Dietary Protein Restriction and Fat Supplementation Diminish the Acidogenic Effect of Exercise During Repeated Sprints in Horses

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ABSTRACT A restricted protein diet supplemented with amino acids and fat may reduce the acidogenic effects of exercise. Twelve Arabian horses were assigned to a 2 × 2 factorial experiment: two fat levels: 0 or 10 g/100 g added corn oil and two crude protein levels: 7.5 g/100 g (supplemented with 0.5% L-lysine and 0.3% L-threonine) or 14.5 g/100 g. The experiment began with a 4-wk diet accommodation period followed by a standard exercise test consisting of six 1-minute sprints at 7 m/s. Horses were interval trained for 11 wk followed by another exercise test with sprints at 10 m/s. Blood samples were taken at rest and during the exercise tests. Plasma was analyzed for PCO₂, PO₂, Na⁺, K⁺, Cl⁻, lactate, pH and total protein. Bicarbonate, strong ion difference and total weak acids were calculated. Data were analyzed using repeated-measures analysis of variance. Venous pH was higher in the low protein group during the first test (P = 0.0056) and strong ion difference became higher (P = 0.022) during sprints in the low protein group. During the second test, venous pH and bicarbonate were higher for the low protein high fat group (P = 0.022 and P = 0.043, respectively) and strong ion difference became higher (P = 0.038) at the end of the exercise in the low protein groups. These results show that restriction of dietary protein diminishes the acidogenic effect of exercise, especially in combination with fat adaptation. J. Nutr. 131: 1959–1964, 2001.

KEY WORDS: • protein • acid base • fat • horses

An optimal range of dietary protein has not been developed for exercising horses (Kronfeld et al. 1994). Increased needs for protein during exercise include maintenance of new muscle mass, repair of existing muscle mass and replacement of any nitrogen that would be lost in sweat (Meyer 1987). The upper limit of dietary protein for exercising horses should minimize thermogenic, ureogenic and acidogenic effects, but the lower limit must avoid protein deficiency.

The current dietary protein recommendations are 9.8, 10.4 and 11.4% crude protein (CP)2 for horses in light, moderate and intense exercise, respectively (National Research Council 1989). Surveys in Michigan (Gallagher et al. 1992a and 1992b) and Australia (Southwood et al. 1993) revealed that thoroughbreds and standardbreds in training were fed an average of 14% CP. Another study in central North Carolina (Honore and Uhlinger 1994) found that 70% of the horses were being fed protein in excess of National Research Council recommendations.

Heat production from higher levels of protein may interfere with exercise by attenuating fatigue (Kronfeld 1996) and an increased acidity will interfere with glycolysis and muscle fiber contraction (Mainwood and Renaud 1985). Restriction of dietary protein may be beneficial during strenuous exercise by diminishing production of heat and acid. A restricted protein diet has been shown to reduce the acidity of exercise when Arabian horses performing repeated sprints resulting in higher pH and bicarbonate levels (Graham-Thiers et al. 1999b).

Fat adaptation refers to the combined effect of training and prolonged consumption of a high fat (HF) diet (Kronfeld and Downey 1981). Fat adaptation has influenced acid-base responses to repeated sprints, mainly by reducing the increase in PCO₂ in venous blood (Kronfeld et al. 1998). An increase in PCO₂ in venous blood has resulted in an acidosis but a concurrent decrease in P CO₂ in arterial blood has resulted in an alkalosis (Taylor and Kronfeld 1995). Other influences on acid-base balance include strong ion difference (SID), which is the difference between strong cations and strong anions in the body, and total weak acids (Ato; Stewart 1981). These factors may be influenced by a reduction in dietary protein that may reduce anions. Separating the effects of PCO₂, SID and total weak acids on hydrogen ion concentration revealed that restricted dietary protein reduced hydrogen ion concentration mainly due to an increase in SID. Restricted dietary protein reduced the level of anions thus improving SID and lowering hydrogen ion concentration (Graham-Thiers et al. 1999c).

Fat supplementation may spare protein during energy demanding states, such as food deprivation or during energy restriction (McCargar et al. 1989). This study tested the hypothesis that fat adaptation may reduce the risk of protein deficiency when dietary protein is restricted and energy demand increased, such as during strenuous exercise. Addition of limiting amino acids may also reduce the risk of protein deficiency. This study also tested the hypothesis that acid-base
responses to repeated sprints will be reduced by the combination of dietary protein restriction and fat adaptation. In another paper, we reported that several indices of protein status were not diminished by the restricted level of crude protein (fortified with lysine and threonine) in this experiment (Graham-Thiers et al. 1999a).

MATERIALS AND METHODS

Horses. Twelve previously unconditioned Arabian horses (6 mares, 6 geldings, ages 5–11 y) were housed in a dry lot with free access to water. General health and feed intake were observed daily, and body weight, body condition and coat condition were monitored every 2 wk. Body condition was evaluated by a standardized system (Henneke et al. 1983). Coat was evaluated on shine and smoothness. The protocol was approved by the Institutional Animal Care and Use Committee.

Diets. Horses were randomly assigned to four complete feeds (HPHF, LPHF, HPLF, LPLF) which were formulated to provide 12.5 mg/kg; folic acid, 66 mg/kg; thiamin, 1,520 mg/kg; biotin, 42 mg/kg. Other components used were: 3 Heartland Lysine, Chicago, IL. Growth was also evaluated on amino acids in HP. The National Research Council currently recommends a dietary CP level of 11.4% for horses in intense exercise (National Research Council 1989). Fat was also at two levels: either 13 g/100 g added corn oil; or 3 g/100 g (no fat supplementation, low fat [LF]). A 4-wk adaptation period began the experiment. Feed intake was adjusted to maintain body condition.

TABLE 1

<table>
<thead>
<tr>
<th>Item</th>
<th>LPLF</th>
<th>HPLF</th>
<th>LPHF</th>
<th>HPHF</th>
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<tr>
<td>Item</td>
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<td>15</td>
<td>3</td>
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</tr>
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<td>Digestible energy, MJ/kg</td>
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<td>12.4</td>
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<tr>
<td>CP, %</td>
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<td>14</td>
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<td>0.54</td>
<td>0.59</td>
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<tr>
<td>L-threonine3 %</td>
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<td>0.47</td>
<td>0.51</td>
<td>0.50</td>
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<tr>
<td>DCAD,4 meq/kg</td>
<td>345.5</td>
<td>424.1</td>
<td>370.0</td>
<td>387.3</td>
</tr>
</tbody>
</table>

1 n = 6.
2 Vitamin/mineral premix provided: Ca, 8%; P, 8%; Mg, 1%; S, 1%; Zn, 0.5%; Cu, 0.12%; Mn, 0.1%; Co, 0.002%; I, 0.002%; Na, 13.65%; Cl, 21.35%; Se, 0.001%; vitamin A, 1,380,000 IU/kg; vitamin D, 258,000 IU/kg; vitamin E, 26,455 IU/kg; riboflavin, 701 mg/kg; niacin, 3,000 mg/kg; folic acid, 66 mg/kg; thiamin, 1,520 mg/kg; biotin, 42 mg/kg.
3 Heartland Lysine Inc., Chicago, IL.
4 DCAD = (Na+ + K+ + Ca2+ + Mg2+) – (Cl– + SO42– + PO43–).

Conditioning. All horses underwent 11 wk of conditioning using a high speed treadmill (Mustang 2000; Kagra, Fahrgauen, Switzerland). The protocol consisted of interval training divided into three phases (Table 2). Horses were interval trained twice a week and were walked at 1.5 m/s for 30 min on a mechanical walker on rest days. Standard exercise test (SET). Before conditioning, but after the dietary accommodation period, all horses performed a standard exercise test (SET-U). The SET consisted of 6 min at the walk (1.5 m/s), followed by six 1-minute sprints at 7 m/s on a 6% slope, separated by 4-min walks (1.5 m/s), and a 30-min recovery period at the walk with no slope. After the conditioning period, two SET were conducted 7 d apart (Fig. 1). These two SET were similar to SET-U except that the sprints were at 10 m/s. This exercise testing was designed to simulate a typical sport horse’s events, often being repetitive in a short period.

Before all SET, horses were deprived of food overnight but had access to water. All tests were performed in a climate-controlled barn with temperature set at 24°C and relative humidity ~50%. A 14-gauge, 150-cm polyethylene catheter (Intramedic polyethylene tubing; Becton Dickinson, Sparks, MD) was fed down the left jugular vein into the pulmonary artery and a 18-guage catheter was placed in the right carotid artery (Angiocath Deseret Medical Inc., Sandy, UT) ~60 min before the SET (Taylor and Kronfeld 1995). Placement of the end of the tubing in the pulmonary artery was facilitated by using an oscilloscope attached to a pressure transducer (Propag 140; Protocol Systems, Beaverton, OR). A thermocouple (Model IT-18; Physitemp Instruments, Clifton, NJ) was inserted to the catheter tip in the pulmonary artery.

Sampling and analysis. During the SET, 10 mL of arterial and 30 mL of mixed venous blood were taken at rest, during the last 15 s of

![FIGURE 1](https://example.com/figure1.png)
sprint 1, 2 and 6 as well as at 5, 10, 20 and 30 min of recovery. Blood samples were taken anaerobically into heparinized syringes (Sherwood Medical, St. Louis, MO) for analysis of PO$_2$, PCO$_2$, pH, [Na$^+$], [K$^+$] and [Cl$^-$.] (STAT Profile 2; Nova Biomedical, Waltham, MA). Bi-carbonate was calculated from pH and PCO$_2$. Additional heparinized blood was centrifuged to obtain plasma. An aliquot of plasma was immediately deproteinized in ice-cold perchlorate for lactate (La$^-$.) assay (Proc. No. 826-UV; Sigma Diagnostics, St. Louis, MO). Atot were estimated from TP using a conversion factor of 0.21 mEq/L (TP; Stämpfli et al. 1999). SID and Atot were estimated from TP using a conversion factor of 0.21 mEq/L (TP; Stämpfli et al. 1999). SID also increased (P 5 0.0038) along with plasma cortisol (P 5 0.0004).

**RESULTS**

All horses remained in good health throughout the study. Body weight was 419 ± 15 kg at the start of the study and 459 ± 15 kg at the end. The amount of weight gained was not affected by diet. No signs of protein deficiency (anorexia, loss of condition, weakness, poor coat) were observed. Feed intake averaged 8.8 ± 0.3 kg/d with no differences in horses fed the two diets. Due to differences in dietary protein, protein intake averaged 711 ± 21 g/d and 1323 ± 45 g/d for horses in LP and HP groups, respectively.

**Rest.** Before SET-1, arterial [La$^-$.] was higher in the LP groups (P 5 0.0022) but lower in the HP groups (P 5 0.011). There were no differences in PO$_2$, PCO$_2$, SID, Atot, [Na$^+$], [K$^+$], [Cl$^-$.], pH or [HCO$_3$$.] Venous [La$^-$.] was also higher for the LP groups (P 5 0.0076) but was not affected by fat level. No differences were found in PO$_2$, PCO$_2$, SID, Atot, [Na$^+$], [K$^+$], [Cl$^-$.], pH or [HCO$_3$$.].

Before SET-2, arterial [K$^+$] was higher in the LP groups (P 5 0.039) as was [HCO$_3$$.] (P 5 0.032). No differences were found in pH, PO$_2$, PCO$_2$, lactate, SID, Atot, [Na$^+$], [K$^+$] or [Cl$^-$.]. Venous [HCO$_3$$.] was higher (P 5 0.024) in the LP groups as well as [K$^+$] (P 5 0.025). Plasma cortisol was lower in the HF groups (P 5 0.0008) as well as in the LP groups (P 5 0.0043). There were no differences for pH, PO$_2$, PCO$_2$, SID, Atot, [La$^-$.], [Na$^+$] or [Cl$^-$.].

**Exercise.** During SET-1, arterial PCO$_2$ was decreased (P 5 0.0001). Increases were observed in [La$^-$.] and [K$^+$] (P 5 0.0001), PO$_2$ (P 5 0.0001), [Na$^+$] (P 5 0.0009) and [Cl$^-$.] (P 5 0.0007) and pH (P 5 0.0001). Exercise had no effect on arterial [HCO$_3$$.] and SID. In venous plasma, decreases were observed in pH, [Cl$^-$.] and PO$_2$ during the SET (P 5 0.0001). Increases were observed in PCO$_2$, [Na$^+$], [K$^+$], [HCO$_3$$.] and [La$^-$.] (P 5 0.0001). Venous SID was unaffected by exercise. During SET-2, arterial [HCO$_3$$.] and PCO$_2$ decreased (P 5 0.0001) along with pH (P 5 0.0086) but [La$^-$.] increased (P 5 0.0001). Plasma [Cl$^-$.] increased (P 5 0.0003) along with [K$^+$] (P 5 0.07), [Na$^+$] (P 5 0.0048) and PO$_2$ (P 5 0.0007). Arterial SID was unaffected by exercise. In venous plasma, decreases were observed in pH and PO$_2$ during the SET (P 5 0.0001). Increases were observed in PCO$_2$, [HCO$_3$$.], [La$^-$.] and [K$^+$] (P 5 0.0001). Plasma [Na$^+$] also increased (P 5 0.0002) along with SID (P 5 0.0038) and plasma cortisol (P 5 0.0004).

**Diet and exercise.** During SET-1, venous pH was higher (P 5 0.0056) in the LP groups (Fig. 2) but was not affected by fat level. Venous [HCO$_3$$.] was not affected by fat or protein level.

Venous PCO$_2$ was also not affected by fat or protein level. Venous PO$_2$ was higher (P 5 0.019) for the LP groups, especially during recovery, but was not affected by fat level. Plasma Atot was not affected by fat or protein level.

Venous SID was affected by a time × protein interaction (P 5 0.022) being higher in the LPHF group and there was not an effect of fat. Venous [La$^-$.] was higher (P 5 0.023) in the LF groups but was not affected by protein level (Fig. 4).

Arterial pH and [HCO$_3$$.] were not affected by fat or protein levels. Arterial PCO$_2$ was not affected by fat or protein level. A fat × protein interaction (P 5 0.0045) occurred for PO$_2$, contrasting increasing levels in the LPLF group but decreasing levels for the LPHF. Arterial SID, [Na$^+$], [K$^+$] and [Cl$^-$.] were not affected by fat or protein level.

During SET-2, venous pH was affected by a fat × protein interaction (P 5 0.022) being higher in the LPHF group and lower in the LPLF group (Fig. 5). Venous [HCO$_3$$.] also had
a fat \times protein interaction (P = 0.043) during recovery, remaining elevated in the LP groups (Fig. 6). Venous PCO2 was higher (P = 0.026) in the LF groups after the first sprint only as well as in the LP groups during the sprints (P = 0.019). Venous PO2 was not affected by fat or protein level. A fat \times protein interaction (P = 0.029) was observed with venous PO2 during recovery with higher levels observed in the LPLF group. Plasma Atot was not affected by fat or protein level.

Venous SID had a time \times protein interaction (P = 0.038) with higher SID in the LP groups during the later stages of the sprints (Fig. 7). Venous [Na+] was higher (P = 0.007) in the LP groups but was not affected by fat. Venous [K+] was higher (P = 0.026) for the LP groups, especially during the sprints, but again, was not affected by fat. Venous [Cl−] was higher (P = 0.046) during the sprints in the HF groups. Venous [La−] was higher (P = 0.05) in the LF groups, especially during the sprints, but was not affected by protein.

Plasma cortisol levels were lower (P = 0.006) in the HF groups and in the LP groups during the sprints (P = 0.048; Fig. 8).

Arterial pH and [HCO3−] were not affected by fat or protein. Arterial PCO2 was higher (P = 0.04) in the HF groups with a time \times fat interaction as the difference between the HP and LF groups became greater during recovery. Arterial PO2 levels were not affected by fat or protein levels. Arterial SID, [La−], [Na+] and [K+] also were unaffected by fat or protein levels. Arterial [Cl−] was higher (P = 0.042) in the HF groups during the sprints but was unaffected by protein.

DISCUSSION

Dietary protein decreased the acidogenic effect of exercise in unconditioned horses (SET-U). There were no main effects of fat with the exception of lower plasma lactate during SET-U and SET-C. However, because the effect of the LP diet during SET-C was only apparent in combination with the HF diet, it seems that there is a positive relationship between protein restriction and fat adaptation. This conclusion is supported by a lack of difference between horses fed the LPLF and HPLF diets.

In the comprehensive physico-chemical model of acid-base status by Stewart (Stewart 1981), the dependent variables,
Protein and fat diminish acidity of exercise

Hydrogen ion and bicarbonate ion, are influenced by changes in the three independent variables, PCO₂, SID and Atot. During SET-U, venous pH was higher for horses in the LP group. This difference in pH was attributable mainly to SID because PCO₂ and Atot were not affected by diet. During SET-C, the lower venous pH in exercising horses fed the two low fat diets could be explained by the higher PCO₂. In studies of intensely exercising horses, PCO₂ was the main determinant of changes in venous or arterial plasma pH (Kronfeld 1996). In contrast, the higher venous pH found in horses fed the LPHF diet could be explained by a combination of reduced PCO₂, due to fat adaptation, and increased SID, due to protein restriction.

The effect of protein restriction on venous plasma pH observed here confirms previous observations (Graham-Thiers et al. 1999a). This earlier study also revealed that the higher pH was influenced through changes in SID (Graham-Thiers et al. 1999c). The lack of effect on pH and PCO₂ in arterial blood is consistent with arterial blood reflecting pulmonary ventilation more than peripheral metabolism (Carlson 1995).

Dietary protein contains sulfur and phosphorus that become oxidized to sulfate and phosphate. The amino acids that are oxidized during exercise (leu, iso, val) do not contain sulfur or phosphorus so dietary protein is not expected to directly affect the acidogenic effect of sprint exercise. Prolonged restriction of dietary protein is expected to exert a chronic anti-acidogenic effect. Oxidation of sulfur-containing amino acids increases the net acid load in the body by 2 mol for every mole of sulfur-containing amino acid oxidized. Endogenous acid is also increased by dibasic and phosphorylated amino acids (Patience 1990). In the present study, venous SID was higher in the LP group over the course of the SET, compared with the HP group. Restricting dietary protein may be an alternative to replacing NaCl with sodium bicarbonate for reducing exercise acidosis, especially in horses consuming a HF diet. This would be beneficial for horses working hard in the heat because HP is thermogenic and will affect water balance with increased urination and possibly higher sweat loss (Meyer 1987).

In a previous study, sodium bicarbonate administration had an additive or, perhaps, synergistic effect with fat adaptation in moderating the blood lactate increase during repeated sprints (Ferrante et al. 1994). Dietary protein restriction had a similar interaction with fat adaptation in moderating the acidogenic effect of repeated sprints in the present experiment.

Dietary fat has also been observed to spare protein, especially under conditions of high energy demand, such as exercise. The present results suggest that protein deficiency may have been avoided partly because of fat adaptation (in addition to added lys and thr) in exercising horses fed a restricted protein diet. Fat adaptation has previously been suggested to improve metabolic regulation as indicated by plasma lactate responses in repeated sprints and incremental exercise. Moreover, fat adaptation increases fatty acid oxidation, which is more efficient than amino acid oxidation (Kronfeld 1996).

Lower plasma cortisol levels in the HF group may provide evidence of less reliance on glucose as fuel and more reliance on fat metabolism, with a diminished need for gluconeogenesis. With less amino acid oxidation, less endogenous acid would be produced in the body (McCargar et al. 1989). This would partly explain the higher pH and [HCO₃⁻] in venous blood of horses in the LPHF group during SET-C.

In conclusion, the acidogenic effect of sprinting exercise was diminished by both LP diets, regardless of fat level before conditioning, but only by the LPHF diet after conditioning. Thus, the combination of protein restriction and fat adaptation was needed for horses in training to reduce the acidogenic effect of intense exercise.

LITERATURE CITED


