

Fluid Balance and Metabolic Response in Athletic Horses Fed Forage Diets

by

Malin Connysson

LICENTIATE THESIS

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences Department of Animal Nutrition and Management Rapport 272 Report

Uppsala 2009 ISSN 0347-9838 ISBN 978-91-86197-16-2

Fluid Balance and Metabolic Response in Athletic Horses Fed Forage Diets

Abstract

Concentrate diets are related to health problems in athletic horses and high-energy forage diets could be an alternative. Forage feeding may alter substrate and hindgut fluid availability which could be beneficial during feed deprivation and dehydration. However, it may also increase bodyweight (BW) and crude protein (CP) intake which may reduce performance. The objective of this thesis was to study trained Standardbred horses on a high oats diet (OD) and a high energy forage-only diet (FD) before and during 12 hour feed deprivation (Study I), and on two CP intakes during exercise and rest (Study II).

In study I ten horses participated. In study II recommended and high CP intakes (diet RP and HP) from forage-only diets were studied in six geldings. BW and TPP were measured in both studies and plasma NEFA, acetate, glucose and insulin concentrations in study I, nitrogen and fluid balance and response to standardised race like exercise in study II.

BW was higher on diet FD than on diet OD (3 kg) but was not affected by CP intake. The weight loss was larger during feed deprivation on diet FD than on diet OD. TPP was lower in diet FD before feed deprivation and the pre-feed deprivation level was maintained for 7 and 11 h on diet OD and FD, respectively. The plasma acetate and NEFA was higher, insulin was lower and urea and glucose unaltered on diet FD compared to diet OD before feed deprivation. During feed deprivation plasma NEFA and urea increased in both diets whereas the acetate decreased on diet FD and plasma insulin on diet OD. Plasma glucose was not affected by feed deprivation. Diet HP caused an increased N excretion, water intake and urine output and a lowered urine pH. CP intake did not affect heart rate, plasma lactate and blood pH during exercise.

In conclusion, feeding horses diet FD resulted in a small increase in BW that diminished after feed deprivation. The plasma volume was larger and maintained longer on diet FD compared to diet OD which suggests that the horses were more resistant to dehydration. The effect of the altered metabolic response during feed deprivation to exercise remains to be investigated but a high CP intake did not adversely affect the response to exercise.

Keywords: Crude protein, Exercise, Feed deprivation, Forage, Fluid balance

Author's address: Malin Connysson, Dept. of Animal Nutrition and Management, SLU, P.O. Box 7024, 750 07 Uppsala, Sweden *E-mail:* malin.connysson@wangen.se

Contents

List of Publications	5
Abbreviations	7
Introduction	9
Influence of diet on health	9
Effects of a forage-only diet on fluid balance	10
Water intake	10
Hindgut and fluid availability	10
Body weight	11
Protein metabolism	11
Exercise and protein metabolism	12
Aim of the Thesis	13
Materials and methods	14
Horses	14
Feeding and sampling	14
Exercise tests (Study II)	15
Statistical analyses	16
Results	17
Body weight, water intake and fluid balance	17
TPP	18
Faecal DM and urine pH	18
Insulin, glucose, NEFA and urea during feed deprivation (Study I)	18
Heart rates, lactate, blood pH and urea during exercise (Study II)	19
General Discussion	20
Body weight, water intake and fluid balace	20
Insulin, acetat, NEFA and urea during feed deprivation (Study I)	21
Heart rates, lactate, blood pH and urea during exercise (Study II)	22
Conclusions	23
Populärvetenskaplig sammanfattning	24

Bakgrund	24
Studie I	25
Studie II	25
Sammanfattning	26
References	27
Acknowledgements	30

List of Publications

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

- I Connysson, M., Lindberg, J E., Essén-Gustavsson, B. and Jansson, A. (2009). Effects of feed deprivation on athletic horses fed a forage-only diet and a 50:50 forage-oats diet.
- II Connysson, M., Muhonen, S., Lindberg, J E., Essén-Gustavsson, B., Nyman, G., Nostell, K. and Jansson, A. (2006). Effects on exercise response, fluid and acid-base balance of protein intake from forageonly diets in Standardbred horses. *Equine Veterinary Journal Supplement* 36, 648-653

Study II are reproduced with the permission of Equine Veterinary Journal.

Abbreviations

ADF	Acid detergent fiber		
	e		
BW	Bodyweight		
СК	Creatine kinase		
СР	Crude protein		
DCP	Digestible crude protein		
DM	Dry matter		
FD	Forage diet		
HP	High protein		
Ν	Nitrogen		
ME	Metabolisable energy		
MJ	Megajoule		
NDF	Neutral detergent fiber		
OD	50:50 oats-forage diet		
RP	Recommended protein		
TPP	Total plasma protein		

Introduction

Horses are grass eaters adapted to a high fibre, low starch diet. Athletic horses require a diet with an energy density of around 11 MJ ME/kg DM (NRC, 2007) and generally, intake of forage alone cannot meet this energy requirement. Therefore, concentrates are included in the diet in order to increase the energy density of the horses feedstuffs.

Influence of diet on health

There are a number of disorders that have been related to feeding horses with concentrate and starch-rich diets or small amounts of forage. Feeding different hay-barley ratios to fistulated ponies decreased cellolytic bacteria as barley ratio increased and this may negatively affect fibre digestion and disturb the microbial ecosystem of the horse (Julliand et al., 2001). In a study by Tinker et al. (1997) the risk for colic increased 5-fold for horses fed 2.5-5 kg concentrate/day and 6-fold for horses fed more than 5 kg concentrate/day compared to horses fed only forage.

High concentrate diets have also been associated with rhabdomyolysis (Mc Leay et al., 1999). In a study on 984 Thorougbred horses it was shown that a concentrate allowance larger than 4.5 kg/day increased the risk for rhabdomyolysis (McLeay et al., 1999). In addition, feeding 2.5 kg compared to 4.6 kg concentrates has been shown to result in lower plasma CK activity at rest (MacLeay et al., 2000). CK is a useful marker for muscle damage and it was concluded that high grain ratios increase the risk for muscle problems in horses.

It has also been shown that horses spend more time chewing wood and on copography when fed only concentrates compared to when they were fed hay (Willard et al., 1977) and in Standardbred and Thoroughbred race horses the risk for stereotypies increased as the amount of concentrates fed

increased (Redbo, 1998). This is in accordance with McGreevy et al., (1995), which showed that feeding less than 6.8 kg forage per day to racehorses increased the risk for stereotypies. Also when studying a small group of cribbing horses there was an increase in cribbing frequency when feeding concentrates compared to when feeding pelleted alfalfa (Gillham et al., 1994). It has also been shown that the frequency of cribbing was lower after feeding a forage meal than after a concentrate meal (Kusunose, 1992).

Based on the known health problems related to high concentrate, high starch diets it is of interest to investigate alternative feeding strategies for athletic horses. High energy forage could be an alternative but information on the effects of feeding high energy forage to athletic horses is scarce.

Effects of a forage-only diet on fluid balance

Water intake

It has been shown that water intake is lower for horses with a limited forage intake compared to when fed more forage (Danielsen et al., 1995; Ellis et al., 2002). An increase in water intake with high fibre diets could partly be due to an increased heat increment of feeding leading to increased evaporative losses. Chewing, swallowing and salivary secretion all effect the heat production during feed utilisation (Blaxter, 1989) and chewing movements per kg feed have been estimated to be higher in horses fed hay compared to oats (3400 vs. 850, Meyer, 1983a). In ruminants, forage diets also increase microbial fermentation which may double heat production compared to digestion of grain (Blaxter, 1989). In addition to these indirect fluid losses, high fibre diets seem to induce an increase in faecal water loss (Fonnesbeck, 1968).

Hindgut and fluid availability

Shorter periods of feed deprivation and lack of water intake are common in athletic horses while at a competition. However, the gut has been suggested to serve as a fluid reservoir during dehydration in horses (Meyer, 1987). The fluid content of the hindgut is affected by diet, there is more fluid in the hindgut of horses fed forage diets than horses fed mixed diets (Meyer, 1995). After exercise TPP levels were lower in horses fed a high hay diet than in horses fed a low hay diet (Danielsen et al., 1995). This indicates a water movement from the gastro-intestinal tract into the plasma volume and an improved ability to maintain plasma volume during exercise.

Body weight

Greater intestinal content increases BW. Horses fed a hay diet had higher total hindgut fill than horses fed a mixed diet and this was related to increased water content in the hindgut (Meyer, 1995). In a study on exercising riding horses fed a diet containing only forage, the horses had a higher BW compared to when horses were fed a diet that consisted of both forage and concentrate (Ellis et al., 2002). The forage-only diet increased heart rate during exercise and recovery compared to when horses where fed a 50:50 (on gross energy basis) forage: concentrate diet (Ellis et al., 2002). However, a study by Jansson & Lindberg (2008) on Standardbred horses in training indicates that a forage-only diet, using early cut high energy forage, will have a limited effect on BW and will not elevate heart rate following exercise compared to a more traditional high concentrate diet. In addition, observations on four Standardbred geldings also suggest that horses fed forage *ad libitum* rapidly lose BW (mean 12 kg) during (8 hour) feed deprivation (Muhonen & Connysson, unpublished data).

Protein metabolism

High energy forage must be cut at an early stage of maturity. Early cut forage can be high in CP as well as in energy, which may result in an excessive CP intake for mature horses. Except for protein synthesised and retained in tissues, the body has a limited capacity to store proteins (Beitz, 2004). Therefore excess protein is degraded to amino acids and then further metabolised and used for energy. The first degradation is called deamination and means that the amino group is removed from the amino acid and converted into ammonia, and the resulting carbon skeleton is either used for the synthesis of other intermediates or metabolised to carbon dioxide and water. Released ammonia is removed from the blood by forming urea:

 $NH_3 + H_2CO \Leftrightarrow NH_4^+ + HCO_3^ 2NH_4^+ + CO_2 \rightarrow 2H^+ + urea + H_2O$

During the formation of urea two hydrogen ions are formed of which some are neutralised by the bicarbonate ions of the first reaction. Some of the ammonia is also carried by glutamine and released and excreted by the kidneys. In horses, increased protein intake results in increased plasma urea (Fonnesbeck & Symons, 1969) and nitrogen excretion in faeces and urea (Freeman et al., 1988; Prior et al., 1974; Slade et al., 1970) The synthesis of

urea is an energy consuming process and thereby a higher protein intake increases heat production (Meyer, 1983b).

Exercise and protein metabolism

In a study on Thoroughbred horses, dry matter intake and crude protein intake were positively correlated with time to finish when the horses were racing (Glade, 1983). For every 1000 g of CP fed in excess of NRC (1978) recommendations times to finish increased by 1 to 3 seconds (Glade, 1983). During exercise, venous pH has also been shown to be lower in horses fed a high protein diet (14.5% CP) compared to a low protein diet (7.5% CP) (Graham-Thiers et al., 2001). This could adversely affect high intensity performance, where anaerobic energy metabolism is important and contribute to fatigue. In contrast to the results by Graham-Thiers et al. (2001), lower heart rates and lower lactate accumulation during exercise with high protein diets has been reported (Pagan et al., 1987). Also, horses fed nearly 1 g digestible crude protein (DCP)/kg BW/day according to NRC (1978) recommendations had a lower recovery pulse than horses fed less protein (Patterson et al., 1985). Other studies found no effects, determinable for exercise performance, of excess protein intakes (Frank et al., 1987; Hintz et al., 1980; Miller & Lawrence, 1988; Miller-Graber et al., 1991). Meyer (1987) suggests that an intake of more than 2 g digestible crude protein (DCP)/kg BW/day should be avoided for exercising horses because water requirements increase, plasma urea level increase and also an excess of protein gives more nitrogen in urine. Several of the above studies have been comparing "high CP diets" with "low CP diets" that provided less CP than the requirements of adult horses. This is not always relevant in practice. In addition, the CP protein sources in these studies have varied but none have studied the effect of CP from forage-only diets.

Aim of the Thesis

The general aim of this thesis was to investigate how trained Standardbred horses were affected by feed deprivation on high concentrate and high forage diets, and how a high CP intake from a forage-only diet affected fluid balance and exercise response. More specifically the aims were:

- To study the effect of a forage-only diet compared to a 50:50 forage-oats diet on BW, plasma volume (TPP), and metabolic response before and during a 12 hour feed deprivation (Study I).
- To study the effect of a recommended (NRC, 1989) and a high (160 % of recommended) CP intake from forage-only diets on BW, fluid and acid-base balance, N metabolism and exercise response (Study II).

Hypotheses:

- Horses on a forage-only diet would be heavier at the start of feed deprivation but that they would loose more weight, have a less variable TPP and metabolic plasma profile than horses fed a high oats diet (Study I).
- A high CP intake can enhance the exercise induced acidosis, alter fluid balance and the response to intensive exercise (Study II).

Materials and methods

The studies in this thesis were performed at the National Trotting School Wången, Nälden, Sweden. The methods used are presented or referred to in the accompanying papers.

Horses

In total 14 trained Standardbred horses (11 geldings and 3 mares) were used in the studies included in this thesis, 4 geldings were used in both studies. Horses were between 5 and 12 years old during the studies and their mean race earnings were 34854 ± 8230 SEK (0-92300 SEK). During the studies their BW ranged between 443-568 kg. Study I was performed in spring 2007. Study II was performed in spring 2005.

Feeding and sampling

In study I the effect of two diets, one forage-only and one high oats diet, was studied at rest and during feed deprivation. In study II the effect of two CP intakes, recommended (NRC, 1989) and high intake (160% of recommended), from forage-only diets was studied at rest and during exercise. Diets and forage properties of both studies are summarised in Table 1.

Study I: Each experimental period was 21 days and followed a 7-day transition period where the new diet was gradually introduced. BW was measured every day at noon. Water intake was noted every morning. During the last day of each experimental period the horses were fasted for 12 hours and every hour BW were measured and blood samples were collected in heparinised tubes from the *vena jugularis*. Two horses were

excluded from the results due to large feed residues on diet FD during the day preceding the feed deprivation (22 and 48 % of the total allowance).

Study II: Each experimental period was 23 days. The first day of each experimental period started with an abrupt change of diet, and all urine and faeces were collected in a collecting harness for 48 hours. All faeces and urine were also collected for 72 hours on days 20 to 22. BW was measured every day before the 12:00 h feeding. Water intake was noted every morning. The horses performed two race-like exercise tests on each diet. At day 19 a standardised test on the treadmill was completed and on day 23 they performed a field test on an oval racetrack. During exercise tests, blood samples were collected in heparinised tubes from a permanent catheter in the *vena jugularis*.

	Diets				
	Stu	ıdy I	Study II		
	FD	OD	RP	HP	
Dry matter ^a	1.9 ± 0.02	1.9±0.01	1.6±0.02	1.8±0.02†	
Metabolisable energy ^b	18.3±0.2	20.4±0.1*	18.3±0.2	21.4±0.2†	
Crude protein ^b	222±3.1	237±1.6	200±2.0	291±2.2†	
NDF^{b}	991±10.6	765±3.0*	778±11.6	893±7.4†	
Ash ^b	184±2.2	127±0.8*	101±0.8	106±0.9†	
Water-soluble carbohydrates ^{b#}	150±1.5	91±0.4 *	84±3.8	169±1.5†	
	Forage chemical composition				
Dry matter ^c	63		45	50	
Metabolisable energy ^d	9.8		11.7	11.7	
Crude protein ^c	12.3		12.8	16.0	
NDF °	538		492	488	
Ash [°]	99		65	58	

Table 1. Average daily intake of dry matter⁴ and dietary components^b from three forage-only diets (FD, RP & HP) and a 50:50 forage:oats diet (OD) mean \pm s.e. Average forage chemical composition during the studies

^a kg/100 kg body weight per day, ^b g/100 kg body weight per day ^c%, ^dMJ/kg DM, ^c g/kg DM. [#]Free glucose, free fructose, sucrose and fructanes. *Significantly different from FD. †Significantly different from RP.

Exercise tests (Study II)

The exercise tests were designed to resemble a race. The standardised treadmill test started with a warm up with 5 minutes walk (1.8 m/s), 3 minutes trot (9 m/s), 45 seconds fast trot (11 m/s), and 4 minutes walk (1.8

m/s), and was designed to correspond to pre-race occurrences. After warmup the horses trotted for 3 min 15 s at 10 m/s with 5 % incline, the test ended with 9.5 m/s for 1 min with no incline.

The field exercise test started with a warm up including 4000 meters slow trot, 2080 meters trot at 10.5 m/s, and 10 minutes walk. After warm up the horses trotted 1600 meters at 11.3–11.5 m/s and then finished for 480-meter as fast as the they could (13.7 - 14.1 m/s). The field test was run in three heats with two horses in each heat, one horse from each diet. The same two drivers drove all the horses and the same driver drove the same horse at both test occasions but was not aware of which diet the horse was on.

Statistical analyses

All data were subjected to analysis of variance (GLM procedure in the Statistical Analysis Systems package 9.1) (SAS Institute Inc. Cary, NC, USA) using the following model;

 $Y_{_{i\,j\,k}} = \mu + \alpha_{_i} + \beta_{_j} + \gamma_{_k} + {}_{_1} + (\beta\gamma)_{_{j\,k}} + e_{_{i\,j\,k\,1}}$

Where Y_{ijk} is the observation, μ the mean value, α_i the effect of animal, β_j the effect of treatment, γ_k the effect of sample, _1 the effect of period, $(\beta \gamma)_{jk}$ the effect of interaction between treatment and sample and e_{ijk-1} the residuals; $e_{ijk-1} \sim IND (0, \delta^2)$. Differences were considered significant within and between treatments at P< 0.05. Values are presented as means \pm standard error of the mean.

Results

Body weight, water intake and fluid balance

BW was higher (3 kg) when feeding only forage compared to feeding 50% oats (Study I) but was not affected by the different CP intakes (Study II) (Table 2). In study I total weight loss was higher on diet FD than diet OD after 12 h of feed deprivation (10.8 \pm 1.0 kg vs. 8.4 \pm 1.0 kg). During neither of the exercise tests in study II there were differences between diet RP and diet HP in BW loss. Water intake was higher when feeding diet FD compared to diet OD and when feeding diet HP compared to diet RP (Table 2). During feed deprivation there was no difference in water intake between diets (diet FD: 3.2 ± 1.0 kg, diet OD: 2.0 ± 0.4 kg).

Table 2. Average body weight (BW) and water intake eating three forage-only diets (RP, HP & FD) and one 50:50 forage-oats diet (OD), mean \pm s.e. Results from each diet are divided in three periods. In study I period 1 = day 1-6, period 2= day 7-12 and period 3=13-18. In study II period 1 = day 1-8, period 2= day 9-16 and period 3=17-25

BW (kg)			Water intake (kg)					
	FD	OD	RP	HP	FD	OD	RP	HP
Period								
1	498±4	493±4*	476±10	484±4	23 ± 0.8	$20\pm0.6\star$	15±0.6	19±0.4†
2	495±4	491±4 *	485±4	484±4	23 ± 0.8	19±0.6*	17±0.7	23±0.8†
3	494±4	493±4 *	479±5	489±5	22 ± 0.7	$19{\pm}0.5{\star}$	16±0.8	20±0.6†

*Significantly different from FD . †Significantly different from RP

The total water intakes (water intake + water intake in feed) were 27.9 kg on diet FD and 22.7 kg on diet OD (Study I) and 25.0 kg on diet RP and 28.6 kg on diet HP (Study II) (Table 3). In study II, there was a

numerical higher (calculated) evaporative loss on diet HP compared to diet RP (Table 3).

Table 3. Average daily water intake (kg/day) by drinking and by feed intake. Average daily water output (kg/day) by faeces and urine during 72 h collection at day 20 to 22 during study II, mean \pm s.e.

	D	viet
	RP	HP
Water intake	16.7±1.4	19.1±1.1†
Water in feed	8.3±0.2	9.5±0.2†
Total water intake	25.0±1.5	28.6±1.1†
Faecal water output	10.8±1.0	12.2±0.7
Urine water output	9.6±0.4	10.5±0.8
Faecal and urine water output	20.4±1.1	22.7±0.8†
Calculated evaporative loss#	4.6±0.5	5.9±0.7

⁺Significantly different from RP. [#]calculated as total water intake-faecal and urine water output.

TPP

During feed deprivation the horses had smaller alterations in TPP after eating FD compared to diet OD and TPP was significantly higher the last 8 hours of feed deprivation on diet OD compared to diet FD (Study I). TPP was not affected by CP intake before, during or after exercise (Study II).

Faecal DM and urine pH

Faecal DM concentration was lower on diet FD (20.9 ± 0.9) compared to diet OD (25.2 ± 1.2) (Study I) and on diet HP (19.5 ± 0.6 %) compared to diet RP (20.9 ± 0.6) (Study II). Urine pH was lower on diet HP (7.03 ± 0.02) compared to RP (7.46 ± 0.04) (Study II).

Insulin, acetate, glucose, NEFA and urea during feed deprivation (Study I)

Plasma insulin was lower on diet FD than on diet OD before, during and after feeding and remained lower during 0 to 5 h of feed deprivation. Plasma NEFA and urea increased on both diets during 12 h of feed deprivation. Plasma NEFA was higher on diet FD than on diet OD at the start of and during feed deprivation. Plasma glucose was not affected by diet or feed deprivation. The plasma acetate concentration was higher on diet FD than on diet OD at 0 and 6 h of feed deprivation and on diet FD it decreased after 12 h of feed deprivation.

Heart rates, lactate, blood pH and urea during exercise (Study II)

During neither of the race-like exercise tests were there any differences between diet RP and diet HP in heart rate during exercise and recovery nor in plasma lactate concentration. During both exercise tests blood urea was higher on diet HP than on diet RP at rest, during exercise and recovery. There were no differences between diets for venous blood pH on either exercise tests.

General Discussion

Body weight, water intake and fluid balace

The increase in BW (3 kg) after eating a forage-only diet was similar to what recently has been reported in Standardbred horses in training (Jansson & Lindberg, 2008). A larger increase in BW (10.6 kg) has previously been reported in riding horses on a 50:50 (on a gross energy basis) concentrate-forage diet compared to on a forage-only diet (Ellis et al., 2002). Forage chemical composition, total energy intake, and training intensity differed between these studies. Increased BW after feeding a forage-only diet has been related to higher gut fill and gut water content than when horses are fed a mixed diet (Meyer, 1995). An increased CP intake in addition to a higher daily energy and DM intake (Table 1) on diet HP did not change BW either during time or compared to diet RP (Study II). This could partly be due to a higher need for energy metabolism of excess protein to urea (Blaxter, 1989) and the excretion of N compounds in the urine on diet HP.

The higher water intake on diet FD agrees with other studies comparing forage-diets with forage-grain diets (Fonnesbeck, 1968). When comparing diet FD to OD (Study I) there was probably more fluid lost in diet FD due to higher evaporative losses caused by more heat produced during chewing, saliva production and fermentation. Increased water content in the hindgut and the extracellular fluid probably also contribute as well as increased losses due to more water content in faeces.

Some of the increased water intake on diet HP compared to diet RP can be explained by increased daily faecal water loss and increased urine volume. A comparison of total water intake and output losses on diet HP and RP, shows however, a numerical larger evaporative output on diet HP (5.9 vs. 4.6 kg/day). This is probably due to the synthesis of urea being energy demanding and thereby increasing heat production and the passive evaporative loss.

Interestingly, an increased forage CP intake increased water intake and lowered faecal DM concentration but did not affect TPP. In contrast, comparing a forage-only diet to a forage-oats diet, water intake also increased and faecal DM decreased, but TPP was lowered on FD. These findings indicate that horses fed a forage-only diet compared to a 50 % oats diet with less fibre have an altered fluid homeostasis. Fibre water holding capacity and water availability to the horse is difficult to predict since the physical and chemical properties of fibre can be altered by fermentation (Eastwood et al., 1983). The delay in dehydration signs during feed deprivation on diet FD compared to diet OD suggests that the FD diet provided a fluid compartment available to maintain fluid balance. The findings agrees with the theory of a larger fluid reservoir in the hindgut of horses fed a diet with a large forage (fibre) content as earlier suggested by Meyer (1987).

Insulin, acetat, NEFA and urea during feed deprivation (Study I)

During feed deprivation the increased plasma urea concentrations are probably due to increased catabolism of body protein for energy production and the increased plasma NEFA concentration due to an increased lipolysis of triacylglycerols from adipose tissues. An increase in plasma NEFA and urea concentrations during feed deprivation has been reported in earlier studies on horses (Baetz & Pearson, 1972; Christensen et al., 1997; Rose & Sampson, 1982; Sticker et al., 1995). The insulin concentrations remained almost unchanged at all times on diet FD but were elevated on diet OD. This indicates that the need for increased insulin secretion was limited on diet FD. The forage-only diet caused small alteration in the insulin response both in connection with feeding and during feed deprivation. Insulin decreases lipolysis in adipose tissue and thereby the release of NEFA. In study I, the plasma NEFA concentration was higher on diet FD than on diet OD even in the fed state, which might be an effect of the low insulin secretion on diet FD. Plasma acetate concentration was also increased on diet FD indicating that the energy substrate profile was altered. The acetate in plasma originates from the fermentation of fibres, the concentration of acetate in colon and faeces fluid has been shown to increase on forage diets compared to grain (starch) diets (Hintz et al. 1971). It can be concluded that the relative contribution of energy from body protein and adipose tissue during feed deprivation appear to be similar on the two diets.

Heart rates, lactate, blood pH and urea during exercise (Study II)

There were marked dietary effects on the concentration of plasma urea, urine N and pH indicating an increased degradation of excess protein when CP intake was increased. In addition, water intake was higher (4 kg/day) indicating that the heat load and evaporative loss was higher on diet HP. However, there were no differences between diets in heart rate, plasma lactate and venous blood pH during the exercise tests. It can be concluded that the high CP diet did not adversely affect the response to intensive exercise but the suggested higher evaporative fluid loss on the high CP diet would not be beneficial during exercise when fluid losses are a limitation.

Conclusions

In conclusion, feeding horses a high-energy forage-only diet will result in a smaller increase in BW than earlier reported for low-energy forage-only diets. The larger BW loss on the forage-only diet during feed deprivation (+2.4 kg) implies that a small weight gain caused by a forage-only diet will not be persistent in connection with racing since many horses, voluntary or as a part of the trainer's management, have a low pre-race feed intake. The low TPP concentrations during feed deprivation on diet FD indicate that the horses had greater potential to use an internal fluid compartment to maintain plasma volume. The increased availability of fluid on diet FD probably originated from increased water content in the hindgut as earlier suggested for high fibre diets. This strategy could prove beneficial for the fluid balance of exercising horses. A high CP intake did not adversely affect the response to race-like exercise tests. However, increased urine and probably also evaporative fluid losses suggests that feeding a HP diet will be a challenge for horses during periods of low water intake and during prolonged exercise when fluid losses might limit performance. Based on the studies included in the present thesis, feeding large ratios of high energy forage to training Standardbred horses still remains an interesting alternative.

Populärvetenskaplig sammanfattning

Bakgrund

Hästar är gräsätare och är därför anpassade för att äta stora mängder vallfoder. För att tillgodose högpresterande hästars energibehov utfodras de ofta med stora mängder kraftfoder. Olika studier har emellertid visat ett samband mellan stora kraftfodergivor och kolik, magsår, muskelproblem och beteendestörningar. Det är därför intressant att belysa hur högpresterande hästar påverkas om en större andel av foderstaten består av vallfoder.

Det finns tidigare studier som tyder på att hästar som bara äter vallfoder blir tyngre än hästar som delvis äter kraftfoder. Den extra kroppsvikten förklaras med en tyngre grovtarm, fylld med vallfoder (fibrer) och vatten. En tidigare studie gjord på ridhästar har visat att hjärtfrekvensen blir högre under arbete på bara vallfoder jämfört med 50 % vallfoder och 50 % kraftfoder. Detta indikerar att arbetet varit tyngre på vallfoder och att prestationen skulle kunna försämras. För de flesta hästar innebär dock tävlingsmomentet att de, mer eller mindre frivilligt, får flera timmars fasta i samband med transport och vistelse på tävlingsplatsen. Preliminära observationer indikerar att en betydande del av hästarnas tarminnehåll då kan avges (i form av träck) och att kroppsvikten därför inte skulle skilja så mycket mellan hästar som fodras med bara vallfoder jämfört med mycket kraftfoder. Det finns dessutom studier som antyder att den extra vätska som finns i hästens grovtarm på en vallfoderrik foderstat skulle kunna utnyttjas som en vätskereserv under perioder med lägre intag av vatten och foder, som till exempel vid en tävling.



Studie I

I första studien användes 12 (tre ston och nio valacker) varmblodiga travhästar i full träning på travskolan Wången. I studien jämfördes en vallfoderdiet med en diet bestående av 50 % av torrsubstansintaget från vallfoder och 50 % från havre. För att motsvara hästarnas höga energibehov var vallfodret förhållandevis energirikt (9,8 MJ/kg ts). Hästarna åt varje diet i ungefär tre veckor och varje period avslutades med 12 timmars fasta.

När hästarna åt bara vallfoder var de 3 kg tyngre än när de åt havre och vallfoder och de drack mer vatten. Att det var så liten skillnad i kroppsvikt mellan dieterna tror vi beror på att det var ett vallfoder med högt energiinnehåll och lågt fiberinnehåll som användes samt att hästarna tränades hårt.

Under fastan förlorade hästarna 2,6 kg mer vikt när de hade ätit vallfoderdieten jämför med havredieten vilket tyder på att den lilla extra kroppsvikten snabbt försvinner när hästen inte äter, till exempel innan en tävling. Under fastan togs blodprov varje timme. Dessa prover visade att hästarna inte kunde behålla sin plasmavolym lika bra under fastan då de hade ätit havre och vallfoder som när de ätit bara vallfoder. Detta tror vi beror på att hästarna kan använda det extra vattnet i grovtarmen för att upprätthålla sin vätskebalans. Blodproven visade också att hästarna fick börja bryta ner kroppsreserver för att kunna energiförsörja sig men det var ingen skillnad mellan dieterna när hästarna började "svälta". Vi kunde också se att insulinutsöndringen var betydligt mindre när hästarna bara hade ätit vallfoder.

Studie II

Ett vallfoder som har ett så högt energiinnehåll att det räcker för att försörja en travhäst som tränar hårt måste skördas tidigt. Ett sådant foder har ofta också ett högt proteininnehåll.-Om hästen får mer protein än den behöver för muskelbyggnad m.m. så bryts överskottsproteinet ner och används som energi. Detta är en energikrävande process och värme, urea och vätejoner bildas som kroppen måste göra sig av med. Det finns tidigare studier som tyder på att ett överskott av protein skulle ge en sämre prestation i lopp.

I studie II jämfördes två vallfoderdieter med olika proteininnehåll. Den ena dieten innehöll rekommenderat dagligt intag (RP) och den andra 160 % av rekommenderat dagligt intag (HP) av råprotein. I studien användes sex varmblodiga travare (valacker) i full träning på travskolan Wången. Hästarna åt varje diet i ungefär tre veckor och varje utfodrings period avslutades med två arbetstest som skulle efterlikna ett travlopp, ett på rullmatta och ett på

1000-meters rundbana. Första två dygnen och tre av de sista dygnen på varje period samlades all urin och träck från hästarna.

I urinen, träcken och blodprov kunde vi se att hästarna fick ett större överskott av protein från HP än från RP men under arbetstesten kunde vi inte se någon skillnad mellan dieterna i puls, blod-pH eller mjölksyrakoncentration i blodplasman. Hästarna drack dock mer vatten på HP än på RP och deras beräknade avdunstning var numeriskt större vilket antyder att det bildats mer värme. En större värmeproduktion och ökat vätskebehov borde inte vara någon fördel under tävlingsformer eller tävlingsförhållanden då vätskebalansen är en begränsande faktor för prestationen.

Sammanfattning

En foderstat med bara energirikt vallfoder verkar underlätta för hästen att behålla sin plasmavolym under perioder utan foderintag utan att öka kroppsvikten jämfört med en kraftfoderrik foderstat. Foderstatens råproteininnehåll verkar inte påverka prestationen under ett kort intensivt arbete som påminner om ett travlopp.

References

- Baetz A.L. & Pearson J.E. 1972. Blood constituent changes in fasted ponies. American Journal of Veterinary Research 33, 1941–1946
- Beitz, D. C. 2004. Protein and amino acid metabolism. In Dukes' physiology of domestic animals 12^{et} Editor Reece, W. O., Cornell University, USA, 535- 552
- Blaxter, K. 1989. Energy metabolism in animals and man. Cambridge University Press, Cambridge, 256-280
- Christensen R. A., Malinowski K., Massenzio A.M., Hafs H. D. & Scanes C. G. 1997. Acute effects of short-term feed deprivation and refeeding on circulating concentrations of metabolites, insulin-like growth factor I, insulin-like growth factor binding proteins, somatroin, and thyroid hormones in adult geldings. *Journal of Animal Science* 75, 1351– 1358
- Danielsen, K., Lawrence, L. M., Siciliano, P., Powell, D. & Thompson, K. 1995. Effect of diet on weight and plasma variables in endurance exercised horses. *Equine Veterinary Journal* 18, 372–377
- Eastwood, M.A., Robertson, J.A., Brydon, W.G. & MacDonald, D. 1983. Measurement of water-holding properties of fibre and their faecal bulking ability in man. *British Journal of Nutrition* 50, 539-547
- Ellis, J. M., Hollands, T. & Allen, D. E. 2002. Effect of forage intake on bodyweight and performance, *Equine Veterinary Journal Supplement* 34, 66-70
- Fonnesbeck, P. V., 1968. Consumption and excretion of water by horses receiving all hay and hay grain diets. *Journal of Animal Science* 27, 1350–1356
- Fonnesbeck, P. V. & Symons, L. D. 1969. Effect of diet on concentration of protein, urea, nitrogen, sugar and cholesterol of blood plasma of horses. *Journal of Animal Science* 28, 216–219
- Frank, N. B., Meacham, T. H. & Fontenot, J. P. 1987. Effect of protein on performance and nutrition. Journal of Equine Veterinary Science 7, 321-322
- Freeman, D. W., Potter G. D., Schelling, G. T. & Kreider, J. L. 1988. Nitrogen metabolism in mature horses at varying levels of work. *Journal of Animal Science* 66, 407-412.
- Gillham, S.B., Dodman N.H., Shuster L., Kream R. & Rand W. 1994. The effect of diet on cribbing behavior and plasma β-endorphin in horses. *Applied Animal Behaviour Science* 41, 147-153

- Glade, M.J, 1983. Nutrition and performance of racing Thoroughbreds. Equine Veterinary Journal 15, 31-36.
- Graham-Thiers, P. M., Kronfeld, D. S., Kline, K. A & Sklan, D. J. 2001. Dietary protein restriction and fat supplementation diminish the acidogenic effect of exercise during repeated sprints in horses. *Journal of Nutrition* 131, 1959-1964
- Hintz, H. F., Argenzio R. A & Schryver H. F.1971. Digestion coefficients, blood glucose levels and molar percentage of volatile acids in intestinal fluid of ponies fed varying forage-grain ratios. *Journal of Animal Science* 33, 992-995
- Hintz, H. F., White, K. K., Short, C. E., Lowe, J. E. & Ross, M. 1980. Effects of protein levels on endurance horses. *Journal of Animal Science Supplement* 51, 202–203
- Jansson A. & Lindberg J. E. 2008 Effects of a forage-only diet on body weight and response to interval training on a track. In: Nutrition of the exercising horse. EAAP Publication No 125, Eds., Saastamoinen MT and Martin-Rosset W, 345-349
- Julliand V., de Fombelle A., Drogoul C. & Jacotot E. 2001. Feeding and microbial disorders in horses: Part 3- effects of three hay:grain rations on microbial profile and activities. *Journal of Equine Veterinary Science* 21(11), 543-546
- Kusunose R. 1992. Diurnal pattern of cribbing in stabled horses. Japanese Journal of Equine Science 3, 173-176
- MacLeay, J.M., Sorum, S.A., Valberg, S.J, Marsh, W.E. & Sorum, M.D. 1999. Epidemiologic analysis of factors influencing exertional rhabdomyolysis in – thoroughbreds. *American Journal of Veterinary Research* 60, 1562-1566
- MacLeay, J.M., Valberg, S. J Pagan J. D., Jinliang L. X., De La Corte F. D. & Roberts J. 2000. Effect of ration and exercise on plasma creatine kinase activity and lactate concentration in Thoroughbred horses with recurrent exertional rhabdomyolysis. *American Journal of Veterinary Research* 61, 1390-1395
- McGreevy, P.D., Cripps, P.J., French, N.P., Green L.E. & Nicol C.J. 1995. Management factors associated with stereotypic and redirected behavior in the Thoroughbred horse. *Equine Veterinary Journal* 27, 86-91
- Meyer, H., 1983a. The pathogenesis of disturbances in alimentary tract in the horse in the light of newer knowledge of digestive physiology. *Proceedings of Horse Nutrition Symposium, Uppsala*, 95–109
- Meyer, H. 1983b. Intestinal protein and N metabolism in the horse. Proceedings of Horse Nutrition Symposium, Uppsala, 113-137
- Meyer, H. 1987. Nutrition of the equine athlete. In Gillespie J. R. & Robinson N. E (eds.) Equine Exercise Physiology 2, ICEEP Publications Davis , CA, 644- 673
- Meyer, H., 1995. Influence of diet, exercise and water restriction on the gut fill in horses. Proceedings in Equine Nutrition Physiology Society 14, 90-91
- Miller, P. A. & Lawrence, L. M. 1988. The effect of dietary protein level on exercising horses. *Journal of Animal Science* 66, 2185–2192
- Miller-Graber, P. A., Lawrence, L. M., Foreman, J. H., Bump, K. D., Fisher, M. G. & Kurcz, E. V. 1991. Dietary protein level and energy metabolism during treadmill exercise in horses. *The Journal of Nutrition* 121, 1462-1469
- National Research Council (NRC). 1978. Nutrient Requirements of Horses. 4th edition, National academy press, Washington D.C. USA.
- 28

- National Research Council (NRC). 1989. Nutrient Requirements of Horses. 5th edition, National academy press, Washington D.C. USA.
- Pagan, J. D., Essén-Gustavsson, B., Lindholm, A. & Thornton, J. 1987. The effect of dietary energy source on exercise performance in Standardbred horses. In Gillespie J. R. & Robinson N. E (eds.) *Equine Exercise Physiology 2*, ICEEP Publications Davis , CA, 686-700
- Patterson, P. H., Coon, C. N. & Hughes, I. M. 1985. Protein requirements of mature working horses. *Journal of Animal Science* 61, 187-196
- Prior, R. L., Hintz, H. F., Lowe, J. E. & Visek, W. J. 1974. Urea recycling and metabolism in ponies. *Journal of Animal Science 38*, 565-571
- Redbo I., Redbo-Torstensson P., Ödberg F. O., Hedendahl A. & Holm J. 1998. Factors affecting behavioural disturbances in race-horses. *Animal Science* 66, 475-481
- Rose R.J. & Sampson D. 1982. Changes in certain metabolic parameters in horses associated with food deprivation and endurance exercise. *Research in Veterinary Science* 32, 198-202
- Slade, L. M., Robinson, D. W. & Casey, K. E. 1970. Nitrogen metabolism in nonruminant herbivores 1. The influence of nonprotein nitrogen and protein quality on the nitrogen retention of adult mares. *Journal of Animal Science 30*, 753-760
- Sticker L.S., Thompson D.L., Jr., Bunting L.D., Fernandez J. M. & DePew C.L. 1995. Dietary protein and(or) energy restriction in mares: Plasma glucose, insulin, nonestrified fatty acid, and urea nitrogen responses to feeding, glucose, and epinephrine. Journal of Animal Science 73, 136-144
- Tinker M. K., White N. A., Lessard P., Thatcher C.D., Pelzer K. D., Davis B. & Carmel D. K. 1997 Prospective study of equine colic risk factors. *Equine Veterinary Journal* 29, 454-458
- Willard J. G., Willard J. C., Wolfram S. A. & Baker J. P. 1977. Effect of diet on cecal pH and feeding behaviour of horses. *Journal of Animal Science*. 45, 87-93

Acknowledgements

Studierna i den här avhandlingen har finansierats genom medel från Stiftelsen Svensk Hästforskning och Travskolan Wången.

Det är så många som varit med och hjälp till under de här två studierna att det kändes som en omöjlig uppgift att skriva det här. Jag är rädd att glömma bort någon eftersom allas bidrag har varit så värdefullt.

Ett stort tack till:

Anna Jansson, för allt du har lärt mig om hästar och hästforskning under de här åren. Speciellt att alltid se möjligheterna och lösningarna på problemen när jag har varit redo att ge upp.

Jan-Erik Lindberg, för att du har varit med och planerat inför mina försök med värdefulla åsikter om upplägg och provtagning samt läst och kommenterat allt jag har skrivit.

Birgitta Essén-Gustavsson, för din aldrig sinande entusiasm för forskning och alla värdefulla kommentarer om försöksupplägg och om det jag har skrivit.

Karin Ericson, för att jag har fått chansen att göra detta och för all värdefull handledning på hemmaplan när det har varit långt till Uppsala.

Elever och studenter på travskolan Wången för att ni hjälpt mig under försöken med den dagliga skötseln av försökshästarna. Utan er skulle det inte gå att göra så bra försök på Wången.



Cajsa Löfqvist, för all hjälp under försöket. Ibland gick allt bra, ibland gick det mindre bra men allt ordnade sig till slut. Och ditt fördjupningsarbete blev klart!

Rebecca Åsebol för all hjälp under proteinförsöket..

Sara Muhonen för samarbetet under proteinförsöket och din hjälp nu under de sista hysteriska dagarna har varit ovärderlig.

All personal på Wången för hjälp med allt från paketskickande, matlådor, hästkörning och fakturering till att koppla in "kylskåp" till hösilaget och skicka hösilage till Frankrike fram och tillbaka. Speciellt tack till Ulf Hedenström (agronom wanna be) min doktorandkollega som ställt upp med veterinär hjälp när det har behövts oavsett tid eller dag.

Laboratoriet på Kungsängen för all hjälp med analyser och speciellt till Börje som sedan har fått svara på alla mina frågor.

Alla på Husdjurens utfodring och vård för all hjälp och att ni har fått mig att känna mig så välkommen dom gånger jag har varit på plats. Speciellt tack till Anna-Greta för hjälp med alla analyser.

Mamma, pappa, systrar och vänner för att ni har stöttat mig och stått ut med mig när hjärnan har varit i samma skick som överkokta snabbmakaroner.

Min familj, Robban, Tova och Albin ni är det bästa som finns.