IJAS Iranian Journal of Applied Animal Science

Effects of Hydrolyzed Cottonseed Protein on Growth Performances, Carcass Traits, Immunity, Microbial and Morphological Responses of the Small Intestine and Total Antioxidant Capacity of Serum, and Small Intestine in Broiler Chickens

Research Article

N. Landy[1*] and F. Kheiri[1]

[1] Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Received on: 13 Aug 2022
Revised on: 21 Sep 2022
Accepted on: 15 Oct 2022
Online Published on: Mar 2023

*Correspondence E-mail: n_landy1984@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

This experiment was designed to assess the efficacy of dietary inclusion of cottonseed protein hydrolysate (CPH) as a substitute to in-feed antibiotics on performances, carcass characteristics, immunity, microbial and morphological respond of the small intestine and total antioxidant capacity (T-AOC) of serum, and small intestine in broiler chickens. A total of four hundreds Ross 308 day-old female broilers were assigned to 4 treatments each with 5 replicate. Three dietaries were formulated to possess 0 (control), 4, and 6 g CPH/kg of diet in confronting with control + 2 mg lincomycin. The broilers receiving 6 g CPH/kg of diet had significantly ($P<0.05$) greater final body weight and feed conversion ratio in contrast with that fed basal diet. The percentage of pancreas and cecum was a premiere in broilers fed diets containing 6 g CPH/kg of diet confront to those fed basal diet ($P<0.05$). Broilers supplemented with lincomycin or 6 g CPH/kg diet had fewer *Coliform* counts than that fed basal diet ($P<0.05$). The T-AOC of jejunum was higher in broilers fed diets containing CPH than those fed diets supplemented with lincomycin ($P<0.05$). The addition of 6 g CPH/kg of diet enhanced antibody titers against avian influenza virus (AIV) compared to other groups ($P<0.05$). In conclusion the outcomes demonstrated that the inclusion of 6 g CPH/kg of diet could induce beneficial impacts on performances by cause of an improvement in small intestine health and it could enhance antibody titers against AIV.

KEY WORDS broiler chicken, cottonseed protein hydrolysate, gut health, immunity, total antioxidant capacity.

INTRODUCTION

The aim in animal husbandry is to obtain high yields (Cilek and Tekin, 2005). For several years, the in-feed antibiotics (IFA) as a growth promoter have been administrated in chicken farms widely at low dosages for high performance and healthiness of the chickens (Nanekarani *et al*. 2012; Fekri Yazdi *et al*. 2014a; Fekri Yazdi *et al*. 2014b; Kheiri *et al*. 2018; Foroutankhah *et al*. 2019). The IFA appears to be efficient to increase the performance criteria of poultry by diminishing the propagation of microorganisms that are capable of causing gastrointestinal diseases in the host, resulting in better digestion, absorption, and metabolism of nutrients in the body (Goodarzi *et al*. 2014; Landy and Kavyani, 2014; Kavyani *et al*. 2012; Shokraneh *et al*. 2016; Gheisari *et al*. 2017). In defiance of the reality that administration of IFA in poultry feeds eventuating in several impressive effects on growth performances parameters of the broiler chickens, the persistent consumption leads to the evolution of multiple resistance of pathogenic bacteria to

antibiotics (Toghyani *et al*. 2015; Froebel *et al*. 2019), an enhance in accumulation of accumulating antibiotics in broilers meat, and dysbacteriosis (Andremont, 2000).

Bioactive peptides are small peptide molecules having lower than 20 numbers of amino acids which have biological activities aside from their nutritional worthiness (Hou *et al*. 2017; Landy and Kheiri, 2021). Several experiments illustrated that bioactive peptides have health-giving profits, including antihypertensive (Zambrowicz *et al.* 2015; Ryder *et al.* 2016), antioxidant (Landy *et al.* 2021), immunomodulatory (Kotzamanis *et al.* 2007; Landy *et al.* 2021), and antimicrobial (Osman *et al.* 2016; Wald *et al.* 2016) activities according to the amino acid (AA) molar mass and AA content (Hou *et al.* 2017). Abdollahi *et al*. (2017) stated that dietary supplementation of soybean protein hydrolysate (SPH) markedly increased growth performances related parameters however they didn't compare its effects with an IFA. Comparably, they stated that the inclusion of SPH in broiler diets could induce affirmative influences on feed conversion ratio (FCR) in broiler chickens (Abdollahi *et al.* 2018). Landy *et al.* (2020) investigated efficacy of cottonseed protein hydrolysate (CPH) supplementation in broiler diets on performance-related parameters, immunity, total antioxidant capacity (T-AOC) of serum, and morphological parameters of jejunum. The results demonstrated that the inclusion of 5 g CPH/kg of diet could markedly increase T-AOC of serum, on the other hand some of the parameters including growth performance specially FCR and immune responses were improved by addition of 6 g CPH/kg of diet. In the mentioned experiment researchers didn't provide sufficient reasons to improve performance. According to the obtained results in the previous study in the present study we investigated the efficacy of supplementing 4 or 6 g CPH/kg of diet in microbial and morphological responses of the small intestine and T-AOC of small intestine as indicators of gut health in broiler chickens.

MATERIALS AND METHODS

Ethics approval

This study was carried out at a broiler unit, not far from Isfahan city next to the Zyar city, Iran. All investigational procedures which include experimenting and slaying of the chickens were carried out according to the Experimental Animal Wellness and Welfare Committee of the Islamic Azad University, Shahrekord Branch, Iran (approval ref no. 2021-003).

Animals and dietary treatments

A total of four hundred Ross 308 day-old female broiler chicks were bought from the Parineh Tashtbandan hatchery located in Amol, and reared in floor pens (120×160×80 cm). The broilers were weighed (average body weight of 38±0.1 g) individually and randomly with a digital balance with an accuracy of 0.1 g and allotted to 4 groups with 5 replicates/group (20 chicks/replicate). Three dietaries based on maize, soybean meal and corn gluten meal were adjusted to meet the nutrient needs of the chickens (Aviagen, 2019), and possess 0 (control), 4, and 6 g CPH/kg of diet as opposed to control + 2 mg lincomycin. The chickens were fed diets having the form of mash within the experiment in 3 growth periods, 0 to 10 days (starter, Table 1), 11 to 24 days (grower, Table 2), and 25 to 38 days (finisher, Table 3). The experiment was carried out under conditions that preclude the influence of extraneous factors and feed and water were supplied *ad libitum* during the trial. The broiler house was closed off on all sides and persistent lighting was offered via electric light. The temperature of the room was around 33 ˚C on day one, and was diminished by 3 ˚C every week to ultimately be constant at 21 ˚C. The thermometers were placed inside broiler house at bird height to control the temperature.

Preparation and analysis of cottonseed protein hydrolysate

The product which has been used in the current experiment was separated from cottonseed protein due to enzymatic hydrolysis. The procedure to obtain CPH from cottonseed protein has been summarized. The dehulled cottonseed protein has been dissolved in a solvent, and sterilized at 105 ˚C for 30 minutes; the temperature of the liquid has been reduced to 105 ˚C afterward. Alkaline protease and calcium hydroxide were used to hydrolyze the protein section of the liquid at 50 ˚C for 10 h; the measured pH after adding calcium hydroxide solution was in the range of 8.0-9.0. The liquid is evaporated after 12 h in drying ovens set at 90 ˚C afterward.

In advance of diet formulation, corn, corn gluten meal, soybean meal, and CPH were considered for the amount of crude protein (AOAC, 2006; method 990.03), and total amino acids (AOAC, 2006; methods 982.30E a, b, and c). The CPH takes the measurements of total phosphorus and calcium via inductively coupled plasma - optical emission spectrometry (AOAC, 1990; method 2011.14) at equipped lab of the university (Table 4). The CPH takes the measurements of molecular weight distribution according to the method pretended by Jung *et al.* (2006).

Performance and carcass components

All measurable factors relevant to growth performances including body weight (BW), daily weight gain (DWG) and daily feed intake (DFI) were calculated at the termination of starter, grower, finisher phases and the whole experiment. FCR was calculated by dividing DFI to DWG.

Table 1 Composition and nutrient levels of dietary treatments in starter phase

Item	Cottonseed protein hydrolysate inclusion (CPH; g/kg)		
	0.0	4.0	6.0
Component, g/kg (as-fed)			
Corn grain (7.5% CP)	508.7	507.9	507.7
Soybean meal solvent (44% CP)	346.6	341.8	339.7
Corn gluten feed (60% CP)	50.0	50.0	50.0
Cottonseed protein hydrolysate (46% CP)	0.0	4.0	6.0
Wheat bran (14.8% CP)	20.1	22.2	22.6
Vegetable oil	26.7	26.7	26.7
L-methionine	2.8	2.8	2.8
L-lysine hydrochloride	4.1	4.1	4.1
L-threonine	1.3	1.3	1.3
Choline chloride	1.2	1.2	1.2
Mono calcium phosphate (15% Ca, 22.5% P)	15.5	15.4	15.4
Limestone	16.5	16.1	16.0
Common salt	1.0	1.0	1.0
Sodium hydrogen carbonate	3.5	3.5	3.5
Mineral supplement[1]	1	1	1
Vitamin supplement[2]	1	1	1
Calculated composition			
Metabolizable energy, kcal/kg	3000	3000	3000
Crude protein (g/kg)	230	230	230
Lysine (g/kg)	14.4	14.4	14.4
Methionine (g/kg)	6.95	6.95	6.94
Methionine + cysteine (g/kg)	10.8	10.8	10.8
Threonine (g/kg)	9.7	9.7	9.7
Tryptophan (g/kg)	2.6	2.6	2.6
Arginine (g/kg)	14.9	15.0	15.0
Valine (g/kg)	11.9	11.9	11.9
Isoleucine (g/kg)	11.4	11.3	11.3
Leucine (g/kg)	21.7	21.7	21.6
Calcium (g/kg)	9.6	9.6	9.6
Available phosphorus (g/kg)	4.8	4.8	4.8
Crude fat (g/kg)	47.7	47.8	47.8
Crude fiber(g/kg)	37.1	36.9	36.9
Analyzed amount			
Crude protein (g/kg)	232	229	231

[1] Supplied the following per kilogram of feed: Manganese: 120 mg; Iron: 20 mg; Copper: 16 mg; Zinc: 110 mg; Selenium: 0.3 mg and Iodine: 1.25 mg.
[2] Supplied the following per kilogram of feed: vitamin A: 12000 IU; vitamin D_3: 5000 IU; vitamin E: 80 IU; vitamin K: 3.2 mg; Thiamin: 3.2 mg; Riboflavin: 8.6 mg; Niacin: 65 mg; Pantothenic acid: 20 mg; Pyridoxine: 4.3 mg; Biotin: 0.22 mg; Folic acid: 2.2 mg and vitamin B_{12}: 0.017 mg.

At 38 d of age, 2 chickens per replicate were carefully chosen to meet the average weight of the group, singly weighed and slaughtered with incision of the jugular vein. Carcass yield was computed by dividing eviscerated weight to live BW. Empty proventriculus, empty gizzard, empty cecum, liver, heart, pancreas, and lymphoid organs including spleen and bursa of Fabricius were separated from carcasses, weighed and reported as a percentage of live BW.

DFI= total consumed feed / number of days during the period

DWG= weight gain during the period / number of days during the period

FCR= total feed consumed / body weight gain

Jejunal morphology

On d 38 of age, ten chickens/treatment were selected depending on the mean of the group, slaughtered and the small intestine was immediately removed from carcasses. After that, approximately 2 cm of proximal segments of jejunum were divided into pieces and stabled in 10% neutral formalin, dehydrated in a serialized ethanol sequences and infixed in paraffin thereafter conforming to the procedure explained by Iji *et al.* (2001). Paraffin pieces at 6 m area were imbued with hematoxylin and eosin, and tested by light microscopy (Olympus Co. Ltd., BX 50, F-3, Tokyo, Japan) sample. Morphological parameters including epithelial thickness, villus height (VH), villi width (VW), and crypt depth (CD) were ascertained as indicated in Figure 1. The ratios of VH to CD were determined by dividing VH to CD.

Table 2 Composition and nutrient levels of dietary treatments in grower phase

Item	Cottonseed protein hydrolysate inclusion (CPH; g/kg)		
	0.0	4.0	6.0
Component, g/kg (as-fed)			
Corn grain (7.5% CP)	527.8	527	526.7
Soybean meal solvent (44% CP)	341.7	336.9	334.8
Corn gluten feed (60% CP)	30.0	30.0	30.0
Cottonseed protein hydrolysate (46% CP)	0.0	4.0	6.0
Wheat bran (14.8% CP)	17.9	19.9	20.4
Vegetable oil	40.9	40.9	40.9
L-methionine	2.5	2.5	2.5
L-lysine hydrochloride	2.6	2.6	2.6
L-threonine	0.8	0.8	0.8
Choline chloride	1.1	1.1	1.1
Mono calcium phosphate (15% Ca, 22.5% P)	13.6	13.6	13.6
Limestone	14.9	14.5	14.4
Common salt	1.5	1.5	1.5
Sodium hydrogen carbonate	2.7	2.7	2.7
Mineral supplement[1]	1	1	1
Vitamin supplement[2]	1	1	1
Calculated composition			
Metabolizable energy, kcal/kg	3100	3100	3100
Crude protein (g/kg)	215	215	215
Lysine (g/kg)	12.9	12.9	12.9
Methionine (g/kg)	6.28	6.27	6.27
Methionine + cysteine (g/kg)	9.9	9.9	9.9
Threonine (g/kg)	8.8	8.8	8.8
Tryptophan (g/kg)	2.5	2.5	2.5
Arginine (g/kg)	14.4	14.5	14.5
Valine (g/kg)	11.3	11.3	11.3
Isoleucine (g/kg)	10.8	10.8	10.8
Leucine (g/kg)	19.8	19.8	19.8
Calcium (g/kg)	8.7	8.7	8.7
Available phosphorus (g/kg)	4.3	4.3	4.3
Crude fat (g/kg)	61.9	62.0	62.0
Crude fiber(g/kg)	36.4	36.2	36.2
Analyzed amount			
Crude protein (g/kg)	216	213	217

[1] Supplied the following per kilogram of feed: Manganese: 120 mg; Iron: 20 mg; Copper: 16 mg; Zinc: 110 mg; Selenium: 0.3 mg and Iodine: 1.25 mg.
[2] Supplied the following per kilogram of feed: vitamin A: 10000 IU; vitamin D_3: 4500 IU; vitamin E: 65 IU; vitamin K: 3 mg; Thiamin: 2.5 mg; Riboflavin: 6.5 mg; Niacin: 60 mg; Pantothenic acid: 18 mg; Pyridoxine: 3.2 mg; Biotin: 0.18 mg; Folic acid: 1.9 mg and vitamin B_{12}: 0.017 mg.

Coliform bacteria count

At termination of the experiment, two broilers from each replicates were killed and ileal digesta of broilers were removed and pooled. *Coliform* bacteria populations from ileum were estimated via serial dilution (10−1 to 10−9) of ileal digesta content in anaerobic dilutors prior to inoculation moving to Petri dishes comprising sterile agar to obtain an incubated plate with an easily countable number of colonies as described by Gunal *et al.* (2007). MacConkey agar (Darmstadt, Germany) was used for growth and the isolation of *Coliforms*.

The plates were incubated in a stationary incubator (Petersime Incubator Co) at 37 ˚C for 24 h. Subsequently, the *Coliform* bacterial colony populations grown on every one of plates were enumerated manually. Colony-forming units were specified as distinguished colonies assaying at least 1 mm in diameter.

Immunity

In summary, the chickens were vaccinated against H9N2 sub-type of type-A Avian Influenza (AIV) and Newcastle disease virus (NDV) at 7 d of age by subcutaneously injection of 0.2 mL/chicken inactivated vaccine. Also, the chickens were orally vaccinated against NDV using attenuated vaccine (B1) at 21 d of age. At 28 d, 2 broilers per pen were marked on the basis of the modest weight of the pen and the blood specimens were taken by venipuncture. The blood specimens were centrifuged for 15 minutes at 3000 rpm to obtain sera for immunoserology tests. Antibody titers against NDV and AIV were carefully determined using the hemagglutination inhibition test (HI) according to the method described by Yadav *et al.* (2018). The obtained antibodies were transformed to $\log_2$ thereafter. Total protein and albumin serum concentrations were measured operating the procedure explained by Kim *et al.* (2015).

Table 3 Composition and nutrient levels of dietary treatments in finisher phase

Item	Cottonseed protein hydrolysate inclusion (CPH; g/kg)		
	0.0	4.0	6.0
Component, g/kg (as-fed)			
Corn grain (7.5% CP)	579.9	579.0	578.9
Soybean meal solvent (44% CP)	287.5	282.7	280.5
Corn gluten feed (60% CP)	40.0	40.0	40.0
Cottonseed protein hydrolysate (46% CP)	0.0	4.0	6.0
Wheat bran (14.8% CP)	9.9	12.0	12.4
Vegetable oil	42.8	42.8	42.8
L-methionine	2.3	2.3	2.3
L-lysine hydrochloride	2.9	2.9	2.9
L-threonine	0.7	0.7	0.7
Choline chloride	1.0	1.0	1.0
Mono calcium phosphate (15% Ca, 22.5% P)	12.6	12.6	12.6
Limestone	14.1	13.7	13.6
Common salt	1.4	1.4	1.4
Sodium hydrogen carbonate	2.9	2.9	2.9
Mineral supplement[1]	1	1	1
Vitamin supplement[2]	1	1	1
Calculated composition			
Metabolizable energy, kcal/kg	3200	3200	3200
Crude protein (g/kg)	200	200	200
Lysine (g/kg)	11.9	11.9	11.9
Methionine (g/kg)	5.96	5.95	5.95
Methionine + cysteine (g/kg)	9.4	9.4	9.4
Threonine (g/kg)	8.1	8.1	8.1
Tryptophan (g/kg)	2.2	2.2	2.2
Arginine (g/kg)	12.9	12.9	12.9
Valine (g/kg)	10.4	10.4	10.4
Isoleucine (g/kg)	9.8	9.7	9.7
Leucine (g/kg)	19.4	19.3	19.3
Calcium (g/kg)	8.1	8.7	8.7
Available phosphorus (g/kg)	4.0	4.3	4.3
Crude fat (g/kg)	65.3	65.3	65.3
Crude fiber(g/kg)	33.1	32.9	32.8
Analyzed amount			
Crude protein (g/kg)	202	201	203

[1] Supplied the following per kilogram of feed: Manganese: 120 mg; Iron: 20 mg; Copper: 16 mg; Zinc: 110 mg; Selenium: 0.3 mg and Iodine: 1.25 mg.
[2] Supplied the following per kilogram of feed: vitamin A: 9000 IU; vitamin D_3: 4000 IU; vitamin E: 55 IU; vitamin K: 2.2 mg; Thiamin: 2.2 mg; Riboflavin: 5.4 mg; Niacin: 45 mg; Pantothenic acid: 15 mg; Pyridoxine: 2.2 mg; Biotin: 0.15 mg; Folic acid: 1.6 mg and vitamin B_{12}: 0.011 mg.

The relative amount of globulin was computed by calculating differentiations between the total protein and albumin concentrations. The albumin to globulin ratios (AGR) were computed as a classic clinical index thereafter.

Total antioxidant activity of serum and small intestine

At the end of trial, 2 broilers per pen were marked and the blood specimens were mustered and the chickens were killed thereafter.

The blood specimens were centrifuged using an MPW 250 centrifuge (MPW MED Instruments, Poland) for 15 minutes at 3000 rpm to obtain serum samples. The samples from jejunum, which were around 3 cm were removed from centric part and cleaned with the precooling physiological saline. In the time following cleaning the filter paper, the jejunum segments were exactly weighed and made ready to nine times voluminosity of physiological saline, produced of 10% texture homogenate milling. The obtained semiliquid mixtures were centrifuged for 10 min at 1,346 rpm, and the supernatant fluids were conveyed to test-tubes and kept at 20 ˚C for future use. T-AOC was measured by Coomassie brilliant blue procedure as explained by Zhao *et al.* (2016).

Statistical analysis

The present trial conducted as a completely randomized design, and collected data were analyzed by one-way ANOVA using procedures of SAS version 9.4 (SAS, 2012). Means were compared applying a post-hoc Tukey test at 5% significance.

Table 4 Composition of a 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

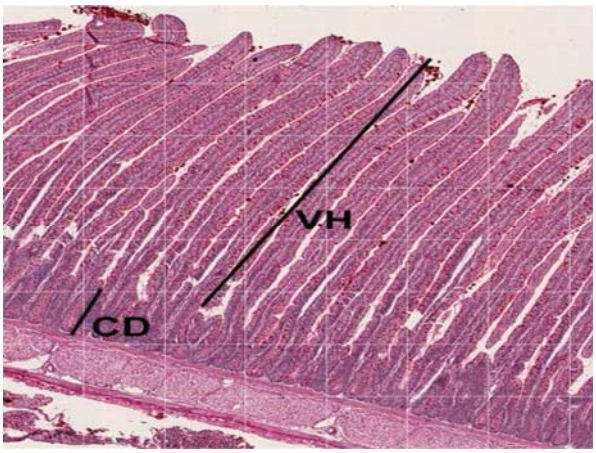

Figure 1 Villus height and crypt depth of broilers at 38 d of age

RESULTS AND DISCUSSION

No mortalities eventuated throughout the trial. The consequences of using non-identical levels of CPH in broiler's diet in comparison with administration of subtherapeutic dosage of lincomycin on growth performance of broilers have been demonstrated in Table 5. At 24 d age broilers receiving lincomycin or 4 and 6 g CPH/kg of diet had higher BW in comparison with broilers fed basal diet; al though the findings were not statistically significant (P>0.05). At 38 d of age broilers receiving lincomycin or 6 g CPH/kg of diet had considerably (P<0.05) higher final BW compared to those fed basal diet or basal diet containing 4 g CPH/kg of diet. In the course of starter phase broilers fed diets containing 6 g CPH/kg of diet had significantly (P<0.05) lower DFI compared to other groups.

During the grower phase broilers fed diets containing lincomycin had considerably (P<0.05) higher DFI compared to those receiving 6 g CPH/kg of diet, but did not incompatible with those fed basal diet or basal diet supplemented with 4 g CPH/kg of diet. Similarly, throughout the finisher phase broilers receiving lincomycin had considerably (P<0.05) higher DFI compared to those fed 4 g CPH/kg of diet, but did not incompatible with those fed basal diet or basal diet supplemented with 6 g CPH/kg of diet. DFI for the total period of trial was considerably (P<0.05) greater in broilers fed diets containing lincomycin compared to those fed diets containing 4 or 6 g CPH/kg of diet. Remarkable discrepancies amongst treatments were recognized in FCR throughout all of the trial periods as well as for the whole experiment.

During the starter phase, broilers fed diets containing 6 g CPH/kg of diet had more effectively FCR compared to other groups (P<0.05). In the course of grower period broilers fed diets containing 4 or 6 g CPH/kg of diet had more effectively FCR in comparison with those fed basal diet or basal diet supplemented with lincomycin (P<0.05). Throughout the finisher phase, broilers fed diets containing 6 g CPH/kg of diet had better FCR value in comparison with those fed basal diet or basal diet supplemented with 4 g CPH/kg of diet, whereas it did not vary from those fed diets containing lincomycin. On the whole experiment broilers receiving 6 g CPH/kg of diet had more effectively FCR value compared to those fed basal diet (P<0.05).

Table 6 demonstrated carcass traits of broiler chickens at 38 d of age. Relative weight of gizzard, liver, heart, spleen, and bursa of Fabricius were not considerably influenced by the dietary treatments. Carcass yield was improved by addition of 4 g CPH/kg of diet compared to those fed basal diet or basal diet supplemented with 6 g CPH/kg of diet (P<0.05).

The percentage of proventriculus was considerably (P<0.05) reduced by addition of 4 or 6 g CPH/kg of diet. The percentage of pancreas was significantly higher in broilers fed diets supplemented with CPH compared to those fed basal diet (P<0.05). The highest percentage of cecum achieved in the group supplemented with 6 g CPH/kg of diet (P<0.05). The percentage of bursa of Fabricius was significantly greater in broilers fed diets containing 6 g CPH/kg of diet, but the findings were not considerably incompatible (P>0.05).

The influences of CPH and lincomycin on intestinal morphology of jejunum at 38 d of age are illustrated in Table 7. Addition of CPH and lincomycin to the basal diet couldn't markedly affect morphological parameters of jejunum at 38 d of age (P>0.05). Inclusion of 6 g CPH/kg of diet increased VH and CD compared other groups, although the differences were not considerably incompatible (P>0.05).

Table 5 Effect of dietary hydrolyzed cottonseed protein inclusion on performance criteria of broiler chickens at different growth phases

Item	Experimental treatments				SEM	P-value
	Control	Lincomycin	4 g CPH/kg	6 g CPH/kg		
Body weight, g						
10 d of age	297	295	292	299	3.15	0.422
24 d of age	1187	1210	1229	1229	14.26	0.159
38 d of age	2320^{b}	2437^{a}	2359^{b}	2434^{a}	17.01	0.001
Daily weight gain, g/d						
1 to 10 d of age	25.9	25.5	25.4	26.1	0.33	0.38
11 to 24 d of age	63.5	65.3	66.9	66.4	0.99	0.12
25 to 38 d of age	80.8^{b}	87.5^{a}	80.7^{b}	85.9^{a}	0.93	0.001
1 to 38 d of age	60.0^{b}	63.1^{a}	61.0^{b}	63.0^{a}	0.44	0.001
Daily feed intake, g/d						
1 to 10 d of age	28.7^{a}	29.6^{a}	28.6^{a}	27.2^{b}	0.26	0.001
11 to 24 d of age	92.0^{ab}	94.3^{a}	91.5^{ab}	90.6^{b}	0.71	0.013
25 to 38 d of age	142.5^{ab}	144.8^{a}	139.6^{b}	140.4^{ab}	1.07	0.017
1 to 38 d of age	93.9^{b}	95.9^{a}	92.7^{c}	92.3^{c}	0.31	0.001
Feed intake: weight gain, g:g						
1 to 10 d of age	1.10^{b}	1.16^{a}	1.11^{b}	1.03^{c}	0.01	0.001
11 to 24 d of age	1.44^{a}	1.44^{a}	1.36^{b}	1.36^{b}	0.01	0.002
25 to 38 d of age	1.76^{a}	1.65^{bc}	1.72^{ab}	1.63^{c}	0.02	0.002
1 to 38 d of age	1.56^{a}	1.51^{ab}	1.51^{ab}	1.46^{b}	0.01	0.001

CPH: cottonseed protein hydrolysate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of dietary hydrolyzed cottonseed protein inclusion on carcass yield and relative weight of organs of broilers at 38 d of age

Relative organ weight	Experimental treatments				SEM	P-value
	Control	Lincomycin	4 g CPH/kg	6 g CPH/kg		
Carcass (%)	79.4^{b}	78.9^{ab}	80.8^{a}	79.3^{b}	0.59	0.04
Proventriculus (%)	0.42^{a}	0.40^{a}	0.37^{ab}	0.35^{b}	0.01	0.008
Gizzard (%)	1.43	1.47	1.30	1.30	0.05	0.10
Liver (%)	2.22	2.23	2.35	2.18	0.10	0.71
Pancreas (%)	0.23^{b}	0.27^{ab}	0.28^{a}	0.28^{a}	0.004	0.009
Cecum (%)	0.39^{b}	0.34^{c}	0.41^{b}	0.46^{a}	0.01	0.001
Heart (%)	0.38	0.38	0.38	0.40	0.02	0.65
Spleen (%)	0.10	0.11	0.10	0.10	0.04	0.91
Bursa of Fabricius(%)	0.18	0.22	0.20	0.25	0.02	0.17

CPH: cottonseed protein hydrolysate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Influence of dietary treatments on intestinal morphology of jejunum in broiler chickens at d 38 d of age

Variables	Experimental treatments				SEM	P-value
	Control	Lincomycin	4 g CPH/kg	6 g CPH/kg		
Villus height (μm)	662	766	780	785	63.38	0.36
Crypt depth (μm)	145	183	216	218	38.02	0.06
Epithelial thickness (μm)	13.3	13.2	13.3	16.6	0.18	0.59
Villus width (μm)	114	155	116	166	1.30	0.06
Villus height to crypt depth ratio	4.56	4.18	3.61	3.60	0.66	0.07

CPH: cottonseed protein hydrolysate.
SEM: standard error of the means.

Table 8 Influence of dietary treatments on ileum coliform counts (log_{10}) of broilers at 38 d

Bacterial count	Experimental treatments				SEM	P-value
	Control	Lincomycin	4 g CPH/kg	6 g CPH/kg		
Coliform	3.12^{a}	1.39^{b}	2.37^{ab}	1.47^{b}	0.75	0.04

CPH: cottonseed protein hydrolysate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 show *Coliform* counts taken from the ileum of broilers at 38 d of age. Counts of *Coliforms* in the ileum of broilers fed diets containing lincomycin or 6 g CPH/kg of diet was considerably (P<0.05) lower than those fed basal diet, but didn't vary from those fed 4 g CPH/kg of diet.

The influences of dietary treatments on antibody titers against Newcastle and Influenza viruses and albumin to globulin ratios are summarized in Table 9. Inclusion of lincomycin or CPH couldn't inspire any remarkable influence on antibody titers against NDV and albumin to globulin ratios (P>0.05). Addition of 6 g CPH/kg of diet markedly increased antibody titers against AIV compared to other groups (P<0.05).

As Table 10 shows, T-AOC of serum wasn't considerably influenced by the dietary treatments; although it tended to increase in boilers fed diets containing 6 g CPH/kg of diet (P>0.05). The T-AOC level of jejunum in boilers fed diets containing 4 or 6 g CPH/kg of diet was considerably higher (P<0.05) than those fed diets containing lincomycin, but didn't vary from those fed basal diet.

In the present study feeding 4 or 6 g CPH/ kg of diet couldn't markedly affect DFI. Similarly, Abdollahi *et al.* (2017) stated that inclusion of varying amounts of SPH couldn't affect DFI of broilers. In agreement, Abdollahi *et al.* (2018) stated that addition of varying amounts of SPH couldn't affect DFI of broiler chickens. In contrast with the obtained findings in the current study Landy *et al.* (2020) stated that addition of 5 g CPH/kg of diet increased DFI of broiler chickens. The reason for the contrast between obtained result in the present trial and the findings achieved by Landy *et al.* (2020) may be due to the amount of feed consumption in the control group. According to Aviagen (2019) the standard DFI for the strain during (1-38 d of age) is 96.3 g/d/chicken. In the current study the obtained DFI was around 93.9 g/d/chicken which is around the reported DFI by Aviagen. In contrary, the obtained results for DFI in the experiment which has been done by Landy *et al.* (2020) was markedly lower than the standard of the breed (79.5 g/d/bird); thus, it seems that additive such as bioactive peptides can't increase DFI when it is around the standard of the breed. In the present study, inclusion of 6 g CPH/kg of diet could improve final BW and FCR of broilers. In disagreement of our findings Abdollahi *et al.* (2017) and Abdollahi *et al.* (2018) stated that addition of SPH in broiler diets couldn't affect final BW. Adjustment with our findings Wang (2005) stated that inclusion of SBH in broiler diet could increase final BW at d 21 post hatch. Landy *et al.* (2020) stated that inclusion of 4 g CPH/kg of diet could improve final BW of broilers as a result of an increment in DFI. In the current trial DFI of broilers was not improved so the BW increased with other mechanism. As indicated in Table 6 inclusion of 4 or 6 g CPH/kg of diet considerably enhanced the relative weight of pancreas as an organ which contains exocrine tissue that secretes pancreatic enzymes. Feng *et al.* (2007) stated that inclusion of fermented soybean meal in broiler diets could increase activation of pancreatic enzymes. Furthermore, as indicated in Table 8 *Coliform* bacteria count of broilers in the ileum was markedly lower in broiler fed diets containing lincomycin or 6 g CPH/kg of diet than those fed basal diet. As reported by Bedford (2000) antibiotics could confine the growth and formation of gut bacteria colonies which may cause higher usage of feed, resultant in higher growth and utilization of the feed. According to Song *et al.* (2020) cottonseed protein hydrolysates perhaps be a favorable provenance of natural antibacterial factors, thus greater final BW and FCR obtained in the current study may be due to antibacterial peptides which can modulate gut microflora. Also, as small intestine is the major organ and area in the digestion and absorption of feed so its health status is very important factor. As indicated in Table 10 the T-AOC level of jejunum was improved in boilers fed diets containing 4 or 6 g CPH/kg of diet. It seems that in the present trial inclusion of 6 g CPH/kg of diet could succeed in favorably influencing on final BW and FCR by improving gut health, and probably higher secretion of pancreatic enzymes.

In the present trial inclusion of 6 g CPH/kg of diet could increase (P<0.05) the relative weight of pancreas and cecum, and its supplementation decreased the relative weight of proventriculus. Comparable to our findings Abdollahi *et al.* (2017) stated that the relative weight of pancreas and cecum tended to increased by addition of SPH and conversely the relative weight of proventriculus tended to decreased. In agreement with archived findings in the present trial, Landy *et al.* (2020) stated that inclusion of CPH in broiler diets reduced the relative weight of proventriculus.

The major function of the pancreas is the production of digestive enzymes related to protein digestion. As reported by Rehfeld (2017) cholecystokinin peptides which are on the whole generated by small intestinal endocrine I-cells can stimulate pancreatic enzyme production and discharge, growth, motility and constriction of gut, and interdict acid secretion from the stomach. Therefore, the mentioned changes observed in the present trial may caused by the presence of such peptides in the CPH.

In the current trial, VH and CD tended to enhance in broilers fed diets supplemented with 4 or 6 g CPH/kg of diet. Likewise, Abdollahi *et al.* (2017) stated that VH and CD tended to increase by increasing the level of SPH in broiler diets. In another trial Osho *et al.* (2019) stated that addition of SPH in broiler diets could potentially succeed in persuading affirmative effects on VH to CD ration in the jejunum.

Table 9 Influence of dietary treatments on antibody titers against Newcastle and Influenza viruses and albumin to globulin ratios at d 28 of broiler chickens

Variables	Experimental treatments				SEM	P-value
	Control	Lincomycin	4 g CPH/kg	6 g CPH/kg		
New Castle(log_2)	4.50	5.16	5.25	5.16	0.31	0.32
Influenza (log_2)	4.00^{b}	3.60^{b}	3.50^{b}	4.83^{a}	0.20	0.001
Albumin to globulin ratio	2.06	2.11	2.00	2.08	0.14	0.95

CPH: cottonseed protein hydrolysate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 10 Effects of dietary treatments on total antioxidant capacity (T-AOC) levels (U/mg prot) of broiler's serum and small intestine

Variables	Experimental treatments				SEM	P-value
	Control	Lincomycin	4 g CPH/kg	6 g CPH/kg		
T-AOC of jejunum	2.80^{ab}	2.37^{b}	2.98^{a}	2.96^{a}	0.128	0.016
T-AOC of serum	3.52	3.58	3.67	3.58	0.094	0.762

CPH: cottonseed protein hydrolysate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

As morphological parameters of small intestine are suitable indexes for indicating the digestive sufficiency of the small intestine its characteristics have been investigated in several experiments. As reported by researchers addition of bioactive peptides in broiler diets can induce beneficial effects on morphological parameters of the small intestine (Liu *et al.* 2008; Bao *et al.* 2009; Wen and He, 2012).

In the present study, counts of *Coliforms* in the ileum of broilers fed diets supplemented with lincomycin or 6 g CPH/kg of diet was lower than those fed basal diet. In agreement with our results, Han *et al.* (2016) stated that CPH prepared by applying trypsin and pepsin indicated antibacterial activity at the relative amount of 100 mg/mL. Song *et al.* (2020) investigated antibacterial activity of peptides obtained from CPH prepared using Alcalase against *Escherichia coli* (*E. coli*); they reported that the CPH containing three peptides (KDFPGRR, LGLRSGIILCNV, and DENFRKF) with high antibacterial activity against *E. coli*. Bioactive peptides could be the cause of membrane lesion via interacting with the superficial of the cell membrane and eventually annihilate the cell. Totally, the findings of the current experiment revealed that CPH might be consumed as a natural antibacterial agent in feed.

In the present study supplementation of lincomycin or CPH couldn't succeed in influencing immune relevant parameters except for antibody titers against AIV which was higher in broilers fed diets supplemented with 6 g CPH/kg of diet. Moreover, the relative weight of bursa of Fabricius tended to enhance in broilers fed diets supplemented with 6 g CPH/kg of diet. Comparably Abdollahi *et al.* (2017) stated that the relative weight of bursa of Fabricius and spleen tended to enhance in broilers fed diets supplemented with SPH. Osho *et al.* (2019) stated that inclusion of SPH in broiler diets could increase the relative weight of spleen.

In chickens the bursa of Fabricius is a primeval specialized organ and the site of hematopoiesis which is necessary for B cell development; therefore, its maturing and enlargement straightly affect the immune function. As explained by Osho *et al.* (2019) bioactive peptides may increase production of TNF-α, IL-8, IL-10, and IL-6 and as a consequence modulate immune responses. It seems that in the current experiment greater antibody titers against AIV are appertaining to enlargement of bursa of Fabricius.

In the current trial T-AOC level of jejunum has been improved by supplementation of CPH. T-AOC of jejunum or serum describes the ability of antioxidant enzymes and the associated to remove preformed free radicals (Ren *et al.* 2012). As stated by Jang *et al.* (2008) bioactive peptides may donate hydrogen from AA to cleave the oxidation chain reaction. In agreement, Girgih *et al.* (2015) stated that peptides which containing aromatic AA have the capacity to become antioxidant by donating electrons. Hisham *et al.* (2018) stated that bioactive peptides obtained from protein of camel milk have the potential to ameliorate oxidative stress by the mechanism of radical-scavenging. According to Davalos *et al.* (2004) peptides which containing hydrophobic AA residues such as Met, Cys and His have radical scavenging effect. As illustrated in Table 4 protein hydrolysate which has been investigated in the present study possess a remarkable quantity of hydrophobic AA. Furthermore, as explained by Wattanasiritham *et al.* (2016) and Ruiz-Ruiz *et al.* (2013) peptides with low molecular weight indicated higher antioxidant activity compared to those have high molecular weight; as demonstrated in Table 4 the percentage of peptides with molecular weight lower than 1000 Dalton is 18, which is remarkable percentage. In the present investigation T-AOC of jejunum significantly increased by addition of CPH in broiler diets whereas T-AOC

of serum tended to increase; it seems that some antioxidant peptides appear to be broken down by cystoplasmic proteases before entering the bloodstream.

CONCLUSION

In conclusion the findings demonstrated that inclusion of 6 g CPH/kg of diet could succeed in influencing growth performance caused by an improvement in small intestine health and it could enhance antibody titers against AIV thus it can be applied in broiler diets as a substitution for IFA.

ACKNOWLEDGEMENT

The authors thank Pishgam Damparvar Sepahan Co. Ltd for supplying broiler house, feed and skill workers.

REFERENCES

AOAC. (1990). Official Methods of Analysis. 10th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

AOAC. (2006). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

Abdollahi M.R., Zaefarian F., Gu Y., Xiao W., Jia J. and 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 M.R., Zaefarian F., Gu Y., Xiao W., Jia J. and Ravindran V. (2018). Influence of soybean bioactive peptides on performance, foot pad lesions and carcass characteristics in broilers. *J. Appl. Anim. Nutr*. **6,** 1-7.

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

Aviagen. (2019). Ross Broiler Management Manual. Aviagen Ltd., Newbridge, UK.

Bao H., She R., Liu T., Zhang Y., Peng K.S., Luo D., Yue Z., Ding Y., Hu Y., Liu W. and 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.

Çilek S. and Tekin M.E. (2005). The environmental factors affecting milk yield and fertility traits of simmental cattle raised at the kazova state farm and phenotypic correlations between these traits. *Turkish J. Vet. Anim. Sci.* **29,** 987-993.

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

Fekri Yazdi F., Ghalamkari G.H., Toghiani M., Modaresi M. and Landy N. (2014a). Anise seed (*Pimpinella anisum*) 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 G.H., Toghiani M., Modaresi M. and Landy N. (2014b). The efficiencyof *Tribulus terrestris* as an antibiotic growth promoter substitute on performance and immune responsesin broiler chicks. *Asian Pac. J. Trop. Dis*. **4(2),** 1014-1018.

Feng J., Liu X., Xu Z.R., Wang Y.Z. and Liu J.X. (2007). Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. *Poult. Sci*. **86,** 1149-1154.

Foroutankhah M., Toghyani M. and Landy N. (2019). Evaluation of *Calendula officinalis* (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.

Froebel L.K., Jalukar S., Lavergne T.A., Lee J.T. and Duong T. (2019). Administration of dietary prebiotics improves growth performance and reduces pathogen colonization in broiler chickens. *Poult. Sci*. **98,** 6668-6676.

Gheisari A., Shahrvand S. and 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,** 249-254.

Girgih A.T., He R., Hasan F.M., Udenigwe C.C., Gill T.A. and Aluko R.E. (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 S.H. and Landy N. (2014). Effect of dietary supplementation with onion (*Allium cepa*) on performance, carcass traits and intestinal microflora composition in broiler chickens. *Asian Pac. J. Trop. Dis*. **4,** S297-301.

Gunal M., Yayli O., Kaya N., Karahan N. and Sulak O. (2007). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *Int. J. Poult. Sci*. **5,** 149-155.

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

Hisham R.I., Isono H. and Miyata T. (2018). Potential antioxidant bioactive peptides from camel milk proteins. *Anim. Nutr*. **4,** 273-280.

Hou Y., Wu Z.H., Dai Z.H, Wang G. and Wu G. (2017). Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. *J. Anim. Sci. Biotechnol*. **8,** 24-26.

Iji P.A., Hughes R.J., Choct M. and Tivey D.R. (2001). Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. *Asian-Australasian J. Anim. Sci*. **14,** 54-60.

Jang A., Liu X.D., Shin M.H., Lee B.D., Lee S.K., Lee J.H. and Jo C. (2008). Antioxidative potential of raw breast meat from broiler chicks fed a dietary medicinal herb extract mix. *Poult Sci*. **87,** 2382-2389.

Jung W.K., Mendis E., Je J.Y., Park P.J., Son B.W., Kim H.C., Choi Y.K. and Kim S.K. (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.

Kavyani A., Zare Shahne A., PorReza J., Jalali Haji-AbadiS M.A. an Landy N. (2012). Evaluation of dried powder of mushroom (*Agaricus bisporus*) as an antibiotic growth promoter substitution on performance, carcass, traits and humoral immune responses in broiler chickens. *J. Med. Plants Res*. **6,** 94-100.

Kheiri F., Faghani M. and 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.

Kim D.W., Mushtaq M.M.H., Parvin R., Kang H.K., Kim J.H., Na J.C., Hwangbo J., Kim J.D., Yang C.B., Park B.J. and Choi H.C. (2015). Various levels and forms of dietary α-lipoic acid in broiler chickens: Impact on blood biochemistry, stress response, liver enzymes, and antibody titers. *Poult. Sci*. **94,** 226-231.

Kotzamanis Y.P., Gisbert E., Gatesoupe F.J., Zambonino Infante J. and Cahu C. (2007). Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to *Vibrio anguillarum* in European sea bass (*Dicentrarchus labrax*) larvae. *Comp. Biochem. Physiol. A*. **147,** 205-214.

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

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

Landy N. and 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. and 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.

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

Nanekarani S., Goodarzi M., Heidari M. and 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,** 1611-1614.

Osho S.O., Xiao W.W. and Adeola O. (2019). Response of broiler chickens to dietary soybean bioactive peptide and coccidia challenge. *Poult. Sci*. **98(11),** 5669-5678.

Osman A., Goda H.A., Abdel-Hamid M., Badran S.M. and Otte J. (2016). Antibacterial peptides generated by Alcalse hydrolysis of goat whey. *LWT-Food Sci Technol*. **65,** 480-486.

Rehfeld J.F. (2017). Cholecystokinin from local gut hormone to ubiquitous messenger. *Front. Endocrinol*. **8,** 47-52.

Ren W.K., Yin Y.L., Liu G., Yu X.L., Li Y.H., Yang G., Li T.J. and Wu G.Y. (2012). Effect of dietary arginine supplementation on reproductive performance of mice with porcine circovirus type 2 infection. *Amino Acids*. **42,** 2089-2094.

Ruiz-Ruiz J., Dávila-Ortíz G., Chel-Guerrero L. and Betancur-Ancona D. (2013). Angiotensin I-converting enzyme inhibitory and antioxidant peptide fractions from hard-to-cook bean enzymatic hydrolysates. *J. Food Biochem*. **37,** 26-35.

Ryder K., Ael-D B., McConnell M. and Carne A. (2016). Towards generation of bioactive peptides from meat industry waste proteins: Generation of peptides using commercial microbial proteases. *Food Chem*. **208,** 42-50.

SAS Institute. (2012). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

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

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

Toghyani M., Mosavi S.K., Modaresi M. and 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.

Wald M., Schwarz K., Rehbein H., Bußmann B. and Beermann C. (2016). Detection of antibacterial activity of an enzymatic hydrolysate generated by processing rainbow trout by products with trout pepsin. *Food Chem*. **205,** 221-228.

Wang F.Q. (2005). Effects of the bioactive peptide as feed additive on the performance, immune function and protein metabolism rate in broiler chicken. PhD Thesis. China Agricultural Univ., China.

Wattanasiritham L., Theerakulkait C., Wickramasekara S., Maier C.S. and Stevens J.F. (2016). Isolation and identification of antioxidant peptides from enzymatically hydrolyzed rice bran protein. *Food Chem*. **192,** 156-162.

Wen L.F. and He J.G. (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. *British Poult. Sci*. **108,** 1756-1763.

Yadav S.P., Kannaki T.R., Mahapatra R.K., Paswan C., Bhattacharya T.K., Sarkar S.K. and Chatterjee R.N. (2018). *In vivo* cell-mediated immune, hemagglutination inhibition response, hematological and biochemical values in native *vs.* exotic chicken breeds. *Poult. Sci*. **97,** 3063-3071.

Zambrowicz A., Pokora M., Setner B., Dąbrowska A., Szołtysik M., Babij K., Szewczuk Z., Trziszka T., Lubec G. and Chrzanowska J. (2015). Multifunctional peptides derived from an egg yolk protein hydrolysate: Isolation and characterization. *Amino Acids*. **47,** 369-380.

Zhao F., Shi B., Sun D., Chen H., Tong M., Zhang P., Guo X. and

Yan S. (2016). Effects of dietary supplementation of *Artemisia argyi* aqueous extract on antioxidant indexes of the small intestine in broilers. *Anim. Nutr*. **2,** 198-203.