Postprint: Biosafety Evaluation of Selenium Yeast in Broiler Chickens

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Abstract

This study was conducted to investigate the effects of dietary supplementation with different levels of yeast selenium on growth performance, blood hemoglobin content and hematocrit, plasma biochemical indices, organ indices, and histological changes in Arbor Acres (AA) broiler chickens, and thereby evaluate the biosafety of yeast selenium in broilers. A single-factor completely randomized design was employed, using 288 1-day-old AA broiler chickens that were randomly allocated into 4 groups with 6 replicates per group and 12 chickens per replicate (half male and half female). Each group was fed a corn-soybean meal basal diet supplemented with 0, 0.4, 2.4, and 4.9 mg/kg (as selenium) of yeast selenium, respectively. The experimental period was 42 days. The results showed that: 1) Compared with the control group, dietary supplementation with 0.4 and 2.4 mg/kg yeast selenium significantly increased the average daily feed intake of broilers during 22-42 days of age ($P<0.05$); dietary supplementation with 0.4, 2.4, and 4.9 mg/kg yeast selenium significantly increased the average daily gain of broilers during 1-42 days of age ($P<0.05$), while no significant differences were observed among the yeast selenium-supplemented groups ($P>0.05$). 2) Except for significant effects on plasma urea nitrogen content and glutathione peroxidase (GSH-Px) activity at 21 days of age and plasma GSH-Px activity at 42 days of age in broilers ($P<0.05$), dietary supplementation with different levels of yeast selenium had no significant effects on other plasma biochemical indices ($P>0.05$). Compared with the control group, plasma GSH-Px activity in broilers at both 21 and 42 days of age increased significantly with increasing dietary yeast selenium levels ($P<0.05$), and dietary supplementation with 2.4 mg/kg yeast selenium significantly decreased plasma urea nitrogen content in broilers at 21 days of age ($P<0.05$). 3) Dietary supplementation with different levels of yeast selenium had no significant effects on blood hemoglobin content and hematocrit in broilers at 21 and 42 days of age, organ indices at 42 days of age ($P>0.05$), and did not cause histological changes in major organs. In

conclusion, when the supplementation level of yeast selenium in broiler diets is 0.4 mg/kg (with total dietary selenium content of 0.41 mg/kg), it possesses a 10-fold safety factor, indicating that dietary supplementation of selenium in the form of yeast selenium is safe for broiler chickens.

Full Text

Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different levels of selenium yeast on growth performance, blood hemoglobin content and hematocrit, plasma biochemical parameters, organ indices, and histopathological changes in Arbor Acres (AA) broilers, thereby evaluating the biological safety of selenium yeast for broiler chickens. The study employed a single-factor completely randomized design. A total of 288 one-day-old AA broilers were randomly divided into four groups with six replicates per group and twelve broilers per replicate (half male and half female). Each group was fed a corn-soybean meal basal diet supplemented with 0, 0.4, 2.4, or 4.9 mg/kg selenium (as selenium yeast). The experimental period lasted 42 days.

The results showed as follows: 1) Compared with the control group, dietary supplementation with 0.4 and 2.4 mg/kg selenium yeast significantly increased the average daily feed intake of broilers during 22–42 days of age ($P<0.05$). Dietary supplementation with 0.4, 2.4, and 4.9 mg/kg selenium yeast significantly increased the average daily gain of broilers during 1–42 days of age ($P<0.05$), while no significant differences were observed among the selenium yeast supplementation groups ($P>0.05$). 2) Except for significant effects on plasma urea nitrogen content and glutathione peroxidase (GSH-Px) activity at 21 days of age and plasma GSH-Px activity at 42 days of age ($P<0.05$), dietary supplementation with different levels of selenium yeast did not significantly affect other plasma biochemical parameters ($P>0.05$). Compared with the control group, plasma GSH-Px activity in broilers at both 21 and 42 days of age increased significantly with increasing dietary selenium yeast levels ($P<0.05$), and dietary supplementation with 2.4 mg/kg selenium yeast significantly decreased plasma urea nitrogen content in 21-day-old broilers ($P<0.05$). 3) Dietary supplementation with different levels of selenium yeast had no significant effects on blood hemoglobin content and hematocrit in broilers at 21 and 42 days of age or on organ indices in 42-day-old broilers ($P>0.05$), and did not cause histopathological changes in major organs. In conclusion, when the dietary supplementation level of selenium yeast is 0.4 mg/kg (with a total dietary selenium content of 0.41 mg/kg), it possesses a 10-fold safety margin, indicating that selenium supplementation in the form of selenium yeast is safe for broiler chickens.

Keywords: selenium yeast; biological safety evaluation; broilers

Introduction

In 1973, the World Health Organization (WHO) and the International Nutrition Organization confirmed selenium as an essential trace element for humans and animals [1]. Selenium primarily exerts its nutritional effects through antioxidant functions by binding to enzyme proteins. Additionally, selenium plays roles in enhancing immune function, promoting growth, improving reproductive performance, lowering blood lipids, resisting stress, delaying aging, and preventing cancer. For a long time, inorganic selenium (sodium selenite) has been commonly used as a selenium source in diets. However, due to its high toxicity and low bioavailability, sodium selenite may pose potential hazards to animals and the environment. Kim et al. [2] observed different toxic reactions in pigs fed diets containing 0–20 mg/kg selenium from selenium yeast versus sodium selenite. When selenium supplementation exceeded 5.0 mg/kg, pigs in the sodium selenite group exhibited more severe poisoning symptoms compared with the selenium yeast group, manifested by greatly reduced feed intake and daily gain, as well as higher incidence of hair loss, staggering gait, separation of the coronary band, and elevated plasma glutamic-oxaloacetic transaminase (GOT) activity. Guo Junrui [3] investigated the feeding safety of selenomethionine at 0–5.0 mg/kg in broiler diets and found that 5.0 mg/kg selenomethionine caused no obvious adverse effects on broilers aged 1–42 days. However, no reports have been published on the safety evaluation of selenium yeast for broilers. This experiment evaluated the effects of supplementing corn-soybean meal diets with different levels of selenium yeast on 1–21-day-old and 22–42-day-old broilers by measuring growth performance, blood physiological and plasma biochemical parameters, organ indices, and histological changes, providing a scientific basis for determining the safety margin of the maximum supplementation level of selenium yeast in broiler diets to ensure its safe application in broiler production.

1.1 Experimental Design and Treatments

This experiment employed a single-factor completely randomized design. Based on previous studies showing that the appropriate supplementation level of selenium from selenium yeast in corn-soybean meal diets for broilers was 0.15–0.50 mg/kg [4–15] and according to the relevant provisions in the "Guidelines for Evaluation of Target Animal Tolerance to Feed and Feed Additives (Trial)" issued by the Ministry of Agriculture, 0.5 mg/kg was regarded as the maximum limit of total selenium content in broiler diets when using selenium yeast as the selenium source. Four groups were established: a control group without selenium yeast supplementation, a maximum limit group, and two high-dose groups at 5 and 10 times the maximum limit, with selenium yeast supplementation levels of 0, 0.5, 2.5, and 5.0 mg/kg (as selenium), respectively. Considering that the basal selenium content in the diet was approximately 0.1 mg/kg, the actual selenium yeast supplementation levels were set at 0, 0.4, 2.4, and 4.9 mg/kg (as selenium).

1.2 Experimental Animals and Diets

A total of 320 healthy commercial one-day-old Arbor Acres (AA) broilers were selected. From these, 288 broilers were chosen based on body weight and sex and randomly allocated into four groups with six replicates (cages) per group and twelve broilers per replicate (half male and half female). The experimental period lasted 42 days and was divided into two phases: 1–21 days and 22–42 days of age. Management and routine immunization were conducted according to the "AA Broiler Management Manual." Broilers had free access to feed and water. Health status was observed and recorded daily. Any dead broilers were immediately necropsied to observe and analyze pathological causes, and feed was weighed accordingly. At days 21 and 42 of the experimental period, broilers were weighed after fasting (feed withdrawal but not water) overnight at the replicate (cage) level, and remaining feed was weighed to calculate average daily feed intake, average daily gain, feed-to-gain ratio, and mortality rate.

Basal corn-soybean meal diets were formulated for broilers aged 1–21 days and 22–42 days according to the nutrient recommendations for broilers in the NRC (1994) [16]. The composition and nutrient levels of the basal diets are shown in Table 1 . Four experimental diets were prepared for each phase by adjusting the amount of corn starch in the basal diets according to the group designations. Selenium yeast was provided by Angel Yeast Co., Ltd., with a selenium content of 2,265 mg/kg. The experimental diets were fed as mash. The analyzed selenium contents in broiler diets are presented in Table 2 .

Table 1. Composition and nutrient levels of basal diets (as-fed basis), %

Items	Ingredients	1–21 days of age	22–42 days of age
	Corn	Content	
	Soybean meal		
	Soybean oil		
	CaHPO		
	CaCO		
	NaCl		
	DL-Met		
	Micronutrients[1]		
	Corn starch + Se		
	Total		
	Nutrient levels[2]		
	ME/(MJ/kg)		
	CP		
	Lys		
	Met		
	Met+Cys		
	Ca		

Items	Ingredients	1–21 days of age	22–42 days of age
	NPP		
	Se/(mg/kg)		

[1] Micronutrients provided the following per kilogram of diet: 1–21 days of age: VA 15,000 IU, VD 4,500 IU, VE 24 IU, VK 3 mg, VB 3 mg, VB 9.6 mg, VB 3 mg, VB 0.018 mg, pantothenic acid calcium 15 mg, nicotinic acid 39 mg, folic acid 1.5 mg, biotin 0.15 mg, choline 700 mg, Cu (as copper sulfate) 8 mg, Mn (as manganese sulfate) 110 mg, Fe (as ferrous sulfate) 60 mg, Zn (as zinc sulfate) 60 mg, I (as potassium iodide) 0.35 mg. 22–42 days of age: VA 10,000 IU, VD 3,400 IU, VE 16 IU, VK 2.0 mg, VB 2.0 mg, VB 6.4 mg, VB 2.0 mg, VB 0.012 mg, calcium pantothenate 10 mg, nicotinic acid 26 mg, folic acid 1.0 mg, biotin 0.1 mg, choline 500 mg, Cu (as copper sulfate) 8 mg, Zn (as zinc sulfate) 40 mg, Mn (as manganese sulfate) 80 mg, Fe (as ferrous sulfate) 60 mg, I (as potassium iodide) 0.35 mg.

[2] CP, Ca, and Se were measured values, while the others were calculated values.

Table 2. Analyzed values of selenium contents in broiler diets, mg/kg

Selenium supplemental level/(mg/kg)	Analyzed value

Analyzed values were mean values based on duplicate determinations.

1.3 Sample Collection and Preparation

During diet preparation, samples were collected on-site, ground to pass through a 200-mesh sieve, and stored in sealed sample bags at low temperature and dry conditions for subsequent analysis of crude protein, calcium, and selenium contents. At 21 and 42 days of age, all broilers were fasted overnight (water allowed) and weighed individually on the following day at 08:00. Two broilers (one male and one female) were selected from each replicate cage based on the average body weight of the cage, and 10–15 mL of blood was collected from the wing vein. One portion of anticoagulated blood was used to determine routine physiological blood indices, while another portion was centrifuged at 3,000 r/min for 20 min to separate plasma, which was aliquoted and stored at -20 °C for plasma biochemical analysis. Whole blood or plasma from the two broilers in each replicate (cage) was pooled as one analytical sample.

After blood collection at 42 days of age, two additional broilers (one male and one female) were selected from each replicate (cage) based on the average body weight of the cage, slaughtered, and the thymus, bursa of Fabricius, spleen, pancreas, heart, lungs, liver, kidneys, glandular stomach, and gizzard were excised. The duodenum, jejunum, and ileum were also removed and their contents

expelled. All organs were weighed to calculate organ indices, and the lengths of the duodenum, jejunum, and ileum were measured to calculate the length indices of each small intestinal segment. Morphological changes in each organ were observed and recorded.

Organ index (%) = (organ weight/live body weight) × 100
Small intestinal segment length index (%) = (length of each segment/total small intestine length) × 100

After weighing the organs, portions of liver, kidney, heart, lung, and spleen were collected from one male broiler in three replicate cages and one female broiler in the other three replicate cages per group, fixed in brown bottles containing 4% formalin solution, and prepared for sectioning to observe histological changes.

1.4 Analytical Methods

1.4.1 Feed Ingredient and Diet Sample Analysis After wet digestion with concentrated nitric acid and perchloric acid, calcium content in feed ingredients and diets was determined using an IRIS Intrepid II plasma emission spectrometer (TE, USA) [17]. Crude protein content in feed ingredients and diets was measured according to the method described in AOAC (1990) [18]. Selenium content in selenium yeast products, feed ingredients, and diets was determined by fluorometric method [19].

1.4.2 Whole Blood, Plasma, and Tissue Index Analysis Routine physiological blood indices (hemoglobin content, hematocrit) were determined using a KX-21 automatic blood cell analyzer (SYSMEX, Japan). Plasma biochemical parameters [lactate dehydrogenase (LDH), GOT, glutamic-pyruvic transaminase (GPT), creatine phosphokinase (CK), alkaline phosphatase (APK) activities, and urea nitrogen (UN), total protein (TP), albumin (ALB), glucose (GLU), total bilirubin (TBILL), creatinine (CREA), total cholesterol (TC), and triglyceride (TG) contents] were measured using a TBA-40FR automatic biochemical analyzer (Toshiba, Japan). Plasma glutathione peroxidase (GSH-Px) activity was determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute).

1.4.3 Histological Examination Histological examination was performed according to the methods reported by Deng et al. [20] and Ashraf et al. [21]. The main procedures were as follows: fixed specimens were washed, cleared, paraffin-embedded, and sectioned at 5 m thickness. After hematoxylin-eosin (HE) staining, histomorphological changes in the liver, kidney, heart, lung, and spleen were observed under a microscope, and corresponding tissue images were captured using an image acquisition system.

1.5 Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS 9.0 software [22]. When significant differences were detected, means were compared using the Least Significant Difference (LSD) method. The significance level for all data in this study was set at $P<0.05$. Data are expressed as means ± standard deviation.

Results

2.1 Effects of Selenium Yeast on Growth Performance and Mortality of Broilers

As shown in Table 3 , dietary supplementation with different levels of selenium yeast had no significant effects on growth performance indices and mortality of broilers during 1–21 days, 22–42 days, and 1–42 days of age ($P>0.05$), except for average daily feed intake during 22–42 days and average daily gain during 1–42 days ($P<0.05$). There was a trend toward increased average daily feed intake during 1–42 days ($P=0.07$). Compared with the control group, dietary supplementation with 0.4 and 2.4 mg/kg selenium yeast significantly increased average daily feed intake during 22–42 days ($P<0.05$), and dietary supplementation with 0.4, 2.4, and 4.9 mg/kg selenium yeast significantly increased average daily gain during 1–42 days ($P<0.05$), with no significant differences among the selenium supplementation groups ($P>0.05$).

These results indicate that dietary supplementation with different levels of selenium yeast can significantly improve average daily feed intake during 22–42 days and average daily gain during 1–42 days in broilers, without significantly affecting other growth performance indices or mortality.

Table 3. Effects of selenium yeast on growth performance and mortality of broilers

Selenium supplemental level/(mg/kg)	ADG/g	ADFI/g	Mortality/%	ADG/g	ADFI/g	Mortality/%	ADG/g	ADFI/g	Mortality/%

In the same column, values with no letter superscripts mean no significant difference ($P>0.05$), while values with different small letter superscripts mean significant difference ($P<0.05$). The same as below.

2.2 Effects of Selenium Yeast on Hemoglobin Content, Hematocrit, and Plasma Biochemical Parameters in Broilers

As shown in Table 4 , dietary supplementation with different levels of selenium yeast had no significant effects on blood hemoglobin content and hematocrit in

broilers at 21 and 42 days of age (P>0.05).

Table 4. Effects of selenium yeast on HGB content and HCT in the blood of broilers

Selenium supplemental level/(mg/kg)	21 days of age	42 days of age	P-value
	HGB/(g/dL)	HCT/%	HGB/(g/dL)

As shown in Table 5 , dietary supplementation with different levels of selenium yeast had no significant effects on other plasma biochemical parameters in broilers at 21 and 42 days of age (P>0.05), except for plasma urea nitrogen content and GSH-Px activity at 21 days and plasma GSH-Px activity at 42 days (P<0.05). Compared with the control group, dietary supplementation with 2.4 mg/kg selenium yeast significantly decreased plasma urea nitrogen content in 21-day-old broilers (P<0.05), but no significant differences were observed among the selenium yeast supplementation groups (P>0.05). Plasma GSH-Px activity in broilers at both 21 and 42 days of age increased significantly with increasing dietary selenium yeast levels (P<0.05).

Table 5. Effects of selenium yeast on plasma biochemical parameters of broilers

Selenium supplemental level/(mg/kg)	Days of age/d	TC/(mmol/L)	TG/(mmol/L)	GSH-Px/(U/mL)

2.3 Effects of Selenium Yeast on Organ Indices of Broilers

As shown in Table 6 , dietary supplementation with different levels of selenium yeast had no significant effects on various organ indices (P>0.05), except for trends toward increased gizzard index and jejunum index in 42-day-old broilers (P=0.07). No abnormal morphological changes were observed in the visceral organs, indicating that under the conditions of this experiment, dietary supplementation with 0.4, 2.4, and 4.9 mg/kg selenium yeast did not affect the development of major visceral organs in broilers.

2.4 Effects of Selenium Yeast on Histological Structure of Major Visceral Organs in Broilers

As shown in Figure 1 [Figure 1: see original paper], no histological changes were observed in the hearts of 42-day-old broilers among groups fed diets with differ-

ent selenium yeast levels. All groups exhibited smooth cardiac capsules, tightly and neatly arranged myocardial cells with uniform staining, no inflammatory cell infiltration in intercalated discs and transverse striations, and round or oval nuclei located centrally within muscle cells.

As shown in Figure 2 [Figure 2: see original paper], no histological changes were observed in the livers of 42-day-old broilers among groups fed diets with different selenium yeast levels. All groups showed hepatic lobules with polygonal prism morphology and intact structure, hepatocytes arranged radially around central veins with clear boundaries and uniform reticular structure, and intact and clear hepatic cords and sinusoids.

As shown in Figure 3 [Figure 3: see original paper], no histological changes were observed in the lungs of 42-day-old broilers among groups fed diets with different selenium yeast levels. Lungs in all groups appeared bright red, with no inflammatory exudate in tertiary bronchial epithelial cells, uniformly distributed microvilli of consistent thickness, flat surfaces of pulmonary atrial epithelium with round or oval nuclei located centrally, uniformly sized respiratory capillaries without foreign matter in the lumen and smooth epithelial cell surfaces, and abundant capillaries surrounding respiratory capillaries.

As shown in Figure 4 [Figure 4: see original paper], no histological changes were observed in the spleens of 42-day-old broilers among groups fed diets with different selenium yeast levels. All groups exhibited clear and intact splenic structure with no changes in the number or diameter of splenic nodules, and no differences in central artery diameter or periarterial lymphatic sheath thickness.

As shown in Figure 5 [Figure 5: see original paper], no histological changes were observed in the kidneys of 42-day-old broilers among groups fed diets with different selenium yeast levels. All groups showed moderate glomerular volume with normal structure and clear capsules in the cortical region, homogeneous red staining of renal tubular epithelial cell cytoplasm, and relatively homogeneous cytoplasm in collecting duct epithelial cells of medullary bodies.

These results demonstrate that under the conditions of this experiment, no microscopic structural or morphological changes were observed in the major visceral organs of broilers fed diets supplemented with different levels of selenium yeast.

Table 6. Effects of selenium yeast on organ indices of 42-day-old broilers

Selenium supplemental level/(mg/kg)	Heart index	Liver index	Kidney index	Pancreas index	Spleen index	Thymus index	Bursa of Fabricius index	Glandular stomach index	Gizzard index	Duodenum index	Jejunum index	Ileum index	Duodenum length	Jejunum length	Ileum length	P-value

Figure 1. Effects of selenium yeast on heart structural changes of broilers at 42 days of age

Control group | 0.4 mg/kg selenium yeast group | 2.4 mg/kg selenium yeast group | 4.9 mg/kg selenium yeast group

Figure 2. Effects of selenium yeast level on liver structural changes of broilers at 42 days of age

Control group | 0.4 mg/kg selenium yeast group | 2.4 mg/kg selenium yeast group | 4.9 mg/kg selenium yeast group

Figure 3. Effects of selenium yeast on spleen structural changes of broilers at 42 days of age

Control group | 0.4 mg/kg selenium yeast group | 2.4 mg/kg selenium yeast group | 4.9 mg/kg selenium yeast group

Figure 4. Effects of selenium yeast on lung structural changes of broilers at 42 days of age

Control group | 0.4 mg/kg selenium yeast group | 2.4 mg/kg selenium yeast group | 4.9 mg/kg selenium yeast group

Figure 5. Effects of selenium yeast on kidney structural changes of broilers at 42 days of age

Control group | 0.4 mg/kg selenium yeast group | 2.4 mg/kg selenium yeast group | 4.9 mg/kg selenium yeast group

Discussion

3.1 Effects of Selenium Yeast on Growth Performance and Mortality of Broilers

Selenium is a major component of iodothyronine deiodinase I (ID-I), which controls the activity of thyroid hormones [triiodothyronine (T) and thyroxine (T)] in the body. ID-I catalyzes the deiodination of T to the more active T , and thyroid hormones play important roles in animal growth and development. Research has shown that selenium deficiency can decrease ID-I activity, thereby reducing the conversion rate of T to T and resulting in increased blood

T content and decreased T content. Therefore, the mechanism by which selenium deficiency impairs animal growth may involve insufficient synthesis of growth hormone, which subsequently affects normal physiological metabolism and growth. Appropriate supplementation of organic selenium in diets can significantly improve growth performance in livestock and poultry. Fan Chun [6] reported that dietary supplementation with 0.1–0.2 mg/kg selenium from selenium yeast enabled fast-growing white-feathered broilers to achieve better daily gain and feed conversion efficiency. Lin Changguang et al. [23] found that dietary supplementation with selenium yeast or nano-selenium significantly increased litter weight at birth and weaning in piglets. Guo Yunxia et al. [24] demonstrated that dietary supplementation with 0.5 mg/kg selenium from selenium yeast during summer high-temperature conditions significantly increased egg production rate in laying hens and that supplementation with 0.5 and 1.0 mg/kg selenium from selenium yeast significantly decreased feed-to-egg ratio. The present study showed that dietary supplementation with different levels of selenium yeast significantly increased average daily feed intake during 22–42 days and average daily gain during 1–42 days in broilers, without significantly affecting other growth performance indices or mortality.

3.2 Effects of Selenium Yeast on Hemoglobin Content and Hematocrit in Broiler Blood

Routine blood indices such as hemoglobin content and hematocrit in animal blood can serve as indicators of overall health status. The results of this experiment showed that dietary supplementation with different levels of selenium yeast had no significant effects on hemoglobin content and hematocrit in broilers at 21 and 42 days of age. Guo Junrui [3] reported that dietary supplementation with high-dose selenomethionine (total selenium content of 5 mg/kg) had no significant effects on hemoglobin content and hematocrit in 42-day-old broilers, which is consistent with the results of this experiment. This indicates that dietary supplementation with selenium yeast does not significantly affect hemoglobin content and hematocrit in broiler blood.

3.3 Effects of Selenium Yeast on Plasma Biochemical Parameters of Broilers

Plasma total protein and albumin contents can reflect protein synthesis and metabolism status in the body [25]. Urea nitrogen is the main end product of protein digestion and metabolism in animals. Plasma urea nitrogen content can reflect protein metabolism level; low plasma urea nitrogen content indicates high protein utilization efficiency. The results of this study showed that compared with the control group, dietary supplementation with 2.4 mg/kg selenium yeast significantly decreased plasma urea nitrogen content in 21-day-old broilers, while having no significant effects on plasma total protein and albumin contents. Sun Chunyang et al. [26] reported that dietary supplementation with a mixture of glucose oxidase and selenium yeast significantly decreased serum urea nitrogen

content in broilers at 21 and 42 days of age, which is similar to the results of this experiment. This suggests that selenium yeast can improve protein utilization efficiency in broilers.

Plasma GPT and GOT are important indicators of liver function. When the liver is damaged, plasma GPT and GOT activities increase. Plasma creatine phosphokinase activity can reflect the degree of myocardial cell damage. Alkaline phosphase is mainly present in bone and liver, where it dephosphorylates corresponding substrates to produce phosphate, which then combines with calcium and deposits in bone. When liver or osteoblasts are damaged, alkaline phosphatase activity increases. The results of this experiment showed that dietary supplementation with different levels of selenium yeast had no significant effects on plasma GPT, GOT, creatine phosphokinase, or alkaline phosphatase activities, which is similar to the findings of Guo Junrui [3]. This indicates that dietary supplementation with 0.4, 2.4, or 4.9 mg/kg selenium yeast has no adverse effects on the liver and heart of broilers.

Selenium is an essential component of GSH-Px, and GSH-Px activity can reflect the antioxidant capacity of the body. Wang et al. [27] reported that dietary supplementation with different selenium sources (sodium selenite and selenium yeast) significantly increased plasma GSH-Px activity in broilers. Wang Qiaoli et al. [28] found that selenium yeast supplementation significantly increased plasma GSH-Px activity in geese. Selenium is the active center of GSH-Px, and 30%–40% of selenium in the body exists in the form of GSH-Px. GSH-Px activity increases with selenium level within a certain range. The results of this study showed that plasma GSH-Px activity in broilers at both 21 and 42 days of age increased significantly with increasing dietary selenium yeast levels. This indicates that dietary supplementation with high levels of selenium yeast can improve the antioxidant capacity of broilers.

3.4 Effects of Selenium Yeast on Organ Indices of Broilers

Immune organ indices and digestive organ indices are important reference indicators for reflecting animal immune and digestive functions. Du Zhen et al. [29] reported that high selenium levels have toxic effects on chicken intestinal mucosa. However, in this experiment, dietary supplementation with 0.4, 2.4, and 4.9 mg/kg selenium yeast had no significant effects on visceral and gastrointestinal organ indices in broilers. The lack of significant effects of high selenium levels on chicken intestinal mucosa in this experiment may be related to differences in selenium form and chicken breed.

3.5 Effects of Selenium Yeast on Histological Changes of Major Organs in Broilers

Qi Zhouyue et al. [30] fed 60-day-old growing chickens with high-selenium diets prepared from high-selenium corn containing 8.06 and 13.08 mg/kg selenium. Histopathological examination of heart, liver, spleen, and breast muscle tissues

revealed that the high-selenium group showed myocardial atrophy and deformation and focal necrosis in the liver as main symptoms. Yin Xiaoping [31] fed Liuzhou black chickens with 12 mg/kg selenium as sodium selenite for 45 days, and pathological changes were mainly manifested as degeneration and necrosis of parenchymal organs such as heart and liver. In this experiment, no histological changes in microstructure or morphology were observed in the heart and other major visceral organs of broilers fed diets supplemented with selenium yeast. These results differ from previous studies [30–31], possibly because the selenium content in the high-level selenium yeast group in this experiment was lower and the selenium form was different. This indicates that dietary supplementation with high levels of selenium yeast (2.4 or 4.9 mg/kg) does not cause histological changes in important organs of broilers.

In this experiment, the measured basal dietary selenium content was lower than 0.10 mg/kg, resulting in a measured total selenium content of only 0.41 mg/kg in the maximum limit group supplemented with 0.4 mg/kg selenium yeast, which is lower than the maximum limit of 0.5 mg/kg for selenium from selenium yeast in complete diets specified in the "Regulations on Safe Use of Feed Additives" (No. 1224) issued by the Ministry of Agriculture in 2009. The results of this experiment showed that dietary supplementation with 0.4, 2.4, and 4.9 mg/kg selenium yeast did not adversely affect growth performance, plasma biochemical parameters, immune organ development, or major organ development in broilers. Therefore, when the dietary supplementation level of selenium yeast is 0.4 mg/kg (with a total dietary selenium content of 0.41 mg/kg), it possesses more than a 10-fold safety margin and is safe for feeding broilers.

Dietary supplementation with 0.4–4.9 mg/kg selenium yeast had no adverse effects on growth performance, blood routine indices, most plasma biochemical parameters, or major organ development in broilers. No histopathological changes were observed in major visceral organs, and no toxic reactions occurred in any broilers. Therefore, when the dietary supplementation level of selenium yeast is 0.4 mg/kg (with a total dietary selenium content of 0.41 mg/kg), it possesses more than a 10-fold safety margin and is safe for feeding broilers.

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