The effect of fat supplementation on nutrient digestibility and fatty acid transport in yearling horses

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STORY IN BRIEF

The effect of fat supplementation on nutrient digestibility has been well documented in both mature and growing horses. Adding fat to the diets of horses has been found to be a safe method to increase the energy density of the diet without the harmful effects feeding excess starch, providing that all nutrients remain in balance. This is particularly important for horses that participate in endurance events which require a significant amount of energy, primarily derived from fatty acids. The effect of fat supplementation on the quantity of proteins responsible for the transport of fatty acids inside the muscle cell has yet to be investigated. The current study attempts to relate the effect of fat supplementation as compared to a traditional high-starch ration on transporter proteins inside the muscle cell of horses. Results of our study found that the quantity of proteins related to the transport of fatty acids inside the muscle cell increased with fat supplementation.

Key Words: FAT/CD36, starch, energy, exercise

INTRODUCTION

At submaximal exercise such as an endurance race, the availability and transport of fatty acids is capable of meeting energy demands (Wolfe, 1998). Conversely, during high intensity exercise, a Quarter Horse race for instance, muscle glycogen breakdown is stimulated and fatty acid transport into muscle mitochondria is inhibited (Wolfe, 1998). Fatty acid oxidation is limited by factors inside the muscle cell (Campbell et. al, 2004) and because muscle is major site of control of the rate of fat oxidation during exercise, the rate of control is the entry of long chain fatty acids (LCFA) into muscle mitochondria (Wolfe 1998). Fatty acid translocase (FAT/CD36) is a protein thought to be involved in the transport of LCFA in the mitochondria (Campbell et al., 2004; van Dam et al., 2004). FAT/CD36 is likely helped by the carnitine-palmitoyl transferase system in the movement of LCFA across the mitochondrial membranes (Campbell et al., 2004). A high fat diet was found to increase FAT/CD36 protein expression in the skeletal muscle of humans (Cameron-Smith et al., 2003; Roepstorff et al., 2004) and rats (Smith et al., 2007). An increased amount of FAT/CD36 available in the muscle cell could be of benefit for the clearance of LCFA during aerobic work as when rates of fatty acid oxidation increased, so did mitochondrial FAT/CD36 content (Campbell et al., 2004). An increase in the amount of FAT/CD36 in the muscle could increase fat utilization during exercise, and as fatty acid substrates remain the most abundant fuel source in the body, the ability to utilize a greater amount of these could be advantageous during exercise.

The current study is the first, according to our knowledge, that has investigated the effect of dietary fat on FAT/CD36 protein expression in horses during an aerobic conditioning program. It is our hypothesis that feeding added dietary fat as compared to a high-starch ration of equal caloric density will promote muscular adaptations that enable greater fat utilization during exercise, will delay the onset of fatigue during endurance exercise and thus would have a

positive impact on endurance by expanding the availability of stored substrates. Our objectives include to: 1) determine if the capacity to transport substrates from the blood stream to the muscle can be increased 2) evaluate the effect of diet on the quantity of transport proteins in the skeletal muscle of horses, 3) evaluate impact of fat supplementation on nutrient digestibility and growth patterns in yearling horses, and 4) determine whether feeding a high-fat diet during conditioning could improve the capacity of horses to utilize fat as a substrate.

MATERIALS AND METHODS

Horses and Diets. This experiment was conducted at the OSU Animal Sciences Equine Center. Seventeen Quarter Horse yearlings (6 geldings and 11 fillies) were utilized in a randomized complete block design. Horses were randomly assigned one of two treatment diets, FAT (n = 8) (12.99% fat, 22.75% starch – DM basis) using 10% soybean oil as the added fat, or STARCH (n = 9) (39.22% starch, 2.84% fat – DM basis) (see Table 1). Rations were formulated to meet NRC recommendations for moderate growth and light exercise for yearling horses. The FAT ration consisted of grain (including 10% soybean oil) and roughage in a 60:40 ratio with the roughage portion consisting of prairie hay and alfalfa cubes in a ratio of 30:10. Horses on the FAT diet were fed at a level of 2.25% of their initial body weight. The STARCH ration consisted of grain and roughage in a 70:30 ratio with the roughage portion consisting of prairie hay and alfalfa cubes in a ratio of 20:10. Horses on the STARCH diet were fed at a level of 2.50% of their initial body weight. Horses were divided into two, 8-wk experimental periods during the summer and were fed treatment diets while being placed on an aerobic exercise program. Body weights and skeletal measurements were taken weekly and a total fecal collection was performed in two periods. Muscle biopsies were taken from the middle gluteal at the beginning (baseline) and the end (post-treatment) of the experiment to evaluate the quantity of FAT/CD36 using Western blot analysis to determine if the capacity to transport fatty acid substrates from the blood stream to the muscle cell can be increased. Animal procedures were approved by Oklahoma State University's Animal Care and Use Committee.

Statistical Analysis. Data collected during the first week of the study were used as baseline measures and diets were compared using analysis of covariance (ANCOVA) methods when the baseline measure is significantly related to the response variable modeling of the correlation (covariance) structure of the measurements across time as appropriate. The SAS/MIXED (2003 SAS Inst. Inc., Cary, NC) procedure was used for these analyses. All tests were performed at the $P \leq 0.05$ level of significance.

Table 1. Composition and chemical analysis of treatment diets

Item[1]	Fat (%)	Starch (%)	Analysis[2]	Fat	Starch
Corn, ground	15.50	33.00	DM, %	91.05	89.16
Wheat middlings	21.00	30.00	DE, Mcal/kg	3.05	2.56
Soybean meal	12.00	4.65	CP, %	13.79	12.32
Limestone	1.00	2.00	ADF, %	19.58	16.39

Trace mineralized salt	0.25	0.35	NDF, %	30.53	27.03
Dicalcium phosphate	0.25	---	Starch, %	22.75	39.22
Soybean oil	10.00	---	Fat, %	12.99	2.84
Alfalfa cubes	10.00	10.00	Ca, %	0.89	1.20
Prairie hay	30.00	20.00	P, %	0.32	0.30
			Mg, %	0.16	0.16
			K, %	0.94	0.83

[1]As-fed basis.

[2]DM basis, except for DM.

RESULTS AND DISCUSSION

Results. There were no differences between the two diets on apparent digestibilities of DM, energy, starch, NDF, ADF, or phosphorus (P); however, FAT horses showed improved digestibility of crude fat, CP and calcium (Ca) (see Table 2). Baseline growth measurements were not different between the FAT and STARCH horses and no differences between dietary treatments existed for skeletal measurements post-treatment, but STARCH horses had significantly heavier body weights (P = 0.023) by the end of the treatment period (data not shown). FAT horses had higher (P = 0.006) FAT/CD36 protein expression post-treatment (see Table 3).

Table 2. Mean apparent nutrient digestibility percentages and observed significance levels by treatment

	DM	Crude Fat	Starch	CP	Energy	ADF	NDF	Ca	P
FAT (n=8)	68.16	86.39[a]	99.31	74.72[a]	67.19	42.37	44.59	54.78[a]	33.76
STARCH (n=9)	70.01	58.91[b]	99.31	61.18[b]	66.28	44.10	44.57	46.11[b]	31.04
P-Value	0.209	<0.001	0.967	<0.001	0.578	0.555	0.995	0.013	0.284

N, number of horses.

[a,b] Means within a column without a common superscript differ ($P < 0.05$).

Table 3. Protein expression of FAT/CD36 equine muscle at baseline (Time 1) and post-treatment (Time 2) (least squares means ± SD), arbitrary units

	FAT/CD36	
	Time 1	Time 2
FAT (n = 7)[1]	89.92 ± 19.74	104.52 ± 8.26 [a]
STARCH (n = 9)	90.06 ± 19.28	78.14 ± 7.73 [b]
P-Value	0.006	

N, number of horses.

[1]Fat group has an n of 7 as one horse was used as a control.

[a,b] Means within a column without a common superscript differ ($P < 0.05$).

Discussion. Feeding supplemental fat to yearlings during an aerobic conditioning program did not cause a negative impact on nutrient digestibility which is consistent with other published research reporting a lack of an associative effect of fat supplementation on nutrient digestibility in growing horses (Scott et al., 1989), although some authors have demonstrated a negative impact of fat supplementation on fiber digestibility (Jansen et al., 2000; Jansen et al., 2002). In fact, supplementing the diets of yearlings in our study with soybean oil enhanced digestibility of crude fat, crude protein, and calcium. The improved digestibility of crude fat is likely caused by the type of fat as well as the level of intake as oil is more highly digestible than fat from grains and forages (Kane et al., 1979; McCann et al., 1987; NRC, 2007) and the relationship between fat intake and fat digestibility is linear (Bush et al., 2001; Lindberg and Palmgren Karlsson 2001). The enhanced digestibility of CP could be explained by the fact that protein quality is a function of amino acid profile and foregut digestibility (NRC, 2007) and a greater portion of the CP fraction of the FAT ration was composed of soybean meal which is highly digestible (Farley et al., 1995). An enhanced digestibility of Ca in a fat supplemented diet has been reported previously (McCann et al., 1987) and could be due in part to the increased availability of Ca in dicalcium phosphate (versus limestone) present in the FAT ration but not in the STARCH ration (Schryver 1975). Lysine also facilitates calcium absorption and the FAT diet contained more soybean meal, therefore more lysine (Schryver, 1975). The ability of fat supplementation to enhance the measured digestibility of crude fat, crude protein, and calcium is likely an effect of the type of fat used for supplementation as well as the increased amount of soybean meal in the FAT diet.

Skeletal growth patterns in the current study did not differ between the two treatment groups, a result echoed by previous researchers with respect to growth patterns in growing horses (Scott et al., 1989). However, horses on the STARCH treatment were significantly heavier by the conclusion of the experiment than those on the FAT treatment despite the higher DE intake for those horses consuming supplemental fat. The increase in body weight for the STARCH horses over the FAT horses could be due to differences in body composition or glycogen content although neither were evaluated in this study.

The novel finding of the current research is the increased quantity of the fatty acid transporter, FAT/CD36 in the skeletal muscle of horses on the Fat treatment. Our findings of increased

expression of FAT/CD36 protein in conjunction with a high-fat diet are consistent with those in human (Cameron-Smith et al., 2003; Roepstorff et al., 2004) and rodent (Smith et al., 2007) skeletal muscle. In horses, FAT/CD36 protein expression has been correlated to specific fiber types (van Dam et al., 2004). Their research found that there was a strong expression of FAT/CD36 protein at the sarcolemma of all equine skeletal muscle fibers types from three different muscle groups (triceps brachii, pectoralis descendens, and vastus lateralis muscles) most likely positioned near capillaries to aid in the uptake of LCFA from the plasma. A low intracellular expression of the protein was observed in type 1 fibers, but no intracellular FAT/CD36 protein expression was observed in type 2 fibers in the same study. Similar findings with respect to fiber type expression of FAT/CD36 occur with rat muscle (Campbell et al. 2004). The selective increase in FAT/CD36 content resulting from the high-fat diet could be due to an increased availability of fat, or a resulting hormonal response due to increased fat consumption.

CONCLUSION

In sum, high fat diets consisting of supplemental soybean oil up to 10% of the total DM intake can be fed as safely and effectively to growing yearlings as a traditional high-starch ration provided that the total diet is balanced and nutrients are sufficiently available for growth and performance. The goal of this study was to determine what adaptations take place within the muscle cell in response to a high-fat diet that might better enable horses to increase their capacity to utilize dietary fat for energy. These data suggest that fat supplementation at the inclusion rate of 10% soybean oil (DM basis) in diets of growing horses subjected to aerobic conditioning could potentially enhance the capacity for transport of fatty acid substrates as defined by an increased expression of the FAT/CD36 protein in the muscle cell. A delay in the onset of fatigue may be possible by expanding the availability of stored substrates with the feeding of fat at this dietary level. The results of this study indicating that increased amounts of FAT/CD36 protein in the muscle cell perhaps allow for another key regulatory step in fatty acid oxidation and should stimulate further research in this area to determine the impact on aerobic performance in horses. The practices and conclusions of this study should stimulate further research to define performance-enhancing benefits of fat supplementation in diets fed to endurance horses.

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