Effects of Branched-Chain Amino Acid Supplementation on Performance and Pre- and Post-Race Plasma Biochemical Parameters in Standardbred Horses During a 1 km Pacing Trial: Postprint

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Abstract

This study investigated the effects of supplemental branched-chain amino acid feeding on 1 km trotting race performance and pre- and post-race plasma antioxidant indices, as well as hormone, creatine, glucose, lactate, and creatinine concentrations in trotting horses, providing reference data for the application of branched-chain amino acids in trotting horse training and competition. Eight Yili horse geldings (trotting race horses) of similar age (approximately 4 years) and body weight [(457$±$50) kg] that had undergone rigorous training were selected and randomly divided into two groups: a control group and a treatment group, with four horses per group. Each horse was fed 3 kg of pelleted concentrate daily, with alfalfa hay provided ad libitum; additionally, the treatment group received a supplemental 72 g of branched-chain amino acids per horse per day (composed of 35 g leucine, 16.6 g isoleucine, and 20.4 g valine). A supplementation and training trial was conducted over a period of 38 days (7-day pre-trial period and 31-day formal trial period). The results showed that branched-chain amino acid supplementation improved race performance in trotting horses, significantly increased plasma total antioxidant capacity at 30 min post-race and plasma superoxide dismutase activity at 24 h post-race ($P<0.05$), but had no significant effect on plasma hormone, creatine, glucose, lactate, or creatinine levels ($P>0.05$). It was concluded that supplemental feeding of branched-chain amino acids can reduce race time and enhance systemic antioxidant capacity in trotting horses, but has no significant effect on plasma hormone, glucose, creatine, lactate, or creatinine concentrations.

Full Text

Effects of Supplemental Feeding Branched-Chain Amino Acids on 1 km Trotting Race Performance and Plasma Biochemical Indices in Trotters

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Abstract

This study investigated the effects of supplemental feeding branched-chain amino acids (BCAA) on 1 km trotting race performance, plasma antioxidant capacity, and concentrations of hormones, creatine, glucose, lactic acid, and creatinine in trotters, providing reference data for BCAA application in training and competition. Eight Yili male horses (trotting race horses) of similar age (approximately 4 years) and body weight [(457$±$50) kg] that had undergone rigorous training were randomly divided into two groups: a control group and a trial group, with four horses per group. Each horse received 3 kg of pelleted concentrate daily with ad libitum access to alfalfa hay. The trial group was additionally supplemented with 72 g BCAA per horse daily (composed of 35 g leucine, 16.6 g isoleucine, and 20.4 g valine). The experiment lasted 38 days, consisting of a 7-day adaptation period followed by a 31-day trial period with concurrent training.

The results demonstrated that BCAA supplementation improved race performance in trotters while significantly increasing plasma total antioxidant capacity (T-AOC) at 30 minutes post-race and plasma superoxide dismutase (SOD) activity at 24 hours post-race ($P<0.05$). However, no significant effects were observed on plasma hormone concentrations or creatine, glucose, lactic acid, and creatinine levels ($P>0.05$). These findings indicate that BCAA supplementation can reduce race time and enhance antioxidant capacity in trotters, though it does not significantly affect plasma hormone, glucose, creatine, lactic acid, or creatinine concentrations.

Keywords: branched-chain amino acids; Yili horses; athletic performance; plasma biochemical indices

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Introduction

Branched-chain amino acids (BCAA), comprising leucine, isoleucine, and valine, serve not only as energy substrates but also participate in muscle cell anabolism, providing energy for muscle contraction. Additionally, BCAA are involved in metabolic regulation, suppressing oxidative stress and modulating hormone levels [1]. Chang et al. [2] confirmed that BCAA can enhance muscle strength and improve athletic performance in humans. Wang et al. [3] reported that BCAA participate in energy supply and glucose metabolism, regulate protein metabolism, combat exercise-induced fatigue, scavenge free radicals, and protect mitochondrial function.

Yili horses, primarily produced in the Ili Kazakh Autonomous Prefecture of Xinjiang, are characterized by uniform conformation, stable genetic performance, combined speed and power capabilities, good reproductive performance, roughage tolerance, and strong disease resistance, making them excellent trotting horses [4]. Performance tests have shown that Yili horses can complete 5 km in 6 minutes 35 seconds. However, compared to world-class trotting breeds, Yili horses exhibit inferior trotting performance, are prone to fatigue during training, and recover slowly—factors that severely constrain their athletic potential. Based on research demonstrating BCAA' s benefits for energy supply, antioxidant function, and post-exercise recovery, this study utilized Yili trotting horses to investigate the effects of BCAA supplementation on athletic performance, antioxidant capacity, and plasma hormone concentrations, providing a reference basis for improving trotting performance.

1.1 Experimental Animals and Design

Eight Yili male horses (trotting race horses) of similar age (approximately 4 years) and body weight [(457$±$50) kg] that had undergone rigorous training were randomly allocated to two groups: a control group and a trial group, with four horses per group. Each horse received 3 kg of pelleted concentrate daily with ad libitum access to alfalfa hay. The trial group was additionally supplemented with 72 g BCAA per horse daily (composed of 35.0 g leucine, 16.6 g isoleucine, and 20.4 g valine, all purchased from Zhongshan Jiahui Food Additive Co., Ltd.). The experimental design and grouping are presented in Table 1 . The 38-day supplementation and training trial consisted of a 7-day adaptation period followed by a 31-day experimental period.

1.2 Feeding Management and Diet Composition

Horses were individually housed in stalls and fed using a roughage-first method. The 3 kg of pelleted concentrate was divided into three equal portions fed at 07:00, 15:00, and 23:00 daily. BCAA supplementation was divided into two equal portions administered at 07:00 and 23:00 daily. Alfalfa hay and water were provided ad libitum. After consuming the pelleted concentrate, horses were moved to exercise paddocks for free activity for 4 hours before training. Stalls were cleaned daily to remove manure and bedding, which was replaced with fresh, dry material.

Horses followed an intermittent training regimen, training one day and resting the next. Training began 4 hours post-feeding and consisted of: one slow walking lap for warm-up, one gradually accelerating walking lap, one maximum-speed trotting lap, and one gradually decelerating lap. After a 5–10 minute rest, this sequence was repeated once to complete the daily training session.

The composition and nutrient levels of the pelleted concentrate are shown in Table 2 . The premix provided per kilogram of concentrate: VA 480 IU, VB1 816.32 mg, VB2 333.2 mg, VB6 48.96 mg, VD 70.4 IU, VE 21,333.36 IU, pantothenic acid 20.46 mg, nicotinamide 484.85 mg, Cu (as copper sulfate) 10.58 mg, Fe (as ferrous sulfate) 5.56 mg, Mn (as manganese sulfate) 33.54 mg, Zn (as zinc sulfate) 30.92 mg, I (as potassium iodide) 2.46 mg, Se (as sodium selenite) 5.93 mg, and Co (as cobalt chloride) 1.11 mg. Digestible energy (DE) was a calculated value, while all other indices were measured values.

1.3 Data Collection

1.3.1 Race Performance Measurement One-kilometer trotting races were conducted on day 0 (before the trial period) and day 31 of the experimental period, with race times measured using a stopwatch.

1.3.2 Heart Rate Measurement On day 31, heart rates were measured using a Polar heart rate monitor placed on the horses' chest at 1 hour pre-race, immediately post-race, and 30 minutes post-race.

1.3.3 Blood Sample Collection On day 31, 5 mL blood samples were collected from the jugular vein at 1 hour pre-race, 30 minutes post-race, and 24 hours post-race into heparinized tubes. Plasma was prepared by centrifugation at 1,500×g for 15 minutes and stored at -20°C for subsequent analysis.

1.4 Measurement Indicators and Methods

Plasma catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities, total antioxidant capacity (T-AOC), and concentrations of malondialdehyde (MDA), insulin (INS), glucagon (Gc), cortisol (COR), glucose (Glu), lactic acid (LA), and creatinine (Cre) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute. Plasma creatine concentration was measured using a kit from Sigma-Aldrich (USA).

1.5 Statistical Analysis

All results are expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA in SPSS 16.0 software. Significant differences were further analyzed using Duncan' s multiple comparison test. P>0.05 indicated no significant difference, P<0.05 indicated significant difference, and P<0.01 indicated highly significant difference.

2.1 Effects of BCAA Supplementation on 1 km Trotting Race Performance

As shown in Table 3 , no significant difference in 1 km race time was observed between groups at the start of the trial (P>0.05). On day 31, the trial group demonstrated improved performance. Throughout the experimental period, the trial group exhibited a marked reduction in race time, shortening by 32.53% compared to the control group (P>0.05).

2.2 Effects of BCAA Supplementation on Heart Rate Before and After the 1 km Trotting Race

Table 4 shows that 1 hour pre-race, all horses were in a stable physiological state with no significant difference in heart rate between groups (P>0.05). Immediately post-race, heart rates increased significantly in all groups, with the control group showing the most pronounced elevation at 12.62% higher than the trial group (P>0.05). Heart rates returned to normal levels in all groups by 30 minutes post-race.

2.3 Effects of BCAA Supplementation on Plasma Antioxidant Indices Before and After the 1 km Trotting Race

Table 5 presents the antioxidant indices. For plasma MDA concentration, no significant difference was observed between groups at 1 hour pre-race (P>0.05). At 30 minutes post-race, MDA concentration increased markedly in the control group (10.97% higher than pre-race). At 24 hours post-race, MDA continued to rise in both groups, though the trial group showed higher values than the control group (P>0.05).

Regarding plasma CAT activity, both groups showed increases at 30 minutes post-race compared to pre-race values. At 24 hours post-race, the control group decreased slightly from the 30-minute value, while the trial group continued to increase. For plasma GSH-Px activity, both groups decreased at 30 minutes post-race compared to pre-race, but at 24 hours post-race, the trial group was 21.43% higher than the control group (P>0.05).

Plasma SOD activity showed a highly significant difference between groups at 24 hours post-race ($P<0.01$), with the trial group 21.00% higher than the control group. For plasma T-AOC, both groups showed dramatic increases at 30 minutes post-race compared to pre-race values, with the trial group significantly higher than the control group ($P<0.05$, 19.69% increase). By 24 hours post-race, T-AOC values had normalized in both groups.

2.4 Effects of BCAA Supplementation on Plasma Hormone Concentrations Before and After the 1 km Trotting Race

Table 6 shows plasma hormone responses. Compared to 1 hour pre-race, plasma INS concentration increased at 30 minutes post-race in both groups, and at 24 hours post-race, the trial group was 10.19% higher than the control group ($P>0.05$). Plasma Gc concentration increased at 30 minutes post-race, with further elevation at 24 hours post-race in both groups; the trial group was 6.50% higher than the control group at 24 hours ($P>0.05$).

For plasma COR concentration, no significant difference was observed between groups at 1 hour pre-race ($P>0.05$). At 30 minutes post-race, COR concentrations decreased in both groups, with continued decline at 24 hours post-race; the reduction was more pronounced in the trial group.

2.5 Effects of BCAA Supplementation on Plasma Creatine and Glucose Concentrations Before and After the 1 km Trotting Race

Table 7 shows that plasma creatine concentration did not differ significantly between groups at 30 minutes post-race ($P>0.05$). However, at 24 hours post-race, the trial group showed a marked increase, being 39.63% higher than the control group ($P>0.05$). No significant differences in plasma glucose concentration were observed between groups at any time point (1 hour pre-race, 30 minutes post-race, or 24 hours post-race) ($P>0.05$).

2.6 Effects of BCAA Supplementation on Plasma Lactic Acid and Creatinine Concentrations Before and After the 1 km Trotting Race

Table 8 reveals that plasma LA concentration increased substantially at 30 minutes post-race in both groups, with the control group showing higher values than the trial group ($P>0.05$). By 24 hours post-race, LA concentrations began to decrease in both groups. For plasma creatinine concentration, no significant differences were observed between groups at any time point (1 hour pre-race, 30 minutes post-race, or 24 hours post-race) ($P>0.05$).

3.1 Effects of BCAA Supplementation on Athletic Performance in Trotters

Extensive research has demonstrated that BCAA not only influence protein synthesis and degradation but also serve as energy substrates and gluconeogenic precursors, regulating muscle protein metabolism. Kim et al. [5] reported that BCAA supplementation before endurance exercise reduces muscle damage, improves endurance duration, and decreases fatigue responses. Watson et al. [6] found that 12 g of BCAA supplementation improved race performance in some athletes.

In the present study, BCAA supplementation enhanced 1 km trotting speed, which can be attributed to BCAA being important and unique energy-supplying amino acids. BCAA undergo vigorous catabolism in muscle, with complete oxidation of one molecule of leucine, isoleucine, and valine producing 42, 43, and 32 molecules of ATP, respectively [7]. The trial horses received BCAA supplementation for 30 consecutive days, allowing BCAA to exert rapid energy supply during competition. Combined with effective trotting training, this contributed to improved race performance.

The normal heart rate range for adult horses is 30–42 bpm [8]. Gehlen et al. [9] reported an average resting heart rate of 31.7 bpm in Thoroughbreds before training. Colahan et al. [10] observed an average heart rate of $(38\$\pm5) bpminsaddledhorsesatrest, increasingto184.23bpmatanaveragespeedof(548\pm\$90)$ m/min. In this study, pre-race heart rates were slightly higher than previously reported, likely due to saddling. Post-race heart rates were lower in the trial group both immediately after and at 30 minutes post-race, indicating that BCAA supplementation not only improved trotting speed but also reduced heart rate during competition. Therefore, BCAA supplementation enhances race speed, reduces heart rate during competition, and facilitates post-race heart rate recovery in trotters.

3.2 Effects of BCAA Supplementation on Plasma Antioxidant Indices Before and After the 1 km Trotting Race

High-intensity exercise induces oxidative reactions in trotters, causing irreversible tissue damage, impairing exercise capacity, and severely compromising athletic performance [11]. Research has demonstrated that BCAA play a significant role in antioxidant processes. Through metabolic pathways, BCAA enter tissue cells via blood circulation, block free radical chain reactions on cell membranes, scavenge excessive free radicals generated during exercise or injury, reduce production of lipid peroxidation end-product MDA [12], and enhance activities of antioxidant enzymes such as SOD and GSH-Px.

In this study, BCAA supplementation increased plasma SOD activity and T-AOC at 30 minutes post-race and enhanced CAT, GSH-Px, and SOD activities along with T-AOC at 24 hours post-race, indicating that BCAA exerted antioxidant effects in trotters. SOD and GSH-Px are protein enzymes whose synthesis and metabolism are influenced by amino acid concentrations during translation and transcription [13]. Some BCAA can enter tissue cells through blood circulation, facilitating free radical scavenging, preventing further lipid oxidation, and protecting the body from damage [14]. The 30-day BCAA supplementation regimen in this study enhanced plasma SOD and GSH-Px activities in trotters.

3.3 Effects of BCAA Supplementation on Plasma Hormone Concentrations Before and After the 1 km Trotting Race

Hormone regulation in animals is influenced by multiple factors, with exercise inducing changes in plasma INS, Gc, and COR concentrations that are directly related to glucose metabolism. Xiao et al. [15] reported that training increased plasma COR concentration while decreasing INS concentration in athletes. Davies et al. [16] investigated plasma COR responses to different exercise intensities, finding that exercise below 50% VO_2max decreased plasma COR, while exercise above 60% VO_2max increased COR concentration.

In this study, plasma INS concentrations at 30 minutes and 24 hours post-race were elevated compared to pre-race values in both groups. This reflects INS' s role as a primary hormone enhancing gluconeogenesis, as trotting horses require substantial glycogen during competition, thereby stimulating INS secretion. Regarding plasma Gc concentration, values at 24 hours post-race were higher than at pre-race and 30 minutes post-race in both groups, likely because the 24-hour post-race period represents recovery, during which increased Gc facilitates restoration of plasma glucose levels. Plasma COR concentration is affected by exercise intensity, with sustained decreases following high-intensity exercise until adrenal cortex secretion restores levels [17]. The continuous decrease in plasma COR at 30 minutes and 24 hours post-race in this study aligns with the findings of Davies et al. [16].

3.4 Effects of BCAA Supplementation on Plasma Creatine and Glucose Concentrations Before and After the 1 km Trotting Race

Glucose and creatine are direct and indirect energy substrates critical for energy supply during exercise. Creatine is synthesized primarily in the liver and kidneys and distributed widely in skeletal muscle, cardiac muscle, and brain tissue. Creatine synthesis utilizes glycine as a backbone, with arginine providing the amidino group under the catalysis of amidinotransferase and methyltransferase. Plasma creatine concentration is influenced by animal breed, dietary amino acid composition, and physiological status. Plasma glucose can be derived from dietary fats and proteins. Sewell and Harris [18] reported plasma creatine concentrations of 8–103 mol/L in resting Thoroughbreds. Falavigna et al. [19] investigated BCAA supplementation in adult rats and found significantly higher pre-exercise plasma glucose concentrations compared to post-exercise values.

In this study, plasma creatine concentrations in the control group showed no significant changes across time points, whereas the trial group exhibited increased creatine at 24 hours post-race compared to pre-race and 30 minutes post-race values. This suggests that BCAA supplementation can increase plasma creatine concentration, likely because BCAA regulate protein synthesis [3], positively influencing creatine synthesis. Plasma glucose concentrations in the trial group were higher than in the control group at all time points, as supplemented BCAA serve as oxidative fuel, particularly during prolonged endurance exercise when BCAA oxidation in muscle increases to participate in energy supply [20], thereby increasing plasma glucose levels.

3.5 Effects of BCAA Supplementation on Plasma Lactic Acid and Creatinine Concentrations Before and After the 1 km Trotting Race

During intense exercise, trotters undergo anaerobic metabolism, producing large amounts of lactic acid that shift the slightly alkaline body fluids toward acidity, impairing cellular nutrient and oxygen uptake, compromising normal cellular function, and severely affecting athletic performance [21]. Falavigna et al. [19] administered different doses of BCAA to adult rats and measured plasma LA concentrations 1 hour post-training and at exhaustion, finding that BCAA supplementation significantly reduced plasma LA. Liu et al. [22] confirmed that BCAA supplementation significantly decreased plasma LA in rowers.

In this study, plasma LA concentrations at 30 minutes and 24 hours post-race were lower in the trial group than in the control group, indicating that BCAA supplementation reduces lactic acid accumulation after exercise and decreases fatigue. This effect may be related to BCAA supplementation maintaining skeletal muscle lactate dehydrogenase activity near normal levels, thereby reducing LA accumulation in muscle and its transfer to serum [14]. Creatinine is the end product of creatine and phosphocreatine metabolism, primarily generated through non-enzymatic reactions of phosphocreatine in muscle, and its production affects exercise capacity. In this study, BCAA supplementation had no significant effect on plasma creatinine concentrations at any time point, possibly due to the physiological status of the trotting horses, which requires further investigation.

Conclusion

Under the conditions of this experiment, BCAA supplementation reduced race time and enhanced antioxidant capacity in trotting horses, but did not significantly affect plasma concentrations of hormones, glucose, creatine, lactic acid, or creatinine.

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Note: Figure translations are in progress. See original paper for figures.