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Effect of Dietary Protein Level on Nitrogen Metabolites in Exercised Quarter Horses

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ABSTRACT Six conditioned Quarter Horse mares were used in a crossover design to assess the effect of dietary protein level on nitrogenous metabolites in venous blood, urine and sweat. After a 2 week adaptation period to isocaloric diets containing 9.0% (control) or 18.5% crude protein (CP), each horse performed an exercise test (ET). The horses were exercised in the absorptive phase of digestion (3–4 hours after a meal) for 15 min on an 11% grade at 4.5 m s^{-1}. Venous blood samples were obtained at rest, during exercise and during a 60 min recovery period. Urine samples were taken at rest and 60 min after exercise. Exercise incresed ($p<0.001$) plasma alanine (ala), glutamine (gln) and the branched chain amino acids (BCAA), while glutamate (glu) decreased ($p<0.05$). Dietary protein level did not ($p<0.05$) alter plasma glu or gln; however, a diet by exercise interaction ($p<0.01$) occurred for plasma ala, being lower in horses receiving 18.5% CP. The BCAA were higher ($p<0.01$) in horses receiving excess protein. Increasing dietary protein stimulated ($p<0.01$) urea production and excretion ($p<0.001$) in sweat. Elevated dietary protein also increased ($p<0.02$) the urinary orotic acid/creatinine ratio which may indicate that an intake of 1 700 g CP d^{-1} exceeds the maximal activity of the urea cycle in the horse. These data indicate that the horse adapts to increased dietary protein by altering nitrogen metabolism at rest, during exercise, and during the subsequent recovery period.

Key words Dietary protein; amino acids; urea; exercise; horses.

INTRODUCTION

Debate exists on whether dietary protein affects performance of the horse. Freeman et al.[10] suggested that exercising horses require a small increase in dietary protein due to increased nitrogen retention. However, Meyer[15] cautioned that because the horse has a low renal urea clearance rate and decreased urine flow rate during exercise, protein intakes in excess of 2 g digestible crude protein per kg body weight should be avoided. In addition, excess protein intake may increase the water requirement and also increase the energy costs associated with ammonia (NH_3) detoxification in the liver.[15] Furthermore, one study of racetrack feeding practices revealed a correlation between dietary protein level and race time such that time to finish increased 1 to 3 s for every kg of crude protein (CP) ingested over recommended levels.[11] In humans and rat, various types of exercise can affect protein catabolism, amino acid degradation and protein synthesis.[12] In addition, exercise will increase the oxidation of amino acids, especially leucine.[30] Very little research has been conducted on amino acid metabolism during exercise in the horse or the interrelationships between dietary protein level and amino acid metabolism. This study was conducted to evaluate the effect of dietary protein level on nitrogenous metabolites in venous blood, urine and sweat during the absorptive phase of digestion.

Table 1. *The composition of the pelleted diets*[a]

	Control (% of diet)	High-protein (% of diet)
Wheat straw	26.6	10.0
Orchardgrass hay	13.2	30.0
Oats	8.6	14.0
Corn	6.2	19.7
Soybean meal (44% CP)	8.4	19.5
Molasses	5.5	5.6
Vitamin-mineral[b]	0.43	0.43
Limestone	0.29	0.44
Dicalcium phosphate	1.06	–
Cornstarch	29.7	–

[a] Control: CP = 9.0%, DE = 2.81 MCal, Ca = 0.50%, P = 0.40%, DM = 91.5%, Ash = 5.51%. High-protein: CP = 18.5%, DE = 2.82 MCal, Ca = 0.50%, P = 0.40%, DM = 91.9%, Ash = 7.16%.

[b] Vitamin-mineral premix (per lb)-vit. A, USP 1 500 000; vit. D_3, USP, 150 000; vit. E, USP, 10 000; vit. K, mg, 1 000; vit. B_{12}, mg, 8; riboflavin, mg, 1 000; D-pantothenic acid, mg 2 750; niacin, mg, 7 500.

MATERIALS AND METHODS

Six mature, conditioned Quarter Horse mares were assigned in a crossover design to either a control (9.0% CP) or a high-protein (18.5% CP) diet. To achieve a level of 18.5% CP, soybean meal (44% CP) was used. For assurance of a similar amino acid composition as a percent of dietary protein intake between diets, the control diet was produced by diluting the high-protein diet with cornstarch and wheat straw (Table 1). The analyzed amino acid compositions of the pelleted diets are shown in Table 2. Each diet was pelleted. Water and trace mineralized salt were available ad libitum. The mares were fed the complete pelleted diets at a rate to maintain body weight (9.4 to 9.6 kg dry matter day^{-1}), resulting in an average daily CP intake of 863 g for the control diet and 1 741 g for the high-protein diet. The horses received 26 to 27 Mcal of digestible energy per day as calculated from the energy values of the feed ingredients. Body weight prior to the study was not different ($p<0.05$) between treatments and did not ($p>0.05$) change during the 2 week adaptation period to the pelleted diets.

In other species, enzyme activities associated with amino acid catabolism in the liver increase within 3 days of altering dietary protein intake.[2,22] Therefore, a 2 week adaptation period to the diets was chosen in this study. At the conclusion of the adaptation period, an exercise test (ET) was conducted which consisted of 2 min warm up at 1.4 m s^{-1}, 15 min at 4.5 m s^{-1} and a 60 min recovery period. Heart rate, oxygen consumption and lactate data reported previously for the ET indicated that the horses were working above 60% VO_2max.[21] On the morning of the exercise test, the horses were allowed access to their morning meal for 2 hours, followed by exercise 1 to 2 hours later. Following the conclusion of the ET, the horses were allowed a 1 week rest period from the experimental diets before being switched to the opposite diet for 2 weeks. The ET was then repeated.

Venous blood was withdrawn through an indwelling catheter in the left jugular vein at rest, at the 5th, 10th and 15th min of exercise and at the 5th, 30th and 60th min of recovery. Plasma was assayed for NH_3, uric acid, alanine (ala), glutamate (glu), glutamine (gln), isoleucine (ile), leucine (leu), valine (val) and urea-N. Serum was obtained at rest and the 60th minute of recovery for evaluation of creatinine levels.

Urine samples were obtained by catheterization of the bladder at rest and at the 60th min of recovery using a 30 French Davol stomach tube (Baxter Hospital Supply, McGaw Park, IL). The urine was immediately frozen for subsequent analysis of creatinine, orotic acid and urea. Sweat was obtained from the left side of the neck within 5 min of completion of the ET. The sample was centrifuged to remove all debris, and frozen until analysis.

Table 2. *Amino acid composition of pelleted diets*[a]

	Control		High-protein	
	% of diet	% of protein	% of diet	% of protein
Alanine	0.43	4.78	0.88	4.76
Arginine	0.52	5.78	1.14	6.16
Aspartic Acid	0.93	10.33	1.89	10.22
Glutamic Acid	1.50	16.67	3.06	16.54
Glycine	0.40	4.44	0.81	4.38
Histidine	0.21	2.33	0.42	2.27
Isoleucine	0.39	4.33	0.78	4.22
Leucine	0.70	7.78	1.45	7.84
Lysine	0.40	4.44	0.90	4.86
Methionine	0.10	1.07	0.17	0.92
Phenylalanine	0.42	4.67	0.90	4.86
Proline	0.48	5.33	0.99	5.35
Serine	0.43	4.78	0.88	4.77
Threonine	0.35	3.89	0.71	3.84
Tyrosine	0.25	2.78	0.55	2.99
Valine	0.41	4.55	0.89	4.81

[a] Control = (9% CP); 863 g CP d^{-1}; High-protein = (18.5% CP); 1741 g CP d^{-1}.

Blood samples for amino acid and urea determinations were drawn into sodium heparin tubes and centrifuged for 20 min at 10°C. Plasma proteins were precipitated by the addition of 3.5% 5-sulfosalicylic acid and centrifuged at 10°C. The protein-free supernatant was frozen until analyzed on a Beckman amino acid analyzer (Model 6300, Beckman Instruments, Palo Alto, CA). Total protein, urine and sweat urea-N, and serum and urine creatinine were analyzed using commercially available kits (Sigma Chemical Company, St. Louis, MO). Urine orotic acid was analyzed by an adaptation of the procedure by Adachi et al.[1]

Total body urea-N was calculated by body weight changes during exercise and recovery and the analyzed plasma urea-N concentrations. For the calculations we assumed total body water is approximately 60% of body weight, urea is freely diffusible in all cellular compartments,[4] body weight loss estimates sweat loss,[3] and 90% of the sweat loss consists of water.[3] Therefore, total body urea-N at rest was calculated as 60% of body weight × the concentration of urea-N (mg kg^{-1}) in plasma. After exercise, total body urea-N was calculated as (60% of body weight before exercise − 90% of weight loss during exercise) × (urea-N (mg kg^{-1}) in plasma at the 60th minute of recovery) + (90% of the weight loss during exercise × the concentration of urea-N in sweat).

Venous blood parameters were analyzed as a split-plot with repeated measures.[26] The whole unit treatment was diet, blocked by period (crossover) and time was the sub-unit treatment. Analysis of variance was used to test for the effects of horse, period, diet, time and the diet by time interaction. Urine and sweat samples were analyzed by an analysis of variance to test for diet and time effects.

RESULTS

At rest ala averaged 37.4 ± 3.6 μmol dl^{-1} in horses receiving the control diet and 31.7 ± 2.5 μmol dl^{-1} in horses receiving the

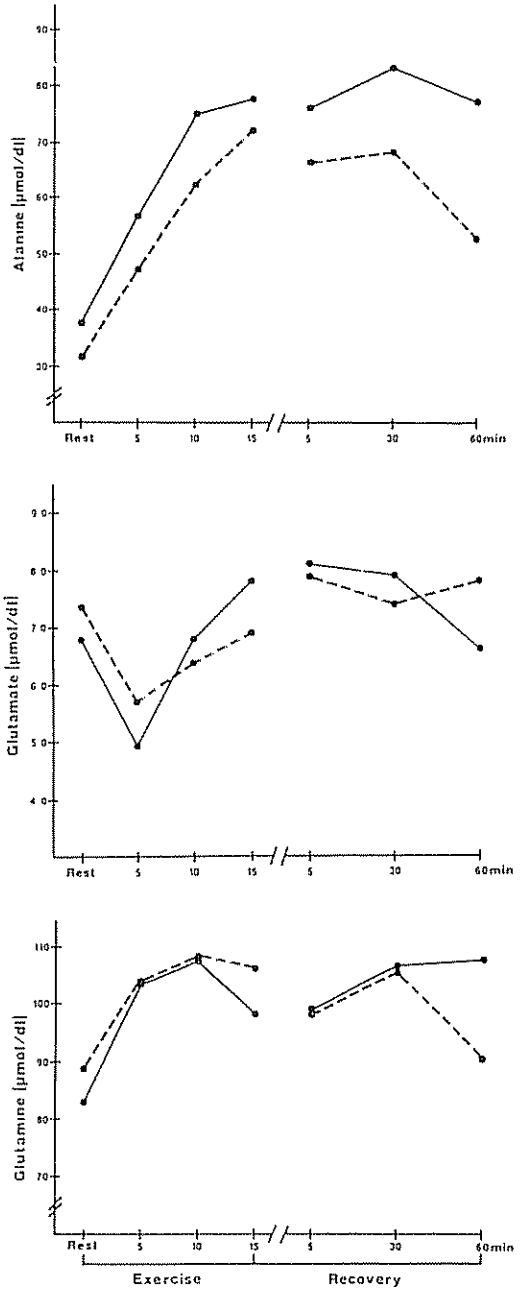

Fig 1 Effect of increased protein intake on alanine, glutamate and glutamine (µmol dl^{-1}) before (rest), during and after exercise. Control: (—): 863 g crude protein (CP) d^{-1}; High-protein (– – –): 1 741 g CP d^{-1}

high-protein diet. Alanine increased ($p<0.001$) to 77.8 ± 6.4 µmol dl^{-1} in the control horses and to 74.4 ± 2.0 µmol dl^{-1} in horse receiving excess protein at the 15th min of exercise and remained elevated in both groups throughout the recovery period. A significant ($p<0.05$) interaction between diet and time occurred as a result of ala being elevated in the control horse at the 5th, 30th and 60th min of recovery (Fig. 1). Plasma gln was not affected by diet ($p>0.05$), however gln increased ($p<0.001$) over time (Fig. 1). At the 15th min of exercise, gln averaged 101.8 ± 10.0 µmol dl^{-1} and 106.4 ± 8.8 µmol dl^{-1} in the control horses and in horses receiving the high-protein diets, respectively (Fig. 1). Initially, glu decreased ($p<0.005$) in both groups; however, by the 10th min of exercise, glu had returned to resting levels. Glutamate was not different ($p>0.05$) from resting levels throughout the recovery period. Dietary protein did not ($p>0.05$) alter the glu concentration.

Plasma val was elevated ($p<0.005$) in horses receiving the high-protein ration at rest, during exercise and during recovery. The exercise bout and the subsequent recovery period elevated ($p<0.001$) plasma val. The concentration of val was highest at the 30th min of recovery, averaging 51.5 ± 4.5 µmol dl^{-1} in the control horses and 67.7 ± 6.4 µmol dl^{-1} in horses receiving 18.5% CP (Fig. 2). A diet by time interaction ($p<0.05$) occurred for plasma ile. Isoleucine was elevated ($p<0.001$) in horses receiving the high-protein ration at rest, during exercise and at the 5th and 30th min of recovery. Isoleucine was not elevated above resting levels until the 5th min of recovery in both groups and remained elevated above resting levels through the 60 min recovery period. Exercise and the recovery period elevated ($p<0.001$) leu levels. In the control horses, at rest, leu averaged 23.6 ± 1.9 µmol dl^{-1}, increasing to 41.3 ± 3.2 µmol l^{-1} at th 30th min of recovery. In horses receiving the high-protein diet, leu averaged 33.8 ± 4.5 µmol dl^{-1} at rest, increasing to 50.0 ± 4.5 µmol dl^{-1} at the 30th min of recovery. Leu-

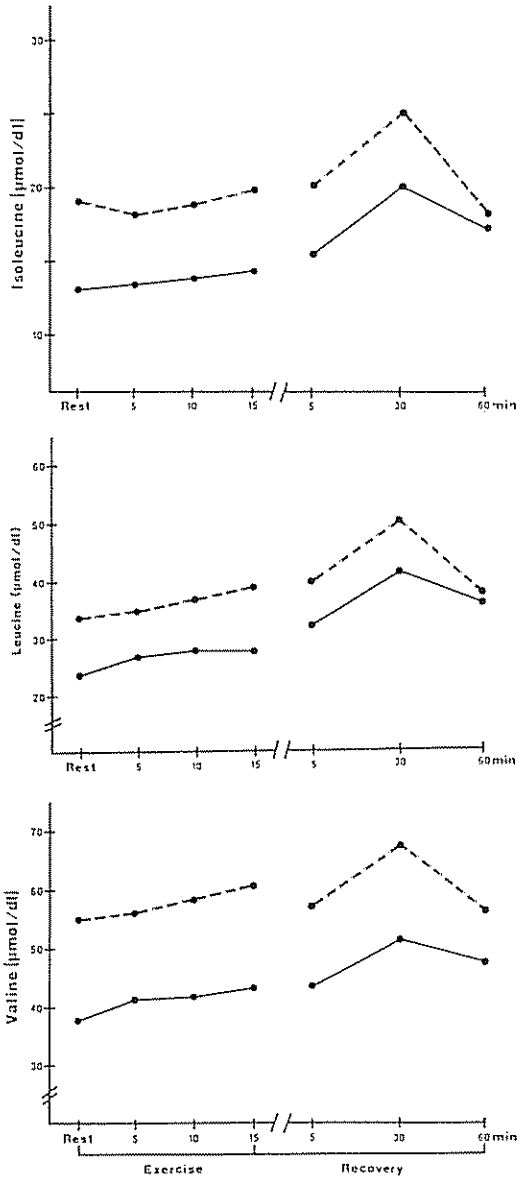

Fig. 2. Effect of increased protein intake on isoleucine, leucine and valine (µmol dl^{-1}) before (rest), during and after exercise. Control: (—): 863 g crude protein (CP) d^{-1}; High protein (– – –): 1 741 g CP d^{-1}.

cine was elevated ($p<0.001$) in horses receiving excess protein at each point, except for the 60th min of recovery (Fig. 2).

Plasma NH_3 was slightly higher ($p<0.07$) in the control horses and significantly

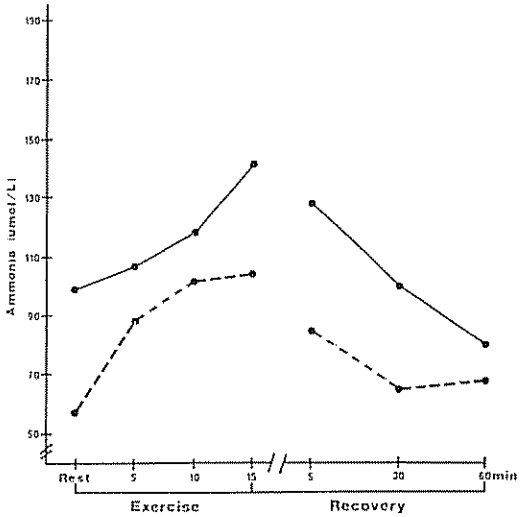

Fig. 3 Effect of increased protein intake on ammonia (µmol l^{-1}) before (rest), during and after exercise. Control: (—): 863 g crude protein (CP) d^{-1}; High protein (– – –): 1 741 g CP d^{-1}.

($p<0.001$) changed over time (Fig. 3). The resting plasma NH_3 concentration averaged 98.9 ± 16.8 µmol l^{-1} in the control horses and 57.7 ± 8.0 µmol l^{-1} in horses receiving 18.5% CP. Exercise resulted in an increase to 141.7 ± 21.0 µmol l^{-1} in horses receiving the control diet and to 104.0 ± 9.1 µmol l^{-1} in horses receiving the high-protein diet. In both groups, NH_3 had returned to resting levels by the 30th min of recovery.

At rest, uric acid averaged 11.2 ± 1.7 µmol l^{-1} in the control group and 13.7 ± 2.8 µmol l^{-1} in horses receiving excess protein. By the 15th min of exercise, uric acid increased to 41.4 ± 6.2 and 35.5 ± 4.0 µmol l^{-1} in the control horses and in horses ingesting the high-protein diet, respectively. Uric acid remained elevated ($p<0.01$) above rest throughout the recovery period. Statistically, uric acid was not ($p>0.05$) affected by diet when all time points were taken into account. However, when comparing individual time points, uric acid was elevated ($p<0.05$) in the control horses at the 5th min of recovery (Fig. 4).

Urea-N was elevated ($p<0.01$) in horses

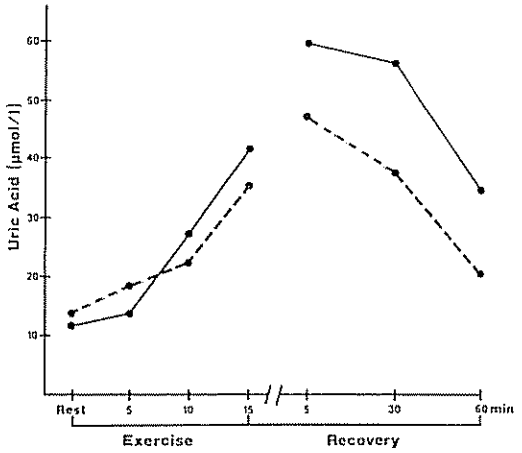

Fig 4 Effect of increased protein intake on uric acid (µmol l^{-1}) before (rest), during and after exercise. Control: (—): 863 g crude protein (CP) d^{-1}; High protein (– – –): 1 741 g CP d^{-1}.

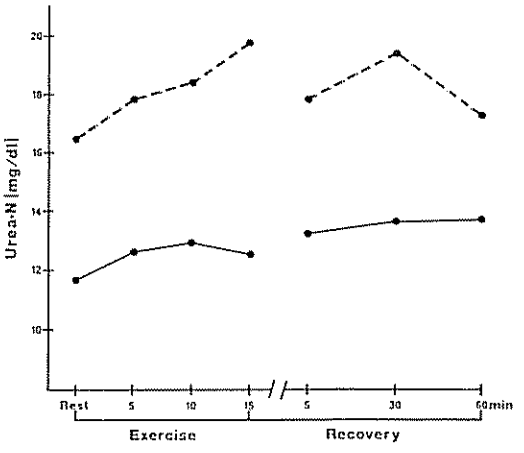

Fig 5 Effect of increased protein intake on urea-N (mg dl^{-1}) before (rest), during and after exercise. Control: (—): 863 g crude protein (CP d^{-1}; High protein (– – –): 1 741 g CP d^{-1}.

receiving the high-protein diet (Fig. 5). Interestingly, urea-N increased ($p<0.05$) over time resulting from an increase in urea-N in horses receiving the high-protein ration. Urea-N averaged 11.7 ± 1.4 mg dl^{-1} and 16.4 ± 1.0 mg dl^{-1} at rest in the control group and in horses receiving the high-protein diet, respectively. Sweat urea-N averaged 20.8 ± 3.5 mg dl^{-1} in the control group which was lower ($p<0.001$) than sweat urea-N in horses receiving 18.5% CP (39.8 ± 4.5 mg dl^{-1}). Body weight loss was not significant between diets, averaging 9.51 ± 1.93 kg in the control horses and 10.91 ± 0.97 kg in horses ingesting the high-protein diet.[19] Total body urea-N after exercise, including sweat losses, increased 5.9 ± 1.2 g in the control horses and 4.3 ± 0.1 g in horses receiving the high-protein ration (Table 3). Interestingly, more ($p<0.005$) urea-N was excreted in sweat in horses receiving the high-protein diet.

Serum creatinine increased ($p<0.005$) over time in both groups. Urinary creatinine was lower ($p<0.001$) in horses receiving the high-protein diet and was not affected ($p>0.05$) over time. Urinary urea-N was not affected ($p>0.05$) by diet or time. However, the urea-N/creatinine ratio (mg mg^{-1}) was higher ($p<0.001$) in horses receiving an increased protein intake, although the ratio was not effected ($p>0.05$) by exercise. The calculated clearance of urea-N[26] was not affected ($p>0.05$) by diet or time. Urinary orotic acid was elevated ($p<0.005$) in the control group in comparison to horses consuming excess protein; however, exercise

Table 3. *Calculated total body urea-N before and after exercise (g)*[a b c]

	Control	High-protein
Rest	37.3 ± 4.7	52.4 ± 3.6*
Post-exercise	41.5 ± 6.1	52.9 ± 3.6*
Sweat	1.7 ± 0.4	3.8 ± 0.2*
Total body urea-N after exercise	43.2 ± 5.9	56.7 ± 3.7*

[a] Mean ± standard error.
[b] Control = (9% CP); 863 g CP d^{-1}; High-protein = (18.5% CP); 1 741 g CP d^{-1}.
[c] Total body urea-N after exercise was elevated ($p<0.05$) above resting levels in both groups.
* Significantly ($p<0.02$) affected by dietary protein level.

Table 4. *Effect of dietary protein level on serum creatinine and urinary metabolites*[a,b]

	Control		High-protein	
	Rest	Rec 60[c]	Rest	Rec 60[c]
Serum creatinine (mg dl^{-1})[d]	1.28±0.06	1.71±0.08	1.25±0.11	1.54±0.13
Urine metabolites				
Creatinine (mg dl^{-1})[d]	361.3±18.80	467.7±54.5	218.3±14.1	267.5±45.7
Urea-N (mg dl^{-1})	1 023.33±135.51	825.50±166.99	1 212.00±271.48	1 191.67±221.62
Urea-N/creatinine ratio (mg mg^{-1})[e]	2.81±0.36	1.93±0.42	5.34±0.96	4.58±0.74
Urea-N clearance (%)	33.53±6.28	26.01±6.77	33.70±8.20	44.19±8.36
Orotic acid (μg ml^{-1})[e]	3.93±0.27	4.89±0.30	3.47±0.15	3.48±0.22
Orotic acid/creatinine ratio (μg mg^{-1})[e]	1.12±0.10	1.12±0.11	1.54±0.08	1.30±0.16

[a] Mean ± standard error.
[b] Control = (9% CP); 863 g CP d^{-1}; High-protein = (18.5% CP); 1 741 g CP d^{-1}.
[c] Rec 60 = 60th min of recovery.
[d] Significantly ($p<0.005$) affected by exercise and the subsequent recovery period.
[e] Significantly ($p<0.02$) affected by dietary protein level.

and the 60 min recovery period did not effect ($p>0.05$) orotic acid. The orotic acid/creatinine ratio (μg mg^{-1}) in urine was higher ($p<0.02$) in horses receiving the high-protein ration (Table 4).

DISCUSSION

Leucine is a ketogenic amino acid which can be completely catabolized to CO_2 and water. The oxidation of leucine increases as the proportion of exercise increases[29] which is associated with an increase in the activity of branched-chain keto acid dehydrogenase activity working muscle.[14] Whether or not leucine oxidation increases during exercise in the horse is not known. Although most attention has focused on leucine as the primary amino acid catabolized during exercise in humans, the other branched chain amino acids (BCAA's) may also be important. If the oxidation of BCAA's in muscle increases during exercise, a decline in plasma levels might be expected.

Valine and leu increased in plasma during exercise, while the subsequent recovery period resulted in an increse in the BCAA's. Branched chain amino acid metabolism is unusual in the sense that metabolism occurs to a greater extent in peripheral tissues than in liver. Therefore, the concentration in plasma reflects production and catabolism by the splanchnic bed, hepatic tissue and muscle. In this experiment the horses were exercised 2 to 3 hours post-feeding when leu levels are still increasing.[24] The slight increase in val and leu during exercise may be due to increased splanchnic outflow[7] exceeding extraction by muscle. The increase in the concentration at the 30th min of recovery could be related to increased hepatic release and (or) decreased extraction by the muscle. There was a consistent trend towards higher BCAA's in the horses receiving the high-protein diet. However, a diet by time interaction was evident only for ile. Although the BCAA changes did not support amino acid oxidation, other responses indicate that pro-

tein catabolism was occurring during exercise.

When intramuscular amino acids are catabolized for energy, the amino group must be transported to the liver for urea synthesis, with transport occurring primarily via ala. The contribution of amino acids to energy production and the change in plasma amino acid levels vary with the intensity and duration of exercise as well as the nutritional status of the individual.[22,30]

In this study, horses working above 60% VO_2max 2 hours after a meal had elevated plasma ala and gln levels during exercise. Plasma ala rose 108% in the control horses and increased 135% in the high-protein horses. In a previous study, fasted horses performing a similar test had a comparable response.[18] However, a brief, mild exercise bout did not alter plasma ala.[24] In humans worked for 40 min, ala release by the working leg increased 55% during mild exercise, 90% during moderate exercise and 500% during intense exercise.[7] Thus ala is important in transferring nitrogen from muscle to liver where the carbon skeleton can be used for synthesis of glucose via gluconeogenesis.[7]

A significant diet by time interaction occurred for plasma ala as a result of ala being lower during recovery in horses receiving excess protein. Other workers have demonstrated that in the liver as protein intake increases, alanine aminotransferase activity increases.[22] Therefore the horses adapted to the high-protein intake may have been able to metabolize ala more efficiently thus clearing plasma ala faster during recovery. There was no effect of diet on gln or glu.

Glutamine levels are influenced by the intensity of work performed. Russell et al.[24] reported unchanged plasma gln levels during mild exercise in horses. In this study, gln increased 22.6% above rest in the control group and 19.7% above rest in horses consuming the high-protein diet. Commencement of exercise increases flux of glu to working muscle,[5] which may have resulted in the initial decrease in glu levels. Collectively, these responses suggest that amino acids were being catabolized during this exercise bout. There was, however, no clear effect of dietary protein level on these responses.

Dietary protein level did affect plasma urea-N concentration and total body urea-N. Both parameters were elevated in horses receiving protein above recommended levels. Similarly, the concentration of urea-N in sweat and the amount excreted in sweat was higher in horses consuming the high-protein diet. Although endurance exercise has been reported to elevate urea-N in systemic blood as a consequene of increased protein catabolism,[25] short-term exercise in horses and humans does not usually alter plasma urea-N.[5,18] In this study, plasma urea-N increased during exercise, particularly in horses ingesting the high-protein diet. In addition, estimated total body urea-N increased after exercise, when sweat urea-N was taken into account. These results suggest that urea production does occur during submaximal, intense exercise, but the increase may not appear in plasma alterations

In the horse, endogenous creatinine clearance is a constant 90%.[27] By relating the concentration of urea to the concentration of creatinine one can obtain the degree of conservation or excretion of urea and correct for concentration differences. Excess protein resulted in an increase in the urea-N/creatinine ratio resulting from an increase in urea production and a need to excrete nitrogen via urea. The unchanged urea-N/creatinine ratio and clearance as a result of exercise and recovery could be the result of less pronounced blood flow shifts and lower dehydration associated with exercise. In addition, water retention after a meal[16] could result in low urea clearance which may influence the results observed in this study.

The evaluation of urea cycle activity has been measured by the excretion of orotic acid.[8] Orotic acid is synthesized in the cytosol by carbamyl phosphate synthetase II. When the urea cycle is impaired, carbamyl phosphate may spill into the cytosol increasing orotic acid synthesis and excretion.[8] In

these horses, the orotic acid/creatinine ratio was higher in horses receiving the high-protein diet. This could suggest that an intake of 1 741 g of CP d^{-1} is in excess of the horses ability to metabolize all of the excess nitrogen into urea efficiently.

Intense exercise, elevates adenosine monophosphate (AMP) deamination resulting in an increase in plasma and muscle NH_3 in the horse.[6,17,20] During exercise, leg muscle shows a net production of NH_3 which effluxes to blood, while splanchnic uptake of NH_3 does not change.[5] Interestingly, plasma NH_3 was lower in horses receiving the high-protein ration at rest, the 15th min of exercise and at the 5th and 30th min of recovery. Adapting animals to high-protein diets may improve their capacity to detoxify systemic NH_3.[9] The increase in plasma urea-N observed in horses consuming protein above recommended levels may have resulted from an improved ability to convert NH_3 into urea.

Adenosine monophosphate deamination also results in inosine monophosphate (IMP) production. Inosine monophosphate may be resynthesized to AMP or can be degraded to uric acid. Uric acid increased in both groups during exercise and peaked during recovery. The levels are similar to values reported by Harris et al.[13] and the response curve is similar (i.e., peaking during recovery). Since NH_3 and uric acid can be produced in the same metabolic pathway, the appearance of uric acid may be a more valid indicator of purine catabolism.[13] The increase in plasma uric acid concentration may also reflect decreased renal clearance during exercise.[23] Interestingly, uric acid was lower in plasma at the 5th min of recovery in the horses ingesting the high-protein diet. Lower levels may be due to increased urinary excretion of uric acid as protein intake is increased[28] or lower uric acid production and (or) more IMP being reamidated to AMP at the 5th min of recovery.

In horses receiving excess dietary protein, worked in the absorptive phase of digestion, the ability to catabolize BCAA's before, during or after exercise was not altered. On the other hand, urea production and excretion increased, which may have resulted in an enhanced ability to remove and metabolize ala and NH_3 before, during and after exercise. However, excess dietary protein above a certain level may exceed the capacity of the urea cycle as indicated by the elevated orotic acid/creatinine ratio in horses receiving 1 741 g of CP day^{-1}. Racing Thoroughbreds ingest between 1 378 and 2 138 g of CP per day.[11] Negative implications of exceeding the horse's ability to excrete excess nitrogen may include intermittent ataxia, irritability and lethargy. Future research is required to determine the optimum level of dietary protein for the exercising horse so that protein catabolizing activity is enhanced and the capability of the urea cycle is not surpassed.

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