Effect of feeding Thoroughbred horses a high unsaturated or saturated vegetable oil supplemented diet for 6 months following a 10 month fat acclimation

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Summary

This study looked at the effect of feeding diets supplemented with either a predominantly saturated or unsaturated vegetable oil over a prolonged period to exercising horses.

Eight Thoroughbred horses were assigned to 2 diet treatments and for 10 months were fed Timothy hay and oats, together with a fortified sweet feed supplemented with either a predominantly unsaturated (Un) or a saturated (S) vegetable oil so that ~19% DE (Digestible Energy) came from dietary fat and ~12% from either the Un or S source (AC). An increased amount of Un or S fortified sweet feed, replacing the oats, was then fed for a further 6 months (HF) so that ~27%DE came from fat and ~20% from the Un or S vegetable oil. Standardised incremental treadmill exercise (8–12 m/s) tests (STEP) and duplicate oral glucose tolerance tests (TOL) were carried out after 3, 6 and 9 months of the AC diet and after 3 and 6 months on the HF diet. There was no significant effect of dietary treatment or when the tests were undertaken (time) on the insulin or lactate responses to the STEP tests. Overall there was a significant ($P<0.05$) effect of time and treatment on the glucose response, but there was no difference between treatments at the first and last tests or between the results for these tests or between the endAC and endHF tests. No significant effect of treatment or time was seen on the TOL glucose response (% change from Time '0') although there was a trend for the glucose concentrations to be lower and the insulin responses higher (nonsignificant) in the S treatment group. No significant effect of treatment on haematological parameters, monitored monthly, was found. Total protein and gamma glutamyl transferase remained within the normal range throughout. There was a significant effect of treatment ($P<0.05$) on cholesterol and triglycerides with higher concentrations in the S group from the first (1 month) sample. Linoleic acid was the main fatty acid in all the 4 plasma lipid classes with slightly, but significant ($P<0.05$), higher concentrations in Un for the cholesterol ester and phospholipid classes. There was no effect of time. Overall, the total resting plasma fatty acid content was significantly higher ($P<0.05$) with S at the sample points (endAC and endHF). No adverse effects of feeding either diet on apparent coat condition or hoof appearance were seen apart from an apparent increase in the grease score. Many of the parameters assessed showed significant improvements with time ($P<0.05$). In conclusion, no apparent adverse effects of feeding a diet supplemented with either an unsaturated or saturated vegetable oil for 6 months at ~20% DE after 10 months at ~12% DE were identified and there were no apparent disadvantages of feeding a saturated vegetable oil supplemented diet compared with an unsaturated one.

Introduction

Horses, which can be considered as monogastric or nonruminant herbivores, evolved to utilise high-fibre diets ingested almost continuously. The provision of energy from such fibre sources is not very efficient and the horse has digestive and metabolic limitations to high-grain, high hydrolysable carbohydrate diets. This has presented nutritional challenges following domestication and the requirement for repetitive, intensive or prolonged exercise (Harris 1997). Work carried out in the mid 1970s, which suggested that horses fed a fat-supplemented diet (9% added corn oil) performed better when ridden over mountainous terrain for 8–10 h (Slade *et al.* 1975), fuelled interest in the potential benefits of feeding supplementary fat to performance horses. Subsequent studies have resulted in variable effects on a range of physiological parameters as well as on athletic performance (partly due perhaps to the variation in the protocols, diets and animals used in the various studies, see Potter *et al.* 1992; Harris 1997). However, feeding fat-supplemented diets to horses has become relatively common today for horses in competitive work. This means that, potentially, horses will be required to train, work and compete while being fed on such diets for prolonged periods. Most of the studies evaluating the effects of fat supplementation have tended to be of short-term duration (usually around 3 months or less) and have concentrated on the use of unsaturated vegetable oils or animal fat. In one previous study, however, no apparent adverse effects were recorded following the feeding of a soybean oil supplemented diet, in which the supplementary fat provided about 12% of digestible energy (DE) intake, to Thoroughbreds for 7 months (Pagan *et al.* 1995). However, it has been

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recommended that, in order to obtain maximum benefit from fat supplementation, higher proportions of fat should be fed (Kronfeld 1996). Soy and corn oil are perhaps the most commonly fed vegetable oils for horses. These are, however, predominantly unsaturated fats and are, therefore, prone to oxidative rancidity. Vegetable oils, high in saturated fatty acids, tend both to be more stable during processing and to have a greater resistance to oxidative rancidity which makes them potentially of high practical value.

The current study was therefore carried out to investigate the effects of feeding a diet in which around 20% of the total DE (~85 MJ/day) came from supplementary fat, either from a predominantly unsaturated or a saturated vegetable oil source, for 6 months following a 10 month acclimation period in which the supplementary fat provided about 12% of the total DE (~85 MJ/day).

Materials and methods

Eight moderately fit, Thoroughbred horses (500–550 kg bwt; age 6–8 years; 6 geldings and 2 mares) were used in this study. All horses had been fed a diet containing 2–4% fat for several weeks before the start of the study. They all initially performed a standardised exercise test (SET) on a high-speed treadmill at 3° (2 min warm-up walk, followed by 800 m trot [~4 m/s], then five 800 m at speeds increasing from 7–11 m/s). Heart rate was recorded (Hippocard PEH 200)[1] during the last 15 s of each speed and a blood sample was taken from an indwelling catheter in the jugular vein into heparinised tubes at the end of each speed. Plasma lactate concentration was measured in each blood sample using an automated L-lactate analyser (YSI, 1500 Sport)[2]. The heart rate velocity relationship was determined by regressing the logarithm of lactate concentration against speed and used to calculate V_{La4}. The speed, at which a heart rate of 200 beats/min was reached, was determined (V_{200}). The horses were then assigned to either of the 2 dietary treatments described below according to their sex, V_{La4} and V_{200}.

Treatments

For the initial 10 month fat acclimation (AC) both groups were fed Timothy hay (~5.5–6.8 kg/day) and oats (~1.5 kg/day) together with a fortified sweet feed (1.5 kg/day) which had been supplemented (15% by weight) either with a saturated vegetable oil (coconut oil - S) or an unsaturated vegetable oil (soy oil - Un) so that ~19% of the daily DE intake (total intake ~85 MJ/day) came from the dietary fat and ~12% from either the unsaturated or saturated vegetable oil source. After this initial period the oats were removed from the daily ration and the horses were fed an increased amount of the Un or S fortified sweet feeds (3 kg/day) for a further 6 months (HF). At this time ~27% of the DE (total intake ~85 MJ/day) came from fat, with approximately 20% coming from either the unsaturated or saturated vegetable oil source. The horses were fed twice daily and housed in individual box stalls where they had access to the Timothy hay. Bodyweight was monitored weekly and feed intake individually adjusted to maintain a fairly constant bodyweight. The estimated daily intakes of the main fatty acids are illustrated in Table 1.

Exercise protocol

Throughout the 16 month study the horses were exercised 5 days a week on either a mechanical exerciser (~10 min walk and 40 min trot ~3–4 m/s) or a high-speed treadmill (walk, trot and 2400 m at a canter ~8–9 m/s). The horses were also allowed daily access to paddocks for several hours a day, but were kept muzzled during this period to prevent grazing. A standardised exercise test (protocol as for the SET but without the collection of blood samples) was carried out monthly to monitor heart rate.

Investigations

Monthly resting blood samples were collected, before the morning feed, for routine haematology (Coulter Counter S560)[3], Triglycerides[4] (TG), cholesterol (Chol), Gamma Glutamyl Transferase (GGT) and Total Protein (TP) (Eppendorf 5060 Automated Analyser)[5]. Resting full plasma fatty acid profiles were determined (following extraction then isolation of the lipid classes (Folch *et al.* 1957; Kaluzny *et al.* 1985) the transmethylated (Park and Goins 1994) fatty acids were then identified using gas chromatography[6], at the end of the AC stage (endAC) and after 6 months of the HF protocol (endHF).

A standardised step exercise test (STEP - as above SET protocol except the five 800 m canters increased stepwise from 8–12 m/s) was carried out 3 h after the morning hay and sweet feed after 3, 6 and 9 months AC and 3 and 6 months HF. Blood samples were taken from an indwelling catheter in the jugular vein before feeding, 1, 2 and 3 h post feeding (3 h post = pre-exercise sample), at the end of each speed and at 15 and 30 min of recovery. These were analysed for lactate (YSI 1500 Sport)[2], glucose using an automated glucose analyser (YSI 2300 Stat)[2] and insulin (using commercially available radioimmunoassay RIA kits[7] which had been validated for specificity and accuracy in equine plasma). V_{La4} and V_{200} were determined. Where appropriate, samples were centrifuged immediately and the plasma harvested, refrigerated and either analysed that day or frozen (-20°C) and normally analysed within a week.

Oral glucose tolerance tests were undertaken on 2 occasions, normally within a 3 day period, after 3, 6 and 9 months (AC) and after 3 , 6 months (HF). The horses were given 2 kg hay at 2200 h the night before the test and no feed on the morning of the test. The baseline blood sample was collected between 0600–0700 h and 1 g/kg bwt glucose as a 20% dextrose solution was then administered via a stomach tube. Blood samples for glucose and insulin were collected every 30 min for 4 h.

General coat and hoof appearance were monitored on 5 occasions throughout the study using independent, trained assessors who had no knowledge of the trial protocol and a semi-quantitative system was used for scoring. All horses were groomed and maintained in a similar manner throughout the trial and no skin, coat, mane or tail preparations were used other than a fly spray, as and when required. The horses were presented to the assessors in a random order under standardised conditions and assessed for general overall appearance; gloss (appearance of reflected light) of the mane, tail and body; softness and greasiness (assessed by running fingers through the full thickness of the coat, etc.) of the mane, tail and coat; scale (assessed by lifting the hairs in the opposite direction of growth and examining the skin and base of the hairs for signs of flaking) in the head, neck, shoulders, chest and hindquarters area as well as the mane and tail. All of the above attributes were scored on a 9 point scale from 1 to 5: where 1 usually represented poor condition for that category (e.g. dull for gloss) and 5 excellent condition (e.g. very glossy). In addition, overall hoof appearance was assessed. Each hoof was evaluated separately and scored on a 9 point scale from 1–5, for coronary cracks, crumbly horn and

TABLE 1 : Estimated typical daily intakes (g/day) of the main fatty acids during both the acclimation study (AC) and the 6 month high-fat study (HF) when Thoroughbred horses were fed a fortified sweet feed supplemented either with a predominantly saturated fat source (S) or an unsaturated fat source (Un) together with hay (HF) or hay and oats (AC). Total daily intake of feed taken as 8.5 kg

Fatty acid	Description	AC		HF	
Type of diet		Un	S	Un	S
Total fat intake based on analysis (g/day)		418	422.5	617.5	626.5
Caprylic	C8:0	1	12	1	24
Capric	C10:0	0.5	9.5	1	19
Lauric	C12:0	4.5	73.5	8	146
Myristic	C14:0	2	29	3	57
Palmitic	C16:0	53	53	71.5	67.5
Palmitolec	C16:1 cis 9	1.5	1.5	1.5	1.5
Stearic	C18:0	13	13	21.5	21
Oleic	C18:1 cis 9	97	74.5	128	83
Linoleic	C18:2n6cis9,12	150	74.5	228	77.5
Linolenic	C18:3n3cis9,12,15	68.5	58	120	100
Behenic	C22:0	2	1.5	3	2
Erucic	C22:1cis 13	1	1	1	1
Lignoceric	C24:0	1.5	1	2	1.5

crumbly sole, where 1 was equal to none of these conditions being present and 5 where the condition was present and severe.

Statistics

Analysis of variance (ANOVA) by repeated measures or *t* tests as appropriate were used to determine the effect of time and treatment on the various parameters measured. Significance was taken at $P<0.05$. Areas under the curve were determined using the trapezoid rule. All data are given as mean ± s.d.

Results

The horses ate all of the diet provided throughout the trial and their bodyweights remained fairly constant being Pre-study: 514 ± 31 kg; EndAC: 514 ± 24 kg; and End HF: 510 ± 25 kg.

Haematological and biochemical analyses

There were no significant effects of treatment on the resting haematological parameters although there was an obvious trend for the white blood cell count to be slightly higher for the majority of the study in the S group. There was no apparent effect of treatment on TP or GGT concentrations, which were within the reference range for the laboratory used. No pre-study results were available for Chol or TG and there was a significant effect of treatment ($P<0.05$) from the first (1 month) sampling point with higher Chol and TG concentrations in the S treatment group. The pattern of changes with sampling period (time) were similar for both treatments. There was a significant increase in Chol concentrations with time from the 1 month point to endAC period followed by a decline during the HF period so that the end of the study results were not significantly different from the 1 month results. The TG concentrations were highly variable between and within individuals.

Plasma fatty acid compositions

The main fatty acid composition of the cholesterol esters (CE), triglycerides, phospholipids (PL) and free fatty acids (FFA) are shown in Table 2. Most of the plasma fatty acids were contained within the CE and PL classes. There was a decrease in total plasma fatty acid content with time in both groups, and in 7 of the 8 horses, but this was not significant. There was a significant effect of treatment at both sampling points with the S group having higher concentrations. With respect to the lipid classes the amount of CE and PL was significantly higher in S. Overall there was a significant decrease with time in the TG and PL content. The significant decrease in the C16:0, C18:0 and C18:3n-3% content contributing to the overall TG decrease. For all the lipid classes the main FA was linoleic acid (C18:2n-6), the proportions of which were not affected by time, but were slightly and significantly higher with Un in the CE and PL. Despite these effects, overall the proportions of the main fatty acids showed relatively little variation, being across both treatments and both sampling occasions for : C18:2n-6 (Linoleic acid) 53.4 ± 2.24; C18:0 (Stearic) 17.1 ± 0.85; C16:0 (Palmitic) 13.4 ± 0.7 and C18:1 (Oleic) 6.7 ± 1.9% total fatty acids recovered. The overall linolenic (C18:3n-3) content was 1.4 ± 0.7%. Higher proportions of C12:0 and C14:0 were found with the S treatment, and an increase with time, but lower levels of C4:0.

Oral glucose tolerance tests

There was no significant difference overall in the responses in the duplicate tests so for comparison the data from both were combined. Overall there was a significant effect ($P<0.05$) of both treatment and time on the baseline glucose concentrations, with both groups showing an increase over the study. However, when the % changes from baseline (Time '0') were compared there was no significant effect of treatment or time. When the areas under the curves were determined and compared there was also no significant effect of treatment and although there was a significant effect of time ($P<0.05$) the pattern was not consistent. There was a trend for the glucose concentrations in response to the oral glucose administration to be lower with the S treatment (Fig 1), which corresponded to a nonsignificant trend for the insulin concentrations to be higher. There was no significant effect of treatment on the baseline insulin concentrations or

TABLE 2: Mean (± s.d.) % more major fatty acids (FA) composition and total plasma concentration (µg/ml) of the plasma cholesterol esters (CE); triglycerides (TG); free fatty acids (FFA) and phospholipids (PL) at the end of the acclimation period (EAC) and end of the high-fat period (EHF) in 2 groups of 4 Thoroughbred horses fed either a diet supplemented with a predominantly saturated vegetable oil (S) or a predominantly unsaturated vegetable oil (Un)

	S		Un		S		Un		S		Un		S		Un	
FA	EAC CE	EHF CE	EAC CE	EHF CE	EAC TG	EHF TG	EAC TG	EHF TG	EAC FFA	EHF FFA	EAC FFA	EHF FFA	EAC PL	EHF PL	EAC PL	EHF PL
4:0	1.61 (0.06)	1.65 (0.23)	2.32 (0.18)	2.21[a] (0.31)	5.06 (2.19)	7.55 (2.56)	6.3 (1.41)	10.3* (1.69)	-	-	-	-	0.9 (0.07)	0.9 (0.08)	1.19 (0.1)	0.74 (0.64)
12:0	0.18 (0.03)	0.32 (0.06)	0	0.04 (0.07)	6.79 (1.77)	6.6 (3.78)	0.57 (0.41)	1.64[a] (0.35)	5.54 (1.12)	8.64 (2.75)	0.98 (1.15)	4.54[a*] (2.87)	0.04 (0.03)	0.09 (0.02)	0.07 (0.15)	0.14 (0.18)
14:0	1.5 (0.12)	1.75 (0.13)	0.48 (0.07)	0.57[a*] (0.05)	5.3 (0.7)	4.89 (1.68)	0.57 (0.41)	1.67[a] (0.26)	9.09 (0.94)	10.79 (1.8)	6.35 (2.6)	4.88[a] (3.65)	0.49 (0.05)	0.62 (0.09)	0.17 (0.01)	0.25[a*] (0.02)
15:0	0.3 (0.06)	0.24 (0.04)	0.28 (0.07)	0.21[a*] (0.01)	0.43 (0.12)	0.32 (0.22)	0.59 (0.06)	0.7[a] (0.2)	-	-	-	-	0.14 (0.04)	0.14 (0.03)	0.17 (0.04)	0.14 (0.02)
16:0	10.5 (0.65)	10.3 (0.48)	11.25 (0.76)	10.24 (0.26)	15.53 (1.8)	13.62 (1.42)	14.2 (2.4)	10.3 (1.83)	-	-	-	-	15.42 (0.9)	15.2 (1.33)	14.2 (0.57)	14.5 (0.58)
16:1	0.79 (0.07)	0.71 (0.49)	0.61 (0.07)	0.42* (0.05)	0.77 (0.11)	0.71 (0.48)	1.08 (0.3)	0.585 (0.41)	-	-	-	-	0.18 (0.02)	0.19 (0.02)	0.15 (0.01)	0.12 (0.01)
17:0	0.67 (0.63)	0.13 (0.02)	0.14 (0.10)	0.15 (0.02)	0.57 (0.125)	0.55 (0.20)	0.72 (0.15)	0.35 (0.24)	0.55 (1.09)	2.1 (3.63)	2.91 (1.33)	2.1 (1.55)	0.51 (0.05)	0.5 (0.13)	0.63 (0.07)	0.51 (0.02)
18:0	0.79 (0.53)	1.51 (0.82)	1.40 (0.63)	1.31 (0.21)	3.27 (2.21)	4.62 (0.64)	4.05 (2.86)	2.98 (2.0)	-	-	-	-	27.6 (0.43)	29.3 (1.3)	27.4 (0.97)	27.4 (0.17)
t18:1	0.13 (0.22)	0.05 (0.04)	0.02 (0.03)	0.035 (0.03)	-	-	-	-	24.5 (1.43)	18.45 (8.81)	28.13 (15.98)	22.48 (7.54)	0.24 (0.33)	0.26 (0.1)	0.3 (0.16)	0.35 (0.07)
c18:1	6.78 (0.27)	6.78 (0.47)	6.40 (0.07)	5.92 (0.14)	13.43 (2.21)	10.4 (0.63)	14.4 (2.86)	10.26* (2.04)					6.67 (0.39)	6.45 (0.26)	6.83 (0.24)	6.93 (0.4)
18:2n6	74.15 (2.4)	75.16 (0.92)	76.05 (1.54)	77.72[a] (0.69)	39 (7.51)	42.5 (6.76)	46.18 (8.2)	55 (3.94)	48.9 (1.01)	46.52 (7.16)	33.01 (23.12)	51.69 (10.68)	43.85 (0.57)	41.6 (2.52)	44.2 (0.53)	44.9[a] (0.49)
20:0	-	-	-	-	0.23 (0.18)	0.15 (0.18)	0.79 (1.03)	-	-	-	-	-	0.675 (0.12)	0.59 (0.12)	0.6 (0.1)	0.62 (0.02)
18:3n3	1.59 (2.5)	0.69 (0.1)	0.34 (0.05)	0.25 (0.02)	6.14 (1.07)	5.67 (1.01)	7.86 (1.76)	3.92* (0.48)	8.43 (2.36)	5.03 (4.47)	5.71 (4.34)	8.54 (4.16)	0.983 (0.07)	1.0 (0.01)	1.0 (0.04)	0.93 (0.07)
20:3n3	-	-	-	-	-	-	-	-	-	-	-	-	0.42 (0.05)	0.43 (0.02)	0.45 (0.05)	0.38 (0.04)
22:1	0.26 (0.17)	0.20 (0.23)	0.20 (0.26)	0.30 (0.05)	2.84 (4.08)	1.44 (0.41)	1.24 (0.55)	1.48 (0.21)	-	-	-	-	0.42 (0.05)	0.11 (0.01)	0.15 (0.02)	0.12 (0.01)
20:4n6	-	-	-	-	-	-	-	-	-	-	-	-	1.11 (0.11)	1.15 (0.13)	1.1 (0.15)	0.98 (0.13)
Total	174 (9)	158 (28)	133 (6)	142[a] (28.5)	64 (16.5)	40 (9.5)	47 (18)	35* (13)	7 (1)	9 (5)	8 (4)	12 (6)	347 (33)	296 (47)	268 (12)	266[a*] (55)

[a] = Significantly different ($P<0.05$) between treatments for that FA and lipid class. * = Significant effect of TIME for that FA and that lipid class.

insulin response. Overall there was a significant effect of time but there was no significant difference in the responses at the endAC and endHF time points.

Exercise tests

The horses remained in fairly constant fitness throughout the study with little change in their V_{La4} or V_{200}s. There was no significant effect of treatment or time on the glucose responses to feeding the diets. There was no significant effect of treatment on the insulin responses to feeding and although there was one for lactate it was inconsistent. There was a small but significant effect of time on both responses. The lactate responses overall tended to decrease and the insulin to increase during the study. There was no effect of treatment or time on the insulin or lactate responses to exercise. However, overall there was a significant effect of both treatment and time on glucose responses, although the responses did not differ significantly between S and Un at the first and last tests or between these time points or endAC and endHF tests as shown in Figure 2. There was no significant effect of treatment on the glucose, lactate or insulin responses in the recovery period. However, there was an effect of time. For both lactate and insulin there was a tendency for the concentrations to increase during the study period but for glucose there was no significant difference between the first and last tests or the endAC and endHF tests.

Coat and hoof appearance

There was no apparent significant effect of treatment on any of the hoof appearance parameters assessed. There was a significant effect of treatment ($P<0.05$) on shoulder, chest and hindquarter scale with better ratings being found with the S treatment. For many of the attributes there was a significant improvement with time with both the treatments (overall appearance; mane, tail and body gloss as well as softness). There was also a significant ($P<0.05$) increase in grease with both treatments over time.

Discussion

This study built upon a previous study which compared the effects of feeding a soy oil supplemented diet for 7 months (Pagan *et al.*

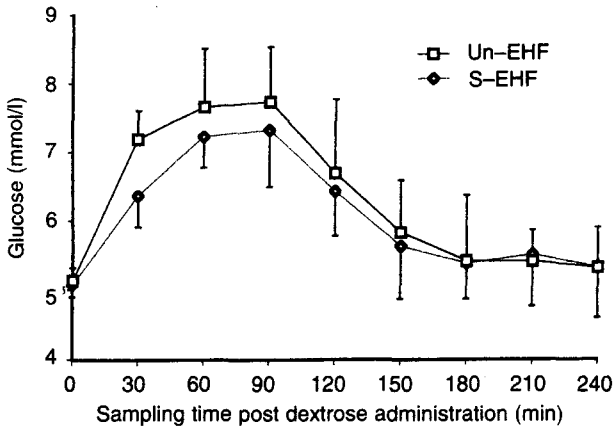

Fig 1: Mean ± s.d. glucose concentrations in response to an oral glucose challenge (1 g/kg bwt) in 2 groups of 4 Thoroughbred horses after 16 months (EHF) of being fed a fortified sweet feed supplemented either with a predominantly saturated fat source (S) or an unsaturated fat source (Un).

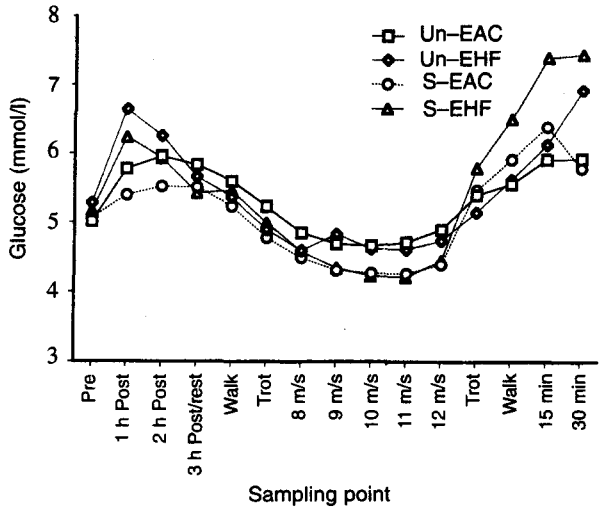

Fig 2: Mean ± s.d. glucose concentrations at rest, pre-feeding (Pre), 1,2 and 3 h post feeding and at each stage of a standardised incremental exercise test on a treadmill in 2 groups of 4 Thoroughbred horses after 10 months (EAC) of being fed a fortified sweet feed supplemented either with a predominantly saturated fat source (S) or an unsaturated fat source (Un) in which the supplemental oil provided ~13% of the DE intake and of being fed for a further 6 months the same supplemental oil but at an increased amount ~20% DE (EHF).

1995) with a control nonfat supplemented diet. It was not intended to compare the effects of a fat supplemented with a non fat supplemented diet, which has been the subject of a number of papers (Potter *et al.* 1992; Harris 1997). For the first 10 months, the dietary fat intakes were kept similar to those used in the previous study, then increased fat intakes were fed for a further 6 months. Both diets were palatable, the horses ate the diets well throughout the trial and no monotony was apparent for the 16 month period. The horses remained healthy, as shown by their haematology and biochemistry, their ability to perform regularly standardised exercise tests with little change in their V_{La4} or V_{200}s as well as the apparent improvement in overall appearance.

Coconut oil contains the highest saturated fatty acid level of any vegetable oil (nearly 90% saturated vs. 15% in soy oil), although it also contains some unsaturated fatty acids (~6% monounsaturated; 4% polyunsaturated vs. 23 and 62% respectively for soy oil). In addition, it has a higher content of medium chain triglycerides (MCT: 4–15% vs. 0% in soy oil). Unlike the longer chain triglycerides, MCT are more watersoluble which facilitates emulsification, hydrolysis and uptake by intestinal mucosa. They are, therefore, digested quickly, require minimal lipase and are transported as free fatty acid (bound to albumin) through the much faster portal circulation rather than through the lymphatic system via lipoproteins (Linscheer and Vergroesen 1994). At least in the species studied, MCT are not stored directly in fat depots but are largely oxidised to acetic acid and do not require the carnitine transport system for mitochondrial entry (Freeman 1983; Linscheer and Vergroesen 1994; Chan *et al.* 1998). Possibly more importantly, MCT do not promote the synthesis of eicosanoids (prostaglandins, thromboxane and leukotrienes) nor do they serve as precursors for oxygen free-radical production (Chan *et al.* 1998). A previous study (Pagan *et al.* 1993) evaluated the short-term effects of feeding coconut oil supplemented diets to horses and suggested that the higher percentage of saturated and medium chain fatty acids in the coconut oil diet might have had some metabolic advantages. In this current study, no marked positive or negative metabolic effects were identified for the S over Un treatment although 'performance' *per se* was obviously not assessed. Significantly higher total resting plasma fatty acids were found with the S and higher concentrations of C12:0 and C14:0, but, interestingly, lower C4:0. In man, orally supplied MCT have not led to the sparing of muscle glycogen during moderate to intense exercise that had been postulated and their effect on performance as ergogenic aids has been questioned. Further work is needed to see if high MCT ingestion in the horse has any performance or inflammatory advantages.

Linoleic (n-6), linolenic (n-3) and arachidonic acid, 3 polyunsaturated fatty acids, are often referred to as essential fatty acids (EFA). Linoleic acid can be converted to arachidonic acid but apart from this, the body cannot synthesise EFAs and they must therefore be absorbed intact. They are, among others, an important structural component of various biomembranes, play a part in lipid transport and, in addition, are precursors for prostaglandins and leukotrienes. Essential fatty acids have been shown to be required by many mammals and it can be assumed that they are also important in the horse but it is not currently known at what level in the diet they may be required. In one study in which ponies were fed very low levels of linoleic acid (control group: 34.4 g/kg feed vs. low fat diet group: 1.42 g/kg feed for 3 months followed by 0.22 g/kg for 4 months), no clinical signs were apparently seen over a 7 month period and there was no visible effect on skin, haircoat or hooves (Sallmann *et al.* 1992). It was suggested that this could just reflect a long biological half life of C18 in the horse due in part to their large stores of essential fatty acids. The potential that the feeding of a highly saturated fat-supplemented diet could have adverse effects on coat condition with time was one of the reasons that this study was undertaken. Feeding either of the fat-supplemented diets seemed to be advantageous for many aspects of coat and hoof appearance. No apparent detrimental effects on coat or hoof appearance and some advantages were apparent with the S diet. The estimated typical daily intakes, for both treatments, of the various major fatty acids (Table 1) were below the equivalent control levels of the Sallmann study (i.e. 8.5[kg

intake] x 34.4 g = 292 g), but well above the equivalent marginal levels (8.5 x 1.42 ~12 g and 8.5 x 0.22 ~1.8 g).

The plasma fatty acid results showed marked differences from a previous study which looked at the plasma fatty acid composition of equine plasma (Luther *et al.* 1981) with higher C18:2 n-6 percentages across all lipid classes and typically a lower C18:1 and C18:3n-3 content regardless of the diet fed. This could, therefore, reflect the breeds used (Thoroughbreds vs. predominantly Quarter Horses), analytical techniques and/or the basal diets. There is still some controversy of the exact role of the n-3 and n-6 Polyunsaturated fatty acids (PUFA) in inflammation and immune function which reflects their multiple sites of action including the alteration of membrane composition and eicosanoid and cytokine production (Deem Morris *et al.* 1989; Chan *et al.* 1998; Yaqoob 1998). The estimated ratios of n-6: n-3 intakes for the Un group during the AC and HF periods were 2.2:1 and 1.9:1 respectively compared with the S groups 1.2:1 and 0.8:1 ratios. The optimal ratio for the horse is currently unknown.

Plasma nonesterified fatty acid (NEFA) patterns have been shown in non ruminants to reflect closely the fatty acid composition of the adipose tissue triacylglycerol which, in turn, closely reflects the dietary fatty acid patterns (Abe *et al.* 1993; McClelland *et al.* 1995; Fontanillas *et al.* 1998) although there may be a limit to the variance that diet can induce (Fontanillas *et al.* 1998). Previous work has shown that the fatty acid content of horse adipose tissue can be altered significantly by the diet fed i.e. from 17% linolenic acid and 4% linoleic in roughage fed horses to 2% linolenic and 22% linoleic in horses fed oats - reflecting the dietary intake of these 2 fatty acids (Shoreland *et al.* 1952). It could be postulated, therefore, that feeding a diet with increased levels of saturated fatty acids could increase their deposition in the adipose tissue resulting in 'harder fat' and a local insulation effect possibly affecting mobilisation of the FFAs and potentially adversely affecting performance. Effects on other biomembranes could also be postulated. However, no apparent significant effect on the parameters monitored in this study was seen. This may reflect the fact that although the S diet provided about 3 times the amount of saturated fat than the high Un diet, there were also substantial amounts of unsaturated fatty acids provided. It has been suggested that altered membrane fluidity may contribute to insulin resistance and changes in the response to a glucose load, as abnormal organisation of membrane lipids and proteins may hinder the action of insulin (Tong *et al.* 1995). For this reason, as a noninvasive way to evaluate any possible major change in membrane composition and function, repeated glucose absorption tests were undertaken throughout the study and compared between the 2 groups. No apparent effect of feeding the S or Un diets was seen on the glucose or insulin responses to a glucose oral challenge although throughout the study the insulins tended to be higher and the glucose concentrations lower with the S treatment group. The nonsignificant difference between the 2 groups in their insulin and glucose concentrations did not become significant as the study progressed. Further work would be needed to evaluate this in more depth. However, from this study it can be concluded that there were no significant adverse effects on the response to an oral glucose challenge of feeding a saturated fat-supplemented diet over an unsaturated one. The changes in the baseline glucose concentrations with time with both treatments could obviously reflect a general time effect, as to the authors' knowledge multiple repeated tests such as this have not been carried out in healthy horses maintained on the same diet, an analytical effect or other unknown and uncontrollable factors. Alternatively as it occurred in both groups it could be a consequence of feeding fat- supplemented diets. As the trend occurred from early on in the study it might be useful for any future studies, which look at the effect of fat supplementation, also to look at the response to an oral glucose challenge test.

One study (Rich *et al.* 1981) evaluating the effect of different fats and dietary fat intake levels on plasma fatty acid concentrations found that although the ponies fed added fat (10%) tended to have higher total fatty acid concentrations, the differences were not significant and the serum linoleic acid concentrations were not significantly affected by the diet, but the highest level was found with the corn oil and the lowest with the tallow or blend. In the current study, despite the much higher linoleic intakes in the Un group, especially during the High F period, only slightly, although significant, higher plasma percentages were seen with Un and there were no effects of time. In this current study, linoleic acid was the major component of all the plasma lipid classes but in some previous work (Luther *et al.* 1981), oleic acid was the major component for TG and FFA. Of the major plasma FAs the overall proportion of linoleic was higher (~53 vs. ~44%) and oleic plus linolenic lower (7 vs. 14.5%; ~1.5 vs. 2.8%) than in this previous study. Further work is needed in this area. There was a significant decrease with both treatments in the 18:1c and 18: 3n-3 triglyceride content between endAC and endHF and an overall decrease in TG content. A decrease in plasma TG content with fat supplementation has been reported by some but not all workers (Duren *et al.* 1987; Grunwald 1991; Kurcz *et al.* 1991; Sallmann *et al.* 1992).

Potentially excessive amounts of unsaturated fatty acids could deplete the vitamin E supply and induce a vitamin E deficiency. In this study plasma vitamin E concentrations were only measured at the end of the study after 4 and 5 months of the HF period (HPLC with a fluorescence detector according to the method of Hidiroglo 1989). The plasma vitamin E concentrations did not differ significantly within the treatment groups (mean ± s.e. 2.97 ± 0.17 and 3.10 ± 0.62 : 4.88 ± 0.99 and 4.91 ± 2.6 for the Un and S group, respectively). The S group tended to have higher concentrations than the Un group (significant at the 4 month stage). The S group concentrations also tended to be higher than the reference range for the laboratory used (1–4 µg/ml) but the Un groups Vitamin E levels were well within this reference range.

In conclusion, this study showed no apparent adverse effects of feeding a fat-supplemented diet for 16 months nor any apparent disadvantages of supplementing with a predominantly saturated vegetable oil rather than the more typically fed predominantly unsaturated oil.

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Manufacturers' addresses

[1]Poplar Electro Inc., Woodbury, New York, USA.
[2]Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, USA.
[3]Coulter Electronics, Hialeati, Florida, USA.
[4]Cobras Mira Plus, Switzerland.
[5]Eppendorf, Gibbstown, New Jersey, USA.

[6]Hewlett Packard, Sunnyvale, California, USA.
[7]BET Labs, Lexington, Kentucky, USA.

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