Effects of Creatine Supplementation on Exercise Performance, Glucose Metabolism, and Antioxidant Capacity in Standardbred Horses (Postprint)

Authors: Li Xiaobin, Ma Jun, Nie Biaobiao, Yang Jingtao, Qiao Chunjiang, YANG Kailun

Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to investigate the effects of creatine supplementation on exercise performance, glucose metabolism, and antioxidant capacity in trotter horses, providing reference data for the application of creatine in trotter horse training and competition. Eight well-trained 4-year-old Yili stallions with an average body weight of (457$±$50) kg and similar performance in 1 km trotting races were selected and randomly divided into two groups (n=4 per group): a control group and a treatment group. Each horse was fed 3 kg of pelleted concentrate daily with free access to dry forage; the treatment group was additionally supplemented with 38 g of creatine per horse per day. A supplementation and training trial was conducted for 38 days (7-day pre-trial period and 31-day formal trial period). The results showed that creatine supplementation improved the race performance of trotter horses in the 1 km trotting race, with the treatment group' s race time being 12.74% shorter than that of the control group ($P > 0.05$). Regarding plasma antioxidant indices, creatine supplementation significantly increased plasma catalase activity and total antioxidant capacity at 30 min post-race, as well as plasma superoxide dismutase activity at 24 h post-race ($P < 0.05$). However, there were no significant differences between the control and treatment groups in pre- and post-race heart rate and plasma concentrations of glucose, lactate, creatine, creatinine, insulin, glucagon, and cortisol ($P > 0.05$). It can be concluded that creatine supplementation can improve the athletic performance of trotter horses and enhance their post-race antioxidant capacity.

Full Text

Effects of Supplemental Creatine Feeding on Athletic Performance, Glucose Metabolism, and Antioxidant Capacity in Trotters

LI Xiaobin, MA Jun, NIE Biaobiao, YANG Jingtao, QIAO Chunjiang, YANG Kailun*

Xinjiang Key Laboratory of Herbivore Nutrition for Meat and Milk Production, Xinjiang Agricultural University, Urumqi 830052, China

Abstract

This study investigated the effects of supplemental creatine feeding on athletic performance, glucose metabolism, and antioxidant capacity in trotters to provide reference data for creatine application in training and competition. Eight well-trained 4-year-old Yili male horses with an average body weight of (457$±$50) kg and similar performance in 1 km trot races were randomly divided into two groups: a control group and a trial group, with four horses per group. Each horse received 3 kg of pelleted concentrate daily with ad libitum access to dry forage; additionally, horses in the trial group received 38 g of creatine per day. The experiment lasted 38 days (7-day adaptation period followed by 31-day collection period) with concurrent training. Results showed that creatine supplementation improved 1 km trot race performance, reducing race time by 12.74% compared to the control group ($P>0.05$). Regarding plasma antioxidant indices, creatine supplementation significantly increased plasma catalase activity and total antioxidant capacity at 30 min post-race, as well as plasma superoxide dismutase activity at 24 h post-race ($P<0.05$). However, no significant differences were observed between groups in pre- and post-race heart rate or plasma concentrations of glucose, lactate, creatine, creatinine, insulin, glucagon, and cortisol ($P>0.05$). These findings indicate that supplemental creatine feeding can enhance athletic performance and improve post-race antioxidant capacity in trotters.

Keywords: creatine; trotters; athletic performance; glucose metabolism; antioxidant capacity

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Creatine, an amino acid derivative, serves not only as a nutrient but also as an energy substrate that participates in muscle cell anabolic metabolism and reserves energy for muscle contraction. It also plays a role in metabolic regulation by inhibiting oxidative stress and modulating hormone levels, thereby facilitating post-exercise recovery. Currently, creatine is widely studied as an ergogenic nutritional supplement for enhancing athletic performance and reducing oxidative stress in humans, rats, and mice. Williams et al. reported enhanced running capacity in mice following creatine supplementation. Araújo et al. found that trained rats receiving 1.5 g/kg BW creatine daily exhibited increased plasma catalase activity. Sewell et al., studying Thoroughbred horses with an average weight of 505 kg, administered 0.05 g/kg BW creatine via drinking water and observed elevated plasma creatine levels without significant effects on muscle creatine content. The Yili horse, a precious breed native to Xinjiang, demonstrates excellent athletic potential with recorded 1 km trot times of 1 minute 11.66 seconds. However, limitations in trotting performance, fatigue susceptibility during training, and slow recovery currently constrain the full expression of their athletic capabilities. Based on reported benefits of creatine in energy provision, antioxidant function, and post-exercise recovery, this study utilized Yili trotters to investigate the effects of creatine supplementation on athletic performance, glucose metabolism, and antioxidant capacity, providing a scientific basis for optimizing performance in trotters.

1. Materials and Methods

1.1 Experimental Animals and Design Eight 4-year-old well-trained Yili male trotters with an average body weight of (457$±$50) kg were randomly assigned to two groups: a control group and a trial group, each comprising four horses. All horses received 3 kg of pelleted concentrate daily with ad libitum dry forage. Based on Sewell et al.' s findings regarding creatine effects on plasma and muscle creatine in Thoroughbreds, the trial group received an additional 38 g of creatine daily (purchased from Zhongshan Jiahui Food Additive Co., Ltd.). The experiment lasted 38 days, including a 7-day adaptation period and 31-day collection period, during which training was conducted concurrently.

1.2 Feeding Management and Diet Composition Horses were housed individually in stalls and fed using a roughage-first approach. The 3 kg of pelleted concentrate was divided into three equal portions fed at 07:00, 15:00, and 23:00 daily. Creatine was divided into two equal portions supplemented at 07:00 and 23:00. Horses had ad libitum access to roughage and water. After concentrate consumption, horses were led to exercise paddocks for free activity for 4 hours before training. Stalls were cleaned daily, with manure and bedding removed and replaced with dry, soft bedding.

The composition and nutrient levels of the pelleted concentrate are presented in Table 1 . The premix provided per kg 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) 35.56 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 was a calculated value, while other nutrients were measured.

1.3 Training Venue Training was conducted at the Xiyu Racecourse in Zhaosu Horse Farm. The track consisted of an oval sand track and grass track.

The sand track, 21 m wide with a circumference of 1,000 m, was composed of fine sand 40 cm deep over an earthen base. The grass track, also 21 m wide with a circumference of 1,100 m, was formed from natural pasture.

1.4 Training Protocol Daily training occurred from 11:30 to 13:00. At 11:30, saddled horses were led to the track for a warm-up consisting of one slow walking lap on the grass track, followed by a gradual acceleration to a jogging lap on the sand track to ensure adequate activity before formal training. The detailed training program is shown in Table 2 .

1.5 Measurement Methods 1.5.1 Trot Race Performance Assessment

One-kilometer trot race performance was measured using a stopwatch on day 0 (before formal experiment) and day 31 of the collection period.

1.5.2 Heart Rate Measurement

On day 31, heart rate was measured using a Polar pulse meter (purchased from Shanghai Yilian Science and Education Equipment Co., Ltd.) placed on the horse' s chest at 1 hour pre-race, immediately post-race, and 30 minutes post-race.

1.5.3 Blood Plasma 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.

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

1.7 Statistical Analysis Data were analyzed using independent samples t-test in SPSS 16.0 software. Results are expressed as mean±SD.

2. Results

2.1 Effects of Creatine Supplementation on 1 km Trot Race Performance The effects of creatine supplementation on trot race performance are shown in Table 3 . No significant difference was observed between groups at day 0 ($P>0.05$). On day 31, the trial group demonstrated reduced race time compared to the control group, with a 12.74% improvement ($P>0.05$). Over the entire experimental period, the trial group showed a marked reduction in race time, shortening by 107.38% compared to the control group (P>0.05).

2.2 Effects of Creatine Supplementation on Pre- and Post-Race Heart Rate Heart rate data are presented in Table 4 . Pre-race heart rates were below 64 bpm with no significant differences between groups (P>0.05). Heart rate increased immediately post-race in both groups, with no significant intergroup differences (P>0.05). At 30 minutes post-race, heart rates returned to normal levels without significant differences between groups (P>0.05).

2.3 Effects of Creatine Supplementation on Plasma Glucose Metabolism Indices Plasma glucose metabolism indices are shown in Table 5 . No significant differences in plasma glucose were observed between groups at 1 hour pre-race (P>0.05). At 30 minutes post-race, glucose levels in the trial group increased by 33.40% compared to the control group (P>0.05), while no significant differences were found at 24 hours post-race (P>0.05). Plasma insulin concentrations showed no significant differences between groups at 1 hour pre-race (P>0.05), but increased at 30 minutes and 24 hours post-race, with the trial group showing 6.92% and 23.37% higher values than the control group, respectively (P>0.05). Plasma glucagon levels were not significantly different between groups at 1 hour pre-race (P>0.05), but were 5.85% and 6.89% higher in the trial group at 30 minutes and 24 hours post-race, respectively (P>0.05). Plasma lactate concentrations were stable pre-race with no significant differences (P>0.05), increased similarly in both groups at 30 minutes post-race (P>0.05), and decreased at 24 hours post-race without significant intergroup differences (P>0.05). Plasma cortisol levels showed no significant differences between groups at any time point (P>0.05).

2.4 Effects of Creatine Supplementation on Plasma Creatine and Creatinine Concentrations Plasma creatine and creatinine concentrations are presented in Table 6 . No significant differences in plasma creatine were observed between groups at 1 hour pre-race (P>0.05). A slight increase occurred at 30 minutes post-race, but without significant differences between groups (P>0.05). At 24 hours post-race, creatine levels decreased in the trial group but remained not significantly different from the control group (P>0.05). Plasma creatinine concentrations were stable pre-race with no significant differences (P>0.05). At 30 minutes post-race, both groups showed elevated creatinine, with the trial group exhibiting a 24.35% higher increase than the control group (P<0.05). Levels returned to normal physiological ranges by 24 hours post-race (P>0.05).

2.5 Effects of Creatine Supplementation on Plasma Antioxidant Indices Plasma antioxidant indices are shown in Table 7 . No significant differences in plasma catalase activity were observed between groups at 1 hour pre-race (P>0.05). Catalase activity increased at 30 minutes post-race in both groups (P>0.05) and returned to normal levels by 24 hours post-race (P>0.05).

Plasma GSH-Px activity showed no significant differences pre-race (P>0.05), decreased at 30 minutes post-race (P>0.05), and increased at 24 hours post-race, with the trial group showing 12.87% higher activity than the control group (P>0.05). Plasma SOD activity was not significantly different between groups pre-race (P>0.05), was higher in the trial group at 30 minutes post-race (P>0.05), and was significantly higher (15.05%) in the trial group at 24 hours post-race (P<0.05). Total antioxidant capacity showed no significant differences pre-race (P>0.05), was significantly higher in the trial group at 30 minutes post-race (P<0.05), and showed no significant differences at 24 hours post-race (P>0.05). Plasma MDA content was higher in the trial group pre-race (P>0.05), decreased in the trial group but increased in the control group at 30 minutes post-race (P>0.05), and increased in both groups at 24 hours post-race without significant differences (P>0.05).

3. Discussion

3.1 Effects of Creatine Supplementation on Athletic Performance in Trotters Certain amino acids not only serve as nutrients but also enhance exercise endurance and delay fatigue. Creatine, an amino acid derivative, functions as an energy substrate that reduces anaerobic metabolism and oxidative stress. Previous studies have reported performance-enhancing effects of creatine in humans and animals. Eckerson et al. found that 5 g/day creatine supplementation for 5 days improved anaerobic work capacity in volunteers. Izquierdo et al. reported significant improvements in sprint performance following 20 g/day creatine supplementation for 5 days. Murphy et al. demonstrated enhanced performance in the final 50 m of a 400 m swim in athletes receiving creatine. However, Cooke et al. found no significant effects on peak power output or fatigue index in untrained men.

In the present study, the trial group exhibited reduced 1 km race times compared to the control group, with greater time reduction from pre-test to day 31, indicating that creatine supplementation improved racing speed. This effect likely occurs because creatine forms phosphocreatine in muscle tissue, which can rapidly convert to ATP when ATP supply is insufficient, providing adequate energy for muscle contraction and enhancing performance. Regarding heart rate, all groups showed values below 64 bpm pre-race, with immediate post-race increases and subsequent decreases at 30 minutes post-race. The trial group consistently showed lower heart rates than the control group, suggesting that creatine supplementation provided energy substrates that prevented functional decline in tissues and organs, facilitating faster physiological recovery after exercise.

3.2 Effects of Creatine Supplementation on Pre- and Post-Race Glucose Metabolism Hormone concentrations are influenced by multiple factors, with exercise altering insulin, glucagon, and cortisol levels. The overall hormonal response to exercise involves reduced hormone reactivity during activity, enabling efficient gluconeogenesis and rapid, economical glucose mobilization. Exercise enhances hepatic glycogenolysis and gluconeogenesis, with epinephrine, glucagon, cortisol, and insulin serving as primary hormones that augment gluconeogenesis. Elevated plasma glucagon during exercise is associated with enhanced hepatic glycogenolysis and plays a crucial role in prolonged exercise. Post-exercise relationships exist between plasma glucose and cortisol, with increased cortisol facilitating glucose recovery.

In this study, plasma glucose in the trial group increased rather than decreased at 30 minutes post-race, likely due to phosphocreatine conversion to ATP. During intense exercise, ATP levels decline rapidly, and muscle phosphocreatine is almost completely converted to ATP via creatine kinase catalysis, thereby delaying glycolytic energy supply. Exercise stimulates glycogenolysis, increasing plasma glucose. The higher efficiency of phosphocreatine-to-ATP conversion compared to glycolysis may explain the modest increase in post-race plasma glucose in the trial group.

Post-race increases in plasma insulin and glucagon at 30 minutes and 24 hours occurred because these hormones are primary regulators of gluconeogenesis, and enhanced exercise intensity strengthened gluconeogenic activity. Higher insulin and glucagon levels in the trial group align with Davies et al.' s findings, possibly due to increased plasma amino acid concentrations from creatine supplementation, which underwent gluconeogenesis to glucose, thereby affecting hormone levels.

Regarding cortisol, levels at 24 hours post-race were lower than at 30 minutes post-race because cortisol is intensity-dependent and decreases after strenuous exercise until adrenal cortex secretion restores levels. Creatine supplementation increased 24-hour post-race cortisol, likely because direct phosphorylation for energy delayed glucose-dependent energy supply, thereby supporting plasma glucose recovery—consistent with higher glucose levels in the trial group at 24 hours post-race.

During intense exercise, trotters undergo anaerobic metabolism, producing large amounts of lactate that shift weakly alkaline body fluids to acidic pH, impairing nutrient and oxygen uptake and compromising cellular function and performance. Studies show that appropriate creatine supplementation during high-intensity interval training can enhance performance and significantly reduce plasma lactate, even during supramaximal training. However, Jin reported increased lactate in serum, myocardium, and skeletal muscle of rats after swimming exercise. In the present study, both groups showed elevated plasma lactate at 30 minutes post-race, reflecting the short-duration, high-intensity nature of trotting races that rely primarily on anaerobic metabolism, producing pyruvate and lactate as intermediates that cannot be expelled via respiration. Higher lactate in the trial group at 30 minutes post-race likely reflects greater exercise intensity due to faster race times. However, lower lactate in the trial group at 24 hours post-race indicates that creatine supplementation significantly enhanced lactate clearance during recovery.

3.3 Effects of Creatine Supplementation on Plasma Creatine and Creatinine Concentrations Creatine significantly improves anaerobic performance, primarily because 95% of body creatine is stored in skeletal muscle. Normal muscle creatine content ranges from 60–160 mmol/kg dry muscle weight, with two-thirds existing as phosphocreatine and one-third as free creatine, providing the basis for exercise energy supply. Sewell et al. reported resting plasma creatine concentrations of 8-103 mol/L in Thoroughbreds, varying by breed and feeding level.

In this study, plasma creatine concentrations ranged from 16–28 nmol/mL, similar to Sewell's findings. Slightly higher pre-race and 30-minute post-race creatine in the trial group indicates that supplementation increased plasma creatine. The decrease at 24 hours post-race may reflect transfer of free creatine from blood to muscle for phosphocreatine resynthesis, restoring stable phosphocreatine levels depleted during exercise.

Creatinine is a metabolite of creatine and phosphocreatine, and post-exercise elevation reflects their utilization during exercise. Higher creatinine in the trial group at 30 minutes post-race suggests greater participation of creatine and phosphocreatine in energy provision.

3.4 Effects of Creatine Supplementation on Antioxidant Capacity Exercise-induced oxidative stress impairs athletic performance, making antioxidant system protection crucial for performance enhancement. The antioxidant system comprises enzymatic (superoxide dismutase, glutathione peroxidase, catalase, peroxidase) and non-enzymatic antioxidants (vitamins, amino acids, trace elements) that scavenge free radicals and enhance endogenous antioxidant enzyme activity. As an amino acid derivative, creatine functions as an exogenous antioxidant that increases antioxidant enzyme activity. Araújo et al. reported increased plasma catalase activity in trained rats receiving 1.5 g/kg BW creatine. Ke et al. demonstrated significantly higher plasma superoxide dismutase activity in swimming rats supplemented with creatine compared to exercise-only controls.

In this study, the trial group showed higher plasma MDA content than the control group at 30 minutes post-race but lower levels at 24 hours post-race. Catalase, superoxide dismutase activities, and total antioxidant capacity were significantly higher in the trial group at 30 minutes post-race, and catalase, glutathione peroxidase, superoxide dismutase activities, and total antioxidant capacity remained higher at 24 hours post-race. These results demonstrate that creatine enhances endogenous antioxidant enzyme activity, facilitating free radical scavenging, reducing exercise-induced cell membrane damage, and promoting post-race recovery in trotters.

Conclusions

Under the experimental conditions, the following conclusions can be drawn: 1. Supplemental creatine feeding improved 1 km trot race performance in trotters. 2. Creatine supplementation had no significant effects on plasma concentrations of glucose, lactate, creatine, creatinine, insulin, glucagon, or cortisol. 3. Creatine supplementation enhanced plasma catalase and superoxide dismutase activities and total antioxidant capacity.

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