Effects of Tribulus terrestris Extract on Growth Performance and Serum Antioxidant Indices in Weaned Piglets (Postprint)

Authors: Li Zelei, Li Meiquan, Liu Shuiling, Che Litao, Jiang Xiaoxu, Guo Rongfu

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Abstract

This experiment aimed to investigate the effects of Tribulus terrestris extract (TTE) on growth performance, nutrient apparent digestibility, serum antioxidant indices, and serotonin (5-HT) content in weaned piglets. One hundred twenty-eight (37±2)-day-old "Duroc × Landrace × Yorkshire" weaned piglets were selected and randomly divided into 4 groups with 4 replicates per group and 8 piglets per replicate. They were fed experimental diets supplemented with 0, 125, 250, and 500 mg/kg TTE in the basal diet, respectively, for a 28-day experimental period. Fasting body weight was measured, and blood and feces were collected at 51 and 65 days of age to determine serum antioxidant indices, 5-HT content, and nutrient apparent digestibility. The results showed that: 1) With increasing dietary TTE supplementation levels, the average daily gain (ADG) and average daily feed intake (ADFI) of weaned piglets increased, while the feed-to-gain ratio (F/G) decreased; compared with the control group, the 500 mg/kg group showed significant increases in ADG by 18.65%, 17.62%, and 18.10% at 37-51, 51-65, and 37-65 days of age, respectively ($P<0.05$). 2) Dietary TTE supplementation improved the apparent digestibility of dry matter (DM), crude protein (CP), and crude ash (Ash) in weaned piglets; compared with the control group, the 250 mg/kg group showed a significant increase in CP apparent digestibility by 5.38% at 49-51 days of age ($P<0.05$), and Ash apparent digestibility was significantly increased by 22.96% and 52.46% at 49-51 and 63-65 days of age, respectively ($P<0.05$). 3) Dietary TTE supplementation increased serum glutathione peroxidase (GSH-Px) and catalase (CAT) activities, and decreased serum malondialdehyde (MDA) content in weaned piglets. Compared with the control group, at 51 days of age, serum CAT activities in the 250 and 500 mg/kg groups were significantly increased by 99.42% and 114.53%, respectively ($P<0.05$), and serum MDA content in the 500 mg/kg group was extremely significantly decreased by 46.98% ($P<0.01$); at 65 days of age, serum

MDA content in the 250 and 500 mg/kg groups was extremely significantly decreased by 53.57% and 57.74%, respectively (P<0.01). 4) Dietary TTE supplementation increased serum 5-HT content in weaned piglets; compared with the control group, serum 5-HT content in the 250 mg/kg group was extremely significantly increased by 33.81% at 65 days of age (P<0.01). In conclusion, dietary supplementation with 500 mg/kg TTE significantly increased ADG and decreased F/G in weaned piglets; supplementation with 250 mg/kg TTE improved CP and Ash apparent digestibility, increased serum 5-HT content, and enhanced antioxidant capacity, thereby reducing the occurrence of stress.

Full Text

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LI Zelei, LI Meiquan*, LIU Shuiling, CHE Litao, JIANG Xiaoxu, GUO Rongfu**

College of Animal Science and Technology, Yunnan Province Key Laboratory of Animal Nutrition and Feed, Yunnan Agricultural University, Kunming 650201, China

Abstract

This experiment was conducted to investigate the effects of Tribulus terrestris extract (TTE) on growth performance, nutrient apparent digestibility, serum antioxidant indexes, and serotonin (5-HT) content in weaned piglets. One hundred twenty-eight "Duroc × Landrace × Yorkshire" weaned piglets at (37±2) days of age were randomly allocated into 4 groups with 4 replicates per group and 8 piglets per replicate. The piglets were fed basal diets supplemented with 0, 125, 250, and 500 mg/kg TTE for a 28-day experimental period. Fasting body weight was measured, and blood and fecal samples were collected at 51 and 65 days of age to determine serum antioxidant indexes, 5-HT content, and nutrient apparent digestibility. The results showed: 1) Average daily gain (ADG) and average daily feed intake (ADFI) increased while feed-to-gain ratio (F/G) decreased with increasing dietary TTE levels. Compared with the control group, ADG in the 500 mg/kg group was significantly increased by 18.65%, 17.62%, and 18.10% during days 37–51, 51–65, and 37–65, respectively (P<0.05). 2) Dietary TTE supplementation improved the apparent digestibility of dry matter (DM), crude protein (CP), and ash in weaned piglets. Compared with the control group, the 250 mg/kg group showed significantly increased CP apparent digestibility by 5.38% at days 49–51 (P<0.05), and ash apparent digestibility by 22.96% and 52.46% at days 49–51 and 63–65, respectively (P<0.05). 3) Dietary TTE increased serum glutathione peroxidase (GSH-Px) and catalase (CAT) activities while decreasing serum malondialdehyde (MDA) content. At 51 days of age, serum CAT activity in the 250 and 500 mg/kg groups was significantly

increased by 99.42% and 114.53%, respectively (P<0.05), while serum MDA content in the 500 mg/kg group was significantly decreased by 46.98% (P<0.01). At 65 days of age, serum MDA content in the 250 and 500 mg/kg groups was significantly decreased by 53.57% and 57.74%, respectively (P<0.01). 4) Dietary TTE increased serum 5-HT content, with the 250 mg/kg group showing a significant increase of 33.81% at 65 days of age compared with the control group (P<0.01). In conclusion, supplementation with 500 mg/kg TTE significantly improved ADG and reduced F/G in weaned piglets, while 250 mg/kg TTE increased CP and ash apparent digestibility, elevated serum 5-HT content, and enhanced antioxidant capacity, thereby reducing stress.

Keywords: Tribulus terrestris extract; weaned piglets; growth performance; antioxidant capacity; serotonin

Introduction

Over the past several decades, antibiotic growth promoters have played an important role in promoting livestock growth and disease prevention. Since the European Union banned antibiotic growth promoters in 2006, natural plant extracts have become a research focus for improving the growth performance of weaned piglets. Tribulus terrestris (TT), also known as puncture vine, is a natural plant with traditional medicinal properties including blood circulation improvement, wind-dispersing and vision-brightening effects, qi-descending and blood-moving actions, and liver-soothing and lipid-lowering benefits. Water or alcohol extraction yields active components such as saponins, alkaloids, polysaccharides, and amino acids, with saponins comprising approximately 90% of the extract. Tribulus terrestris extract (TTE) exhibits broad antioxidant effects and can scavenge free radicals and exert antibacterial properties. Current research on TTE primarily focuses on antioxidant effects under pathological conditions and alleviation of glucose metabolism disorders. Liu Sheqin found that TTE could alleviate the decline in blood insulin content and promote growth hormone secretion in exercise-trained rats. Studies on convict cichlid and tilapia demonstrated that appropriate TTE levels could improve fish growth performance. Li Mingjuan et al. and Sharawy et al. reported that TTE significantly inhibited gluconeogenesis and decreased serum triglyceride levels in animals. Research also indicated that 125 mg/kg TTE significantly increased serum 5-HT content in depressed rats. To date, TTE application research has predominantly focused on aquatic economic animals, with limited reports on its effects on growth performance and antioxidant capacity in weaned piglets. This experiment aimed to investigate the effects of different dietary TTE levels on growth performance, nutrient apparent digestibility, serum antioxidant indexes, and 5-HT content in weaned piglets to provide scientific evidence for TTE application in weaned piglet diets.

Materials and Methods

1.1 Experimental Design and Diets

The main component of TTE was Tribulus saponins at 90% concentration, with remaining components including polysaccharides, amino acids, and alkaloids. The raw herb was harvested in autumn. The experiment was conducted at the Guangdong Feisaidi Ecological Agriculture Company farm. One hundred twenty-eight "Duroc × Landrace × Yorkshire" weaned piglets at (37±2) days of age were selected and randomly divided into 4 groups based on body weight, with 4 replicates (pens) per group and 8 piglets per replicate. The control group was fed a basal diet formulated according to NRC (2012) nutrient requirements for piglets as a powdered complete feed. The basal diet composition and nutrient levels are shown in Table 1 . The experimental groups were fed the basal diet supplemented with 125, 250, and 500 mg/kg TTE, respectively. The trial lasted 28 days, during which piglets had ad libitum access to feed and water.

1.2 Growth Performance Measurement

Initial fasting body weight (BW) was recorded before the experiment began, and daily feed consumption per pen was recorded thereafter. Fasting body weight was measured again at 51 and 65 days of age (days 14 and 28 of the experiment). Average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated.

1.3 Nutrient Apparent Digestibility Measurement

Fecal samples were collected from each group during days 49–51 (days 12–14 of the experiment) and days 63–65 (days 26–28 of the experiment). Daily fecal samples were mixed with 10 mL of 10% sulfuric acid per 100 g of feces, thoroughly mixed, dried at 65°C to constant weight, and ground for analysis. Dry matter (DM), crude protein (CP), ash, ether extract (EE), and the endogenous indicator acid-insoluble ash content were determined to calculate nutrient apparent digestibility. Acid-insoluble ash content was determined according to GB/T 23742−2009/ISO 5985:2002, while DM, CP, ash, and EE contents were determined according to Zhang Liying' s "Feed Analysis and Feed Quality Detection Technology" .

1.4 Serum Antioxidant Index Measurement

Before each weighing, 2 piglets per pen were randomly selected for blood collection from the anterior vena cava. Blood samples were left to stand for 1 hour, then centrifuged at 3,500 r/min for 10 minutes. The supernatant was carefully transferred and stored at -20°C. Serum glutathione peroxidase (GSH-Px) and catalase (CAT) activities and malondialdehyde (MDA) content were determined according to kit instructions.

1.5 Serum 5-HT Content Measurement

A portion of serum was thawed on ice and mixed well. Serum 5-HT content was determined according to the porcine enzyme-linked immunosorbent assay (ELISA) kit instructions.

1.6 Statistical Analysis

Experimental data were initially processed using Excel 2016 and analyzed using SPSS 17.0 for one-way ANOVA, with Duncan' s multiple range test for post-hoc comparisons. Results are expressed as "mean ± standard deviation" . $P<0.05$ was considered statistically significant, $P<0.01$ was considered highly significant, and $P>0.05$ was considered not significant.

Results

2.1 Effects of TTE on Growth Performance of Weaned Piglets

As shown in Table 2 , dietary TTE supplementation tended to increase ADG and ADFI in weaned piglets ($P>0.05$). During days 37-51, ADG in the 500 mg/kg group was significantly increased by 18.65% compared with the control group ($P<0.05$). ADFI increased with TTE supplementation level but showed no significant differences among groups ($P>0.05$). F/G tended to decrease with increasing TTE levels ($P>0.05$). During days 51-65, ADG in the 250 and 500 mg/kg groups was significantly higher than the control group by 9.22% and 17.62%, respectively ($P<0.05$). ADFI in all TTE-supplemented groups was higher than the control group ($P>0.05$), and F/G tended to decrease with increasing TTE levels ($P>0.05$). During days 37-65, ADG in the TTE-supplemented groups was increased by 2.22% ($P>0.05$), 8.92% ($P>0.05$), and 18.10% ($P<0.05$) compared with the control group. ADFI showed an increasing trend but with no significant differences among groups ($P>0.05$), and F/G also tended to decrease in the TTE-supplemented groups ($P>0.05$).

2.2 Effects of TTE on Nutrient Apparent Digestibility of Weaned Piglets

As shown in Table 3 , during days 49-51, DM apparent digestibility in all TTE-supplemented groups showed an increasing trend compared with the control group but without significant differences ($P>0.05$). The 250 mg/kg group showed significantly increased CP and ash apparent digestibility by 5.38% and 22.96%, respectively ($P<0.05$), while EE apparent digestibility in the 250 and 500 mg/kg groups was significantly decreased ($P<0.05$). During days 63-65, CP apparent digestibility in all TTE-supplemented groups showed an increasing trend ($P>0.05$). Ash apparent digestibility in the 250 mg/kg group was significantly increased by 52.46% ($P<0.05$). EE apparent digestibility showed no significant differences among groups ($P>0.05$) but tended to decrease with increasing TTE levels.

2.3 Effects of TTE on Serum Antioxidant Indexes of Weaned Piglets

As shown in Table 4 , TTE tended to increase serum GSH-Px and CAT activities and decrease serum MDA content in weaned piglets (P>0.05). At 51 days of age, serum GSH-Px activity was increased in all TTE-supplemented groups (P>0.05), with the highest activity observed in the 250 mg/kg group. Serum CAT activity in the 250 and 500 mg/kg groups was significantly increased by 99.42% and 114.53%, respectively (P<0.05). Serum MDA content decreased with increasing TTE levels, with the 500 mg/kg group showing a highly significant decrease of 46.98% (P<0.01). At 65 days of age, the 250 mg/kg group showed the highest serum GSH-Px activity, which was 10.62% higher than the control group (P>0.05). Serum CAT activity in the TTE-supplemented groups was increased by 57.56%, 60.00%, and 74.63% compared with the control group (P>0.05). Serum MDA content in the 250 and 500 mg/kg groups was highly significantly decreased by 53.57% and 57.74%, respectively (P<0.01).

2.4 Effects of TTE on Serum 5-HT Content of Weaned Piglets

As shown in Table 5 , at 51 days of age, serum 5-HT content in the TTE-supplemented groups was increased by 5.42%, 4.40%, and 4.40% compared with the control group (P>0.05). At 65 days of age, the 250 mg/kg group showed a highly significant increase in serum 5-HT content of 33.81% (P<0.01), while other groups showed increasing trends without significant differences (P>0.05).

Discussion

3.1 TTE Improved Growth Performance of Weaned Piglets

Weaning stress in piglets can lead to decreased feed intake and impaired growth performance. The present results demonstrate that TTE can improve the growth performance of weaned piglets. After a 28-day feeding trial, supplementation with 500 mg/kg TTE significantly increased ADG in weaned piglets. This finding is consistent with Yeganeh et al., who reported that 1 g/kg TTE supplementation in convict cichlid feed significantly increased weight gain and reduced F/G in juvenile animals. Gultepe et al. found that 400 mg/kg TTE significantly improved ADG and reduced F/G in tilapia. Insulin and insulin-like growth factor-I (IGF-I) are important hormones for growth and development in weaned piglets. TTE has been shown to increase insulin levels, regulate insulin sensitivity, and significantly elevate blood IGF-I content and IGF-I receptor expression in muscle. Therefore, the improved growth performance observed with TTE supplementation may be attributed to enhanced nutrient absorption from the diet and promotion of tissue synthesis and utilization.

Intestinal morphology and microbial flora balance also influence weaned piglet growth performance. Sahin et al. reported that TT significantly affected the weight of various digestive tract segments in broilers, decreasing small intestine proportion while increasing large intestine proportion. The active components of

TTE also exhibit antibacterial activity against Escherichia coli and Staphylococcus aureus, while promoting the growth of probiotic bacteria such as Bifidobacterium longum and Bifidobacterium adolescentis. Thus, the growth-promoting effects of TTE may also stem from its influence on intestinal development and microecological health.

3.2 TTE Enhanced Digestive Function in Weaned Piglets

Intensive production systems demand high nutritional efficiency, which depends on nutrient digestion, absorption, and utilization. Under ad libitum feeding conditions, improving nutrient absorption efficiency is a key approach to enhance animal growth performance and reduce costs. The present study found that TTE improved CP apparent digestibility and showed a tendency to increase DM and ash apparent digestibility in weaned piglets. Previous studies have demonstrated that various plant extracts can improve CP digestibility in weaned piglets. However, limited research exists on TTE effects on nutrient apparent digestibility in piglets, and the underlying mechanisms require further investigation.

Fat is a major energy source for animals, and dietary fat supplementation primarily addresses energy deficiency and provides functional fatty acids. TTE has demonstrated significant lipid- and cholesterol-lowering effects under pathological conditions. In this experiment, EE apparent digestibility in weaned piglets decreased with increasing TTE supplementation levels. Dietary EE includes triglycerides and lipids (such as phospholipids and cholesterol). The effect of TTE on EE apparent digestibility may involve two mechanisms: 1) Triglyceride digestion primarily depends on pancreatic lipase hydrolysis, and Ercan et al. found that TTE significantly inhibited human pancreatic lipase activity in vitro, thereby reducing triglyceride absorption; 2) Cholesterol undergoes both absorption and excretion during digestion, with excess cholesterol excreted into digesta as bile acids and salts. Studies have shown that diosgenin, an active component of TTE, can alleviate estrogen-induced cholestasis in mice and promote bile acid formation and bile secretion, thereby enhancing cholesterol excretion. It is therefore hypothesized that TTE reduces EE apparent digestibility by inhibiting intestinal fat hydrolysis into fatty acids and glycerol while promoting hepatic bile acid excretion.

3.3 TTE Enhanced Antioxidant Capacity in Weaned Piglets

The active components of TTE can protect vital organs by enhancing antioxidant capacity. GSH-Px and CAT are important antioxidant enzymes that scavenge peroxides in the body. The present study demonstrated that dietary supplementation with 250 and 500 mg/kg TTE significantly increased serum CAT activity in 51-day-old weaned piglets. Serum GSH-Px and CAT activities increased with TTE supplementation levels at both 51 and 65 days of age, consistent with results from pathological model studies.

MDA is a product of lipid peroxidation initiated by free radical attack on polyunsaturated fatty acids in cell membranes. Its content directly reflects the degree of lipid peroxidation, indirectly indicates cell damage severity, and is a major factor affecting mitochondrial respiratory chain complexes and key mitochondrial enzyme activities. The present results showed that TTE decreased serum MDA content, which aligns with findings reported by Sharma et al. and Liu Sheqin. In this experiment, serum MDA content in the 250 and 500 mg/kg groups was highly significantly reduced at 65 days of age compared with the control group, suggesting that TTE effectively reduced membrane lipid peroxidation and protected cell membranes. This effect may be attributed to TTE' s ability to significantly reduce reactive oxygen species (ROS) production. Ghareeb et al. and Vangalapati et al. demonstrated that TTE possesses free radical scavenging activity. The reduced MDA content observed in this study suggests that TTE may decrease free radical peroxide production, reduce consumption of antioxidant enzymes (GSH-Px, CAT), minimize cell damage, and maintain cell membrane integrity and normal physiological functions.

3.4 Effects of TTE on Serum 5-HT Content in Weaned Piglets

Serotonin (5-HT) is an important neurotransmitter and regulatory substance involved in various physiological processes and is a key factor regulating feed intake and intestinal function. Koopmans et al. reported that increased hypothalamic 5-HT content reduced aggressive and feeding behaviors while increasing resting behavior in piglets, thereby decreasing activity and stress and facilitating nutrient deposition and utilization. Approximately 95% of blood 5-HT originates from the intestine, primarily from enterochromaffin cells in the intestinal mucosa that contain the enzymes required for tryptophan conversion to 5-HT. Tryptophan is a crucial precursor for 5-HT and also functions as a feed intake stimulant in pigs, showing strong positive correlation with feed intake and daily gain. The present study demonstrated that dietary TTE supplementation generally increased serum 5-HT content in weaned piglets. At 65 days of age, the 250 mg/kg group showed a highly significant increase of 33.81% in serum 5-HT content compared with the control group, accompanied by a significant 9.22% increase in ADG and 4.21% increase in ADFI during days 51–65. This suggests that TTE may increase tryptophan intake through elevated feed consumption, thereby enhancing 5-HT synthesis. Increased 5-HT secretion may subsequently reduce piglet activity and nutrient expenditure, promoting growth. Zhang Wei et al. reported that TTE could also inhibit expression of indoleamine 2,3-dioxygenase (IDO), a tryptophan-metabolizing enzyme in the brain that is activated by inflammatory factors to convert tryptophan to toxic kynurenine, thereby reducing 5-HT synthesis. The present results suggest that TTE may increase 5-HT content by inhibiting IDO expression, though the specific regulatory mechanisms require further investigation.

Conclusions

1. Dietary supplementation with 500 mg/kg TTE effectively improved growth performance in weaned piglets, while 250 mg/kg TTE increased apparent digestibility of CP and ash.
2. Dietary supplementation with 250–500 mg/kg TTE enhanced serum GSH-Px and CAT activities, reduced serum MDA content, and improved antioxidant capacity in weaned piglets.

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