Effects of honeycomb extract on the growth performance, carcass traits, immunity, antioxidant function and intestinal microorganisms of yellow bantam broilers

W. J. Song [*,1], Q. L. Song,[*,2] X. L. Chen,[*,1] G. H. Liu [†,*] Z. H. Zou,[*] J. Tan,[*] L. X. Liu,[*] and Y. B. Zeng[*]

[*]Institute of Animal Husbandry and Veterinary Science, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, P. R. China; and [†]Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China

ABSTRACT Although many studies have already described the physiological effects of bee products, such as honey, propolis, pollen, and royal jelly, on livestock farming, the health benefits of the honeycomb are still not fully understood. The problem of drug residues and bacterial resistance caused by the abuse of antibiotics is becoming increasingly serious. For this reason, a safe, green substitute has to be sought. We conducted a comparative study of honeycomb extract (**HE**) and an antibiotic on growth performance, carcass traits, immunity, antioxidant function and intestinal microorganisms of yellow bantam broilers. A total of four hundred eighty 21-day-old female yellow bantam broilers were randomly divided into 5 groups of 6 replicates of 16 birds each. The 5 groups were as follows, with birds receiving a basal diet supplemented with 150 ppm (mg/kg) of chlortetracycline (**CTE**), a basal diet without HE (control group), and a basal diet with 0.1%, 0.15%, or 0.2% HE for 60 days. The results showed that HE addition significantly increased average daily feed intake (**ADFI**), average daily gain (**ADG**), decrease feed gain ratio (**F/G**) from 21 to 80 and 51 to 80 days of age compared to the control group, with all 3 HE addition groups having statistically identical values to the antibiotic group. HE implementation dramatically increased spleen index, serum immunoglobulin A (**IgA**), immunoglobulin M (**IgM,**), glutathione peroxide (**GSH-Px**), superoxide dismutase (**SOD**), total antioxidant capacity (**T-AOC**), and total cecum bacteria and *Lactobacillus* compared to the control group, numerically at the same level as, or even better than, the antibiotic group. HE and CTE both markly reduced serum malondialdehyde (**MDA**) concentration compared to the control group, with higher concentrations of HE reducing the effect more dramatically than antibiotics. Both HE and CTE significantly raised dressed yield compared to the control group. In summary, HE, as a potential antibiotic alternative, improved growth performance, carcass traits, immune function, serum antioxidant capacity and intestinal microorganisms in yellow bantam broilers. According to the cubic regression analyses, the recommended supplemental dose of HE was calculated to be 0.15 to 0.17% for female yellow bantam broilers between 21 and 80 d of age.

Key words: honeycomb extract, growth performance, immune function, intestinal microorganisms, yellow bantam broilers

INTRODUCTION

The widespread use of antibiotics has greatly improved the growth performance of livestock and poultry, while the misuse of antibiotics in animal feed may result in antibiotic residues in animal products, making bacteria more resistant to drugs, and the consequences of this are a threat to people's health (Dipendra et al., 2017; Seal et al., 2013). Broilers are one of the fastest growing and very heavily farmed species in the livestock industry, yet the addition of antibiotics in farming has long been a common means used to address intestinal problems in broilers (Jadhav et al., 2015), but with the development of the times, the disadvantages of antibiotic use have been realized. Therefore, the search for alternatives to antibiotics in broiler feed is an increasing concern (Tayeri et al., 2018; He et al., 2019).

Honeycomb also called *Nidus Vespae* or *Beehive* (the hive of *Polistes olivaceous*, *P. japonicus* Saussure and *Parapolybiavaria fabricius*) is a nontoxic and natural resinous bee byproduct. Honeycomb contains many kinds of residues of bee products, such as beeswax, cocoons, honey, pollen, propolis, and royal jelly, and has been recognized to have several physiological and biochemical properties, including antibacterial, antioxidant, antimutagenic, antitumor, anti-inflammatory, and bactericidal properties (Gekker et al., 2005; Li et al., 2008; Alvarez-Suarez et al., 2010; Cheng et al., 2011; Astani et al., 2013; Talas et al., 2014; Zhao et al., 2015). These properties may be attributed to the active components in honeycombs, such as flavonoids, polysaccharides and polyphenols (Alani et al., 2019). In recent years, with the large-scale development of apiculture, bee products have been widely used in livestock and poultry production. Many studies have shown that the use of bee byproducts as additives in animal feed can improve the growth and productive performance of the animals. A significant increase in body weight, feed consumption, and feed conversion ratio and a significant decrease in mortality was observed when chicks were fed propolis (Shalmany and Shivazad, 2006). Propolis can relieve the injury caused by heat stress and improve the quality of animal products under heat stress conditions (Seven, 2008). Inspite of bee byproducts's broad applications, few studies have investigated the effect of honeycomb extract (**HE**) on animals, particularly in poultry.

Many countries, such as China, Turkey, Canada, Argentina, and Iran, are large bee producers with large-scale bee colonies (Shahbandeh, 2021). In addition, honeycombs have a certain cost advantage compared with other bee byproducts. The substantial honeycomb yields provided the material resources for the utilization of honeycomb residue as an antibiotic substitute in broilers.

The yellow bantam broiler is a breed bred from the introduction of the *dw* gene (Stewart et al., 1984) into high-quality yellow bantam broilers, which has characteristics such as high fertility, feed conversion and disease resistance, low space occupation higher. Yellow bantam broilers are popular with Chinese consumers for their tender meat and unique flavor. The effect of HE on the growth of yellow bantam broilers has not been reported yet. Previous results from our laboratory have shown that HE can improve the growth performance of broiler (data unpublished). Therefore, the aim of this study was to evaluate the effects of HE on growth performance, immune and antioxidant capacity and intestinal microorganisms of yellow bantam broiler, as well as its alternative role to antibiotics, to provide a theoretical basis and reference for further application of HE. In addition, research on the substitution of HE for antibiotics can improve food safety and quality and promote green, healthy and sustainable development of livestock and poultry farming.

MATERIALS AND METHODS

Animals and Raw Material Preparation

Animal care and procedures were carried out in accordance with the Chinese Animal Welfare Guidelines and approved by the Animal Care and Use Committee of the Jiangxi Academy of Agricultural Sciences under approval number 2010-JXAAS-XM-01. The original honeycombs used in this study were provided by the Jiangxi Academy of Agricultural Sciences, China. The honeycomb was mixed with alcohol in the ratio of 1:2, incubated overnight, 100°C for 1 h, then filtered, cooled and concentrated to obtain HE. The contents of total flavonoids, polysaccharides and polyphenols in HE was 8.63 mg/g, 0.490 g/100 g, and 98.6 mg/g, respectively.

Birds, Diets, and Management

A total of four hundred eighty 1-day-old female yellow bantam broilers were purchased from a local hatchery (Nanchang, Jiangxi), and after an acclimatization period ($0-20$ days of age), the birds were randomly allocated to 5 treatment groups with 6 replicates, each containing 16 birds. The treatments were as follows: the antibiotic group received a basal diet complemented with 150 ppm (mg/kg) chlortetracycline (**CTE**), and the other groups received a basal diet supplemented with 0.0% (control group), 0.1%, 0.15%, and 0.2% HE, respectively. The basal diet (in pellet form) was developed according to the Nutrition Research Council (1994) that met the requirements for native breed broilers (Table 1). Throughout the whole experimental

Table 1. Composition and nutrient levels of the basal diet (air-dry basis).

Items	Contents	
	$21-50$ d	$51-80$ d
Basal ingredients		
Corn, %	64.00	65.00
Soybean oil, %	3.00	4.00
Soybean meal (42.0% CP), %	26.00	26.00
Fish meal, %	2.00	-
Premix[1], %	5.00	5.00
Total, %	100.00	100.00
Nutrient level[2]		
Metabolism energy, MJ/kg	12.66	13.43
Crude protein, %	18.38	17.15
Calcium, %	0.90	0.70
Total phosphorus, %	0.60	0.45
Lysine, %	1.17	1.07
Methionine, %	0.45	0.42
Cysteine, %	0.31	0.30
Tryptophan, %	0.21	0.19
Threonine, %	0.70	0.64
Arginine, %	1.21	1.13

[1]The premix provided the following per kilogram of diet: vitamin A: 12500 IU; vitamin D_3: 500 IU; vitamin E: 25 mg; vitamin K_3: 3 mg; vitamin B_1: 3 mg; vitamin B_2: 8 mg; vitamin B_6: 7 mg; vitamin B_{12}: 0.03 mg; D-pantothenic acid: 20 mg; niacin: 50 mg;biotin:0.1 mg; folic acid:1.5 mg; Fe:100 mg;Cu:8 mg;Zn:100 mg;Mn:100 mg; I: 0.6 mg; Se: 0.16 mg.
[2]Estimated from the Chinese feed database, which provides tables of feed composition and nutritive values in China (2015 26th edition).

period (60 d), the birds were fed in individual cages with nets and housed in naturally ventilated windowed coops with temperatures between 23°C and 26°C, relative humidity between 65 and 75%, and illumination for 24 h/d (20 lux). Diets and water were provided ad libitum. Immunization and disinfection were carried out as per routine procedures.

Sample Collection and Carcass Trait Evaluation

All birds were fasted (water available) for 12 h and the body weight and feed intakes of each replicate was recorded on 21, 51, and 80 d. The average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed to gain ratio (**F/G**) were calculated from the body weight and feed intake data. Daily mortality records (if available) were maintained for each treatment. At the end of the experimental period, two broilers were randomly selected from each replicate based on their average body weight after a 12 h fast. Blood samples were collected from wing veins. These samples were placed in test tubes and centrifuged at $3,000g$ for 15 min to separate the serum, which was then stored at $-80°C$ for future determination of serum parameters.

Serum Parameters

The concentration of serum Immunoglobulin A (**IgA**), Immunoglobulin G (**IgG**), Immunoglobulin M (**IgM**) were determined using a BS-420 automatic biochemical analyser (Mindray, Shenzhen, China) with the appropriate detection kits (BioSino Bio-Technology & Science Inc., Beijing, China). The activities of superoxide dismutase (**SOD**), glutathione peroxidase (**GSH-Px**), the total antioxidant capacity (**T-AOC**), and the concentration of malondialdehyde (**MDA**) were determined with commercial assay kits (Jiancheng Bioengineering, Nanjing, China) using a DR-200BS enzymatic analyser (Hiwell diatek instruments Co., LTD, Wuxi, China) according to the protocols of the manufacturers. Sample quality control and testing parameter settings were strictly in accordance with the product specification. The results were normalized to liter of serum and are shown as U/L, nmol/L, and g/L, respectively.

Carcass Traits and Immune Organ Index

To assess carcass traits, at the end of the experiment (80 d of age), 2 birds of average weight were randomly selected from each replicate, weighed individually and sacrificed after 12 h of feed deprivation. Birds were dissected manually to determine carcass, breast muscle, leg muscle, and abdominal fat weights and yields. All yields were calculated as follows. Dressed weight = body weight - blood weight - feathers weight; half-eviscerated weight = dressed weight - (trachea + esophagus + crop + intestine + spleen + pancreas + gall bladder + reproductive organs + gizzard contents and membranes)

weights; eviscerated weight = half-eviscerated weight - (heart + liver + proventriculus + gizzard + fat around abdomen and gizzard + head + neck + claws) weights. Dressed yield = dressed weight/body weight × 100%; half-eviscerated yield = half-eviscerated weight/body weight × 100%; eviscerated yield = eviscerated weight/body weight × 100%; breast muscle yield = breast muscle weight/eviscerated weight × 100%; leg muscle yield = thigh muscle weight/eviscerated weight × 100%; abdominal fat yield = abdominal and gizzard/eviscerated weight × 100%. The immune organ index was recorded and expressed relative to body weight as follows: immune organ index = immune organ weight/body weight × 100%.

Intestinal Microbial Count

Following the collection of blood samples, the birds were euthanized by cervical dislocation and a postmortem examination was carried out. In the lab, on a sterile operating table, a sample of approximately 0.5 g of the chyme in cecum was collected, mixed with 10 mL of 0.9% NaCl solution and then diluted to different gradient concentrations. Finally, 100 μl of the bacterial dilutions were inoculated onto the corresponding agar medium plates. Total bacteria, *E. coli, Lactobacillus* and yeast were coated on nutrient agar (**NA**), eosin methylene blue agar (**EMB**), man rogosa sharpe medium (**MRS**), and yeast peptone dextrose adenine (**YPDA**) (Qingdao Hope Bio-Technology Co., Ltd. Qingdao, China) solid media, respectively. Total bacteria and *E. coli* were incubated for 24 h at 37°C, yeast was incubated for 36 h at 30°C in SPX-250BSH-II incubator (Xinmiao, Shanghai, China) and *Lactobacillus* for 36 h in a 37°C YQX-II anaerobic incubator (Shanghai Haixiang Inareumenr & Equipment Factory, Shanghai, China). At the end of the incubation, 2 replicates of each sample were counted. The number of colonies was determined by the following formula: colonies per gram of intestinal contents = Lg [(average number of colonies × dilution times × 10 mL/0.1 mL)/weight of the organism].

Statistical Analysis

Statistical analyses were performed by SPSS 24.0 (SPSS Inc., Chicago, IL). Data were subjected to one-way ANOVA, linear, quadratic, and cubic tests of variance and Levene's test. After ANOVA was significant, significant differences between treatments were determined by Tukey's multiple range test. The polynomial comparison method was used to test for linear and quadratic effects at the supplementary HE level. A cubic regression ($Y = b3 \times X^3 + b2 \times X^2 + b \times X + constant$) was fitted to the response of the dependent variable to the level of HE supplemented in the diet. The extreme response of HE was defined according to the derived equation of the cubic regression. Each broiler was considered as one experimental unit in each replicate. The

significance level was set at $P < 0.05$. All values are expressed as mean and standard error of the mean (**SEM**).

RESULTS

Growth Performance

Growth performance data are presented in Table 2. Results showed that dietary HE and CTE supplementation had significant increase in ADFI, ADG but a decrease in F/G, compared to the control group ($P < 0.05$), within d 21 to 80 and d 51 to 80. There was no difference in ADFI, ADG, and F/G between the 0.15% and 0.2% HE supplemented groups and the antibiotic group ($P > 0.05$).

Carcass Traits

Carcass traits of broilers are shown in Table 3. Compared to the control group, broiler dressed yield increased significantly ($P < 0.05$) in the 0.1% and 0.2% HE groups and half-eviscerated yield increased significantly ($P < 0.05$) in the all 3 HE groups. The dressed yield and half-eviscerated of broilers increased linearly and quadratically ($P < 0.05$) with higher HE addition. No significant differences in dressed yield or half-

eviscerated was observed between the 0.15%, 0.2% HE added, and antibiotic groups ($P > 0.05$). All treatments did not have any statistically significant effects on eviscerated yield, breast muscle yield, leg muscle yield, or abdominal fat yield ($P > 0.05$).

Immune Organ Index

As shown in Table 4, broilers receiving dietary supplementation of 0.1%, 0.15%, 0.2% HE or CTE exhibited a higher spleen index compared to the control group ($P < 0.05$) and there was no significant difference between broilers fed CTE or different concentrations of HE ($P > 0.05$). The varying treatments of the diets did not affect the thymus index and the bursa index ($P > 0.05$).

Serum Immunoglobulin Content

Serum immunoglobulin levels in broilers are summarized in Table 5. Serum IgA levels were significantly ($P < 0.05$) increased in broilers supplemented with 0.1%, 0.15%, 0.2% HE or CTE in the diet compared to the control group. Of which, dietary supplementation of 0.15% HE had the most significant effect on raising serum IgA levels ($P < 0.05$). Serum IgM was significantly increased in broilers supplemented with 0.15, 0.2% HE or CTE in the diet compared to the control

Table 2. Effect of honeycomb extract on the growth performance of broilers.

| Item | AB | Honeycomb extract, % | | | | SEM | P-Value | | |
		0.0(CON)	0.1	0.15	0.2		ANOVA	Linear	Quadratic
21−50 d									
ADFI, g/d	50.90	50.47	50.52	50.60	50.57	0.076	0.415	0.614	0.880
ADG, g/d	20.23	19.09	20.04	19.92	20.32	0.154	0.065	0.007	0.022
F/G	2.52	2.65	2.53	2.54	2.49	0.020	0.116	0.012	0.038
51−80 d									
ADFI, g/d	79.12[a]	72.52[b]	78.77[a]	78.25[a]	77.46[a]	0.589	0.001	0.005	<0.001
ADG, g/d	20.59[a]	16.56[b]	19.88[a]	20.28[a]	20.51[a]	0.389	0.002	<0.001	<0.001
F/G	3.86[b]	4.43[a]	3.98[b]	3.86[b]	3.79[b]	0.065	0.013	0.001	0.003
21−80 d									
ADFI, g/d	64.91[a]	61.91[b]	63.38[ab]	64.58[a]	63.40[ab]	0.302	0.010	0.168	0.034
ADG, g/d	20.36[a]	18.00[c]	19.45[b]	19.80[ab]	19.98[ab]	0.175	<0.001	<0.001	<0.001
F/G	3.19[b]	3.45[a]	3.26[b]	3.26[b]	3.17[b]	0.023	<0.001	<0.001	<0.001

Values are represented as the mean and SEM (n = 6).
AB: antibiotic group; ADG: average daily gain; ADFI: average daily feed intake; CON: control group; HE: honeycomb extract; F/G: feed to gain ratio; SEM: standard error of the mean.
[a-c]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 3. Effect of honeycomb extract on slaughter performance of broilers.

| Item | AB | Honeycomb extract, % | | | | SEM | P-Value | | |
		0.0(CON)	0.1	0.15	0.2		ANOVA	Linear	Quadratic
Dressed yield, %	88.57[ab]	88.09[b]	89.33[a]	89.19[ab]	89.61[a]	0.181	0.048	0.011	0.030
Half-eviscerated yield, %	81.66[ab]	80.66[b]	82.03[a]	82.50[a]	82.29[a]	0.193	0.018	0.004	0.008
Eviscerated yield, %	66.62	65.98	66.73	67.52	66.88	0.202	0.197	0.068	0.115
Breast muscle yield, %	31.35	32.45	30.74	32.00	31.07	0.370	0.599	0.307	0.495
Leg muscle yield, %	36.34	38.04	37.48	36.55	37.34	0.419	0.705	0.444	0.675
Abdominal fat yield, %	9.17	8.63	10.29	9.62	9.95	0.308	0.474	0.218	0.314

Values are the means and SEM of 12 broilers (2 broilers per replicate).
AB: antibiotic group; CON: control group; HE: honeycomb extract; SEM: standard error of the mean.
[a-b]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 4. Effects of honeycomb extract on the organ indices of broilers.

Item	AB	Honeycomb extract, %				SEM	P-Value		
		0.0(CON)	0.1	0.15	0.2		ANOVA	Linear	Quadratic
Spleen index, %	0.45[a]	0.27[b]	0.38[a]	0.40[a]	0.42[a]	0.015	<0.001	<0.001	0.001
Thymus index, %	0.25	0.23	0.28	0.27	0.25	0.009	0.422	0.386	0.132
Bursa index, %	0.14	0.13	0.15	0.15	0.15	0.005	0.588	0.225	0.229

Values are the means and SEM of 12 broilers (2 broilers per replicate).
AB: antibiotic control group; CON: control group; HE: honeycomb extract; SEM: standard error of the mean.
[a-b]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

group ($P < 0.05$). Serum IgM was increased in broilers supplemented with 0.15%, 0.2% HE or CTE diets compared to the control group ($P < 0.05$). There was no difference in serum IgA, IgG, and IgM between the HE and CTE supplemented groups ($P > 0.05$).

Serum Antioxidant Activity of Broilers

Table 6 shows the effect of HE supplementation in the diet on the activity of serum antioxidant enzymes and MDA content in broilers. All 3 HE-addition groups and antibiotic group had significant effects on GSH-Px, T-AOC, SOD, and MDA ($P < 0.05$). Serum GSH-Px, T-AOC, and SOD activities increased linearly and quadratically ($P < 0.05$) with increasing HE levels, MDA concentration decreased linearly and quadratically with increasing HE levels. Dietary supplementation with 0.15% HE resulted in the greatest effect on reducing serum MDA compared to the antibiotic group ($P < 0.05$), and the 0.15% and 0.2% HE supplemented groups showed higher GSH-Px, T-AOC, and SOD activities ($P < 0.05$).

Cecal Microorganisms

The data in Table 7 show the effect of HE on broiler cecal microorganisms. The total number of bacteria in the cecal increased significantly ($P < 0.05$) with the addition of HE to the diet compared to the control group. *Lactobacillus* was higher in the 0.2% HE added group than that in the control group ($P < 0.05$). For the above bacteria count, the antibiotic group had similar results, but the abundance of total cecal bacteria, *E. coli*, yeast and *Lactobacillus* did not change between the antibiotic and HE added groups in broilers ($P > 0.05$).

Optimal HE Level

As shown in Table 8, the data of the ADG from 21 to 50 d; ADFI, ADG, and F/G from 51 to 80 d; ADFI, ADG, and F/G from 21 to 80 d, dressed yield, half-eviscerated yield; spleen index; serum IgA, IgM, GSH-Px, MDA, T-AOC, and SOD levels, cecum total bacteria, *Lactobacillus* count were selected for further analysis by cubic regressions related to the HE level. The optimal HE levels that minimized F/G from 51 to 80 d, F/G

Table 5. Effects of dietary honeycomb extract on the serum immunoglobulin contents of broilers.

Item	AB	Honeycomb extract, %				SEM	P-Value		
		0.0(CON)	0.1	0.15	0.2		ANOVA	Linear	Quadratic
IgA, g/L	2.26[b]	2.10[c]	2.20[b]	2.35[a]	2.23[b]	0.016	<0.001	<0.001	<0.001
IgG, g/L	4.21	4.09	4.17	4.31	4.28	0.027	0.078	0.007	0.027
IgM, g/L	1.63[abc]	1.57[c]	1.61[bc]	1.70[a]	1.65[ab]	0.011	0.004	0.001	0.003

Values are the means and SEM of 12 broilers (2 broilers per replicate).
AB: antibiotic group; CON: control group; HE: honeycomb extract; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; SEM: standard error of the mean.
[a-c]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 6. Effects of honeycomb extract on serum antioxidant capacity in broilers.

Item	AB	Honeycomb extract, %				SEM	P-Value		
		0.0(CON)	0.1	0.15	0.2		ANOVA	Linear	Quadratic
GSH-Px, U/mL	522.70[c]	372.46[e]	443.08[d]	668.19[a]	603.89[b]	15.413	<0.001	<0.001	<0.001
MDA, nmol/mL	4.69[bc]	5.37[a]	4.94[ab]	3.99[d]	4.23[cd]	0.113	<0.001	<0.001	<0.001
T-AOC, U/mL	10.07[b]	8.03[c]	9.40[b]	12.94[a]	12.53[a]	0.303	<0.001	<0.001	<0.001
SOD, U/mL	86.40[b]	74.52[b]	82.09[b]	113.87[a]	102.40[a]	2.641	<0.001	<0.001	<0.001

Values are the means and SEM of 12 broilers (2 broilers per replicate).
AB: antibiotic group; CON: control group; GSH-Px: glutathione peroxide; HE: honeycomb extract; MDA: malondialdehyde; SEM: standard error of the mean; SOD: superoxide dismutase; T-AOC: total antioxidant capacity.
[a-e]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 7. Effects of honeycomb extract on cecum microorganisms of broilers.

Item	AB	Honeycomb extract, %				SEM	P-Value		
		0.0(CON)	0.1	0.15	0.2		ANOVA	Linear	Quadratic
Total bacteria, lg (CFU/g)	8.12[ab]	7.69[b]	8.28[a]	8.31[a]	8.49[a]	0.086	0.032	0.003	0.011
Escherichia coli, lg (CFU/g)	7.00	7.30	6.84	7.08	6.97	0.072	0.371	0.192	0.258
Yeast, lg (CFU/g)	7.46	7.10	7.28	7.59	7.80	0.081	0.057	0.003	0.010
Lactobacillus, lg (CFU/g)	8.12[b]	7.69[b]	8.51[ab]	8.48[ab]	9.09[a]	0.142	0.030	0.001	0.007

Values are the means and SEM of 12 broilers (2 broilers per replicate).
AB: antibiotic group; CON: control group; HE: honeycomb extract; lg: base-10 logarithm. SEM, standard error of the mean.
[a-b]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 8. Optimal HE level based on the equation model parameter.

Stages HE level /%	Items	Parameter estimate				R[2]	P	Optimal
		Constant	b1	b2	b3			
51−80 d	ADFI	72.520	143.439	−1025.833	2161.111	0.564	0.001	0.104
	ADG	16.562	59.654	−330.617	655.444	0.564	0.001	0.200
	F/G	4.426	−6.591	24.567	-37.556	0.440	0.010	0.200
21−80 d	ADFI	61.906	104.339	−1067.517	2915.222	0.365	0.031	0.177
	ADG	18.002	60.437	−596.450	1718.778	0.699	<0.001	0.156
	F/G	3.446	−5.255	48.817	−147.000	0.580	0.001	0.129
	Dressed yield	88.094	38.192	−364.083	1055.000	0.154	0.059	0.149
	Half-eviscerated yield	80.655	8.851	102.125	−528.056	0.196	0.021	0.163
	Spleen index	0.266	2.146	−12.560	28.651	0.437	0.003	0.200
	IgA	2.096	−5.483	99.750	−345.000	0.499	<0.001	0.160
	IgM	1.566	−2.860	50.458	−170.278	0.302	0.001	0.163
	GSH-Px	372.464	−8063.872	129295.333	−415951.111	0.869	<0.001	0.169
	MDA	5.367	30.571	−515.792	1672.500	0.542	<0.001	0.170
	T-AOC	8.027	−111.853	1839.917	−5840.556	0.700	<0.001	0.173
	SOD	74.515	−1226.401	19212.875	−61918.611	0.498	<0.001	0.167
	Total bacteria	7.692	14.436	−118.417	330.556	0.357	0.029	0.200
	Lactobacillus	7.685	28.403	−296.250	947.222	0.452	0.011	0.134

The data were fitted by a cubic equation, the dependent variables were the Items, and the independent variable was the HE level. b1, b2, and b3 indicate the coefficients of the cubic equation. R^2, fitting degrees of regression model.
ADFI: average daily feed intake; ADG: average daily gain; F/G: feed to gain ratio; GSH-Px: glutathione peroxide; HE: honeycomb extract; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; MDA: malondialdehyde; SOD: superoxide dismutase; T-AOC: total antioxidant capacity.

from 21 to 80 d and serum MDA content in broilers were 0.200%, 0.129%, and 0.170%, respectively. The maximum responses of ADG, ADFI from 51 to 80 d, ADG, ADFI from 21 to 80 d, dressed yield, half-eviscerated yield, spleen index, serum IgA, IgM, GSH-Px, T-AOC, SOD levels, and cecum total bacteria, *Lactobacillus* count were observed at 0.10%, 0.20%, 0.18%, 0.16%, 0.15% 0.16%, 0.20%, 0.16%, 0.16%, 0.17%, 0.17%, 0.17%, 0.20%, and 0.134%, respectively. Based on the above indices, the optimal HE level was 0.15 to 0.17%.

DISCUSSION

The honeycomb and its extracts have a variety of pharmacological activities, including antibacterial, anti-inflammatory, antiviral, antitumor, and anesthetic effects (Jin, 2007), and have been widely used in traditional Chinese medicine (**TCM**). HE contains flavonoids, polysaccharides, enzymes, peptides and other unique active ingredients that have growth-promoting effects on animals (Yan et al., 2006). In this study, improved feed intake and growth performance may have been associated with good health, digestion and absorption (Tayeb et al., 2014).

Broilers have an underdeveloped sense of taste but a relatively acute sense of smell. HE has an aromatic odor and a stimulating effect on salivary and gastric glands, and the addition of HE to the diet increased the ADFI of broilers, suggesting that HE may have some food-inducing effects. Many herbal extracts can slow down the emptying of the gastrointestinal tract (Manzanilla, 2004), stimulate the secretion of digestive juices (saliva, bile, mucus) (Platel, 2004) and increase the activity of digestive enzymes (Platel, 1996), G of the animals, which i/G of the animals, which is one of the possible reasons why HE leads to an increase in ADG and FCR in broilers. Many studies have confirmed that the addition of substances such as palygorskite-based antibacterial agent (Zha et al. 2021) and cetylpyridinium-montmorillonite (Ke et al. 2014) to feed can improve feed utilization in broilers in the later and whole stages of growth. We also found in our study that the number of intestinal probiotics became more abundant with the addition of HE, which may also promote the absorption of nutrients in the feed.

Flavonoids (Kamboh, 2013), polyphenols (Denli, 2004) and polysaccharides (Yang, 2019) contained in HE have been studied for growth promotion. In addition, nutrients are fully and completely digested (Seven, 2008;

Abass et al., 2017) with the help of components such as 4-hydroxybenzoic acid and benzoic acid in HE, so broilers fed HE supplements show higher ADG and lower F/G. Bee products have beneficial effects on broiler intestinal morphophysiology by increasing the contact area between the small intestine and chime (Prakatur et al., 2019), thus promoting feed absorption. Most importantly, HE maintains broiler health by altering the microbial community structure and creating a microbial balance in the gastrointestinal tract. Previous studies have reported that diets supplemented with propolis, royal jelly, honey, and bee pollen can improve the growth performance of quails, and that adding honey to the drinking water of broilers can increase weight gain and feed intake and reduce feed conversion ratio (Babaei et al., 2016; Emmanuel et al., 2016). The findings of the above study corroborate the results we obtained.

In this study, HE showed a cumulative effect on broiler growth performance as time progressed. During d 21 to 50, HE had no effect on ADFI and F/G, with a modest increase in ADG. From d 51 to 80, HE increased ADFI, ADG, and decreased F/G. These results suggest that the increase in early growth performance may be due to nutrient deposition and that the increase in later growth performance may be mainly due to feeding stimulation. The growth-promoting effect of HE in yellow bantam broiler was linearly and quadratically correlated with the level of supplementation, and it may be related to the concentration of different levels of active ingredients such as flavonoids. Studies have shown that high concentrations of flavonoids can be directly absorbed in the intestine and then exert relevant effects in gastrointestinal epithelial cells, endocrine cells, and immune cells, but low concentrations of flavonoids cannot be directly absorbed and must be metabolized by microbial breakdown into substances such as valerolactone and phenolic acids before they can be absorbed by intestinal cells (Appeldoorn, 2009; Zhang, 2016).

Carcass traits are a major indicator to evaluate poultry productivity and meat production performance. The honeycomb contains propolis, bee pollen, flavonoids, and polysaccharides, therefore, the suggested enhancement of slaughter performance in broilers may be related to the influence of these active ingredients. In the present study, HE showed linear and quadratic improvements in dressed yield and half-eviscerated yield in broilers, and these effects may be closely related to improved growth performance. Previous studies have pointed out that polysaccharides can significantly dressed yield and half-eviscerated yield of broilers (Sun et al., 2017), flavonoids can modify carcass traits in broilers (Yang et al., 2014), propolis can significantly boost carcass yield and breast weight in broilers (Seven et al., 2008), and the addition of bee pollen can significantly lift dressed yields with or without edible bowels (Abood and Ezzat, 2018).

With the ban on antibiotics in poultry diets, there will be a greater reliance on the poultry's own immune system to combat the various threats that may be encountered during growth. The thymus, bursa and spleen are the most important immune organs in poultry and it is generally accepted that immunosuppression is associated with a decrease in the relative weight of immune organs, while an increase in the relative weight of immune organs reflects an increase in immunity (Leticia et al., 2006). In this study, the addition of HE to the broiler diet had no effect on the thymus and bursa, which was somewhat of a departure from the previous findings (Emmanuel et al., 2016). May be due to differences in age. The bursa begins to develop from hatching and reaches its maximum size at 8 to 10 wk of age. It then enters the regression process, which is completed at 6 to 7 mo of age (Cazaban et al., 2015). In contrast, a linear and quadratic increase in spleen index was observed with the addition of HE to the diet. This is similar to the relevant results of previous authors who found that HE can enhance immune function by directly stimulating the proliferation of thymus and spleen cells (Wei et al., 1996). HE promotes spleen growth and development, increases spleen index, raises serum IgG, IgA, and IgM levels and enhances immune function in broilers. Honey bee by-products can fight viral infections through immunomodulation and can significantly improve humoral and cellular immunity by inhibiting the formation of prostaglandins, which are considered to be a highly immunoreactive substance (Taheri et al., 2005). Therefore, our findings suggest that HE significantly increases serum IgA and IgM levels, thus enhancing the immune function of broiler chickens, which is also similar to the findings of some previous studies on honeycomb (Zhu et al., 1999).

The antioxidant activity of HE in vitro (Hou et al., 2011) has been confirmed by previous studies. Honeycomb has favorable antioxidant and free oxygen radical scavenging abilities, including the ability to scavenge superoxide anions and hydroxyl radicals in vitro (Cheng et al., 2012). Both the aqueous and ethanolic extracts of honeycomb have beneficial free radical scavenging abilities. The fat-soluble and water-soluble components of honeycomb possess antioxidant activity. This study showed that HE significantly increased the enzyme activities in broiler serum, which is consistent with the findings of Wan (2013), that concluded that honeycomb significantly increased the activity of SOD and GSH-Px in serum, reduced MDA content and improved the antioxidant capacity of broilers. Propolis, royal jelly, and honey have good antioxidant properties (Kumazawa et al., 2004; Hang et al., 2008). The antioxidant activity of HE may be due to propolis and other antioxidant components. This is because of the presence of antioxidant peptides (Mckibben and Engeseth, 2002; Guo et al., 2009), as well as phenolic compounds including flavonoids, phenolic acids, caffeic acid, phenethyl esters, and terpenoids. They contribute to the metabolism and enhance the organic acids influenced by total polyphenols, thus playing an important role in the neutralization and absorption of free radicals (Balkanska et al., 2017).

HE has a good antibacterial effect, which has been studied and confirmed by many authors. HE has an inhibitory effect on the growth of *Staphylococcus aureus*, and this inhibition increases with increasing HE concentration (Gong et al., 2008). The aqueous extract of honeycomb showed some inhibitory effect on *Bacillus brevis, Staphylococcus aureus, E. coli, Bacillus cereus, Bacillus subtilis*, and *Salmonella* (Zhu et al., 1999). The alcoholic extract of the honeycomb showed good concentration-dependent inhibition of *S. aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Streptococcus haemolyticus* B, and *Streptococcus pneumoniae* (Cheng, 2012). The cecum is the part of the intestine with the highest probability of microbial colonization (Shi, 2019), so this experiment investigated the effect of HE on the microbiology of the cecum of yellow bantam broilers. The balance of the intestinal flora of livestock plays an essential role in optimizing production performance. The intestinal microorganisms consist mainly of pathogenic and probiotic bacteria. Most pathogenic bacteria are aerobic, while probiotic bacteria are anaerobic. In this study, HE promote the proliferation of *Lactobacillus* in the cecum of broiler, which is in general agreement with Zhang, 2011, who found that honeycomb could promote the proliferation anaerobic bacteria in the gut of broiler. Higher doses of honeycomb in the diet significantly reduced the number of *Enterobacteria* isolated from the chicken crop (Krocko et al., 2012). Our results showed that HE had a very slight inhibitory effect on *E. coli* in the cecum of broiler, which may be due to the level of HE in the diet. In addition, *E. coli* is a Gram-negative bacterium and the honeycomb showed better inhibitory effect on Gram-positive bacteria than Gram-negative bacteria (Sforcin et al., 2002; Chu and Hu, 2012; Bílikova et al., 2015). Therefore, the mechanisms by which HE improves growth performance may be regulation of the gut microbiota, nutrient savings due to reduced numbers of competing microorganisms, reduction or elimination of microorganisms that cause subclinical infections, and reduction of growth-inhibiting toxins or metabolites produced by the gut microbiota.

It is worth noting that the above indicators do not exist independently of each other, meaning that there is an interaction between them. The increase in the number of intestinal probiotic bacteria breaks down the nutrients in the feed more completely, and the products obtained from the complete breakdown provide the intestinal probiotic bacteria with a greater and more adapted source of energy. The increased immunity also provides for the growth and intestinal microorganisms of broilers. The end result is that the addition of HE improves all evaluation indicators in broilers and is no less effective than the commonly used antibiotics.

Finally, as this experiment did not clarify the chemical components of HE by techniques such as mass spectrometry or nuclear magnetic resonance spectroscopy, it is not possible to determine which substances play an active role in HE. This also provides a theoretical basis and research direction for our subsequent in-depth study.

CONCLUSION

In summary, this study shows that dietary supplementation of HE can improve growth performance, carcass traits and serum antioxidant capacity, modulate immune function and increase intestinal probiotic populations in female yellow bantam broilers. The effect of HE in broiler diets was at the same level as the addition of antibiotics to broiler diets, and even performed better in some indicators. It is a potential and valuable alternative to antibiotics. According to cubic regressions, most indicators were in the best zone when HE was added in the range of 0.15 to 0.17%.

AUTHOR CONTRIBUTIONS

Wenjing SONG, Qiongli SONG and Xiaolian CHEN designed the experiment and the analyzed data. Wenjing SONG, Qiongli SONG and Linxiu LIU conducted the experiments. Wenjing SONG and Jia TAN drafted the article. Guohua LIU and Zhiheng ZOU supervised the experiments and revised the manuscript. All the coauthors have seen a draft of the manuscript and agree with its publication.

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DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "Effects of honeycomb extract on the growth performance, carcass traits, immunity, antioxidant function and intestinal microorganisms of yellow bantam broilers".

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101811.

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