changes in BW (LSM ± SE) within treatments (○ filled line, high energy allowance; Δ dotted line, restricted energy allowance). W1, W2, W3, W4, W5 are weeks 1, 2, 3, 4 and 5, respectively. Filled markers are significantly (P<0.05) different from the BW in the first week within treatment; difference between the same sample between treatments = ** shows significant (P<0.05) difference; *** shows significant (P<.0001) difference.

Table 8. *Parameters for body condition measurements (LSM ± SE) during both treatments and level of significance (P-value)*

	High (n=9)	**Restricted (n=9)**	***P-value***
BW (kg)	405.6 ± 1.2	388.8 ± 1.2	<.0001
Body fat (%)	14.6 ± 0.1	14.05 ± 0.1	0.021
Neck	6.0 ± 0.1	5.7 ± 0.1	0.003
Back	4.7 ± 0.0	4.6 ± 0.0	0.058
Rump	5.2 ± 0.0	5.1 ± 0.0	0.014
Tailhead	5.7 ± 0.0	5.4 ± 0.0	0.001
Ribs	6.9 ± 0.1	6.5 ± 0.1	0.001
Elbow area	7.5 ± 0.1	7.1 ± 0.1	0.004
BCSmb	6.0 ± 0.0	5.7 ± 0.0	<.0001
BCSub	6.5 ± 0.0	6.2 ± 0.0	<.0001
Overall picture	7.1 ± 0.1	6.5 ± 0.1	0.007
Cresty neck score	2.3 ± 0.0	2.34 ± 0.0	0.078
Girth circumference (cm)	174.1 ± 0.1	171.7 ± 0.1	<.0001
Neck circumference (cm)	103.4 ± 0.8	102.2 ± 0.8	0.351

BW = body weight

All evaluated BCS parameters, except neck circumference and cresty neck score, were positively correlated to mean BCSub and BW (Table 9). Plasma insulin concentration, both from before sample and mean value from feeding samples, were not in relation to BCS parameters and BW.

Table 9. *Correlations and P-values between overall changes in treatments showed with the use of Pierson´s coefficient*

	BW	*P*-value	BCSub	*P*-value
BW	.	.	0.904	<.0001
Neck	0.758	0.0003	0.846	<.0001
Back	0.632	0.0049	0.576	0.0124
Tailhead	0.822	<.0001	0.831	<.0001
Rump	0.598	0.0088	0.521	0.0265
Ribs	0.801	<.0001	0.918	<.0001
Elbow area	0.801	<.0001	0.875	<.0001
Girth circumf.	0.851	<.0001	0.758	0.0003
Overall picture	0.898	<.0001	0.778	0.0001

BW = body weight

4.3 Feeding trial

All horses finished eating the 1 kg of DM, leaving no leftovers.

4.3.1 Metabolic responses to feed intake

There was an overall significant difference in plasma glucose between treatments (P<0.05) and horses adapted to restricted energy allowance had higher mean plasma glucose concentration than horses fed high energy allowance (6.3 ± 0. 13 mmol/L vs. 5.7 ± 0.13 mmol/L, respectively). Plasma glucose levels did not differ (P>0.05) at resting state between treatments. There was a significant difference between treatments 20 minutes (P<0.05) and 90 minutes (P<.0001) after feeding. Horses adapted to high energy allowance reached peak plasma glucose concentration 60 minutes after feeding while horses fed restricted energy allowance reached peak values 90 minutes after (Figure 4).

A significant difference was seen in plasma insulin concentration between treatments (P<0.05) where mean plasma insulin concentrations was higher for horses adapted to high energy allowance than for horses fed restricted energy allowance (0.33 ± 0.02 µg/L vs. 0.26 ± 0.02 µg/L, respectively). There was a tendency (P<0.1) for a significant difference between treatments at resting state. In both treatments plasma insulin reached its peak concentration 60 minutes after feeding (Figure 4).

There was a significant difference in plasma NEFA concentration between treatments (P<.0001; n=72) where horses adapted to restricted allowance had higher plasma concentration than horses adapted to high allowance (0.27 ± 0.01 mmol/L vs. 0.13 ± 0.01 mmol/L, respectively). Plasma NEFA concentration significantly decreased (P<0.05) in response to feed intake in both treatments. At resting state and 20 minutes after feeding, plasma NEFA levels were significantly (P<.0001) different between treatments, but horses adapted to restricted energy allowance showed higher plasma NEFA concentration. This significant difference was also observed 60-, and 90 minutes (P<0.05) after feeding (Figure 4).

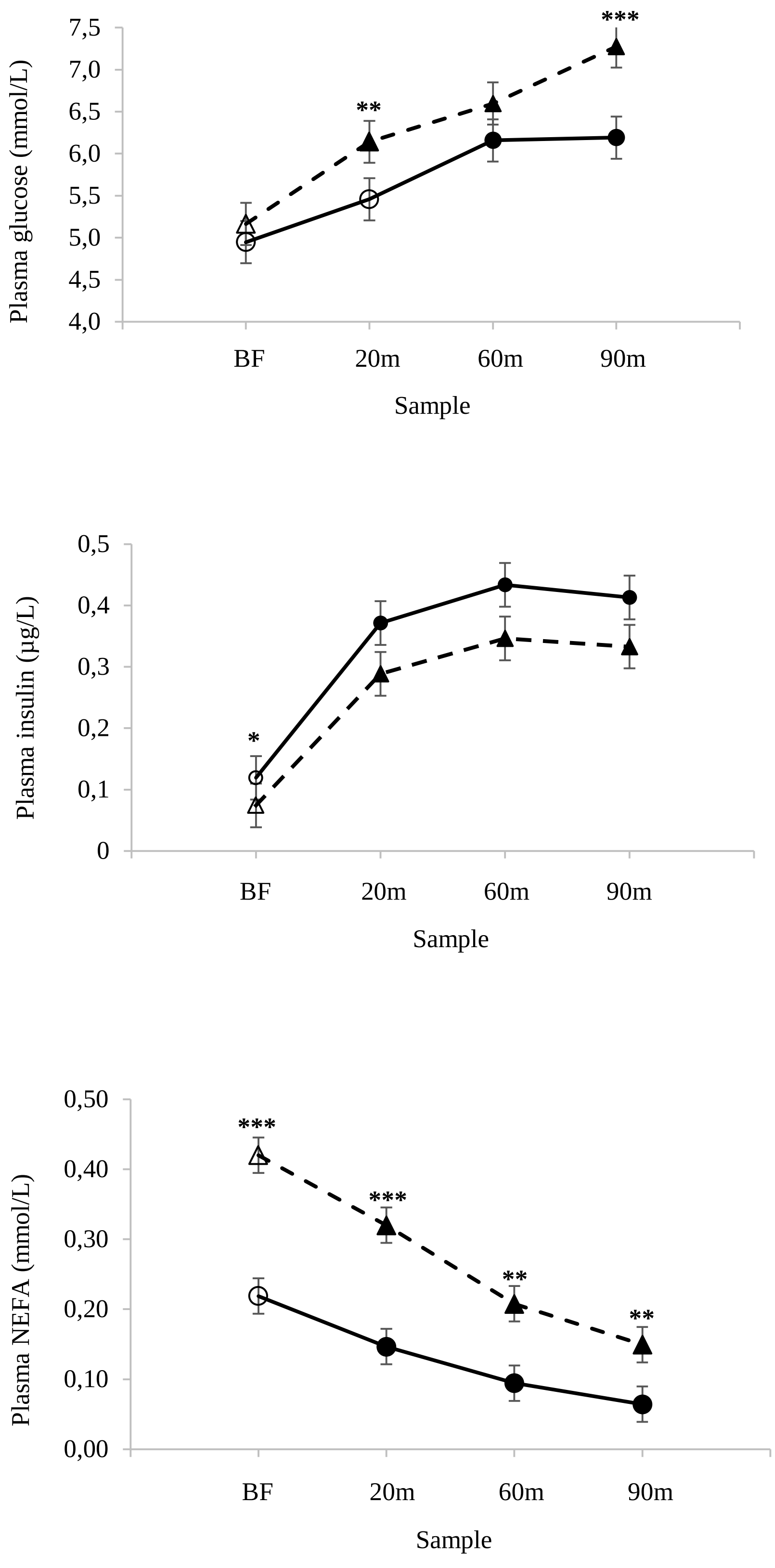

Figure 4. Plasma glucose, insulin and NEFA concentration (LSM ± SE) before, 20-, 60-, and 90 min after feed intake of 1 kg DM of forage (○ filled line, high energy allowance; Δ dotted line, restricted energy allowance). BF is before sampling; 20m, 60m and 90m are minutes after receiving feed of 1 kg DM. Filled markers are significantly ($P<0.05$) different from the BF sample within treatment; difference between the same sample between treatments = * shows a tendency ($P<0.1$) for difference; ** shows significant ($P<0.05$) difference; *** shows significant ($P<.0001$) difference.

4.4 Standardised treadmill exercise test

All horses were able to perform the four incremental steps in both treatments.

4.4.1 Physiological responses to exercise

There was a tendency for higher overall HR on high energy allowance treatment (P<0.1), peak HR in both treatments was 202 ± 2 beats/min and HR increased linearly with exercise intensity. In both treatments, there was a rapid decrease in HR during cool-down on walk until 30 minutes post exercise, HR was not significantly different from its resting values (Figure 5).

Mean plasma glucose concentration for horses on restricted allowance was higher than for those adapted to high allowance (5.3 ± 0.1 mmol/L vs. 4.8 ± 0.1 mmol/L, respectively). During the second and third step in the SET, there was a significant difference (P<0.05) between treatments and horses adapted to high energy allowance had lower plasma glucose concentration. Plasma glucose values 15,- and 30 minutes post exercise were not significantly different from resting values and between treatments there was not a significant difference (Figure 5).

Mean plasma NEFA concentration was higher for horses adapted to restricted allowance than for horses on high allowance (0.34 ± 0.02 mmol/L vs. 0.19 ± 0.02 mmol/L, respectively). Plasma NEFA concentration did significantly (P<.0001) differ between treatments at resting state. During SET, there was not a significant (P>0.05) difference between treatments, but 15- and 30 minutes post exercise plasma NEFA concentrations had increased significantly in both treatments and were significantly higher in the restricted energy allowance treatment (P<0.05). For restricted energy allowance group, plasma NEFA levels 15-, and 30 minutes post exercise had reached resting values collected and for high energy allowance horses, plasma NEFA was significantly higher than at resting state (Figure 5).

Horses fed the restricted energy allowance had lower mean plasma lactate concentration than horses fed high allowance (2.3 ± 0.1 vs. 2.6 ± 0.1 mmol/L, respectively) during SET. Horses adapted to high allowance reached maximal plasma lactate level of 6.5 ± 0.2 mmol/L and horses fed restricted allowance reached maximal plasma lactate of 5.6 ± 0.2 mmol/L (P<0.05). Plasma lactate levels did not differ (P>0.05) between treatments at resting state. During SET, there was a tendency for a significant (P<0.1) difference between treatments at step one and a significant difference (P<0.05) between treatments at step four, and 15-, and 30 minutes post-exercise plasma lactate values had not reached resting values (Figure 5).

There was a significant difference in V_{La2} between treatments (P<0.05) and horses fed high allowance reached lower V_{La2} than horses fed on restricted allowance (4.1 ± 0.1 m/s vs. 4.5 ± 0.1 m/s).

There was a significant difference in V_{La4} between treatments (P<0.05) and horses adapted to high allowance reached lower V_{La4} than horses on restricted allowance (5.3 ± 0.1 m/s vs. 5.6 ± 0.1 m/s).

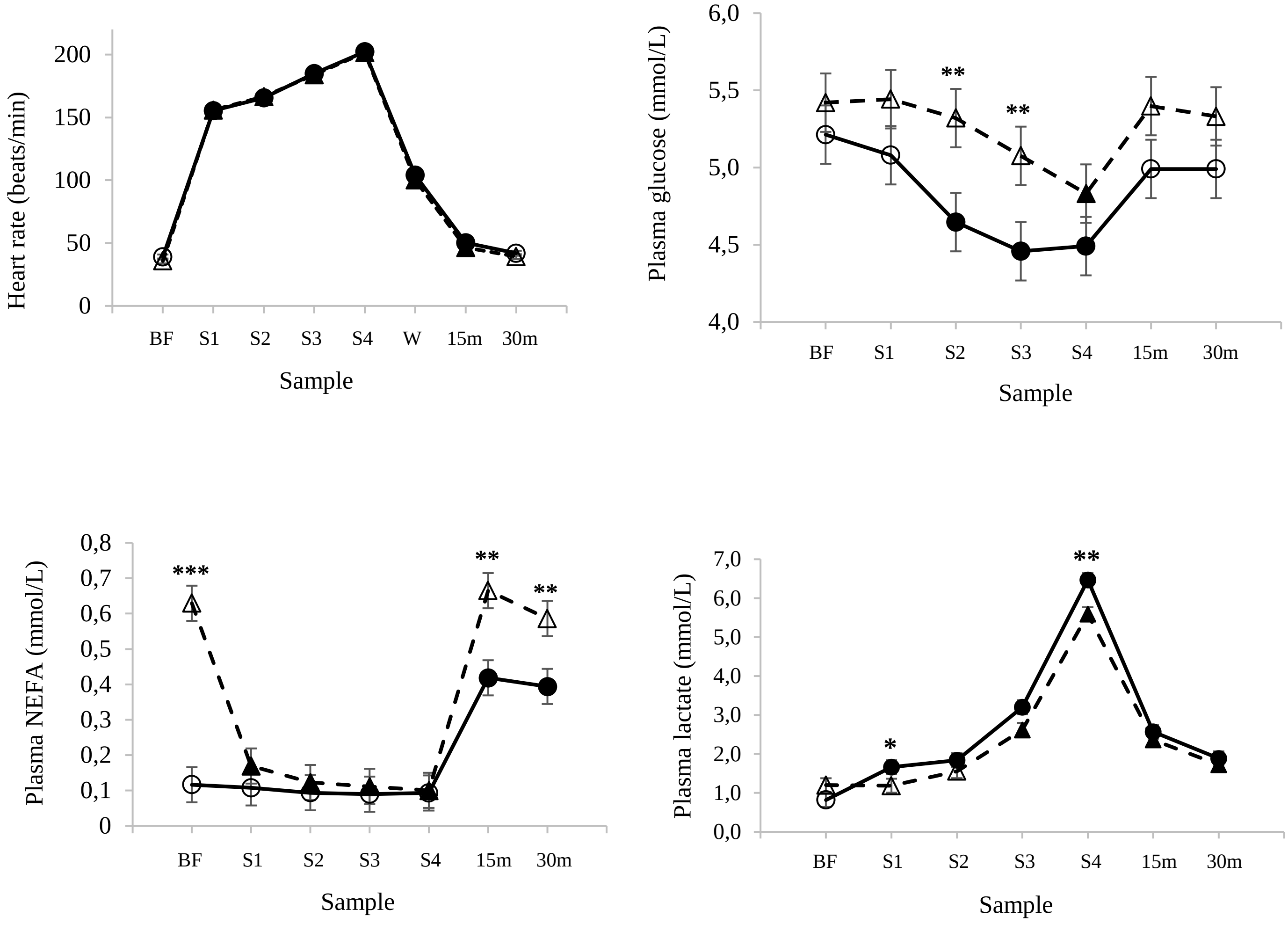

Figure 5. Heart rate, plasma glucose, NEFA and lactate concentrations (LSM ± SE) during a standardised exercise treadmill test (SET) (○ filled line, high energy allowance; Δ dotted line, restricted energy allowance). BF= before sampling; S1, S2, S3 and S4 indicate step 1, 2, 3 and 4, respectively; W = walk (cool-down); 15m = 15 minutes post exercise; 30m = 30 minutes post exercise. Filled markers are significantly different (P<0.05) from the BF sample within treatment; difference between the same sample between treatments = * shows a tendency (P<0.1) for difference; ** shows a significant (P<0.05) difference; *** shows a significant (P<.0001) difference.

Indoor temperature during the SET was higher when horses adapted to high allowance were tested compared to horses fed restricted allowance (12.5 ± 0.1 °C vs. 11.6 ± 0.1 °C, respectively). Temperature was negatively correlated to plasma glucose (r = -0.36, P<.0001) but not to plasma NEFA (r = 0.03, P>0.05) or lactate (r = -0.06, P>0.05) during the SET.

5 Discussion

5.1 General

The main findings in the thesis are that increased BW and BCS alter the metabolic- and physiological responses to feeding and exercise, respectively. When horses were scored in lower body condition they exposed a greater catabolic state prior to, during and after feed consumption of 1 kg DM, allowing them to utilise stored energy in the form of triglycerides to a greater extent for maintenance. During exercise, the same pattern was seen as horses that were scored in a lower body condition were in a higher catabolic state and showed greater aerobic capacity, enabling them to reach higher performance level in contrast to when they were scored with higher BCS and increased BW.

5.2 Body condition score

The results from the study showed that alterations in the horses BW, body condition and fat content were successful within both treatments and changes were seen after 28 adaption days within treatment. When horses were adapted to restricted energy allowance they were considered to be in a negative energy balance due to BW loss and lowered BCS, but in positive energy balance when adapted to high energy allowance due to BW gain and increased BCS (Figure 3). Interestingly, the difference between treatments was significant even though not dramatic, as mean difference was 0.3 points and 16.8 kg in BCS and BW, respectively. All horses were accustomed to a standardised training program, indicating that the results in BW and BCS variations was due to feeding state, restricted vs. high energy allowance and not training. Prior to the study all horses were scored in a high body condition and were considered to be near the desired BCS for the high energy allowance treatment. If they had entered the study in a more moderate BCS, the changes in BW and BCS between treatments could have been more dramatic, showing greater difference in the results.

The Henneke scale was initially created on mature Quarter Horse mares (Henneke *et al.*, 1983), however, the results in the present study indicate that the Henneke-scale is a useful tool for BCS and BW estimation in the Icelandic horse. This is in accordance to another study where the Henneke-scale was applied on both horses and ponies (Carter *et al.,* 2009). In the present study, BW was highly correlated to BCS (r = 0.9; P<.0001) which is consistent with Henneke *et al.* (1983) and Carroll and Huntington (1988) results, indicating that it is possible to give fairly accurate estimation on horses BCS by knowing the BW and vice versa. The results from our study showed a highly positive correlations between fat accumulation in various assessed body parts, i.e. neck and elbow area, tail head, ribs and overall body picture, to both BW and BCS which highlights the importance of capturing the overall impression of the horse to increase estimation accuracy. Today, BCS of the Icelandic horse is estimated with the use of a specific scale that has been adapted to the breed. Within this scale high emphasis is put on palpating the rib area and an eye-assessment on the horse overall impression, for evaluation on the subcutaneous fat layer and hence the horse BCS (Stefánsdóttir and Björnsdóttir, 2001). In the present study, ribs had the strongest correlation (r=0.92; P<.0001) to BCS which indicates that if only one body part should be palpated and used for BCS assessment, subcutaneous fat layer over the ribs gives an accurate estimation. The results, showed a great variation in fat deposition in different body parts both within the horse and between individuals, which corresponds to measurements performed by Westervelt *et al.* (1976). Therefore, to increase the accuracy of the estimated BCS and changes in body fat content, more body parameters should be included when the Icelandic-scale is applied or the Henneke-scale should be used.

5.2.1 Total body fat percentage and body weight

According to the results in the present study, no correlation was found between TBFP and BCS, but this relation has been observed in other studies (Henneke *et al.,* 1983; Leleu and Cotrel, 2006; Ragnarsson and Jansson, 2011). However, the horses used in the study by Ragnarsson and Jansson (2011) were Icelandic and Standardbred horses, with mean BCS of 7.5 and 4.5, respectively, and horses in Leleu and Cotrel (2006) ranged from scores 2-4 in body condition. The greater the variation is in BCS, a higher correlation can be seen and since horses in our study were more homogenous in BCS and individual effects were eliminated with the use of a change-over study, it is expected that limited or no correlation is found between the variables. Another factor influencing the lack of correlation between TBFP and BCS could be due to measurements frequency, few animals and relatively short adaptation periods to both treatments.

In the present study, TBFP was found with the use of Westervelt *et al.* (1976) equation that was adapted to find the correlation between rump fat thickness and total body fat percentage in horses. Westervelt *et al.* (1976) applied another equation to find the correlation between those parameters within Shetland ponies. The former equation adapted to horses was used in the present study as it gave more realistic measurements on TBFP in the Icelandic horse compared to what was seen with the latter equation. There was a linear regression between rump fat thickness and body fat percentage according to Westervelt *et al.* (1976) which corresponds to 2.2 kg or 80 g/d in weight gain in the form of body fat between treatments in the present study. However, assuming an exponential relationship between BCS and TBFP as presented by Dugdale *et al.* (2011a) the mean difference in BW in the form of body fat is 7.5 kg or 270 g/d between treatments which gives a more logical estimation on body fat changes in the present study. Relatively small changes in BCS can give an indication on substantial changes in TBFP, however, when horses become overweight or obese the accuracy of estimated TFBP is reduced (Dugdale *et al.*, 2010; 2011a; 2012). With the use of Dugdale *et al.* (2011a) equation the results in the present study showed relatively large changes in TBFP over short period of time even though changes in BCS were limited. It is believed that distribution of WAT is equal between the horse internal and external sites (Dugdale *et al.*, 2011b), therefore inaccurate BCS estimation can result in dramatic over- or underestimation on TBFP (Dugdale *et al.*, 2010).

Dugdale *et al.* (2011a) believed that body fat represents the greater proportion when horses gain BW. With the use of Dugdale *et al.* (2011a) equation approximately half of the BW gain between treatments in the present study is derived from increased body fat content and the rest could be in relation to gut fill. Horses adapted to high energy allowance consumed at least double the amount of forage provided to horses adapted to the restricted energy allowance treatment. Water intake increases when horses are provided forage-only diet (Connysson *et al.*, 2010; Jansson and Lindberg, 2012) and with increased kg of ingested DM (Pagan and Harris, 1999) which can alter the hind gut homeostasis. From the information on the haylage VOS% used in the study, it is possible to estimate that the difference in undigested feed in the gastrointestinal tract between treatments was approximately 0.5 kg/DM/day. In the present study, water intake was not measured and horses had *ad libitum* access to water. However, according to Pagan and Harris (1999) results it is possible that in the present study, horses adapted to high energy allowance had higher water intake since they ingested more kg DM per day compared to the restricted energy allowance treatment which could explain part of the BW gain.

5.2.2 Morphometric measurements and cresty neck score

In the present study, girth circumference was positively correlated with both BW and BCS which is comparable to previously performed studies (Carroll and Huntington, 1988; Dugdale *et al.,* 2010; 2011a; Matthíasdóttir, 2012). In the Icelandic horse, girth circumference is positively correlated to BW (Matthíasdóttir, 2012), this has also been observed among other pony breeds, where girth circumference gave an accurate estimation on BW (Dugdale *et al.,* 2010; 2011a) and BCS (Dugdale *et al.,* 2011a). In contrast to girth circumference, neck circumference was not correlated to BW nor BCS in the present study. Frank *et al.* (2006) results showed that neck circumference was a good indicator for horses' physical state, as it was positively related to IR. This is in contrast to Dugdale *et al.* (2011a) results where mid neck circumference did not provide an accurate estimation on body mass and BCS. However, apparent neck adiposity was highly related to BW and BCS in the present study, indicating that difficulties in standardising the exact area for neck circumference measurements as well as the horse neck position along with short adaptation period to treatments have influenced the results in the present study.

Cresty neck score did not correlate to BW, BCS nor plasma insulin concentration in the present study and therefore, it is not an accurate indices for the horse physical state. This is in contradiction to Carter *et al.* (2009) results, where cresty neck score was in positive relation to physiological variables, i.e. plasma insulin concentration. A potential explanation that supports these results could be insufficient measurements as cresty neck score was only assessed in the end of both treatments. However, since all body parameters, except neck circumference and cresty neck, were positively correlated to BW and BCS it raises the question whether excessive body fat starts to accumulate earlier in other regions before it gradually increases in the crest of the neck within the Icelandic horse. This variation has been seen among other breeds as it is believed that increased body fat content is accumulated first in the neck area of Thoroughbred horses and later in withers and loin areas (Suagee *et al.,* 2008).

5.2.3 Effects of management on the domesticated horse

In the present study, horses adapted to high energy allowance gained BW and increased their BCS but did not however, show any symptoms of being insulin resistant even though increased body fat content is believed to decrease insulin sensitivity (Hoffman *et al.,* 2003) and promote the development of laminitis (Treiber *et al.,* 2006b; Geor, 2008). A possible explanation supporting this indication is the horses' physical activity state as they were trained at a relatively high exercise intensity throughout both treatments. This is accordance to Powell *et al.* (2002) results on obese insulin resistant mares where insulin sensitivity increased significantly with exercise. However, horses that are overweight or obese are in a greater risk of becoming obese and have poorer health status if their physical activity is limited (Hoffman *et al.,* 2003), especially if there are kept in a positive energy balance all year around (Scheibe and Streich, 2003) and cannot compensate for their increased accumulation on body fat during winter by entering a negative energy balance state (Dawson *et al.,* 1945). It is important that horses BCS is monitored and maintained both through feeding and exercise to ensure its health and durability as an athlete.

5.3 Metabolic responses to feed intake

5.3.1 Plasma glucose and insulin concentrations

There was no difference observed in daily plasma glucose concentration between treatments and only a tendency for daily plasma insulin levels. Glucose is the body primary energy source and essential for the function of the body mechanism (Sjaastad *et al.,* 2016). Therefore, the body tries to maintain its plasma glucose levels in a balance to keep the whole machine going, irrespective of body condition. The fine balance is noticeable in the present study as daily plasma glucose levels were not significantly different between treatments even though the horses BW and BCS were altered. This is in an agreement to previously performed studies, where there was no difference in daily plasma glucose concentration between horses adapted to restricted energy allowance and horses fulfilling their energy requirements (Sticker *et al.,* 1995a), horses that were either feed deprived or fed prior to exercise (Sticker *et al.,* 1995b) and lean and obese horses (Hoffman *et al.,* 2003; Dugdale *et al.,* 2011a).

Plasma glucose and insulin gradually increased the first minutes after the horses were offered the feed and reached peak values after 1 hour in horses in positive energy balance. Within horses in negative energy balance plasma insulin concentration reached peak value 1 hour after feeding, but plasma glucose continued to rise until 90 minutes after feeding. It is unknown if this was the actual peak value or if glucose levels continued to rise after the final sampling, 90 minutes after feeding. The trend of the increase in plasma glucose and insulin concentration after feed consumption corresponds to results from previously performed studies (DePew *et al.,* 1994; Sticker *et al.,* 1995a). However, DePew *et al.* (1994) measured the difference in plasma variables when horses were either fed forage-concentrates or feed deprived. Their results showed that following feeding, plasma glucose and insulin rose significantly for couple of hours. Sticker *et al.* (1995a) results showed that plasma glucose and insulin concentration increased following feeding and reached peak values after 2 hours and no difference was between treatments. One explanation for the difference in total absorption time between the present study and DePew *et al.* (1994) and Sticker *et al.* (1995a) even though following the same trend, could be due to differences in feed composition, as horses in our study received 1 kg DM of forage and horses in the other studies a mixture of forage-concentrate diets. It is also highly likely that forage content differed between studies in i.e. DM, CP and water soluble carbohydrates (WSC). Overall, horses in positive energy balance had higher plasma insulin levels during all sampling periods and lower plasma glucose concentrations, but only a significant difference was observed in glucose levels 20-, and 90 minutes after feeding between treatments. This trend is somewhat similar to what Connysson *et al.* (2010) measured between horses fed forage-only (F) and forage-oats (FO) diets as horses adapted to FO diet had higher plasma insulin levels following feed consumption but no difference was marked between treatments and plasma glucose.

In the present study, all horses irrespective of treatment, showed normal metabolic reactions to feed consumption indicating that horses adapted to high energy allowance, even though overweight and with increased BCS, did not show symptoms of being IR. According to Jansson *et al.* (2015) plasma insulin increases parallel with altered BW and BCS. Horses adapted to high energy allowance showed more efficient plasma glucose uptake which corresponds to their higher plasma insulin levels, as insulin promotes plasma glucose absorption, both for utilisation and storage. However, since these horses showed normal metabolic responses to feed intake it is uncertain why they responded to the same amount of DM as provided in the restricted energy treatment with increased plasma insulin secretion. One possible explanation could be that horses with higher body fat content and BCS are more sensitive to WSC (Ragnarsson and Jansson, 2011) and CP (Ragnarsson and Jansson, 2011; Ringmark and Jansson, 2013a) in the forage compared to horses with lower BW and BCS. Another reason behind this difference could be related to increased sensitivity of epithelial cells in the small intestine (SI). When feed enters the SI, epithelial cells produce the hormones GIP and GLP-2, which diffuse into the blood stream and are transported to the pancreas where they stimulate β-cells for insulin production and secretion. This metabolic response is initiated even though limited amount of glucose has been absorbed from the feed (Sjaastad *et al.*, 2016).

There was no correlation found between plasma insulin concentration and BW or BCS in the present study which is in contrast to Ragnarsson and Jansson (2011) results where plasma insulin was positively correlated both to BCS and total rump fat thickness. A potential explanation supporting the difference between studies could be that horses in the present study where homogenous as BCS was similar between individuals and individual effects were eliminated. In the study by Ragnarsson and Jansson (2011), they compared horses of different breeds with greater variation in BCS. If the horses used in the present study would have been less homogenous, i.e. BCS ranging from 4-8, plasma insulin would probably have been positively correlated with BW and BCS as reported by Dugdale *et al.* (2011a).

5.3.2 Plasma NEFA concentrations

Daily plasma NEFA concentration differed significantly between treatments in the present study, where horses adapted to restricted energy allowance had double the amount of plasma NEFA compared to horses adapted to high energy allowance. This is in accordance to what Sticker *et al.* (1995a; 1995b) results showed, where horses fed restricted energy allowance or feed deprived prior to exercise had significantly higher plasma NEFA levels compared to horses fulfilling their energy requirements or fed prior to an exercise. From these results it is clear that horses in negative energy balance rely on plasma NEFA utilisation to a greater extent for maintenance compared to horses in positive energy balance. In the present study, horses in positive energy balance relied on plasma NEFA utilisation to some extent, but one explanation for the significant difference between treatments could be that their internal fat storage was greater and provided the body mechanisms with necessary fuel substrate which reduced the rate of lipolysis in the adipose tissue, resulting in less plasma NEFA concentrations.

Following feed intake, plasma NEFA concentrations decreased in both treatments but the drop was greater in horses adapted to restricted energy allowance. Plasma NEFA concentrations are influenced by plasma insulin level which stimulates triglyceride synthesis and inhibits lipolysis. The results are in relation to the relationship between those variables, as horses adapted to higher energy allowance had higher plasma insulin level and lower plasma NEFA concentration throughout the study. The metabolic pattern of plasma NEFA in respond to feed intake is comparable to what has previously been observed even though those horses were adapted to forage-concentrate diet (DePew *et al.,* 1994; Sticker *et al.,* 1995a). Horses fed during the day showed a significant drop in plasma NEFA concentrations following feed consumption (DePew *et al.,* 1994) and horses fed restricted energy allowance had a greater drop in plasma NEFA concentration following feeding compared to horses adapted to energy fulfilling diet (Sticker *et al.,* 1995a). In the present study, the greater drop in horses adapted to restricted energy allowance could be related to higher lipolytic activity. Elevated plasma insulin concentrations had greater effect on the lipolytic activity and total plasma NEFA concentrations as was seen among horses in positive energy balance that showed lower lipolytic activity prior to feeding, as previously explained by Sticker *et al.* (1995a). Connysson *et al.* (2010) results showed a similar pattern in the metabolic profile of plasma NEFA during feed deprivation, as horses fed FO had higher plasma insulin and lower plasma NEFA concentration, indicating reduced lipolytic activity compared to F fed horses. From these information it is clear that horses in positive energy balance adapted to forage-only diet show a similar trend in metabolic response to feed intake as horses adapted to forage-concentrate diet, having reduced capacity of fulfilling their maintenance energy requirements through lipolysis when compared to horses in negative energy balance fed forage-only diet.

5.4 Physiological responses to exercise

5.4.1 Adaptation to a standardised exercise treadmill test

Mean HR during SET did not differ between treatments but was significantly higher in period 1 compared to period 2. Before the initiation of the present study, all horses were accustomed to the treadmill with four training sessions. However, a probable explanation for the variation in mean HR between periods could be due to insufficient number of training sessions. It has previously been measured that horses show increased stress when changes occur in their usual surroundings (Irvine and Alexander, 1994) or during stressful situations resulting in increased plasma cortisol levels and HR (Schmidt *et al.*, 2010). When the horses performed SET in both treatments, their gait consistency on the board was recorded and it appeared that consistency on trot was greater in period 2. This is somewhat in agreement with Buchner *et al.* (1994) results where horses required at least three training sessions on the treadmill to become consistent on trot. However, few seconds after being accustomed to the SET in both periods, horses reached consistency and relaxed. In the present study, HR increased linearly with exercise intensity which is in an accordance to previously performed studies (Persson and Ullberg, 1974; McKeever *et al.*, 1993; Eaton *et al.*, 1995). The results from our study did not show a significant difference between treatments in HR during SET which is in relation to the results by Lawrence *et al.* (1995). This indicates that mean difference in measured plasma variables in response to exercise between treatments was greatly affected by the horses altered BW and BCS.

5.4.2 Plasma glucose concentration in response to exercise

In the present study, there was not a significant difference between treatments in plasma glucose concentration before exercise onset. According to Lawrence *et al.* (1993) results, horses that consumed corn before exercise had higher initial levels of plasma glucose compared to feed deprived horses which is in contrast to our results where horses in negative energy balance showed higher glucose levels. According to Jansson *et al.* (2015) plasma insulin level increases parallel to horses increased BW and BCS, allowing the assumption, since plasma insulin was not measured in the present study, that horses in negative energy balance had lower plasma insulin levels that reflected in the concentration of plasma glucose. However, the lack of difference between treatments in initial plasma glucose levels further highlights the body importance of maintaining balance in plasma glucose, irrespective of its physical state.

During the first three exercise steps in SET horses adapted to the restricted energy allowance showed higher plasma glucose concentration compared to horses adapted to high energy allowance, but at the final and highest intensity exercise step they had a significant drop in plasma glucose. Similar trend in plasma glucose metabolism in response to exercise has been observed in other previously performed studies (Lawrence *et al.*, 1993; 1995; Pagan and Harris, 1999; Jansson and Lindberg, 2012). Pagan and Harris (1999) measured a greater rate in glucose disappearance during exercise in horses adapted to grain-based diet compared to horses adapted to forage-only diet or feed deprived, which showed relatively stable glucose utilisation during an exercise test and is in agreement to Lawrence *et al.* (1993). Lawrence *et al.* (1993) results showed a greater decrease in plasma glucose concentration in horses fed grains prior to exercise while plasma glucose levels remained fairly constant in feed deprived horses. However, during the exercise high intensity step, no difference in glucose levels was marked between treatments in the present study. Another explanation supporting the plasma glucose concentration in response to exercise is the concentration of plasma NEFA prior to exercise. Horses in negative energy balance had significantly higher plasma NEFA concentrations which allowed them to perform the first steps of the SET in a glucose sparing state, but following the first two steps in the SET they started to utilise plasma glucose to a greater extent as an energy source donor when plasma NEFA availability reduced.

Exercise is believed to increase glucose effectiveness irrespective of horses feeding state (Treiber *et al.*, 2006a) and is probably due to increased number of GLUT-4 transporters in the cell membrane, which enable increased glucose uptake to fulfil the muscles energy requirements (Stewart-Hunt *et al.*, 2006). It is possible to estimate that number of GLUT-4 increased in horses within both treatments as they followed similar plasma glucose metabolic trend in response to exercise even though there was a significant difference between them. However, endurance is believed to increase with higher plasma glucose concentration (Lacombe *et al.*, 2001; Jansson and Lindberg, 2012) which is in agreement with the results from the present study, as horses in negative energy balance reached higher performance level.

In the present study, room temperature was positively correlated with plasma glucose concentration during SET in both treatments. The mean difference in room temperature was around 1 °C and even though not dramatic it was significantly different between treatments, as it was higher when horses in positive energy balance performed SET. Treiber *et al.* (2006a) measured a significant difference (5 °C) in room temperature when horses adapted to difference feeding strategies where exercised on a treadmill. However, since there was no difference between treatments in mean HR or body temperature during the SET, Treiber *et al.* (2006a) concluded that difference in room temperature did not affect metabolic responses to exercise. Room temperature was not in relation to the horses' body temperature during SET in the present study, and along with HR results there was nothing that indicated that room temperature influenced plasma glucose response to exercise, more likely altered BW and BCS.

5.4.3 Plasma NEFA concentration in response to exercise

In the present study, plasma NEFA concentration before exercise onset was significantly higher when horses were in negative energy balance compared to when in positive energy balance. This is in accordance to previously performed studies where feed deprived horses (Lawrence *et al.,* 1993; 1995) and horses adapted to forage-only diet (Jansson and Lindberg, 2012) showed significantly greater plasma NEFA concentration compared to horses fed grain-based diet (Lawrence *et al.,* 1993; 1995; Jansson and Lindberg, 2012) prior to exercise. However, Mittendorfer *et al.* (2004) results on lean, overweight and obese men were in contradiction to our results and the reason for the difference is unclear and needs further investigation. As previously mentioned, plasma insulin was not measured in the present study, but it is believed that horses in negative energy balance had lower plasma insulin levels compared to horses in positive energy balance, due to increased BW and BCS (Jansson *et al.*, 2015). Therefore, the significant difference in plasma NEFA could have resulted from plasma insulin levels and increased lipolysis activity when adapted to the restricted energy allowance treatment where plasma NEFA were utilised to a greater extent for maintenance.

There was a significant drop in plasma NEFA concentrations between pre-exercise sample and after the first step in SET when horses were in negative energy balance, but the decrease continued over all exercise steps and reached lowest value during the fourth and highest intensity exercise level. This was in contrast to what was observed when horses were in positive energy balance where plasma NEFA concentration was relatively stable prior to and during all exercise steps in the SET. A similar trend in plasma NEFA metabolic response to exercise has been observed in other studies where horses have been adapted to different feeding strategies (Lawrence *et al.*, 1993; 1995; Pagan and Harris, 1999; Jansson and Lindberg, 2012). One explanation supporting this difference is that lipolytic activity was lower when horses were in positive energy balance influencing reduced plasma NEFA availability prior to and during the exercise. The results from plasma glucose and NEFA concentrations in response to exercise clearly demonstrate that horses in negative energy balance have greater lipolytic activity prior to exercise, enabling them to utilise plasma NEFA to a greater extent as an energy source donor for the contracting muscles. A similar metabolic response has also been observed among human athletes in different physical state (Mittendorfer *et al.,* 2004). As mentioned by Jansson and Lindberg (2012) plasma NEFA has glucose sparing effect which is observed in the present study. Horses in negative energy balance started to gradually increase plasma glucose utilisation during the final high intensity exercise step compared to horses in positive energy balance which showed significant decrease in plasma glucose after the second exercise step. The results indicate that horses in negative energy balance, lower in BW and BCS, had greater aerobic capacity during exercise.

Plasma NEFA concentrations gradually increased following the exercise and reached peak values 15 minutes post SET. This is comparable to what Snow and MacKenzie (1977) results showed after horses had performed a field exercise test. According to Treiber *et al.* (2006a) exercise increases insulin sensitivity. Reduced plasma insulin concentration stimulates increased lipolytic activity which could explain the significant increase in plasma NEFA that occurred in both treatments following the SET. In the present study, samples were not collected after 30 minutes post exercise, but pre-exercise values imply that plasma NEFA concentration reduces significantly more in horses adapted to the high energy allowance compared to horses adapted to restricted energy allowance. This is supported by the results from Jansson and Lindberg (2012) from samples collected the day after exercise test as horses fed forage-only diet appeared to rely on plasma NEFA to a greater extent as an energy source for maintenance.

6.4.3. Plasma lactate concentration in response to exercise

Plasma lactate concentration did not differ before SET between treatments but from pre-exercise values to the end of the exercise first step, there was a significant increase in plasma lactate when horses were in positive energy balance. For horses in negative energy balance, plasma lactate did not show a significant increase until after the third exercise step in the SET. However, plasma lactate concentration increased exponentially in relation to exercise intensity, both speed and treadmill incline, in both treatments and is in agreement to previously performed studies (Seeherman and Morris., 1990; Eaton *et al.,* 1995; Treiber *et al.,* 2006a). The results from the present study showed that when horses were in positive energy balance they performed more work when exercising at same intensity level as they did when in negative energy balance. This is comparable to Pagan and Harris (1999) results where horses fed *ad libitum* hay the night before exercise test showed greater plasma lactate concentration in comparison to feed deprived horses. A similar trend was seen by Treiber *et al.* (2006a) where horses adapted to diet rich in starch and sugars showed significantly higher plasma lactate concentration during high intensity exercise compared to horses adapted to fat and fibrous diet. However, other studies have not measured an altered response in plasma lactate concentration when horses are feed deprived or fed grain-based diet prior to exercise (Lawrence *et al.*, 1993; 1995) or adapted to different feeding frequency (Jansson *et al.*, 2006).

During the SET in the present study, plasma lactate concentration showed a similar trend in response to exercise as plasma glucose. When plasma glucose concentration started to decrease, irrespective of treatment, concentrations of plasma lactate increased and reached peak values after the fourth and final exercise step. Blood samples were not collected after 30 minutes post-exercise in the present study but according to Seeherman and Morris (1990) results it is not unlikely that horses reached their peak values in the first minutes after finishing the SET. During the first 30 minutes post-exercise there was not a significant difference measured between treatments, in contrast to what Jansson and Lindberg (2012) results showed between horses with different feeding regimes.

There was a significant difference in V_{La2} and V_{La4} between treatments where horses in negative energy balance reached greater speed before reaching plasma lactate concentration of 2 mmol/L and 4 mmol/L. These results are in relation to Leleu and Cotrel (2006) results where TBFP was negatively correlated with V_{La4} , implying reduced aerobic capacity when TFBP increases. It has previously been observed that horses adapted to forage-only diet have a tendency for greater V_{La4} compared to horses adapted to forage-concentrate diet (Jansson and Lindberg, 2012). Treiber *et al.* (2006a) did not measure a significant difference between treatments but horses adapted to high starch and sugars rich diets reached lower speed when reaching the lactate threshold compared with horses adapted to fat and fibrous diet. With increased body mass there is an extended need for energy expenditure to promote locomotion and fulfil contracting muscles energy requirements (McMiken, 1983; Pagan and Hintz, 1986). It has previously been shown that fat free mass is positively correlated with aerobic capacity (Kearns *et al.*, 2002a) and performance (Kearns *et al.*, 2002b) while TBFP (F%) is negatively correlated with performance level in horses performing a field exercise test (Kearns *et al.*, 2002b).

Overall, there was a significant difference in plasma lactate concentration, V_{La2} and V_{La4} during SET between treatments as horses in positive energy balance showed higher lactate levels. A probable explanation behind this significant difference is that plasma lactate metabolism was more efficient through gluconeogenesis when horses were in a negative energy balance. Another potential explanation to the concentration difference in plasma lactate is that lactate production was lower in horses in negative energy balance as they did not work at as high intensity to perform the same exercise due to less weight bearing in the form of fat content and gut fill. However, to be able to conclude the weight bearing statement it would have been necessary to perform a weight bearing SET, where additional load is added on the horses during exercise and their metabolic response measured.

5.5 The study methodologies strength and weaknesses

In this thesis a unique study that was performed on Icelandic horses is presented. As far as the author knows, this is the first time that metabolic effects from altered body condition and fat content have been recorded in the Icelandic horse in relation to feeding and exercise performed on a high speed incremental treadmill. In relation to the feeding trial, another study has previously been performed on the Icelandic horse where its digestibility and metabolic response was compared to the Standardbred horse when fed two grass haylages harvested at different maturity stage. However, this is the first time where the Icelandic horse metabolic- and physiological responses to feed intake is observed when the horse body condition and fat content is altered. This is also the first time, where study emphasis on applying a standardised training protocol where all individuals are introduced to exactly the same training in two separated treatments. By performing a study of this kind it is possible to gain increased knowledge on the Icelandic horse metabolic- and physiological response towards feeding and exercise when its physical state is altered and how it affects its performance capacity and durability as an athlete.

In the present study, the greatest challenge was to create a standardised training protocol for the horses. This was also one of the study weaknesses as five trainers were included in the horses training which made it difficult to ensure that all trainers approached the training idea in the same way, according to intensity and duration. However, the training did not differ significantly between treatments, indicating that overall the standardisation between treatments was successful. Another weakness of the study were the short adaptation periods to treatments. Prior to the study all horses used in the study had a relatively high BCS which made it impossible to start adapting them to the treatments from a moderate BCS and due to a short adaptation periods, the difference in BW and BCS was not dramatic. However, alterations in body content and body fat were successful as the difference between treatments was significant and had significant effect on horses' metabolic and physiological responses. During the feeding trial, exact time should have been recorded when the horses finished consuming all the feed as it would have provided a better estimation for stress related factors. Water intake should also have been recorded between treatments. It would also have been beneficial to measure plasma insulin response to exercise during SET to gain increased knowledge on how its concentration affected other plasma variables.

6 Conclusion

Overall conclusions from the results presented in the thesis are that altered body condition and fat content affects the metabolic response to feeding and metabolic- and physiological responses to exercise in the Icelandic horse.

Detailed conclusions in the feeding trial were that:

- Altered body condition and fat content affect the horse metabolic response to feeding
- Horses in negative energy balance/lower BCS show increased plasma NEFA availability that is utilised to a greater extent for maintenance.
- All horses showed normal and expected metabolic response in relation to alterations in BW and BCS.
- High physical activity state is believed to promote normal metabolic response which highlights the importance of exercise when horses are in a positive energy balance.

Detailed conclusions from the standardised treadmill exercise test were that:

- Altered body condition and fat content affects the horse metabolic- and physiological response to exercise
- When horses are in a positive energy balance/high energy allowance and have increased BCS they perform more work at the same exercise intensity compared to their respond to exercise when in negative energy balance. This indicates that with increased body mass energy expenditure needed to fulfil contracting muscles energy requirements to stimulate locomotion is greatly extended.
- Horses in negative energy balance and closer to moderate BCS rely to a greater extent on plasma NEFA for energy utilisation during exercise which has glucose sparing effects.
- Horses in negative energy balance and closer to moderate BCS show greater aerobic capacity as they can work at higher speed before reaching the lactate threshold.

The results presented in this thesis highlight the importance of keeping horses in a moderate BCS and physically active. Their feeding strategy and physical activity should be adapted to each other and maintained at a fine balance. This balance is essential to minimize the risk of horses becoming overweight or obese that influences the development of various and painful metabolic syndromes, i.e. laminitis that can affect the horse quality of life. When horses are kept physically active and in a moderate BCS their athletic abilities are also positively affected, enabling them to reach and maintain higher performance capacity in a healthy and sound state.

7 Future research

Further studies that emphasise on how alterations in the Icelandic horse physical state, body condition and fat content affects its health and performance capacity are needed.

In the feeding study, forage-only diet and a homogenous group of horses were used, geldings around the same age in similar BCS used for education. To gain a better understanding on how altered BW and BCS affects metabolic responses to feeding this methodology should be applied to a more heterogeneous group of horses with different roles, i.e. competition horses with high performance capacity, horses that will perform a breeding evaluation test, leisure horses, stallions and breeding mares at different breeding periods, fetal growth, pregnancy and lactation. Today, feeding forage-concentrate mixed diets is becoming increasingly popular among Icelandic horse trainers. However, the knowledge regarding concentrate effects on the Icelandic horse is insufficient and therefore, it would be of great interest to perform a similar feeding trial where horses are adapted to forage-only vs. forage-concentrate diets. Metabolic responses to feeding should be investigated in a heterogeneous group of horses, both in moderate BCS and when their BW and BCS have been altered.

In the study where horses performed a SET, the same group of forage-only adapted horses as in the feeding trial were used. The methodology used in the present study should be applied to a more heterogeneous group of horses, greater variation in age and BCS, with different roles i.e. competition horses, horses entering a breeding field evaluation test, stallions and leisure horses. This study should also be applied on the same heterogeneous group adapted to low-quality forage vs. high-quality forage as well as groups adapted forage-only vs. forage-concentrate diets. Metabolic and physiological responses to exercise should be observed both in moderate BCS and when BW and BCS have been altered. By feeding a high quality forage-only diet it is possible to estimate the effects from feeding strategy on horses performing at a high capacity, i.e. in competitions or when at breeding shows in comparison to low-quality forage-only or forage-concentrate diets. Additional studies where the effects from increased load added to the exercising horse to mimic increased BW and BCS should be observed. Those information would provide additional and important knowledge on the real effect that increased weight load has on the horse performance capacity. Muscle biopsies should be performed in all studies, for evaluation on mean difference in muscle glycogen storage and how it is affected by feeding strategy and altered body condition and fat content.

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References

Akers, R. & Denbow, D. M. (2008). *Anatomy and Physiology of Domestic animals*. USA: Blackwell Publishing.

Adalsteinsson, S. (1981). Origin and conservation of farm animal populations in Iceland. *Journal of Animal Breeding and Genetics (Zeitschrift fur Tierzuchtung und Zuchtungsbiologie),* 98, pp. 258-264.

Arnold, W., Ruf, T. & Kuntz, R. (2006). Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (Equus ferus przewalskii) II. Energy expenditure. *Journal of Experimental Biology,* 209(22), pp. 4566-4573.

Árnason, Th., Jensen, P., Klemetsdal, G., Ojala, M. & Philipsson, J. (1994). Experience from application of animal breeding theory in Nordic horse breeding. *Livestock Production Science,* 40, pp. 9-19.

Bergman, R.N., Ider, Y.Z., Bowden, C.R. & Cobelli, C. (1979). Quantitative estimation of insulin sensitivity. *American Journal of Physiology-Endocrinology and Metabolism,* 236(6), pp. E667 - E677.

Berry, D. C., Stenesen, D., Zeve, D. & Graff, J. M. (2013). The developmental origins of adipose tissue. *Development,* 140(19), pp. 3939 - 3949.

Bitschnau, C., Wiestner, T., Trachsel, D.S., Auer, J.A. & Weishaupt, M.A. (2010). Performance parameters and post exercise heart rate recovery in Warmblood sport horses of difference performane levels. *Equine Veterinary Journal,* 42(38), pp. 17-22.

Björnsson, G. B. & Sveinsson, H. J. (2006). *The Icelandic horse.* Reykjavík, Iceland: Mál og Menning.

Blaxter, K. L. (1989). *Energy Metabolism in Animal and Man.* Cambridge: Cambridge University Press.

Buchner, H.H.F., Savelberg, H.H.C.M., Schamhardt, H.C., Merkens, H.W. & Barneveld, A. (1994) Habituation of horses to treadmill locomotion. *Equine Veterinary Journal,* 26(S17), pp. 13-15.

Carroll, C.L. & Huntington, P.J. (1988). Body condition scoring and weight estimation of horses. *Equine veterinary journal,* 20(1), pp. 41-45.

Carter, R.A., Geor, R.J., Staniar, W.B., Cubitt, T.A. & Harris, P.A. (2009). Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. *The Veterinary Journal,* 179, pp. 204-210.

Connysson, M., Essén-Gustavsson, B., Lindberg, J.E. & Jansson, A. (2010). Effects of feed deprivation on Standardbred horses fed a forage-only diet and a 50:50 forage-oats diet. *Equine Veterinary Journal,* 42(38), pp. 335-340.

Couroucé, A. (1999). Field exercise testing for assessing fitness in French Standardbred trotters. *The Veterinary Journal,* 157(2), pp. 112-122.

Couroucé, A. Geffroy, O., Barrey, E., Auvinet, B. & Rose, R.J. (1999). Comparison of exercise tests in French trotters under training track, racetrack and treadmill conditions. *Equine Veterinary Journal*, 31, pp. 528-532.

Dawson, W.M., Phillips, R.W. & Speelman, S.R. (1945). Growth of horses under western range conditions. *Journal of Animal Science,* 4(1), pp. 47-54.

DePew, C.L., Thompson, D.L., Fernandez, J.M., Sticker, L.S. & Burleigh, D.W. (1994). Changes in concentrations of hormones, metabolites, and amino acids in plasma of adult horses relative to overnight feed deprivation followed by a pellet-hay meal fed at noon. *Journal of Animal Science*, 72(6), pp. 1530-1539.

Dionne, R.M., Vrins, A., Doucet, M.Y. & Paré, J. (2003). Gastric ulcers in Standardbred racehorses: Prevalence, Lesion, Description, and risk factors. *Journal of Veterinary Internal Medicine,* 17, pp. 218-222.

Dugdale, A.H.A., Curtis, G.C., Cripps, P., Harris, P.A. & Argo, C.McG. (2010). Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. *Equine Veterinary Journal,* 42(7), 600-610.

Dugdale. A.H.A., Curtis, G.C., Cripps, P.J., Harris, P.A. & Argo, C.McG. (2011a). Effects of season and body condition on appetite, body mass and body composition in ad libitum fed pony mares. *The Veterinary Journal,* 190, pp. 329-337.

Dugdale, A.H.A., Curtis, G.C., Harris, P.A. & Argo, C.Mc. (2011b). Assessment of body fat in the pony: Part I. Relationships between the anatomical distribution of adipose tissue, body composition and body condition. *Equine Veterinary Journal,* 43(5), pp. 552-561.

Dugdale, A.H.A., Grove-White, D., Curtis, G.C., Harris, P. A. & Argo, C. McG. (2012). Body condition scoring as a predictor of body fat in horses and ponies. *The Veterinary Journal,* 194, 173-178.

Eaton, M., Evans, D.L., Hodgson, D.R. & Rose, R.J. (1995). Effect of treadmill incline and speed on metabolic rate during exercise in Thoroughbred horses. *Journal of Applied Physiology,* 79(3), pp. 951-957.

Essén-Gustavsson, B. & Jensen-Waern, M. (2002). Effect of an endurance race on muscle amino acids, pro- and macroglycogen and triglycerides. *Equine Veterinary Journal,* 34, pp. 209-213.

Evans, D. (2008). Exercise testing in the field. In: Hinchcliff, K.W., Geor, R.J. & Kaneps, A.J., (eds). *Equine Exercise Physiology. The science of exercise in the athletic horse.* Philadelphia, USA: Elsevier. pp. 12-27.

Evans, D.L., Harris, R.C. & Snow, D.H. (1993). Correlation of racing performance with blood lactate and heart rate after exercise in Thoroughbred horses. *Equine Veterinary Journal,* 25(5), pp. 441-445.

Evans, D.L., Rainger, J.E., Hodgson, D.R., Eaton, M.D. & Rose, R.J. (1995). The effects of intensity and duration of training on blood lactate concentrations during and after exercise. *Equine Veterinary Journal,* 18, pp. 422-425.

Evans, D.L. & Rose, R.J. (1988). Cardiovascular and respiratory responses to submaximal exercise training in the Thoroughbred horse. *Pflugers Arch,* 411, pp. 316-321.

FEIF (2017). Facts and figures. https://www.feif.org/FEIF/Factsandfigures.aspx [Assessed 12 April 2017]

Ferrier, D.R. (2014). *Lippincott´s illustrated reviews: Biochemistry.* 6th ed. Philadelphia: Lippincott Williams & Wilkins.

Frank, N., Elliot, S.B., Brandt, L.E. & Keisler, D.H. (2006). Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. *Journal of the American Veterinary Medical Association,* 228(9), pp. 1383-1390.

Frederickson, I., Drevemo, S., Dalin, G., Hjertbn, G., Bjorne, K., Rynde, R. & Franzen. G. (1983) Treadmill for equine locomotion analysis. *Equine Veterinary Journal,* 15(2), pp. 111-115.

Garlinghouse, S.E. & Burrill, M.J. (1999). Relationship of body condition score to completion rate during 160 km endurance races. *Equine Veterinary Journal,* 30, pp. 591-595.

Geor, R.J. (2008). Metabolic predispositions to laminitis in horses and ponies: Obesity, insulin resistance and metabolic syndromes. *Journal of Equine Veterinary Science,* 28(12), pp. 753-759.

Gerard, M.P., Graaf-Roelfsema, E. DE., Hodgson, D.R. & van der Kolk, J.H. (2014). Energetic considerations of exercise. In: Hodgson, D.R., McKeever, K.H. & McGowan, C.M. (eds). *The athletic horse, principles and practice of equine sports medicine.* 2nd ed. USA: Elsevier Saunders, pp. 19-33.

Gesta, S., Tseng, YH. & Kahn, C.R. (2007). Developmental origin of fat: Tracking obesity to its source. *Cell,* 131, pp. 242-256.

Gudmundsson, O. & Dyrmundsson, O.R. (1994). Horse grazing under cold and wet conditions: a review. *Livestock Production Science,* 40, pp. 57-63.

Gunn, H.M. (1979). Total fibre numbers in cross sections of the semitendinosus in athletic and non-athletic horses and dogs. *Journal of Anatomy*, 128(4), pp. 821-828.

Hargreaves, B.J., Kronfeld, D.S. & Naylor, J.R.J. (1999). Ambient temperature and relative humidity influenced packed cell volume, total plasma protein and other variables in horses during an incremental submaximal field exercise test. *Equine Veterinary Journal,* 31(4), pp. 314-318.

Harris, P. & Snow, D.H. (1988). The effects of high intensity exercise on the plasma concentration of lactate, potassium and other electrolytes. *Equine Veterinary Journal,* 20(2), pp. 109-113.

Henneke, D.R., Potter, G.D., Kreider, J.L. & Yeates, B.F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Veterinary Journal,* 15(4), pp. 371-372.

Henneke, D.R., Potter, G.D. & Kreider, J.L. (1984). Body condition during pregnancy and lactation and reproductive efficiency of mares. *Theriogenology*, 21(6), pp. 897-909.

Henriksen, E.J., Bourey, R.E., Rodnick, K.J., Koranyi, L.A.S.Z.L.O., Permutt, M.A. & Holloszy, J.O. (1990). Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *American Journal of Physiology-Endocrinology and Metabolism*, 259(4), pp. E593-E598.

Hetland, M.L., Haarbo, J. & Christiansen, C. (1998). Regional body composition determined by dual-energy x-ray absorptiometry. Relation to training, sex hormones, and serum lipids in male long-distance runners. *Scandinavian Journal of Medicine and Science in sports,* 8(2), pp. 102-108

Hodgson, D R. & McGowan, C. (2014). An overview of performance and sports medicine. In: Hodgson, D.R., McKeever, K.H. & McGowan, C.M. (eds). *The athletic horses, principles and practice of equine sports medicine.* 2nd ed. USA: Elsevier Saunders. pp. 1-8.

Hoffmann, G., Bentke, A., Rose-Meierhöfer, S., Ammon, C., Mazetti, P. & Hardarson, G.H. (2013). Estimation of the body weight of Icelandic horses. *Journal of Equine Veterinary Science*, 33, pp. 893-895.

Hoffman, R.M., Boston, R.C., Stefanovski, D., Kronfeld, D.S. & Harris, P.A. (2003). Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. *Journal of Animal Science*, 81(9), pp. 2333-2342.

Hreiðarsdóttir, G.E. & Hallsson, J.H. (2007). The origin of the Icelandic horse. *Fræðaþing landbúnaðarins,* 4, pp. 97-103.

Irvine, C.H.G. & Alexander, S.L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology,* 11(2), pp. 227-238.

Jansson. A., Sandin, A. & Lindberg, J.E. (2006). Digestive and metabolic effects of altering feeding frequency in athletic horses. *Equine and Comparative Exercise Physiology,* 3(2), 83-91.

Jansson, A. & Lindberg. J.E. (2012). A forage-only diet alters the metabolic response of horses in training. *Animal,* 6(12), pp. 1939-1946.

Jansson, A., Stefánsdóttir, G.J., Torres, J.C.R. & Ragnarsson, S. (2015). Plasma insulin concentration is affected by body condition in Icelandic horses. *Acta Veterinaria Scandinavica,* 57(1), 01.

Jensen. R.B., Danielsen, S.H. & Tauson, A.H. (2016). Body condition score, morphometric measurements and estimation of body weight in mature Icelandic horses in Denmark. *Acta Veterinaria Scandinavica,* 58(1), pp. 59.

Kang, OD., Ryu, YC., Yun, YM. & Kang, MS. (2012). Effects of cooldown methods and durations on wquine physiological traits following high-intensity exercise. *Livestock Science,* 143, pp. 70-76.

Karlsson, J., Nordesjö, L.O., Jorfeldt, L. & Saltin, B. (1972). Muscle lactate, ATP, and CP levels during exercise after physical training in man. *Journal of Applied Physiology,* 33(2), pp. 199-203.

Kearns, C.F., McKeever, K.H., John-Alder, H., Abe, T. & Brechue, W.F. (2002a). Relationship between body composition, blood volume and maximal oxygen uptake. *Equine Veterinary Journal,* 34(34), pp. 485-490.

Kearns, C.F., McKeever, K.H., Kumagai, K. & Abe, T. (2002b.) Fat-free mass is related to one-mile race performance in elite Standardbred horses. *The Veterinary Journal,* 163(3), pp. 260-266.

Keenan, D.M. (1979). Changes of blood metabolites in horses after racing, with particular reference to uric acid. *Australian Veterinary Journal,* 55, pp. 54-57.

Kline, H. & Foreman, J.H. (1991). Heart and spleen weights as a function of breed and somatotype. *Equine Exercise Physiology,* 3, pp. 17-21.

Krzywanek, H., Wittke, G., Bayer, A. & Borman, P. (1970). The heart rates of Thoroughbred horses during a race. *Equine Veterinary Journal,* 2(3), pp. 115-117.

Lacombe, V.A., Hinchcliff, K.W., Geor, R.J. & Baskin, C.R. (2001). Muscle glycogen depletion and subsequent replenishment affect anaerobic capacity of horses. *Journal of Applied Physiology,* 91, pp. 1782-1790.

Lafontan, M. & Langin, D. (1995). Cellular aspects of fuel mobilization and selection in white adipocytes. *Proceedings of the Nutrition Society*, 54, pp. 49-63.

Lawrence, L., Soderholm, L.V., Roberts, A., Williams, J. & Hintz, H. (1993). Feeding status affects glucose metabolism in exercising horses[1,2]. *The Journal of Nutrition*, 123(12), pp. 2152-2157.

Lawrence, L.M., Williams, J., Soderholm, L.V., Roberts, A.M. & Hintz, H.F. (1995). Effect of feeding state on the response of horses to repeated bouts of intense exercise. *Equine Veterinary Journal*, 27(1), pp. 27-30.

Leleu, C. & Cotrel, C. (2006). Body composition in young Standardbreds in training: relationships to body condition score, physiological and locomotor variables during exercise. *Equine Veterinary Journal,* 36, pp. 98-101.

Lindholm, A., Bjerneld, H. & Saltin, B. (1974). Glycogen depletion pattern in muscle fibres of trotting horses. *Acta Physiologica*, 90(2), pp. 475-484.

Lindholm, A. & Piehl, K. (1974). Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscles of Standardbred horses. *Acta Veterinaria Scandinavica*, 15, pp. 287-309.

Lindholm, A. & Saltin, B. (1974). The physiological and biochemical response of Standardbred horses to exercise at varying speed and duration. *Acta Veterinaria Scandinavica,* 15, pp. 310-324.

Lucke, J.N. & Hall, G.N. (1980). Further studies on the metabolic effects of long distance riding: Golden Horseshoe Ride 1979. *Equine Veterinary Journal,* 12(4), pp. 189-192.

Markusfeld, O., Galon, N. & Ezra, E. (1997). Body condition score, health, yield and fertility in dairy cows. *The Veterinary Record*, 141(3), pp.67-72.

Marsland, W.P. (1968). Heart rate response to submaximal exercise in the Standardbred horse. *Journal of Applied Physiology,* 24(1), pp. 98-101.

Matthíasdóttir, S. (2012). Lífþungi íslenskra reiðhrossa áætlaður út frá skrokkmálum. Unpublished B.Sc. Thesis. The Agricultural University of Iceland, Hvanneyri and Hólar University College.

McGowan, C. & Hodgson, D.R. 2014. Hematology and Biochemistry. In: Hodgson, D.R., McKeever, K.H. & McGowan, C.M. (eds). *The athletic horse, principles and practice of equine sports medicine.* 2 ed. USA: Elsevier Saunders. pp. 56-68.

McKeever, K.H., Hinchcliff, K.W., Reed, S.M. & Robertson, J.T. (1993). Role of decreased plasma volume in haematocrit alterations during incremental treadmill exercise in horses. *American Journal of Physiology*, 265(2), pp. 404-408.

McMiken, D.F. (1983). An energetic basis of equine performance. *Equine Veterinary Journal*, 15(2), pp. 123-133.

Ministry of Fisheries & Agriculture (2011). Regulation on the origin and breeding of the Icelandic horse. https://www.feiffengur.com/documents/RegulationOriginBreeding.pdf [Assessed 12 April 2017]

Mittendorfer, B., Fields, D.A. & Klein, S. (2004). Excess body fat in men decreases plasma fatty acid availability and oxidation during endurance exercise. *American Journal of Physiology-Endocrinology and Metabolism,* 286(3), pp. E354-362.

NRC (2007). *Nutrient Requirements of Horses.* 6th revised edition. Washington, D.C: National Academies Press.

Owers, R. & Chubbock, S. (2013). Fight the fat! *Equine Veterinary Journal,* 45, pp. 5.

Pagan, J.D. & Hintz, H.F. (1986). Equine energetics. II. Energy expenditure in horses during submaximal exercise. *Journal of Animal Science,* 63(3), pp. 822-830.

Pagan, J.D. & Harris, P.A. (1999). The effects of timing and amount of forage and grain on exercise response in Thoroughbred horses. *Equine Veterinary Journal,* 30, pp. 451-457.

Pagan, J.D., Martin, O.A., Crowley, N.L. & Hooks, K.L. (2009). Relationship between body condition and metabolic parameters in sport horses, pony hunters and polo ponies. *Journal of Equine Veterinary Science,* 29(5), pp. 418-420.

Parker, M., Goodwin, D., Eager, R.A., Redhead, E.S. & Marlin, D.J. (2010). Comparison of Polar® heart rate interval data with simultaneously recorded ECG signals in horses. *Comparative Exercise Physiology,* 6(4), pp. 137-142.

Persson, S.G.B. (1967). On blood volume and working capacity in horses. *Acta Veterinaria Scandinavica,* supplement, 19, 1-189.

Persson, S.G.B. (1968). Blood volume state of training and working capacity of race horses. *Equine Veterinary Journal,* 1(2), pp. 52-62.

Persson, S.G.B. & Ullberg, L.E. (1974). Blood volume in relation to exercise tolerance in trotters. *Journal of the South African Veterinary Association*, 45(4), pp. 293-299.

Persson, S.G.B. (1997). Heart rate and blood lactate responses to submaximal treadmill exercise in the normally performing Standardbred trotter—age and sex variations and predictability from the total red blood cell volume. *Transboundary and Emerging Diseases*, 44, pp. 125-132.

Powell, D.M., Reedy, S.E., Sessions, D.R. & Fitzgerald, B.P. (2002). Effect of short-term exercise training on insulin sensitivity in obese and lean mares. *Equine Veterinary Journal*, 34, pp. 81-84.

Pratt, S.E., Geor, R.J. & McCutcheon, L.J. (2006). Effects of dietary energy source and physical conditioning on insulin sensitivity and glucose tolerance in Standardbred horses. *Equine Veterinary Journal*, 36, pp. 579-584.

Pösö, A.R., Viljanen-Tarifa, E., Soveri, T. & Oksanen, H.E. (1989). Exercise-Induced Tran Hyperlipidemia in the Racehorse. *Journal of Veterinary Medicine Series A*, 36(1-10), pp. 603 611.

Ragnarsson, S. & Jansson, A. (2011). Comparison of grass haylage digestibility and metabolic plasma profile in Icelandic and Standardbred horses. *Journal of Animal Physiology and Animal Nutrition*, 95, pp. 273-279.

Ringmark, S. & Jansson, A. (2013a). Insulin response to feeding forage with varying crude protein and amino acid content in horses at rest and after exercise. *Comparative Exercise Physiology*, 9(3-4), pp. 209-217.

Ringmark, S., Roepstorff, L., Essén-Gustavsson, B., Revold, T., Lindholm, A., Hedenström, U., Rundgren, M., Ögren, G. & Jansson, A. (2013b). Growth, training response and health in Standardbred yearling fed a forage-only diet. *Animal*, 7(5), pp. 746-753.

Ringmark, S., Lindholm, A., Hedenström, U., Lindinger, M., Dahlborn, K., Kvart, C. & Jansson, A. (2015). Reduced high intensity training distance had no effect on V_{La4} but attenuated heart rate response in 2-3-year-old Standardbred horses. *Acta Veterinaria Scandinavica*, 57(1), pp. 17.

RML (2017). Breeding goal for the Icelandic horse breed. https://www.rml.is/static/files/Hrossaraekt_RML/raektunarmarkmid.pdf [Assessed 12 April 2017]

Rose, R.J., Ilkiw, J.E. & Hodgson, D. (1979). Electrocardiography, heart score and haematology of horses competing in an endurance ride. *Australian Veterinary Journal*, 55, pp. 247-250.

Rose, R.J., Hodgson, D.R., Kelso, T.B., McCutcheon, L.J., Reid, T.A., Bayly, W.M. & Gollnick, P.D. (1988). Maximum O2 uptake, O2 debt and deficit, and muscle metabolites in Thoroughbred horses. *Journal of Applied Physiology*, 64(2), pp.781-788.

Scheffer, C.J.W. & Sloet van Oldruitenborgh-Oosterbaan, M.M. (1996). ECG recording in the horse during exercise. *Veterinary Quarterly,* 18(1), pp. 2-7.

Scheibe, K.M. & Streich, W.J. (2003). Annual rythm of body weight in Przewalski horses (Equus ferus przewalski). *Biological Rythm Research,* 34(4), 383-395.

Schmidt, A., Möstl, E., Wehnert, C., Aurich, J., Müller, J. & Aurich, C. (2010). Cortisol release and heart rate variability in horses during road transport. *Hormones and Behaviour*, 57(2), pp. 209-215.

Seeherman, H.J. & Morris, E.A. (1990). Application of a standardised treadmill exercise test for clinical evaluation of fitness in 10 Thoroughbred racehorses. *Equine Veterinary Journal,* 22(S9), pp. 26-34.

Sejian, V., Maurya, V.P., Naqvi, S.M.K., Kumar, D. & Joshi, A. (2010). Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment. *Journal of Animal Physiology and Animal Nutrition,* 94, pp. 154-161.

Selk, G.E., Wettemann, R.P., Lusby, K.S., Oltjen, J.W., Mobley, S.L., Rasby, R.J. & Garmendia, J.C. (1988). Relationships among weight change, body condition, and reproductive performance of range beef cows. *Journal of Animal Science,* 66(12), pp. 3153-3159.

Sexton, W.L. & Erickson, H.H. (1990). Effects of treadmill evaluation on heart rate, blood lactate concentration and packed cell volume during graded submaximal exercise in ponies. *Equine Veterinary Journal,* 22(S9), pp. 57-60.

Sjaastad, Ø.V., Sand, O. & Hove, K. (2016). *Physiology of Domestic Animal*. 3rd ed. Oslo: Scandinavian Veterinary Press.

Sloet van Oldruitenborgh-Oosterbaan, M.M. & Clayton, H.M. (1999). Advantages and disadvantages of track vs. treadmill tests. *Equine Veterinary Journal,* 31(S30), pp. 645-647.

Snow, D.H. & MacKenzie, G. (1977). Some metabolic effects of maximal exercise in the horse and adaptations with training. *Equine Veterinary Journal,* 9(3), pp. 134-140.

Stefánsdóttir, G.J. & Björnsdóttir, S. (2001). Body condition scoring of horses (Mat á holdafari hrossa). *Eiðfaxi-Ræktun,* special issue, Reykjavík, Iceland. Vol. 1, pp. 60-65.

Stefánsdóttir, G.J., Ragnarsson, S., Gunnarsson, V. & Jansson, A. (2014). Physiological response to a breed evaluation field test in Icelandic horses. *Animal,* 8(3), pp.431-439.

Stefánsdóttir, G.J., Ragnarsson, S., Gunnarsson, V., Roepstorff, L. & Jansson, A. (2015). A comparsion of the physiological response to tölt and trot in the Icelandic horse. *Journal of Animal Science,* 93(8), pp. 3862-3870.

Steel, J.D. & Stewart, G.A. (1974). Electrocardiography of the horse and potential performance ability. *Journal of the South African Veterinary Association,* 45(4), pp. 263-268.

Stewart, G.A. (1981). The heart score theory in the racehorse. *Australian Veterinary Journal,* 57(9), pp. 422-428.

Stewart-Hunt, L., Geor, R.J. & McCutcheon, L.J. (2006). Effects of short-term training on insulin sensitivity and skeletal muscle glucose metabolism in Standardbred horses. *Equine Veterinary Journal,* 36, pp. 226-232.

Sticker, L.S., Thompson, D.L., Bunting, L.D., Fernandez, J.M. & DePew, C.L. (1995a). Dietary protein and(or) energy restriction in mares: Plasma glucose, insulin, nonesterified fatty acid, and urea nitrogen responses to feeding glucose, and epinephrine[1]. *Journal of Animal Science,* 73(5), pp. 136-144.

Sticker, L.S., Thompson, D.L., Bunting, L.D., Fernandez, J.M., DePew, C.L. & Nadal, M.R. (1995b). Feed deprivation of mares: Plasma metabolite and hormonal concentrations and responses to exercise[1]. *Journal of Animal Science,* 73(12), pp. 3696-3704.

Stull, C.L. & Rodiek, A.V. (1988). Responses of blood glucose, insulin and cortisol concentrations to common equine diets. *The Journal of nutrition*, 118(2), pp.206-213.

Suagee, J.K., Burk, A.O., Quinn, R.W., Petersen, E.D., Hartsock, T.G. & Douglass, L.W. (2008). Effects of diet and weight gain on body condition scoring in Thoroughbred geldings. *Journal of Equine Veterinary Science,* 28(3), pp. 156-166.

Treiber, K.H., Hess, T.M., Kronfeld, D.S., Boston, R.C., Geor, R.J., Friere, M., Silva, A.M.G.B. & Harris, P.A. (2006a). Glucose dynamics during exercise: dietary energy sources affect minimal model parameters in trained Arabian geldings during endurance exercise. *Equine Veterinary Journal,* 36, pp. 631-636.

Treiber, K.H., Kronfeld, D.S., Hess. T.M., Byrd. B.M., Splan, R.K. & Staniar, W.B. (2006b). Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *Journal of the American Veterinary Medical Association,* 228(10), pp. 1538-1545.

Valberg, S.J. (2014). Muscle anatomy, physiology, and adaptations to exercise and training. In: Hodgson, D.R., McKeever, K.H. & McGowan, C.M. (eds). *The athletic horse, principles and practice of equine sports medicine.* 2 ed. USA: Elsevier Saunders. pp. 174-201.

Valberg, S., Essén-Gustavsson, B., Lindholm, A. & Persson, S. (1985). Energy metabolism in relation to skeletal muscle fibre properties during treadmill exercise. *Equine Veterinary Journal*, 17(6), pp. 439-444.

Vermeulen, A.D. & Evans, D.L. (2006). Measurements of fitness in Thoroughbred racehorses using field studies of heart rate and velocity with global positioning system. *Equine Veterinary Journal,* 38(36), pp. 113-117.

Von Wittke, P., Lindner, A., Deegen, E. & Sommer, H. (1994). Effects of training on blood lactate running speed relationship in thoroughbred racehorses. *Journal of Applied Physiology,* 77(1), pp. 298-302.

Webb, A.I. & Weaver, B.M.Q. (1979). Body composition of the horse. *Equine Veterinary Journal,* 11(1), pp. 39-47.

Wesserman, K., Whipp, B.J., Koyal, S.N. & Beaver, W.L. (1973). Anaerobic threshold and respiratory gas exchange during exercise. *Journal of Applied Physiology,* 35(2), pp. 236-243.

Westervelt, R.G., Stouffer, J.R., Hintz, H.F. & Schryver, H.F. (1976). Estimating fatness in horses and ponies. *Journal of Animal Science,* 43(4), pp. 781-785.