# Physiological responses in trained Standardbred horses to forage diets, transport and housing

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## Abstract

This thesis describes the effects of forage-only and forage-oats diets, different levels of crude protein intake, road transport and different housing systems on body weight, fluid shifts, metabolic response and exercise response in trained Standardbred horses. A total of 28 horses participated in the research.

Body weight was significantly higher on the forage-only diet (mean 496 kg) than the forage-oats diet (mean 492 kg) in one study, but a 12 h feed deprivation period diminished the small increase in body weight on the forage-only diet. The forage-only diet resulted in lower plasma protein concentration, indicating greater potential to use an internal fluid compartment to maintain plasma volume. In another study assessing these two diets and transportation, there were no differences between the diets (forage-only 502 kg, forage-oats 501 kg). There was also no effect of high crude protein intake (160% of recommended level) on response to exercise, but the intake was high enough to increase plasma urea. High protein intake also increased water excretion through faeces and urine and thereby exerted an unnecessary load on the racing horse. Overall, the forage-only diet decreased exercise-induced effects on extracellular fluid regulation.

Aerobic energy supply was elevated during exercise following a period of road transport and forage-only diet further increased the usage of fat (acetate) as energy substrate. However, this positive effect on aerobic energy supply needs to be studied further in terms of transport timing and duration, in order to confirm whether this can be used as an efficient part of pre-race preparations

It was found that keeping the horses in an active group housing system had positive effects on appetite and recovery of energy balance and post-race joint swelling compared with housing in a conventional box stall system.

In conclusion, this thesis demonstrates that a forage-only diet and a free-range housing system are good management options for racing Standardbred horses and can form part of a sustainable horse management system that supports performance, health and animal welfare.



*Keywords:* NEFA, acetate, exercise, forage-only



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## Abstract

Syftet med avhandlingen var att undersöka effekterna av grovfoderdieter, högt proteinintag, transport och uppstallningssystem på kroppsvikt, plasmavolym, metabolisk respons och arbete hos travhästar i full träning.

Totalt användes 28 tränade travhästar i studierna (19 valacker och 9 ston). I studie I var hästarnas kroppsvikt högre på en grovfoderdiet än på en grovfoder-havre diet (496 vs. 492 kg SEM 10). I studie III var det ingen skillnad på kroppsvikt mellan en grovfoderdiet och en grovfoder-havre diet (grovfoder: 502 kg, grovfoder-havre: 501 kg, SEM 26). När hästarna fastades i 12 timmar tappade de mer vikt på grovfoderdieten vilket gjorde att den lilla viktskillnaden mellan dieterna försvann. Under fastan hade hästarna lägre plasmaproteinkoncentration på grovfoderdieten vilket tyder på att de hade bättre möjlighet att bibehålla sin plasmavolym. Troligtvis beror detta på en större vätskevolym i grovtarmen. Ett högt proteinintag (studie II) påverkade inte prestationsparametrar under och efter ett arbetstest även om proteinintaget var högt nog att höja plasmaurea. Det höga proteinintaget ökade vätskeförluster i träck och urin vilket är en onödig belastning för en högpresterande häst. Transport (studie III) påverkade energiomsättningen mot mer aerob metabolism under travlopp, likaså gjorde en grovfoderdiet. En grovfoderdiet jämfört med en grovfoder-havre diet minskade arbetstestets effekter på plasma volymen. En aktiv grupphästhållning (studie IV) hade en positiv effekt på foderintag, användning av kroppsfettreserven och svullnad runt hästarnas bakkotor jämfört med boxuppstallning.

Sammanfattningsvis visar studierna i den här avhandlingen att en grovfoderdiet och en aktiv grupphästhållning är ett bra alternativ för tränande travhästar. De positiva effekterna av transporten skulle behöva studeras ytterligare för att utvärdera tid i förhållande till lopp och hur lång transporten behöver vara för att får en effekt innan vi vet hur och om det skulle kunna användas som en effektiv del av förberedelserna före tävling.



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# Dedication

To the One I love

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

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

II Connysson, M., S. Muhonen, J.E. Lindberg, B. Essén-Gustavsson, G. Nyman, K. Nostell and A. Jansson (2006). Effects on exercise response, fluid and acid-base balance of protein intake from forage-only diets in Standardbred Horses. *Equine Veterinary Journal* 38 (S36), 648-653.

III Connysson, M., S. Muhonen and A. Jansson (2017). Road transport and diet affect metabolic response to exercise in horses. *Journal of Animal Science* 95 (11), 4869-4879.

IV Connysson, M., M. Rhodin, A. Bergh and A. Jansson. Effects of free-range housing and individual box housing on exercise recovery in horses (*manuscript*).

Papers I-III are reproduced with the permission of the publishers.

# Related publications not included in the thesis

Nostell K., P. Funkqvist, G. Nyman, B. Essén-Gustavsson, M. Connysson, S. Muhonen and A. Jansson (2006). The physiological responses to stimulated race tests on a track and on a treadmill in Standardbred trotters. *Equine Veterinary Journal* 38 (S36), 123-127.

Muhonen S., M. Connysson, J.E. Lindberg, V. Julliand, J. Bertilsson and A. Jansson (2008). Effects of crude protein intake from grass silage-only diets on the equine colon ecosystem after an abrupt feed change. *Journal of Animal Science* 86, 3465-3472.

Essén-Gustavsson B, M. Connysson and A. Jansson (2010). Effects of crude protein intake from forage-only diets on muscle amino acids and glycogen levels in horses in training. *Equine Veterinary Journal* 42 (38), 341-346.

Bergh A., M. Svernhage and M. Connysson (2018). Assessment of swelling in the equine fetlock joint area. Comparative exercise physiology, (*accepted*).

# Abbreviations

| | |
|---|---|
| ADF | Acid detergent fibre |
| AST | Aspartate amino transferase |
| bpm | Beats per minute |
| BW/bwt | Body weight |
| CK | Creatine kinase |
| CP | Crude protein |
| CT | Control test, not transported |
| DCP | Digestible crude protein |
| DM | Dry matter |
| FD | Forage diet |
| FONLY | Forage-only diet |
| FOATS | Forage-oats diet |
| HP | High protein |
| HR | Heart rate |
| N | Nitrogen |
| ME | Metabolisable energy |
| MJ | Megajoule |
| MNT | Mechanical nociceptive threshold |
| NDF | Neutral detergent fibre |
| NEFA | Non-esterified fatty acids |
| OD | 50:50 oats-forage diet |
| RP | Recommended protein |
| RR | Respiratory rate |
| TPP | Total plasma protein |
| TT | Transport test |
| VFA | Volatile fatty acids |

Table 1. *The thesis at a glance*

| Paper | Horses | Treatments | Measurements | Main conclusions |
|---|---|---|---|---|
| I | Twelve adult trained Standardbred trotters (9 geldings, 3 mares). | 12-hour feed deprivation, two diets in a crossover design; one forage-only (FD) and one 50:50 forage:oats diet (OD) | BW, water intake, TPP, NEFA, urea, acetate, glucose, insulin | Basal TPP was maintained for longer during feed deprivation on FD, indicating greater potential to use an internal fluid compartment to maintain plasma volume. Small increase in BW on FD compared with OD (496 vs. 492, SEM 10 (P=0.008)) |
| II | Six adult trained Standardbred trotters. Geldings. | Two forage-only diets; one providing recommended CP intake (100%) and one providing 160 % in a crossover design. Two exercise tests on each diet, one treadmill and one race track test. | BW, water intake, TPP, urea, lactate, HR, RR, Na, P, Cl, $TCO_2$, blood pH. Faecal water, DM, N and pH. Urine quantity, N and pH. | CP intake did not affect the acute response to intensive exercise, but increased fluid losses. The increased fluid losses suggest that feeding a 160% CP diet prior to exercise will pose an unnecessary challenge in athletic horses. |
| III | Six adult trained Standardbred trotters. Mares | 100 km transportation. Two diets; one forage-only and one 50:50 forage:oats diet in a crossover design. Two exercise tests on each diet, one with transportation and one without. | BW, water intake, TPP, NEFA, urea, acetate, glucose, insulin, cortisol, lactate and HR. | Transport affected more metabolic parameters than diet. Aerobic energy supply was most likely elevated by transportation and by the forage-only diet. The forage-only diet also decreased exercise-induced effects on extracellular fluid regulation. |
| IV | Eight adult trained Standardbred trotters. Geldings | Two housing systems. One traditional box stall and one free-range group housing. | BW, feed intake, TPP, NEFA, urea, lactate, HR, AST, CK, MNT, fetlock region swelling | Free-range group housing had positive effects on appetite and recovery of energy balance compared with housing in a traditional box stall system and abolished post-race joint swelling. However, the importance of swelling observed around distal joints for health needs further investigation. |

# 1 Background

The Swedish Standardbred trotter originates mainly from the American Standardbred trotter. However, there is influence from the French trotter, while a little influence from the Russian Orlov trotter can still be found. In Sweden, the Standardbred trotter is the second largest breed (HNS, 2017). There are 33 official harness race tracks in Sweden, located from Bodentravet in the north (65.8ºN) to Jägersro in the south (55.6ºN). Most races are performed on oval 1000-1600 m gravel tracks. The most common race distances are 1640, 2140 and 2640 m and most races are harness races where the driver sits in a sulky behind the horse. During races, the horses trot at a velocity of 12.5-14.3 m/s, which means that the duration of races is between 2 and 3.5 minutes.

The harness racing industry differs from some other horse disciplines since there is a lot of money involved, both as prize money and in betting. Horse betting leads to media exposure, with races broadcast on 364 days of the year, which generates public interest and opinion on horse appearance and performance. The number of people involved in the daily feeding and management of harness racing horses in Sweden is approximately 5500 (grooms, licensed trainers and drivers) (Svensk Travsport, 2017). It is likely that they aim to provide the optimal conditions for their horses to perform at their best. However, knowledge of feeding and management routines that actually make a difference in racing horses is limited.

# 2 Introduction

Racing Standardbred horses are athletes that train and race at a high level of physical activity during most of their career and, apart from the obvious aim of winning races, the goal is for them to have a long career. In a study where 16 Standardbred trotters were followed from first training at the age of 1.5 years, the mean distance covered in intensive training (with heart rate (HR) >180 bpm) before the first race at 3 years of age was found to be 267±42 km (Ringmark, 2014). During 2017, there were 13,768 Standardbred trotters in training in Sweden and 47% of these were young horses (2-4 years old), while only 53% of older horses (5-12 years) remained in training (Svensk Travsport, 2017). This probably reflects both the distribution of race prize money and older horses being retired due to injury. The performance of Standardbred horses is affected by many different factors, such as genetic capacity, conformation, training, injuries, nutrition and management. This thesis focuses on management and nutrition.

## 2.1 Management

### 2.1.1 Housing

Most horses in Sweden (Jordbrukverket, 2012) and other countries (Bachmann & Stauffacher, 2002; Petersen *et al*., 2006; Henderson, 2007; Hotchkiss *et al*., 2007) are currently housed in individual box stalls in the same way as they have been for the past hundred years.

Living indoors in stables, with limited ability to move, has been shown to negatively affect bone quality in young horses compared with young horses kept on pasture (Hoeckstra *et al*., 1999). In the Hoeckstra et al. (1999) study, bone quality was measured both during 84 days without training and during 56 days of training and there was a negative effect of housing on bone quality during the whole study, irrespective of training.

Exposure to dust from feed and bedding, combined with insufficient ventilation, increases the risk of horses experiencing problems with their respiratory system (Couëtil *et al*., 2016).

Box stalls facilitates the supervision of horses, individual feeding, and grooming of the horses, but obviously limit their scope for free movement and social interaction. An alternative is free-range housing system where horses are kept in groups in paddocks with shelter and lying areas, and with individual feeding controlled by transponders. Anecdotal information indicates concerns among trainers and horse owners that a lack of rest in such systems delays recovery and affects competition performance negatively.

### 2.1.2 Pre-competition management and transportation

Taking part in a competition can involve long transport distances for horses. Access to feed and water is limited during transportation, but limiting water and feed intake can also be a part of trainers' preparation prior to competition (Pinchbeck *et al*., 2004; Burke & Williams, 2008). In a French study on pre-race (harness racing) routines, most trainers (95%) modified the concentrate meal on race day and excluded hay and straw completely or partly (Fortier, 2013). In horses, road transportation has been shown to increase heart rate (Smith *et al*., 1996; Doherty *et al*., 1997; Schmidt *et al*., 2010), plasma lactate (Stull, 1999; Werner & Callo, 2007) and cortisol concentration (White *et al*., 1991; Smith *et al*., 1996; Stull & Rodiek, 2000; Fazio *et al*., 2008; Stull *et al*., 2008; Schmidt *et al*., 2010). These physiological changes connected to transportation may have the potential to change the response of horses during exercise, but had not been studied prior to the work in this thesis.

Intense exercise can lead to increased synovial effusion in joints and tendon sheaths. Swelling is associated with inflammation, and joint-related lameness is a common problem in Standardbred trotters (Vigre *et al*., 2002). However, swelling in the joint or structures around it can also be due to impaired lymph flow secondary to low physical activity. The importance of this condition for wellbeing and locomotion 'fitness' is unknown. A common practice in competition stables is to try to reduce swelling and/or increase blood flow with bandages, liniment and massage to hasten healing (recovery). However, knowledge of the effect of different treatments in horse limbs is limited.

## 2.2 Nutrition

Standardbred trotters in full training have energy requirements that are double, or more than double, those of a horse of similar size that is not in training. The requirement for other nutrients is also elevated compared with that in non-training animals. To meet the high energy requirements of athletic horses, the feed offered must be energy-dense. Traditionally, this need for feed rich in energy has been met by large amounts of high energy concentrates. A review on Standardbred race horses in the USA, Germany, Australia and Sweden between 1979 and 2007 reported concentrate allowances of 6.8±0.4 kg/day and forage allowances of 5.8 ±0.4 kg/day (Jansson & Harris, 2013). More recent data (2015) from a voluntary internet survey involving 452 Swedish amateur and professional harness race trainers suggest that the majority (54%) of trainers feed 1-3 kg concentrate/day, 37% feed 3.5-5 kg, 3% feed more than 5.5 kg concentrate and 6% feed less than 1 kg or no concentrate (Hurtes, 2015). In the same study, 65% of trainers reported feeding more than 10 kg forage (dry matter (DM) content unspecified). The difference between the study of Jansson & Harris (2013) and Hurtes (2015) might be a effect of the last years intense research on forage-only diets in Sweden but it might also just reflect study design. People that answer surveys, used in Hurtes (2015), tend to be more interested in the area. However, in Hurtes (2015) study there was a clear difference between Swedish and British trainers.

The problem with feeding a large amount of concentrate (starch-rich diet) is that it is known to increase the risk of colic (Tinker *et al*., 1997; Hudson *et al*., 2001). It may also be linked to gastric ulcers (Luthersson *et al*., 2009), rhabdomyolysis (McLeay *et al*., 2000) and stereotypic behaviours (Kusunose, 1992; Gillham *et al*., 1994; Redbo *et al*., 1998).

To decrease the risks associated with to feeding large amounts of concentrate, the diet can be adjusted to contain more/only forage. However, feeding a forage-only diet to an athletic horse requires a forage that is sufficiently energy-rich to meet the high energy requirements. A forage that contains enough energy to fill the requirements of athletic horses may also contain high protein levels. There is limited empirical evidence on how increasing the forage content in the diet affects trained Standardbred trotters.

### 2.2.1 Forage

Horses are grazing animals and well adapted to a diet consisting of grass. Grass comprises water, cell walls and cell contents. The cell wall part consists of cellulose, lignin, hemicellulose and pectin, and is commonly analysed as neutral detergent fibre (NDF). The cell content part consists of water-soluble carbohydrates (mono and disaccharides, oligosaccharides and fructan polysaccharides), proteins, peptides, lipids, vitamins and minerals. As grass matures, the proportion of NDF increases and the cell contents decrease. In the horse, digestion of the fibre component (cellulose and hemicellulose) is carried out by microorganisms in the hindgut, while the cell contents are enzymatically and chemically digested in the stomach and small intestine. The proportions of different microorganisms in the hindgut are related to diet (Julliand *et al*., 2001). The hindgut microorganisms produce volatile fatty acids (VFAs), primarily acetic, propionic and butyric acid, that are transported to the blood through the mucosa in the caecum and colon (Argenzio *et al*., 1974b). In horses consuming a diet consisting mainly of hay, it has been estimated that 80% of the horse's energy demand is met by VFAs as the energy source (Vermorel *et al*., 1997). In a study by Jansson & Lindberg (2012) the plasma lactate concentrations during recovery was lower in horses fed a forage-oats diet compared with a forage-only diet.

### 2.2.2 Exercise energy requirements

Aerobic energy utilisation during exercise can be estimated by measurements of gas exchange and oxygen consumption. Energy expenditure depends on a number of factors, such as speed (Evans & Rose, 1987; Rose *et al*., 1990), draught load (Gottleib-Vedi & Lindholm, 1997), slope (Thornton *et al*., 1987) and track surface. Therefore the actual energy expenditure by one individual horse during one training or competition session is difficult to estimate. Exercise energy can be estimated using heart rate measurements to estimate oxygen utilisation by applying the formula: Oxygen utilisation (mL $O_2$/kg BW/min) = 0.0019 x $HR^{2.0653}$ (NRC, 2007 revised from Coenen, 2005). Estimating energy expenditure for horses exercising at an average heart rate of 188 bpm using this formula gives 1.92 kJ/min/kg body weight (1 L $O_2 \approx$ 20.35 kJ) (NRC, 2007). According to this formula, energy expenditure by a 500-kg horse during a harness race (223 bpm, 3 min total race time) would be 4.1 MJ and the energy used during warm-up and cool-down (30 min, HR 150 bpm) would be 3.6 MJ. These values for energy expenditure are similar to estimates obtained in a study where values from a portable respiratory gas analyser were used to calculate exercise energy expenditure during ordinary harness race training in French trotters (Fortier *et al*., 2015).

Studies on elevated post-exercise metabolism in humans suggest that the intensity and duration of exercise increase energy expenditure during recovery (LaForgia *et al*., 2006). After intense exercise, oxygen consumption in that study was found to remain elevated for up to nine hours but, even for the most intense exercise tests, the recovery did not exceed 14% of the energy cost of the exercise (LaForgia *et al*., 2006). Little is known about post-exercise metabolism in horses, but the elevated heart rate measured during transportation (Smith *et al*., 1996; Doherty *et al*., 1997; Schmidt *et al*., 2010) suggests that metabolism is elevated over the maintenance level during transportation.

To transform exercise energy losses into feed intake energy to compensate for those energy losses, the efficiency of use of feed intake energy must be known. During intensive exercise, NRC (2007) suggests an efficiency of 20% of digestible energy intake.

### 2.2.3 Energy substrates

Different substrates can be used by the muscle cell to produce ATP. The energy substrate metabolised depends on the availability of different substrates and the intensity of exercise. At rest and during low intensity exercise, fat oxidation is the major energy source (Hodgson *et al*., 2014). As exercise intensity increases, the increased energy expenditure is mainly covered by upregulation of blood glucose and glycogen metabolism. During exercise at 30% of $VO_{2max}$, approximately 30% of the total energy expenditure is from muscle glycogen (and lactate). During exercise at 60% of $VO_{2max}$, approximately 65% of the total energy expenditure is from muscle glycogen (and lactate), while fat metabolism remains almost unchanged (Geor *et al*., 2000). Availability of energy substrate also seems to determine the energy substrate used in muscle cells. Increased concentrations of free fatty acids during exercise result in higher plasma glucose concentrations after exercise in rats, due to decreased glucose muscle uptake and increased energy supply from fatty acid oxidation (Rennie *et al*., 1976). Elevated post-exercise levels of free fatty acids have been shown to lower lactate production and glycogen utilisation during exercise at 68% of $VO_{2max}$ (Costill *et al*., 1977). In addition, in training humans increased free fatty acid concentration has been found to increase fat oxidation at 85% $VO_{2max}$ (Romijn *et al*., 1995). Increasing fat availability by adding fat to the diet has also been shown to reduce the production and utilisation of glucose by 30% during low intensity exercise in horses (Pagan *et al*., 2002).

### 2.2.4 Acetate

Compared with grain-based (starch) diets, forage diets increase the concentration of the VFA acetate in colon and faeces fluid (Hintz *et al.*, 1971). Most cells in the body can absorb acetate and use it as energy after conversion to acetyl-CoA and metabolism via the citric acid cycle. In horses, acetate has been shown to be the major metabolite in the hind limb at rest (Pethick *et al*., 1993). In addition, Pratt *et al*. (2005) found that acetate clearance is accelerated by exercise, suggesting that acetate may be used as an energy source during exercise.

### 2.2.5 Insulin and glucose

Insulin is involved in the regulation of carbohydrate, fat and protein metabolism. Starch-rich diets results in higher blood glucose and higher insulin concentrations in connection with feeding than forage diets (fibre diets) (Pagan *et al*., 1987; Williams *et al*., 2001; Jensen *et al*., 2016). In a study by Jansson & Lindberg (2012), starch-rich diets elevated insulin concentrations during warm-up and recovery compared with a forage-only diet and plasma glucose was elevated for the forage-only diet during warm-up and at the end of an incremental exercise test. In addition to the diet effects, plasma glucose concentrations are also affected by differences in the timing of feeding in relation to exercise (Duren *et al*., 1999; Pagan & Harris, 1999). There is also evidence that intensity of exercise affects plasma glucose concentration (Judson *et al*., 1983; Snow *et al*., 1983). Elevated exercise plasma glucose concentration has been associated with increased performance (Lacombe *et al*., 2001).

### 2.2.6 Glycogen

High intensity exercise decreases muscle glycogen in horses (Lindholm & Saltin, 1974; Valberg *et al*., 1989; Hyyppä *et al*., 1997; Schuback *et al.*, 2000; Jansson & Lindberg, 2012) and reduced glycogen levels are associated with reduced exercise performance (Lacombe *et al*. 2001). During intense exercise, muscle glycogen is the main energy source, with contributions from muscle triglyceride, plasma non-esterified fatty acids (NEFA) and plasma glucose. The substrate for re-synthesis of muscle glycogen is glucose-6-phosphate. In humans, the replenishment of glycogen is rapid (up to 24 h) and depends largely on glucose supply to the muscle (Burke *et al*., 2016). In horses, re-synthesis of muscle glycogen is a process that has been shown to take up to 72 h (Hyyppä *et al*., 1997). Not only does the replenishment of muscle glycogen seems to be slower in horse muscle than human muscle, but oral supplementation of glucose does not increase re-synthesis of glycogen in horses (Geor *et al*., 2006). A study examining the effect of corn starch consumption after exercise found that this did not enhance muscle glycogen re-synthesis during the first 6 h of recovery (Jose-Cunilleras, 2006).

Provision of acetate, to save glucose, has been shown to increase the rate of glycogen replenishment during the first 4 h of recovery (Waller *et al*., 2009). However, in a study comparing a forage-only diet with a forage-concentrate diet, muscle glycogen before and after exercise was found to be higher after the forage-concentrate diet, although the forage-only diet resulted in higher plasma acetate concentrations (Jansson & Lindberg, 2012). The difference in glycogen content was however, small (13 %) and the relevance of this for performance remains to be shown. Interestingly, when horses were feed a forage-only diet with high crude protein (CP) glycogen concentrations after intensive exercise was higher than when they were feed a forage-only diet with recommended crude protein (Essén-Gustavsson *et al*., 2010).

### 2.2.7 Non-esterified fatty acids

Non-esterified fatty acids originate from lipolysis of adipose tissue. Insulin plays an important role in regulating lipolysis in adipose tissue, as increased insulin concentration results in decreased lipolysis and thereby decreased release of NEFA. Upregulation of lipolysis is stimulated by the hormones noradrenalin, adrenalin, glucagon and adrenocorticotropic hormone. Non-esterified fatty acids are used as an energy substrate by most body tissues. In horses, NEFA concentrations have been shown to increase during and after exercise (Rose & Sampson, 1982; Pösö *et al*., 1983; Jansson & Lindberg, 2012). In addition, an increase in plasma NEFA in ponies and horses during feed deprivation has been shown in numerous studies (Baetz & Pearson, 1972; Rose & Sampson, 1982; Sticker *et al*., 1995; Christensen *et al*., 1997). Elevated NEFA values as a result of road transport have been detected in other animals (calves, heifers, dromedary camels and sheep) (Locatelli *et al*., 1989; Earley & Murray, 2010; Saeb *et al*., 2010; Zhong *et al*., 2011).

### 2.2.8 Protein as energy source

A forage that is energy rich often has a high protein content. The body has a limited capacity to store protein that is not synthesised and retained in tissues. If the protein intake is higher than required, it is further metabolised from amino acids and used for energy. Amino acid degradation starts with deamination, where the amino group is removed and converted into ammonia. The ammonia released is removed from the body by forming urea in the liver. The carbon skeleton that remains from the amino acid is metabolised to pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate. Degradation of amino acids is mainly located in the liver, but during starvation and prolonged exercise muscle cells can use branched-chain amino acids for energy. Muscle cells lack the enzymes to form urea, so after deamination the nitrogen is released, in non-toxic form, to the blood, where it is absorbed by the liver and converted into urea. In athletic horses, even short periods without feed, such as those caused by a feeding strategy with two feedings per 24-h period, result in higher plasma urea concentrations than when horses are fed six times per 24-h period (Jansson *et al*., 2006).

*Excessive protein and performance*

Studies on horses have shown that increased intake protein results in increased plasma urea (Fonnesbeck & Symons, 1969) and excretion of nitrogen through faeces and urine (Slade *et al*., 1970; Prior *et al*., 1974; Freeman *et al*., 1988).

High crude protein intake has been associated with slower racing in a study on Thoroughbred horses, where racing times increased by 1 to 3 s for every 1000 g of crude protein fed above the NRC (1978) recommendation (Glade, 1983). However, that study did not record body weight or the effect trainer, and higher body weight could also be an explanation for the decreased performance (Ellis *et al*., 2002; Kearns *et al*., 2002) as well as the effect of trainer. Feeding a high protein diet (14.5% CP) has been shown to lower venous pH during exercise compared with a low protein diet (7.5% CP (under recommended intake for maintenance)) (Graham-Thiers *et al*., 2001). Decreased pH is considered to be one factor in muscle fatigue (Grassi *et al*., 2016). In contrast, Pagan *et al*. (1987) reported lower heart rate and lower lactate during exercise with high protein diets. In other studies, excess protein intakes have been found to have no effects on heart rate and lactate during exercise (Miller & Lawrence, 1988; Miller-Graber *et al*., 1991). Meyer (1987) suggests that feeding excess protein to exercising horses should be avoided, because water requirements increase, plasma urea levels increase and an excess of protein gives more nitrogen in the urine, which could elevate ammonia levels in indoor air. In addition, since synthesis of urea from protein is an energy-consuming process Meyer (1983b) suggests that increased heat production occurs when feeding excess protein.

Dietary protein intake is a hot topic for human athletes (Jäger *et al*., 2017). With studies on humans that show effects of protein supplementation on strength and muscle development and muscle protein synthesis. In addition, supplementing with amino acids combined with carbohydrates after exercise has been suggested to increase glycogen synthesis in humans (Zawadzki *et al*., 1992; van Loon *et al*., 2000). In a study on horses in which leucine and glucose were administered by nasogastric tube post-exercise, there was no difference in muscle glycogen synthesis compared with a placebo (tap water) (Bröjer *et al*., 2012).

### 2.2.9 Water intake

Feeding horses a diet containing a large amount of forage has been shown to increase water intake compared with a diet with limited forage intake (Danielsen *et al*., 1995; Ellis *et al*., 2002; Jansson & Lindberg, 2012). In addition, the amount of water intake by drinking is also related to dry matter content of the forage, as a high dry matter content (hay) increases water intake, while a lower dry matter content (silage) decreases it (Muhonen *et al*., 2008). The increased water intake with high fibre diets can be explained by an increased heat increment of feeding, leading to increased evaporative fluid losses. Forage-only diets increase microbial fermentation in ruminants, which may increase heat production compared with digestion of concentrate (Blaxter, 1989). Studies on maintenance-fed ponies comparing heat production during forage-only diets and forage-concentrate diets found higher heat production for forage-only diets Vermorel *et al*. (1997). In addition, chewing and salivary secretion affect heat production during digestion (Blaxter, 1989). Chewing movements have been estimated to be much higher in horses fed hay (3400 per kg feed) compared with oats (850 per kg feed) (Meyer, 1983a). Forage-only diets also seem to increase faecal water losses (Fonnesbeck, 1968).

The gut is suggested to be a fluid reservoir in horses during dehydration (Meyer, 1987). This suggestion is supported by the finding that exercise and post-exercise total plasma protein levels are lower in horses fed a high forage diet than in horses fed a low forage diet (Danielsen *et al*., 1995; Jansson & Lindberg, 2012). These studies indicate fluid movement from the hind gut into the plasma volume, and thereby improved ability to maintain plasma volume during exercise.

### 2.2.10 Fluid balance and electrolytes

High intensity exercise results in loss of water and electrolytes, mainly through sweat. Horses can lose up to 10-15 L of sweat per hour during exercise (Carlson, 1987). Horse sweat has higher electrolyte concentrations than plasma and human sweat, *e.g*. it is reported to contain 116-135 mmol sodium (Na), 25-42 mmol potassium (K) and 142-156 mmol chlorine (Cl) per litre, with the range being due to ambient temperature and humidity (McCutcheon *et al*., 1995). Horses can replenish potassium losses by eating forage, or other horse feed, since forage contains more potassium than is lost through sweat. The content of sodium and chloride in horse feed is low and has to be supplemented to horses as salt (NaCl). Sodium and potassium are the two most important electrolytes in regulating the fluid balance. For thirst regulation, sodium is the most important electrolyte and sodium depletion can result in decreased feed intake and lowered plasma volume.

Measuring total plasma protein concentration is a way of monitoring fluid shifts in the body, since fluids can easily move to and from plasma and thereby change the plasma protein concentration. Total plasma protein concentration increases due to feeding, when fluid moves to the gastro-intestinal tract (Argenzio *et al*., 1974a), and during exercise (Hyyppä & Pösö, 1998), when fluid shifts to the contracting skeletal muscle.

### 2.2.11 Body weight and body composition

There are different ways to detect a negative or positive energy balance. Measuring body weight is one way to monitor whether the mass of the horse is going up or down during a period of time. However, with body weight measurements some consideration of timing is needed, since body weight may change quickly due to factors such as feeding state, diet (Ellis *et al*., 2002) and fluid losses (Snow *et al*., 1982). Total body weight is correlated with the proportion of body fat (Lohman, 1971), and adipose tissue has been shown to be a variable component of the equine body (Webb & Weaver, 1979).

Another way is to detect energy balance is to evaluate fat stores. In practice, this is done by body condition scoring (BCS) protocols (Henneke *et al*., 1983; Carroll & Huntington, 1988), the "cresty neck score" protocol (Carter *et al*., 2009) or ultrasonic measurement of rump fat thickness (Westervelt *et al*., 1976). Body composition scoring using the Henneke *et al*. (1983) protocol has been shown to give a good estimate of body fat content (Dugdale *et al*., 2012). However, body condition scoring has been found not to be useful for detecting early weight loss in obese ponies (Dugdale *et al*., 2010). On the other hand, in a study with Thoroughbreds, body condition score increased every month along with a monthly increase in body weight (9.9 kg/month on average) (Suagee *et al*., 2008).

In Standardbred race horses, body fat percentage, estimated by ultrasonic rump fat measurements, shows a significant positive correlation to running performance ($r=0.70$, $P<0.05$) and fat-free mass is negatively correlated with running performance ($r=-0.65$, $P<0.05$) (Kearns *et al*., 2002). In addition, Ringmark (2014) showed a negative correlation between both body condition score and subcutaneous fat thickness and velocity at lactate threshold (VLa4). Increased body weight has also been shown to increase exercise heart rate (Ellis *et al*., 2002).

## 2.3 Exercise response

The success or failure of a race horse in a race depends on factors such as start position, race course characteristics, competitors and 'will-to-win', which makes it difficult to standardise real races when evaluating different treatments. Using exercise tests on a treadmill makes it possible to control velocity and ambient temperature and provides more opportunities to monitor the horses during the exercise tests. However, use of a treadmill changes stride length (Couroucé *et al*., 1999) and needs to be done with an incline (Couroucé *et al*., 2002) or draught weight (Gottlieb-Vedi & Lindholm, 1997) to impose enough work-load (to simulate a race). It also gives different physiological data during exercise than field testing (Nostell *et al*., 2006). Field exercise tests can also be designed to simulate a race, but the success depends greatly on experienced drivers (to keep the horses trotting and maintain velocity) and track characteristics, and there are limited possibilities for sampling during the exercise test. In exercise, physiology parameters such as heart rate, lactate and velocity at heart rate 200 bpm ($V_{200}$) and at lactate threshold (4 mmol/l) ($V_{la4}$) are measurements used to estimate performance.

### 2.3.1 Heart rate

Peak heart rate during exercise testing gives information on whether the intensity of the exercise test is comparable to that in a competitive race. Maximal heart rate of Standardbreds during harness racing is reported to be 223 bpm (range 210-238 bpm) (Hodgson *et al*., 2014). Maximal heart rate is a repeatable measurement in the individual horse and is not affected by training (Evans & Rose,1988). It is therefore not a good measurement of changes in fitness, but heart rate at a given submaximal work load indicates how well the cardiovascular system is adapted to the exercise intensity (Couroucé *et al*., 2002). Heart rate during exercise has a linear relationship with velocity until the horse reaches maximal heart rate, where the velocity can still increase but heart rate will not (Hodgson *et al.,* 2014). Maximal heart rate has been shown to decrease with age (Betros *et al*., 2002; Stefánsdóttir *et al*. 2017).

During the first minute after exercise, the heart rate drops rapidly, while thereafter it shows a gradual drop. One previous study on Standardbreds found a correlation between race performance and recovery heart rate (Marsland, 1968). In young horses (2-3 years), heart rate at 10 minutes of recovery has been shown to reflect amount of training, with horses subjected to more high intensity training showing lower recovery heart rates (and higher haematocrit) (Ringmark *et al*., 2015). Delayed heart rate recovery could be a sign of illness or lameness (Hodgson *et al.,* 2014).

### 2.3.2 Lactate

Lactate is produced during almost all intensities of exercise when pyruvate is utilised anaerobically in the muscle cells. As exercise intensity increases, more fast twitch type IIA muscle fibres, and later also type IIX fibres, are recruited and the anaerobic energy supply becomes more important. During maximal exercise, anaerobic energy supply has been shown to contribute as much as 30% of total energy utilised in Thoroughbred horses (Eaton *et al.*, 1995). The proportion of type IIX fibres in the muscle is correlated to lactic acid production (Valberg, 1987). After exercise, most of the lactate is transported to the liver, where it is converted back to glucose by gluconeogenesis. The post-exercise decrease in blood lactate can be hastened by submaximal exercise compared with standing still or walking (Marlin *et al.*, 1987). Acidity in muscle during intense exercise is believed to be a contributor to muscle fatigue, since it can negatively affect glycolysis and the respiratory capacity in the mitochondria, causing decreased ATP concentration in muscle (Hodgson *et al.*, 2014). However, there is reported to be no direct relationship between muscle lactate concentration and race performance (Valberg, 1987; Roneus *et al.*, 1993).

### 2.3.3 Delayed-onset muscle soreness

Intense exercise can cause exercise-induced muscle damage that in humans is described as causing delayed-onset muscle soreness (DOMS), reduced range of motion in the affected limb and release of muscle enzymes (CK) (Peake *et al.*, 2017). These symptoms commonly occur 1-3 days post exercise in humans (Peake *et al.*, 2017), but little is known about DOMS in horses. One way to measure soreness in muscle is by pressure algometry, which quantifies muscle response by mechanical nociceptive threshold values instead of the more subjective palpation. Pressure algometry has been shown to be a useful tool for quantifying muscle response changes in horses with suspected sacroiliac dysfunction (Varcoe-Cocks *et al.*, 2006), but there is a lack of data on use of the method in athletic horses. Muscle enzyme (CK and aspartate amino transferase (AST)) levels have been shown to be increased in Standardbreds and Thoroughbreds after racing (Pösö *et al.*, 1983), indicating muscle damage and/or increased leakage. In humans, there has been a large number of studies on different treatments to increase muscle blood flow and thereby hasten muscle recovery (Peake *et al.*, 2017). The most effective means of alleviating pain during DOMS is exercise, although the analgesic effect is temporary (Cheung *et al.*, 2003).

# Aims of the thesis

The general aim of this thesis was to investigate how the response to exercise and recovery in trained Standardbred horses are affected by a forage-only diet, crude protein intake, road transport and housing system. In addition, one study investigated how response to feed deprivation was affected by a forage-only diet.

Specific objectives of the different studies were to:

- Examine the effect of a 12-hour feed deprivation in horses adapted to either a forage-only diet or a 50:50 forage-oats diet on body weight, fluid shifts and metabolic response (Paper I).
- Examine the effect of recommended and high crude protein intake from a forage-only diet on body weight, fluid and acid-base balance, nitrogen metabolism and exercise response (Paper II).
- Determine the effect of road transport and a forage-only diet or a 50:50 forage:oats diet during a subsequent race-like test on body weight, fluid shifts, exercise response and metabolic response (Paper III).
- Assess whether examine whether recovery of energy balance, body weight, swelling in the hind fetlock region and MNT after competition-like exercise (Paper IV)

Hypotheses:

- Paper I: Horses on a forage-only diet are heavier at the start of feed deprivation, but lose more weight and have a less variable total plasma protein concentration and metabolic plasma profile than horses fed a high oats diet.
- Paper II: A high crude protein intake enhances exercise-induced acidosis and alters fluid balance and the response to intensive exercise.
- Paper III: Transportation affects exercise response, but a forage-only diet alters transportation-induced metabolic changes and fluid balance less than a forage-oats diet.
- Paper IV: A free-range housing system does not delay recovery.

# 3 Materials and methods

The studies in this thesis were performed at Wången, the Swedish national centre for education in trotting. They were all approved by Umeå local ethics committee, and carried out in compliance with the laws and regulations governing experiments on live animals in Sweden.

## 3.1 Horses

A total of 28 trained Standardbred horses (19 geldings and nine mares) were included in the studies presented in this thesis, with four geldings were used in two of the studies (Papers I and II). The mean age of the horses during the studies was 9 (5-13) years, mean race earnings were 76,965 SEK (0-495,798 SEK) and mean number of races performed was 26 (0-70). Their mean body weight was 493 kg (388-576 kg).

## 3.2 Exercise tests

Exercise tests were performed and presented in Papers II-IV and all these tests were designed to be race-like in velocity and duration.

In Paper II, two different exercise tests were performed on each diet. The first was a standardised exercise test on a treadmill that all horses performed, one by one and the second was a field exercise test performed in pairs.

In Paper III, two exercise tests were performed on each diet. The horses were transported for 100 kilometres before and after the first exercise test, while they were kept in their box before and after the second exercise test. All five horses did the test together in one group.

In Paper IV, the horses were transported to an official race track (Östersunds travbana, Sweden, 1000-m oval, banked, gravel race track). The horses performed the exercise test in two groups, with four horses in each group

## 3.3 Diets

In Papers I and III, the effect of one forage-only and one high oats diet was studied. In Paper II, the effect of two crude protein intake levels, the recommended (NRC, 1989) and high intake (160% of recommended), from forage-only diets was studied. In Paper IV, the horses were fed forage *ad libitum* in two different housing systems. Properties of the forage used in all four studies are summarised in Table 2.

Table 2. *Average forage chemical composition during the studies reported in Papers I-IV. HP = high protein intake, RP = recommended protein intake*

| | Paper I | Paper II | | Paper III | Paper IV |
|---|---|---|---|---|---|
| | | HP | RP | | |
| Dry matter[a] | 63 | 45 | 50 | 76 | 78 |
| Metabolisable energy[b] | 9.8 | 11.7 | 11.7 | 9.5 | 11.2 |
| Crude protein[c] | 12.3 | 12.8 | 16.0 | 10.9 | 14.3 |
| Neutral detergent fibre[c] | 538 | 492 | 488 | 534 | 482 |

[a]%, [b]MJ/kg DM, [c]g/kg DM

## 3.4 Experimental design and sampling

The studies were performed during spring 2007 (Paper I), spring 2005 (Paper II), spring 2011 (Paper III) and spring 2015 (Paper IV). Average ambient temperature in the area in May is 7.2$^{o}$ C. All four studies were carried out in a cross-over design. In Papers I-III, all horses were randomly allocated to different diets, while in Paper IV all horses were randomly allocated to different housing systems.

### *Paper I (feed deprivation)*

In Paper I, the two experimental periods were 21 days each. Body weight and water intake was measured every day. On day 21, the horses were fasted for 12 hours, body weight was measured, and blood samples were collected every hour. Two horses were excluded from the results due to large feed residues (22 and 48 % of the total allowance).

### *Paper II (crude protein diets)*

In Paper II, the two experimental periods were 23 days each. On days 1 and 20-22, all urine and faeces were collected in collection harnesses. Body weight was measured every day before the 12:00 h feeding. Water intake was recorded every morning. Blood samples were collected from the jugular vein during the exercise tests.

*Paper III (transportation and forage-only vs forage-oats diets)*

In Paper III, each experimental period lasted 29 days. On days 21 and 26, the horses performed an exercise test. Before and after the exercise test on day 21, the horses were subjected to road transport for 100 km (approximately 1.5 h). Body weight and daily water intake was measured during days 14-29. The body condition score of all horses was evaluated according to Carroll and Huntington (1988). During the days of the exercise tests and 24-h post exercise, blood samples were collected from the jugular vein. Heart rate was recorded from rest until after the second transport.

*Paper IV (housing)*

In Paper IV, each treatment was performed for 21 days. The treatments consisted of box stall housing and a free-range housing system. The horses performed two exercise tests during each treatment, one at day 7 and at day 14. The horses were fed forage *ad libitum* and feed intake was measured. Body weight was measured every morning. Blood samples were taken, and heart rate was recorded during the exercise test and until 420 minutes of recovery. Before and three days after the exercise tests, the synovial swelling in the equine fetlock region was recorded with measuring tape and slide calliper and mechanical nociceptive threshold (MNT) in back muscles was measured with an algometer.

## 3.5 Chemical analyses

Full details of the methods used in chemical analyses are presented in Papers I-IV.

## 3.6 Blood and plasma analyses

In all studies, the blood samples were taken in heparinised tubes and kept on ice until centrifuged and then frozen (-20 °C). Analysis of total plasma protein was performed in the field using a handheld refractometer (Atago, Tokyo, Japan). In Paper II, blood samples were analysed for concentrations of sodium (Na), potassium (K), chloride (Cl), total carbon dioxide ($TCO_2$), pH, glucose and urea, using an i-STAT®1 analyser and cartridges (portable clinical analyser and i-STAT®1 cartridges CG8+ and EC8+).

Plasma lactate concentration was analysed in Papers II-IV using an enzymatic and spectrophotometric method (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). Plasma acetate (Papers I and III), plasma urea (Papers I and III) and plasma glucose (Papers I and III) concentrations were analysed with an enzymatic colorimetric/UV-method (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). In Paper IV, plasma urea concentration was analysed with a spectrophotometric method (Urea Assay Kit, Cell Biolabs Inc., San Diego, USA). Plasma cortisol (Paper III) concentration was analysed using ELISA (IBL International, Hamburg, Germany). Plasma insulin (Papers I and III) concentration was analysed using ELISA (Mercodia Equine Insulin Kit, Mercodia, Uppsala, Sweden). For quantitative determination of non-esterified fatty acids (NEFA) in plasma in Paper IV, an enzymatic colorimetric method was used (ACS-ACOD method, Wako Chemicals GmbH, Neuss, Germany).

## 3.7 Statistical analyses

In Papers I and II, the data were subjected to analysis of variance with the GLM procedure in SAS (version 9.1) (SAS Institute Inc. Cary, NC, USA). In Papers III and IV, the data were subjected to analysis of variance with the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). Additional statistical analyses in this thesis essay were performed with the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC), with a statistical model including fixed effects treatment, sample and the interaction between these. The model for an observed variable of horse *i* in treatment *j*, sample *k*, was:

$$Y_{ijk} = \mu + \eta_i + \pi_j + \gamma_k + (\pi\gamma)_{ik} + e_{ijk}$$

The model components are the overall mean $\mu$, the effect of horse $\eta$i, the effect of treatment $\pi$j, the effect of sample $\gamma$k, the effect of the interaction between treatment and sample ($\pi\gamma$)ik, and the random error eijk. The random part included horse, horse x treatment and period. Observations within each horse x period x treatment combination were modelled as repeated measurements. Values from Papers I and II are presented as mean ± standard error of the mean and values in Papers III, IV and this thesis essay are presented as least square means (LSM) with the pooled standard error of the mean (SEM). Differences were considered statistically significant at $P<0.05$. In Papers III, IV and the thesis essay, *post hoc* comparisons were adjusted for multiplicity using the Bonferroni method. Values from Papers I and II presented in this thesis essay were re-processed with the MIXED model above. This means that some of the mean values and significant differences differ from those published in Papers I and II.

# 4 Results

## 4.1 General effects of diet, transportation and housing

### 4.1.1 Body weight (Papers I, II, III and IV)

In Paper I, body weight was higher in horses on the forage-only diet (FONLY) than in those on the forage-oats diet (FOATS) (496 vs. 492 SEM 10; P<0.01). In Paper III, there were no differences between the diets in terms of body weight (FONLY: 502 kg, FOATS: 501 kg, SEM 26). In Paper IV, horse body weight was higher in the free-range (FR) housing system than the box (B) housing system (FR:499 vs. B:493 SEM 13 kg; P=0.030). Body weight was lower on recommended (RP) than high (HP) protein diet (RP: 483 kg, HP: 488 kg SEM 13, P<0.01).

*Body weight before exercise and during recovery (Papers II, III and IV)*

Body weight was higher in horses on the high crude protein diet than in those receiving the recommended crude protein level the day before the exercise in both exercise tests (Paper II). It remained higher during recovery after the standardised exercise test (SET), but not after the field exercise test (FT) (Table 3). Body weight was lower during recovery than before transport in the transport test (TT) but not the control test (CT) and after the exercise tests in Paper IV (Table 3).

Table 3. *Body weight day before exercise tests and after 24 h and 48 h of recovery.*

| Study | Treatment | Before | 24 hour recovery | 48 hour recovery | SEM |
|---|---|---|---|---|---|
| Study II FT | HP | 491* | 486 | | 13 |
| | RP | 483 | 485 | | 13 |
| Study II SET | HP | 486* | 488 | | 13 |
| | RP | 481 | 481 | | 13 |
| Study III TT | Forage-only | 506 | 497[a] | 497[a] | 28 |
| | Forage-oats | 506 | 499[a] | 500 | 28 |
| Study III CT | Forage-only | 500 | 495 | 499 | 28 |
| | Forage-oats | 497 | 497 | 499 | 28 |
| Study IV | Free-range housing | 510 | 504[a] | 504[a] | 3 |
| | Box housing | 505 | 498[a] | 498[a] | 3 |

* Significant difference (P<0.05) between diets within test. aMean differs significantly from before values in the same row (P<0.05).

### 4.1.2 Water intake (Papers I, II and III)

Daily water intake was greater in horses on the FONLY diet than in those on the FOATS diet (Paper I: 23.0 ± 0.4 L vs. 20.0 ± 0.3 L (P<0.01); Paper III: 27 L vs. 18 L, SEM 1 (P<0.01)). During feed deprivation, there was no difference in 12 h water intake between the diets and there was no effect of transportation on water intake. Crude protein level increased water intake (HP:20 L vs. RP:17 L, SEM 1 (P=0.02)).

*Fluid excretion*

There was a tendency (P<0.1) for higher fluid excretion (urine+feces) and calculated evaporative losses (total fluid intake-fluid excreation) on HP compared to RP (HP: 22.3 L, RP: 20.4 L SEM 1 and HP: 5.9 L, RP: 4.6 L, SEM 0.5).

## 4.2 Exercise and recovery

### 4.2.1 Heart rate, lactate and velocity during exercise tests

The velocities during the field exercise tests varied between 11.5 and 12.9 m/s. The velocity during the standardised exercise test was 10 m/s with a 5% incline (Table 4). All exercise tests increased heart rate to a mean peak level of over 200 bpm and raised plasma lactate concentration to near 20 mmol/L (Table 4).

Table 4. *Exercise tests applied in Papers I-IV. FT = field test, SET = standardised test. SE/SEM =standard error/standard error of the mean*

| | Peak heart rate (bpm) | SE/SEM | Finish line lactate (mmol/L) | SE/SEM | Velocity (m/s) |
|---|---|---|---|---|---|
| II FT | 219 | 5 | 21.6 | 2.8 | 11.8-12.0 |
| II SET | 215 | 6 | 18.0 | 2.8 | 10 (5 % incline) |
| III | 212 | 2 | 21.7 | 1.5 | 11.6-12.7 |
| IV | 223 | 2 | 20.9 | 1.4 | 11.5-12.9 |

During the exercise tests, there was no difference in peak heart rate or plasma lactate concentration between housing systems, transport treatments, diets (FONLY vs. FOATS) or crude protein intakes (Table 4).

### 4.2.2 Total plasma protein

Total plasma protein concentration in horses was higher at the finish line compared with rest during all exercise tests and was also higher at 10-15 min of recovery for all treatments except the standardised exercise test and the high crude protein diet. During the transport test in Paper III, total plasma protein concentration was lower with diet FONLY than diet FOATS at the finish line and after 10 min of recovery. In Paper IV, plasma total plasma protein concentration was lower in group-housed horses than in box-housed horses at 20-44 h of recovery after the first exercise test.

### 4.2.3 Metabolic response

Plasma acetate concentration was greater for horses on the FONLY diet than the FOATS diet in both Papers I and III (Paper I: 0.40 vs. 0.29 mmol/L, SEM 0.03 (P=0.01); Paper III: 0.40 vs. 0.20 mmol/L, SEM 0.02 (P<0.01)). Plasma acetate concentration decreased after 12 hours of feed deprivation in horses on FONLY, but remained unchanged in horses receiving FOATS. During the control test, but not the transport test, plasma acetate concentration remained higher in horses on FONLY than in horses on FOATS at the finish line and after 10 min of recovery (Paper III).

In Paper IV, plasma non-esterified fatty acid (NEFA) concentration was lower in horses in free-range housing than in horses in box stall housing at 20-44 hours of recovery. Plasma NEFA concentration was lower during free-range housing than during in box housing during recovery and 24 and 48 h recovery values in free-range house treatment were lower than pre-test values. In Paper III, plasma

NEFA concentration was higher at 24 h recovery compared with before in horses on the FONLY diet during the transport test.

Plasma insulin concentration (Paper III) was lower in horses on the FONLY diet than in horses on the FOATS diet (0.06 vs. 0.10 µg/L, SEM 0.01 (P=0.02)). In Paper III, plasma insulin concentration was lower in horses on the FONLY diet than in those on FOATS during rest in both the transport test and the control test, and after 10 min of recovery in only the transport test.

Blood urea concentration was higher in horses on the high protein diet than in those on the recommended protein diet both at rest and during exercise (Paper II). When housed in box stalls (Paper IV), plasma urea concentration in horses was elevated at the finish line and 10 min recovery compared with rest.

In horses on the FOATS diet (Paper IV), plasma glucose concentration was higher in the transport test than the control test at both the finish line and after 10 min of recovery. In horses on the FONLY diet, there was also a tendency for elevated plasma glucose during the transport test at 10 min of recovery.

### 4.2.4 Acid base balance (Paper II)

For both crude protein intakes, blood pH was lower at the finish line and after 15 min of recovery compared with at rest for both diets in the field test, but only for the finish line in the standardised exercise test.

### 4.2.5 Cortisol (Paper III)

Transportation increased overall plasma cortisol concentration, which was higher in the transport test than in the control test (181 vs. 144 mmol/L, SEM 15 (P=0.020)). During the transport test, plasma cortisol concentration was higher in horses on the FOATS diet than in those on the FONLY diet after the second transportation (227 vs 141 mmol/L, SEM 24 (P=0.017)). Cortisol levels were elevated at the finish line compared with rest values for both diets and tests.

### 4.2.6 Swelling in the hind fetlock (Paper IV)

Overall circumference and diameter of hind fetlock region were lower in G horses than B horses (circumference 26.3 and 26.7 cm, respectively, SEM 0.4 (P=0.045), diameter 4.9 and 5.1 cm, respectively, SEM 0.9 (P=0.017)).

Mechanical nociceptive threshold (MNT) was not affected by the race (before compared with the days after race).

## 4.3 Feed deprivation (Paper I)

Total weight loss during feed deprivation was greater in horses on the FONLY diet than in horses on the FOATS diet after 12 h of feed deprivation (10.9 vs. 8.2 kg, SEM 0.8 (P=0.01).

Total plasma protein concentration was lower in horses on FONLY than in horses on FOATS at 9 h of feed deprivation (Table 5). Plasma glucose concentration was not affected either by diet or feed deprivation (Table 5). Plasma insulin concentration was lower in horses on FONLY than in those on FOATS at 0 h of feed deprivation (Table 5). Plasma NEFA concentration was higher with the FONLY diet than with FOATS at 6 and 12 h of feed deprivation. The NEFA concentration was elevated relative to the 0 h concentration at both 6 and 12 h feed deprivation in horses on the FONLY diet but only after 12 h feed deprivation in horses on the FOATS diet (Table 5). Plasma urea concentration was elevated at 12 h of feed deprivation in horses on both diets (Table 5). Plasma acetate concentrations was higher in horses on FONLY than FOATS at 0 h of feed deprivation. Plasma acetate concentration was lower at 12 h feed deprivation than at 0 h feed deprivation in horses on diet FONLY (Table 5).

Table 5. *Concentrations of total plasma protein (TPP), plasma glucose, insulin, non-esterified fatty acids (NEFA), urea and acetate in horses on the forage-only diet (FONLY) and 50:50 forage-oats diet (FOATS) at 0 h (finished feed intake), 6 h and 12 h of feed deprivation. SEM = standard error of the mean*

| | | FONLY | FOATS | SEM | *P*-value |
|---|---|---|---|---|---|
| TPP (g/L) | | | | | |
| | 0 h | 63.8 | 63.7 | 1.0 | 1.00 |
| | 6 h | 59.4[a] | 60.9[a] | 1.0 | 0.70 |
| | 9 h | 58.9[a] | 62.5 | 1.0 | 0.03 |
| | 12 h | 61.4[a] | 63.7 | 1.0 | 0.17 |
| Glucose (mmol/L) | | | | | |
| | 0 h | 4.8 | 4.7 | 0.1 | 1.00 |
| | 6 h | 4.8 | 4.7 | 0.1 | 1.00 |
| | 12 h | 4.8 | 4.8 | 0.1 | 1.00 |
| Insulin (µg/L) | | | | | |
| | 0 h | 0.07 | 0.13 | 0.01 | <0.01 |
| | 6 h | 0.04 | 0.06[a] | 0.01 | 0.27 |
| | 12 h | 0.04 | 0.06[a] | 0.01 | 0.61 |
| NEFA (mmol/L) | | | | | |
| | 0 h | 0.09 | 0.04 | 0.02 | 0.18 |
| | 6 h | 0.17[a] | 0.06 | 0.02 | <0.01 |
| | 12 h | 0.27[a] | 0.19[a] | 0.02 | <0.01 |
| Urea (mmol/L) | | | | | |
| | 0 h | 5.8 | 6.1 | 0.5 | 1.00 |
| | 6 h | 6.1 | 6.5 | 0.5 | 1.00 |
| | 12 h | 6.9[a] | 7.1[a] | 0.5 | 1.00 |
| Acetate (mmol/L) | | | | | |
| | 0 h | 0.47 | 0.24 | 0.04 | <0.01 |
| | 6 h | 0.42 | 0.30 | 0.04 | 0.08 |
| | 12 h | 0.30[a] | 0.22 | 0.04 | 0.41 |

[a]Mean differs significantly from before values in the same column (P<0.05).

# 5 Discussion

The results presented in this thesis show that Standardbred trotters can train and recover on a feeding strategy without the conventional feeding of concentrates. The results also show that some horse management practices like such as transportation affect metabolic parameters as much or more than diet and that a free-range housing system can be positive for recovery.

The aim of the research presented in this thesis was to test some of the speculations concerning how trained Standardbred trotters should be fed and managed. This was done by performing exercise tests simulating a 2140 m harness race in terms of duration and velocity. All the horses included in the research were trained to the same extent as other Standardbred trotters in Sweden. The mean peak heart rate in the studies was 212-223 bpm, which is within the range reported for horses in competitive harness race rates (210-238 bpm) (Hodgson *et al.*, 2014). The duration of the exercise test 'races' in the studies was approximately 3 min and the velocity was 11.5-12.9 m/s. The latter is equivalent to 1.18-1.27 min/km, which is slower than the velocity in competitive harness races in Sweden. The main reason for this was track conditions, as the race track at Wången is estimated by experienced drivers to be 0.3 m/s slower than a competition race track and the race track at Östersund (Paper IV) was muddy and slow on one occasion due to heavy rainfall. Plasma lactate concentration measured directly after the finish line was 18.0-21.7 mmol/L, which is similar to the range reported in horses after competitive harness races (15.0-42.7 mmol/L) (Ronéus *et al.*, 1999).

The hind fetlock region was consistently more swollen in the traditional box housing during the days following racing, indicating more swelling of the joint region, although the numerical differences were small (Paper IV). The importance of this remains to be evaluated. A common practice in racing stables with box-housed horses is to put bandages and liniment on the distal parts of the legs (from carpus/hook to pastern) to prevent or reduce swelling. There was a lack of post-exercise response in MNT in the back that indicated that the ET in

Paper IV did not cause any measurable DOMS in the area evaluated. The lack of post-exercise response in the horses was somewhat surprising, but indicates that they were well prepared for the task.

## 5.1 Response to exercise

Plasma lactate concentration during exercise and recovery was not affected by forage inclusion level, forage crude protein content, transport or housing system. That contradicts findings by Jansson & Lindberg (2012) of higher plasma lactate concentrations at 5 and 15 minutes of recovery in horses fed a forage-oats diet compared with a forage-only diet. They also found that that the velocity at lactate threshold ($V_{la4}$) tended (P=0.086) to be higher in horses on a forage-only diet compared with a conventional oats-forage diet (8.0±0.1 vs. 7.6±0.1 m/s). Palmgren-Karlsson *et al*. (2002) also found higher peak lactate values (during a simulated harness race on a treadmill) in horses fed a forage-oats diet than in horses fed a forage-beet pulp diet.

Excess crude protein intake did not affect heart rate and lactate during exercise, which is in accordance with findings in other studies (Miller & Lawrence, 1988; Miller-Graber *et al*., 1991). In contrast, Pagan *et al*. (1987) recorded lower heart rate and lower lactate during a long slow exercise with horses on high protein diets. The lack of effect on blood pH following exercise is in contrast to observations made by Graham-Thiers *et al*. (2001). The effect of transportation and housing system on exercise heart rate and lactate concentrations has not been studied previously, to the best of my knowledge, so comparisons are not possible.

The lack of effect of diet on exercise response in the studies included in this thesis could possibly be attributable to the design of the exercise tests. Three of four exercise tests reported in Papers I-IV were performed in the field and all tests were designed to simulate real harness races. Small differences such as those which can be expected in these tests probably need a more standardised exercise test (in terms of velocity, track conditions and sampling time) and possibly also monitoring of $V_{200}$, $V_{la4}$ or oxygen consumption.

## 5.2 Metabolic response

In Paper IV, there was a distinct increase in plasma NEFA concentrations at 10 min of recovery for both housing systems, whereas in Paper III only one of the treatments (control test, FOATS diet) showed a small increase in plasma NEFA value. Increased NEFA concentration in response to exercise has been reported in earlier studies (Pösö *et al*., 1983; Jansson & Lindberg, 2012). One explanation for the difference in NEFA response between Papers III and IV might be that the exercise test in Paper IV was conducted on an official race track, which might have made the horses more excited and thereby increased cortisol and lipolysis of adipose tissue.

High forage crude protein intake increased blood urea concentration, confirming findings by Fonnesbeck & Symons (1969). Plasma acetate concentration was higher in horses on the FONLY diet than in horses on the FOATS diet when the horses were not transported (control test). However, when transported (transport test), plasma acetate concentration in horses dropped markedly after exercise. One explanation for this decrease in plasma acetate could be increased utilisation of acetate during exercise in transported horses, stimulated by the high availability of acetate together with increased fatty acid utilisation initiated by the long period of elevated NEFA before exercise (transport time 1.5-2 h). Increased utilisation of acetate in horses on a forage-only diet during exercise has been suggested previously by Jansson & Lindberg (2012).

Increased performance has been associated with high plasma glucose concentration during exercise in previous studies (Lacombe *et al.*, 2001). Increased fatty acid (NEFA and acetate) utilisation during exercise could explain why transport increased the plasma glucose concentration at finish line and 10 min recovery compared with no transport, especially with diet FOATS. Interestingly, there was no difference in exercise glucose concentration related to diet (FONLY vs FOATS or crude protein intake).

Non-esterified fatty acid concentration was elevated for the FONLY diet at 24 h after the exercise test when the horses were box stalled and transported (Paper III). This is in accordance with observations by Jansson & Lindberg (2012) on box stalled and transported FONLY fed horses but in contrast with the decreased NEFA at 24 h and 48 h post-exercise when the horses were transported but group-housed (Paper IV). This was probably due to the higher voluntary feed intake in group-housed horses. This is interesting, since trainers often argue that racing horses must eat concentrate-rich diets to perform and to maintain energy balance.

## 5.3 Fluid shifts

Total plasma protein concentration was elevated by exercise during all treatments and in all exercise tests, and remained elevated for all samples except those from horses on the high protein diet at 15 min recovery during the standardised exercise test. This is interesting, since the high protein intake probably increased heat production and challenged fluid balance by increasing water intake and increasing fluid loss in faeces and urine (Paper II).

When the horses were transported, total plasma protein concentration was lower with diet FONLY than FOATS at the finish line and after 10 minutes of recovery. A similar diet effect was shown in Paper I during feed deprivation and could be explained by a larger gastrointestinal fluid reservoir available (Meyer, 1995) on the FONLY diet. Interestingly, however, this diet effect only occurred when horses were subjected to the extra effort of transportation.

Lower body weight after exercise only occurred when the horses were transported to the exercise test (transport test treatment in Paper III and both treatments in Paper IV). The ambient temperature was in the range 1.3-9.8°C during the exercise tests but temperature inside the horse trailers was not measured. It has been suggested that 90% of post-exercise weight loss is due to sweat losses (Carlson, 1987), and, since diet effects on total plasma protein concentration only occurred when the horses were transported, a connection between body weight loss and fluid balance seems likely. The FONLY diet increased water intake during recovery compared with the FOATS diet, as also found in Paper I and reported by Cymbaluk (1989), Danielsen *et al*. (1995), Ellis *et al*. (2002), Fonnesbeck (1968) and Jansson and Lindberg (2012). As an extra challenge to the fluid balance, the water intake during the exercise test day (8 h) in Paper III was 1-5 L and with the 12 h feed deprivation in Paper I it was 3 L.

In conclusion, this thesis shows that forage-only diets and a free-range housing system are a good alternative to the conventional management regime for racing Standardbred horses and can form part of a sustainable horse management system that supports performance, health and animal welfare. The positive effects of transportation need to be confirmed in further studies examining the timing and duration of transport before transport can be used as an efficient part of pre-race preparations. However, the new knowledge this thesis provides on how much transportation affects the metabolic response in horses makes it important to include transportation as part of the experimental design.

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# Popular science summary

The Standardbred trotter is the second largest horse breed in Sweden, where it is mainly used in harness racing. Racing Standardbred horses are athletes that train and race at a high level of physical activity during most of their careers and, apart from the obvious goal of winning races, the aim is to have a long career. The performance and longevity of these horses is affected by many different factors, such as genetic capacity, confirmation, training, injuries, nutrition and management. This thesis examined management and nutrition factors and their effects on exercise and recovery.

Standardbred trotters in full training require twice as much energy as horses of similar size that are not training. To meet the high energy requirements of athletic horses, the feed offered must be energy-rich and therefore conventional feeds for racing trotters contain large amounts of energy-rich, starch-rich concentrates. The problem with feeding horses large amounts of starch-rich concentrates is that they increase the risk of health disturbances (e.g. colic, gastric ulcers and tying-up). To avoid such problems, it may be possible to feed a forage-only diet. Among trainers there is a concern that feeding large amounts of forage would decrease the horse's performance due to increased bodyweight. However, for an athletic horse the forage needs to be sufficiently energy-rich to meet the high energy requirements and a forage that contains enough energy may also contain high protein levels. The latter may negatively affect fluid losses, heat production and maybe performance in the horse.

Going to a competition may involve long transport distances for horses. Access to feed and water is limited during transportation, but limiting water and feed intake can also be part of the trainers' regime prior to competition. Knowledge is lacking on how the horse responds to this period of feed deprivation when fed different diets (forage-only or forage-oats). Moreover, the effect of transportation on exercise response and whether this is affected by diet are not well studied.

Most horses in Sweden and other countries are currently housed in individual box stalls, which decreases their opportunity for movement and social interaction with other horses. Horse owners becoming more aware of this and horse housing systems that provide the animals with the possibility for free movement in groups are therefore becoming more popular. However, few competition horses are kept in group housing systems, perhaps because owners and trainers are unsure about this form of housing system could affect house performance and recovery.

Some of these questions were studied in this thesis.

Feeding a forage-only diet compared with a 50:50 forage-oats diet had little or no effect on body weight. This shows that increased body weight, and thereby decreased performance, is not a problem in race horses fed a high-energy forage. During 12-hour of feed deprivation, the horses were able to maintain blood plasma volume better when they were fed a forage-only diet than when they were fed a forage-oats diet. The 12-hour feed deprivation resulted in a 11 kg weight loss when horses were fed the forage-only diet and an 8 kg weight loss when they were fed the forage-oats diet.

Feeding a high crude protein amount had no effect on response to exercise in the horses even though the protein intake was high enough to affect blood parameters. However, high crude protein increased water excretion through faeces, urine and evaporation and thereby exerts an unnecessary load on a racing horse, especially during warm conditions.

Horses transported 100 km used fat as an energy substrate to a greater extent during a subsequent simulated race than horses which were not transported. This resulted in higher glucose in the blood at the finish line in transported horses, which is a positive effect since higher blood glucose concentrations have been associated with better performance. In addition to the transportation, the horses were fed two different diets (forage-only and forage-oats). The forage-only diet improved how the horses maintained their blood plasma volume during transportation and racing and also further increased the usage of fat (acetate) as energy substrate. The forage-oats diet increased the cortisol response to transportation compared with the forage-only diet, indicating higher stress levels since cortisol is a hormone connected to stress.

Keeping horses in a free-range group housing system compared with conventional box stalls seemed to have some positive effect on recovery. During recovery, the need to use adipose tissue for energy was lower in the free-range system, indicating that energy intake was sufficient. This was confirmed by higher voluntary feed intake in the free-range system, a finding that is especially interesting for racehorses since they can have periods with a lack of appetite.

The free-range system also resulted in less swelling in the hind fetlock area of the horses. The importance of this remains to be evaluated.

In conclusion, this thesis shows that forage-only diets and a free-range housing system are a good alternative to the conventional management regime for racing Standardbred horses and can form part of a sustainable horse management system that supports performance, health and animal welfare. The positive effects of transportation need to be confirmed in further studies examining the timing and duration of transport before transport can be used as an efficient part of pre-race preparations. However, the new knowledge this thesis provides on how much transportation affects the metabolic response in horses makes it important to include transportation as part of the experimental design.

# Populärvetenskaplig sammanfattning

Travhästar är atleter som tränar och tävlar på en hög nivå av fysisk aktivitet under sin karriär. Ett mål är, förutom att vinna lopp, att hästen ska vara frisk och få en lång karriär. En hästs prestationer och hållbarhet påverkas av många olika faktorer som genetisk kapacitet, exteriör, träning, tid till återhämtning, skador, utfodring och hästhållning. Denna avhandling fokuserar på vilka effekter utfodring, hästhållning och rutiner i samband med tävling har på kroppsvikt, blodplasmavolym, metabol- och arbetsrespons hos travhästar i full träning.

Travhästar i full träning har ett energibehov som är dubbelt eller mer än dubbelt så stort som en häst av samma storlek som inte tränas. För att fylla den tränande travhästens höga energibehov måste fodret vara energirikt. Historiskt har detta behov av energirikt foder tillgodosetts med stora mängder kraftfoder. Att utfodra stora mängder kraftfoder (stärkelserik diet) ökar risken för kolik, magsår och korsförlamning. Ett alternativ är att utfodra med en grovfoderdiet, men då krävs ett grovfoder som är energirik nog för att fylla de höga energibehoven. Travtränare kan vara skeptiska till att fodra hästar med mycket grovfoder då det misstänkts öka hästens kroppsvikt och sänka prestationsförmågan. Ett grovfoder som innehåller tillräckligt med energi för att uppfylla energibehovet hos högpresterande hästar kan ibland också ha ett högt proteininnehåll vilket skulle kunna öka hästens vätskeförluster, värmeproduktion och kanske påverka prestationen negativt.

Tävlingsmomentet kan innebära att hästarna måste transporteras långa sträckor. Oftast är hästarnas tillgång på foder och vatten under transporten begränsad, men en begränsning av vatten- och foderintag kan också vara en del av tränarens skötselstrategi. Det har hittills inte varit känt hur hästar påverkas av ett längre utfodringsuppehåll vid olika typer av dieter, som den traditionellt kraftfoderrika eller en med bara grovfoder. Det har heller inte undersökts hur transport påverkar travhästar i samband med travlopp och om diet kan påverka den eventuella effekten av transport.

De flesta hästar i Sverige är uppstallade i individuella boxar i traditionella stall. Individuell boxhållning minskar möjligheten till rörelse och social interaktion med andra hästar. Det finns en ökad medvetenhet om detta och hästhållningssystem där hästarna kan gå fritt har blivit mer populära. Det är få tävlingshästar som hålls i sådana system och en av orsakerna kan vara att ägare och tränare är osäkra på hur det skulle påverka hästarnas återhämtning efter fysisk aktivitet. Detta har inte undersökts före denna avhandling.

Resultaten i denna avhandling visar att travhästar i full träning kan prestera med en utfodringsstrategi utan traditionell utfodring med kraftfoder. Den visar också att transport påverkar vissa metabola parametrar lika mycket eller mer än diet och att uppstallningssystem.

En grovfoderdiet jämfört med en traditionell grovfoder-havre diet hade liten (studie I) eller ingen (studie III) effekt på kroppsvikten. Det visar att tävlingshästar som utfodras med ett energirikt grovfoder inte ökar kroppsvikten och detta är därför inte en faktor som skulle kunna försämra prestation. När hästarna fastades i 12 timmar resulterade det dessutom i en viktminskning på 11 kg när hästar hade ätit grovfoderdieten och 8 kg när hästarna hade ätit grovfoder-havre dieten. Hästarna kunde också bibehålla blodplasmavolymen bättre när de utfodrades med en grovfoderdiet än när de åt en grovfoder-havre diet.

Överutfodring med protein gav ingen effekt på hjärtfrekvens och mjölksyra under arbetstest, även om proteinintaget var tillräckligt högt för att kunna observeras i blodet (ökning av plasmaurea). Ett högt proteinintag ökade vätskeförlusterna i träck, urin och värmeavgivning vilket borde vara en onödig belastning för en högpresterande häst, särskilt under varma förhållanden.

En 10 mil lång transport gjorde att hästarna använde fett som energisubstrat i större utsträckning under ett travlopp än när de inte hade transporterats. Den ökade fettanvändningen att resulterade i högre blodglukos vid mållinjen och högre blodglukos i är förknippat med en förbättrad prestation. Förutom att transporteras så utfodrades hästarna med två olika dieter, en grovfoderdiet och en grovfoder-havrediet. Grovfoderdieten förbättrade hästarnas förmåga att bibehålla sin blodplasmavolym under transport och lopp samt ökade användningen av fett (acetat) som energisubstrat. Grovfoder-havredieten ökade kortisolnivån (stresshormon) vid transport jämfört med grovfoderdieten.

Att hålla hästar i ett system där de kan röra sig fritt i grupp (aktiv grupphästhållning) jämfört med i ett traditionellt boxstall hade liten effekt på återhämtningen men de skillnader som fanns indikerar att denna typ av hästhållning kan vara positiv för återhämtning. Under återhämtningen efter lopp var hästarnas behov att använda fettvävnad för energi litet i den aktiva hästhållningen vilket tyder på att intaget av energi genom foder var tillräckligt. Det bekräftades också av ett större foderintag i den aktiva hästhållningen. Detta är intressant eftersom hästar i hård träning kan ha perioder med låg aptit. Den aktiva hästhållningen resulterade också i att hästarnas bakkotor var mindre svullna men betydelsen av detta är oklar.

Sammanfattningsvis visar studierna i den här avhandlingen att en grovfoderdiet och en aktiv grupphästhållning är ett bra alternativ för tränande travhästar. De positiva effekterna av transporten skulle behöva studeras ytterligare för att utvärdera tid i förhållande till lopp och hur lång transporten behöver vara för att få en effekt, innan vi vet hur det skulle kunna användas som en effektiv del av förberedelserna före tävling. Med den här kunskapen om hur mycket transport påverkar det metabolaa svaret hos hästar bör framtida studier ta med transport som en del av försöksupplägget för att vara riktigt relevant för tävlingshästar.

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