Effects of High-Dose Ethoxyquin on Growth Performance, Serum Biochemical Indices, Antioxidant Performance, Organ Indices, and Meat Quality in Growing-Finishing Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of ethoxyquin on growth performance, serum biochemical indices, antioxidant capacity, organ indices, and meat quality in growing-finishing pigs. A total of 180 crossbred (Duroc × Landrace × Yorkshire) growing pigs with a body weight of (31.98±2.34) kg were randomly allocated to 5 groups with 6 replicates per group and 6 pigs per replicate. The experiment utilized a corn-soybean meal basal diet supplemented with 0, 150, 300, 750, and 1,500 mg/kg ethoxyquin for a period of 98 days. The results showed: 1) Dietary supplementation with 150–1,500 mg/kg ethoxyquin had no significant effects on growth performance, organ indices, or meat quality in growing-finishing pigs ($P>0.05$). 2) On day 70 of the experiment, serum alkaline phosphatase activity and total bilirubin (TBIL) content decreased linearly with increasing dietary ethoxyquin supplementation (linear $P<0.05$). On day 98, serum TBIL content increased linearly with increasing dietary ethoxyquin supplementation (linear $P<0.05$). 3) On days 70 and 98, serum total antioxidant capacity and activities of glutathione peroxidase and catalase decreased linearly with increasing dietary ethoxyquin supplementation (linear $P<0.05$). On day 98, serum malondialdehyde content increased quadratically with increasing dietary ethoxyquin supplementation (quadratic $P<0.05$). In conclusion, dietary supplementation with 150–1,500 mg/kg ethoxyquin had no significant effect on growth performance or meat quality in growing-finishing pigs; dietary supplementation with 750 and 1,500 mg/kg ethoxyquin induced hepatocellular injury; and dietary supplementation with 300–1,500 mg/kg ethoxyquin reduced serum antioxidant capacity. Therefore, the recommended supplementation level of ethoxyquin in diets for growing-finishing pigs is 150 mg/kg.

Full Text

Effects of High Dose of Ethoxyquin on Growth Performance, Serum Biochemical Indices, Antioxidant Capacity, Organ Index and Meat Quality of Growing-Finishing Pigs

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Abstract: This experiment was conducted to investigate the effects of dietary supplementation with different levels of ethoxyquin on growth performance, serum biochemical indices, antioxidant capacity, organ index and meat quality of growing-finishing pigs. A total of 180 crossbred (Duroc×Landrace×Yorkshire) growing pigs with an average body weight of (31.98±2.34) kg were randomly allocated to 5 groups with 6 replicates per group and 6 pigs per replicate. The pigs were fed corn-soybean meal basal diets supplemented with 0, 150, 300, 750 and 1 500 mg/kg ethoxyquin for a 98-day trial period. The results showed that: 1) dietary supplementation with 150–1 500 mg/kg ethoxyquin had no significant effects on growth performance, organ index or meat quality of growing-finishing pigs ($P>0.05$). 2) On day 70 of the trial, serum alkaline phosphatase activity and total bilirubin (TBIL) content decreased linearly with increasing dietary ethoxyquin supplementation ($P<0.05$). On day 98, serum TBIL content increased linearly with increasing dietary ethoxyquin supplementation ($P<0.05$). 3) On both day 70 and day 98, serum total antioxidant capacity and activities of glutathione peroxidase and catalase decreased linearly with increasing dietary ethoxyquin supplementation ($P<0.05$). On day 98, serum malondialdehyde content increased quadratically with increasing dietary ethoxyquin supplementation ($P<0.05$). In conclusion, dietary supplementation with 150–1 500 mg/kg ethoxyquin had no significant effects on growth performance or meat quality of growing-finishing pigs. However, supplementation with 750 and 1 500 mg/kg ethoxyquin caused liver cell damage, while supplementation with 300–1 500 mg/kg ethoxyquin reduced serum antioxidant capacity. Therefore, the recommended dosage of ethoxyquin in diets for growing-finishing pigs is 150 mg/kg.

Key words: ethoxyquin; growing-finishing pigs; growth performance; serum biochemical indices; antioxidant capacity; organ index; meat quality

Ethoxyquin is a widely used antioxidant in feed production that scavenges free radicals generated during feed storage and is extensively employed to prevent oxidation of vitamins and lipids in feed. Early studies demonstrated that supplementation with 125 mg/kg ethoxyquin in pig and broiler diets could enhance antioxidant capacity. However, other research indicated that dietary ethoxyquin supplementation in dogs and rats could cause health-related issues, with notable side effects on the liver and gastrointestinal tract. Although responses to ethoxyquin vary among animal species, most animals exhibit symptoms such as reduced daily weight gain, liver and kidney damage, and digestive tract lesions when ethoxyquin exceeds permitted levels. While ethoxyquin has been approved as a feed additive in many countries and regions, with recommended supplementation rates of 150 mg/kg in complete feed in both the United States and European Union, China has also adopted similar standards, establishing a maximum limit of 150 mg/kg in compound feed for livestock (excluding dogs). In practice, the appropriate dosage of ethoxyquin in diets is influenced by animal species, age, and dietary composition. Currently, no studies have reported the effects of high-dose ethoxyquin supplementation on tolerance and meat quality in growing-finishing pigs. To further validate the applicability of the 150 mg/kg limit for growing-finishing pigs and evaluate the effects of high-dose supplementation, this study was conducted according to the "Guidelines for Evaluation of Target Animal Tolerance of Feed and Feed Additives for Livestock and Poultry" issued by the Ministry of Agriculture. Using growing-finishing pigs as the target animal, we investigated the effects of effective and high-multiple doses of ethoxyquin on growth performance, serum biochemical indices, antioxidant indices, organ index and meat quality to provide a scientific basis for the safe and effective use of ethoxyquin in growing-finishing pig production.

1.1 Experimental Material

Ethoxyquin was purchased from Jiangsu Zhongdan Group Co., Ltd., with an ethoxyquin content of 95%.

1.2 Experimental Animals and Design

One hundred eighty crossbred (Duroc×Landrace×Yorkshire) growing pigs with a body weight of (31.98±2.34) kg were selected and randomly divided into 5 groups according to body weight and sex in a randomized complete block design. Each group comprised 6 replicates with 6 pigs per replicate (half barrows and half gilts). The pigs were fed corn-soybean meal basal diets supplemented with 0, 150 (recommended dose), 300 (2× dose), 750 (5× dose) and 1 500 mg/kg (10× dose) ethoxyquin for a 98-day experimental period.

1.3 Experimental Diets

The basal diets were formulated according to NRC (2012) nutrient requirements for swine. The composition and nutrient levels are presented in Table 1 . During diet preparation, ethoxyquin was sequentially diluted and amplified with soybean oil, mixed thoroughly, and then blended with other ingredients.

Table 1 Composition and nutrient levels of the basal diets (as-fed basis)

Note: The premix provided the following per kg of diets: VA 6 000 IU, VD3 1 500 IU, VE 15 IU, VK3 1.5 mg, VB1 0.9 mg, VB2 3 mg, VB6 1.5 mg, VB12 10 g, nicotinic acid 17 mg, pantothenic acid 9 mg, folic acid 0.32 mg, biotin

0.02 mg, choline chloride 350 mg, Fe (FeSO4) 90 mg, Cu (CuSO4) 8 mg, Mn (MnSO4) 20 mg, Zn (ZnSO4) 50 mg, I (KI) 0.25 mg, Se (Na2SeO3) 0.30 mg. ME was a calculated value, while the others were measured values.

1.4 Feeding Management

The trial was conducted from January to April 2017 at the Fengning Animal Experimental Station of China Agricultural University (Fengning, Hebei). Before the experiment, pig houses were thoroughly disinfected, feed troughs and waterers were cleaned, and experimental pigs received routine immunization and deworming. House temperature was maintained at approximately 21 °C. Pigs had ad libitum access to feed and water. Regular disinfection procedures were followed according to standard farm management protocols.

1.5.1 Nutrient Analysis

Dietary dry matter, crude protein, ether extract, crude fiber, crude ash, calcium and total phosphorus contents were determined according to GB/T 6435–2014, GB/T 6432–2014, GB/T 6433–2006, GB/T 6434–2006, GB/T 6438–2007, GB/T 6436–2002 and GB/T 6437–2002, respectively. Dietary lysine content was determined according to GB/T 18246–2000.

1.5.2 Growth Performance Measurement

Initial body weight of each pig was recorded at the beginning of the trial. On days 70 and 98, pigs were weighed after overnight fasting and feed consumption was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F/G).

1.5.3 Serum Biochemical and Antioxidant Indices

On days 70 and 98, one pig with body weight closest to the replicate average was selected from each replicate for blood collection via anterior vena cava puncture. Ten milliliters of non-anticoagulated blood was collected, placed at room temperature for 30 minutes, and centrifuged at 3 000 r/min for 15 minutes to separate serum, which was stored at -20 °C for subsequent analysis. Serum clinical biochemical indices including glucose (GLU), urea nitrogen (UN), creatinine (CREA), total protein (TP), albumin (ALB), total bilirubin (TBIL), and activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using colorimetric methods according to kit instructions (Zhongsheng Beikong Co., Ltd., China) with a Mindray BS-420 automatic biochemical analyzer. Serum antioxidant indices including total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity and malondialdehyde (MDA) content were determined using colorimetric methods according to kit instructions (Beijing Huaying Biological Research Institute, China) with an A6 semi-automatic biochemical analyzer.

1.5.4 Organ Index Measurement

On day 98, one pig with body weight closest to the replicate average was selected from each replicate, fasted for 24 hours, weighed to obtain live weight before slaughter, and then slaughtered. The heart, liver, spleen, lung and kidney were removed and weighed after surface fluid was absorbed with filter paper. Organ index was calculated using the following formula: Organ index (g/kg) = organ weight (g) / live weight before slaughter (kg).

1.5.5 Meat Quality Measurement

Meat quality sampling and measurement were performed according to NY/T 1333–2007 "Determination of Meat Quality in Livestock and Poultry." Measured parameters included drip loss, cooking loss, shear force, pH at 45 min and 24 h postmortem, and meat color. pH was measured using a portable pH meter (OPTP-STAR, Matthaus, Germany). Meat color parameters (L, *a*, b*) of the longissimus dorsi muscle were measured at 45 min postmortem using a Minolta colorimeter (CR-410, Minolta, Japan). Shear force was determined using a muscle tenderness meter (CLM3B, Beijing Tianxiang Feiyu Instrument Equipment Co., Ltd., China).

1.6 Data Processing and Statistical Analysis

Experimental data were analyzed using one-way ANOVA with SAS 9.2 statistical software. Duncan' s multiple range test was used for multiple comparisons. Linear and quadratic polynomial models were used to evaluate the effects of ethoxyquin supplementation. Differences were considered significant at $P<0.05$.

2.1 Effects of Dietary Ethoxyquin on Growth Performance of Growing-Finishing Pigs

The effects of dietary ethoxyquin on growth performance are presented in Table 2 . No significant differences were observed among treatment groups in ADG, ADFI or F/G ($P>0.05$).

Table 2 Effects of dietary ethoxyquin on growth performance of growing-finishing pigs

Note: In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while values with the same small letter or no superscript indicate no significant difference (P>0.05). The same applies to the following tables.

2.2 Effects of Dietary Ethoxyquin on Serum Biochemical Indices of Growing-Finishing Pigs

The effects of dietary ethoxyquin on serum biochemical indices are shown in Table 3 . On day 70, serum ALP activity and TBIL content decreased linearly with increasing dietary ethoxyquin supplementation (P<0.05). On day 98, serum TBIL content increased linearly with increasing dietary ethoxyquin supplementation (P<0.05), while serum ALP activity decreased quadratically (P<0.05).

Table 3 Effects of dietary ethoxyquin on serum biochemical indices of growing-finishing pigs

2.4 Effects of Dietary Ethoxyquin on Serum Antioxidant Indices of Growing-Finishing Pigs

The effects of dietary ethoxyquin on serum antioxidant indices are presented in Table 4 . On day 70, serum T-AOC and activities of SOD, GSH-Px and CAT decreased linearly with increasing dietary ethoxyquin supplementation (P<0.05). Compared with the control group, dietary supplementation with 300–1 500 mg/kg ethoxyquin significantly reduced serum T-AOC and activities of SOD, GSH-Px and CAT (P<0.05). On day 98, serum T-AOC and activities of GSH-Px and CAT decreased linearly with increasing dietary ethoxyquin supplementation (P<0.05), while serum MDA content increased quadratically (P<0.05). Compared with the control group, dietary supplementation with 750 mg/kg ethoxyquin significantly reduced serum T-AOC and activities of GSH-Px and CAT (P<0.05), and supplementation with 750 and 1 500 mg/kg ethoxyquin significantly increased serum MDA content (P<0.05).

Table 4 Effects of dietary ethoxyquin on serum antioxidant indices of growing-finishing pigs

2.5 Effects of Dietary Ethoxyquin on Organ Indices of Growing-Finishing Pigs

The effects of dietary ethoxyquin on organ indices are shown in Table 5 . Compared with the control group, dietary supplementation with 150–1 500 mg/kg ethoxyquin had no significant effects on organ indices of growing-finishing pigs (P>0.05).

Table 5 Effects of dietary ethoxyquin on organ indices of growing-finishing pigs

2.6 Effects of Dietary Ethoxyquin on Meat Quality of Growing-Finishing Pigs

The effects of dietary ethoxyquin on meat quality are presented in Table 6 . No significant differences were observed among treatment groups in drip loss, cooking loss, shear force, pH or meat color (P>0.05).

Table 6 Effects of dietary ethoxyquin on meat quality of growing-finishing pigs

3.1 Effects of Dietary Ethoxyquin on Growth Performance of Growing-Finishing Pigs

Few studies have reported the effects of dietary ethoxyquin on pig growth performance. Previous research indicated that supplementation with 150 and 1 500 mg/kg ethoxyquin significantly reduced growth performance (ADG and ADFI) in weaned piglets. Additionally, Lu et al. found that dietary supplementation with 135 mg/kg ethoxyquin and propyl gallate mixture had no significant effects on weaned piglet performance but significantly reduced ADFI and feed conversion ratio in growing-finishing pigs. The present study demonstrated that dietary supplementation with 150–1 500 mg/kg ethoxyquin had no significant effects on growth performance of growing-finishing pigs.

3.2 Effects of Dietary Ethoxyquin on Serum Biochemical Indices of Growing-Finishing Pigs

Abnormal serum ALP activity may indicate problems related to liver, gallbladder or bone, and increased liver stress may elevate blood ALP activity. Bilirubin is produced through normal heme catabolism in vertebrates, and elevated TBIL content may indicate certain diseases. Current literature reports inconsistent results regarding ethoxyquin' s effects on bilirubin. Some studies reported no significant effects of 150–1 500 mg/kg ethoxyquin on serum TBIL in weaned piglets, while others found increased serum TBIL in rats fed diets containing 200, 400, 500 and 1 000 mg/kg ethoxyquin. According to the Merck Veterinary Manual, normal serum ALP activity in pigs ranges from 118–395 U/L, and normal TBIL content ranges from 0–17.1 mol/L. In this trial, although serum TBIL on day 70 and serum ALP on day 98 were affected by dietary ethoxyquin, their levels remained within normal reference ranges. However, when diets contained 750 and 1 500 mg/kg ethoxyquin, serum ALP activity on day 70 (below normal) and serum TBIL content on day 98 (above normal) fell outside normal reference ranges. Previous studies have reported that the degree of TBIL elevation is proportional to hepatocellular damage. Therefore, dietary supplementation with 750–1 500 mg/kg ethoxyquin may cause hepatocellular damage in growing-finishing pigs.

3.3 Effects of Dietary Ethoxyquin on Serum Antioxidant Indices of Growing-Finishing Pigs

Antioxidant status plays a crucial role in maintaining animal health, growth and reproductive performance. The antioxidant status must maintain a certain balance, as either excessive or insufficient levels can affect animal health. T-AOC reflects the combined action of various oxidative and antioxidant substances and serves as a comprehensive indicator of overall antioxidant capacity. SOD is a ubiquitous endogenous antioxidant enzyme that scavenges free radicals. MDA is a product of lipid peroxidation metabolism and an important indicator of lipid peroxidation. Ethoxyquin has been reported to exert antioxidant effects in rainbow trout and poultry. Lu et al. found that supplementation with 135 mg/kg ethoxyquin and propyl gallate mixture improved plasma and liver antioxidant capacity in growing-finishing pigs. The present study found that serum antioxidant capacity decreased with increasing dietary ethoxyquin supplementation, with significant differences observed when 300–1 500 mg/kg ethoxyquin was added compared with the control group, indicating diminished capacity to resist oxidative damage and potential adverse effects on growing-finishing pigs. Other studies have shown that like other antioxidants, excessive dietary ethoxyquin may exert pro-oxidant effects. Błaszczyk et al. demonstrated that ethoxyquin in solution may partially exist as free radicals, and thus ethoxyquin nitroxide radicals possess oxidant properties like other nitroxide radicals. Therefore, high-dose ethoxyquin may lead to increased formation of free oxygen, resulting in decreased antioxidant enzyme activities and increased MDA content, though the specific mechanism requires further investigation.

3.4 Effects of Dietary Ethoxyquin on Organ Indices of Growing-Finishing Pigs

Organ indices are biological characteristic indicators that reflect organism functional status and are important for both theoretical and practical applications. Parke et al. reported increased liver index in rats fed ethoxyquin-supplemented diets, and Bailey et al. found that 1 000 mg/kg ethoxyquin increased liver index in chickens. However, the present study showed that dietary supplementation with 150–1 500 mg/ethoxyquin had no significant effects on organ indices of growing-finishing pigs, possibly due to differences in animal species. Additionally, our laboratory simultaneously evaluated the effects of ethoxyquin on organ indices in weaned piglets, finding that 750 and 1 500 mg/kg ethoxyquin significantly increased liver index by 11.16% and 27.09% compared with the control group (unpublished data). Thus, organ index results are also affected by pig age.

3.5 Effects of Dietary Ethoxyquin on Meat Quality of Growing-Finishing Pigs

Meat quality is commonly evaluated using parameters including meat color, pH, drip loss, cooking loss and shear force, which collectively reflect sensory properties and palatability and determine consumer preference. Meat color is primarily determined by myoglobin and hemoglobin contents, which are influenced by various factors, causing color variation from gray-white to dark red. Tavárez et al. reported that supplementation with 135 mg/kg antioxidant mixture (ethoxyquin and propyl gallate) had no significant effects on breast meat color in Ross 308 broilers, though no studies have reported ethoxyquin' s effects on meat quality of growing-finishing pigs. In this trial, dietary supplementation with 150–1 500 mg/kg ethoxyquin had no significant effects on meat quality of growing-finishing pigs.

3.6 Recommended Dosage

Given that dietary supplementation with 300–1 500 mg/kg ethoxyquin reduced serum antioxidant capacity and that 750 and 1 500 mg/kg ethoxyquin caused hepatocellular damage in growing-finishing pigs, the recommended dosage of ethoxyquin in diets for growing-finishing pigs is 150 mg/kg.

4 Conclusions

1. Dietary supplementation with 150–1 500 mg/kg ethoxyquin had no significant effects on growth performance or meat quality of growing-finishing pigs.
2. Dietary supplementation with 750 and 1 500 mg/kg ethoxyquin caused hepatocellular damage in growing-finishing pigs.
3. Dietary supplementation with 300–1 500 mg/kg ethoxyquin reduced serum antioxidant capacity in growing-finishing pigs.
4. The recommended dosage of ethoxyquin in diets for growing-finishing pigs is 150 mg/kg.

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