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Time of feeding and fat supplementation affect plasma concentrations of insulin and metabolites during exercise

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Summary

Six Thoroughbreds were used to evaluate time of feeding on changes in exercise response in horses receiving either a textured feed or a fat-supplemented textured feed. Using a crossover design, 3 horses were fed a fat-supplemented diet while 3 horses received a control ration of textured feed. Horses performed a standardised exercise test (SET) on a high speed treadmill. The SET was performed at 3 different times: 1) following an overnight 12 h fast, 2) 3 h after feeding and 3) 8 h after feeding. The SET consisted of a 2 min walk at 1.4 m/s, 800 m trot at 4.2 m/s, 800 m gallop at 7.7 m/s, 1600 m gallop at 11 m/s, 800 m trot at 4.2 m/s and 2 min walk at 1.4 m/s. Jugular blood samples were taken before feeding, hourly until the beginning of the SET, at the end of each exercise step, 15 min post exercise and 30 min post exercise. During the SET, heart rate was measured and blood samples collected for analysis of glucose, lactate, insulin and nonesterified fatty acids (NEFA). Feeding horses 3 h prior to exercise resulted in elevated concentrations of plasma glucose and insulin ($P<0.01$) at rest. Elevated concentrations of insulin in horses fed 3 h prior to exercise decreased plasma glucose ($P<0.01$) during exercise and appeared to have suppressed fat oxidation during exercise because horses that were either fasted or fed 8 h post prandial had a net disappearance of NEFA in the plasma during exercise. This study indicates that beginning exercise with elevated plasma insulin appeared to be of no benefit during the exercise conducted in this experiment.

Introduction

Improving athletic performance of horses through dietary manipulation has been the objective of numerous studies (Duren *et al.* 1987; Greiwe *et al.* 1989; Oldham *et al.* 1989; Scott *et al.* 1991). These studies often describe in detail the type of diet fed, but rarely mention the time of feeding in relation to exercise performance. The importance of controlling the post prandial interval when evaluating exercise was established by Duren *et al.* (1992) when they reported that feeding a meal to ponies prior to exercise changed the haemodynamic response to exercise. Several recent studies have concentrated on manipulation of post prandial interval and on diet as a means of altering energy substrate availability. Stull and Rodiek (1995) reported that timing of a meal prior to exercise can be manipulated to influence glycaemic and lipaemic metabolites available in blood during moderate exercise. Lawrence *et al.* (1995a) found that time of feeding prior to exercise can affect metabolic responses of horses to exercise. In both studies, a pre-exercise meal of corn only was used to alter the plasma concentrations of nutrients and hormones. Although straight corn grain has the advantage of being a homogeneous substance, it does not constitute a normal or a traditional grain concentrate fed to a performance horse.

Many studies involving human subjects have been conducted which debate the positive or negative effects of various types of exercise performed after a meal. Costill (1985) and Williams (1989) suggested exercise conducted in a fed state, during a period of elevated insulin concentration, is detrimental to performance due to the potential of hypoglycaemia. Other studies have reported improvements in exercise performance when subjects consumed a pre-exercise carbohydrate meal (Sherman *et al.* 1991; Wright *et al.* 1991). Data are limited regarding the potential effects of post prandial exercise in horses. The objectives of this study were to further define the impact of feeding prior to exercise and to determine if replacing a portion of the carbohydrate from a typical performance horse diet with fat would alter hormone and substrate concentrations during exercise.

Materials and methods

Six trained Thoroughbreds (age 3 years; 3 fillies and 3 geldings) were used. The horses had been introduced to exercise on a high-speed treadmill[1] in several previous studies, and each horse maintained fitness by galloping 1200 m twice a week for a minimum of one month prior to the start of this experiment. Using two 3 x 3 Latin square in a crossover design, 3 horses were fed a fat-supplemented textured feed (FAT) while 3 horses received a control diet of textured feed (CON) (Table 1). Each horse received its respective textured diet and a mix of alfalfa (hay 1) and grass (hay 2) forage daily for a one month adjustment period prior to the start of data collection (Table 1).

Each week, during period one, the horses performed a standardised exercise test (SET) on a high speed treadmill inclined to 3°. The SET was performed at 3 different times: 1) after a 12 h overnight fast (FAST), 2) 3 h after eating (3H), and 3) 8 h after feeding (8H). The time of feeding was assigned within each dietary treatment in a 3 x 3 Latin square design so each horse

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TABLE 1: Nutrient concentrations and intakes of experimental feeds

Nutrient	Textured feed[2]	Hay 1	Hay 2	Supplement pellet	Soybean oil
Dry matter g/kg	849.0	927.0	930.0	909.0	1000.0
Crude protein g/kg[1]	151.0	189.0	65.0	285.0	-
Acid detergent fibre g/kg[1]	68.0	332.0	443.0	122.0	-
Neutral detergent fibre g/kg[1]	140.0	442.0	730.0	229.0	-
Ether extract g/kg[1]	53.0	32.0	18.0	38.0	1000.0
Calcium g/kg[1]	8.9	11.7	3.6	26.1	-
Phosphorus g/kg[1]	7.7	4.7	2.8	16.6	-
Magnesium g/kg[1]	2.5	3.3	1.7	4.6	-
Potassium g/kg[1]	9.8	20.8	19.1	15.2	-
Sodium %[1]	2.1	0.4	0	6.4	-
Iron mg/kg[1]	268	117	53	799	-
Copper mg/kg[1]	44	9	5	115	-
Zinc mg/kg[1]	126	21	16	357	-
FAT intake (g/d)	2642	5434	1358	226	340
CONTROL intake (g/d)	3736	5434	1358	0	0

[1]Dry matter basis.
[2]42% oats, 31% corn, 19% supplement pellets, 8% molasses.

was tested weekly at a different feeding time. The horses were not exercised the day before each SET, and they received their evening textured diet ($^1/_2$ of daily amount) at 1700 h and 2.72 kg of hay 2 at 2200 h. On the morning of the SET, a catheter was placed in each horse's jugular vein and a 0700 h fasting sample taken. The textured diet was fed to the 3H and 8H groups after the fasting sample was taken. FAT horses received 1590 g of textured feed, 150 g of supplement pellet, and 200 ml (170 g) soybean oil. CON horses received 2273 g of textured feed. The FAT diet was formulated so the intake of textured feed, supplement pellet, and soybean oil provided the same amount of calories, protein, vitamins, and minerals as supplied by the control diet. No hay was fed to either group of horses on the day of the SET.

The fasted horses were tested at 0900 h. Hourly blood samples were taken from the 3H group until the SET at 1000 h. Blood samples were taken at 1, 2, 3, 4, 5, 6 and 8 h after feeding from the 8H group before the SET at 1500 h. At the end of period one, the diets were switched and horses continued in training for a one month adjustment phase. At the end of the adjustment phase, the horses repeated the three week SET as in period one.

Standardised exercise test

All exercise was performed on a high-speed treadmill inclined to 3°. The SET consisted of a 2 min walk at 1.4 m/s, 800 m trot at 4.2 m/s, 800 m gallop at 7.7 m/s, 1600 m gallop at 11 m/s, 800 m trot at 4.2 m/s, and a 2 min walk at 1.4 m/s. Heart rate was

TABLE 2: Heart rate (beats/min) mean ± s.e. for diet and time of feeding

Speed (m/s) Distance (m)	Mean control	Mean fat	Mean fast	Mean 8H	Mean 3H
1.4	81.1[A]	75.4[B]	77.5	78.7	78.5
500 m	± 2.2	± 2.1	± 2.7	± 2.6	± 2.7
4.2	138.2	134.4	136.6	134.6	137.8
800 m	± 2.3	± 2.2	± 2.8	± 2.7	± 2.8
7.7	180.1	179.3	177.5[a]	178.5[a]	183.1[b]
800 m	± 1.5	± 1.4	± 1.8	± 1.7	± 1.8
11	206.7	207.3	205.9	208.2	207.0
800 m	± 0.8	± 0.7	± 0.9	± 0.9	± 0.9
11	213.0	214.6	212.7	215.2	213.5
800 m	± 0.6	± 0.6	± 0.8	± 0.8	± 0.8
4.2	134.1	135.4	134.2[a]	131.5[a]	138.6[b]
800 m	± 1.5	± 1.4	± 1.8	± 1.7	± 1.8
1.4	94.3	92.1	91.8	92.1	95.7
500m	± 1.1	± 1.0	± 1.3	± 1.2	± 1.31

[A,B]Means in rows with different superscripts are significantly different (P<0.10).
[a,b]Means in rows with different superscripts are significantly different (P<0.10).
8H, 3H = 8 and 3 h after eating.

recorded during the last 15 s of each speed, and blood samples were taken, placed in sterile tubes containing EDTA, and centrifuged immediately. The plasma was pipetted into glass tubes and analysed immediately for glucose and lactate. Plasma glucose was measured using an automated glucose analyser (YSI, 2300 STAT)[2]. Lactate levels were measured using an automated L-lactate analyser (YSI, 1500 Sport)[2]. The remaining plasma was frozen. At the conclusion of the study, frozen plasma samples were analysed for insulin and glycerol with pre-determined samples (rest, post gallop and warm down) analysed for NEFA. Insulin was analysed using a commercially available radioimmunoassay (RIA) kit[3] which had been validated for specificity and accuracy in equine plasma. Nonesterified fatty acids and glycerol concentrations were determined by enzymatic methods using commercially available reagents[4,5]. Analysis of variance was performed using animal, period, diet, and time of feeding as main effects. Interactions between diet and time of feeding were also tested. When significant F ratios were obtained, individual treatment differences were evaluated using Fisher's LSD tests.

Results

Heart rate response to the SET is shown in Table 2. Differences in heart rate as a result of dietary treatment were present during the first 2 min of exercise when the horses were walking. Horses fed the fat-supplemented diet tended to have lower (P<0.10) heart rates compared with horses eating the control diet. Differences in heart rate during the SET associated with time of feeding were recorded at 7.7 m/s and again during the warm down at 4.2 m/s. At both times, horses that were fed 3 h prior to exercise tended to have higher (P<0.10) heart rates compared to the 8 h post prandial and fasted horses. No time vs. diet interactions were found.

Plasma lactate concentrations (Table 3) increased with exercise intensity but were not affected by dietary treatment. Time of feeding did not influence lactate concentration during exercise. A difference (P<0.05) in lactate concentration following 15 min of recovery from exercise was observed in this

TABLE 3: Plasma lactate (mmol/l) means ± s.e. for diet and time of feeding

Speed (m/s) Distance (m)	Mean control	Mean fat	Mean fast	Mean 8H	Mean 3H
Rest	0.60	0.61	0.65	0.57	0.60
	± 0.02	± 0.02	± 0.03	± 0.03	0.03
4.2	0.69	0.62	0.65	0.72	0.60
800 m	± 0.04	± 0.41	± 0.05	± 0.05	± 0.05
7.7	1.96	1.85	1.94	1.98	1.78
800 m	± 0.11	± 0.11	± 0.14	± 0.13	± 0.14
11	5.51	5.22	5.46	5.42	5.21
800 m	± 0.16	± 0.15	± 0.19	± 0.19	± 0.19
11	9.10	8.83	8.92	8.19	9.07
800 m	± 0.17	± 0.16	± 0.21	± 0.20	± 0.21
4.2	5.46	5.70	5.67	5.70	5.37
800 m	± 0.22	± 0.21	± 0.27	± 0.25	± 0.27
15-P	1.67	1.88	1.91[a]	1.91[a]	1.52[b]
	± 0.10	± 0.09	± 0.12	± 0.11	± 0.12
30-P	1.07	1.24	1.12	1.21	1.15
	± 0.07	± 0.07	± 0.09	± 0.08	± 0.09

[a,b]Means for time of feeding in rows with different superscripts are significantly different (P<0.05); 8H, 3H = 8 and 3 h after eating; 15-P, 30-P = 15 and 30 min post exercise

TABLE 4: Plasma insulin (μu/ml) means ± s.e. for dietary treatments

Speed (m/s) Distance (m)	Mean control	Mean fat
Rest	51.69	47.22
	± 4.03	3.68
4.2	54.54[a]	43.09[b]
800 m	± 3.72	± 3.51
7.7	51.94[a]	41.99[b]
800 m	± 3.55	± 3.35
11	44.49	41.17
800 m	± 2.38	± 2.24
11	45.78[a]	40.89[b]
800 m	± 1.91	± 1.71
4.2	57.49[a]	50.79[b]
800 m	± 2.34	± 2.21
15-P	60.55[a]	53.89[b]
	± 2.69	± 2.53
30-P	49.36	54.65
	± 3.17	± 2.99

[a,b]Means in rows with different superscripts are significantly different (P<0.10); 15-P; 30-P = 15 and 30 min post exercise

study with horses fed 3 h prior to exercise having lower lactate concentrations compared with the other groups of horses. No time vs. diet interactions were found.

Fat supplementation was found to affect plasma glucose concentration post exercise. Plasma glucose concentrations were higher (P<0.05) 15 min post exercise (6.22 vs. 5.80 mmol/l) and 30 min post exercise (5.68 vs. 5.17 mmol/l) for fat-supplemented horses compared with control horses. Time of feeding influenced plasma glucose concentrations during exercise (Fig 1). Horses fed 3 h prior to exercise began the SET with higher (P<0.01) plasma glucose concentrations compared with either fasted or 8 h post prandial horses. The elevated plasma glucose values in the 3 h post prandial horses declined during exercise and were lower (P<0.01) than either the fasted or 8 h post prandial horses following the final gallop phase of exercise. Plasma glucose remained lower (P<0.01) during recovery from exercise in horses fed 3 h prior to exercise compared to horses in the other feeding groups. No time vs. diet interactions were found.

Plasma insulin concentrations (Fig 2) were higher (P<0.01) at the onset of exercise and during exercise (P<0.01) when horses were fed 3 h prior to exercise. Fat supplementation did not affect (P>0.05) plasma insulin concentration at rest, but fat-supplemented horses tended to have lower (P<0.10) plasma insulin concentrations during exercise (Table 4). No time vs. diet interactions were found.

Differences in NEFA concentrations were observed during exercise due to time of feeding and dietary treatment (Fig 3a,b). NEFA concentrations were higher at rest in fasted and 8 h post prandial horses compared to horses fed 3 h prior to exercise. Fat-supplemented horses had higher (P<0.05) NEFA concentrations at rest and during the final stage of exercise compared to horses on the control diet. Glycerol concentrations were lower at rest, following the gallop and during the warm down from exercise in horses fed 3 h prior to exercise (Fig 4a,b) compared with both fasted and 8 h post prandial horses. Fat supplementation resulted in higher (P<0.05) glycerol concentrations at rest and during the warm down from exercise compared with horses receiving the control diet. Neither NEFA or glycerol were found to have a time vs. diet interaction.

Discussion

A primary goal of this study was further to define changes in metabolite and insulin concentrations during post prandial exercise. The difference between this study and others evaluating post prandial exercise is that this study evaluated feeding and exercise schedules typical of field conditions. Horses were exercised following a 12 h overnight fast, 3 h post feeding or 8 h post feeding. The second goal of this study was to determine if replacing a portion of the carbohydrate from a typical performance horse diet with fat would alter metabolite and insulin concentrations during post prandial exercise. The diets used in this study were formulated to use ingredients and nutrient profiles representative of field conditions. Other studies have used single grains and/or forages to test post prandial diet effects (Lawrence *et al.* 1993; Lawrence *et al.* 1995a,b; Stull and Rodiek 1995). These diets are homogeneous and produce predictable changes in blood metabolites and hormone profiles but do not simulate a typical diet fed to performance horses. In this study, a textured feed fortified with protein, vitamins and minerals was provided as the control diet. This same textured feed supplemented with additional soybean oil and balanced for protein, vitamins and minerals served as a fat-supplemented diet.

The SET utilised in this study elicited a maximum heart rate response of approximately 214 beats/min. During peak exertion, no differences in heart rate were observed as a result of feeding state or dietary treatment. The horses fed 3 h prior to exercise tended to have higher heart rates during the warm-up and warm-down portions of exercise compared to horses with longer post prandial phases. Duren (1990) reported similar differences in heart rate associated with exercise in fed and fasted ponies. In the present study, lactate increased 15-fold over resting levels. Lactate was not affected by dietary treatment and time of feeding had only minor influences on lactate concentrations during recovery from exercise. The magnitude of change in both

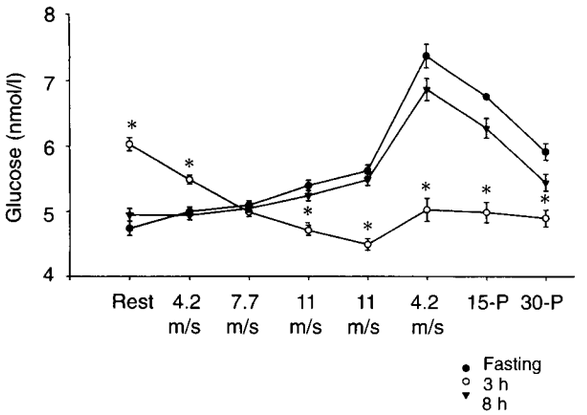

Fig 1: Mean (± s.e.) plasma glucose (mmol/l) for horses performing a SET. Horses were fasted, fed 3H or fed 8H prior to exercise. Means with a superscript asterisk are significantly different (P<0.05) from means without asterisk. 15-P, 30-P = 15 and 30 min post exercise.

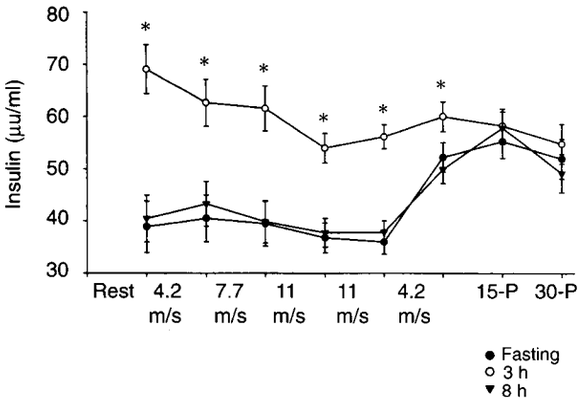

Fig 2: Mean (± s.e.) plasma insulin (μu/ml) for horses performing a SET. Horses were fasted, fed 3H or fed 8H prior to exercise. Means with a superscript asterisk are significantly different (P<0.05) from means without an asterisk. 15-P, 30-P = 15 and 30 min post exercise.

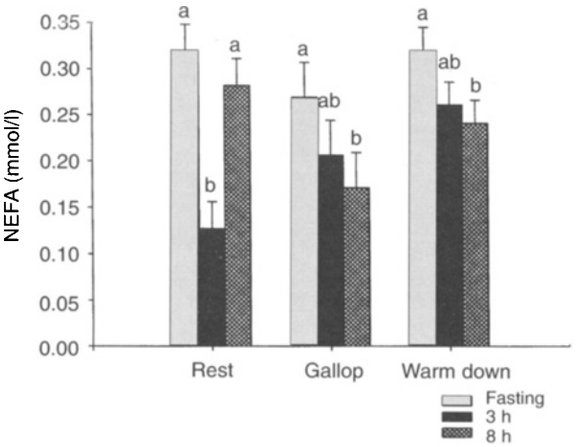

Fig 3a: Mean (± s.e.) plasma NEFA (mmol/l) for horses performing a SET. Horses were fasted, fed 3H or fed 8H prior to exercise. Means within sample time with unlike superscripts are significantly different (P<0.05).

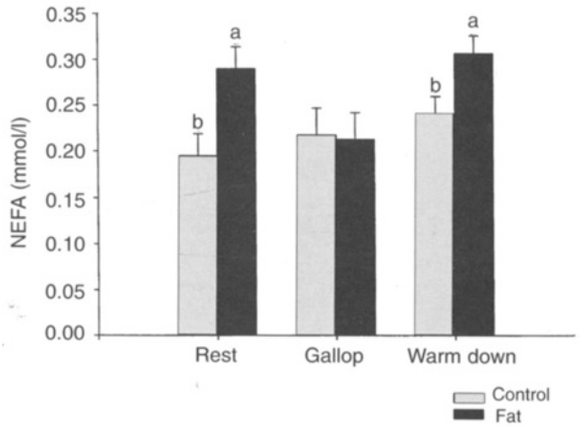

Fig 3b: Mean (± s.e.) plasma NEFA (mmol/l) for horses performing a SET. Horses were fed a fat-supplemented diet or a control diet prior to exercise. Means within sample time with unlike superscripts are significantly different (P<0.05).

lactate concentrations and heart rate indicate a submaximal, but anaerobic intensity SET.

Time of feeding markedly influenced plasma glucose and insulin concentration measured during exercise. Horses that were fed 3 h prior to exercise began exercise with higher plasma glucose concentrations compared to horses with longer post prandial intervals. During exercise, plasma glucose dropped in the horses fed 3 h prior to exercise. Similarly, insulin concentrations were elevated at rest and during exercise in the horses fed 3 h prior to exercise. Stull and Rodiek (1995) and Lawrence *et al.* (1993, 1995a), reported similar plasma glucose and insulin patterns in horses fed within 4 h of exercise. Several possible explanations exist for the decline in blood glucose. The drop may be attributed to a combination of increased insulin-mediated glucose disposal due to hyperinsulinaemia, increased exercise-mediated glucose disposal and suppression of hepatic glucose production (Ploug *et al.* 1992; McConell *et al.* 1994; Kirwan *et al.* 1998). Support of decreased hepatic glucose production was provided in horses by Lawrence *et al.* (1993) when they reported hyperinsulinaemia resulted in decreased hepatic glycogenolysis in horses fed 2.5–3 h prior to exercise. Reports of horses with hypoglycaemia as a result of exercise are limited. Robertson *et al.* (1983) reported that a horse with a markedly reduced blood glucose concentration collapsed at the end of a 42 km event. Farris *et al.* (1995) speculated that horses may be susceptible to hypoglycaemic episodes during prolonged exercise. Further, methods of carbohydrate delivery that would minimise hypoglycaemia have an ergogenic effect during prolonged treadmill exercise (Farris *et al.* 1995). Information from the present study indicates that preventing hyperinsulinaemia caused by pre-exercise grain feeding (textured grain meal fed within 3 h prior to exercise) appeared to protect against the decline in plasma glucose concentration during exercise.

In this study, fat supplementation resulted in lower insulin concentrations during exercise. Despite the lower insulin concentrations, plasma glucose at rest and during exercise was not affected by dietary treatment. These results do not agree with a previous study reported by Pagan *et al.* (1995) in which replacing carbohydrate with fat-reduced post feeding peaks of insulin and glucose and thus reduced the fall in blood glucose during strenuous exercise. In the present study, soybean oil provided about 12% of the digestible energy (DE) of the fat-

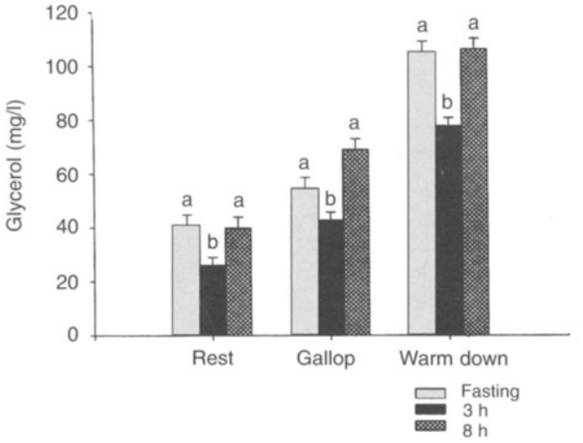

Fig 4a: Mean (± s.e.) plasma gylcerol (mg/l) for horses performing a SET. Horses were fasted, fed 3H or fed 8H prior to exercise. Means within sample time with unlike superscripts are significantly different (P<0.05).

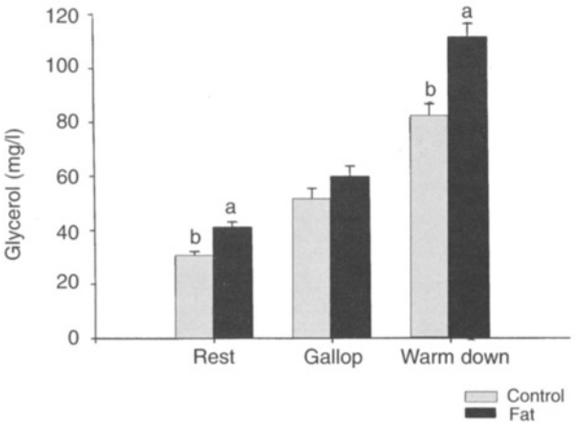

Fig 4b: Mean (± s.e.) plasma glycerol (mg/dl) for horses performing a SET. Horses were fed a fat-supplemented diet or a control diet prior to exercise. Means within sample time with unlike superscripts are significantly different (P<0.05).

supplemented diet. This diet was nearly identical to the diet utilised by Pagan *et al.* (1995). These apparent conflicting results indicate a need to study further the influence of fat supplementation on plasma glucose and insulin concentrations during exercise.

Plasma NEFA and glycerol concentrations were influenced by time of feeding and dietary treatment. Horses fed 3 h prior to exercise had the lowest NEFA concentrations at rest and during warm down from exercise. These low NEFA concentrations in horses fed 3 h prior to exercise corresponded with low glycerol values and high insulin concentrations during the same time periods. Several recent studies in man (Coyle *et al.* 1997; Horowitz *et al.* 1997; Kirwan *et al.* 1998) and in horses (Lawrence *et al.* 1993, 1995a; Stull and Rodiek 1995) have reported that small elevations in plasma insulin before exercise suppress lipolysis during exercise to the point of apparently limiting fat oxidation. A reduction in fat oxidation during exercise after ingestion of carbohydrate requires a compensatory increase in carbohydrate oxidation to maintain energy production (Horowitz *et al.* 1997). In this study, horses exercised 3 h post prandial had lower blood glucose levels, indicating blood glucose may be used to maintain energy production. Glycogen, another source of energy during reduced fat oxidation, was not measured in this study. However, Lawrence *et al.* (1995a) reported that muscle glycogen utilisation was lowest when horses were fasted compared to being fed prior to exercise. In this study, horses that were either 12 h fasted or fed 8 h prior to exercise appear to have utilised fat as an energy source during exercise as indicated by the net disappearance of NEFA from resting values to post gallop values. Horses exercised 3 h post prandial did not show a net disappearance in NEFA from rest to post gallop samples.

In this study, fat supplementation resulted in higher NEFA and glycerol concentrations at rest and during warm down from exercise compared with horses eating the control diet. Glycerol and NEFA are released into circulation with mobilisation of triglycerides from adipose tissue (Stull and Rodiek 1995). It appears that fat-supplemented horses were more efficient in their ability to utilise fat at rest and during exercise.

The present study used a training regime and an exercise test that were more comparable in intensity to those used in sport horses than in Thoroughbred racing. Under these conditions, feeding horses a textured feed 3 h prior to exercise increased plasma glucose and insulin at rest. The combination of exercise and hyperinsulinaemia resulted in a drop in blood glucose and interference with fat metabolism in the horses fed 3 h prior to exercise. Adding fat to the diet resulted in lower plasma insulin values during exercise which appeared to increase the utilisation of fat compared with horses receiving the control diet. The ultimate objective of this type of research is to further the understanding of whether, when or what to feed horses prior to exercise. Results from this study and from other studies that have investigated pre-exercise feeding indicate that time of feeding has an influence on circulating blood metabolites available for use during exercise. Whether providing a textured feed 3 h prior to exercise may be beneficial is subject to interpretation. On one hand, starting exercise with a high plasma glucose concentration may be viewed as a readily available energy source. However, the potential of hyperinsulinaemia negatively to influence plasma concentrations of nutrients and hormones appears to outweigh the potential benefit of feeding horses 3 h prior to exercise.

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Manufacturers' addresses

[1]Beltalong, Euroa, Australia.
[2]YSI, Yellow Springs, Ohio, USA.
[3]BET Labs, Lexington, Kentucky, USA.
[4]Wako Chemical USA, Inc. Richmond, Virginia, USA.
[5]Eppendorf EPOS, Gibbstown, New Jersey, USA.

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