

## Effects of Feed Processing Technology and Compound Microecological Preparation on Growth Performance and Immune Function in Broiler Chickens: Postprint

**Authors:** Ge Chunyu, Li Junguo, Duan Haitao, Yang Jie, Han Qing, Zhang Jiaqi, Qin Yuchang

**Date:** 2018-12-25T00:00:00+00:00

### Abstract

The present experiment was conducted to investigate the effects of different feed processing techniques on the survival rate of microorganisms in compound microecological preparations and feed pellet quality, and to explore the influences of feed processing techniques and compound microecological preparations on growth performance and immune function of broiler chickens. A total of 864 one-day-old Arbor Acres (AA) broiler chickens were randomly allocated to 8 groups with 6 replicates per group and 18 birds per replicate, following the principle of consistent sex ratio, for a 42-day feeding trial. Two feed processing techniques were employed for broiler diet manufacturing: ordinary conditioning pelleting (OT) and high-temperature conditioning of macro-ingredients with low-temperature pelleting (ET). For each processing technique, four treatments were established based on different supplementation levels of chlortetracycline and compound microecological preparation: 0 and 0 mg/kg (0/0 group), 600 and 0 mg/kg (600/0 group), 300 and 200 mg/kg (300/200 group), and 0 and 200 mg/kg (0/200 group). The results showed: 1) Regarding pellet feed processing quality, the ET technique exhibited significantly superior effects on microbial survival rate of compound microecological preparations and feed pellet quality in broiler diets compared to the OT technique ( $P < 0.05$ ). 2) Regarding broiler growth performance, under OT conditions, supplementation levels of chlortetracycline and compound microecological preparation had no significant effect on broiler growth performance ( $P > 0.05$ ); under ET conditions, the final body weight and average daily gain of broilers in the 0/0 group during the early growth period were significantly higher than those in the 300/200 and 0/200 groups ( $P < 0.05$ ); irrespective of supplementation levels of chlortetracycline and compound microecological preparation, broiler growth performance in the OT

group was significantly higher than that in the ET group ( $P < 0.05$ ). 3) Regarding broiler immune function, no significant differences were observed in immune organ indices or intestinal microbial counts among all groups ( $P > 0.05$ ). 4) Regarding broiler plasma biochemical indices, under the same processing condition, supplementation levels of chlortetracycline and compound microecological preparation had no significant effect on plasma biochemical indices ( $P > 0.05$ ); irrespective of supplementation levels of chlortetracycline and compound microecological preparation, no significant difference was observed in the effects of the two processing techniques on broiler plasma biochemical indices ( $P > 0.05$ ). The results indicated that: compared with the OT technique, the ET technique significantly improved the survival rates of *Bacillus subtilis*, *Bacillus licheniformis*, and coated lactic acid bacteria in feed, and significantly increased the starch gelatinization degree of pellet feed; compared with the OT technique, the ET technique reduced broiler growth performance, and the increased feed starch gelatinization degree failed to improve growth performance; under the same processing condition, supplementation levels of chlortetracycline and compound microecological preparation had no significant effects on broiler growth performance, immune organ indices, intestinal microbial counts, or plasma biochemical indices.

## Full Text

### Effects of Feed Processing Technology and Compound Probiotics on Growth Performance and Immune Function of Broilers

GE Chunyu<sup>1,2</sup>, LI Junguo<sup>1,3</sup>, DUAN Haitao<sup>1,2</sup>, YANG Jie<sup>1,3</sup>, HAN Qing<sup>1,3</sup>, ZHANG Jiaqi<sup>1,3</sup>, QIN Yuchang \*

<sup>1</sup>Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

<sup>2</sup>Institute of Food and Nutrition Development, Ministry of Agriculture, Beijing 100081, China

<sup>3</sup>Key Laboratory of Feed Biotechnology, Ministry of Agriculture, Beijing 100081, China

Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China

---

## Abstract

This experiment was conducted to investigate the effects of different feed processing technologies on the survival rate of compound probiotics and feed pellet quality, and to explore the impacts of feed processing technology and compound probiotics on growth performance and immune function in broilers. A total of 864 one-day-old white-feathered Arbor Acres (AA) broiler chicks were randomly

allocated to 8 groups with 6 replicates per group and 18 birds per replicate, following a 42-day feeding trial. Two feed processing technologies were employed: ordinary conditioning and pelleting (OT) and efficient conditioning at high temperature for major ingredients followed by low-temperature pelleting (ET). Each processing technology included four treatments with varying additions of chlortetracycline and compound probiotics: 0 and 0 mg/kg (0/0 group), 600 and 0 mg/kg (600/0 group), 300 and 200 mg/kg (300/200 group), and 0 and 200 mg/kg (0/200 group). The results showed: (1) In terms of pellet feed processing quality, ET significantly improved the survival rate of compound probiotics in broiler diets compared to OT ( $P < 0.05$ ). (2) Regarding broiler growth performance, under OT conditions, different additions of chlortetracycline and compound probiotics had no significant effects ( $P > 0.05$ ); under ET conditions, the final body weight and average daily gain during the early growth period in the 0/0 group were significantly higher than those in the 300/200 and 0/200 groups ( $P < 0.05$ ). Regardless of chlortetracycline and compound probiotic addition, the growth performance of broilers in the OT group was significantly higher than that in the ET group ( $P < 0.05$ ). (3) For immune function, no significant differences were observed in immune organ indices or intestinal microorganism counts among groups ( $P > 0.05$ ). (4) For plasma biochemical indices, under the same processing technology, different additions of chlortetracycline and compound probiotics showed no significant effects ( $P > 0.05$ ); regardless of additive levels, the two processing technologies did not differ significantly in their effects on plasma biochemical indices ( $P > 0.05$ ). In conclusion, compared with OT, ET significantly improved the survival rates of *Bacillus subtilis*, *Bacillus licheniformis*, and coated lactic acid bacteria, and significantly increased the starch gelatinization degree of pelleted feed. However, ET reduced broiler growth performance compared to OT, and increased starch gelatinization did not improve growth performance. Under the same processing technology, different additions of chlortetracycline and compound probiotics had no significant effects on growth performance, immune organ indices, intestinal microorganism counts, or plasma biochemical indices.

**Keywords:** compound probiotics; broilers; growth performance; immune function; plasma biochemical indices

---

## Introduction

The addition of antibiotics to feed can promote livestock growth, improve feed conversion efficiency, and prevent diseases. However, long-term and extensive use of antibiotics leads to drug resistance in pathogenic bacteria and antibiotic residues in animal products, which can cause allergic reactions and poisoning in humans, posing health risks [1]. Currently, microecological preparations are widely used as antibiotic alternatives. Xie et al. [2] found that broilers fed diets supplemented with 2% microecological preparations showed significantly improved growth performance compared to control and antibiotic groups. At

17 and 24 days of age, serum immunoglobulin G levels in the 2% microecological preparation group increased by 24.3% and 16.2%, respectively, compared to the antibiotic group. Hu et al. [3] reported that broilers fed microecological preparations had significantly higher numbers of *Escherichia coli* and lactic acid bacteria in the cecum compared to the control group. Newbold et al. [4] reported that microecological preparations can improve beneficial intestinal flora, inhibit harmful bacteria, promote animal growth, and improve feed utilization while overcoming the disadvantages of drug residues and resistance. However, microecological preparations also suffer significant losses during feed processing. Wang [5] found that conditioning time significantly affected the viable counts of lactic acid bacteria, yeast, and bacilli in microecological preparations without protective additives. Li et al. [6] reported that after pelleting, *Bacillus* showed high survival rates, while lactic acid bacteria were more vulnerable and could not withstand processing conditions such as temperature and pressure. Previous studies have focused on either losses of microecological preparations during feed processing or their effects on broiler performance, but few have investigated how feed processing technologies affect probiotic losses and their subsequent impacts on broiler growth performance and immune function. Therefore, this study examined the effects of ordinary conditioning and pelleting (OT) versus efficient conditioning at high temperature for major ingredients followed by low-temperature pelleting (ET) on the activity of compound probiotics and feed quality, as well as the effects of different processing technologies and compound probiotics as antibiotic alternatives on broiler growth performance and immune function, providing reference for the application of microecological preparations in broiler feed and processing technology selection.

---

## 1. Materials and Methods

**1.1 Experimental Diets** The composition and nutrient levels of the basal diets are shown in Table 1 .

**Table 1** Composition and nutrient levels of the basal diets (air-dry basis)

*Note: The mineral premix provided the following per kg of diet: for 1-21 days, Fe (as ferrous sulfate) 100 mg, Cu (as copper sulfate) 8.0 mg, Zn (as zinc sulfate) 100 mg, Mn (as manganese sulfate) 120 mg, I (as potassium iodide) 0.7 mg, Se (as sodium selenite) 0.3 mg; for 22-42 days, Fe 80 mg, Cu 8.0 mg, Zn 80 mg, Mn 100 mg, I 0.7 mg, Se 0.3 mg. The vitamin premix provided the following per kg of diet: for 1-21 days, VA 10,000 IU, VD 1,000 IU, VE 20 IU, VK 0.5 mg, VB 2.0 mg, VB 8.0 mg, pantothenic acid 10.0 mg, niacin 35.0 mg, VB 3.5 mg, biotin 0.05 mg, folic acid 0.55 mg, VB 0.01 mg; for 22-42 days, VA 8,000 IU, VD 750 IU, VE 15 IU, VK 0.5 mg, VB 2.0 mg, VB 5.0 mg, pantothenic acid 10.0 mg, niacin 30.0 mg, VB 3.5 mg, biotin 0.05 mg, folic acid 0.55 mg, VB 0.01 mg. Crude protein was a measured value, while others were calculated values.*

**1.2 Experimental Design** The experiment employed two feed processing technologies: OT and ET. Each processing technology included four treatments with different additions of chlortetracycline and compound probiotics, totaling 8 groups as shown in Table 2. The compound probiotics contained *Bacillus subtilis* at 10.41 log(CFU/g), *Bacillus licheniformis* at 10.26 log(CFU/g), and coated lactic acid bacteria at 9.77 log(CFU/g).

**Table 2** Experimental groups

*Processing technology details: For OT, all ingredients were ground, proportioned, mixed, conditioned, and pelleted. The early-stage feed was ground using a 2.0 mm sieve, and the late-stage feed using a 2.5 mm sieve. The pellet mill die had a 3 mm diameter with a length-to-diameter ratio of 10:1, conditioning temperature was 75°C, and conditioning time was approximately 30 s. For ET, major ingredients were first ground and mixed (same grinding size as OT), then conditioned at high temperature for maturation. After cooling, they were mixed with premix and probiotics, conditioned at 60°C for approximately 30 s, and then pelleted at low temperature.*

**1.3 Experimental Animals and Management** The feeding trial was conducted at the Nankou Experimental Base of the Chinese Academy of Agricultural Sciences. A total of 864 one-day-old white-feathered Arbor Acres (AA) broiler chicks with an initial body weight of (48.00±0.05) g were randomly divided into 8 groups with 6 replicates per group and 18 birds per replicate. The 42-day trial consisted of two phases: early phase (1-21 days) fed crumbles and late phase (22-42 days) fed pellets. Management followed the AA broiler management manual. Lighting, temperature, and ventilation were controlled; regular disinfection, manure removal, and cleaning were performed; vaccination programs were implemented; bird conditions and mortality were recorded; and feed consumption was monitored with ad libitum access to feed and water.

**1.4 Measurements**

**1.4.1 Total Viable Count and Microbial Survival Rate** *Bacillus subtilis* was detected according to GB/T 26428-2010, *Bacillus licheniformis* according to NY/T 1461-2007, and coated lactic acid bacteria using capsule dissolution solution followed by 10-fold serial dilution with physiological saline. Appropriate dilutions were plated on sterilized agar plates and incubated at 37°C for 48 h before counting.

Microbial survival rate (%) =  $100 \times (\text{viable count after conditioning or pelleting}) / (\text{viable count before conditioning or pelleting})$

**1.4.2 Pellet Hardness** Pellet hardness was determined according to the method described in *Feed Inspection and Testing Personnel* [7].

**1.4.3 Pellet Durability Index (PDI)** PDI was measured using the American Society of Agricultural Engineers standard method [8].

**1.4.4 Starch Gelatinization Degree** Starch gelatinization degree was determined using the simplified enzymatic method commonly employed in the American feed industry [9].

**1.4.5 Growth Performance** Feed was withdrawn 24 h before weighing on days 21 and 42, with water provided ad libitum. Birds were weighed individually, and average body weight was calculated per replicate. Daily feed intake was recorded accurately, and feed consumption was calculated by weighing remaining feed when mortality occurred.

Average daily feed intake (ADFI) = total feed consumption / (number of birds × days)

Average daily gain (ADG) = total weight gain / (number of birds × days)

Feed-to-gain ratio (F/G) = total feed consumption / total weight gain

**1.4.6 Immune Organ Indices** At 42 days, one bird per replicate was randomly selected, euthanized by jugular venipuncture, and the spleen and bursa of Fabricius were excised and weighed after removing fat to calculate immune organ indices.

Immune organ index (mg/g) = immune organ weight (mg) / live body weight (g)

**1.4.7 Cecal Microorganism Counts** At 42 days, one bird per replicate was euthanized, and the cecum was aseptically isolated. Cecal contents were collected, and lactic acid bacteria, *Salmonella*, and *Escherichia coli* were counted using plate culture methods. Microbial counts were expressed as log(CFU/g) of cecal content.

**1.4.8 Plasma Biochemical Indices** At 42 days, one bird per replicate was euthanized, and blood samples were collected in procoagulant tubes. Plasma was separated by centrifugation, and biochemical indices including urea nitrogen, total protein, albumin, immunoglobulin content, and activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute).

**1.5 Data Processing** Data were expressed as mean ± standard deviation. All data were analyzed using SAS 9.2 software for one-way ANOVA and two-way ANOVA. Duncan's multiple comparison test was used to examine significant differences, with significance level at  $P < 0.05$  and extremely significant level at  $P < 0.01$ .

## 2. Results

**2.1 Effects of Feed Processing Technology on Probiotic Activity** The effects of feed processing technology on probiotic activity are shown in Table 3 . During the early growth period, the total viable counts and survival rates of *Bacillus subtilis* and coated lactic acid bacteria after conditioning were significantly higher in ET than in OT ( $P < 0.05$ ), while no significant differences were observed for *Bacillus licheniformis* ( $P > 0.05$ ). After pelleting, the total viable counts and survival rates of *B. subtilis* and *B. licheniformis* were significantly higher in ET than in OT ( $P < 0.05$ ), but no significant differences were found for coated lactic acid bacteria ( $P > 0.05$ ). During the late growth period, the total viable counts and survival rates of coated lactic acid bacteria after conditioning were significantly higher in ET than in OT ( $P < 0.05$ ), with no significant differences for *B. subtilis* and *B. licheniformis* ( $P > 0.05$ ). After pelleting, the total viable counts and survival rates of coated lactic acid bacteria were significantly higher in ET than in OT ( $P < 0.05$ ), while no significant differences were observed for *B. subtilis* and *B. licheniformis* ( $P > 0.05$ ).

*Note: Uppercase and lowercase letters distinguish differences between two data sets. In the same row, values with the same letter or no letter superscripts indicate no significant difference ( $P > 0.05$ ), while different letters indicate significant difference ( $P < 0.05$ ). The same applies to Table 4.*

**2.2 Effects of Feed Processing Technology on Feed Pellet Quality** The effects of feed processing technology on feed pellet quality are presented in Table 4 . During both early and late growth periods, pellet hardness, starch gelatinization degree, and pellet durability index (PDI) were significantly higher in ET than in OT ( $P < 0.05$ ).

**2.3 Effects of Feed Processing Technology and Compound Probiotics on Broiler Growth Performance** The effects of feed processing technology and compound probiotics on broiler growth performance are shown in Table 5 . Under OT conditions, different additions of chlortetracycline and compound probiotics had no significant effects on growth performance ( $P > 0.05$ ). Under ET conditions, final body weight and ADG during the early growth period in group 5 were significantly higher than those in groups 7 and 8 ( $P < 0.05$ ), while other indices showed no significant differences ( $P > 0.05$ ). When chlortetracycline and compound probiotic additions were 0 and 0 mg/kg, the two processing technologies showed no significant differences in growth performance ( $P > 0.05$ ). Regardless of additive levels, the mean values of final body weight, ADG, and ADFI during the early growth period were significantly higher in OT than in ET ( $P < 0.05$ ), while F/G was significantly lower ( $P < 0.05$ ). During the late growth period, mean final body weight was significantly higher in OT than in ET ( $P < 0.05$ ), with no significant differences in ADG, ADFI, or F/G ( $P > 0.05$ ). Over the entire growth period, mean ADG and ADFI were significantly higher in OT than in ET ( $P < 0.05$ ), with no significant difference in F/G ( $P > 0.05$ ).

Two-way ANOVA revealed that processing technology had extremely significant or significant effects on early growth performance, late-phase final body weight and ADFI, and overall ADG and ADFI ( $P < 0.01$  or  $P < 0.05$ ), but no significant effects on other indices ( $P > 0.05$ ). The addition levels of chlortetracycline and compound probiotics had extremely significant effects on early-phase final body weight, ADG, and ADFI ( $P < 0.01$ ), but no significant effects on early-phase F/G, late-phase, or overall growth performance ( $P > 0.05$ ). The interaction between processing technology and additive levels had extremely significant or significant effects on early-phase final body weight, ADG, F/G, and late-phase ADFI ( $P < 0.01$  or  $P < 0.05$ ), but no significant effects on other growth performance indices ( $P > 0.05$ ).

*Note: In the same column, values with the same letter or no letter superscripts indicate no significant difference ( $P > 0.05$ ), while different letters indicate significant difference ( $P < 0.05$ ). The same applies below.*

**2.4 Effects of Feed Processing Technology and Compound Probiotics on Immune Organ Indices and Cecal Microorganism Counts** The effects of feed processing technology and compound probiotics on immune organ indices and cecal microorganism counts are presented in Table 6. Under both OT and ET conditions, different additions of chlortetracycline and compound probiotics showed no significant effects on immune organ indices or cecal microorganism counts ( $P > 0.05$ ). When chlortetracycline and compound probiotic additions were 0 and 0 mg/kg, the two processing technologies showed no significant differences in these parameters ( $P > 0.05$ ). Regardless of additive levels, the two processing technologies had no significant effects on mean values of immune organ indices or cecal microorganism counts ( $P > 0.05$ ), though ET showed slightly better results than OT.

Two-way ANOVA indicated that processing technology, additive levels, and their interaction had no significant effects on immune organ indices or cecal microorganism counts ( $P > 0.05$ ).

**2.5 Effects of Feed Processing Technology and Compound Probiotics on Plasma Biochemical Indices** The effects of feed processing technology and compound probiotics on plasma biochemical indices are shown in Table 7. Under both OT and ET conditions, different additions of chlortetracycline and compound probiotics had no significant effects on plasma biochemical indices ( $P > 0.05$ ). When chlortetracycline and compound probiotic additions were 0/0 mg/kg, the two processing technologies showed no significant differences ( $P > 0.05$ ). Regardless of additive levels, the two processing technologies had no significant effects on mean plasma biochemical indices ( $P > 0.05$ ).

Two-way ANOVA revealed that processing technology, additive levels, and their interaction had no significant effects on plasma biochemical indices ( $P > 0.05$ ).

### 3. Discussion

**3.1 Effects of Feed Processing Technology on Compound Probiotic Activity** Compound probiotics have been used as alternative growth promoters in poultry, swine, and ruminant feeds to improve growth rate, immunity, and feed conversion efficiency [10]. However, their application is constrained by many factors, as they are susceptible to environmental conditions during feed processing and transportation. Hao et al. [11] reported that *B. subtilis* mortality rates were 1.4% at 60°C for 12 min, 21.8% at 70°C for 10 min, and 21.1% at 80°C for 5 min. Liu et al. [12] found that *B. subtilis* survival rate decreased by 10-29% at 70-80°C, *B. licheniformis* loss rate was below 50% at 80°C, while lactic acid bacteria suffered greater losses, with thermotolerance ranking as *B. subtilis* > *B. licheniformis* > *Streptococcus lactis*. This study demonstrated that ET improved viable microbial survival rates compared to OT after conditioning and pelleting. This is because OT uses a conditioning temperature of 80°C, which partially inactivates probiotics, whereas ET first conditions major ingredients (excluding probiotics) at temperatures above 85°C, then cools and mixes them with probiotics before low-temperature pelleting at 60°C, effectively preserving probiotic activity.

**3.2 Effects of Feed Processing Technology on Feed Pellet Quality** As feed formulations become increasingly sophisticated, processing technology plays a crucial role in feed quality. Sun [13] reported that expanded major ingredient low-temperature pelleting significantly improved pellet durability, starch gelatinization, and hardness compared to conventional pelleting. This study found that ET significantly increased hardness, starch gelatinization degree, and PDI compared to OT during both early and late growth periods. This is because ET conditions major ingredients above 85°C, allowing sufficient gelatinization that transforms compact  $\alpha$ -starch into gelatinized  $\alpha$ -starch, denatures proteins, and increases material adhesiveness. Low-temperature pelleting further enhances material binding, resulting in higher hardness and durability than OT [13-16].

**3.3 Effects of Feed Processing Technology and Compound Probiotics on Broiler Growth Performance** Compound probiotics can promote animal growth and enhance immunity. Wang et al. [17] reported that adding 0.2% compound probiotics and 2.5% astragalus polysaccharides improved broiler ADG and F/G. Hao et al. [18] found that probiotic-supplemented groups showed decreased growth performance in weeks 1-2 but improved in week 3 compared to controls. Chen et al. [19] observed that 50 mg/kg *B. licheniformis* improved growth performance, while 200 mg/kg increased F/G at 28 days. This study showed that compound probiotics did not significantly improve broiler growth performance, possibly because early-phase probiotic supplementation induced strong immune responses in young chicks, and immune stress may have masked growth improvements. Additionally, Wang et al. [20] reported that only ADFI was significantly higher in the 16.92% extruded corn group compared to the 5.64% group, with no significant differences in other performance indices, in-

dicating that increased starch gelatinization did not significantly affect broiler growth performance. This study found OT outperformed ET in growth performance, contradicting Wang et al. [20]. This discrepancy may be due to broilers' unique physiological characteristics: higher starch gelatinization improves digestibility and increases chyme passage rate, reducing nutrient absorption time and preventing growth performance improvements.

### 3.4 Effects of Feed Processing Technology and Compound Probiotics on Immune Organ Indices and Cecal Microorganism Counts

Immune organs include central and peripheral immune organs. The spleen is the largest peripheral immune organ in poultry, participating in systemic cellular and humoral immune responses, while the bursa of Fabricius is the unique central humoral immune organ and primary site for B cell differentiation. The functional capacity of central and peripheral immune organs determines overall immune levels, and immune organ indices reflect immune system maturation and functional strength [21]. Cheng et al. [22] found that traditional Chinese medicine immune enhancers increased immune organ indices compared to controls. Wang et al. [23] reported that at 35 days, bursa and spleen indices in probiotic-supplemented groups increased by 40.12% and 19.30%, respectively, compared to controls, demonstrating that probiotics can enhance immune function. Xu et al. [24] found that at 33 days, spleen index in a probiotic-supplemented group increased by 55.1% compared to controls, and at 26 days, bursa index increased by 65.7%, both significantly. This study found no significant differences in spleen indices among groups, though the highest values occurred when chlortetracycline and compound probiotics were added at 0 and 200 mg/kg under the same processing technology, indicating that compound probiotics can enhance immune capacity to some extent. The two processing technologies showed no significant differences in immune organ indices, though ET performed slightly better than OT, possibly because ET improved probiotic retention and thus immune capacity.

Yuan et al. [25] reported that adding 1.5 g/kg compound probiotics reduced cecal *E. coli* counts by 37.51% and increased lactic acid bacteria counts by 1.92% compared to controls. Hu et al. [3] found that adding 2.0% compound probiotics and 0.5% adjuvant significantly increased cecal lactic acid bacteria and decreased *E. coli* counts compared to controls. In this study, under the same processing technology, the 300/200 mg/kg group showed the lowest *E. coli* and *Salmonella* counts and highest lactic acid bacteria counts, possibly because antibiotics also inhibit harmful intestinal bacteria and regulate flora [26], and combined with probiotics at appropriate levels, produce better immune effects. The two processing technologies showed no significant differences in intestinal microorganism counts, though ET performed slightly better than OT, possibly because ET maintained higher probiotic activity. *Bacillus* in compound probiotics can create anaerobic environments for lactic acid bacteria growth, reduce redox potential, inhibit harmful microorganisms like *E. coli*, and regulate intestinal microecological balance for antibacterial disease prevention [27].

**3.5 Effects of Feed Processing Technology and Compound Probiotics on Plasma Biochemical Indices** Serum total protein, albumin, and globulin reflect immune function status. Total protein indicates dietary crude protein digestibility and utilization, representing the sum of albumin and globulin. Elevated total protein and albumin indicate vigorous protein metabolism, improved amino acid and protein absorption, enhanced hepatic protein synthesis, and increased tissue protein deposition, thereby improving animal performance. Globulin, secreted by plasma cells, reflects resistance levels. The albumin-to-globulin ratio reflects spleen function and measures immune status [28-29]. A decreased ratio indicates enhanced specific immune response and improved disease resistance.

Abdulrahim et al. [30] reported that probiotics can increase serum total protein, albumin, and globulin contents. Chen et al. [31] found that 2.0% compound probiotics significantly increased serum total protein at 14, 21, 28, and 35 days. Xie et al. [32] reported that compound probiotics increased serum total protein, albumin, and globulin while decreasing the albumin-to-globulin ratio. This study found that the 300/200 mg/kg group performed slightly better under the same processing technology, possibly because appropriate combinations of probiotics and antibiotics promote amino acid metabolism, reduce protein catabolism, and enhance anabolism, favoring protein accumulation [28-30]. The two processing technologies showed no significant differences in plasma biochemical indices, though ET performed slightly better than OT, possibly because ET improved probiotic retention in feed.

---

#### 4. Conclusion

1. Compared with OT, ET significantly improved the survival rates of *Bacillus subtilis*, *Bacillus licheniformis*, and coated lactic acid bacteria, and significantly increased the starch gelatinization degree of pelleted feed.
2. Compared with OT, ET reduced broiler growth performance, and increased feed starch gelatinization did not improve growth performance.
3. Under the same processing technology, different additions of chlortetracycline and compound probiotics had no significant effects on broiler growth performance, immune organ indices, intestinal microorganism counts, or plasma biochemical indices.

---

#### References

- [1] He LQ. Discussion on the hazards and control of antibiotics in animal husbandry[J]. China Animal Health Inspection, 2010, 27(4): 11-12.
- [2] Xie QX, Cui SF, Xu HY, et al. Effects of compound probiotics and feed antibiotics on growth performance, immune function and antioxidant indices of

- broilers[J]. Chinese Journal of Animal Nutrition, 2012, 24(7): 1336-1344.
- [3] Hu SZ, Zhang JM, Xie QX, et al. Effects of compound probiotics on production performance, intestinal flora, antioxidant indices and immune function of broilers[J]. Chinese Journal of Animal Nutrition, 2012, 24(2): 334-341.
- [4] Newbold CJ, Wallace RJ, McIntosh FM. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants[J]. British Journal of Nutrition, 1996, 76(2): 249-261.
- [5] Wang C. Study on the effect of processing technology on probiotics and the application effect of probiotics[D]. Master' s thesis. Changsha: Hunan Agricultural University, 2008.
- [6] Li X, Li J, Zhang DX, et al. Effects of feed processing and storage on probiotic activity[J]. China Feed, 2011(7): 37-40.
- [7] Gu JH, Hu GD. Feed Inspection and Testing Personnel[M]. Beijing: China Agriculture Press, 2010: 89-90.
- [8] Han GZ. Pellet feed durability index tester and its application in feed quality detection[J]. Feed and Animal Husbandry (New Feed), 2011(3): 26-30.
- [9] Xiong YQ. Determination of feed starch gelatinization degree[J]. Feed Industry, 2000, 21(3): 30-31.
- [10] Oso AO, Idowu OMO, Haastrup AS, et al. Growth performance, apparent nutrient digestibility, caecal fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing probiotics and prebiotics[J]. Livestock Science, 2013, 157(1): 184-190.
- [11] Hao SH, Gu CT, Sa RN, et al. Effects of high temperature treatment on three probiotic strains[J]. Feed Industry, 2004, 25(6): 27-28.
- [12] Liu CQ, Wang P, Chang J, et al. Study on tolerance of probiotics to temperature, pH and antibiotics[J]. Feed Research, 2016(12): 19-25.
- [13] Sun J. Comparative study on pellet feed processing technology for weaned piglets[D]. Master' s thesis. Beijing: Chinese Academy of Agricultural Sciences, 2014.
- [14] Zhang XL. Study on effects of conditioning temperature and grinding particle size on pellet feed quality and utilization in broilers[D]. Master' s thesis. Beijing: Chinese Academy of Agricultural Sciences, 2013: 5-12.
- [15] Duan HT, Li JG, Ge CY, et al. Effects of efficient conditioning and low-temperature pelleting on pellet feed processing quality and vitamin E retention rate[J]. Chinese Journal of Animal Nutrition, 2017, 29(11): 4101-4107.
- [16] Hu YR. Effects of different conditioning temperatures on pellet feed quality and broiler performance[D]. Master' s thesis. Nanchang: Jiangxi Agricultural University, 2011.

- [17] Wang HL, Liu DD, Jiang SW, et al. Effects of compound probiotics and astragalus polysaccharides on growth performance, intestinal flora and immune function of broilers[J]. Feed Industry, 2014, 35(6): 10-14.
- [18] Hao SH. Effects of probiotic activity after pelleting on broiler performance, blood biochemical indices and intestinal microorganisms[D]. Master' s thesis. Taigu: Shanxi Agricultural University, 2004.
- [19] Chen JX, Zhang RY, Wang QX, et al. Effects of *Bacillus licheniformis* on growth performance, antioxidant indices and blood biochemical indices of broilers[J]. Chinese Journal of Animal Nutrition, 2010, 22(4): 1019-1023.
- [20] Wang H, Yu JB, Yu ZQ, et al. Effects of feed starch gelatinization degree on growth performance and in vitro protein digestibility of broilers[J]. Feed Industry, 2017, 38(6): 35-40.
- [21] Ma CQ. Study on the development of probiotic preparation and its immunological effects and mechanisms in chicks[D]. Doctoral thesis. Beijing: China Agricultural University, 2004.
- [22] Cheng XC, Zhang CJ, Li YJ, et al. Study on the effects of traditional Chinese medicine immune enhancers on immune organ development and immune active cells in broilers[J]. Chinese Journal of Traditional Veterinary Science, 2002(3): 6-8.
- [23] Wang M, Zhang JM, Nie HM, et al. Effects of microecological preparations on growth performance, immune function and intestinal mucosa of chicks[J]. Chinese Journal of Veterinary Science, 2017, 37(9): 1785-1789.
- [24] Xu HY, Xin GM, Wang H, et al. Effects of compound probiotics on growth performance and immune function of broilers[J]. Animal Husbandry and Feed Science, 2013, 34(4): 45-48, 51.
- [25] Yuan N, Chen Q, Liu CM, et al. Effects of compound probiotics on ammonia concentration, nutrient absorption and intestinal flora in layer breeder houses[J]. Feed Industry, 2010, 31(20): 42-44.
- [26] Tong JM, Gao X, Sa RN. Effects of chlortetracycline on growth and intestinal microbial reproduction in broilers[J]. China Feed, 1998(17): 10-11.
- [27] Gu J, Zhou WR, Yan JS, et al. Study on the regulation of intestinal flora by microecological preparations in chickens[J]. Feed Research, 2010(1): 16-18.
- [28] Lin Q, Dai QZ, Bin SY, et al. Synergistic effects of probiotics and enzyme preparations on blood biochemical indices and immune performance of yellow-feathered broilers[J]. Feed Industry, 2012, 33(14): 31-36.
- [29] Han YC, He YG, Zhang XH, et al. Effects of Chinese herbal compound microecological preparations on growth performance and blood biochemical indices of weaned piglets[J]. Jiangsu Agricultural Sciences, 2014, 42(8): 218-221.

[30] Abdulrahim SM, Haddadin MSY, Hashlamoun EAR, et al. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk[J]. British Poultry Science, 1996, 37(2): 341-346.

[31] Chen J, Xie QX, Liu NZ, et al. Effects of compound probiotics and feed antibiotics on serum biochemical indices and intestinal enzyme activities of broilers[J]. Animal Husbandry and Feed Science, 2012, 33(3): 15-18.

[32] Xie WT, Zhong RC, Xu CH. Effects of compound probiotics on serum biochemical indices and duodenal histomorphology of broilers[J]. China Animal Husbandry and Veterinary Medicine, 2010, 37(8): 13-17.

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

*Source: ChinaXiv –Machine translation. Verify with original.*