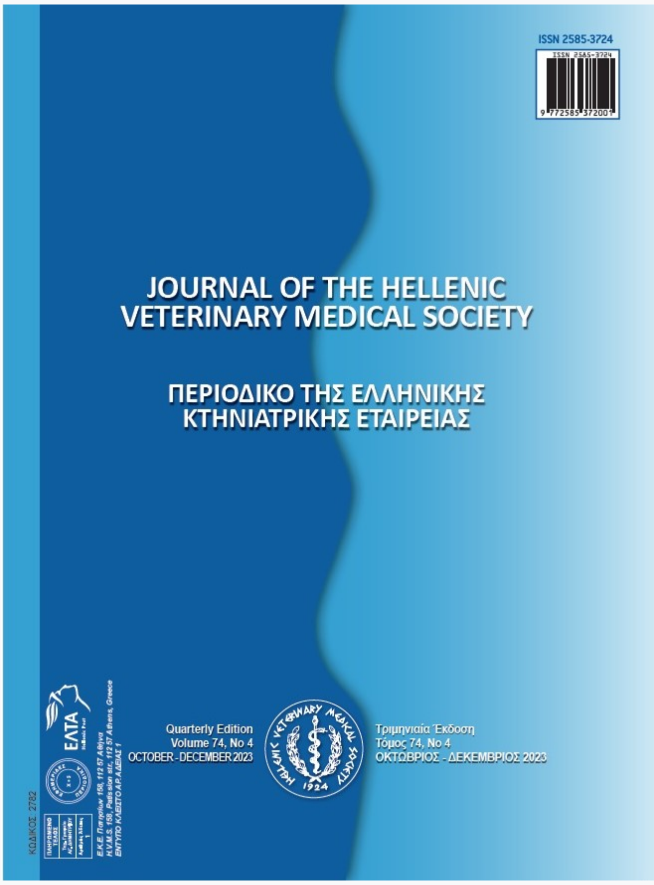
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 1 (2024)



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

H Moradpour, F Kheiri, M Faghani, N Landy

doi: [10.12681/jhvms.33448](https://doi.org/10.12681/jhvms.33448)

To cite this article:

Moradpour, H., Kheiri, F., Faghani, M., & Landy, N. (2024). Influence of dietary hydrolyzed cottonseed protein supplementation on physiology and metabolism of Japanese quails. *Journal of the Hellenic Veterinary Medical Society*, *75*(1), 6925–6936. https://doi.org/10.12681/jhvms.33448

J HELLENIC VET MED SOC 2024, 75 (1): 6925-6936
ΠΕΚΕ 2024, 75 (1): 6925-6936

Research article
Ερευνητικό άρθρο

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

H. Moradpour, F. Kheiri*, 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

Date of initial submission: 04-02-2023
Date of acceptance: 18-07-2023

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; Nanekarani 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 a*ccumulation* 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* Wellness 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.

	Cottonseed protein hydrolysate inclusion (CPH; g/kg)			
Item	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
L-lysine hydrochloride	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[1]	1.0	1	1	1
Vitamin supplement[2]	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

[1] 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. [2] Supplied the following per kilogram of feed: vitamin A, 1,650 IU; vitamin D_3, 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_{12}, 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 molecular 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 beginning 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
Arginine	41.2
Histidine	12.0
Isoleucine	16.0
Leucine	26.2
Lysine	30.6
Methionine	9.4
Cysteine	8.0
Phenylalanine	21.0
Threonine	14.2
Valine	19.5
Glycine	17.3
Alanine	17.5
Proline	22.7
Serine	23.5
Aspartic acid	58.5
Glutamic acid	93.0
Tyrosine	12.8
Tryptophan	6.0
Calcium	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.

$$Yij = m + ti + eij$$

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		
Body weight, g								
35 d of age	185	187	192	192	185	186	3.74	0.58
Daily feed intake, g/d								
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
Feed conversion ratio, g:g								
7 to 35 d of age	2.71^{ab}	2.43^{b}	2.71^{ab}	2.54^{ab}	2.74^{ab}	2.87^{a}	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^{ab}	0.37^{a}	0.29^{ab}	0.29^{ab}	0.26^{b}	0.31^{ab}	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^{ab}	0.10^{ab}	0.09^{b}	0.14^{a}	0.08^{b}	0.11^{ab}	0.001	0.02

* Cottonseed protein hydrolysate. ** Standard error of mean.a-b 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^{a}	13.0^{b}	17.0^{ab}	17.5^{ab}	15.0^{ab}	15.0^{ab}	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. $^{a-b}$ 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^{a}	11^{b}	35^{ab}	26^{ab}	34^{ab}	10^{b}	17.9	0.02

* Cottonseed protein hydrolysate. ** Standard error of mean. $^{a-b}$ 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^{b}	0.76^{a}	0.89^{a}	0.88^{a}	0.88^{a}	0.87^{a}	0.22	0.64

* Cottonseed protein hydrolysate. ** Standard error of mean. $^{a-b}$ 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. Sayrafi 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 miroflora. 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 researchers stated that separated peptides such as KDFPGRR, 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-α, 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 current 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**.**

ACKNOWLEDGEMENTS

The authors thank Shahrekord branch of Islamic Azad University for provision of a breeding house and university laboratories.

CONFLICT OF INTEREST

The authors disclose that there is no conflict of interest.

REFERENCES

Afiouni A, Toghyani M, Landy N (2023) Performance, morphological responses of the small intestine, and humoral immunity of broilers fed oak acorn (*Quercus brantii*) as a substitution for antibiotic growth promoters. Iran J Appl Anim Sci 13: 321-331.

AOAC (1965) Official methods of analysis. 10th ed. Washington (DC): AOAC.

AOAC (2006) Official methods of analysis. 18th ed. Washington (DC): AOAC.

Abdollahi MR, Zaefarian F, Gu Y, Xiao W, Jia J, Ravindran V (2017) Influence of soybean bioactive peptides on growth performance, nutrient utilisation, digestive tract development and intestinal histology in broilers. J Appl Anim Nutr 5:1-7.

Abdollahi MR, Zaefarian F, Gu Y, Xiao W, Jia J, Ravindran V (2018) Influence of soybean bioactive peptides on performance, foot pad lesions and carcass characteristics in broilers. J Appl Anim Nutr 0:1-7.

Aguiar GC, Freitas ER, Watanabe PH, Figueiredo CWS, Silva LP, Nascimento GAJ, Lima RC, Nepomuceno RC, S´a NL (2017) Lighting programs for male and female meat quails (*Coturnix coturnix*) raised in equatorial region. Poult Sci 96:3122-3127.

Andremont A (2000) Consequences of antibiotic therapy to the intestinal ecosystem. Ann Fr Anesth Reanim 19:395-402.

Baco N, Oslan SNH, Shapawi R, Mohhtar RAM, Noordin WNM, Huda N (2022) Antibacterial activity of functional bioactive peptides derived from fish protein hydrolysate. Earth Environ Sci 967: 012019.

Bafundo KW, Cox LA, Bywater R (2003) Review lends perspective to recent scientific findings on virginiamycin, antibiotic resistance debate. Feedstuffs 75:26-27.

Ball C, Forrester A, Herrmann A, Lemiere S, Ganapathy K (2019) Comparative protective immunity provided by live vaccines of Newcastle disease virus or avian metapneumovirus when co-administered alongside classical and variant strains of infectious bronchitis virus in day-old broiler chicks. Vaccine 10: 7566-7575.

Bao H, She R, Liu T, Zhang Y, Peng KS, Luo D, Yue Z, Ding Y, Hu Y, Liu W, Zhai L (2009) Effects of pig antibacterial peptides on growth performance and intestine mucosal immune of broiler chickens. Poult Sci 88:291-297.

Bedford M (2000) Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. Worlds Poult Sci J 56:347-365.

Brenes A, Smith M, Guenter W, Marquardt RR (1993) Effect of enzyme supplementation on the performance and digestive tract size of broiler chickens fed wheat- and barley-based diets. Poult Sci 72: 1731-1739.

Cannon DC, Olitzky I, Inkpen JA, Henry RJ, Winkelman JE (1974) Protein Cli Chem. In: Clinical chemistry principles and techniques. 2th ed. Harper and Row, Hagerstown, Md: pp 407-421.

Chen Y, Zhou L, Yu Q, Li E, Xie J (2023) Effects of sulfamethoxazole and florfenicol on growth, antioxidant capacity, immune responses and intestinal microbiota in pacific white shrimp *Litopenaeus vannamei* at low salinity. Antibiotics 12:575.

Davalos A, Miguel M, Bartolome B, Lopez-Fandino R (2004) Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. J Food Prot 67:1939-1944.

Doumas BT, Watson WA, Bigg HG (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31: 87-96.

Fekri Yazdi F, Ghalamkari GH, Toghiani M, Modaresi M, Landy N (2014a) Anise seed (*Pimpinella anisum* L.) as an alternative to antibiotic growth promoters on performance, carcass traits and immune responses in broiler chicks. Asian Pac J Trop Dis 4:447-451.

Fekri Yazdi F, Ghalamkari GH, Toghiani M, Modaresi M, Landy N

(2014b) Efficiency of *Tribulus terrestris* L. as an antibiotic growth promoter substitute on performance and immuneresponses in broiler chicks. Asian Pac J Trop Dis 4(Suppl. 2):S1014-8.

Feng J, Liu X, Xu ZR, Wang YZ, Liu JX (2007) Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. Poult Sci 86:1149-1154.

Ferket PR (2004) Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. In: Nutritional Biotechnology in the Feed and Food Industries. ed. Nottingham Univ. Press, Nottingham, UK : pp 57-67.

Foroutankhah M, Toghyani M, Landy N (2019) Evaluation of *Calendula officinalis* L. (marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers. Anim Nutr 5:314-318.

Gheisari A, Shahrvand S, Landy N (2017) Effect of ethanolic extract of propolis as an alternative to antibiotics as a growth promoter on broiler performance, serum biochemistry and immune responses. Vet World 10:249-54.

Girgih AT, He R, Hasan FM, Udenigwe CC, Gill TA, Aluko RE (2015) Evaluation of the *in vitro* antioxidant properties of a cod (*Gadus morhua*) protein hydrolysate and peptide fractions. Food Chem 173:652-659.

Goodarzi M, Nanekarani SH, Landy N (2014) Effect of dietary supplementation with onion (*Allium cepa* L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. Asian Pac J Trop Dis 4:S297-301.

Goodarzi M, Landy N, Nanekarani S (2013) Effect of onion (Allium cepa L.) as an antibiotic growth promoter substitution on performance, immune responses and serum biochemical parameters in broiler chicks. Health 5 : 1210.

Hancock R and Sahl H (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 24: 1551-1557.

Han X, Bao Y, Xin F, Kong Z, Blecker C, Dai X (2016) Preparation and antimicrobial activity of cottonseed protein hydrolysate. Sci Agric Sin 49: 3019-3029.

Harmon DL (2009) Understanding Starch Utilization in the Small Intestine of Cattle. Asian-Australas J Anim Sci 22: 915-922.

Hou Y, Wu ZH, Dai ZH, Wang G, Wu G (2017) Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. J Anim Sci Biotechno 8:24.

Iji PA, Hughes RJ, Choct M, Tivey DR (2001) Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. Asian - Australas J Anim Sci 14:54-60.

Jung WK, Mendis E, Je JY, Park PJ, Son BW, Kim HC, Choi YK, Kim SK (2006) Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (Limanda aspera) frame protein and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 94:26-32.

Kheiri F, Faghani M, Landy N (2018) Evaluation of thyme and ajwain as antibiotic growth promoter substitutions on growth performance, carcass characteristics and serum biochemistry in Japanese quails (*Coturnix japonica*). Anim Nutr 4:79-83.

Landy N, Ghalamkari GH, Toghyani M (2011) Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (Azadirachta indica) as alternative for an antibiotic growth promoter. Livest Sci 142: 305-309.

Landy N, Kavyani A (2014) Effect of using multi-strain probiotic on performance, immune responses, and cecal microflora composition in broiler chickens reared under heat stress condition. Iran J Appl Anim Sci 3:703-708.

Landy N, Kheiri F, Faghani M (2021) Effects of periodical application of bioactive peptides derived from cottonseed on performance, immunity, total antioxidant activity of serum and intestinal development of broilers. Anim Nutr 7: 134-141.

Landy N, Kheiri F (2021) Evaluation of cottonseed bioactive peptides supplementation on performance, egg quality, and total antioxidant activity of serum in laying hens. Bull Natl Res Cent 45: 1-7.

Landy N, Kheiri F (2023) Effects of hydrolyzed cottonseed protein on growth performance, carcass traits, immunity, microbial and morphological responses of the small intestine and total antioxidant capacity of serum, and small intestine in broiler chickens. Iran J Appl Anim Sci. 13: 121-132.

Landy N, Kheiri F, Faghani M (2020) Evaluation of cottonseed bioactive peptides on growth performance, carcase traits, immunity, total antioxidant activity of serum and intestinal morphology in broiler chickens. Ital J Anim Sci 19: 1375-1386.

Liu T, She R, Wang K, Bao H, Zang Y, Luo D, Hu Y, Ding Y, Wang D, Peng K (2008) Effect of rabbit sacculus rotundus antimicrobial peptides on the intestinal mucosal immunity in chicken. Poult Sci 87:250-254.

Mahmoud KZ, Hijazi AA (2007) Effect of vitamin A and/or E on plasma enzymatic antioxidant systems and total antioxidant capacity of broiler chickens challenged with carbon tetrachloride. J Anim Physiol Anim Nutr 91:333-340.

Maxey BW, Page RK (1977) Efficacy of Lincomycin Feed Medication for the Control of Necrotic Enteritis in Broiler-type Chickens. Poult Sci 56:1909-1913.

Markovicv R, D Šefer D, Krsticv M, Petrujkicv B (2009) Effect of different growth promoters on broiler performance and gut morphology. Arch Med Vet 41: 163-169.

Miles RD, Butcher GD, Henry PR, Littell RC (2006) Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult Sci 85: 476-485.

National Institute of Health (2002) Public health service policy on human care and use of laboratory animals, USA.

Nanekarani S, Goodarzi M, Heidari M, Landy N (2012) Efficiency of ethanolic extract of peppermint (Mentha piperita) as an antibiotic growth promoter substitution on performance, and carcass characteristics in broiler chickens. Asian Pac J Trop Biomed 2: S1611-S1614.

Narain R., Lyman CM, Deyoe CW, Couch JR (1961) Paper electrophoresis and albumin/globulin ratios of the serum of normal chickens and chickens fed free gossypol in the diet. Poult Sci 40:21-25.

National Research Council [NRC] (1994) Nutrients requirements of poultry (9th. ed.). Washington, DC: National Academy Press.

Niu ZY, Min YN, Liu FZ (2018) Dietary vitamin E improves meat quality and antioxidant capacity in broilers by upregulating the expression of antioxidant enzyme genes. J Appl Poult Res 46: 397-401.

Osho SO, Xiao WW, Adeola O (2019) Response of broiler chickens to dietary soybean bioactive peptide and coccidia challenge. Poult Sci 0:1-10.

Pavlicevic M , Marmiroli N , Maestri E (2022) Immunomodulatory peptides-A promising source for novel functional food production and drug discovery. Peptides 148: 170696.

Ren WK, Yin YL, Liu G, Yu XL, Li YH, Yang G, Li TJ, Wu GY (2012) Effect of dietary arginine supplementation on reproductive performance of mice with porcine circovirus type 2 infection. Amino Acids 42:2089-2094.

SAS, SAS for Windows. Copyright © 2012 by SAS Institute Inc., Cary, NC, US.

Sayrafi R, Shahrooz R, Soltanalinejad F, Rahimi S (2011) Histomorphometrical study of the prebiotic effects on intestine morphology and growth performance of broiler chickens. Vet Res Forum 2:45-51.

Shokraneh M, Ghalamkari G, Toghyani M, Landy N (2016) Influence of drinking water containing Aloe vera (Aloe barbadensis Miller) gel on growth performance, intestinal microflora, and humoral immune responses of broilers. Vet World 9: 1197-1203.

Song J, Xiao K, Ke Y, Jiao L, Hu C, Diao Q, Shi B, Zhou X (2014) Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult. Sci 93:581-588.

Song W, Kong X, Hua Y, Chen Y, Zhang C, Chen Y (2020) Identification of antibacterial peptides generated from enzymatic hydrolysis of cottonseed proteins. *Food Sci Technol* 125: 1091-1099.

Song Y, Yu J, Song J, Wang S, Cao T, Liu Z, Gao X, Wei Y (2021) The antihypertensive effect and mechanisms of bioactive peptides from Ruditapes philippinarum fermented with Bacillus natto in spontaneously hypertensive rats. J Funct Foods 79 : 104411.

Szott V, Peh E, Friese A, Roesler U, Kehrenberg C, Ploet M, Kittler S (2022) Antimicrobial effect of a drinking water additive comprising four organic acids on Campylobacter load in broilers and monitoring of bacterial susceptibility. Poult Sci 101:102209.

Toghyani M, Mosavi SK, Modaresi M, Landy N (2015) Evaluation of kefir as a potential probiotic on growth performance, serum biochemistry and immune responses in broiler chicks. Anim Nutr 1:305-309.

Wang FQ (2005) Effects of bioactive peptide as feed additive on the performance, immune function and protein metabolism rate in broiler chicken. Dissertation, China Agricultural University.

Wen LF, He JG (2012) Dose-response effects of an antimicrobial peptide, a cecropin hybrid, on growth performance, nutrient utilisation, bacterial counts in the digesta and intestinal morphology in broilers. Br Poult Sci 108: 1756-1763.

Xu M, Du S, Wang J, Yu ZP, Harmon DL, Yao JH (2009) Influence of rumen escape starch on pancreatic exocrine secretion of goats. J Anim Physiol Anim Nutr 93: 122-129.

Yaqoob MU, Abd El-Hack ME, Hassan F, El-Saadony MT, Khafag AF, Batih GE (2021) The potential mechanistic insights and future implications for the effect of prebiotics on poultry performance, gut microbiome, and intestinal morphology. Poult Sci 100:101143.

Zhao F, Shi B, Sun D, Chen H, Tong M, Zhang P, Guo X, Yan S (2016) Effects of dietary supplementation of Artemisia argyi aqueous extract on antioxidant indexes of small intestine in broilers. Anim Nutr 2: 198-203.

Zhang J, Zhang H, Wang L, Guo X, Wang X, Yao H (2010) Isolation and identification of antioxidative peptides from rice endosperm protein enzymatic hydrolysate by consecutive chromatography and MALDI-TOF/TOF MS/MS. Food Chem 119: 226-234.

Zhong X, Lin P, Yao Y, Liu Z, Zhou X, Guan X, Huang J (2022) Effects of dietary supplementation with bioactive peptides derived from rapeseed protein on the growth performance, serum biochemistry and faecal micro-organism composition of weaned piglets. J Anim Physiol Anim Nutr doi.org/10.1111/jpn.13796