
Abstract

A restricted protein diet supplemented with amino acids and fat may reduce undesired effects of protein metabolism on exercise but risk protein deficiency. Supplementation with limiting amino acids and fat may facilitate the efficacy of utilization of a restricted protein diet. Twelve Arabian horses were assigned randomly to 2 X 2 factorial experiment: two fat levels 3 or 13% (10% added corn oil) and two protein levels: 7.5 (supplemented with lysine and threonine) or 14.5% crude protein (CP) on a dry matter basis. Horses were placed on the diets for a four week accommodation period then performed a standard exercise test (SET). The SET began with a warm-up followed by six one minute sprints at 7 m/s separated by 4 minute walks and concluded with a 30 minute recovery at the walk. All horses were trained for eleven weeks followed by another SET. This SET was like the first SET except the sprints were at 10 m/s instead of 7 m/s. After SET-2, horses were observed for eight weeks o deconditioning. The entire experiment lasted 26 weeks, including three weeks o SET's. Blood samples were taken every 2 weeks and during SETs. Plasma was analyzed for albumin, total protein, urea-N and creatinine. Urine samples were obtained from mares every 2 weeks and analyzed for urea, creatinine and uric acid. Horses were observed for clinical signs of protein deficiency and genera health. Data were summarized as least squares means and standard errors. Analysis of variance with repeated measures was used to evaluate the effects of fat, protein, time and interactions.

During the accommodation and conditioning periods, no differences were found in plasm albumin due to fat level \( P = 0.58 \) or protein level \( P = 0.63 \) or in total protein due to protei level \( P = 0.21 \). Total protein was higher for those receiving the fat supplemented diets \( P = 0.067 \). Plasma urea-N levels were higher for horses in fat-supplemented groups \( P = 0.090 \) as well as for horses in high protein groups \( P = 0.006 \). There was a fat X protein interaction for
creatinine \( (P = 0.072) \) with higher creatinine observed with low protein only in combination with a fat-supplemented diet. There was no difference when low protein was in combination with a low fat diet. Despite the observed differences, all blood and urine values were within reference ranges.

During the exercise tests, no differences were found during SET-1 or SET-2 in concentrations of albumin due to fat \( P = 0.17 \) and 0.40 respectively) or protein \( P = 0.28 \) and 0.80 respectively). Total protein during SET-1 was also unaffected by fat \( P = 0.31 \) or protein \( P = 0.36 \). During SET-2, however, higher total protein was associated with fat-supplementation \( P = 0.084 \) and high protein \( P = 0.005 \). During SET-1 and 2, creatinine was not affected by fat \( P = 0.80 \) and 0.47 respectively). Creatinine was not affected by protein level during SET-1 \( P = 0.20 \) however, a fat X protein interaction was observed during SET-2 \( P = 0.008 \). Low protein resulted in higher creatinine \( (P = .0001) \) but the magnitude of the response was greater in the fat supplemented diets, causing an interaction. Plasma urea-N was higher for those in the high protein group \( P = 0.005 \) but not affected by fat \( P = 0.39 \) during SET-1. A fat X protein interaction existed for plasma urea-N during SET-2 \( P = 0.055 \) with higher levels seen in combination with fat-supplementation but not with the low fat diet.

These observations revealed no detrimental effect of the lower level of dietary protein on protein status over the course of 26-week experiment. Higher plasma urea-N levels simply reflected the level of nitrogen in the diet. Higher creatinine levels during training and SET-2 suggested that the fat-supplemented low protein diet supported additional muscle development.

**Introduction**

Restriction of dietary protein may be beneficial during strenuous exercise by moderating production of heat and acid. Contrarily, protein is needed during physical conditioning to suppor
muscle hypertrophy and repair, and to replete nitrogen lost in sweat (Meyer, 1987). The risk of protein deficiency with a low protein diet may be reduced by fortification with limiting amino acids and by supplementary fat, which may have a protein-sparing action (Steffens, 1996). This study compares the protein status of horses fed a typical protein diet with a restricted protein diet supplemented with amino acids and fat. A companion paper addresses acid-base status of this experiment.

Materials and methods

**Horses.** Twelve Arabian horses (6 mares, 6 geldings, age 5 to 11 years) were housed in a dry lot with free access to water. Twice daily they were brought into box stalls where they 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 four complete feeds (HPHF, LPHF, HPLP, LPLF), which were formulated to provide 3 Mcal DE/kg dry matter (DM)(Table 1). Protein was at two levels: high protein (HP, 14.5% CP) and low protein (LP, 7.5% CP), which was supplemented with crystalline lysine (0.5%) and threonine (0.3%) (Heartland Lysine Inc., Chicago, IL) to match the amino acids levels in HP. Two levels of fat were also fed: low fat (LF, 3%) and high fat (HF, 13% including 10% added corn oil). A 4-week accommodation period began the experiment.

**Conditioning.** All horses underwent eleven weeks of conditioning using a high-speed treadmill (Mustang 2000, Kagra Inc., Fahrwangen, Switzerland). The protocol consisted of interval training divided into three phases. The first phase lasted 3 weeks, phases two and three were four weeks each (Table 2). Horses were interval trained twice a week and were walked a 1.5 m/s for 30 minutes on non-exercised days on a mechanical walker.
Standard Exercise Test. A standard exercise test (SET-1) followed the accommodation period. It 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 7 m/s on a 6% slope separated by four minute walks (1.5 m/s), and a 30 minute recovery period at the walk with no slope. Following the conditioning period, SET-2 and SET-3 were conducted. These SETs were similar to SET-1 except the sprints were at 10 m/s.

For all SETs, horses were fasted overnight but had access to water. The climate-controlled barn had 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-guage, 150 c 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 antiseptic 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 was anesthetized with a subcutaneous injection (lidocaine hydrochloride injectable 2%, VEDCO, St. Joseph, MO). A 10-guage 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 by 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) and 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).

Deconditioning: Following the last 2 SETs, horses were placed on stall rest for eight weeks. They were walked at 1.5 m/s for 30 minutes twice a day.
Sampling and Analysis. Throughout the experiment, 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 (2, 7 mL 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 using 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 SETs, 30 mL of mixed venous blood was taken at rest, during the last seconds of sprint 1, 2 and 6 as well as at 5, 10, 20 and 30 minutes of recovery. Blood samples were taken using syringes (Sherwood Medical, St.Louis, MO), placed into 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. 640, 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 fat level, protein level, time and interactions. Data from SET-2 and SET-3 were combined if there was no effect of SET. Paired t-tests were used to evaluate changes within diet from the start and finish of the
experiment, changes in resting values from SET 1 to SET 2 and changes within each SET (SAS, 1991). Significance was set a $P < .05$ and trends set a $P < .15$.

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 419 ± 15 kg and ending weight was 459 ± 15 kg. All horses gained weight ($P = .010$), however, no differences ($P = .41$) in weight due to diet were observed. Bod condition score (BCS) was lower ($P = .009$) in the LPHF group (Figure 1). Feed intake of 8.8 ± 0.3 kg/day was not different between diets ($P = .87$) but average intake of CP was 711 ± 21 g/d in the LP groups and 1323 ± 45 g/d in the HP groups ($P = .001$).

Diet: Throughout the experiment, no differences in plasma albumin due to fat level ($P = 0.58$) or protein level ($P = 0.63$)(Figure 2) and no difference in total protein due to protein level ($P = 0.21$)(Figure 3B) were observed. Total protein tended to be higher ($P = 0.067$) in the HF groups (Figure 3A). Plasma urea-N levels were higher for horses in the HF group ($P = 0.090$)(Figure 4A) as well as for horses in the HP group ($P = 0.008$)(Figure 4B). Plasma urea-N, during the conditioning period, developed a fat X protein interaction ($P = .070$) with the HP group increasing only with the HF group. There was a fat X protein interaction for creatinine ($P = 0.072$)(Figure 5) with higher creatinine observed in the LPHF group. Despite the observed differences, all values were within reference ranges (Wilson et al., 1986; Weideman et al., 1995).

Comparing the start of the accommodation period to the end of conditioning period values of main effects, albumin was increased by 8.9% in the HF groups ($P = .071$) as well as by 10.7% in the HP groups ($P = .031$). Total protein was also increased by 16% in the HF groups ($P = .007$) and on both levels of protein (LP, 8% increase, $P = .10$ and HP, 16% increase, $P = .011$) compared to initial values. Plasma urea-N was decreased on both fat and protein levels ($P = .0001$) however, it was unchanged comparing week 2 to the end of conditioning. Creatinine was
increased by 63% in the LP group and by 55% in the HF group ($P = .072$) as well as by 39% in the LF group ($P = .026$) compared to initial values.

During the exercise tests, no differences were found during SET-1 or SET-2 in concentrations of albumin due to fat ($P = 0.17$ and 0.40, respectively) or protein ($P = 0.28$ and 0.80, respectively) (Figure 6 and 7). Total protein during SET 1 was unaffected by fat ($P = 0.31$) or protein ($P = 0.36$) (Figure 8). During SET-2 however, higher total protein was observed in the HF groups ($P = 0.084$) (Figure 9A) especially during the sprints ($P = .004$) as well as in the HP groups ($P = 0.005$) (Figure 9B). During SET-1 and 2, creatinine was not affected by fat ($P = 0.80$ and 0.47 respectively) (Figure 10 and 11). Creatinine was not affected ($P = 0.20$) by protein level during SET-1 (Figure 10) however, a fat X protein interaction was found during SET-2 ($P = 0.008$) (Figure 12). Creatinine was higher ($P = .0001$) in the LP groups regardless of fat level (Figure 11) but the magnitude of the difference was greater for the HF diet, thus causing the interaction. A time X fat X protein interaction also existed for creatinine ($P = .047$) because creatinine became higher in the LPLF group than the HPLF group at the end of exercise and during recovery. Plasma urea-N was not affected by fat ($P = 0.39$) (Figure 13) during SET-1 but was higher ($P = 0.005$) for those in the HP group (Figure 13). A fat X protein interaction existed for plasma urea-N during SET-2 ($P = 0.055$) (Figure 14) with higher ($P = .016$) levels during sprints in the HF group but not in the LF group.

Urine urea was affected by protein ($P = .004$) but not fat level ($P = .81$) (Figure 15). Urine uric acid and creatinine were not affected by either fat ($P = .78$ and .15, respectively) or protein level ($P = .21$ and .57, respectively) (Figure 16 and 17).

**Training:** Over the conditioning period, albumin decreased ($P = .0001$) about 10% (Figure 2) and urea-N decreased ($P = .0001$) about 40% (Figure 4). Also total protein increased ($P = .0001$) about 7% (Figure 3) and creatinine increased ($P = .0001$) about 30% (Figure 5).
Deconditioning: During the 8 weeks of stall rest, albumin and total protein were unchanged; creatinine increased \((P = .012)\) about 17\% (Figure 5) but plasma urea-N decreased \((P = .0043)\) about 25\% (Figure 4).

SET exercise: Exercise resulted in an average increase of 14\% in albumin concentration, 10.3\% for total protein and 27\% for creatinine \((P = .0001)\). It had no effect on plasma urea-N \((P = .39)\). Exercise during the first SET did not cause any differences in the changes observed in albumin, urea or creatinine between diets. There was a trend \((P = .11)\) for total protein to increase more in the HP group versus the LP group. During SET-2, there were no effects of diet on the changes in total protein, albumin, creatinine or urea-N. There were also no differences between SETs in resting values for albumin, total protein, creatinine or urea-N.

The increases in concentrations of albumin and total protein during exercise reflect changes in plasma volume, which can be estimated to decrease about 10-14\%.

Discussion

This study revealed no detrimental effect of restricted dietary protein or fa supplementation on protein status over 26 weeks or during the sprint exercise tests. Previously protein level of 8.4\% CP was inadvertently fed to Arabian horses in similar training and exercise tests without adverse effects (Taylor et al., 1995). A crude protein level as low as 5.5 \% CP was shown to be adequate for exercising horses based on albumin and total protein levels (Patterson, et al., 1985). A higher level of 8.5\% CP was recommended since the author felt these horses had better protein reserves with this level of protein as demonstrated by an increase in urea levels during a 4 day fast. One study even claimed that growing two year olds had adequate protein during exercise with dietary protein of 6\% CP (Orton et al., 1986). The author suggested that protein utilization improved with exercise. In contrast, an increase in nitrogen retention was demonstrated during conditioning as well as following this period in horses (Freeman et al.,
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 with exercise.

Current recommendations are 9, 10.4 and 11% CP in rations 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 Thoroughbreds and Standardbreds were being fed an average of 14% CP. Surveys conducted in central North Carolina (Honore et al., 1994) indicated that 70% of the horses were being fed excess protein in relation to NRC recommendations.

Higher protein levels have shown detrimental effects, not exclusive to the exercising horse, that include increasing the water requirement due to increased urea production and urination, increasing amounts of ammonia in the confined horses’ stalls which would stress the respiratory track of these animals (Meyer, 1987). Higher protein levels will also increase the heat of digestion (Kronfeld, 1996). Several researchers have not found specific detrimental effects of high protein in heart rate and blood lactate in the exercising horse (Miller and Lawrence, 1988; Frank et al., 1989; Miller et al., 1991). It seems likely however, excess high protein that produces more heat, urea and acid would be detrimental to exercise and lead to earlier fatigue.

The use of fat supplementation increases the energy density of the diet. By decreasing feed intake, and thus protein intake, the quality of the protein may need to be improved by supplementation with amino acids to ensure adequate intake of essential amino acids. Lysine and threonine have been shown to be limiting amino acids for growth in the horse (Ott et al., 1981 and Graham et al., 1994). Feeding a fat-supplemented diet to weanlings had no detrimental effects on total protein or urea levels in the blood (Saastamoinen et al., 1994). Dietary fat may actually improve digestibility of protein. Ileal digestibilities of amino acids in growing swine were improved with the addition of fat presumably by delaying gastric emptying (Li and Sauer, 1994). This would theoretically improve the amino acid profile in the bloodstream since amino acids are
not readily absorbed from the hindgut of the horse (Mason, 1984). Exercise has also been shown to increase protein digestibility in the horse (Worth et al., 1987) and the combination of amino acid supplementation, added dietary fat and interval training may have contributed to the results seen in this study.

Higher plasma urea-N levels were associated with the HP diet in this study and are generally a reflection of nitrogen intake. Fat may have increased nitrogen availability thus increasing the urea-N level in the HPHF group. The increase in N availability may have contributed to the higher TP in the HF group as well. Albumin was not affected by fat or protein level. Albumin is not considered a sensitive index of protein status for the horse. The length of the experiment (26 weeks) should have been long enough to result in any detrimental effects on albumin synthesis since its half life is about 18 days (Young et al., 1990). Creatinine reflects muscle mass and may be an indication that the LPHF diet supported additional muscle development or turnover, which is also supported by a lower BCS. Unfortunately, the difference in creatinine may have been a result of the random assignment to the groups since the difference persisted throughout the experiment.

The difference in urea-N during the first SET is a reflection of different nitrogen intakes. The second SET following conditioning had results similar to those reported during the conditioning period. Albumin was not affected by diet. Higher total protein in the HF group and in the HP group as well as higher urea-N in the HP group, especially in the presence of HF, may be due to increased nitrogen availability with fat increasing digestibility. Again, no detrimental effects were observed and it appears that the LPHF diet may support additional muscle development or turnover. Lower cortisol levels in the HF group may also reflect less stress and resulted in higher TP, with increased globulins, since albumin was not different.

Higher excretion of urea-N was associated with the HP group. It would lead to release of ammonia in the stalls as suggested by Meyer (1987).
The observed training effects were most likely due to changes in plasma volume and muscle hypertrophy. Deconditioning effects may have been due to a reduced stress level with little forced exercise. The drop in urea during deconditioning in the HP group is due to feed restriction imposed during this phase to prevent digestive disturbances. Increases in metabolites during SET exercise are partly due to changes in plasma volume with intense exercise and an increase in hematocrit. The tendency for a larger increase in total protein in the HP group during the first SET may be due to a larger change in plasma volume. This may be related to increased sweat loss and a change in hydration status in the untrained horse. Training may have altered this response because differences between diets were not observed in the second SET conducted after conditioning.

In conclusion, it appears that the LP diet was not detrimental to protein status of the horse regardless of fat level. In fact, HF may have improved the utilization of the protein and reduced stress and may have contributed to the increase in TP observed in the HF group, high urea-N in the HPHF group only and an increase in creatinine for horses fed LPHF. This in combination with lower BCS and presumably less fat deposition may suggest that this level of protein is no detrimental and its benefit in minimizing the detrimental effects of high protein may be enhanced by fat supplementation. A companion study also suggests that low protein may be beneficial to the acid-base status of the exercising horse.

**Literature Cited**


Steffens, W. 1996. Protein sparing effect and nutritive significance of lipid supplementation


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

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Analysis

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* Standard analytical procedures were followed by the Virginia Tech Forage Laborator; N=6.

** Heartland Lysine Inc., Chicago, IL
Table 2. Protocol for interval training over 11 weeks. Each horse exercised twice weekly and each phase is additive to the one before. Phase one was week 0-3, phase two week 4-7 and phase three weeks 8-11.

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Figure 1. Body Condition Score during the 26-week experiment was affected by a fat x protein interaction ($P = .15$) in which scores were lower for the HFLP group (contrast $P = .009$).
Figure 2. Plasma albumin at rest during the 26-week experiment was decreased during the conditioning period ($P = .0001$) but was unaffected by either fat ($P = .58$) or protein ($P = .63$) level.
Figure 3. Plasma total protein at rest during the 26-week experiment was decreased during the conditioning period ($P = .0001$), tended to be higher in the HF groups ($P = .067$) but was unaffected by protein level ($P = .21$).
Figure 4. Plasma urea-N at rest during the 26-week experiment was decreased throughout the conditioning and deconditioning periods ($P = .0001$). Those horses in the HF group and HP group had higher levels ($P = .090$ and $P = .008$, respectively).
Figure 5. Plasma creatinine at rest during the 26-week experiment was increased during the conditioning and deconditioning periods ($P = .0001$) and was affected by a fat X protein interaction ($P = .072$). Those horses in the HFLP group had higher levels compared to the others (contrast $P = .0081$).
Figure 6. Plasma albumin at rest (R), sprints 1 (S1), 2 (S2) and 6 (S6) as well as at 5,10, 20 and 30 minutes of recovery (R1-R4) was increased by exercise \((P = .0001)\) but was unaffected by fa \((P = .17)\) or protein \((P = .28)\) level during SET 1. Time points marked with an asterisk are different from rest \((P < .05)\).
Figure 7. Plasma albumin at rest (R), sprints 1 (S1), 2 (S2) and 6 (S6) as well as at 5, 10, 20 and 30 minutes of recovery (R1-R4) was increased by exercise ($P = .0001$) but was unaffected by fa ($P = .40$) or protein ($P = .80$) level during SET 2. Time points marked with an asterisk are different from rest ($P < .05$).
Figure 8. Plasma total protein at rest (R), sprint 1 (S1), 2 (S2) and 6 (S6) as well as at 5, 10, 20 and 30 minutes of recovery (R1-R4) was increased by exercise ($P = .0001$) but was unaffected by fat ($P = .31$) or protein ($P = .36$) level during SET 1. Time points marked with an asterisk are different from rest ($P < .05$).
Figure 9. Plasma total protein at rest (R), sprint 1 (S1), 2 (S2) and 6 (S6) as well as at 5, 10, 20 and 30 minutes of recovery (R1-R4) was increased by exercise ($P = .0001$) and in the HP group ($P = .005$) and tended to be higher ($P = .084$) in the HF group during SET 2. Time points marked with an asterisk are different from resting values ($P < .05$).
Figure 10. Plasma creatinine at rest (R), sprint 1 (S1), 2 (S2) and 6 (S6) as well as at 5, 10, 20 and 30 minutes of recovery (R1-R4) was increased during exercise ($P = .0001$) but was unaffected by fat ($P = .80$) or protein ($P = .20$) levels during SET 1. Time points marked with an asterisk are different from rest ($P < .05$).
Figure 11. Plasma creatinine at rest (R), sprint 1 (S1), 2 (S2) and 6 (S6) as well as at 5, 10, 20 and 30 minutes of recovery (R1-R4) was increased during exercise ($P = .0001$) and in the LP group ($P = .0001$) but was unaffected by fat ($P = .47$) level during SET 2. Time points marked with an asterisk are different from resting values ($P < .05$).
Figure 12. Plasma creatinine at rest (R), sprint 1 (S1), 2 (S2) and 6 (S6) as well as at 5, 10, 20 and 30 minutes of recovery (R1-R4) was affected by a fat X protein interaction ($P = .008$) with higher levels in the LPHF group (contrast $P = .0004$) during SET 2. All time points are different from their respective resting value ($P < .05$).