The effect of diet and exercise on plasma metabolite and hormone concentrations in horses measured before and after exercise

R.B. Jensen[1,4], D. Blache[2], K.E. Bach Knudsen[3], D. Austbø[4] and A.-H. Tauson[1,4]

[1]University of Copenhagen, Department of Veterinary and Animal Sciences, Grønnegaardsvej 3, 1870 Frederiksberg C, Denmark
[2]University of Western Australia, School of Animal Biology, 35 Stirling Highway, Crawley WA 6009, Australia
[3] Aarhus University, Department of Animal Science, Blichers Allé 20, 8830 Tjele, Denmark
[4] Norwegian University of Life Sciences, The Department of Animal and Aquacultural Sciences, Postboks 5003, 1432 Ås, Norway

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Corresponding author:
Rasmus Bovbjerg Jensen
Norwegian University of Life Sciences,
Department of Animal and Aquacultural Sciences, Campus Ås
Postboks 5003
1432 Ås
Norway
Mail: rasmus.bovbjerg.jensen@nmbu.no

Abstract

Exercise influences different endocrine and metabolic parameters, and information in the literature is sparse for some of these hormones and metabolites in the exercising horse. The aim of the present experiment was to study the metabolic response to exercise when feeding diets with varying carbohydrate composition (fibre and starch) under experimental conditions where diet and exercise were standardized and controlled. The response was investigated in a 4 x 4 Latin square design experiment using four Norwegian Coldblooded trotter horses. The dietary treatments were two fibre based diets, hay only and hay (85% of dry matter intake (DMI)) supplemented with molassed sugar beet pulp (mSBP) (15% of DMI), or two starch based diets of hay (68% of DMI) and barley (32% of DMI), and hay (68% of DMI), barley (26% of DMI) and mSBP (6% of DMI). Each diet was fed for 28 days; 16 days of adaptation followed by 12 days of data collection. Four hours after the morning feeding at 06:00 the horses performed a standardized exercise test (SET) lasting 45 minutes. Blood samples were taken before feeding the morning meal at 06:00, before the SET (10:00), after the SET (10:45) and after recovery from exercise (15:00), and plasma samples were analysed for relevant metabolites and hormones. Plasma leptin concentrations increased after exercise but were not affected by diet, whereas diet and exercise had no effect on the plasma concentrations of ghrelin and insulin like growth factor 1 (IGF-1). Furthermore, diet influenced the plasma concentrations of short-chained fatty acids (SCFA) more than exercise. The results provide important comparative information that can be useful in studies where diet and exercise cannot be controlled, e.g. in field studies.

Keywords: carbohydrate, equine, ghrelin, leptin, nutrition

Introduction

It is well known that diet and exercise influence different endocrine and metabolic parameters because of mobilization of energy yielding substrates such as glucose from glycogen and non-esterified fatty acids (NEFA) from fat depots during exercise. Starch rich grains have traditionally been included in the ration to performance horses as an energy source, even though it is well known that feeding large amounts of grain reduces the microbial stability in the hindgut (Willing *et al*., 2009), and increases the risk of diseases like laminitis (Garner *et al.*, 1975), colic (Hudson *et al.*, 2001) and gastric ulcers (Luthersson *et al.*, 2009). Recent research has therefore focused on forage only diets (Jansson and Lindberg, 2012) or alternative feedstuffs rich in easily fermentable fibre like sugar beet pulp (Moore-Colyer *et al.*, 2002; Palmgren Karlsson *et al.*, 2002) and soybean hulls (Coverdale *et al.,* 2004). However, the knowledge on the metabolic response to feeding fibre based diets to performance horses is limited, especially plasma concentrations of the individual short-chained fatty acids (SCFA).

Performance horses need to be in energy balance when in training, so that they do not lose or gain body weight. Energy balance is not only determined by energy intake and expenditure, it is also under endocrine control. Hormones like leptin and ghrelin are related to maintenance of energy balance (Spiegelman and Flier, 2001) and feed intake (Wren *et al.*, 2001), respectively. However, little information exists on concentrations of these hormones in exercising horses (Gordon and McKeever, 2005).

The aim of the present experiment was to study the metabolic response to exercise when feeding diets with varying carbohydrate composition in terms of starch and readily fermentable fibre under experimental conditions where diet and exercise could be standardized and controlled. It was hypothesised that plasma concentrations of glucose and SCFA would be affected by diet and exercise, and subsequently affect the hormonal response. The results from this study provide important comparative information to be used when

evaluating results of metabolite and hormone analyses in studies where diet and exercise cannot be controlled, e.g. in field studies.

Materials and methods

Experimental design

The experimental design was a 4 x 4 Latin square with four experimental periods of 28 days each consisting of 16 days of adaptation to a diet and 12 days of data collection. After ended data collection on day 28, the transition from one diet to another was done gradually over the first two days in the next period. Data in this study collected were on day 25 and 26, and results from data collected on the other days are reported elsewhere (Jensen *et al.* 2014 and 2016). All horses remained healthy throughout the study, and they were cared for according to the laws and regulations concerning experiments on live animals in Norway (i.e., the Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance concerning Experiments on Animals of January 15, 1996).

Animals and housing

Four 6- to 15-year-old cecum cannulated Norwegian Cold-blooded trotter horse geldings, with an initial body weight (BW) of 542 (SD: ±17 kg) were used in the experiment. The horses were housed in an unheated barn in 3 x 3 m individual stalls with wood shavings as bedding material. Throughout the adaptation period they were allowed access to a dirt paddock (~2,300 m^2) for approximately 6 hours after the morning feeding, whereas during data collection they were allowed access to the dirt paddock for a few hours after the daily measurements on days 17-18, 21-23 and 28. Before the start of the experiment all horses had a health inspection by a veterinarian, including floating of the teeth.

Feeding

The horses were fed four iso-energetic diets; two fibre based diets based on hay only (mainly mature timothy) and hay (85% of dry matter intake (DMI)) supplemented with molassed sugar beet pulp (mSBP) (15% of DMI) and two starch based diets based on hay (68% of DMI) and barley (32% of DMI), and hay (68% of DMI), barley (26% of DMI) and mSBP (6% of DMI). The ingredient and nutrient composition of the total daily ration is shown in Table 1, and ingredient and nutrient composition in the morning meal fed before exercise is shown in Table 2. Detailed information on the nutrient content of individual feedstuffs can be found in Jensen *et al.* (2014). The main nutrient fractions in hay, barley and mSBP, respectively were: DM (%): 0.88, 0.88. 0.89 and nutrients (% of DM): ash: 0.06, 0,03, 0,08; CP: 0.10, 0.11, 0.11; fat: 0.03, 0.03, 0.02; sugar (water soluble carbohydrates (WSC)): 0.10, 0.04, 0.23; starch: -, -, 0.58, and dietary fibre: 0.68, 0.20, 0.50.

Table 1. Composition of the total daily ration (kg DM) when hay only (Hay), hay and molassed sugar beet pulp (mSBP) (Hay+mSBP), hay and barley (Barley) or hay, barley and mSBP (Barley+mSBP) were fed to horses.

	Experimental diets			
	Hay	Hay+mSBP	Barley	Barley+mSBP
Ingredients	Total daily ration			
Hay	10.05	7.95	5.73	5.73
Barley	-	-	2.76	2.24
Molassed sugar beet pulp	-	1.41	-	0.52
NaCl	0.05	0.05	0.05	0.05

Vitamins and minerals mix[1]	0.10	0.10	0.10	0.10
Nutrients	Total daily ration			
Dry matter	10.20	9.51	8.64	8.64
Dietary fibre	6.86	6.13	4.47	4.62
Starch	-	-	1.61	1.31
Sugar	0.95	1.08	0.65	0.75
Crude protein	1.05	0.97	0.90	0.90
Fat	0.26	0.23	0.24	0.23

[1]The vitamin mineral mix provided per kg: Ca, 135 g; P, 30 g; Na, 38 g; Mg, 23 g; Zn, 3000 mg; Mn, 1500 mg; Fe, 1500 mg; Cu 675 mg; Co; 8 mg; I, 41 mg; Se, 15 mg; vitamin A, 225,000 IU; vitamin D3, 22,500; vitamin E (alpha tocopherol acetate), 7500 mg; vitamin B1, 188 mg; vitamin B2, 150 mg; vitamin B6, 150 mg; vitamin B12, 2 mg; D-pantothenic acid, 450 mg; nicotinic acid, 375 mg; folic acid, 150 mg; choline chloride, 1125 mg; biotin, 23 mg; vitamin C, 375 mg.

The daily ration (Table 1) was divided into four individual meals and fed at 06:00, 07:00, 16:00 and 22:00. Barley (2g starch/kg BW) and SBP (50% of the daily ration) were fed at 06:00 and the rest at 22:00. The amount of hay was divided into three meals and fed at 07:00, 16:00 and 22:00, except for the Hay diet where the morning meal was divided into two meals fed at 06:00 and 07:00 (Table 2). A vitamin–mineral blend (Champion Multitilskudd; Felleskjoebet Agri) and NaCl was included in the meal at 22:00, at levels of 100 and 50 g, respectively.

Table 2. Composition of the morning meals fed at 06:00 and 07:00 (kg DM) when hay only (Hay), hay and molassed sugar beet pulp (mSBP) (Hay+mSBP), hay and barley (Barley) or hay, barley and mSBP (Barley+mSBP) were fed to horses, and the nutrient content (kg) of the 06:00 and 07:00 meal.

	Experimental diets			
	Hay	Hay+mSBP	Barley	Barley+mSBP
Ingredients	Morning feeding at 06:00			
Hay	1.75	-	-	-
Barley	-	-	1.88	1.88
Molassed sugar beet pulp	-	0.71	-	0.27
Ingredients	Morning feeding at 07:00 h			
Hay	1.75	1.75	1.75	1.75
Nutrients	Morning feeding at 06:00 and 07:00			
Dry matter	3.50	2.46	3.63	3.90
Dietary fibre	2.39	1.55	1.57	1.71
Starch	0	0	1.10	1.10
Sugar	0.29	0.31	0.21	0.28
Crude protein	0.36	0.26	0.39	0.42

Fat	0.09	0.06	0.11	0.11

Exercise

The horses were exercised in an outdoor rotary exerciser (intervals of trotting and walking for 30 minutes at speeds from 1.8 m/s to 5 m/s) four to five times a week during the 16 days of adaptation and two times during the 12 days of data collection (same days in each experimental period). The exercise program started 8 weeks before the experiment to standardize fitness across the horses, and during the experiment, the exercise program aimed to maintain fitness. A standardized exercise test (SET) was performed on an inclined (3 %) treadmill (Haico 4000, Loimaa, Finland) four hours after the morning meal. Two horses performed the SET on day 25 and two horses on day 26. The morning feeding for one of the horses tested was postponed for one hour in order to standardize the time for the SET to be exactly four hours after the morning meal.

The horses carried a heart rate (HR) monitor (Polar Equine RS800CX, 135 Kempele, Finland), a safety harness and pulling belt (Haico, Loimaa, Finland), where pulling force was adjusted (+1 bar) during the SET to increase workload without increasing speed. The SET consisted of 5 minutes of walk (1.8 m/s), 5 minutes of trot (3.4 m/s), 5 minutes of trot (3.8 m/s), 5 minutes of trot (3.8 m/s + 1 bar), 5 minutes of walk (1.5 m/s), 1 minute of trot (3.4 m/s + 1 bar) and 5 minutes of trot (3.8 m/s + 1 bar). During the last 5 minutes of the SET the average HR was 163 beats/minute (SD: ±7). After the SET the horses walked (1.5 m/s) on the treadmill (0 % incline) until HR was below 90 beats/minute.

Sample collection and analyses

Samples of feedstuffs were collected regularly during each data collection period, and stored in sealed plastic bags for later analyses. All samples of feeds were analysed in duplicate except for DF. The DM content of the feedstuffs was determined by drying to constant weight (24h at 105°C). Samples were freeze-dried and analyses were performed after milling the samples to pass a 1 mm screen (DM, ash, CP and fat) or a 0.5 mm screen (DF, starch, sugars and fructan). Ash content was determined by incineration at 525°C for 6 h. The feedstuffs fed in each period were analysed separately, except for DF, starch, sugars and fructan for which a pooled sample of each feedstuff was made. Nitrogen was determined by the Kjeldahl technique (Tecator-Kjeltec system 1030; Tecator AB, Höganäs, Sweden) and CP calculated as N×6.25. Fat content of feedstuffs was determined by petroleum ether extraction in a Soxtec system after HCl hydrolysis (SoltexTM 2043; Foss, Hillerød, Denmark). Starch was analysed by an enzymatic colorimetric method according to Bach Knudsen (1997) and sugars (glucose, fructose and sucrose) and fructan in feedstuffs by the enzymatic colorimetric method of Larsson and Bengtsson (1983). Dietary fibre was analysed as described by Bach Knudsen (1997). Detailed information on feedstuff analyses can also be found in Jensen *et al.* (2014).

Blood samples were collected on day 25 or 26 (two horses each day) by jugular vein puncture into 10ml heparinized tubes before feeding the morning meal at 06:00, before the SET (10:00), after the SET (10:45) and after recovery from exercise (15:00). The blood samples were centrifuged immediately after sampling at 3,000 x g for 10 minutes and plasma was harvested and stored at -20°C for later analysis of plasma metabolites and hormones. Detailed information on procedures for plasma analyses of metabolites (glucose, total protein, urea, lactate, triglyceride, non-esterified fatty acids (NEFA), β-OH-butyrate (BOHB), total SCFA, acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and caporate) and hormones (insulin, cortisol, leptin, ghrelin and insulin like growth factor (IGF-1)) can be found in Jensen *et al.* (2016).

Statistics

Statistical analyses of plasma hormone and metabolite concentrations were performed as repeated measurements analysis using the MIXED procedure in SAS® (Version 9.4, SAS Institute Inc., Cary, North Carolina, USA), with a model comprising the fixed effect of diet (fibre based or starch based), inclusion of mSBP (with or without mSBP), time (06:00, 10:00, 10:45 or 15:00) and interactions (diet and inclusion of mSBP; diet and time; diet, inclusion of mSBP and time) and the random effect of horse, and the interaction between horse and period (period in the Latin square). Serial correlation over the interaction between horse and period was modelled using a spatial Gaussian correlation structure. The effect of period was non-significant and it was removed from the model. Results are presented as least square means (LS-means) and standard error of the mean (SEM) is reported. Effects were considered significant if $P<0.05$.

Results

All horses accepted the experimental diets and there were no refusals. The four different diets were arranged into two groups, two fibre based (Hay and Hay+mSBP) and two starch based (Barley and Barley+mSBP) diets. The effects of diet and time on plasma metabolite and hormone concentrations are presented in Figures 1-3.

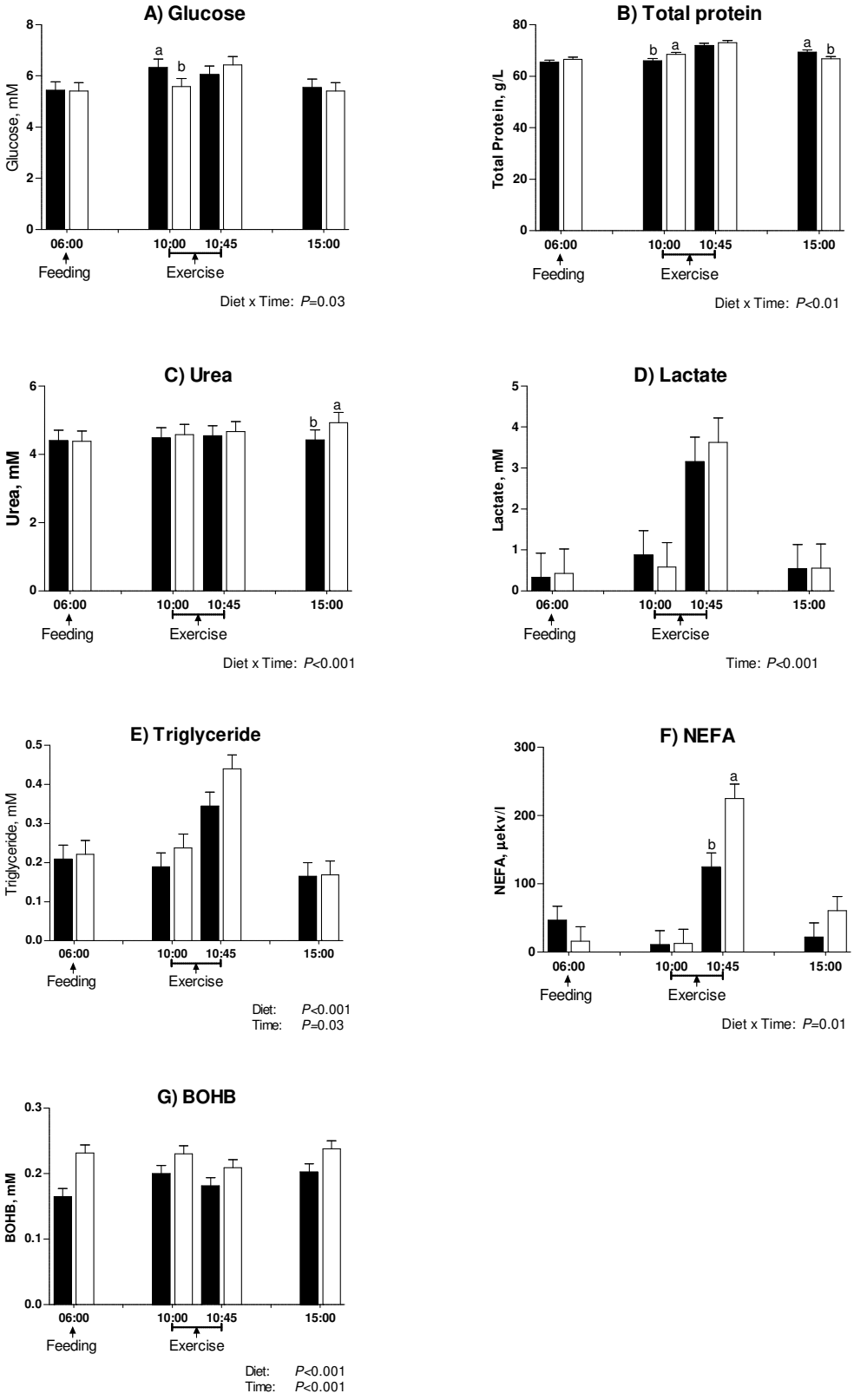
Metabolites

Plasma glucose concentration increased after feeding the starch based diets compared to the fibre based diets and an interaction between diet and time was present ($P=0.03$). After exercise there was no effect of diet on plasma glucose concentration (Figure 1A). Plasma total protein concentration was higher after feeding the fibre based diets than when feeding the starch based diets. However, higher total protein in plasma was measured after recovery from exercise when feeding the starch based diets than the fibre based diets, and an interaction between diet and time ($P<0.01$) was found (Figure 1B). An interaction between diet and time ($P<0.001$) was also found for plasma urea concentration, where higher concentrations were measured on the fibre based diets than the starch based diets after recovery form exercise (Figure 1C). Plasma lactate concentration was affected by time ($P<0.001$) and the concentration peaked after exercise (Figure 1D). The concentration of plasma triglyceride was affected by both time ($P<0.001$) and diet ($P=0.03$), with concentration increasing after exercise and the highest concentration was found for the fibre based diets (Figure 1E). An interaction between diet and time ($P=0.02$) was found for plasma NEFA concentration, and the concentration peaked after exercise with the largest increase for the fibre based diets (Figure 1F). The concentration of plasma BOHB was affected by time ($P<0.01$) and diet ($P<0.01$) with the BOHB concentration being lowest after exercise compared to other time points, and concentration on the fibre based diets in general being higher than on the starch based diets (Figure 1G).

Total SCFA and acetate concentrations in plasma were affected by diet ($P=0.03$ and $P=0.02$, respectively) with the fibre based diets resulting in higher concentrations than the starch based diets (Figure 2A and 2B, respectively). Plasma propionate concentration was affected by time ($P=0.03$) with higher concentration after exercise than at other time points (Figure 2C). An interaction between diet and time ($P=0.04$) was found for plasma butyrate with higher concentration for the fibre based diets than for the starch based diets before feeding and after exercise (Figure 2D). Plasma iso-byturate concentration was affected by time ($P<0.001$), diet ($P<0.01$) and mSBP inclusion ($P=0.01$), and the highest concentration was found after exercise, and the fibre based diets resulted in higher concentration than the starch based diets while inclusion of mSBP had a negative effect on the concentration (Figure 2E). There were no effects on plasma valerate concentration (Figure 2F), but an interaction between diet and

237 time was found for plasma iso-valerate concentration with higher concentration for the fibre
238 based diets than the starch based diets. The lowest concentration was found after recovery
239 from exercise (Figure 2G). There were no effects on plasma caporate concentration (figure
240 2H).
241

242 Figure 1



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244 Figure 2
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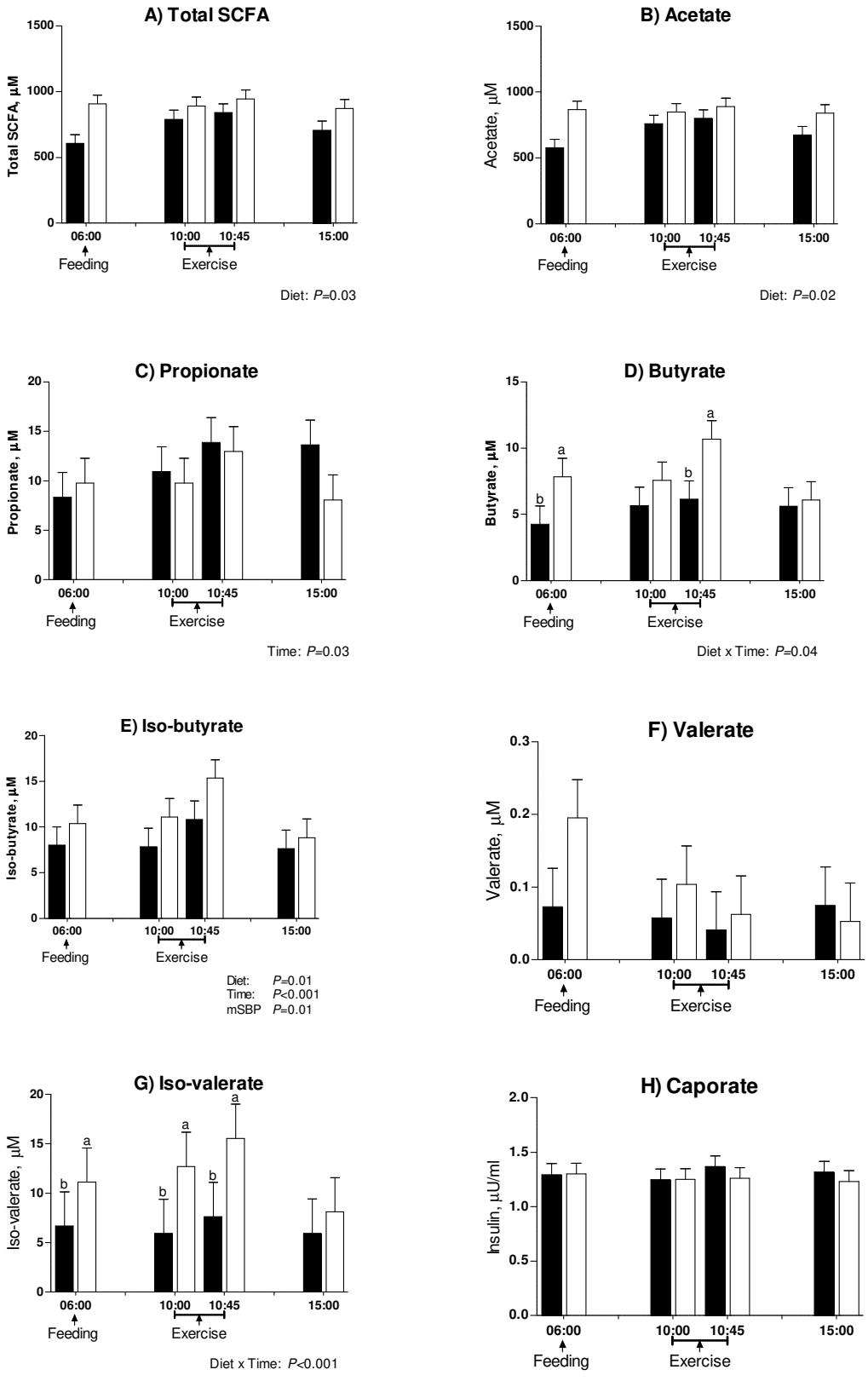
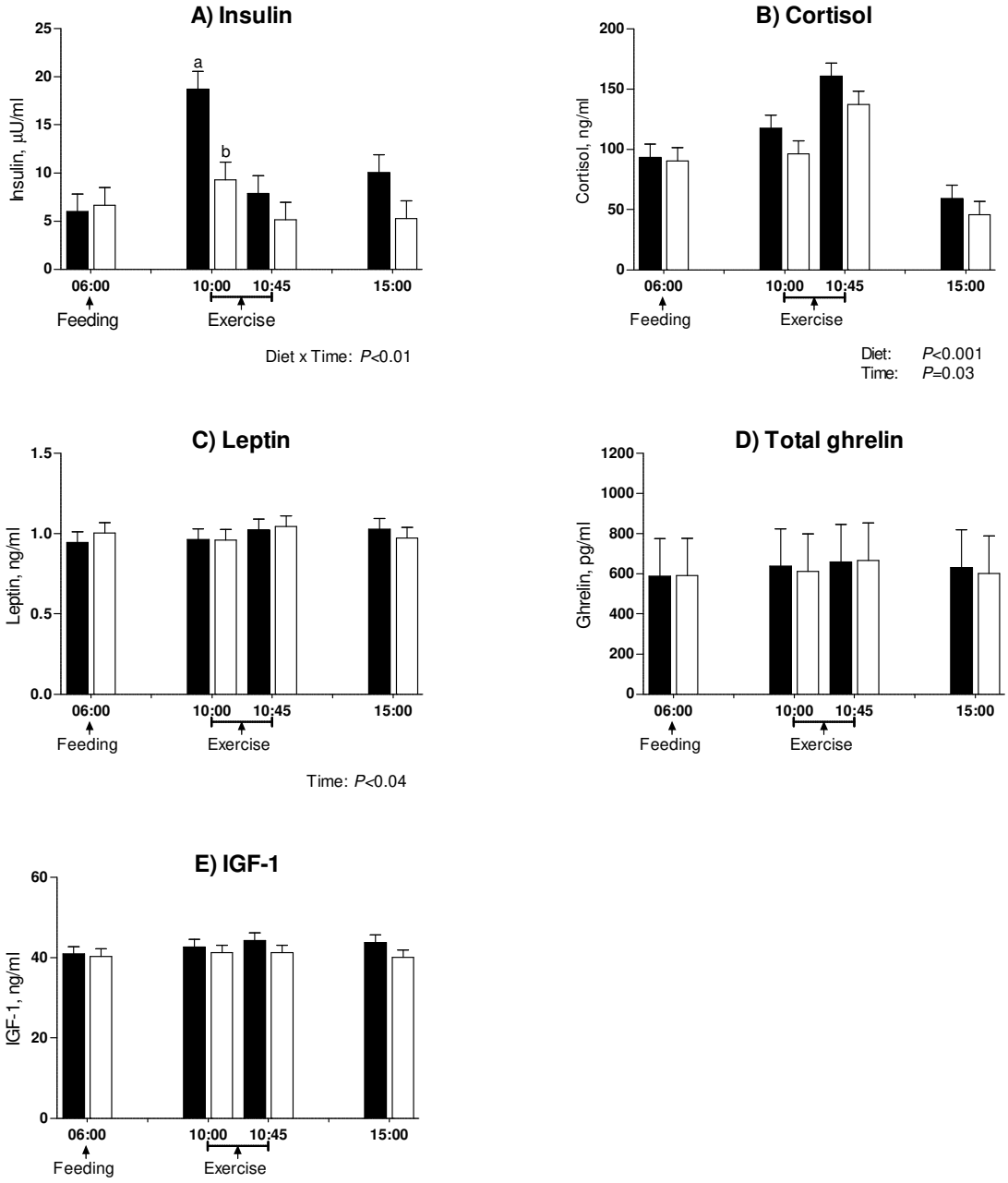


246

247 *Hormones*
248 There was an interaction between diet and time on plasma insulin concentration (P<0.01),
249 with plasma insulin concentration increasing after feeding the starch based diets, but
250 remaining stable when feeding the fibre based diets. After exercise, plasma insulin
251 concentration returned to the pre-prandial level (Figure 3A). Plasma cortisol concentration
252 was affected by time (P<0.001) with increasing concentrations after exercise on all diets, and
253 the lowest concentration was measured after recovery from exercise. Diet effects were
254 significant (P=0.03) with higher plasma concentration when feeding the starch based than the
255 fibre based diets (Figure 3B). Plasma leptin concentration was highest (P=0.04) after the SET
256 compared to the other time points (Figure 3C). There was no effect of diet or exercise on the
257 plasma concentrations of ghrelin (Figure 3D) and IGF-1 (Figure 3E).
258
259 Figure 3



Discussion

The main objective of the present investigation was to study the metabolic response to exercise when feeding diets with varying carbohydrate composition, fibre and starch, under experimental conditions where diet and exercise were standardized and controlled.

The most important substrates to meet the increased energy demands of exercise are glucose and fatty acids from the degradation of glycogen and triglycerides, respectively. The starch based diets, because of the provision of exogenous glucose from dietary starch, increased plasma glucose and insulin concentrations; however, after exercise there was no difference in plasma glucose and insulin concentrations between diets. It is well documented that the insulin response during exercise is supressed (Jose-Cunilleras *et al.*, 2002; McKeever, 2011)

as also supported by the data of the current study, and during exercise muscle contractions stimulate glucose transportation into the muscle cells (van der Kolk, 2014).

Plasma triglyceride and NEFA concentrations increased as a response to exercise, and the fibre based diets caused a larger peak in NEFA than the starch based diets because of relatively more energy provided as ketogenic energy, i.e. acetate and butyrate, from the fermentation processes. Therefore, more blood glucose is likely available for the muscle cells when feeding starch based diets (Jose-Cunilleras *et al.*, 2002). However, insulin is known to inhibit lipolysis and fatty acid oxidation in skeletal muscles (Jose-Cunilleras *et al*., 2002), and insulin concentrations were greatest before the SET when the starch based diets were fed, which might have affected lipid oxidation during exercise. This might have caused the lower concentrations of NEFA when feeding the starch based diets than the fibre based diets.

The higher levels of plasma total SCFA and acetate concentrations when feeding the fibre based diets than the starch based diets reflected fermentation of fibre in the hindgut with subsequent absorption of SCFA as energy yielding substrates. Jansson and Lindberg (2012) did also find higher plasma concentrations of acetate when feeding performance horses forage only diets compared to traditional grain supplemented diets. Propionate is not used as an energy source directly as it is converted to glucose via gluconeogenesis in the liver (Ford and Simmons, 1985), and a fraction of butyrate is metabolized by the intestinal cells, cleared to a high degree in the liver and converted to BOHB (Von Engelhardt *et al*., 1989; Ingerslev *et al*., 2014). This was also reflected in the low concentrations of propionate and butyrate in plasma compared to acetate, and lower plasma concentrations of butyrate than BOHB. The higher concentrations of butyrate and BOHB when feeding the fibre based diets than the starch based diets might be a reflection of a generally higher production of SCFA in the hindgut of the horses fed the fibre based diets.

The horses performed submaximal aerobic work during the SET with an average HR of 163 beats/minute during the final step of the SET as also reflected in the plasma lactate concentration which was below the lactate threshold of 4 mmol/l. Different SETs are used in different studies and this might cause different changes in subsequent metabolite and hormone concentrations. The SET used in the present study was designed to reflect aerobic work like dressage and show-jumping, and the diets provided the horses with on average 97.5 MJ digestible energy per day (Jensen *et al.,* 2014), which corresponds to 128% of the requirements for maintenance according to NRC (2007), equivalent to the energy requirements for horses in light to moderate work. The response in cortisol measured after exercise was as expected, as cortisol induces substrate mobilization by enhancing gluconeogenesis and free fatty acid release during exercise (McKeever, 2011). The increased plasma concentrations of triglyceride and NEFA also reflected this mobilization of nutrients. Cortisol was also higher when feeding the starch based diets than the fibre based diets, however, the significance of this difference is not clear.

There were no effects of diet or time on plasma ghrelin concentration in this study. In the literature, results are conflicting as Gordon and McKeever (2006) found active ghrelin to decrease after intravenous administration of dextrose, whereas ghrelin increased after meal feeding (Gordon and McKeever, 2005). It has been suggested that the fermentation of fibre in the hindgut and subsequent absorption of SCFA might cause a more stable and continuous energy supply rather than the postprandial fluctuations associated with bolus feeding (like in humans) and thereby giving rise to less fluctuations in ghrelin in horses (Gordon and McKeever, 2005, Jensen *et al.*, 2016). Exercise did not affect ghrelin concentrations in this study in accordance with a previous study where exercise intensity was greater (Gordon et al., 2007).

Leptin is an adipocyte derived hormone that promotes satiety and decreases feed intake (Spiegelman and Flier, 2001), and it has been found that plasma leptin concentration

increased with increasing body condition score in horses (Buff et al., 2002). There was no effect of diet on plasma leptin concentration in this study when the horses were measured under resting conditions (Jensen *et al*., 2016), and feeding has been found to both increase (Cartmill *et al*., 2005) and decrease (Gordon and McKeever, 2005) plasma leptin concentration. In this study, exercise increased plasma leptin concentration. Similar results have been reported by Gordon *et al.*, (2006), who suggested that leptin might be involved in suppressed feed intake in horses in training. However, no differences in feed intake wereobserved in the present study in relation to exercise.

This study presents some basic results where feeding and exercise were standardized. Future research should focus on the effect of different diets and feeding management pre- and post-exercise in relation to different types of performance (e.g. aerobic vs. anaerobic exercise or short vs. long term exercise) and recovery from exercise (e.g glycogen replenishment). Knowledge on the factors affecting fluctuations in metabolite and hormone concentrations is important e.g. when interpreting results from field studies, where this cannot be controlled. This study showed that time of sampling in relation to diet and exercise had no effect on the plasma concentrations of ghrelin and IGF-1, whereas exercise increased the plasma concentration of leptin. Furthermore, diet influenced differences in the plasma concentration of SCFA more than exercise did.

References:

Bach Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology 67: 319–338.

Buff, P.R., Dodds, A.C., Morrison, C.D., Whitley, N.C., McFadin, E.L., Daniel, J.A., Djiane, J. and Keisler, D.H., 2002. Leptin in horses: Tissue localization and relationship between peripheral concentrations of leptin and body condition. Journal of Animal Science 80: 2942-2948.

Cartmill, J.A., Thompson Jr., D.L., Storer, W.A., Crowley, J.C., Huff, N.K., Waller, C.A., 2005. Effect of dexamethasone, feeding time, and insulin infusion on leptin concentrations in stallions. Journal of Animal Science 83: 1875-1881.

Coverdale, J.A., Moore, J.A., Tyler, H.D. and Miller-Auwerda, P.A., 2004. Soybean hulls as an alternative feed for horses. Journal of Animal Science 82: 1663-1668.

Ford, E.J.H. and Simmons, H.A., 1985. Gluconeogenesis from caecal propionate in the horse. British Journal of Nutrition 53: 55-60.

Garner, H.E., Coffman, J.R., Hahn, A.W., Hutcheson, D.P. and Tumbleson, M.E., 1975. Equine laminitis of alimentary origin: an experimental model. American Journal of Veterinary Research 36: 441-444.

Gordon, M.E. and McKeever, K.H., 2005. Diurnal variation of ghrelin, leptin, and adiponectin in Standardbred mares. Journal of Animal Science 83: 2365-2371.

Gordon, M.E. and McKeever, K.H., 2006. Oral and intravenous carbohydrate challenges decrease active ghrelin concentrations and alter hormones related to control of energy metabolism in horses. Journal of Animal Science 84: 1682-1690.

Gordon, M.E., McKeever, K.H., Bokman, S., Betros, C.L., Manso-Filho, H., Liburt, N. and Streltsova, J., 2006. Interval exercise alters feed intake as well as leptin and ghrelin concentrations in Standardbred mares. Equine Veterinary Journal 38: 596-605.

Hudson, J.M., Cohen, N.D., Gibbs, P.G. and Thompson, J.A., 2001. Feeding practices associated with colic in horses. Journal of American Veterinary Medical Association 219: 1419-1425.

Ingerslev, A.K., Theil, P.K., Hedemann, M.S., Lærke, H.N. and Bach Knudsen, K.E., 2014. Resistant starch and arabinoxylan augment SCFA absorption, but affect postprandial glucose and insulin responses differently. British Journal of Nutrition 111: 1564-1576.

Jansson, A. and Lindberg, J.E., 2012. A forage-only diet alters the metabolic response of horses in training. Animal 6: 1939-1946.

Jensen, R.B., Austbø, D., Blache, D., Bach Knudsen, K.E. and Tauson, A.H., 2016. The effect of feeding barley or hay alone or in combination with molassed sugar beet pulp on the metabolic responses in plasma and caecum of horses. Animal Feed Science and Technology 214: 53-65.

Jensen, R.B., Austbø, D., Bach Knudsen, K.E. and Tauson, A.H., 2014. The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. Animal 8: 1788-1796.

Jose-Cunilleras, E., Hinchcliff, K.W., Sams, R.A., Devor, S.T. and Linderman, J.K., 2002. Glycemic index of a meal fed before exercise alters substrate use and glucose flux in exercising horses. Journal of Applied Physiology 92: 117-128.

Karlsson, C.P., Jansson, A., Essèn-Gustavsson, B. and Lindberg, J.E., 2002. Effect of molassed sugar beet pulp on nutrient utilisation and metabolic parameters during exercise. Equine Veterinary Journal 34: 44-49.

Larsson, K., Bengtsson, S., 1983. Bestämning av lättilgängeliga kolhydrater i växtmaterial (Determination of readily available carbohydrates in plant material). Chemistry Methods Report no. 22. National Laboratory of Agricultural Chemistry, Uppsala, Sweden.

Luthersson, N., Nielsen, K.H., Harris, P. and Parkin, T.D.H., 2009. Risk factors associated with equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. Equine Veterinary Journal 41: 625-630.

McKeever, K.H., 2011. Endocrine Alterations in the Equine Athlete: An Update. Veterinary Clinics of North America: Equine Practice 27: 197-218.

Moore-Colyer, M.J.S., Hyslop, J.J., Longland, A.C. and Cuddeford, D., 2002. The mobile bag technique as a method for determining the degradation of four botanically diverse fibrous feedstuffs in the small intestine and total digestive tract of ponies. British Journal of Nutrition 88: 729-740.

NRC 2007. Nutrient requirements of horses, 6th edition The National Academies Press, Washington, DC, USA.

Spiegelman, B.M. and Flier, J.S., 2001. Obesity and the regulation of energy balance. Cell 104: 531-543.

van der Kolk, J.H., 2014. Endocrine function during exercise and response to training. In: Hinchcliff, K.W., Kaneps, A.J., Geor, R.J. (ed). Equine sports medicine and surgery. Sunders, London, UK. pp. 769-785.

Vervuert, I., Voigt, K., Hollands, T., Cuddeford, D. and Coenen, M., 2009. Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. The Veterinary Journal 182: 67-72.

Von Engelhardt, W., Rönnau, K., Rechkemmer, G. and Sakata, T., 1989. Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. Animal Feed Science and Technology 23: 43-53.

Willing, B., Vörös, A., Roos, S., Jones, C., Jansson, A. and Lindberg, J.E., 2009. Changes in faecal bacteria associated with concentrate and forage-only diets fed to horses in training. Equine Veterinary Journal 41: 908-914.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillo, W.S., Ghatei, M.A. and Bloom, S.R., 2001. Ghrelin enhances appetite and increases food intake in humans. Journal of Clinical Endocrinology and Metabolism 86: 5992-5997.

Figure 1 Feeding fibre based diets (open bars □) or starch based diets (solid bars ■) to horses and the effects on concentrations of (a) plasma glucose (mM), (b) plasma total protein (g/L), (c) plasma urea (mM), (d) plasma lactate (mM), (e) plasma triglyceride (mM), (f) plasma NEFA (µekv./L) and (G) plasma BOHB (mM). Results are presented as least square means and standard error of the mean. The horses are fed at 06:00 and exercised from 10:00 to 10:45 on a treadmill. [a,b] indicate differences between diets at specific time points.

Figure 2 Feeding fibre based diets (open bars □) or starch based diets (solid bars ■) to horses and the effects on concentrations of (a) plasma total SCFA (µM), (b) plasma acetate (µM), (c) plasma propionate (µM), (d) plasma butyrate (µM),(e) plasma iso-butyrate (µM), (f) plasma valerate (µM), (g) plasma iso-valerate (µM) and (h) plasma caporate (µM) after feeding. Results are presented as least square means and standard error of the mean. The horses are fed at 06:00 and exercised from 10:00 to 10:45 on a treadmill. [a,b] indicate differences between diets at specific time points.

Figure 3 Feeding fibre based diets (open bars □) or starch based diets (solid bars ■) to horses and the effects on concentrations of (a) plasma insulin (µU/ml), (b) plasma cortisol (ng/ml), (c) plasma leptin (ng/ml), (d) plasma ghrelin (pg/ml) and (e) plasma IGF-1 (ng/ml). Results are presented as as least square means and standard error of the mean. The horses are fed at 06:00 and exercised from 10:00 to 10:45 on a treadmill. [a,b] indicate differences between diets at specific time points.