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*H Moradpour, F Kheiri, M Faghani, N Landy*

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## Influence of dietary hydrolyzed cottonseed protein supplementation on physiology and metabolism of Japanese quails

H. Moradpour, F. Kheiri\*<sup>ID</sup>, M. Faghani, N. Landy

Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, 8813733395, Iran

**ABSTRACT:** The current study was carried out to examine the effects of dietary supplementation of cottonseed protein hydrolysate (CPH) as an alternative to in-feed antibiotics on growth performance, carcass characteristics, immunity, jejunum morphology, coliform bacteria of ileum, and total antioxidant capacity (T-AOC) of serum in Japanese quails. A total of one hundred and ninety two 7-d-old mixed-sex broiler quails (*Coturnix japonica*) were allotted to 6 treatments each with 4 replicate pens of 8 chicks. Four dietary were formulated to contain 0 (basal diet as a control), 5, 6, and 7 g CPH/kg of diet in comparison with basal diet + 15 IU vitamin E/kg or basal diet + 2 mg lincomycin. At 35 d of age final body weights of broiler quails were not significantly affected by the dietary treatments although it tended to increase in broiler quails supplemented with vitamin E or 5 g CPH/kg of diet ( $P > 0.05$ ). The FCR of broiler quails fed diets containing antibiotic was significantly improved ( $P < 0.05$ ). The relative weight of bursa of fabricius was significantly higher in broiler quails supplemented with 5 g CPH/ kg of diet ( $P < 0.05$ ). The broiler quails fed diets containing antibiotic or 7 g CPH/kg of diet had significantly lower coliform bacteria counts ( $P < 0.05$ ). The T-AOC of serum was higher in boiler quails fed diets supplemented with vitamin E or different levels of CPH compared to those fed basal diet ( $P < 0.05$ ). In conclusion the findings of the present study indicated that supplementation of lincomycin could markedly improve FCR although; supplementation of 5 g CPH/kg of diet could induce favorable influences on coliform bacteria counts and T-AOC of serum and it can be used in broiler quails diet without any adverse effect on growth related parameters

**Keywords:** Antibiotic; Bird; Immunity; Hydrolyzed cottonseed protein; Total antioxidant capacity.

*Corresponding Author:*

Farshid Kheiri, Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, 8813733395, Iran  
E-mail Address: farshid\_kheiri@yahoo.com

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## INTRODUCTION

Different kind of antibiotics widely have been used as *growth promoters* in poultry farming at low levels for their positive effects on performance traits and health status of poultry (Landy et al., 2011; Nane-karani et al., 2012; Kheiri et al., 2018; Foroutankhah et al., 2019). The antibiotic growth promoters (AGPs) such as avilamycin, flavophospholipol and *virginiamycin* appearing to be capable to enhance performance traits of the birds by limiting the proliferation of the pathogenic bacteria which are strong of causing harm in the birds which a parasite or commensal organism lives, eventuating in greater nutrient digestion and absorption (Afiouni et al., 2023; Fekri Yazdi et al., 2014a,b; Shokraneh et al., 2016; Gheisari et al., 2017). In contrast to the fact that inclusion of AGPs in modern feeds for poultry resulting in multiple effects on performance traits, the constant supplementation of AGPs conducting to evolvement of numerous resistance bacteria which can cause disease (Toghyani et al., 2015), an increase in *accumulation* of *antibiotics* in poultry meat, and an imbalance in the microbiota (Andremont, 2000). As a result of quest for novel alternate *replacements* to AGPs, organic acids (Szott et al., 2022), probiotics (Landy and Kavyani, 2014), prebiotics (Yaqoob et al., 2021), phytochemicals and *natural* compounds derived from *medicinal herbs* (Goodarzi et al., 2013, 2014) and bioactive peptides (Landy et al., 2021; Landy and Kheiri, 2021) *had given* remarkable consideration.

Bioactive peptides are a group of peptides which *possess* biological activities besides their nutritional values (Hou et al., 2017). Numerous trials indicated that bioactive peptides possess *benefits* for *health*, which includes antioxidant (Landy et al., 2021), antihypertensive (Song et al., 2021), antibacterial (Baco et al., 2022) and immunomodulatory (Pavlicevic et al., 2022) activities according to the amino acid (AA) profiles, compositions and AA molecular mass (Hou et al., 2017). Zhong et al. (2022) stated that dietary supplementation of bioactive peptides derived from rapeseed protein improved growth performance, immune responses and antioxidant function of piglets. Abdollahi et al. (2018) reported that inclusion of bioactive peptides derived from soybean protein (PSP) in broiler chickens diet improved feed conversion ratio (FCR). Landy et al. (2021) reported that supplementation of cottonseed protein hydrolysate (CPH) in laying hen diets could improve egg quality and total antioxidant capacity (T-AOC) of egg. Landy et al. (2020) stated that inclusion of CPH in broiler diets

could improve performance traits, immune responses and T-AOC of serum. Despite these findings there has been dearth of information on the effect of bioactive peptides on broiler quail's nutrition. The aim of the current trial was to evaluate the effects of dietary CPH supplementation on performance traits, carcass characteristics, immunity, jejunum morphology, coliform bacteria of ileum, and total antioxidant capacity of serum in Japanese quails.

## MATERIALS AND METHODS

### Ethics Approval

The current trial was conducted at research farm of the Shahrekord University in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institute of Health, 2002) and all experimental methods were performed according to Experimental *Animal* Welfare and *Welfare Committee* of the Islamic Azad University, Shahrekord Branch, Iran (approval ref no. 2022-08).

### Animals and Dietary Treatments

A total of one hundred and ninety two 7-d-old mixed-sex broiler quails (*Coturnix japonica*) were allotted to 6 treatments with 4 replicates. Four dietary based on corn, soybean meal, corn gluten feed, and wheat bran were formulated according to the recommendations of

National Research Council (NRC, (1994)) to contain 0 (basal diet as a control), 5, 6, and 7 g CPH/kg (Fortide, Chengdu Mytech Biotech Co. Ltd., Chengdu, Sichuan, China) of diet in comparison with basal diet + 15 IU vitamin E/kg or basal diet + 2 mg lincomycin (recommended dosage by Maxey and Page, 1977). The broiler quails were fed dietary treatments in powder form during 7 to 35 d of age (Table 1). The experiment was carried out in such a way that the birds were raised under conditions that prevent from influence of environmental conditions. The feed and water were available *ad libitum*. The quail breeding house was closed off in every direction and the lighting program includes: 23h lighting, and 1h darkness d (Aguiar et al., 2017). The quail breeding house temperature was kept at 35°C, at the beginning of the experiment and gradually decreased by 3 °C/week, and finally fixed at 24 °C thereafter.

### Analysis of Cottonseed Protein Hydrolysate

Before the setting trial diets, corn, soybean meal,

**Table 1.** Composition and nutrient provided by dietary treatments in starting and growing periods.

Item	Cottonseed protein hydrolysate inclusion (CPH; g/kg)			
	0.0	5.0	6.0	7.0
Component, g/kg (as-fed)				
Mize (7.5% CP)	510.91	511.24	511.1	511.23
Soybean meal solvent (44% CP)	387.3	383.5	382.7	382.0
Corn gluten meal (55.4% CP)	30.0	30.0	30.0	30.0
Cottonseed protein hydrolysate (46% CP)	0.0	5.0	6.0	7.0
Wheat bran (14.9% CP)	30.0	29.0	29.0	28.70
Vegetable oil	8.60	8.59	8.64	8.60
L-methionine	0.21	0.17	0.16	0.16
<i>L-lysine hydrochloride</i>	1.12	1.06	1.05	1.04
L-threonine	1.43	1.43	1.43	1.43
Choline chloride	1.85	1.85	1.85	1.85
Mono calcium phosphate (15% Ca, 22.5% P)	8.76	8.79	8.80	8.81
Limestone	13.63	13.19	13.10	13.01
Common salt	1.4	1.42	1.42	1.43
Sodium hydrogencarbonate	2.79	2.76	2.75	2.74
Mineral supplement <sup>1</sup>	1.0	1	1	1
Vitamin supplement <sup>2</sup>	1.0	1	1	1
Calculated composition				
Metabolizable energy, kcal/kg	2,803	2,803	2,803	2,803
Crude protein (g/kg)	232.0	232.0	232.5	232.6
Lysine (g/kg)	13.0	13.0	13.0	13.0
Methionine (g/kg)	3.73	3.71	3.71	3.71
Methionine + Cysteine (g/kg)	7.50	7.50	7.50	7.50
Threonine (g/kg)	10.20	10.20	10.20	10.20
Tryptophan (g/kg)	2.79	2.80	2.80	2.80
Arginine (g/kg)	15.08	15.16	15.17	15.19
Valine (g/kg)	10.85	10.86	10.86	10.86
Isoleucine (g/kg)	9.80	9.80	9.80	9.80
Leucine (g/kg)	20.50	20.50	20.50	20.50
Calcium (g/kg)	8.0	8.0	8.0	8.0
Available phosphorus (g/kg)	3.0	3.0	3.0	3.0
Crude fat (g/kg)	36.45	36.33	36.28	36.31
Crude fibre (g/kg)	33.70	33.43	33.37	33.33
Sodium (g/kg)	1.58	1.58	1.58	1.58
Chlorine (g/kg)	1.67	1.67	1.67	1.67
Potassium (g/kg)	9.66	9.64	9.56	9.54
DCAB	268	268	266	265
Analyzed amount				
Crude protein (g/kg)	232	230	231	230

<sup>1</sup> Supplied the following per kilogram of feed: *Manganese*, 60 mg; *Iron*, 120 mg; *Copper*, 5 mg; *Zinc*, 25 mg; *Selenium*, 0.2 mg; *Iodine*, 0.3 mg. <sup>2</sup> Supplied the following per kilogram of feed: *vitamin A*, 1,650 IU; *vitamin D<sub>3</sub>*, 750 IU; *vitamin E*, 12 IU; *vitamin K*, 1.0 mg; *thiamin (B1)*, 2.0 mg; *riboflavin (B2)*, 4.0 mg; *niacin*, 40 mg; *pantothenic acid*, 10 mg; *pyridoxine*, 3 mg; *biotin*, 0.3 mg; *folic acid*, 1 mg; *vitamin B<sub>12</sub>*, 0.003 mg.

corn gluten feed, wheat bran and CPH were considered for the amount of crude protein (AOAC, 2006), and the content of total AAs (AOAC, 2006). The CPH take the measurements of total phosphorus and calcium of by Inductively coupled plasma - optical emission spectrometry (AOAC, 1965). The molecu-

lar weight distribution of the BPC was measured by a Superdex peptide HR 10/30 column as described by Jung et al. (2006).

#### Performance and Carcass Traits

The body weight (BW) was measured at the be-

ginning and end of the experiment. Average daily feed intake (DFI) and daily weight gain (DWG) during the experiment (7-35 d) were determined at the end of experiment. The feed conversion ratios (FCR) were determined by dividing DFI to DWG. At 35 d of age two male broiler quails were selected to meet middle weight of the pen, separately weighed and slaughtered by cutting of the neck. Carcass yield and the relative weight of internal organs and lymphoid organs were computed by dividing eviscerated weight, internal organs and lymphoid organs (spleen and bursa of fabricius) weight to live BW.

### Jejunal Morphology

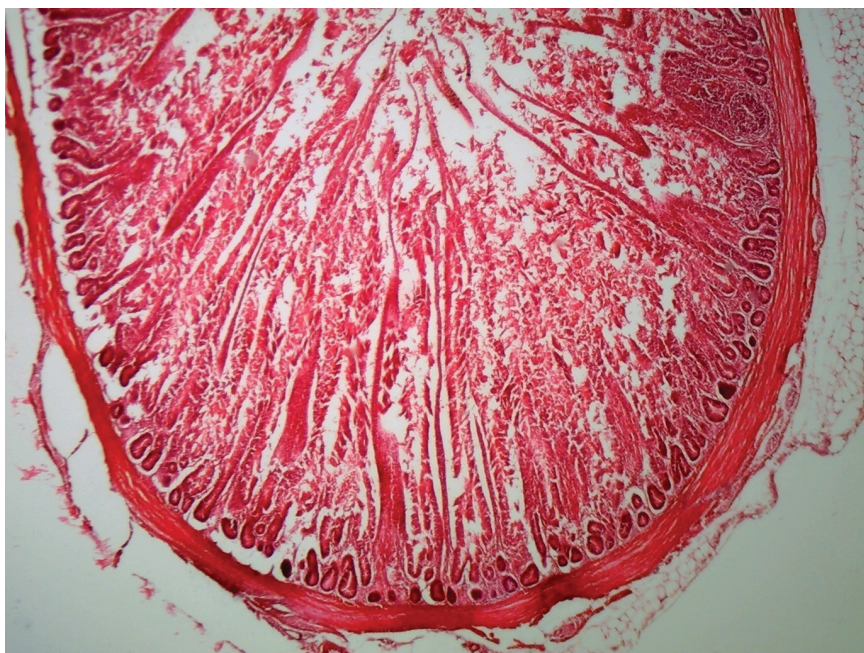
At 35 d of age, two male broiler quails were chosen to meet middle weight of the pen, slaughtered and the small intestine without any intervening time were separated from the carcasses. Shortly after removing of small intestine nearly 2 cm of *proximal jejunum* were collected and the samples were stabled in 10% neutral formalin, dehydrated in a serialized ethanol subsequence to *fix firmly in* paraffin and according to the method explicated by Iji et al. (2001). Paraffin sections were stained with hematoxylin and eosin at 6 m and examined by light microscopy (Olympus Co. Ltd., BX 50, F-3, Tokyo, Japan). Morphological parameters consist of epithelial thickness, villus width (VW), crypt depth (CD), and villus height (VH) were determined as shown in Figure 1. The ratios of VH to CD were computed by dividing VH by CD (Song et al., 2014).

### Coliform Bacteria Count

At 35 d of age, two broiler quails from each replicate were slaughtered and *digesta samples from ileum were removed and pooled*. Coliform bacteria population from the ileum were roughly calculated by serial dilution (10-1 to 10-9) of the contents of the ileum digest in anaerobic diluents before transferring the inoculum to Petri dishes containing sterile agar to obtain an incubated plate with a colony count of countable convenience was estimated as explicated by Gonal et al. (2007). MacConkey Agar has been used for the *isolation and differentiation of* coliforms. After incubation, for 24 h to 48 h at 37 °C, coliform bacterial colony populations were enumerated.

### Immunity

In 10 d of age, the birds were orally vaccinated against Newcastle disease virus (NDV) using a commercial *B-1 strain vaccine*. At 18 d of age the *blood samples were taken from brachial vein and NDV-specific titers in the serum were measured by* hemagglutination inhibition (HI) test as explicated by Ball et al. (2019). The measured antibodies obtained from HI test were converted to  $\log_2$ . At 35 d of age, total protein and albumin concentrations in serum samples were measured according to the methods explicated by Cannon (1974) and Doumas et al. (1971), respectively. The quantity of globulin in serum was measured by computing difference between the content of total protein and albumin of sera (Narain et al., 1961).



**Fig. 1** Villus height and crypt depth of broilers at 35 d of age.

**Table 2.** Analysis of the cottonseed protein hydrolysate.

Composition of cottonseed protein hydrolysate, g/kg	
Total protein (N × 6.25)	464.4
Peptides with molecular weight < 1000 Da	180.0
<i>Arginine</i>	41.2
<i>Histidine</i>	12.0
<i>Isoleucine</i>	16.0
<i>Leucine</i>	26.2
<i>Lysine</i>	30.6
<i>Methionine</i>	9.4
<i>Cysteine</i>	8.0
<i>Phenylalanine</i>	21.0
<i>Threonine</i>	14.2
<i>Valine</i>	19.5
<i>Glycine</i>	17.3
<i>Alanine</i>	17.5
<i>Proline</i>	22.7
<i>Serine</i>	23.5
<i>Aspartic acid</i>	58.5
<i>Glutamic acid</i>	93.0
<i>Tyrosine</i>	12.8
<i>Tryptophan</i>	6.0
<i>Calcium</i>	33.0

### Total Antioxidant Activity of Serum

At end of the experiment, 2 broiler quails/pen were chosen and the *blood samples* were collected and the broiler quails were slaughtered afterward. The *serum samples* were separated from the bloods by centrifuging for 15 minutes at 3,000 rpm. The samples from mid *jejunal* segments were separated, and cleaned via precooling physiological saline. After filter paper cleaning, pieces of jejunum were accurately weighed and prepared with 9 times the volume of physiological saline made from ground 10% tissue homogenate. The obtained semi-liquid mixtures were centrifuged at 1,346 rpm for 10 min and the supernatants were transferred to test tubes and stored at 20 °C for future use. The obtained samples take the measurements of the Coomassie brilliant blue method as described by Zhao et al. (2016).

### Statistical Analysis

The current experiment carried out as a completely randomized design, and *obtained* data were examined methodically using the General Linear Model procedures of SAS (SAS Institute Inc., Cary, NC, 2012). The obtained means were considered using a post-hoc Tukey test at 5% significance.

$$Y_{ij} = m + t_i + e_{ij}$$

## RESULTS

### Performance and Carcass Traits

No mortalities occurred during the experiment. The effects of supplementation of different levels of CPH in broiler quail's diet in comparison with inclusion of excessive level of vitamin E or subtherapeutic dosage of lincomycin on performance criteria of broiler quails have been indicated in Table 3. Final BW of broiler quails were not significantly affected by the dietary treatments although it tended to increase in broiler quails supplemented with 5 g CPH or excessive level of vitamin E ( $P > 0.05$ ). DFI of broiler quails were not affected by the addition of excessive level of vitamin E, antibiotic or CPH. The FCR of broiler quails fed diets containing lincomycin significantly reduced compared to those fed 7 g CPH/kg of diet, also the FCR of broiler quails fed diets containing 5 g CPH/kg tended to improve whereas the results were not statistically significant ( $P > 0.05$ ).

Table 4 indicated carcass yield and relative weight of internal organs at 35 d of age. Carcass yield and relative weight of proventriculus, gizzard, liver, cecum, and heart were not markedly affected by inclusion of additives. The relative weight of pancreas was significantly increased by inclusion of lincomycin and the lowest relative weight of pancreas obtained in the group supplemented with 6 g CPH/kg of diet

**Table 3.** Effect of dietary lincomycine, Vitamin E and hydrolyzed cottonseed protein inclusion on average daily weight gain, average daily feed intake, and feed conversion ratios of broiler quails during the experiment.

Item	Experimental treatments						SEM**	P-value
	Control	Lincomycine	Vitamin E	5 g CPH*/kg	6 g CPH*/kg	7 g CPH*/kg		
<b>Body weight, g</b>								
35 d of age	185	187	192	192	185	186	3.74	0.58
<b>Daily feed intake, g/d</b>								
7 to 35 d of age	15.5	14.1	16.1	15.2	15.5	16.4	0.72	0.32
Daily weight gain, g/d								
7 to 35 d of age	5.7	5.8	5.9	6.0	5.7	5.7	0.12	0.49
<b>Feed conversion ratio, g:g</b>								
7 to 35 d of age	2.71 <sup>ab</sup>	2.43 <sup>b</sup>	2.71 <sup>ab</sup>	2.54 <sup>ab</sup>	2.74 <sup>ab</sup>	2.87 <sup>a</sup>	0.08	0.03

\* Cottonseed protein hydrolysate. \*\* Standard error of mean.

**Table 4.** Effect of dietary lincomycine, Vitamin E and hydrolyzed cottonseed protein inclusion on carcass yield and relative weight of organs of broiler quails at 35 d of age.

Relative organ weight	Experimental treatments						SEM**	P-value
	Control	Lincomycine	Vitamin E	5 g CPH*/kg	6 g CPH*/kg	7 g CPH*/kg		
Carcass (%)	69.5	70.5	73.3	70.5	70.1	72.3	2.07	0.77
Proventriculus (%)	0.43	0.47	0.41	0.46	0.42	0.48	0.03	0.42
Gizzard (%)	2.13	2.10	1.91	2.27	2.05	2.29	0.14	0.5
Liver (%)	2.41	2.51	2.34	2.53	2.36	2.20	0.14	0.63
Pancreas (%)	0.33 <sup>ab</sup>	0.37 <sup>a</sup>	0.29 <sup>ab</sup>	0.29 <sup>ab</sup>	0.26 <sup>b</sup>	0.31 <sup>ab</sup>	0.02	0.05
Cecum (%)	0.78	0.66	0.55	0.74	0.66	0.62	0.06	0.17
Heart (%)	0.71	0.77	0.79	0.76	0.77	0.80	0.06	0.92
Spleen (%)	0.07	0.07	0.05	0.09	0.07	0.08	0.01	0.39
Bursa of Fabricius(%)	0.11 <sup>ab</sup>	0.10 <sup>ab</sup>	0.09 <sup>b</sup>	0.14 <sup>a</sup>	0.08 <sup>b</sup>	0.11 <sup>ab</sup>	0.001	0.02

\* Cottonseed protein hydrolysate. \*\* Standard error of mean. <sup>a-b</sup> Values in the same row not sharing a common superscript differ  $P < 0.05$ .

( $P < 0.05$ ). The relative weight of bursa of fabricius was significantly higher in broiler quails supplemented with 5 g CPG/ kg of diet ( $P < 0.05$ ). The percentage of spleen as a lymphoid organ was not affected by the dietary treatments although it tended to increase in broiler quails supplemented with 5 g CPH/kg of diet ( $P > 0.05$ ).

### Morphometric Analysis of the Jejunum

The effects of CPH, lincomycin and excessive level of vitamin E on morphology of jejunum at 35 d of age are summarized in Table 5. The VH was not markedly affected by the dietary treatments whereas it tended to increase in broiler quails supplemented with lincomycin or 5 g CPH/kg of diet ( $P > 0.05$ ). CD and epithelial thickness were not affected by the dietary treatments. Villus width was significantly higher in broiler quails fed basal diet compared to those fed diets containing lincomycin ( $P < 0.05$ ). VH to CD ratios were not affected by the dietary treatments although it tended to improve in broiler quails supplemented with lincomycin, or 7 g CPH/kg of diet ( $P > 0.05$ ).

### Coliform Bacteria Count

Table 6 indicated coliform counts of samples from the ileum of broiler quails at 35 d of age. The lowest coliform counts obtained in the groups fed diets containing antibiotic or 7 g CPH and the highest obtained in the group fed basal diet ( $P < 0.05$ ).

### Immune Responses

The effects of antibiotic, CPH and excessive level of vitamin E on antibody titers against NDV and albumin to globulin ratios have been shown in Table 7. Assessed parameters were not affected by the dietary treatments.

### Total Antioxidant Capacity of Serum

As illustrated in Table 8, T-AOC of serums were significantly higher in broiler quails fed diets containing lincomycin, CPH and excessive levels of vitamin E ( $P < 0.05$ ). The highest T-AOC obtained in the group supplemented with excessive level of vitamin E furthermore it doesn't markedly different from those fed different levels of CPH.

**Table 5.** The effects of dietary lincomycine, Vitamin E and hydrolyzed cottonseed protein supplementation on morphological responses of jejunum in broiler quails at d 35 d of age.

Variables	Experimental treatments						SEM**	P-value
	Control	Lincomycine	Vitamin E	5 g CPH*/kg	6 g CPH*/kg	7 g CPH*/kg		
Villus height (µm)	67	90	71	84	79	65	7.65	0.22
Crypt depth (µm)	9.5	16.5	13.0	12.0	13.0	12.5	1.50	0.11
Epithelial thickness (µm)	1.0	1.5	1.0	1.0	1.5	1.5	0.20	0.18
Villus width (µm)	18.0 <sup>a</sup>	13.0 <sup>b</sup>	17.0 <sup>ab</sup>	17.5 <sup>ab</sup>	15.0 <sup>ab</sup>	15.0 <sup>ab</sup>	0.95	0.02
Villus height to crypt depth ratio	7.1	5.4	5.9	7.9	6.0	5.18	1.19	0.57

\* Cottonseed protein hydrolysate. \*\* Standard error of mean. <sup>a-b</sup> Values in the same row not sharing a common superscript differ  $P < 0.05$ .

**Table 6.** Influence of dietary lincomycine, Vitamin E and hydrolyzed cottonseed protein inclusion on ileum coliform counts ( $\log_{10}$ ) of broiler quails at 35 d.

Bacterial count	Experimental treatments						SEM**	P-value
	Control	Lincomycine	Vitamin E	5 g CPH*/kg	6 g CPH*/kg	7 g CPH*/kg		
Coliform	90 <sup>a</sup>	11 <sup>b</sup>	35 <sup>ab</sup>	26 <sup>ab</sup>	34 <sup>ab</sup>	10 <sup>b</sup>	17.9	0.02

\* Cottonseed protein hydrolysate. \*\* Standard error of mean. <sup>a-b</sup> Values in the same row not sharing a common superscript differ  $P < 0.05$ .

**Table 7.** Efficacy of dietary lincomycine, Vitamin E and hydrolyzed cottonseed protein supplementation on antibody titers against Newcastle virus and albumin to globulin ratios at 35 d of age of broiler quails.

Variables	Experimental treatments						SEM**	P-value
	Control	Lincomycine	Vitamin E	5 g CPH*/kg	6 g CPH*/kg	7 g CPH*/kg		
New castle ( $\log_2$ )	3.25	3.62	3.50	3.75	3.37	3.37	0.22	0.64
Albumin***	0.97	1.09	1.10	1.03	0.81	0.99	0.15	0.75
Globulin***	1.18	1.09	1.17	1.03	0.88	0.99	0.18	0.86
Albumin to globulin ratio	0.92	1.03	0.99	1.04	0.98	1.03	0.18	0.99

\* Cottonseed protein hydrolysate. \*\* Standard error of mean. \*\*\*mg/100 ml

**Table 8.** Effects of dietary treatments on total antioxidant capacity (T-AOC) levels (U/mg prot) of broiler quail's serum at 35 d of age.

Variables	Experimental treatments						SEM**	P-value
	Control	Lincomycine	Vitamin E	5 g CPH*/kg	6 g CPH*/kg	7 g CPH*/kg		
T-AOC of serum	0.46 <sup>b</sup>	0.76 <sup>a</sup>	0.89 <sup>a</sup>	0.88 <sup>a</sup>	0.88 <sup>a</sup>	0.87 <sup>a</sup>	0.22	0.64

\* Cottonseed protein hydrolysate. \*\* Standard error of mean. <sup>a-b</sup> Values in the same row not sharing a common superscript differ  $P < 0.05$ .

## DISCUSSION

### Performance and Carcass Characteristics

In the current experiment supplementation of different levels of CPH couldn't induce any marked effect on DFI. In agreement with our results, Abdollahi et al. (2017, 2018) stated that supplementation of different levels of PSP couldn't affect DFI of broiler chickens. In the current trial, addition of additives couldn't significantly affect final BW of broiler quails although it tended to increase in broiler quails fed diets containing lincomycin or 5 g CPH/kg. Similarly, Abdollahi et al. (2017, 2018) reported that supplementation of broiler

chickens diet with PSP couldn't markedly affect final BW. Wang (2005) reported that addition of bioactive peptides in broiler chickens diet could improve final BW at termination of the experiment (21 d of age). Landy et al. (2020) reported that supplementation of 5 g CPH in broiler chickens diet increased DFI resulting in increments in final BW. In the present experiment, no clear relationship between DFI and final BW was observed, so the observed tendency to improve final BW was probably happened by other reasons. Feng et al. (2007) reported that supplementation of fermented soybean meal to the diet of broiler chickens increased

the activity of enzymes secreted from the pancreas. Unfortunately, in the present experiment efficacy of CPH on the activity of pancreatic enzymes have not been investigated; thus further investigations are required to declare efficacy of bioactive peptides on the activity of pancreatic enzymes. As indicated in Table 6, coliform bacteria count of ileum in broiler quails fed diets containing lincomycin or 7 g CPH were significantly lower than the control group. As reported by Bedford (2000) antibiotics can reduce the growth and colonization of gut bacteria colonies, which can improve the health of the digestive system, improves growth and FCR. Antimicrobial peptides may be rapid-acting and potent, and contain an unusually broad spectrum of activity; consequently, it seems that they may be used as new antibiotics in the future; although the studies which have been done to date have proven the topical efficacy of these compounds (Hancock and Sahl, 2006). According to Song et al. (2020) the CPH may be a favorable source of natural antibacterial so the observed tendency to reduce FCR in the present trial may be due to antibacterial peptides which can modulate gut microflora.

In the current study the relative weight of pancreas significantly decreased by addition of 6 g CPH/kg of diet; it also tended to decrease in broiler quails fed diets containing 5 g CPH/k. Abdollahi et al. (2017) reported that inclusion of different levels of PSP in broiler chickens diet couldn't induce any significant effects on relative weight of internal organs. Similarly, Landy et al. (2020) stated that supplementation of different levels of CPH had not any significant effects on carcass yield, and relative weight of proventriculus, gizzard, liver, pancreas, small intestinal, heart, and spleen. The main function of the pancreas is to secrete digestive enzymes which are especially effective in protein digestion. It has been reported that there is a close relationship between the secretion of digestive enzymes from the pancreas and the digestion and absorption of nutrients (Harmon, 2009; Xu et al., 2009). Brenes et al (1993) reported that supplementation of enzyme in boiler chickens diet significantly reduced the relative weight of pancreas. It seems that there is a relationship between required enzyme for digestion of feed and relative weight of pancreas; thus it seems that the lower relative weight of pancreas which have been observed in the present experiment may be due to higher pancreatic enzyme activity. In agreement, Feng et al. (2007) reported that supplementation of fermented soybean meal to the diet of broiler chickens increased the activity of enzymes secreted from the pancreas.

### Morphometric Analysis of the Jejunum

In the current trial, addition of lincomycin in broiler diet reduced VW compared to other groups. Sayra-

fi et al (2011) reported that supplementation of AGP in broiler diet had not any marked effects on VW in different segments of small intestine. In contrast with our findings, Markovic et al (2009) reported that AGP induce enlargement of VW which can be correlated with beneficial effects of AGP on intestinal microflora. In the present study, VH tended to enhance in broiler quails supplemented with lincomycin or 5 g CPH/kg of diet. Similarly, Landy and Kheiri (2023) reported that VH and CD tended to increase in broiler chickens fed diets containing 5 or 6 g CPH/kg of diet. In agreement with findings of the present study, Abdollahi et al. (2017) reported that the length of VH and CD tended to enhance in broiler chickens fed diets supplemented with different levels of PSP. Osho et al. (2019) reported that inclusion of PSP in broiler chickens diet could improve VH/CD ratio of jejunum. Since, morphological parameters of jejunum, such as VH, CD, and VH to CD ratio are correlated with its digestive capacity thus its traits have been evaluated in many experiments. The positive effects of bioactive peptides on small intestine morphology have been reported in several trials (Liu et al., 2008; Bao et al., 2009; Wen and He, 2012). VH to CD ratio tended to reduce in broiler chickens fed diets containing lincomycin. Miles et al (2006) reported that supplementation of bacitracin methylene disalicylate (BMD) or virginiamycin (VM) in broiler diet could induce favorable influences on intestinal morphology. The beneficial effects of feeding AGPs, such as VM and BMD, have been reported by several researchers (Bafundo et al., 2003; Ferket, 2004) and will not be repeated herein.

### Coliform bacteria count

In the present study supplementation of lincomycin in broilers diet could reduce the coliform bacteria counts of ileum. As reported by Ferket (2004) antibiotics can limit the growth and proliferation of pathogenic and non-pathogenic bacteria in the digestive tract of poultry. In the current trial addition of antibiotic or 7 g CPH in broiler quails diet could decrease coliform bacteria counts of ileum. Similarly, Landy and Kheiri (2023) stated that inclusion of antibiotic or 6 g CPH in broiler chickens diet could markedly decrease coliforms counts of ileum. Findings of an experiment indicated that bioactive peptides derived from hydrolysis of cottonseed protein have antibacterial activity (Han et al., 2016). Song et al. (2020) considered antibacterial activity of cottonseed protein hydrolysate against *Escherichia coli* (*E. Coli*); the re-

searchers stated that separated peptides such as KD-FPGRR, LGLRSGIILCNV, and DENFRKF indicated high antibacterial activity against *E. Coli*. Bioactive peptides can cause membrane damage through interaction with the surface of the cell membrane and eventually kill the cell. In summary, our data indicated that the CPH can be use as a natural antibacterial in feed.

### Immune Responses

In the current trial inclusion of different levels of CPH and antibiotic had not any marked effects on antibody titers against NDV and albumin to globulin ratios whereas the relative weight of bursa of fabricius has been increased in broiler quails fed diets containing 5 g CPH/ kg of diet. Landy and Kheiri (2023) reported that addition of CPH in broiler chickens diet couldn't induce any marked effects on immune related parameters except for antibody titers against AIV which was greater in broiler chickens fed diets containing 6 g CPH/kg of diet; furthermore, they reported that the relative weight of bursa tended to increase in broiler chickens fed diets containing 6 g CPH/kg of diet. Similarly, Abdollahi et al. (2017) reported that the relative weight of bursa and spleen tended to increase in broiler chickens fed diets containing PSP. In birds, the bursa of fabricius is a specialized organ which is essential for the development of B cells; therefore, its maturation and growth directly affects the function of the immune system. As reported by Osho et al. (2019) bioactive peptides may increase the production of TNF- $\alpha$ , IL-8, IL-10, and IL-6, thereby modulating immune responses, but it not happened in the current study; which may be due to the lack of effective stimulation of the immune system.

### Total Antioxidant Capacity of Serum

In the current trial addition of excessive levels of vit E (as a positive control) and lincomycin could markedly increase T-AOC of serum in broiler quails. Mahmoud et al. (2007) investigated the effect of vitamin E on antioxidant status of broiler chickens; the results indicated that vitamin E supplementation elevated antioxidant enzyme activities. Similarly, Niu et al. (2018) reported that dietary vitamin E improves meat quality and antioxidant capacity in broilers by upregulating the expression of antioxidant enzyme genes. In agreement with obtained results in the cur-

rent study Chen et al. (2023) investigated the effects of sulfamethoxazole (SMZ) and florfenicol (FLO) on antioxidant capacity in pacific white shrimp *Litopenaeus vannamei* ; the results indicated that the glutathione S-transferase activity of shrimp exposed to SMZ or FLO was significantly higher than that in the control group. In the present experiment T-AOC of serum in broiler quails has been increased by addition of different levels of CPH. Similarly, Landy and Kheiri (2023) reported that supplementation of CPH in broiler chickens diet increased T-AOC of jejunum and serum. T-AOC of jejunum or serum describes the ability of total enzymes related to antioxidant activity and related to the removal of free radicals (Ren et al., 2012). As reported by Zhang et al. (2010) bioactive peptides which have potential to increase T-AOC may donate hydrogen from AA to terminate the oxidation chain reaction. Consistently, Girgih et al. (2015) reported that peptides containing aromatic AA have the ability to become antioxidants by donating electrons. Davalos et al. (2004) reported that peptides containing hydrophobic AA residues such as Met, Cys, and His have radical scavenging effects. As shown in Table 2, the protein hydrolyzate investigated in the present study has a significant amount of hydrophobic AA.

### CONCLUSIONS

In conclusion, supplementation of CPH in broiler quails diet had no significant effect on growth performance, intestinal morphology, and immune related parameters, on the other hand supplementation of lincomycin could markedly improve FCR during the experiment; the results indicated that supplementation of 5 g CPH/kg of broiler quails diet could induce favorable influences on coliform bacteria counts and T-AOC of serum and it can be used in broiler quails diet without any adverse effect on growth related parameters.

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### CONFLICT OF INTEREST

The authors disclose that there is no conflict of interest.

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