
Abstract

A restricted protein diet reduces undesired metabolic effects during exercise but risks protein deficiency when fed chronically. Ten Arabian horses were assigned to 2 diets in a completely randomized design: fat 12% (10% added corn oil) and crude protein 7.5 or 14.5% on a dry basis. Horses were conditioned for 9 weeks, then an exercise test was performed. It began with a warm-up followed by six, one minute sprints at 10 m/s separated by 4 minutes of walking on a 6% slope. It was concluded with a 30 minute recovery at the walk. Blood samples were taken every 2 weeks during the conditioning period as well as at rest, during the last 15 seconds of each sprint and at 5, 10, 20 and 30 minutes of recovery during the exercise test. Urine samples were obtained from mares every 2 weeks. Blood samples were analyzed for albumin, total protein, plasma urea-N (PUN) and creatinine. Urine was analyzed for urea, creatinine and uric acid. Horses were observed daily for clinical signs of protein deficiency. Effects of diet and time were evaluated by analysis of variance with repeated measures.

During the conditioning period, there was no effect of diet on plasma albumin ($P = .25$), total protein ($P = .72$) or creatinine ($P = .21$). All values were within the normal ranges reported for horses. There was an effect of diet on PUN ($P = .0001$) with horses in the high protein group exhibiting greater PUN levels than horses in the low protein group. No difference in urine creatinine levels ($P = .78$) were observed. Urine urea ($P = 0.011$) as well as uric acid ($P = 0.0001$) were greater in the high protein group. These differences are expected simply as a reflection of the different protein levels in the diet. During the exercise test, no difference in albumin ($P = .32$), total protein ($P = .81$) or creatinine levels ($P = .39$) were observed. A greater PUN persisted in the high protein group ($P = .0001$) but this was expected due to the difference in dietary nitrogen. No detrimental effect of the lower level of protein on protein status was observed during interval training over the course of the
experiment. In a companion study, the lower level of protein moderated the acid-base responses to repeated sprints

**Introduction**

The upper limit of dietary protein for the exercising horse avoids thermogenic, ureogenic and acidogenic effects. The lower limit must avoid protein deficiency and previous studies have yielded inconsistent results. Increased needs for protein during exercise include maintenance of new muscle mass, repair of existing muscle mass and replacement of an nitrogen that would be lost in sweat (Meyer, 1987). The current dietary protein recommendations are 9, 10.4 and 11% crude protein (CP) for horses in light, moderate and intense exercise, respectively (NRC, 1989). Surveys in Michigan (Gallagher et al., 1992a and b) and Australia (Southwood et al., 1993) revealed that Thoroughbreds and Standardbreds were fed an average of 14% CP. Another study in central North Carolina (Honore et al., 1994) found that 70% of the horses were being fed excess protein in relation to NRC recommendations.

We propose that a low protein diet fortified with limiting amino acids may reduce undesired effects of protein without risking protein deficiency. This study evaluated the effects of dietary protein level on protein status of Arabian horses during interval training and a repeated sprint test. A companion study evaluated the acid-base status of these horses.

**Materials and methods**

_Horses._ Ten Arabian horses (5 mares, 5 geldings, age 7 to 10 years) were housed in a dry lot with free access to water. Twice daily they were brought into box stalls where the were given three hours to consume their feed. General health and feed intake were observed daily, and body weight, body condition, and coat condition were monitored every two weeks. The protocol was approved by the Institutional Animal Care and Use Committee.
Diets. Horses were randomly assigned to two complete feeds formulated to provide 3.3 Mcal DE/kg dry matter (DM) (Table 1) and were similar except for two protein levels: HP, 14.5% CP and LP, 7.5% CP, supplemented with .5% crystalline lysine and .3% threonine (Heartland Lysine Inc., Chicago, IL) to match the amino acids levels in HP. Three mares were fed LP and two mares were fed HP.

Conditioning: All horses underwent nine weeks of conditioning using a high-speed treadmill (Mustang 2000, Kagra Inc., Fahrwangen, Switzerland). The interval training protocol consisted of three phases, each lasting 3 weeks (Table 2). Horses were interval trained twice a week and were walked at 1.5 m/s for 30 minutes on non-exercised days on a mechanical walker.

Standard Exercise Test. After the nine weeks of conditioning, all horses performed a standard exercise test (SET). The SET consisted of six minutes at the walk (1.5 m/s, 3 minutes on zero slope and 3 minutes on a 6% slope), a five minute warm up at the trot (3.5 m/s) followed by six, one minute sprints at 10 m/s on a 6% slope, and a 30 minute recovery period at the walk (1.5 m/s) with zero slope. Horses were fasted overnight but had access to water before the SET. All tests were performed in a climate-controlled barn with temperature set at 24°C and relative humidity approximating 50%. Heart rate was recorded using a digital monitor (Polar Pacer, Port Washington, NY). A 14-gauge, 150 c polyethylene catheter (Intramedic polyethylene tubing, Becton Dickinson, Sparks, MD) was placed in the left jugular vein approximately 60 minutes before the SET. The area over the left jugular vein was surgically prepared by shaving and washing with an iodine solution (7.5% iodine, Operand, Redi Products, Inc., Prichard, WV). A small incision was made through the skin over the area for placement of the catheter in the jugular vein after the area had been anaesthetized with a subcutaneous injection (lidocaine hydrochloride injectable 2%, VEDCO, St. Joseph, MO). 10-gauge needle was inserted into the jugular vein and the catheter was passed into the pulmonary artery. Placement of the end of the tubing in the pulmonary artery was ensured b
observing pressure waves on an oscilloscope attached to a pressure transducer (Propaq 140, Protocol Systems Inc.). Once the catheter was placed, it was secured by sutures (Z-O, 60mm, Dermalon, Davis & Geck). A 5-mL extension set (Baxter Healthcare Corp., Deerfield, IL) was attached to the catheter to facilitate sampling during exercise. The catheter was kept patent with heparinized saline (10 units/mL) (heparin: Elkins-Sinn Inc., Cherry Hill, NJ; saline: VEDCO, St. Joseph, MO).

**Sampling and Analysis.** During the conditioning period, weight, body condition score, blood and urine samples were taken every two weeks approximately 3 hours after the morning meal. Horses were weighed on an electronic scale (EZ-weigh, Dyco, Cave Creek, AZ). Body condition score (BCS) was evaluated using a standardized system (Henneke et al., 1983). Blood samples were taken via jugular puncture into heparinized tubes (Vacutainers, Becton Dickinson, Rutherford, NJ). Plasma was separated and frozen for later analysis of urea-N (Proc. No. 67-UV, Sigma Diagnostics, St. Louis, MO), albumin (Proc. No. 631, Sigma Diagnostics, St. Louis, MO), total protein (Proc. No. 541, Sigma Diagnostics, St. Louis, MO) and creatinine (Proc. No. 557, Sigma Diagnostics, St. Louis, MO). Urine samples were taken from mares only. A catheter was placed into the urethra after washing the mare with an iodine solution (7.5% iodine, Operand, Redi Products Inc., Prichard, WV) and urine flow was initiated by suction from a syringe placed on the end of the catheter. Urine was collected, divided into aliquots and frozen for later analysis of urea (Proc. No. 640, Sigma Diagnostics, St. Louis, MO), creatinine (Proc. No. 557, Sigma Diagnostics, St. Louis, MO) and uric acid (Proc. No. 685-11, Sigma Diagnostics, St. Louis, MO).

During the SET, 30 mL of mixed venous blood was taken at rest, during the last seconds of each sprint and at 5, 10, 20 and 30 minutes of recovery. Blood samples were taken using syringes (Sherwood Medical, St. Louis, MO), placed in heparinized tubes (Vacutainers, Becton Dickinson, Rutherford, NJ) and centrifuged to yield plasma. Aliquots were frozen for later analysis of total protein (TP) (Proc. No. 541, Sigma Diagnostics, St. Louis, MO),
albumin (Proc. No. 631, Sigma Diagnostics, St. Louis, MO), creatinine (Proc. No. 557, Sigma Diagnostics, St. Louis, MO) and urea-N (Proc. No. 67-UV, Sigma Diagnostics, St. Louis, MO).

Statistics: Data were summarized as least squares means and standard errors. Analysis of variance with repeated measures was used to evaluate the effects of diet and time (conditioning) during the conditioning period, and diet, time (exercise and/or recovery) and interactions during the SET using the GLM procedure of SAS (SAS, 1991). Paired t-tests were conducted to evaluate changes within diet from the start and finish of the experiment (SAS, 1991). Significance was determined to be a $P < .05$ and tendencies at $P < .15$.

Results

Horses remained in good condition throughout the study. No signs of protein deficiency (anemia, weakness, poor condition or poor coat condition) were observed during the study. Average starting weight was 436±17 kg and ending weight was 445±17 kg. All horses gained weight, however, no differences in weight ($P = .53$) or body condition score ($P = .33$)(BCS) (Figure 1) were observed between diets. Feed intake was not different between diets ($P = .28$) but due to protein level differences, average intake of CP was 578 ± 29 g/d on the LP diet and 1,117 ± 65 g/d on the HP diet ($P = .001$).

Diet: During the conditioning period, no effect of diet was observed in plasma albumin ($P = 0.53$)(Figure 2), total protein ($P = 0.93$)(Figure 3) or creatinine ($P = 0.29$)(Figure 4). All values were within reference ranges (Wilson et al., 1986; Weldemen et al., 1995). Plasma urea-N concentration and the urea-N:creatinine ratio were greater ($P = 0.0001$)(Figure 5) in the HP group compared to the LP group. Plasma creatinine was similar ($P = 0.29$) in both groups. Urine urea (Figure 6) and uric acid (Figure 7) concentrations as well as urine urea:creatinine and uric acid:creatinine ratios were greater ($P = .011$ and .0002 respectively) in the HP group. No differences were noted in urine creatinine levels ($P = .78$)(Figure 8).
During the exercise test, no differences were found in plasma concentrations of albumin ($P = .32$)(Figure 9), total protein ($P = .81$)(Figure 10) or creatinine ($P = .39$)(Figure 11). Plasma urea-N as well as the urea-N:creatinine ratio (Figure 12) were greater ($P = .0001$) in the HP group compared to the LP group.

**Conditioning:** Training resulted in a 14% increase in plasma concentrations of albumin ($P = .005$) and creatinine ($P = .033$). Total protein was decreased by 11% as a result of training ($P = .021$). Over the course of training, there was a training X diet interaction in plasma urea-N ($P = .004$) with the level increasing in the HP group and decreasing in the LP group over the course of the experiment.

A diet X training interaction also existed for urine uric acid ($P = .056$) concentration increasing in the HP group but decreasing in the LP group. No effects of training on urine creatinine and urine urea levels were observed.

Considering changes within each group over the conditioning period, total protein was decreased below the initial value for both the LP group ($t$-test, $P = .0005$) and the HP group ($t$-test, $P = .023$) by 12 and 9.6% respectively. Albumin was increased over the course of the experiment for both the LP and HP groups ($t$-test, $P = .002$) by 16 and 13% respectively. Creatinine was increased by 18% for the LP group ($t$-test, $P = .03$) compared to the initial value while the HP group was unchanged (1% increase) ($t$-test, $P = .92$) compared to the beginning of the experiment. Plasma urea-N was increased in the HP group ($t$-test, $P = .01$) and decreased in the LP group ($t$-test, $P = .01$) compared to initial values.

**SET exercise:** Exercise resulted in a 10% increase in plasma total protein ($P = .001$) and a 29% increase in creatinine ($P = .001$). Exercise also resulted in a 9.4% increase in plasma albumin ($P = .004$). Plasma urea-N was relatively unchanged in the HP group increasing by 7% and in the LP group decreasing by 9% however, the urea-N:creatinine ratio
was decreased by 18% during exercise \((P = .001)\). This difference was due to the increase in creatinine since when urea-N alone was reviewed the change in its level during exercise was much less. Since albumin and total protein were both increased by approximately 10%, a 10% loss of water from plasma can be estimated from this data.

**Discussion**

The results revealed no detrimental effect of the lower level of dietary protein on protein status during interval training over the course of this experiment or during a standard exercise test. Lower levels of protein have been fed to exercising horses without detrimental effects. A level of 8.4% CP was inadvertently fed to Arabian horses undergoing similar exercise and SET to this study without any detrimental effects (Taylor et al., 1995). A crude protein level as low as 5.5% CP was shown to be adequate for exercising horses by Patterson et al. (1985). This conclusion was based on normal albumin and total protein levels as well as a lack of outward signs of protein deficiency. The authors recommended a protein level of 8.5% CP however, since the horses fed this level of protein appeared to have better protein reserves as demonstrated by increased plasma urea levels during a four day fast. A level of 6% CP was found to be adequate for growing two year olds suggesting improved use of the dietary protein with exercise (Orton et al., 1986). An increase in nitrogen retention in exercising horses was observed during conditioning as well as following the period of conditioning (Freeman et al., 1988). It was concluded that more protein (above maintenance) was needed to build and support additional muscle mass that developed as a result of muscle hypertrophy seen with exercise training.

Total protein and albumin in this study were not affected by diet however, over time, albumin increased and total protein decreased. With the main two components of total protein being albumin and globulins, an overall decrease in TP and increase in albumin suggest a decrease in globulin level which has been observed as a result of exercise stress. Despite the
fact that albumin has not been regarded as a sensitive index of protein status in the horse, the
length of the experiment (nine weeks) would appear to have been long enough to observe an
adverse effect of dietary protein on albumin synthesis since the half life of albumin is about 18
days (Young et al., 1990).

The changes that occurred during exercise in this study show an increase in albumin,
TP, urea-N and creatinine with a decrease during recovery. These changes are most likely
results of changes in plasma volume during exercise with an increase in hematocrit seen during
strenuous exercise. The increase in plasma creatinine and its failure to return to resting values
by 30 minutes of recovery may reflect an increase in muscle activity as a result of exercise.
The lack of increase in urea-N during exercise and recovery indicates that energy and protein
were adequate, and did not require additional catabolism of muscle protein.

Excess protein leads to many detrimental effects including increasing the water
requirement due to increased urea excretion, an increase of ammonia in the bloodstream as
well as in urine increasing respiratory stress from exposure to ammonia fumes (Meyer, 1987).
This would require protein to be at a minimum without risking deficiency, which has been
observed in this study. The difference in PUN as well as urine urea are reflections of the leve
of nitrogen in the diets and demonstrate the increased excretion of urea in the HP group
contributing to the detrimental effects of high protein as described by Meyer (1987).

Despite the fact that several researchers have not found specific detrimental effects o
high protein for the exercising horse (Miller and Lawrence, 1988; Frank et al., 1989; Miller e
al., 1991), it seems logical that high protein producing more heat, urea and acid, would lead to
earlier fatigue. Those researchers determined that higher levels of protein were not detrimenta
to exercise based on measurements of heart rates and lactate levels. However, other
parameters of exercise such as acid-base balance may be affected by high protein and warrants
further investigation.
Protein quality is a function of the amino acid profile as well as digestibility. The addition of lysine and threonine to the LP diet improved the amino acid profile of that diet in relation to requirements. Lysine and threonine have been shown to be the limiting amino acids for growth in the horse (Ott et al., 1981; Graham et al., 1994). Exercise has shown to increase protein digestibility in the horse and increased protein efficiency for gain in two year olds. (Worth et al., 1987; Orton et al., 1986).

The lack of a detrimental effect of the LP diet in this study may have been due to the improved amino acid profile of the LP diet, improved utilization of the dietary protein due to exercise or both. Fat level may also affect digestibility. Improved ileal digestibility of amino acids was observed in growing pigs fed a fat supplemented diet. Fat may delay gastric emptying and thus improve digestibility. Improved ileal digestibility has improved the amino acid profile of plasma despite the fact that overall tract digestion of amino acids was no improved (Li and Sauer, 1994). The fact that the diets in this study were high in fat content may have contributed to a more efficient use of the protein through improved digestibility.

Overall, the suggestion can be made that the LP diet supplemented with limiting amino acid can support normal protein status during a nine week conditioning program and a repeated sprint test. A companion study suggested a favorable effect of the low protein diet on acid-base responses in the sprint tests.

**LITERATURE CITED**


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## Table 1. Ingredient and chemical composition of experimental diets, as-fed basis*

<table>
<thead>
<tr>
<th>Item, %</th>
<th>High Protein</th>
<th>Low Protein</th>
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<tbody>
<tr>
<td>Orchardgrass ha</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Oats</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Corn oil</td>
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<td>10</td>
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<td>5</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>Corn grain</td>
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<td>17</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
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<td>.75</td>
</tr>
<tr>
<td>Limestone</td>
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<td>0.55</td>
</tr>
<tr>
<td>Sal</td>
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<td>0.5</td>
</tr>
<tr>
<td>Vitamin/Mineral mix</td>
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<td>1</td>
</tr>
<tr>
<td>L-Lysine/Threonine</td>
<td>0</td>
<td>.5/.3</td>
</tr>
<tr>
<td>Moisture (%)</td>
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<td>7.3</td>
</tr>
<tr>
<td>DE (Mcal/kg)</td>
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<td>3.22</td>
</tr>
<tr>
<td>Ether Extract (%)</td>
<td>13.1</td>
<td>13.3</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
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<td>7.6</td>
</tr>
<tr>
<td>L-Lysine (%)**</td>
<td>0.69</td>
<td>0.61</td>
</tr>
<tr>
<td>L-Threonine (%)**</td>
<td>0.59</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* Standard analytical procedures were followed by the Virginia Tech Forage Laboratory; N=4

** Heartland Lysine Inc., Chicago, IL
Table 2. Protocol for interval training over 9 weeks. Each horse exercised twice weekly and each phase is additive to the one before. Phase one was week 0-3, phase two week 4-6 and phase three weeks 7-9.

<table>
<thead>
<tr>
<th>Training (week)</th>
<th>Exercise (minutes)</th>
<th>Gait</th>
<th>Speed (m/s)</th>
<th>Slope (%)</th>
</tr>
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<tbody>
<tr>
<td>0-3</td>
<td>0-2.5</td>
<td>Walk</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>0-3</td>
<td>2.5-5</td>
<td>Walk</td>
<td>1.5</td>
<td>6</td>
</tr>
<tr>
<td>0-3</td>
<td>5-9</td>
<td>Trot</td>
<td>3.5</td>
<td>6</td>
</tr>
<tr>
<td>0-3</td>
<td>9-12</td>
<td>Canter</td>
<td>7.0</td>
<td>6</td>
</tr>
<tr>
<td>0-3</td>
<td>12-15</td>
<td>Trot</td>
<td>3.5</td>
<td>6</td>
</tr>
<tr>
<td>4-6</td>
<td>15-18</td>
<td>Canter</td>
<td>8.0</td>
<td>6</td>
</tr>
<tr>
<td>4-6</td>
<td>18-21</td>
<td>Trot</td>
<td>3.5</td>
<td>6</td>
</tr>
<tr>
<td>7-9</td>
<td>21-24</td>
<td>Canter</td>
<td>9.0</td>
<td>6</td>
</tr>
<tr>
<td>7-9</td>
<td>24-27</td>
<td>Trot</td>
<td>3.5</td>
<td>6</td>
</tr>
<tr>
<td>0-9</td>
<td>Additional 2 minutes</td>
<td>Walk</td>
<td>1.5</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 1. Body condition scores during the nine week experiment were increased with time, presumably training ($P = .01$) but were unaffected by diet ($P = .33$).
Figure 2. Plasma albumin at rest during the nine week training period increased with time ($P = .53$), but was unaffected by diet ($P = .53$).
Figure 3. Plasma total protein at rest during the nine week training period decreased over time
\((P = .021)\) but was unaffected by diet \((P = .928)\).
Figure 4. Plasma creatinine at rest during the nine week training period was affected by time ($P = .033$) but was not different between diets ($P = .29$). It was increased ($P = .031$) in the LP group compared to day 0 but remained unchanged ($P = .92$) in the HP group compared to day 0.
Figure 5. Plasma urea-N was lower \((P = .0001)\) in the LP group than in the HP group. It exhibited a time X diet interaction \((P = .004)\), increasing in the HP group \((P = .043)\) but decreasing in the LP group \((P = .012)\). Response of the urea-N:creatinine ratio were similar because creatinine changes were small (Figure 4).
Figure 6. Urine urea and urea:creatinine ratio at rest during the nine week conditioning period was affected by diet ($P = .011$) but was unaffected by time ($P = .56$). Urine was obtained from mares only (n=5; 3 LP and 2 HP).
Figure 7. Urine uric acid levels and uric acid:creatinine ratio at rest during the nine week conditioning period resulted in a diet X time interaction ($P = .056$) where the LP group decreased over time and the HP group remained unchanged. There was also a main effect of diet ($P = .0002$). Urine was obtained from mares only (n=5; 3 LP and 2 HP).
Figure 8. Urine creatinine levels at rest during the nine week training period were unaffected by time and diet \((P = .78)\). Urine was obtained from mares only \((n=5)\).
Figure 9. Plasma albumin at rest, during 6 sprints (S1-S6) and at 5, 10, 20 and 30 minutes of recovery (R1-R4). It was unaffected by diet ($P = .32$) but increased during exercise ($P = .004$). Time points denoted with an asterisk are different from resting values ($P < .05$).
Figure 10. Plasma total protein at rest, during 6 sprints (S1-S6) and at 5, 10, 20 and 30 minutes of recovery (R1-R4). It was unaffected by diet ($P = .81$) but increased during exercise ($P = .001$). Time points denoted with an asterisk are different from resting values ($P < .05$).
Figure 11. Plasma creatinine at rest, during 6 sprints (S1-S6) and at 5, 10, 20 and 30 minutes of recovery (R1-R4). It was unaffected by diet ($P = .39$) but increased during exercise ($P = .001$). Time points denoted with an asterisk are different from resting values for both groups ($P < .05$).
Figure 12. Plasma urea-N and urea-N:creatinine ratio at rest, during 6 sprints (S1-S6) and a 5, 10, 20 and 30 minutes of recovery (R1-R4) were affected by diet ($P = .0001$). Plasma urea-N was unaffected by exercise however the urea-N:creatinine ratio decreased during exercise ($P = .001$). Time points denoted with an asterisk are different from resting values ($P < .05$).