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Original Article

# Co-Supplementation of Dietary Seaweed Powder and Antibacterial Peptides Improves Broiler Growth Performance and Immune Function

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## ABSTRACT

The feasibility of *Laminaria japonica* powder (LJP) combined with cecropin as a dietary supplement to enhance broiler growth performance and immune function was evaluated in this study. In total, 648 one-day-old Arbor Acres broiler chicks were randomly distributed into nine numerically-equal treatment groups: $T_1$ (control group; fed a basal diet); $T_2$ (fed the basal diet supplemented with 1% LJP);$T_3$ (fed the basal diet supplemented with 300mg cecropin/kg); and $T_4$,$T_5$,$T_6$,$T_7$,$T_8$ and $T_9$, individually fed with the dietary supplemented with varying levels of LJP and cecropin). Compared with the control, dietary of LJP or cecropin supplementation slightly improved feed conversion ratio (FCR). However, the dietary supplementation of LJP combined with cecropin significantly improved broiler growth performance during the periods of 1-21,21-42, and 1-42 days ($p$<0.05).The dietary supplementation of 3% LJP combined with 300 mg/kg cecropin significantly increased FCR, and serum Newcastle disease antibody titers and lymphocyte numbers during the periods of 1-21, 21-42, and 1-42 days ($p$<0.05). Cecal microorganisms were cultivated and the number of *Escherichia coli* and *Lactobacillus* colonies were counted. The dietary supplementation of LJP combined with cecropin remarkably inhibited *E. coli* growth and increased *Lactobacillus* growth. The results of this study demonstrate the feasibility of using LJP and cecropin as feed supplement for improving the growth performance and enhancing the immune function of broilers.

## INTRODUCTION

There has been growing interest to control the use of antibiotics in poultry feeds over recent years because the high daily intake of antibiotic growth promoters (AGP) is considered a possible risk factor for the increasing microbial resistance against antibiotics, and the presence of AGP residues in animal products may cause serious health problems to the consumers (Castañon, 2007). Therefore, the need for AGP alternatives in poultry production is increasing and the contribution of seaweed may be considerable (Patterson & Burkholder, 2003). *Laminaria japonica*, a species of seaweed native to coastal waters, is extensively used in the food, animal feed, and pharmaceutical industries due to its abundant nutrient contents, including proteins, soluble dietary fibers, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids (Mohamed *et al.*, 2012; Abudabos *et al.*, 2013; Kulshreshtha *et al.*, 2014; Nitschke & Stengel, 2015). Moreover, *Laminaria japonica* has potential medicinal effects, as shown by the enhancement of the resistance of finishing pigs to gastrointestinal tract pathogens, of humoral immune function in piglets, and regulation of the gastrointestinal function and growth of pigs (Wang *et al.*, 2000; Leonard *et al.*, 2010). Bai *et al.* (2013) reported that the dietary addition of *Laminaria japonica* powder (LJP) enhanced the growth performance and improved the immune function

of chickens during the entire rearing period, and Wang *et al.* (2014) showed that LJP in broiler diets increased their metabolic and growth rates. However, annually, *Laminaria japonica* is mostly used as food and as raw material in pharmaceutical industry, and only a small volume is used in live stock production. Moreover, the growth period of *Laminaria japonica* is long.

Alternatively, antibacterial peptides not only can overcome these disadvantages mentioned above, but also have additional advantages, such as resistance to high temperature, safety, easy degradation and does not promote drug resistance. Antibacterial peptides have attracted great interest in the poultry industry over the past few decades (Izadpanah & Gallo, 2005; Rajanbabu & Chen, 2011; Li *et al.*, 2011; Abraham *et al.*, 2014; Park *et al.*, 2015). Previous reports on antibacterial peptides as feed additives have presented contradictory results because of their complex production process and high production cost. In order to overcome these disadvantages, Wang *et al.* (2011) proposed that dietary supplementation of antibacterial peptides combined with other additive could be more effective in improving the growth performance and immune function of poultry. However, until now, co-supplementation of dietary LJP and cecropin for improving the growth performance and immune function of broilers was rarely reported.

Cecropin extracted from *Bombyx mori* (silkworm) can improve the growth performance and immune function of poultry (Park *et al.*, 2015). Wen & He (2012) reported that cecropin had a positive quadratic effect on weight gain and increased nutrient utilization for both grower and finisher broilers. Therefore, in the current study, the influence of co-supplementation of dietary LJP and cecropin on the growth performance and immune function of broilers was first examined.

## MATERIALS AND METHODS

All experimental procedures were performed according to the National Institutes of Health Guidelines for Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Lüliang University, China (NIH Pub. No. 85-23, revised 1996).

### Materials

*Laminaria japonica* powder (LJP) was purchased from Qingdao Hanfeng Biotechnology Corporation (Shandong, China). The cecropin extracted from *Bombyx mori* was purchased from Shanghai Livestock Biotechnology Corporation (Shanghai, China).

### Bird management

A total of 648 one-day-old Arbor Acres broiler chicks (1 male:1 female) were purchased from Da Xiang company (Shanxi, China). Chicks were randomly distributed into nine treatment groups, with 6 replicates of 6 males and 6 females each (Table 1): $T_1$, fed the basal diet formulated to meet the nutritional requirement of chicks according to the NRC (1994) recommendations, Table 2); $T_2$, fed the basal diet and 1% LJP; $T_3$, fed the basal diet and 300mg cecropin/kg; and $T_4$, $T_5$, $T_6$, $T_7$, $T_8$ and $T_9$, each fed the basal diet supplemented with varying combinations of LJP and cecropin levels, as described in Table 1. The chicks were reared on the poultry farm of WenshuiJinKe Biological Ecology company (Shanxi, China), under well-controlled conditions and according to standard management practices. Birds were fed *adlibitum* for 42 days.

**Table 1 –** The distribution of 168 chicks in ninetrial groups

| Treatments | Number of chickens | Composition |
|---|---|---|
| $T_1$ | 12×6 | Control (basal diet) |
| $T_2$ | 12×6 | Basal diet +1%LJP |
| $T_3$ | 12×6 | Basal diet +300mg/kgcecropin |
| $T_4$ | 12×6 | Basal diet+1%LJP+300 mg/kgcecropin |
| $T_5$ | 12×6 | Basal diet+3%LJP+300 mg/kgcecropin |
| $T_6$ | 12×6 | Basal diet+5%LJP+300 mg/kgcecropin |
| $T_7$ | 12×6 | Basal diet+1%LJP+600 mg/kgcecropin |
| $T_8$ | 12×6 | Basal diet+3%LJP+600 mg/kgcecropin |
| $T_9$ | 12×6 | Basal diet+5%LJP+600 mg/kgcecropin |

### Growth performance

Growth performance was evaluated as described by Bai *et al.* (2013). The total body weight of each group was measured on d 1, 21, and 42, respectively, and expressed in g. Average daily gain (ADG) was calculated using the following equation:

$$ADG = \frac{M_2 - M_1}{7_{2n}}$$

$M_1$: total body weight of each measured on d 0;

$M_2$: total body weight of each group measured on d 21 and 42;

n: number of days of the rearing period (21 and 42 days)

The total feed intake of each group was individually determined by measuring feed residue on d 21 and 42, and expressed in g. Average daily feed intake (ADFI) was calculated using the following equation:

$$ADG = \frac{m_2 - m_1}{7_{2n}}$$

**Table 2 –** Composition of the basal diet.

| Ingredients | Content (%) | | Nutrient levels[2] | | |
|---|---|---|---|---|---|
| | 1-21d | 22-42d | | 1-21d | 22~42d |
| Corn | 57.90 | 62.51 | ME (MJ/kg) | 12.55 | 12.85 |
| Rapeseed meal | 3.00 | 4.00 | CP (%) | 21.49 | 21.38 |
| Cotton seed meal | 3.00 | 4.00 | Ca (%) | 1.00 | 0.80 |
| Soybean meal | 30.00 | 23.00 | Met (%) | 0.52 | 0.40 |
| Soybean oil | 1.50 | 2.20 | Lys (%) | 1.20 | 1.00 |
| $Ca(HCO_3)_2$ | 1.60 | 1.50 | | | |
| Limestone | 1.37 | 1.30 | | | |
| Salt | 0.30 | 0.30 | | | |
| Premix[1] | 1.00 | 1.00 | | | |
| Met | 0.19 | 0.10 | | | |
| Lys | 0.14 | 0.09 | | | |
| Total | 100.00 | 100.00 | | | |

[1]Provided per kilogram of diet: 8 mg copper,60 mg iron,40 mg zinc,50 mg manganese,0.2 mg selenium,0.2 mg iodine,10 000 IU vitamin A,2 750 IU vitamin D,20 IU vitamin E,2 mg vitamin K,1.5 mg thiamin,6 mg riboflavin,3.54 mg calcium,0.42 mg phosphorus,20 mg niacin,2 mg pyridoxine,0.5 mg; folacin,0.2 mgbiotin,200 mg choline chloride.

[2]Nutrient levels were the calculated values. Abbreviation: ME: metabolic energy; CP: crude protein; Ca: Calcium; Met: methionine; Lys: Lysine.

$m_1$: feed offer weight of each group on d 0;

$m_2$: feed residue weight of each group in the feeder on d 21 and 42;

n: number of days of the rearing period (21 and 42 days)

Feed conversion ratio (FCR) was calculated using the following equation:

$$FCR = \frac{X}{Y}$$

X: total feed intake (FI);

Y: total body weight gain (BWG).

## Newcastle disease immunization

Inactivated New castle disease virus (INDV) vaccine and Newcastle disease virus IV strain vaccine (NDV-IV) were purchased from Heilongjiang biological product Co., Ltd, Haerbin, P.R. China. Birds were immunized according to the procedure of Huang *et al.* (2014): 7-d-old birds were firstly vaccinated against NDV via intramuscular injection and against IV via eyedrop.

## Serum collection and processing

On d 21 and 42, two birds per replicate were randomly selected for blood collection from their wing veins. The sera were centrifuged and frozen at -20 °C. The serum was prepared according to the procedure of Virden *et al.*(2004)

## Newcastle disease antibody titering

Newcastle disease (ND) antibody titers were determined by hemagglutination and hemagglutination inhibition assays (Wang *et al.*, 2014). The above-mentioned serum samples were 2-fold serially diluted in a 96-well V-shaped bottom microtiter plate with 50 µL phosphate buffered solution (pH 7.4) in each well. Then, 50 µL of NDV antigen (4 HA units) was added into each well, except for the last row, which served as the control. Serum dilutions ranged from 1:2 to 1:2048. The plate was incubated at 37 ºC for 10 min, after which 50 µL of 1% rooster erythrocyte suspension was added to each well, and plates were re-incubated for 30 min. Positive and negative serum samples, erythrocytes, and antigens were also included as controls. The highest dilution of the serum that caused complete inhibition was considered the endpoint. Mean titer was expressed as reciprocal log2 values of the highest dilution that displayed hemagglutination inhibition assays.

## Nonspecific acid esteraselymphocyte assay

Nonspecific acid esteraselymphocyte percentage was determined by the acid a-naphthalene acetic acid esterase staining method (Ma *et al.*, 2015). At 21 and 42 day of age, the blood of two randomly selected birds per replicate was collected from the wing veins. One drop blood was uniformly smeared on a glass slide, which was dried by cool wind. The slide was immersed in a methanal-acetone solution (1:50) for 1minat 4°C, washed with double-distilled water for 3 min, and dried by cool wind at room temperature. Slides were then incubated in the incubation solution for 1.5 h at 37°C, washed with double-distilled water for 3 min, immersed in methyl green stain for 1 min, washed with double-distilled water for 3 min, dried at room temperature and the number of cells was counted under a microscope. Nonspecific acid esterase lymphocyte percentage (NAELP) was calculated using the following equation:

$$NAELP = \frac{N_1}{N_0} \times 100\%$$

$N_1$: number of acid-esterase positive cells;

$N_0$: total number of lymphocytes.

### Serum lysozyme activity assay

Serum lysozyme activity was measured using an ELISA kit purchased from Nanjing Institute of Biological Engineering. Serum samples were diluted 1:500 and incubated in 96-well microtiter plates, and the assay was performed according to the manufacturer's recommendations.

### *Escherichia coli* and *Lactobacillus* spp counts and immune organ index

The number of *E. coli* and *Lactobacillus* colonies as counted according to the method of Liu *et al.* (2013).

A volume of 0.5 g of cecal contents were recursively diluted in 4.5 mL sterile water under sterile conditions, and placed into a sterile incubator at 37°C. *E. coli* was cultured in Makanke media for 24 h and *Lactobacillus* was in MRS media for 48 h, respectively. After incubation, colonies were counted, and bacterial number was expressed as an algorithm relative to the entire bacterial community in 1 g intestinal contents [lg (CFU/g)].

On d 42, all birds were killed by cervical dislocation, and the spleen, bursa of Fabricius and thymus were collected and weighed to calculate immune organ index (g/kg) as the fresh weigh of the immune organ relative to live weight.

### Statistical analysis

The data were statistical analyzed using SAS 9.0. Treatments were compared using two-way analysis of variance (ANOVA), followed by Duncan's multiple comparison tests. For all statistical analysis, $p<0.05$ was considered to be significant.

## RESULTS

### Effect of the dietary co-supplementation of LJP and cecropin on broiler growth performance

In order to understand the effects of the dietary co-supplementation of LJP and cecropin on the growth performance, broiler BWG, ADG, ADFI and FCR were measured and the results are shown in Table 3.

**Table 3 –** Effects of dietary supplementation of LJP and cecropin on body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broilers.

| Treatments | | BWG, g | | ADG, g | | | ADFI, g | | FCR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1d | 21d | 42d | 1~21d | 22~42d | 1~42d | 1~21d | 22~42d | 1~42d | 1~21d | 22~42d | 1~42d |
| $T_1$ | 48.18±0.08 | 915.52±12.36 | 2560.56±31.32 | 38.18±1.22 | 81.72±3.12 | 109.99±5.78 | 55.52±2.36 | 166.04±3.24 | 215.22±1.22 | 1.56±0.04$^a$ | 1.92±0.01$^a$ | 1.88±0.05$^a$ |
| $T_2$ | 48.48±0.1 | 915.92±14.36 | 2630.56±35.32 | 38.38±1.63 | 82.81±3.16 | 111.99±5.63 | 55.81±2.63 | 166.36±3.64 | 215.18±1.12 | 1.56±0.04$^a$ | 1.90±0.01$^a$ | 1.87±0.05$^a$ |
| $T_3$ | 48.38±0.12 | 916.02±13.38 | 2635.56±38.31 | 38.48±1.22 | 83.22±3.1 | 112.29±6.05 | 56.12±2.45 | 166.28±2.94 | 215.24±1.21 | 1.56±0.04$^a$ | 1.89±0.01$^a$ | 1.86±0.05$^a$ |
| $T_4$ | 48.68±0.07 | 916.46±12.64 | 2642.55±41.24 | 38.68±2.10 | 83.82±1.24 | 113.22±6.54 | 56.46±2.64 | 166.52±2.20 | 215.30±1.04 | 1.55±0.03$^b$ | 1.88±0.03$^c$ | 1.85±0.04$^b$ |
| $T_5$ | 49.25±0.06 | 916.86±13.21 | 2664.54±25.44 | 39.25±1.02 | 83.94±2.54 | 114.32±5.24 | 56.86±3.21 | 167.94±2.44 | 216.40±1.00 | 1.54±0.02$^c$ | 1.87±0.01$^d$ | 1.81±0.03$^c$ |
| $T_6$ | 50.05±0.04 | 927.04±12.10 | 2786.51±28.46 | 40.05±1.24 | 86.96±1.16 | 119.38±4.22 | 57.04±2.10 | 171.44±1.58 | 218.84±1.56 | 1.51±0.01$^f$ | 1.86±0.01$^e$ | 1.72±0.06$^g$ |
| $T_7$ | 48.54±0.10 | 918.60±14.20 | 2664.54±34.42 | 38.54±3.20 | 83.98±5.14 | 118.13±4.84 | 56.60±4.20 | 168.54±2.84 | 217.62±1.82 | 1.54±0.05$^c$ | 1.89±0.04$^b$ | 1.79±0.04$^d$ |
| $T_8$ | 48.96±0.09 | 920.98±13.56 | 2682.53±48.24 | 38.96±2.40 | 84.34±3.22 | 115.45±7.22 | 56.98±3.56 | 169.36±3.06 | 217.12±1.72 | 1.53±0.04$^d$ | 1.88±0.03$^c$ | 1.78±0.01$^e$ |
| $T_9$ | 49.68±0.07 | 917.00±12.11 | 2690.52±38.94 | 39.68±1.56 | 86.12±1.40 | 117.35±5.12 | 57.00±2.11 | 169.62±2.56 | 217.86±1.64 | 1.52±0.05$^e$ | 1.87±0.05$^d$ | 1.76±0.03$^f$ |
| Principal effect analysis of LJP and cecropin | | | | | | | | | | | | |
| LJP addition | 1 | 48.61 | 917.53 | 2653.54 | 38.61 | 83.90 | 115.67 | 56.53 | 167.53 | 216.46 | 1.54$^a$ | 1.88$^a$ | 1.82$^a$ |
| | 3 | 49.10 | 918.92 | 2673.53 | 39.10 | 84.14 | 114.88 | 56.92 | 168.65 | 216.76 | 1.53$^b$ | 1.87$^b$ | 1.80$^b$ |
| | 5 | 49.86 | 922.02 | 2738.51 | 39.86 | 86.54 | 118.36 | 57.02 | 170.53 | 218.35 | 1.51$^c$ | 1.86$^c$ | 1.74$^c$ |
| cecropin addition | 300 | 49.33 | 920.12 | 2697.86 | 39.33 | 84.91 | 115.64 | 56.78 | 168.63 | 216.84 | 1.53 | 1.87$^b$ | 1.79$^a$ |
| | 600 | 49.06 | 918.86 | 2679.20 | 39.06 | 84.81 | 116.98 | 56.86 | 169.17 | 217.53 | 1.53 | 1.88$^a$ | 1.77$^b$ |
| *p*-value | | | | | | | | | | | | | |
| LJP | | 0.7214 | 0.6386 | 0.6682 | 0.6452 | 0.7124 | 0.7312 | 0.4386 | 0.5422 | 0.0762 | 0.0482 | 0.0312 | 0.0352 |
| cecropin | | 0.9842 | 0.9789 | 0.8674 | 0.8678 | 0.8688 | 0.8320 | 0.9789 | 0.8214 | 0.0854 | 0.3674 | 0.0320 | 0.0386 |
| LJP× cecropin | | 0.4518 | 0.5784 | 0.4264 | 0.5918 | 0.5287 | 0.6148 | 0.5784 | 0.2718 | 0.0652 | 0.0264 | 0.0248 | 0.0312 |

$^{a-g}$ values represent mean± SEM. Values within a column with different superscripts differ significantly ($p<0.05$).

Compared with the control, the BWG, ADG and ADFI of $T_2$ (basal diet + 1% LJP), $T_3$ (basal diet + 300 mg/kg cecropin), $T_4$ (basal diet + 1% LJP and 300 g/kg cecropin), $T_5$ (basal diet + 3% LJP and 300 g/kg cecropin), $T_6$ (basal diet + 5% LJP and 300 g/kg cecropin), $T_7$ (basal diet + 1% LJP and 600 g/kg cecropin), $T_8$ (basal diet + 3% LJP and 600 g/kg cecropin), $T_9$ (basal diet + 5% LJP and 600 g/kg cecropin) birds was no significantly different on d 21 and 42 ($p$>0.05). The FCR of the experimental groups fed different LJP levels combined with cecropin were not statistically different on d 21 ($p$>0.05), but were statistically difference on d 42 ($p$<0.05).The LJP and cecropin interaction did not significantly affect BWG, ADG, or ADFI on d 1, 21, and 42, but FCR significantly decreased from 1.82 to 1.77 at each stage ($p$<0.05).

No BWG, ADWG, or ADFI differences ($p$>0.05) were detected among treatments on d 21 or 42. However, considering the entire rearing period (d 1-42), the dietary supplementation of LJP and cecropin tended ($p$<0.10) to increase ADFI compared with the control treatment. Relative to FCR results, no differences were detected ($p$>0.05) among$T_1$ (basal diet), $T_2$ (basal diet + 1% LJP), $T_3$ (basal diet + 300 mg/kg cecropin) groups during none of the evaluated periods. However, during the period of 1-21 d, significantly lower FCR ($p$<0.05) was obtained with $T_6$, followed by $T_9$ (basal diet + 5% LJP and 600 mg cecropin/kg), $T_8$ (basal diet + 3% LJP and 600 mg cecropin/kg), $T_4$ (basal diet + 1% LJP and 300 mg cecropin/kg).

## Effects of dietary LJP and cecropin on serum ND antibody titers and immune organ index

The effect of dietary LJP and cecropin addition on the immune organ index of broilers was evaluated by serum NDV antibody titers and the results are shown in Table 4.

The dietary supplementation of LJP did not significantly influence HI or ANAE values determined on d 21and d 42 ($p$>0.05). However, the dietary supplementation of cecropin significant increased both HI and ANAE values on d 21 and d 42 ($p$<0.05). Moreover, the dietary supplementation of 1% LJP plus300 mg/kg cecropin, 3% LJP plus 300 mg/kg cecropin, 5% LJP plus 300 mg/kg cecropin, 1% LJP plus 600 mg/kg cecropin , 3% LJP plus 600 mg/kg cecropin and 5% LJP plus 300 mg/kg cecropin significantly increased Newcastle disease antibody titers and ANAE lymphocyte number on d 21 and 42 ($p$<0.05).

**Table 4 –** Effects of dietary LJP and cecropin on serum Newcastle disease antibody titers.

| Treatments | | 21d | | 42d | |
|---|---|---|---|---|---|
| | | HI $log_2N$ | ANAE$^+$% | HI $log_2N$ | ANAE$^+$% |
| $T_1$ | | 1.75±0.50$^d$ | 29.25±2.02$^d$ | 5.00±1.00$^d$ | 32.25±2.65$^d$ |
| $T_2$ | | 1.85±0.50$^{cd}$ | 30.45±2.12$^{cd}$ | 5.12±0.50$^{cd}$ | 33.65±2.36$^{cd}$ |
| $T_3$ | | 2.12±0.50$^{cd}$ | 31.10±2.32$^{cd}$ | 5.18±0.50$^{cd}$ | 34.10±2.42$^{cd}$ |
| $T_4$ | | 2.25±0.50$^{cd}$ | 31.50±2.52$^{cd}$ | 5.25±0.50$^{cd}$ | 34.50±2.52$^{cd}$ |
| $T_5$ | | 2.50±1.00$^c$ | 33.75±2.08$^c$ | 5.50±0.50$^c$ | 36.50±2.53$^c$ |
| $T_6$ | | 3.00±0.40$^a$ | 40.50±1.26$^a$ | 6.50±0.50$^a$ | 44.75±2.42$^a$ |
| $T_7$ | | 2.50±0.50$^c$ | 35.25±2.50$^{bc}$ | 5.50±1.00$^c$ | 37.50±2.40$^{bc}$ |
| $T_8$ | | 2.75±0.45$^b$ | 37.25±2.06$^b$ | 6.00±1.15$^b$ | 39.50±2.60$^b$ |
| $T_9$ | | 2.90±1.00$^{ab}$ | 39.25±2.52$^{ab}$ | 6.25±1.00$^{ab}$ | 42.50±2.50$^{ab}$ |
| Principal effect analysis of LJP and cecropin | | | | | |
| LJP addition | 1 | 2.37$^c$ | 33.37$^c$ | 5.37$^c$ | 36.00$^c$ |
| | 3 | 2.62$^b$ | 35.50$^b$ | 5.75$^b$ | 38.00$^b$ |
| | 5 | 2.95$^a$ | 39.87$^a$ | 6.37$^a$ | 43.62$^a$ |
| cecropin addition | 300 | 2.58$^b$ | 35.25$^b$ | 5.75$^b$ | 38.58$^b$ |
| | 600 | 2.72$^a$ | 37.25$^a$ | 5.92$^a$ | 39.83$^a$ |
| *p*-value | | | | | |
| LJP | | 0.0012 | 0.0076 | 0.0052 | 0.0062 |
| cecropin | | 0.0002 | 0.0001 | 0.0010 | 0.0001 |
| LJP× cecropin | | 0.0004 | 0.0001 | 0.0002 | 0.0001 |

$^{a-d}$ values represent mean± SEM. Values within a column with different superscripts differ significantly ($p$<0.05).

The results of the effect of LJP and cecropin dietary supplementation on immune organ (bursa, spleen, and thymus) indexes are shown in Table 5. Broilers fed diets supplemented with LJP (1%,3% and 5%), cecropin (300 mg/kg and600 mg/kg ) or 1% LJP plus 300 mg/kg cecropin, 3% LJP plus 300 mg/kg cecropin, 5% LJP plus 300 mg/kg cecropin, 1% LJP plus 600 mg/kg cecropin, 3% LJP plus 600 mg/kg cecropin and 5% LJP

**Table 5 –** Effect of dietary supplementation of LJP and cecropin on the immune function of broilers (g/kg).

| Treatments | | 21d | | | 42d | | |
|---|---|---|---|---|---|---|---|
| | | Bursal index | Spleen index | Thymus index | Bursal index | Spleen index | Thymus index |
| $T_1$ | | 2.48±0.06 | 0.93±0.17$^{c}$ | 4.48±0.14$^{c}$ | 1.56±0.09$^{e}$ | 1.25±0.17 | 5.05±0.14 |
| $T_2$ | | 2.49±0.04 | 0.94±0.15$^{c}$ | 4.79±0.14$^{c}$ | 1.58±0.09$^{e}$ | 1.26±0.14 | 5.08±0.14 |
| $T_3$ | | 2.50±0.03 | 0.95±0.14$^{c}$ | 4.82±0.13$^{c}$ | 1.61±0.08$^{e}$ | 1.27±0.13 | 5.10±0.13 |
| $T_4$ | | 2.52±0.02 | 0.96±0.12$^{bc}$ | 5.12±0.13$^{bc}$ | 1.64±0.08$^{d}$ | 1.28±0.12 | 5.12±0.13 |
| $T_5$ | | 2.58±0.02 | 0.99±0.15$^{bc}$ | 5.14±0.12$^{bc}$ | 1.78±0.05$^{c}$ | 1.30±0.15 | 5.24±0.12 |
| $T_6$ | | 2.76±0.04 | 1.06±0.11$^{a}$ | 6.45±0.11$^{a}$ | 2.16±0.04$^{a}$ | 1.38±0.11 | 5.64±0.11 |
| $T_7$ | | 2.65±0.06 | 1.02±0.13$^{b}$ | 5.21±0.15$^{b}$ | 1.81±0.06$^{bc}$ | 1.32±0.13 | 5.46±0.15 |
| $T_8$ | | 2.68±0.03 | 1.03±0.12$^{ab}$ | 6.05±0.14$^{ab}$ | 1.88±0.03$^{bc}$ | 1.35±0.12 | 5.53±0.14 |
| $T_9$ | | 2.72±0.06 | 1.04±0.11$^{ab}$ | 6.09±0.15$^{ab}$ | 1.95±0.06$^{b}$ | 1.36±0.11 | 5.56±0.15 |
| Principal effect analysis of LJP and cecropin | | | | | | | |
| LJP addition | 1 | 2.58 | 0.99$^{c}$ | 5.16$^{c}$ | 1.73$^{c}$ | 1.30 | 5.29 |
| | 3 | 2.63 | 1.01$^{b}$ | 5.60$^{b}$ | 1.83$^{b}$ | 1.33 | 5.38 |
| | 5 | 2.74 | 1.05$^{a}$ | 6.27$^{a}$ | 2.06$^{a}$ | 1.37 | 5.60 |
| Ceropin addition | 300 | 2.62 | 1.00$^{b}$ | 5.57$^{b}$ | 1.86$^{b}$ | 1.32 | 5.33 |
| | 600 | 2.68 | 1.03$^{a}$ | 5.78$^{a}$ | 1.88$^{a}$ | 1.34 | 5.52 |
| *p*-value | | | | | | | |
| LJP | | 0.9382 | 0.0376 | 0.0282 | 0.0211 | 0.0942 | 0.0914 |
| ceropin | | 0.9312 | 0.0382 | 0.0412 | 0.0358 | 0.0928 | 0.0956 |
| LJP×ceropin | | 0.8204 | 0.0284 | 0.0264 | 0.0201 | 0.0876 | 0.0902 |

$^{a-e}$ values represent mean± SEM. Values within a column with different superscripts differ significantly ($p$<0.05).

plus 300 mg/kg cecropin significantly increased spleen and thymus indexes on d21and bursa of Fabricius index on d 42 ($p$<0.05), but did not affect broilers' bursa of Fabricius index on d 21 and spleen index and thymus index on d 42 ($p$>0.05). The interaction of LJP and cecropin significantly increased broilers' spleen index and thymus index in broilers on days 21 and 42 ($p$<0.05).

## Effects of dietary LJP and cecropin on *E. coli* and *Lactobacillus* spp counts

*E. coli* and *Lactobacillus* results are shown in Table 6. The dietary supplementation of LJP did not significantly inhibit *E. coli* growth ($p$>0.05) and promoted *Lactobacillus* growth in the ceca of broilers on d 21 and 42 ($p$<0.05). The dietary supplementation of cecropin

**Table 6 –** Effect of dietary supplementation of LJP and cecropin on *E. coli* and *Lactobacillus* (lg CFU/g) in the cecum of broilers.

| Treatments | | 21d | | 42 d | |
|---|---|---|---|---|---|
| | | *E. coli* | *Lactobacillus* | *E. coli* | *Lactobacillus* |
| $T_1$ | | 7.24±0.05$^{a}$ | 7.85±0.04$^{c}$ | 8.14±0.02$^{a}$ | 8.50±0.03$^{d}$ |
| $T_2$ | | 7.04±0.04$^{a}$ | 8.05±0.04$^{c}$ | 8.04±0.04$^{a}$ | 8.53±0.05$^{d}$ |
| $T_3$ | | 6.85±0.03$^{a}$ | 8.10±0.04$^{c}$ | 7.74±0.05$^{a}$ | 8.55±0.05$^{d}$ |
| $T_4$ | | 6.70±0.02$^{ab}$ | 8.13±0.08$^{bc}$ | 7.51±0.06$^{b}$ | 8.54±0.08$^{cd}$ |
| $T_5$ | | 6.54±0.02$^{bc}$ | 8.17±0.03$^{bc}$ | 7.32±0.04$^{bc}$ | 8.76±0.05$^{bc}$ |
| $T_6$ | | 6.26±0.08$^{d}$ | 8.34±0.02$^{a}$ | 7.05±0.04$^{d}$ | 9.12±0.06$^{a}$ |
| $T_7$ | | 6.64±0.06$^{b}$ | 8.19±0.03$^{b}$ | 7.35±0.06$^{bc}$ | 8.62±0.02$^{c}$ |
| $T_8$ | | 6.42±0.04$^{bc}$ | 8.25±0.02$^{ab}$ | 7.20±0.02$^{c}$ | 8.83±0.02$^{b}$ |
| $T_9$ | | 6.30±0.02$^{c}$ | 8.28±0.04$^{ab}$ | 7.08±0.06$^{cd}$ | 8.94±0.06$^{ab}$ |
| Principal effect analysis of LJP and ceropin | | | | | |
| LJP addition | 1 | 6.67$^{a}$ | 8.16$^{c}$ | 7.43$^{a}$ | 8.58$^{c}$ |
| | 3 | 6.48$^{b}$ | 8.21$^{b}$ | 7.26$^{b}$ | 8.79$^{b}$ |
| | 5 | 6.28$^{c}$ | 8.31$^{a}$ | 7.06$^{c}$ | 9.03$^{a}$ |
| Cecropin addition | 300 | 6.50$^{a}$ | 8.21$^{b}$ | 7.29$^{a}$ | 8.81$^{b}$ |
| | 600 | 6.45$^{b}$ | 8.24$^{a}$ | 7.21$^{b}$ | 8.88$^{a}$ |
| *p*-value | | | | | |
| LJP | | 0.0222 | 0.0276 | 0.0271 | 0.0292 |
| cecropin | | 0.0302 | 0.0242 | 0.0413 | 0.0426 |
| LJP×ceropin | | 0.0204 | 0.0184 | 0.0251 | 0.0218 |

$^{a-d}$Values represent the mean. Values within a column with different superscripts differ significantly ($p$<0.05).

significantly inhibited *E. coli* growth on d 21 and 42 ($p$<0.05), but not significantly influence *Lactobacillus* growth ($p$>0.05).The dietary supplementation of 5% LJP combined with300 mg cecropin/kg and 5% LJP combined with 600 mg cecropin/kg significantly reduced *E. coli* counts and increased *Lactobacillus* counts on d 21 and 42 ($p$<0.05). The dietary supplementation of LJP (1-3%) combined with 300 mg cecropin/kg or 600 mg cecropin/kg significantly reduced *E. coli* counts on d 21 and 42 ($p$<0.05). However, the dietary supplementation of LJP (1-3%) did not significantly increased *Lactobacillus* counts ($p$>0.05).

# DISCUSSION

### Growth performance assays

The dietary supplementation of LJP significantly improved broiler FCR during all evaluated periods, and increased ADFI from 22 to 42 days and from 1 to 42 days. These results suggest that the dietary supplementation of LJP may have increased dietary energy content during the stage of rapidly increasing body weight (from day 22 to day 42), as previously demonstrated by Wong & Cheung (2001). Literature studies report that *Laminaria japonica* is rich in proteins, soluble dietary fibers, minerals, vitamins, phytochemicals and polyunsaturated fatty acids (Abudabos *et al.*, 2013; Kulshreshtha *et al.*, 2014; Mohamed *et al.*, 2012; Nitschke & Stengel, 2015). Moreover, the dietary supplementation of cecropin significantly improved broiler FCR from 22 to 42 days and from 1 to 42 days of age in the present experiment. Cecropin accelerates nutrient metabolism and improves nutrient utilization rate in broilers (Ganz, 2003). Wen & He (2012) reported that cecropin had a positive quadratic effect on weight gain and increased nutrient utilization in both grower and finisher broilers. Cecropin also reduces diarrhea incidence in chickens and enhances the absorption of ingested nutrients (Li *et al.*, 2008). Wang *et al.* (2007) reported that cecropin was more efficient than antibiotics for the prevention and control diarrhea of weaned piglets. Wu *et al.* (2012) reported that cecropin improved pig performance by enhancing their immune status and nitrogen and energy retention, as well as reducing intestinal pathogens in weaned piglets. Based on these results, it can be concluded that the dietary supplementation of LJP and cecropin may reduce diarrhea and enhance the absorption of ingested nutrients of broilers during the feeding stage.

The co-supplementation of LJP with cecropin significantly decreased broiler FCR during the periods of 1-21, 21-42, and 1-42 days of age ($p$<0.05). This result indicates that the supplementation of LJP and cecropin was able to accelerate nutrient absorption and utilization, and to enhance the live performance during the entire production cycle (Bouchet *et al.*, 2014), and demonstrate the synergistic effect of LJP and cecropin.

### Immune organ growth, ND titers and lymphocytes of broiler

The dietary supplementation of cecropin significantly increased the indexes of the spleen and thymus in 21-d-old broilers and of the bursa of Fabricius index in 42-d-old broilers, which consistent with the results of Michailidis *et al.* (2012). These results were also observed with the co-supplementation of LJP and cecropin. In 21- and 42-d-old broilers, the dietary supplementation of LJP or cecropin, and their co-supplementation increased spleen, thymus, and bursa indexes, indicating the synergistic effect of LJP and cecropin on immune organ indexes. It might be caused by the stimulation of cecropin leading to the development of the thymus, spleen and bursa of fabricius.

The dietary supplementation of LJP, cecropin and LJP combined with cecropin significantly increase ND titers and lymphocyte numbers on days 21 and 42. According to literature, the active substance of LJP, stimulates lymphocytes, changing their cell structure, which affects immunity (Immanuel *et al.*, 2012).In addition, because cecropin causes membrane permeabilization of both gram-positive and gram-negative bacteria, it kills the bacteria in the intestinal tract, improving the immune function (Hui *et al.*, 2002). These results demonstrated that cecropin and LJP act in synergy to enhance the immune function of broilers during the rearing period.

### The effect of LJP and cecropin on *E. coli* and *Lactobacillus* growth

The normal flora distribution in the intestines ensures the healthy growth of broilers (Crhanova *et al.*, 2011). The dietary supplementation of LJP, cecropin, or cecropin combined with LJP reduced the number of *E. coli* colonies, and increased the number of lactic-acid bacterial colonies in the ceca during the feeding period, which may be explained by the synergic antiseptic effect of LJP and cecropin in inhibiting *E. coli* growth. *Laminaria japonica* as the potential medicine

may enhance the resistance against gastrointestinal tract pathogens in fattening pigs, improve the humoral immune function of piglets, regulate gastrointestinal function and promote the growth of pigs (Wang *et al.*, 2000; Leonard *et al.*, 2010). It shown that cecropin causes membrane permeabilization of both gram-positive and gram-negative bacteria, killing these bacteria in intestinal tract (Hui *et al.*, 2002).On the other hand, the nutritional components of LJP may accelerate the replication of *Lactobacillus*, and optimize the intestinal microecological environment (Siahaan *et al.*, 2014; Radulovich *et al.*, 2015). In the present study, cecropin effectively inhibited the growth of competitive bacteria and increased lactic-acid bacteria number in the ceca of broilers. Previous literature studies reported that cecal microorganisms may be affected by the supplementation of either LJP or/and cecropin (Abdelsalam *et al.*, 2010; Radulovich *et al.*, 2015). It can be concluded that dietary supplementation of LJP combined with cecropin regulated the balance of the intestinal microbiota.

## CONCLUSION

This study demonstrated that the co-supplementation of 5% LJP combined with 300 mg/kg cecropin LJP and cecropin in conventional diets improved the growth performance and enhanced the immune function of broilers. The study indicated that the dietary supplementation of LJP combined with cecropin is a technically viable strategy for broiler production.

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