Effects of Letrozole Supplementation on Exercise Performance, Plasma Antioxidant Indices, Hormone Levels, and Body Weight in Pacing Horses: Postprint

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Abstract

This experiment aimed to investigate the effects of supplementary feeding of letrozole on exercise performance, plasma antioxidant indices, hormone levels, and body weight in pace horses. Ten well pace-trained Yili horse geldings with an average age of 2.5 years and an average body weight of (398.16$±$25.34) kg were selected and randomly divided into 2 groups, namely a control group and an experimental group, with 5 horses in each group. All horses were fed 10 kg of mixed forage and 3 kg of pelleted concentrate daily; on this basis, horses in the experimental group were supplemented with 5 mg of letrozole per horse per day, and a 30-day feeding and training experiment was conducted. Blood samples were collected from the jugular vein in the early morning under fasting conditions on days 0, 10, 20, and 30 of the experiment to determine plasma hormone indices; on days 0 and 30 of the experiment, body weight was measured in the morning under fasting conditions, and a 1,000 m pace race was conducted; blood samples were collected from the jugular vein at 1 h before the race, immediately after the race, 20 min after the race, and 2 h after the race to determine blood acid-base related indices and plasma antioxidant indices. The results showed that supplementary feeding of letrozole could improve the 1,000 m pace race performance of the experimental horses; at 2 h post-race, the plasma activities of catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and uric acid (UA) content in the experimental group horses were all higher than those in the control group, with GSH-Px activity and T-AOC reaching extremely significant ($P<0.01$) and significant levels ($P<0.05$), respectively, while plasma malondialdehyde (MDA) content was significantly lower than that in the control group ($P<0.05$). Regarding blood acid-base related indices, the bicarbonate (HCO3-) content in the experimental group was lower than that in the control group immediately

after the race, 20 min after the race, and 2 h after the race (P>0.05), while base excess (BEecf) and pH were higher than those in the control group (P>0.05). On days 10, 20, and 30 of the experiment, the plasma levels of testosterone (T) and androstenediol (AD) in the experimental group horses were higher than those in the control group (P>0.05). In addition, supplementary feeding of letrozole could also increase the body weight of pace horses. Therefore, supplementary feeding of letrozole at 5 mg/(horse · d) to pace horses can shorten the race time in 1,000 m pace races, improve the body' s antioxidant capacity and acid-base buffering capacity, increase plasma T and AD levels, and simultaneously increase the body weight of pace horses.

Full Text

Effects of Supplemental Feeding Letrozole on Athletic Performance, Plasma Antioxidant Indices and Hormone Levels, and Body Weight of Trotters

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Abstract

This experiment investigated the effects of supplemental feeding letrozole on athletic performance, plasma antioxidant indices, hormone levels, and body weight in trotters. Ten well-trained Yili male horses aged 2.5 years with an average body weight of (398.16$±$25.34) kg were randomly divided into two groups: a control group and a trial group, with five horses per group. All horses received 10 kg of mixed pasture and 3 kg of pelleted concentrate daily. The trial group was additionally supplemented with 5 mg letrozole per horse per day for 30 days of feeding and training. Blood samples were collected from the jugular vein on days 0, 10, 20, and 30 of the trial to measure plasma hormone levels. Body weight was recorded on days 0 and 30 before morning feeding. A 1,000 m trot training race was conducted on days 0 and 30, with blood samples collected from the jugular vein at 1 hour pre-race, immediately post-race, 20 minutes post-race, and 2 hours post-race to determine plasma antioxidant indices and blood acid-base parameters.

The results demonstrated that letrozole supplementation improved 1,000 m trot race performance. At 2 hours post-race, the trial group exhibited higher plasma activities of catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and uric acid (UA) content compared to the control group, with GSH-Px activity and T-AOC reaching extremely significant (P<0.01) and significant (P<0.05) differences, respectively. Meanwhile, plasma malondialdehyde (MDA) content was significantly lower in the trial group (P<0.05). Regarding acid-base parameters, the trial

group showed lower blood bicarbonate ($HCO3^{-}$) content and higher base excess (BEecf) and pH compared to the control group at immediately post-race, 20 minutes post-race, and 2 hours post-race, though these differences were not statistically significant ($P>0.05$). Plasma testosterone (T) and androstenedione (AD) levels in the trial group were higher than those in the control group on days 10, 20, and 30 ($P>0.05$). Additionally, letrozole supplementation increased body weight. Therefore, supplementing trotters with 5 mg letrozole per horse per day can shorten 1,000 m race times, enhance antioxidant capacity and acid buffering capacity, elevate plasma T and AD levels, and increase body weight.

Keywords: letrozole; trotters; athletic performance; antioxidant indices; hormone; body weight

Introduction

Letrozole (LE) is a synthetic, highly efficient, selective non-steroidal benzyltriazole derivative that functions as a non-steroidal aromatase inhibitor. It reversibly inhibits aromatase activity by binding to iron atoms in ferrous hemoglobin and competing with endogenous substrates for the enzyme' s active site, thereby selectively suppressing estrogen biosynthesis and effectively blocking the conversion of androstenedione and testosterone to estrogen. This indirectly increases androgen levels in muscle tissue, improves myogenic cell development, and promotes muscle growth and strength enhancement. Previous research has demonstrated that letrozole supplementation can increase androgen levels and body weight, with higher androgen levels positively influencing athletic performance and maintenance. Bai et al. reported that administering 2.5 mg/d letrozole to obese men with oligoasthenozoospermia for three months resulted in significantly higher plasma testosterone levels compared to controls. Zhao et al. found that dihydrotestosterone promoted skeletal muscle hypertrophy in male SD rats, significantly increasing muscle cross-sectional area, wet weight, and major histocompatibility complex (MHC) content. Liu showed that daily oral administration of 1 mg/kg BW letrozole to rats increased body weight by 32.69% compared to controls. However, long-term training can lead to decreased testosterone levels in athletic horses, reducing anabolic effects and causing muscle loss that diminishes training efficacy. The Yili horse is an excellent Chinese breed for sport, but some trained Yili horses experience negative weight gain, particularly during trot training, where they exhibit fatigue, poor exercise persistence, and slow recovery, severely limiting performance improvement. Based on letrozole' s biological actions, this study supplemented letrozole to trained Yili horses to investigate its effects on athletic performance, plasma antioxidant indices, hormone levels, and body weight, providing a reference for enhancing trotter performance.

1. Materials and Methods

1.1 Experimental Period and Location The experiment was conducted from September 6 to October 9, 2014, at Zhaosu Horse Farm in Ili Kazakh Autonomous Prefecture, Xinjiang.

1.2 Animal Selection and Experimental Design Ten well-trained Yili male horses of similar age (2.5 years) and body weight [(398.16$±$25.34) kg] were randomly divided into two groups: a control group and a trial group, with five horses per group. All horses received 10 kg of mixed pasture (alfalfa hay:mountain grass = 1:1) and 3 kg of pelleted concentrate daily. The trial group was additionally supplemented with two letrozole tablets (2.5 mg per tablet, purchased from Jiangsu Hengrui Medicine Co., Ltd.) per horse per day for 30 days of feeding and training.

1.3 Feeding Management During the trial, all horses were housed individually and fed using a roughage-first method. Each horse received 10 kg of mixed pasture (alfalfa hay:mountain grass = 1:1) and 3 kg of pelleted concentrate daily. The 10 kg of mixed pasture was divided into three feedings at 07:00 (3 kg), 13:00 (4 kg), and 20:00 (3 kg). The 3 kg of pelleted concentrate was divided equally into two portions fed at 07:00 and 20:00. Two letrozole tablets were mixed with the pelleted concentrate at 07:00 for trial group horses. All horses had free access to water. After feeding, horses were led to exercise paddocks for free movement. Stables were cleaned daily to remove manure and bedding, which was replaced with dry, soft material.

The diet composition and nutrient levels are shown in Table 1 . The premix provided per kg of diet: 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, Co (as cobalt chloride) 1.11 mg.

1.4 Training Venue Training was conducted at the Xiyu Racecourse of Zhaosu Horse Farm. The track consisted of an oval sand track and grass track. The sand track was made of fine sand 40 cm deep with an earthen base, 21 m wide, and 1,000 m in circumference. The grass track was formed from natural pasture, 21 m wide, and 1,100 m in circumference.

1.5 Training Protocol Daily training occurred from 11:30 to 13:00. At 11:30, horses were saddled and led to the track, where they first walked one lap on the grass track, then gradually increased speed to jog one lap on the sand track for adequate warm-up before formal training. The training protocol is detailed in Table 2 .

Table 2 Training Protocol - Days 1-7: 80% of full speed[1] - Days 8-14: 80% of full speed - Days 15-21: 80-90% of full speed[2] - Days 22-30: 80-90% of full speed

^{1}80% of full speed means riders controlled horses to complete 1,000 m trot training in 200 s.
^{2}80-90% of full speed means riders controlled horses to complete 1,000 m trot training in 175 s.

1.6 Data Collection **1.6.1 Body Weight Measurement** Body weight was measured on day 0 (pre-trial) and day 30 before morning feeding by leading fasted horses onto a platform scale.

1.6.2 Race Performance Measurement A 1,000 m trot training race was conducted on days 0 and 30, with race times measured using a stopwatch to calculate performance.

1.6.3 Blood Sample Collection On days 0, 10, 20, and 30, 5 mL fasting blood samples were collected from the jugular vein into heparinized tubes, centrifuged at 1,500×g for 15 min, and plasma was separated and stored at -20°C for hormone analysis. On day 30, additional blood samples were collected at 1 hour pre-race, immediately post-race, 20 minutes post-race, and 2 hours post-race into both regular and heparinized tubes. Heparinized samples were processed as above for antioxidant analysis, while whole blood from regular tubes was immediately used for acid-base parameter determination.

1.7 Analytical Methods Plasma levels of testosterone (T), androstenedione (AD), estrone (E1), estradiol (E2), estriol (E3), gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured by Beijing Huaying Biotechnology Research Institute.

Plasma activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC), and contents of uric acid (UA) and malondialdehyde (MDA) were determined using assay kits from Beijing Huaying Biotechnology Research Institute according to manufacturer instructions.

Whole blood pH, total carbon dioxide (TCO_2) content, partial pressure of carbon dioxide (PCO_2), bicarbonate (HCO_3^-) content, and base excess (BEecf) were measured using an i-STAT blood gas analyzer (Shanghai Qiwei Industrial Co., Ltd.) with CG8+ cartridges purchased from Beijing Lidehongao Company.

1.8 Statistical Analysis Data were initially processed using Excel 2010 and expressed as mean±SD. Statistical analysis was performed using SPSS 18.0 software with independent samples t-tests.

2. Results

2.1 Effects of Letrozole Supplementation on 1,000 m Trot Race Performance As shown in Table 3 , after 30 days of letrozole supplementation, the average speed of trial group horses was faster than that of the control group, though the difference was not significant (P>0.05).

Table 3 Effects of Supplemental Feeding Letrozole on Performance of 1,000 m Trot Training Race of Trotters (n=5) | Item | Day 0 Average Speed (m/s) | Day 30 Average Speed (m/s) | |—|————————|————————-| | Control group | 7.15$±1.30|7.25±1.42||*Trialgroup*|7.34±2.08|7.92±$2.30 |

In the same column, values of the same item with different capital letter superscripts indicate extremely significant difference (P<0.01), different small letter superscripts indicate significant difference (P<0.05), and same or no letters indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Letrozole Supplementation on Plasma Antioxidant Indices Before and After 1,000 m Trot Race Table 4 shows that at 1 hour pre-race, no significant differences were observed between groups in CAT, GSH-Px, SOD activities, T-AOC, or UA and MDA contents (P>0.05). Immediately post-race, the trial group showed higher CAT and SOD activities, UA content, and T-AOC, with lower MDA content compared to controls (P>0.05). At 20 minutes post-race, the trial group exhibited higher GSH-Px and SOD activities, UA content, and T-AOC, with lower MDA content (P>0.05). At 2 hours post-race, the trial group demonstrated higher CAT and SOD activities and UA content (P>0.05), with GSH-Px activity and T-AOC being extremely significantly (P<0.01) and significantly (P<0.05) higher than controls, respectively, while MDA content was significantly lower (P<0.05).

Table 4 Effects of Supplemental Feeding Letrozole on Plasma Antioxidant Indices Before and After 1,000 m Trot Training Race of Trotters (n=5)

Item	1 h before race	End of race	20 min after race	2 h after race
CAT (U/mL)				

Item	1 h before race	End of race	20 min after race	2 h after race
Control group	44.78$±1.50	44.11±4.87	51.51±1.63	47.71±1.85
	$*GSH - Px(U/mL)*			
	*||||||Controlgroup	826.67±83.59	799.77±25.54	826.28±3.51	807.52±8.83^{B}
	$*SOD(U/mL)*			
	*||||||Controlgroup	98.93±22.41	80.24±26.88	78.76±25.75	77.43±10.79
	$*UA(\mu mol/L)*			
	*||||||Controlgroup	37.32±2.31	52.68±9.22	45.19±8.64	34.26±3.22
	$*T - AOC(U/mL)*			
	*||||||Controlgroup	9.24±0.36	9.86±0.33	10.58±0.28	10.43±0.22^{b}
	$*MDA(nmol/mL)*			
	*||||||Controlgroup	5.08±0.34	5.31±0.40	5.35±0.08	5.33±0.11^{a}

Item	1 h before race	End of race	20 min after race	2 h after race
Trial group	42.27±0.76	47.48±3.00	41.91±0.69	48.77
	*||Trialgroup	854.42±80.58		
	*||Trialgroup	101.65±23.61	105.	
	*||Trialgroup	38.80±3.50	54.57±10.2	
	*||Trialgroup	8.69±0.05	10.67±0.57	11
	*||Trialgroup	4.76±0.22	5.16±0.35	5.09

2.3 Effects of Letrozole Supplementation on Blood Acid-Base Parameters Before and After 1,000 m Trot Race Table 5 shows that no significant differences were observed between groups in TCO_2 content or PCO_2 at any time point (P>0.05). The trial group exhibited lower HCO_3^- content and higher BEecf and pH than the control group immediately post-race, 20 minutes post-race, and 2 hours post-race, though these differences were not significant (P>0.05).

Table 5 Effects of Supplemental Feeding Letrozole on Blood Acid-Base Related Indices Before and After 1,000 m Trot Training Race of Trotters (n=5)

Item	1 h before race	End of race	20 min after race	2 h after race
TCO_2 content (mmol/L)				
Control group	31.67$±2.31	24.00±3.00	28.00±0.00	28.67±2.08
	$*PCO_{2}$ (mmHg)**			
Control group	45.63$±3.36	38.05±2.62	38.10±1.25	42.70±4.38
	$*HCO_{3}$^- (mol/L)**			
Control group	30.50$±2.43	24.35±2.62	26.93±0.32	28.37±1.46
	$*BEecf(mol/L)*			
	*||||||Controlgroup	6.00±2.65	— 1.33±3.79	3.00±1.00	4.00±1.73
	$*pH *			
	*||||||Controlgroup	7.43±0.02	7.42±0.04	7.45±0.02	7.44±0.01

Item	1 h before race	End of race	20 min after race	2 h after race
Trial group	31.00±2.65	25.67±3.06	28.00±4.58	28.00
	*||Trialgroup	44.70±1.85	35.13±1.33	38.50±2.34	41.33
	*||Trialgroup	29.73±2.23	22.90±1.98	28.30±1.97	26.95
	*||Trialgroup	7.00±0.00	0.67±4.51	5.50±2.12	5.50±2.12 ||*
	*||Trialgroup	7.43±0.01	7.44±0.06	7.47±	

2.4 Effects of Letrozole Supplementation on Plasma Hormone Levels Table 6 shows that on days 10, 20, and 30, plasma T and AD levels in the trial group were higher than those in the control group, while E1 and E2 levels were lower (P>0.05), though none of these differences were significant (P>0.05). No significant differences were observed between groups in GnRH, FSH, LH, or E3 levels at any time point (P>0.05).

Table 6 Effects of Supplemental Feeding Letrozole on Plasma Hormone Levels of Trotters (n=5)

Item	Day 0	Day 10	Day 20	Day 30
T (ng/ml)				
Control group	0.14$±0.02|0.12±0.03|0.16±0.04|0.13±0.01||*Trialgroup*|0.15±0.01|0.45±0.38|0.36±0.24|0.51±0.2			
	AD(nmol/L)			
	*||||||*Controlgroup*|11.59±0.56|10.52±1.42|11.82±1.52|10.43±0.91||*Trialgroup*|13.52±4.08|21.96			
	E2(pg/mL)			
	*||||||*Controlgroup*|35.26±2.23|39.18±5.85|35.40±15.96|35.51±9.34||*Trialgroup*|34.82±3.04|28.19			
	E3(ng/mL)			
	*||||||*Controlgroup*|5.61±1.83|4.97±1.36|7.82±1.32|6.63±0.51||*Trialgroup*|6.14±2.38|6.46±0.47|6			
	E1(pmol/L)			
	*||||||*Controlgroup*|100.28±2.80|105.13±16.72|93.56±43.26|86.6±15.39||*Trialgroup*|93.85±11.87|			
	GnRH(pg/mL)			
	*||||||*Controlgroup*|51.39±2.35|47.72±0.88|50.23±0.32|48.15±0.07||*Trialgroup*|47.08±0.08|46.23			
	FSH(mIU/mL)			
	*||||||*Controlgroup*|7.61±0.26|7.59±0.45|8.75±1.00|8.34±0.09||*Trialgroup*|8.19±1.08|8.32±1.07|9			
	LH(mIU/mL)			
	*||||||*Controlgroup*|20.89±0.51|18.40±1.44|19.65±0.80|21.74±0.62||*Trialgroup*|19.93±0.73|22.19			

2.5 Effects of Letrozole Supplementation on Body Weight Table 7 shows that both groups exhibited increasing body weight during the trial. The trial group showed a 59.49% higher growth rate compared to the control group (P>0.05).

Table 7 Effects of Supplemental Feeding Letrozole on Body Weight of Trotters (n=5)

Item	Initial weight (kg)	Final weight (kg)	Weight gain (kg)	Growth rate (%)
Control group	412.33$±51.81|426.00±44.54|13.67±10.02|3.53±0.03||*Trialgroup*|384.00±90.51|404.00±82.02|20.00±8.4			

3. Discussion

3.1 Effects of Letrozole Supplementation on Athletic Performance in 1,000 m Trot Race Athletic performance in horses depends not only on breed, training methods, and dietary nutrients, but also on muscle mass and strength. The work capacity of equine muscle is a crucial indicator of performance, making muscle growth and strength enhancement essential for improving athletic ability. Muscle development and strength gain are influenced by dietary protein levels, training intensity, hormone levels, and Na^+/K^+ content. Griggs et al. demonstrated that androgens stimulate formation of new myofilaments in myofibrils, causing hypertrophy and division that promotes protein synthesis, muscle development, strength enhancement, and reduced fat accumulation.

Our results indicate that 30 days of letrozole supplementation improved speed in the 1,000 m trot race, likely by modulating androgen metabolism. Letrozole binds to iron atoms in ferrous hemoglobin, competing with endogenous substrates for the aromatase active site to reversibly inhibit enzyme activity, thereby selectively suppressing estrogen biosynthesis and effectively blocking androgen conversion to estrogen. This causes transient androgen increases that act on myogenic cells to promote muscle growth and strength. Long-term training can decrease testosterone levels in athletic horses, reducing anabolic effects and muscle mass while diminishing training efficacy. Our findings show that letrozole supplementation increased plasma T and AD levels, helping maintain androgen levels during competition and improve performance. While letrozole causes side effects in humans (headache, hot flashes, nausea, bone pain, weight gain), few reports exist on side effects in animals, warranting further investigation.

3.2 Effects of Letrozole Supplementation on Plasma Antioxidant Indices High-intensity or prolonged endurance exercise alters blood distribution in horses, shunting blood to active organs while temporarily reducing flow to less active tissues. In ischemic tissues, coenzyme Q dissociates from the electron transport chain and undergoes auto-oxidation, generating oxygen free radicals. Additionally, dissociated ubisemiquinone radicals ($QH\cdot$) can abstract hydrogen from adjacent polyunsaturated fatty acids to form lipid radicals ($LO\cdot$), initiating lipid peroxidation that produces abundant free radicals and alters the antioxidant system. Therefore, eliminating free radicals and enhancing antioxidant capacity are critical concerns in equine training and competition. Androgens have been shown to mitigate oxidative stress. Ren et al. demonstrated that testosterone improved antioxidant capacity and reduced plasma MDA in rabbits. Ma et al. found that dehydroepiandrosterone decreased MDA content in serum and liver while increasing hepatic SOD activity in rats. Keymel et al. observed oxidative stress imbalance in patients undergoing androgen-deprivation therapy, suggesting androgens are important oxidative stress inhibitors.

In this study, letrozole supplementation increased post-race plasma CAT, SOD, GSH-Px activities, UA content, and T-AOC while decreasing MDA content.

This likely occurred because letrozole elevated plasma T and AD levels, which exerted antioxidant effects by increasing GSH-Px activity and T-AOC while inhibiting lipid peroxidation to reduce MDA content. These findings indicate that letrozole enhances antioxidant enzyme activity, scavenges free radicals, and improves antioxidant capacity in trotters. Thus, letrozole supplementation can improve performance while reducing exercise-induced oxidative stress and promoting post-exercise recovery. However, due to limited animal numbers, these results require validation in larger populations.

3.3 Effects of Letrozole Supplementation on Blood Acid-Base Parameters Blood acid-base status reflects gas transport capacity and acid-base balance maintenance. Key indicators include pH, TCO_2, PCO_2, HCO_3^-, and BEecf. Decreased pH and HCO_3^- with increased TCO_2 and PCO_2 indicate acid ion accumulation, while reduced BEecf suggests compromised buffering capacity. In rowers, high-intensity exercise increased blood lactate and decreased pH and HCO_3^-, but immediate post-exercise oxygen inhalation significantly reduced lactate and restored pH and HCO_3^-.

In our study, the trial group exhibited higher BEecf and pH with lower HCO_3^- post-race, indicating stronger gas transport and acid-base regulation capacity with more oxygen available for aerobic metabolism and reduced lactate accumulation. This may result from: (1) elevated androgens promoting protein synthesis, reducing protein breakdown, enhancing muscle development and strength, and decreasing energy expenditure; and (2) increased plasma T acting as an anabolic hormone to enhance amino acid uptake and promote nucleic acid and protein synthesis, providing more protein for oxidative function. Therefore, letrozole supplementation improves gas transport and acid-base buffering capacity while helping eliminate acid ion accumulation.

3.4 Effects of Letrozole Supplementation on Plasma Hormone Levels Hormone regulation is a complex process. GnRH reaches the anterior pituitary via the portal system to promote FSH and LH secretion. LH and FSH stimulate sex hormone secretion from theca and Leydig cells. FSH activates granulosa cell aromatase to convert AD and T to E1 and E2, while excess sex hormones negatively feedback on LH, FSH, and GnRH secretion. Testosterone can be converted to E2 by aromatase, raising estrogen levels. Elevated E2 and T then inhibit GnRH release, reducing LH and FSH secretion and ultimately decreasing sex hormone production to maintain balance.

Letrozole binds to iron atoms in ferrous hemoglobin, competing with endogenous substrates for the aromatase active site to reversibly inhibit enzyme activity, selectively suppressing estrogen biosynthesis and preventing androgen conversion while transiently increasing androgen levels. Liu et al. reported that 2.5 mg/d letrozole in women with poor ovarian response significantly increased plasma testosterone. Tao et al. found that 2.5 mg/d letrozole in pubertal boys increased plasma T levels by 61.6% compared to baseline, with significant differ-

ences from controls. In our study, letrozole supplementation increased plasma T and AD while decreasing E1, E2, and E3 trends, without affecting GnRH, FSH, or LH levels, confirming that letrozole acts peripherally without disrupting the hypothalamic-pituitary axis.

3.5 Effects of Letrozole Supplementation on Body Weight Androgens are important anabolic hormones that promote protein synthesis, muscle growth, and bone development. Testosterone and AD are primary androgens that stimulate anabolism, promoting amino acid uptake, nucleic acid and protein synthesis, and myofiber and bone growth. Letrozole inhibits aromatase activity, preventing androgen conversion to estrogen (primarily T and AD to estrogen), causing transient androgen accumulation that stimulates insulin-like growth factor I. This factor promotes cell proliferation and differentiation through RNA and DNA synthesis regulation, enhancing mitosis and protein synthesis while inhibiting degradation, ultimately increasing protein deposition. Liu reported that 1 mg/kg·BW letrozole in rats increased body weight by 32.69% compared to controls. Wang et al. also found that 6 mg/d dehydroepiandrosterone sulfate significantly increased rat body weight.

In this study, letrozole supplementation increased trotter body weight, likely directly related to elevated T and AD levels. Absorbed letrozole enters circulation and inhibits androgen-to-estrogen conversion, increasing T and AD levels that promote anabolism, amino acid uptake, nucleic acid and protein synthesis, and myofiber and bone growth, resulting in weight gain.

Conclusion

Supplementing trotters with 5 mg letrozole per horse per day can shorten 1,000 m race times, enhance antioxidant capacity and acid buffering capacity, elevate plasma testosterone and androstenedione levels, and increase body weight.

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