A forage-only diet alters the metabolic response of horses in training

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Running head: Exercise response in horses on forage-only diet.

Abstract

Most athletic horses are fed a high-starch diet, despite the risk of health problems. Replacing starch concentrate with high-energy forage would alleviate these health problems, but could result in a shift in major substrates for muscle energy supply from glucose to short-chain fatty acids (SCFA) due to more hindgut fermentation of fibre. Dietary fat inclusion has previously been shown to promote aerobic energy supply during exercise, but the contribution of SCFA to exercise metabolism has received little attention. This study compared metabolic response to exercise and lactate threshold (V_{La4}) in horses fed a forage-only diet (F) and a more traditional

high-starch, low-energy forage diet (FC). The hypothesis was that diet F would increase plasma acetate concentration and increase V_{La4} compared with diet FC. Six Standardbred geldings in race training were used in a 29 day change-over experiment. Plasma acetate, non-esterfied fatty acids (NEFA), lactate, glucose and insulin concentrations and venous pH were measured in samples collected before, during and after a treadmill exercise test (ET, day 25) and muscle glycogen concentrations before and after ET. Plasma acetate concentration was higher before and after exercise in horses on diet F compared with diet FC and there was a tendency (P=0.09) for increased V_{La4} on diet F. Venous pH and plasma glucose concentrations during exercise were higher in horses on diet F than diet FC, as was plasma NEFA on the day after ET. Plasma insulin and muscle glycogen concentrations were lower for diet F, but glycogen utilisation was similar for the two diets. The results show that a high-energy, forage-only diet alters the metabolic response to exercise and, with the exception of lowered glycogen stores, appears to have positive rather than negative effects on performance traits.

Keywords: acetate, blood pH, exercise, insulin, muscle glycogen

Implication

It is a serious animal welfare issue that diets associated with gastrointestinal disorders and abnormal and stereotypical behaviour are fed to horses today. There is an urgent need for diets that support the natural digestive function and behaviour of horses. Such diets would reduce welfare problems and healthcare costs for the horse industry.

Introduction

Current practice in feeding performance horses world-wide is to use fairly late-cut forage, whereas for example in dairy production early-cut, high-energy forage is used. The high energy requirements of many performance horses means that supplementation with more energy-dense feeds is necessary and more than 40% of the diet commonly consists of cereal-based, high-starch concentrates (Glade, 1983;

53 Redbo *et al.*, 1998; Williamson *et al.*, 2007). This is a serious animal welfare issue,
54 since low forage to concentrate ratios and high starch intake are associated with
55 reduced gut microbial stability (Willing *et al.*, 2009), gastrointestinal disorders (Tinker
56 *et al.*, 1997; Hudson *et al.*, 2001; Luthersson *et al.*, 2009) and abnormal and
57 stereotypical behaviour (Gillham *et al.*, 1994, Redbo *et al.*, 1998; Waters *et al.*,
58 2002). In addition, rhabdomyolysis has been associated with high concentrate
59 intakes (MacLeay *et al.*, 1999). We have shown in earlier studies that Standardbred
60 horses in training can maintain body weight and condition on high-energy, forage-
61 only diets (Connysson *et al.* 2006; Muhonen *et al.,* 2009; Connysson *et al.,* 2010).
62 We found no detrimental effect of increased crude protein (CP) intake (which may be
63 associated with the use of high-energy forages) on plasma lactate concentration and
64 pH during exercise, but urinary pH decreased and evaporative losses tended to
65 increase (Connysson *et al.*, 2006). However, the effect on exercise performance of
66 forage-only diets compared with the traditional forage-concentrate diet has not yet
67 been studied.

68 From a physiological point of view the horse is adapted to continuous grazing of
69 a forage-only diet (low in starch content) and has a well-developed symbiosis, with

70 the hindgut microbiota fermenting the forage fibres. This results in the production of
71 energy-yielding substrates in the form of short-chain fatty acids (SCFA) and the
72 proportion of acetate increases and propionate decreases when forage:concentrate
73 ratio is increased (Hintz *et al.*, 1971; Willard *et al.*, 1977). This suggests that horses
74 are well-adapted to rely on fat metabolism and aerobic energy supply at rest, but
75 probably also during exercise. The adaptation to, and importance of, aerobic
76 substrate utilisation during exercise in Thoroughbred and Standardbred horses has
77 been documented in a number of studies (Lindholm and Phiel, 1974; Wilson *et al.*,
78 1987; Essén-Gustavsson *et al.*, 1989) and is also confirmed by the correlation
79 between the performance of Standardbred horses and plasma lactate threshold
80 (V_{La4}) (Persson, 1983). Equine muscle also shows high plasticity with respect to its
81 adaptation to energetic demands as a result of exercise training (Voiton *et al.*, 2007).
82 Therefore, it appears reasonable to assume that the substrate profile for efficient
83 muscle energy metabolism in horses will comprise glucose, long-chain FA from the
84 diet or from body lipid stores (Pagan *et al.*, 2002; Geor, 2006) and SCFA from
85 hindgut fermentation of dietary fibre (Palmgren-Karlsson *et al.*, 2002). The aim of this
86 study was to compare the effects of a high-energy forage-only diet with those of a

50:50 (DM basis) forage:concentrate (starch-rich) diet in terms of lactate threshold (V_{La4}), muscle glycogen concentration and metabolic plasma profile in Standardbred horses in training. The hypothesis was that the forage-only diet would increase plasma acetate concentration and V_{La4}.

Materials and methods

Horses

Six Standardbred geldings in race training were used, aged 6.5 ± 0.4 years (mean ± SD). The average number of races in which the horses had competed was 27 ± 8 and the average racing record was 77.3 ± 0.8 s/1000 m. They had an initial body weight (BW) of 515 ± 21 kg. The horses were kept at a training camp for harness racing 20 km south of Uppsala, Sweden. They were housed in individual stalls on wood shavings during the night and were kept together in a sand/clay paddock between 08:00-15:00 h on days without training. All horses had passed a flexure test prior to the study and were regarded as healthy. The experiment was approved by

103 the Uppsala local ethics committee and was conducted in the period October-
104 December 2007.
105
106 **Experimental design**
107 *Diets*
108 The horses were offered a forage-only diet (F) consisting of early-cut haylage
109 (timothy, meadow fescue mixture) (Table 1) and a mixed diet (FC) consisting of late-
110 cut haylage (timothy, meadow fescue mixture) supplemented with concentrate (50:50
111 dry matter basis) in a change-over design experiment with 29-day experimental
112 periods. Feed allowance was based on individual BW and was 13-17.4 kg haylage
113 and 180-240 g sugar (only to ensure complete intake of the salt, mineral and vitamin
114 supplements) for diet F and 6.3-8.4 kg haylage, 5.3-7.1 kg oats, 0.9-1.2 kg soy bean
115 meal, 0.18-0.24 kg wheat bran and 90-120 g sugar for diet FC. The diets were
116 estimated to be iso-caloric and iso-nitrogenous, and provided energy and nutrients
117 according to requirements specified by NRC (1989). Horses on both diets were
118 offered a mineral and vitamin supplement (51 ± 2 g/day, Miner Röd, Krafft,
119 Falkenberg, Sweden), NaCl (36 ± 1 g/day) and those on diet FC ground chalk

120 (calcium carbonate, 34 ± 1 g/day) to meet mineral and vitamin requirements specified
121 by NRC (1989). Water was provided *ad libitum* from graded buckets. The forage
122 allowance was fed in the afternoon and the concentrate and mineral and vitamin
123 supplement (diet FC) at 15.00, 23.00 and 06.00 h. With diet F, the mineral and
124 vitamin supplement was fed at 23.00 and 06.00. Diet FC was introduced gradually
125 during the experimental period (on days 1 and 2, horses were fed 50% of the F diet
126 and 50% of the FC diet, and then the FC diet was increased by 10% per day until the
127 full ration was reached on day 7). Diet F was introduced abruptly on day 1.
128
129 *Training*
130 The horses were given sub-maximal warm-up (3.5-5 km, heart rate < 200 beats/min)
131 and intensive training (heart rate > 200 beats/min) on an oval or straight field track
132 (approx. 0.6% incline) on days 2, 5, 7, 9, 13, 17, 21, 25 and 29 in order to maintain
133 but not improve fitness. The intensive training consisted of interval training (4
134 intervals of 600 m) or 1600-2000 m heats and the training protocol was exactly the
135 same in both periods.
136

137 *Exercise test*

138 On day 25 of each experimental period, the horses performed a standardised
139 incremental exercise test (ET) on a treadmill (Säto, Knivsta, Sweden) located at a
140 veterinary clinic 25 km from the training camp. The horses were transported to the
141 clinic by trailer. All horses had prior experience of exercise on this treadmill (minimum
142 2 occasions).

143 Prior to ET, the horses were kept together in the paddock from 07:30 to 10:30 h
144 and were then offered 1 kg of forage (diet F) and 1 kg of oats (diet FC). After this,
145 water but no feed was offered until the horses returned to the stable at about 24:00 h.
146 This design was chosen because practical experience indicated that many horses do
147 not consume any feed while at the racetrack. On the day after ET, all horses were
148 fed at 06.00 h as usual, but were kept in their stalls until 11:00 h.

149 Exercise testing consisted of two phases. The first phase (warm-up, walk at 2
150 m/s for 3 minutes, trot 6 m/s for 5 min, trot 9.5 m/s for 5 min and walk 2 m/s for 3 min,
151 no incline) was performed 25 min prior to the second phase, which consisted of 5
152 minutes of walk (0% incline) and then a stepwise increase in velocity (6.0, 7.0, 8.0,
153 9.0, 9.5 m/s) at an incline of 6.3% every second minute until the horses reached or

exceeded a heart rate of 200 beats/min. Four horses reached 9.0 m/s and two reached 9.5 m/s. The horses walked for 5 min on the treadmill after the final step.

Sampling

Feed and water intake, body weight and condition

Samples of concentrate were collected from each batch used in every period and samples of forage from each new bale opened and kept frozen at -20ºC until analysis. The forage samples were pooled and analysed as one sample per feed and period. Feed leftovers were weighed and eliminated every day. Daily nutrient and energy intake was calculated using feed intake data and analysis of feeds and leftovers. Water intake (drinking) was measured on days 19 to 28.

Body weight was recorded before each training session, before the warm-up prior to ET, immediately after ET, on the day after ET (at 11.00 h) and on days 27-29 (afternoon). Changes in body condition were assessed by a simple recording of whether the ribs were easy to palpate or not and whether they were visible during motion.

Exercise test

Heart rate was recorded by a heart frequency meter (Polar RS800, Kempele, Finland) during exercise and manually post-exercise with a stethoscope. Heart rate was measured before warm-up, during warm-up (9.5 m/s), immediately after warm-up, after 25 minutes of rest, during each incremental step, after 5 minutes walking on the treadmill and 10, 25, 55 and 85 minutes after the end of walking.

Breathing frequency was recorded before warm-up, directly after warm-up, after 25 minutes of rest, after 5 minutes walking on the treadmill and 10, 25, 55 and 85 minutes after the end of walking. Rectal temperature was recorded before warm-up, directly after warm-up, after 25 minutes of rest and 10, 25, 55, 85 minutes after the end of walking.

A jugular catheter was introduced under local anaesthesia (Carbocaine 20 mg/ml, Astra Zeneca AB, Sweden) before transport to the clinic and blood samples (20 ml/sample) were collected in Li-heparinised tubes before warm-up, immediately after warm-up, after 25 minutes of rest, at the end of each incremental step, after 5 minutes walking on the treadmill and 10, 25, 55 and 85 minutes after the end of walking. A final blood sample was taken on the day after ET, at 11:00 h. The blood

188 samples were kept chilled until centrifuged and frozen at -20C° for later analysis.
189 Samples collected for analysis of blood pH, TCO_2 and HCO_3 were analysed within
190 10 min of collection.

191 Muscle biopsies were taken from *m. gluteus medius* at a depth of approximately 6
192 cm according to the method described by Lindholm and Piehl (1974). A local
193 anaesthetic (Carbocain 20 mg/ml, Astra Zeneca AB, Sweden) was applied to the
194 area and a nose twitch was used. Biopsies were taken before warm-up and
195 immediately after ET, frozen in liquid nitrogen and stored at -80ºC until analysis.

196

197 **Analyses**

198 Preparation and conventional chemical analyses (dry matter (DM), nitrogen (N),
199 neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, water-soluble
200 carbohydrate (WSC) and ash) of feeds were performed as described by Palmgren-
201 Karlsson *et al.* (2000). Minerals were analysed with inductively coupled plasma
202 optical emission spectrometry (ICP-OES) (SS-EN 14538:2006, Ametek Spectro,
203 Kleve, Germany). The metabolisable energy content of the forage was estimated by
204 an *in vitro* method (Lindgren, 1979).

205

206 *Analyses of plasma, blood and muscle*

207 Plasma insulin was analysed using an ELISA method (Mercodia equine insulin kit,
208 Mercodia, Uppsala, Sweden). For quantitative determination of non-esterified fatty
209 acids (NEFA), an enzymatic colorimetric method was used (ACS-ACOD method,
210 Wako Chemicals GmbH, Neuss, Germany). Plasma urea, acetate and glucose
211 concentrations were analysed with an enzymatic colorimetric/UV-method (Boehringer
212 Mannheim/R-Biopharm, Darmstadt, Germany) and lactate using an ELISA method
213 (R-Biopharm GmbH, Darmstadt, Germany). Venous pH, TCO_2 and HCO_3 were
214 analysed using an i-STAT®1 analyser (Abbot Laboratories, Abbot Park, Illinois, USA).
215 Total plasma protein (TPP) concentration was measured by refractometer (Atago,
216 Sur-Ne, Tokyo, Japan) in samples taken before warm-up, before ET, 55 min after
217 walking and the day after walking.

218 Muscle samples for glycogen analysis were freeze-dried and dissected free
219 from visible blood, connective tissue and fat under a microscope before analysis. A
220 sample of about 1-2 mg muscle fibre was boiled in 1 M HCl and glucose residues
221 were determined by fluorescence (Lowry and Passonneau, 1973).

222

223 ### Calculations and statistical analysis

224 The velocity at plasma lactate concentration 4 mmol/l (V_{La4}) was calculated
225 individually from an exponential curve fitted by Microsoft Office Excel 2007.

226 All data were subjected to analysis of variance (GLM procedure in the Statistical
227 Analysis Systems package 9.1) (SAS Institute Inc. Cary, NC, USA) using the
228 following model: $Y_{i\ j\ k} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_l + (\beta\gamma)_{j\ k} + e_{i\ j\ k\ l}$, where $Y_{i\ j\ k}$ is the
229 observation, μ the mean value, α_i the effect of animal, β_j the effect of diet, γ_k the
230 effect of sample, ε_l the effect of period, $(\beta\gamma)_{j\ k}$ the effect of interaction between diet
231 and sample and $e_{i\ j\ k\ l}$ the residuals; $e_{i\ j\ k\ l} \sim IND\ (0, \delta^2)$. The value used for
232 significance within and between treatments was $P<0.05$. Values are presented as
233 least square mean $\pm$ standard error of the mean.

234

235 ## Results

236 *General observations*

237 All horses completed the study. No health problems were observed, except for one
238 horse which developed a fever two days into period 1 (resulting in three missed

239 training sessions). There were leftovers on both diets (Table 2) and forage intake
240 corresponded to 69% of the allowance on both diets, resulting in a
241 forage:concentrate ratio of 40:60 for diet FC. A comparison of the *in vitro* digestible
242 organic matter (IVDOM) of the individual leftovers and the feed samples collected on
243 days 20-22 showed consistently lower IVDOM in the leftovers compared with the
244 feed for diet F, but not diet FC (Diet F: feed=78.3%, leftovers=73.7-76.9%; diet FC:
245 feed=66.9%, leftovers=63.7-69.6%), indicating selective feed intake in all individuals
246 on diet F.

247 Body condition was similar in four individuals on both diets but two individuals
248 had higher body condition on diet FC (horse 1 changed from 'ribs visible at motion' to
249 'not visible but easy to palpate' and horse 2 changed from 'ribs not visible at motion
250 but easy to palpate' to 'not visible, not easy to palpate'). Mean BW was higher
251 (P=0.0006) for horses on diet F (519.0 ± 0.5 kg) than diet FC (516.0 ± 0.5 kg). Water
252 intake was higher with diet F than diet FC (30.0 ± 0.5 *vs* 19.0 ± 0.5 l/day, P<0.0001).

253

254 *Exercise test*

255 There was no significant difference in V_{La4} between diets, although there was a
256 tendency (P=0.086) for higher V_{La4} in horses on diet F (8.0 ± 0.1 *vs* 7.6 ± 0.1 m/s).
257 Plasma lactate concentration was higher (P<0.05) in horses on diet FC compared
258 with diet F at the end of the 5-min walk and 10 min after the walk on the treadmill
259 (diet FC: 8.1 ± 0.4 and 4.4 ± 0.4 *vs* diet F: 5.7 ± 0.4 and 3.2 ± 0.4 mmol/l). Venous
260 pH (Figure 1), TCO_2 and HCO_3 (P=0.0001) in connection with exercise were higher
261 in horses on diet F compared with diet FC (pooled data for TCO_2: 31.2 ± 0.2 *vs* 30.3
262 ± 0.2 mmol/l and HCO_3: 30.0 ± 0.2 *vs* 29.1 ± 0.2 mmol/l). Acetate concentration was
263 higher with diet F than diet FC (P=0.0001) and plasma NEFA concentration on the
264 day after ET was higher (P<0.05) with diet F compared with diet FC (Figure 2).
265 Plasma insulin concentration was higher (P=0.0001) in horses on diet FC compared
266 with diet F (Figure 2) and glucose concentration was higher (P<0.05) with diet F
267 immediately after warm-up and at 9 m/s, but lower (P<0.05) on the day after ET
268 (Figure 2). Muscle glycogen content was higher (P<0.05) in horses on diet FC than
269 diet F both before (644 ± 22 *vs* 560 ± 22 mmol glucosyl units/kg dry weight) and after
270 exercise (546 ± 22 *vs* 473 ± 22 mmol glucosyl units/kg dry weight). There were no
271 differences between the diets in heart rate (109 ± 1 and 111 ± 1 beats/min in F and

272 FC, respectively), breathing frequency (62 ± 3 and 68 ± 3 beats/min in F and FC,
273 respectively), or rectal temperature (38.5 ± 0.1 and 38.5 ± 0.1 beats/min in F and FC,
274 respectively), before, during and after exercise (pooled data).

275 There were no differences in BW between diets before (diet FC: 509 ± 1 and
276 diet F: 511 ± 2 kg) and after warm-up and ET (diet FC: 501 ± 1.0 and diet F: 500 ± 1
277 kg). The pre-exercise BW was not recovered until day 28 on both diets. Mean TPP
278 during ET day and on the day after was lower with diet F than diet FC (64.6 ± 0.6 *vs*
279 67.0 ± 0.6 g/l).

280

281 Discussion

282 Horses fed the forage-only (F) diet showed an altered metabolic response during
283 exercise compared with horses on the forage-concentrate diet and few, if any, effects
284 likely to impair performance were observed. There was an increase in plasma
285 acetate concentration on the F diet, as expected. Moreover, with this diet plasma
286 lactate concentration was significantly lower post-exercise and there was a tendency
287 for higher V_{La4}, indicating that aerobic energy utilisation may have improved. In
288 addition, venous pH increased during exercise in horses on the F diet, which could

289 counteract the acidosis induced by the exercise typically performed by racehorses.
290 This indicates that high-energy, forage-only diets are an interesting alternative to
291 conventional diets.
292
293 The increased plasma levels of acetate in horses on the F diet can be explained by
294 more extensive hindgut fermentation due to higher fibre intake, and subsequent
295 production of SCFA (Hintz *et al.*, 1971; Willard *et al.*, 1977). SCFA absorbed from the
296 hindgut can be used as substrates in body tissue metabolism. Propionate may be
297 primarily used to produce glucose in the gluconeogenetic pathway, while acetate and
298 butyrate can be transformed to acetyl-CoA and then utilised as substrates for aerobic
299 energy metabolism in the tri-carboxylic pathway in the muscle (Voiton *et al.*, 2007).
300 The latter also applies to long-chain fatty acids (LCFA) that are metabolised through
301 β-oxidation in the muscle. It has been suggested that long-term feeding of
302 supplemental fat to exercising horses increases the mobilisation and speed of
303 mobilisation of free fatty acids (FA), increasing the speed of uptake into muscle of
304 free FA, lowering lactic acid production, imparting a glycogen-sparing effect and
305 increasing pre-exercise muscle glycogen levels (Potter *et al.*, 1992; Harris and

306 Harris, 2005). However, there is a large variation in the effects reported in the
307 literature and only a few studies report direct benefits on performance traits (Geor,
308 2006). Pagan *et al.* (2002) used a stable glucose isotope and showed that a high-fat
309 diet can be glucose-sparing by increasing FA oxidation from body lipid stores during
310 exercise, suggesting that performance could be improved. In the present study no
311 clear effect of the F diet on performance was observed, but there was a lowering of
312 post-exercise plasma lactate concentration, confirming previous findings for a high-
313 fibre diet (Palmgren-Karlsson *et al.*, 2002).
314
315 Waller and Lindinger (2007) reported profound plasma alkalosis, i.e. a decrease in
316 venous plasma hydrogen concentration and an increase in plasma TCO_2 and HCO_3^-,
317 after post-exercise oral administration of sodium acetate. These results were
318 confirmed in the present study, where venous pH, TCO_2 and HCO_3^- concentrations
319 were higher in horses on the F diet. The alkalising effect of a forage-only diet may
320 also be a direct result of the increased plant cell and organic acid intake typical of
321 herbivores (Houpt, 1989). Plant cells contain organic anions (citric, oxalic, malonic
322 and fumaric acids) that are electrically balanced by potassium and other cations.

323 Citrate and other organic anions are oxidised to CO_2 and H_2O but their breakdown
324 requires hydrogen ions, which are derived from the hydration of carbon dioxide.
325 Thus, as organic anions are oxidised, HCO_3 is produced, which has an alkalotic
326 effect on body fluid pH (Houpt, 1989). The possibility of counteracting the acidosis
327 induced by intensive exercise by oral supplementation (generally with salts of HCO_3^-)
328 has been studied for a long time, but the effects on performance are not unequivocal
329 (Kelso *et al.*, 1987; Lawrence *et al.*, 1987; Schuback *et al.*, 2002). This may be the
330 first study to show that there is a natural way of achieving this effect, although a
331 positive effect on performance remains to be proven.
332
333 Plasma glucose levels were higher during exercise in horses on the F diet. The origin
334 and importance of this elevation in glucose concentration for exercise performance is
335 not clear, but endurance during both maximal (Lacombe *et al.*, 2001) and
336 submaximal (Farris *et al.*, 1998) exercise is improved by glucose infusion, indicating
337 that high blood glucose availability could improve performance. Alterations in glucose
338 metabolism (both in tissue uptake and release from the liver) are likely to occur in
339 horses on forage-only diets, based on the low insulin levels observed here and

340 earlier (Connysson *et al.*, 2010), and might be the reason for the elevated glucose
341 levels during exercise. However, on the day after exercise tests the plasma glucose
342 concentration was lower in horses on the F diet and the NEFA concentration was
343 higher. The importance of this is also unclear, but it might reflect the demand for
344 glucose for resynthesis of glycogen and low dietary glucose availability (total daily
345 intake of WSC and starch was approximately 860 g and 3100 g/day on diet F and
346 FC, respectively) and increased utilisation of body fat for maintenance.
347
348 The low WSC intake might also have affected muscle glycogen synthesis (Lacombe
349 *et al.*, 2004). Muscle glycogen content was lower (-13%) before and after ET in
350 horses on the F diet, while relative glycogen depletion was similar. It is known that
351 glycogen synthase is activated by insulin (Devlin and Horton, 1985) and the low
352 insulin levels on the F diet might have been a limiting factor for glycogen synthesis.
353 Further studies are needed to determine whether total muscle glycogen storage
354 capacity is reduced on a forage-only diet or whether it is only the rate of synthesis
355 that is reduced. It is important to note that all horses had trained approximately 96 h
356 before ET and glycogen recovery might not have been complete. It is well-

357 documented that post-exercise recovery of glycogen stores takes several days in
358 horses (Snow *et al.*, 1987; Hyyppä *et al.*, 1997, Lacombe *et al.*, 2004). While an
359 exercise-induced reduction in muscle glycogen content of 80% has been shown to
360 impair endurance during high-intensity exercise (Lacombe *et al.*, 2001), the
361 importance of a smaller reduction, such as that induced by the F diet in the present
362 study, is not known. Interestingly, oral acetate supplementation of a typical hay-grain
363 diet has been shown to enhance the rate of glycogen re-synthesis during the initial 4-
364 h recovery period after muscle glycogen depletion (Waller *et al.*, 2009), but our data
365 suggest that increased acetate availability does not maximally support glycogen
366 repletion between training sessions. However, a recent study (Essén-Gustavsson *et*
367 *al.*, 2012) of horses on forage-only diets with different CP content showed that high
368 CP forage increased muscle glycogen content compared with a forage providing the
369 CP intake recommended by NRC (2007). This shows that CP is important for
370 glycogen content and might have increased the glycogen content in horses on diet
371 FC in the present study.
372

373 One argument against using forage-only or high-forage diets for performance horses
374 is the risk of an unwanted increase in BW. This is probably based on the common
375 perception that fibre is ‘bulk’ and perhaps also on knowledge of the water-holding
376 capacity of plant fibres. In a study on riding horses (Ellis *et al.*, 2002), a forage-only
377 diet increased body weight and heart rate during submaximal exercise, suggesting
378 that performance might be impaired. However, it should be noted that the energy
379 content of the forage in that study was not high enough to support the energy needs
380 of horses in race training and, accordingly, the digestibility was not high enough,
381 thereby causing ‘bulk weight gain’. The present study showed no (ET days) or a
382 limited (3 kg in pooled data) increase in BW in horses fed a high-energy forage diet.
383 There was also no effect on heart rate and breathing frequency. It is likely that
384 differences in chemical composition between forages are the reason for the
385 differences in BW change in these studies. In the present study, the forage in diet F
386 was early-cut and had high fibre digestibility, as reported by Ragnarsson and
387 Jansson (2011) for the same batch of forage.
388

389 Part of the increase in BW observed on a forage-only diet could also be due to
390 increased plasma volume, as indicated by the lowered TPP. It has been suggested
391 that the hindgut serves as a fluid reservoir and that fibre-rich diets increase this
392 reservoir (Meyer, 1987). The change in TPP with altered fibre intake could therefore
393 reflect changes in the equilibrium between the gut and the extracellular fluid. It has
394 been shown that increased forage intake lowers TPP (Danielsen *et al.*, 1995) and
395 also that signs of dehydration (increased TPP) following feed deprivation are delayed
396 in horses on a forage-only diet compared with those on a mixed diet (Connysson *et*
397 *al.*, 2010). However, BW recovery was similar on both diets in the present study. The
398 results also showed that it may take two to three days for horses transported to an
399 exercise event to recover their body weight. The loss of BW on ET day was due to
400 lack of feed intake (less than 60% of the allowance) and to fluid and faeces losses
401 during transportation and exercise.
402
403 There is also anecdotal information that forage intake capacity is limited in
404 performance horses. Horses in the present study consumed forage corresponding to
405 1.95% of BW and four of the horses maintained similar body condition on both diets.

406 It is possible that the other two horses would have been maintained on a forage diet
407 with slightly higher energy content (around 11 MJ ME/kg dry matter). In all horses on
408 the forage-only diet, the maintenance of energy intake and body condition was to
409 some extent due to selection of the forage offered, as reflected in lower digestibility of
410 leftovers from this diet. This contributed to higher energy intake on the forage-only
411 diet and was probably an attempt to maximise energy intake.
412
413 Another concern about a forage-dominated diet for athletic horses is the increased
414 heat increment of feeding. In the present study this was probably reflected in the
415 higher water intake on diet F. However, body temperature was similar, as would have
416 been expected in a homeothermic animal, and it was not possible to draw any
417 conclusions about evaporative fluid losses during exercise from the results, since
418 only BW was measured before and after exercise (including sweat, faecal and
419 respiratory losses).
420
421 No feed-related clinical or behavioural disturbances very observed during the study.
422 Exercise temperament, novel object reaction, voluntary motion and post-exercise

423 feeding (Jansson, 2010) were also evaluated in the present study and numerical, but
424 not statistically significant, differences were detected, with the exception of
425 observations on post-exercise feed intake, which was more common on diet F.
426 However, subjectively more aggression was observed in horses fed diet FC,
427 especially in the afternoon when horses were fetched from the paddock (one at a
428 time) to be put in their boxes, where feed was available. Therefore, our impression is
429 that the FC diet might have affected behaviour (making horses more active and
430 reactive), although the recording system used could not verify this.
431
432 In conclusion, the present study indicates that a high-energy, forage-only diet alters
433 the metabolic response to exercise and, with the exception of lowered glycogen
434 stores, appears to have positive rather than negative effects on performance traits of
435 Standardbred horses.
436

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588 USA.

589 Table 1. Dry matter (DM, %), estimated energy (MJ ME/kg DM), chemical (g/kg DM) and

590 microbial composition (cfu[1]/g fresh matter) of feeds in the experimental diets

	Forage		Concentrate
	Diet F	Diet FC	Diet FC
DM	80	78	90
ME	10.4	8.8	11.4
Ash	75	56	37
Crude protein	104	61	174
Neutral detergent fibre	605	600	203
Acid detergent fibre	363	370	117
Lignin	51	69	27
EEC-fat	19	15	60
WSC[2]	79	147	
Free glucose	35	22	3
Free fructose	31	57	0
Fructans	4	51	5
Starch	0	0	358
Yeast	$\underline{<}$2.5	$\underline{<}$3.6	-
Mould	<2.0	$\underline{<}$2	-

591 [1]Colony-forming units, [2]Water-soluble carbohydrates

592

593 Table 2. Daily feed allowance, feed intake (kg) and nutrient (g) and estimated metabolisable
594 energy intake (MJ ME) during 29 days on a high-energy, forage-only diet (F) and a mixed
595 forage-concentrate diet (FC) (LSmeans ± SE)
596

	Diet F	Diet FC
Forage allowance	14.56 ± 0.04	7.00 ± 0.04
Forage intake	10.07 ± 0.09	4.88 ± 0.09
Concentrate allowance	0.29 ± 0.03	7.31 ± 0.03
Concentrate intake	0.29 ± 0.04	7.07 ± 0.04
CP intake[1]	1132 ± 87	1467 ± 66
NDF intake	6588 ± 507	3885 ± 270
Starch intake	0	2503 ± 108
WSC intake	861 ± 66	605 ± 60
Energy intake[2]	110 ± 6	116 ± 6

597 [1]Corresponds to 113-146% of the requirements for very heavy exercise suggested by NRC
598 (2007), [2]Corresponds to 90-94% of the requirements for very heavy exercise suggested by
599 NRC (2007).
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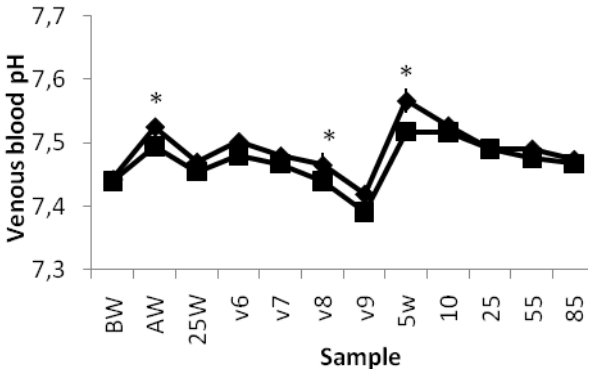
Figure legends

Figure 1. Venous pH before, during and after an incremental exercise test. Values (LSmeans ± SE) for six Standardbred geldings on a high-energy, forage-only diet (F; diamonds) and a 40:60 forage-concentrate diet (FC; squares). BW=before warm-up, AW=after warm-up, 25W=25 min after warm-up, v6-v9=incremental exercise test at velocities 6, 7, 8 and 9 m/s (treadmill incline 6.3%), 5w=after 5 min walk, 10, 25, 55, 85 min after the walk. The effect of diet was significant (ANOVA, P=0.0001). * indicates significant difference (P<0.05) between diets for single samples.

Figure 2. Plasma glucose[a], insulin[a], acetate[a] and non-esterified fatty acid (NEFA) concentrations before, during and after an incremental exercise test. Values (LSmeans ± SE) from six Standardbred geldings on a high-energy, forage-only diet (F; diamonds) and a 40:60 forage-concentrate diet (FC; squares). BW=before warm-up, AW=after warm-up, 25W=25 min after warm-up, v6-v9=incremental exercise test at velocities 6, 7, 8 and 9 m/s (treadmill incline 6.3%), 5w=after 5 min walk, 10, 25, 55, 85 min after the walk and DA=day after at 11.00. [a] =no analyses for v6-v8 available. The effect of diet was significant for acetate and insulin (ANOVA, P<0.0001). * indicates significant difference (P<0.05) between diets for single samples.

633 Figure 1



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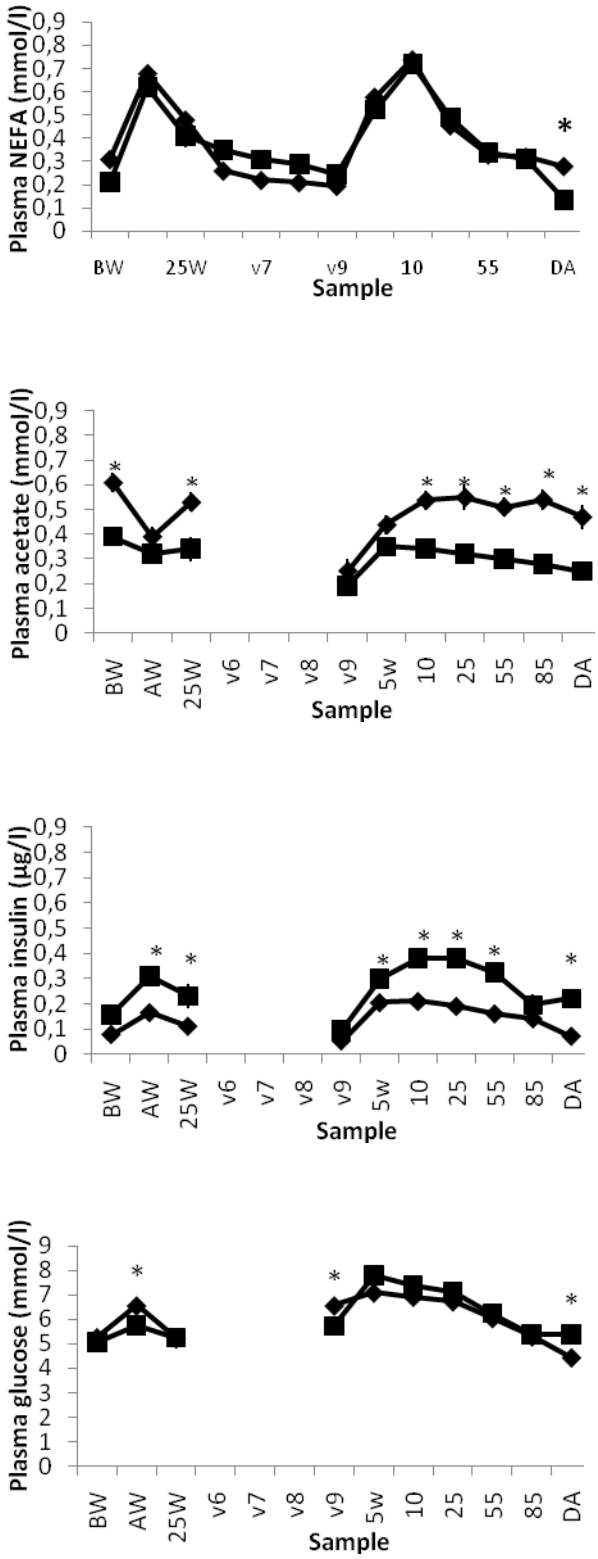
636 Figure 2



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