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Original Research Article

Effects of periodical application of bioactive peptides derived from cottonseed on performance, immunity, total antioxidant activity of serum and intestinal development of broilers

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ABSTRACT

This experiment aimed to examine the effect of periodical application of bioactive peptides derived from cottonseed (BPC) in comparison with using sub-therapeutic doses of lincomycin and the excessive inclusion of vitamin E on performance, immunity, total antioxidant capacity of serum and intestinal morphology of broiler chickens. A total of 240 one-d-old male broiler chicks with similar initial weight (Ross strain) were randomly assigned to 6 groups (8 chicks/pen): non-treated group (basal diet), basal diet supplemented with 2 mg/kg lincomycin, basal diet supplemented with 50 IU vitamin E, basal diet supplemented with 6 g BPC/kg in starter period, basal diet supplemented with 6 g BPC/kg in starter and grower periods and basal diet supplemented with 6 g BPC/kg throughout the whole experiment. The highest final body weight was obtained in the group supplemented with BPC in starter and grower periods. In the finisher phase, broilers fed the diet containing BPC in the starter period and in the whole trial had significantly ($P < 0.05$) better feed conversion ratios (FCR). Jejunal villus height was significantly elevated in broilers supplemented with antibiotic ($P < 0.001$), furthermore it tended to be greater in broilers fed BPC in the starter period. The jejunal villus height-to-crypt depth ratio was significantly ($P < 0.01$) higher in broilers fed the diet containing antibiotic in comparison to other groups. Humoral immune response against Newcastle disease vaccine tended to be elevated in broilers fed the diet containing BPC in the whole trial ($P > 0.05$). Broilers supplemented with BPC in starter and grower, and in the whole trial had significantly ($P < 0.05$) higher antibody titers against sheep red blood cells (SRBC). The highest total antioxidant capacity was obtained in broilers supplemented with the excessive level of vitamin E, furthermore it tended to improve in broilers fed the diet containing BPC in the whole trial. In summary, the results of the study indicated that addition of BPC in broiler diets in the whole trial could improve FCR, immune responses and total antioxidant activity of serum, and BPC could be used in broiler diets as an alternative to in-feed antibiotics.

1. Introduction

Sub-clinical levels of in-feed antibiotics (IFA) have been included into livestock feed for many years for their positive effects on performance and health status of farm animals (Feanti et al., 1971; Fekri Yazdi et al., 2014a, 2014b; Goodarzi et al., 2014; Kavyani et al., 2014; Foroutankhah et al., 2019). IFA was purported to promote growth performance of broilers via reducing the proliferation of pathogenic microorganisms in the intestine, resulting in further digestion, absorption and metabolism of feed ingredients (Kheiri et al., 2018; Gheisari et al., 2017). However, widespread supplementation of IFA in broiler's diets resulted in the appearance of resistant microorganisms (Sorum and Sunde, 2001), and antibiotic residuum in poultry meat (Andremont, 2000). As a result of above mentioned problems, alternatives are being introduced to poultry feed such as probiotics (Landy and Kavyani et al., 2014; Toghyani et al., 2015), prebiotics (Ceylan and Çiftçl, 2003), and herbal products (Landy et al., 2012), and bioactive peptides (Dhama et al., 2014) have received increased attention.

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Bioactive peptides can be produced from different protein sources by acid, alkaline or enzymatic hydrolysis and microbial fermentation (Muir et al., 2013). Several experiments indicated that based on the molecular weight and amino acid profile (Hou et al., 2017) bioactive peptides have therapeutic benefits, including antimicrobial (Wald et al., 2016; Osman et al., 2016), antioxidant (Power et al., 2013; Ibrahim et al., 2018), antihypertensive (Ryder et al., 2016; Zambrowicz et al., 2015), and immunomodulatory benefits (Kotzamanis et al., 2007). Opioid peptides can affect the gut function due to binding to the opioid receptors in the brain, furthermore, they can be used in animal feed to alleviate stress, control pain and sleep, and modulate satiety (Fernstrom, 2013; San Gabriel and Uneyama, 2013). Abdollahi et al. (2017, 2018) reported that continuous supplementation of 6 g/kg of soybean bioactive peptide (SBP) improved production performance of broiler chickens through improving feed conversion ratio (FCR). In another trial, continuous supplementation of 6 g/kg of bioactive peptides derived from cottonseed (BPC) in broiler diets could result in desirable outcomes on growth performance, relative weight of bursa of Fabricius, antibodies against sheep red blood cells (SRBC) and Newcastle disease virus (NDV) antigens and total antioxidant activity of serum (Landy et al., 2020). Contrary to mentioned findings, there has been a shortcoming of knowledge regarding comparing the efficacy of continuous or periodical application of bioactive peptides in poultry. Apart from an insignificant clearance of peptides from plasma by erythrocytes, circulating peptides remain in plasma and may not be hydrolyzed (Lochs et al., 1990; Odoom et al., 1990); thus, this study was carried out to examine the possibility of periodical application of BPC in comparison to IFA and the excessive level of vitamin E on broiler growth performance, carcass characteristics, gut development, total antioxidant activity of serum and immune responses.

2. Materials and methods

2.1. Ethical matters

The present study was performed in Pishgam Damparvar Sepahan company research farm in Zyar city. All experimental procedures including sampling and killing broilers were conducted in accordance with University of Shahrekord ethical guidelines for animals (approval ref no. 2019 to 064).

2.2. Animals and dietary treatments

A total of 240 one-d-old male broiler chicks (Ross strain) with similar initial weight were randomly assigned to 6 groups (8 chicks/pen): non-treated group (basal diet), basal diet supplemented with 2 mg lincomycin/kg, basal diet supplemented with 50 IU vitamin E/kg, basal diet supplemented with 6 g BPC/kg of diet (Fortide, Chengdu Mytech Biotech Co. Ltd., Chengdu, Sichuan, China) in starter period, basal diet supplemented with 6 g BPC/kg in starter and grower periods and basal diet supplemented with 6 g BPC/kg entire the whole trial. The dietary treatments in 3 growth periods (Tables 1–3) were iso-caloric and iso-nitrogenic. Diets were composed of maize, corn gluten meal, and soybean meal to meet Ross 308 strain (Ross, 2019) nutrient requirements and were fed in mash form. The broiler house was completely controlled in respect of temperature, lighting and other recommended rearing parameters; furthermore, feed and fresh water were presented on an ad libitum basis for the whole trial. At d 1 the temperature of the broiler house was maintained at 33 °C, and was reduced step-by-step to 21 °C by 21 d of age and controlled by temperature sensors. The broiler house was windowless and broilers were raised on continuous lighting.

Table 1
Ingredients and composition of starter diets (as-fed, g/kg).

Item	Cottonseed bioactive peptide inclusion, g/kg	
	0	0.6
Ingredients		
Corn (7.5% CP)	508.7	507.7
Soybean meal (44% CP)	346.6	339.7
Corn gluten meal (60% CP)	50.0	50.0
Cottonseed bioactive peptides (46% CP)	0.0	6.0
Wheat bran (14.8% CP)	20.1	22.6
Soybean oil	26.7	26.7
DL-methionine	2.8	2.8
L-lysine	4.1	4.1
L-threonine	1.3	1.3
Choline chloride	1.2	1.2
Mono calcium phosphate (15% Ca, 22.5% P)	15.5	15.4
Calcium carbonate	16.5	16.0
Sodium chloride	1.0	1.0
Sodium bicarbonate	3.5	3.5
Trace mineral premix[1]	1	1
Vitamin premix[2]	1	1
Calculated composition		
Metabolizable energy, kcal/kg	3,000	3,000
Crude protein	230	230
Lysine	14.4	14.4
Methionine	6.95	6.94
Methionine + cysteine	10.8	10.8
Threonine	9.7	9.7
Tryptophan	2.6	2.6
Arginine	14.9	15.0
Valine	11.9	11.9
Isoleucine	11.4	11.3
Leucine	21.7	21.6
Ca	9.6	9.6
Available P	4.8	4.8
Ether extract	47.7	47.8
Crude fibre	37.1	36.9
Analyzed content		
Crude protein	231	233

[1] Provided the following per kilogram of diet: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 110 mg; Se, 0.3 mg; I, 1.25 mg.
[2] Provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 5,000 IU; vitamin E, 80 IU; vitamin K, 3.2 mg; thiamin, 3.2 mg; riboflavin, 8.6 mg; nicotinic acid, 65 mg; pantothenic acid, 20 mg; pyridoxine, 4.3 mg; biotin, 0.22 mg; folic acid, 2.2 mg; vitamin B_{12}, 0.017 mg.

2.3. Analysis of bioactive peptides derived from cottonseed

Before formulating diets, maize, soybean meal, corn gluten meal, wheat bran and BPC were evaluated for the level of crude protein (Method 990.03; AOAC, 2006), and the amount of total amino acids (Methods 982.30 E a, b, and c; AOAC, 2006). Calcium and total P of BPC were measured by inductively coupled plasma - optical emission spectrometry (Method 2011.14; AOAC, 1965) at the Shahrekord University Laboratories (Table 4). The molecular weight distribution of the BPC was measured by a Superdex peptide HR 10/30 column as described by Jung et al. (2006).

2.4. Performance and carcass components

The body weight (BW) was measured on a pen basis at 10, 24 and 40 d of age, average daily weight gain (DWG) was calculated in starter, grower, and finisher periods, and throughout the experiment. Daily feed intake (DFI) was determined per pen and adjusted for dead broilers. FCR was also computed as the DFI: DWG.

At the end of the trial, 2 broilers per pen were selected, individually weighed and killed by cutting the jugular vein. Eviscerated weight, empty proventriculus, empty gizzard, liver, pancreas,

Table 2
Ingredients and composition of grower diets (as-fed, g/kg).

Item	Cottonseed bioactive peptide inclusion, g/kg	
	0	0.6
Ingredients		
Corn (7.5% CP)	527.8	526.7
Soybean meal (44% CP)	341.7	334.8
Corn gluten meal (60% CP)	30.0	30.0
Cottonseed bioactive peptides (46% CP)	0.0	6.0
Wheat bran (14.8% CP)	17.9	20.4
Soybean oil	40.9	40.9
DL-methionine	2.5	2.5
L-lysine	2.6	2.6
L-threonine	0.8	0.8
Choline chloride	1.1	1.1
Mono calcium phosphate (15% Ca, 22.5% P)	13.6	13.6
Calcium carbonate	14.9	14.4
Sodium chloride	1.5	1.5
Sodium bicarbonate	2.7	2.7
Trace mineral premix[1]	1	1
Vitamin premix[2]	1	1
Calculated composition		
Metabolizable energy, kcal/kg	3,100	3,100
Crude protein	215	215
Lysine	12.9	12.9
Methionine	6.28	6.27
Methionine + cysteine	9.9	9.9
Threonine	8.8	8.8
Tryptophan	2.5	2.5
Arginine	14.4	14.5
Valine	11.3	11.3
Isoleucine	10.8	10.8
Leucine	19.8	19.8
Ca	8.7	8.7
Available P	4.3	4.3
Ether extract	61.9	62.0
Crude fibre	36.4	36.2
Analyzed content		
Crude protein	218	217

[1] Provided the following per kilogram of diet: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 110 mg; Se, 0.3 mg; I, 1.25 mg.
[2] Provided the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 4,500 IU; vitamin E, 65 IU; vitamin K, 3.0 mg; thiamin, 2.5 mg; riboflavin, 6.5 mg; nicotinic acid, 60 mg; pantothenic acid, 18 mg; pyridoxine, 3.2 mg; biotin, 0.18 mg; folic acid, 1.9 mg; vitamin B_{12}, 0.017 mg.

Table 3
Ingredients and composition of finisher diets (as-fed, g/kg).

Item	Cottonseed bioactive peptide inclusion, g/kg	
	0	0.6
Ingredients		
Corn (7.5% CP)	579.9	578.9
Soybean meal (44% CP)	287.5	280.5
Corn gluten meal (60% CP)	40.0	40.0
Cottonseed bioactive peptides (46% CP)	0.0	6.0
Wheat bran (14.8% CP)	9.9	12.4
Soybean oil	42.8	42.8
DL-methionine	2.3	2.3
L-lysine	2.9	2.9
L-threonine	0.7	0.7
Choline chloride	1.0	1.0
Mono calcium phosphate (15% Ca, 22.5% P)	12.6	12.6
Calcium carbonate	14.1	13.6
Sodium chloride	1.4	1.4
Sodium bicarbonate	2.9	2.9
Trace mineral premix[1]	1	1
Vitamin premix[2]	1	1
Calculated composition		
Metabolizable energy, kcal/kg	3,200	3,200
Crude protein	200	200
Lysine	11.9	11.9
Methionine	5.96	5.95
Methionine + cysteine	9.4	9.4
Threonine	8.1	8.1
Tryptophan	2.2	2.2
Arginine	12.9	12.9
Valine	10.4	10.4
Isoleucine	9.8	9.7
Leucine	19.4	19.3
Ca	8.1	8.7
Available P	4.0	4.3
Ether extract	65.3	65.3
Crude fibre	33.1	32.8
Analyzed content		
Crude protein	203	201

[1] Provided the following per kilogram of diet: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 110 mg; Se, 0.3 mg; I, 1.25 mg.
[2] Provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 4,000 IU; vitamin E, 55 IU; vitamin K, 2.2 mg; thiamin, 2.2 mg; riboflavin, 5.4 mg; nicotinic acid, 45 mg; pantothenic acid, 15 mg; pyridoxine, 2.2 mg; biotin, 0.15 mg; folic acid, 1.6 mg; vitamin B_{12}, 0.011 mg.

empty small intestine, heart, spleen and bursa of Fabricius were removed from carcasses, weighed and calculated as a percentage of live BW.

2.5. Jejunal histology

At 40 d of age, 2 broilers per cage were selected, killed and their gastrointestinal tracts were removed from the carcasses. The proximal parts of each jejunum were fixed in 10% neutral formalin and dehydrated in a graded ethanol series before being embedded in paraffin. Jejunum sections were stained using hematoxylin and eosin according to the method described by Iji et al. (2001). Stained segments were photographed by light microscopy (Olympus Co. Ltd., BX 50, F-3, Tokyo, Japan) to determine villus height (VH), crypt depth (CD) and villus width (VW).

2.6. Immunity

The current study was performed to examine serologic immune responses of broilers vaccinated with a commercially available live vaccine of NDV at 7 (B 1), 14 (B 1) and 21 (LaSota) d of age. At 25 d of

Table 4
Composition of the protein hydrolysates of cottonseed (g/kg).

Item	Content
Total protein (N × 6.25)	464.4
Peptides with molecular weight < 1,000 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
Asparagine	58.5
Glutaurine	93.0
Tyrosine	12.8
Tryptophan	6.0
Ca	33.0

age, 2 chicks per pen were intravenously injected with 1 mL of 1% suspension of SRBC antigen. Antibody responses to injected SRBC were measured at 6 d post-inoculation by the microtiter method as described by Landy et al. (2011a,b). Antibody titers were expressed as the Log_2 of the reciprocal of the highest dilution. At 7 d after post vaccination (28 d) broilers were bled by puncture of brachial vein to determine the hemagglutination-inhibition (HI) antibody titers against NDV, and HI antibody responses were converted to log2 (Landy et al., 2011a,b).

At the termination of the experiment, 2 broilers per pen were bled via vena brachialis to obtain blood samples into syringes containing heparin. Blood smears were prepared using May−-Greenwald−Giemsa stain (Lucas and Jamroz, 1961). One hundred leukocytes, per sample including nongranular and granular cells, were enumerated below an optical microscope (Nikon, Tokyo, Japan). The heterophil-to-lymphocyte (H:L) ratio was computed by dividing heterophil counts to lymphocyte counts (Gross and Siegel, 1983). The packed cell volume (PCV) was determined by micro-hematocrit method as described by Kececi et al. (1998). Furthermore, total counts of white blood cell (WBC) were measured via brilliant cresyl blue dye (Haddad and Mashaly, 1990).

2.7. Total antioxidant activity of serum

At 40 d of age, blood samples were collected via vena brachialis, and samples were centrifuged to obtain serum. Total antioxidant capacity (T-AOC) of serum was measured via BioAssay Systems Commercial kits.

2.8. Statistical analysis

The experiment was carried out as a completely randomized design, and combined variable data were analyzed using ANOVA (SAS Inst. Inc., Cary, NC). Significant differences between means were detected using a post-hoc Tukey test at 5%.

3. Results and discussion

3.1. Performance and carcass traits

No mortalities occurred during the trial. The BW that was obtained in the current trial was less than the breed standard (Table 5). The research was performed in Pishgam Damparvar Sepahan company research farm in Zyar city with an altitude of 1,590 m. Julian (2007) mentioned that pressure of oxygen drops nearly 2.5% per 1,000 m increase in altitude. Beker et al. (2003) reported that for broiler chickens reared under low pressure of oxygen, the BW reduced via a depression in DFI. Besides rearing in high altitude, feeding a mash diet could account for the reduced BW at various ages. At 10 d of age, the highest BW was obtained in the group supplemented with antibiotic ($P < 0.01$). The BW obtained in the group supplemented with BPC in the whole trial tended to be significantly higher in comparison to those fed the basal diet. At 24 d of age, treatments had no effect on BW, however, it tended to improve in broilers fed the diet supplemented with antibiotic ($P > 0.05$). At 40 d of age, the highest final BW was obtained in the group supplemented with BPC in the starter and grower periods; though it didn't statistically differ from other groups except for broilers fed the basal diet supplemented with the excessive level of vitamin E. During starter phases (1 to 10 d of age), grower phases (11 to 24 d of age), and in the whole trial (1 to 40 d of age) treatments had no effects on the DFI ($P > 0.05$). During the finisher period, broiler chickens fed the diet containing the excessive level of vitamin E had a significantly ($P < 0.05$) greater DFI in comparison to those fed the diet containing BPC in the starter period. In the finisher period, broilers fed the diet containing BPC had a higher DFI in comparison to those fed the basal diet, though the differences were not statistically significant. During the starter period the best FCR was obtained in the group supplemented with antibiotic ($P < 0.05$). In the starter period, supplementation with BPC did not have any significant effect on the FCR value in comparison to those fed the basal diet; however, it tended to be better in broilers fed the diet containing BPC throughout the whole trial. In the grower phase, treatments had no significant effect on FCR ($P > 0.05$). In the finisher phase, broilers fed the diet containing BPC in the starter period and in the whole trial had significantly ($P < 0.05$) better FCR values in comparison to those fed the excessive level of vitamin E, but didn't statistically differ from other groups. Abdollahi et al. (2017) reported that continuous application of 6 g SBP/kg of diet in broilers' diet improved FCR via improvement in intestinal histology and consequently better digestion of nutrients. Similarly, results of experiment performed by Abdollahi et al. (2018) indicated that continuous supplementation of 5 and 6 g SBP/kg of diet improved feed efficiency of broilers. These are in agreement with the outcomes obtained by Wang et al. (2011) and Mateos et al. (2014), who reported that continuous application of bioactive peptides in broilers' diet improved performance criteria. According to Feng et al. (2007) bioactive peptides can improve intestinal enzyme activities; unfortunately, in the present experiment we didn't measure intestinal enzyme activities. Since, in the present trial, continuous application of 6 g BPC/kg of diet improved FCR and final BW and in the mentioned treatment the absorptive capacity of the gut was not improved, it seems that the obtained FCR may have been improved by higher intestinal enzyme activities. In the current trial, the highest final BW was obtained in the group supplemented with BPC in the starter and grower periods, whereas the BW obtained in broilers fed the diet containing BPC throughout the whole trial tended to be significant. According to Webb (1990) di- and tri-peptides may remain intact in the circulatory system and act in their role. Similarly, Lochs et al. (1990) reported that apart from an insignificant elimination of peptides via erythrocytes, circulating peptides remain in the plasma and perform their physiological functions. In the current trial, the results indicated that circulatory peptides have the potential to use periodical treatments to enhance their benefits on performance parameters.

In the present experiment, the final BW of broilers supplemented with antibiotic tended to be significant in that the addition of antibiotic to broilers' diet significantly improved VH-to-CD (VH:CD) ratio. As reported by Bedford (2000) antibiotics can control and limit the formation of bacterial colonies in the gastrointestinal tract of the birds. This can lead to higher feed utilization, resulting in better performance and feed efficiency.

Table 6 shows the effects of dietary treatments on carcass yield and development of internal organs as a percentage of live BW. Treatments did not have any significant effect on carcass yield and development of organs ($P > 0.05$). The effect of supplementing BPC in the whole trial on carcass yield tended ($P > 0.05$) to be significant. The relative weight of the small intestine tended to be lower in broilers fed the diet containing the excessive level of vitamin E, antibiotic and BPC in the starter period ($P > 0.05$). Similar to our results, Abdollahi et al. (2017) reported that continuous supplementation of different levels of SBP in broiler diets did not have any significant effects on carcass characteristics. In several trials, researchers reported that the percentage of small intestine of broilers tended to decrease when IFA was included in the diet (Landy et al., 2011a, 2011b, 2012). This may be due to the restriction of growth and the colonization of pathogenic and none-pathogenic bacteria and consequently reduction in gastrointestinal infections. Similar to antibiotics, special bioactive peptides have antimicrobial effects, as mentioned for some endogenous peptides in the small intestine

Table 5
Influence of dietary treatments on performance indices of broiler chickens at different ages.

Item	Experimental treatments						SEM	*P*-value
	Control	Vitamin E	Lincomycine	6 g BPC/kg in starter	6 g BPC/kg in starter and grower	6 g BPC/kg in the whole trial		
Body weight, g								
10 d of age	218[b]	220[b]	238[a]	222[ab]	225[ab]	235[ab]	4.04	0.007
24 d of age	933	866	965	924	934	921	22.00	0.129
40 d of age	2,120[ab]	2,069[b]	2,142[ab]	2,114[ab]	2,156[a]	2,147[ab]	17.93	0.026
Daily feed intake, g/d								
1 to 10 d of age	22.6	22.9	23.9	24.0	23.5	23.9	0.68	0.643
11 to 24 d of age	72.4	71.4	73.7	71.3	71.9	71.0	1.00	0.487
25 to 40 d of age	121.2[ab]	125.8[a]	120.7[ab]	120.0[b]	124.7[ab]	122.7[ab]	1.17	0.010
1 to 40 d of age	79.4	81.0	80.1	78.9	80.9	79.9	0.63	0.209
FCR								
1 to 10 d of age	1.25[ab]	1.25[ab]	1.19[b]	1.30[a]	1.25[ab]	1.21[ab]	0.02	0.061
11 to 24 d of age	1.42	1.54	1.42	1.42	1.42	1.45	0.04	0.256
25 to 40 d of age	1.63[ab]	1.67[a]	1.63[ab]	1.61[b]	1.63[ab]	1.59[b]	0.01	0.002
1 to 40 d of age	1.52[b]	1.59[a]	1.52[b]	1.52[b]	1.52[b]	1.51[b]	0.01	0.002

BPC = bioactive peptide derived from cottonseed; SEM = standard error of mean; FCR = feed conversion ratio.
[a, b] Values in the same row not sharing a common superscript differ ($P < 0.05$).

Table 6
Influence of dietary treatments on carcass yield and internal relative organ weight of broilers at 40 d of age (%).

Item	Experimental treatments						SEM	*P*-value
	Control	Vitamin E	Lincomycine	6 g BPC/kg in starter	6 g BPC/kg in starter and grower	6 g BPC/kg in the whole trial		
Carcass	78.8	78.5	79.2	79.1	78.2	80.5	1.14	0.77
Proventriculus	0.47	0.45	0.41	0.39	0.38	0.38	0.03	0.22
Gizzard	1.47	1.69	1.54	1.39	1.56	1.22	0.10	0.11
Liver	2.8	2.4	2.5	2.5	2.6	2.8	0.12	0.21
Pancreas	0.27	0.27	0.26	0.27	0.27	0.27	0.01	0.99
Small intestine	8.1	7.3	7.1	7.4	7.9	8.1	0.55	0.71
Heart	0.44	0.42	0.43	0.37	0.41	0.41	0.01	0.22
Spleen	0.11	0.12	0.13	0.11	0.10	0.11	0.01	0.84
Bursa of Fabricius	0.20	0.19	0.20	0.20	0.20	0.21	0.01	0.98

BPC = bioactive peptide derived from cottonseed; SEM = standard error of mean.

(Bevins and Salzman, 2011). So reduction in the percentage of small intestine when antimicrobial peptides are supplemented to diets is descriptive.

3.2. Morphometric analysis of the jejunum

The effects of experimental treatments on the morphology of the jejunum are summarized in Table 7. Broilers fed the diet containing antibiotic had the highest VH ($P < 0.001$). The VH tended to be greater in broilers fed BPC in the starter period in comparison to those fed the basal diet, the basal diet supplemented with vitamin E, the basal diet supplemented with BPC in the starter and grower periods, and in the whole trial. Treatments had no significant effect on CD. VW tended to be significantly ($P > 0.05$) lower in broilers fed diet the excessive level of vitamin E and BPC in the starter period. The VH:CD ratio was statistically significant ($P < 0.01$) and greater in broilers fed diets containing antibiotic in comparison to those fed the basal diet, the basal diet supplemented with BPC in the starter and grower phases and in the whole trial. In several trials, the affirmative influences of bioactive peptides on chickens' gut has been documented (Liu et al., 2008; Bao et al., 2009; Wen and He, 2012). Adjustment with the obtained results in the present experiment Abdollahi et al. (2017) reported that supplementation of 3 or 6 g SBP/kg enhanced VH in broilers, whereas supplementation of SBP did not have any positive effects on CD, epithelial thickness, and goblet cell number in the duodenum. Osho et al. (2019) reported that inclusion of SBP in broilers' diet could induce favorable influences on the histology of small intestine.

3.3. Immune responses and hematology

The effect of treatments on immune related parameters has been shown in Table 8. At 40 d of age no differences ($P > 0.05$) were observed for H:L ratio ($P > 0.05$). Treatments had no significant

Table 7
Influence of dietary treatments on villus height, villus width, crypt depth, villus height-to-crypt depth ratio and epithelial thickness in jejunum of broiler chickens at 40 d of age.

Item	Experimental treatments						SEM	*P*-value
	Control	Vitamin E	Lincomycine	6 g BPC/kg in starter	6 g BPC/kg in starter and grower	6 g BPC/kg in the whole trial		
Villus height, μm	633[bc]	666[bc]	1035[a]	950[ab]	633[bc]	470[c]	80.8	0.001
Crypt depth, μm	276	286	245	276	266	260	23.8	0.863
Villus width, μm	166	123	175	130	153	158	12.6	0.06
Villus height-to-crypt depth ratio	2.25[c]	2.30[c]	4.2[a]	3.4[ab]	2.4[bc]	1.79[c]	0.25	0.01

BPC = bioactive peptide derived from cottonseed; SEM = standard error of mean.
[a–c] Values in the same row not sharing a common superscript differ ($P < 0.05$).

Table 8
Influence of dietary treatments on humoral immune responses and serum antioxidant status of broiler chickens.

Item	Experimental treatments						SEM	*P*-value
	Control	Vitamin E	Lincomycine	6 g BPC/kg in starter	6 g BPC/kg in starter and grower	6 g BPC/kg in the whole trial		
Antibody titers against New castle, $\log_2$	2.75	2.75	2.25	2.4	2.6	3.4	0.36	0.330
Antibody titers against SRBC, $\log_2$	6.5^{b}	7.5^{ab}	7.6^{ab}	6.5^{b}	8.6^{a}	8.2^{a}	0.75	0.050
Heterophil-to-lymphocyte ratio	0.36	0.37	0.43	0.32	0.32	0.43	0.03	0.067
Total antioxidant activity of serum[1]	363^{b}	498^{a}	455^{ab}	386^{b}	353^{b}	432^{ab}	24	0.001

BPC = bioactive peptide derived from cottonseed; SEM = standard error of mean; SRBC = sheep red blood cells.
[a-b] Values in the same row not sharing a common superscript differ (P < 0.05).
[1] Expressed as μmol/L Trolox Equivalents.

Table 9
Influence of dietary treatments on hematological responses of broiler chickens (%).

Item	Experimental treatments						SEM	*P*-value
	Control	Vitamin E	Lincomycine	6 g BPC/kg in starter	6 g BPC/kg in starter and grower	6 g BPC/kg in the whole trial		
PCV	27.6	31.0	29.6	26.1	28.0	26.6	1.16	0.083
WBC, $\times 10^3/\mu L$	19.9	18.1	18.9	17.4	18.4	19.6	0.66	0.152
Heterophil	24.6	25.0	28.6	22.6	22.8	28.1	1.50	0.06
Lymphocyte	67.6	67.6	63.4	69.8	69.5	65.0	1.74	0.123
Monocytes	4.6	4.0	4.2	3.8	4.0	3.8	0.42	0.843
Eosinophils	1.8	2.2	2.2	2.3	2.1	1.8	0.28	0.748
Basophil	1.4	1.2	1.6	1.5	1.5	1.1	0.20	0.665

BPC = bioactive peptide derived from cottonseed; SEM = standard error of mean; PCV = packed cell volume; WBC = white blood cell.

effect on antibody titers against NDV, whereas the effect tended to increase in broilers supplemented with BPC in the whole trial ($P > 0.05$). Broilers supplemented with BPC in the starter and grower periods, and in the whole trial had significantly ($P < 0.05$) higher antibody titers against SRBC in comparison to those fed the basal diet and the basal diet supplemented with BPC in the starter period. It seems that to improve immune responses in birds it is necessary to use BPC in the diet continuously; because there is a clearance of circulatory peptides by erythrocytes in plasma (Lochs et al., 1990; Odoom et al., 1990). Hou et al. (2017) reported that SBP contains antibodies which increase the immune responses of animals and consequently can improve the health status of animals. Similarly, Osho et al. (2019) reported that inclusion of SBP in broilers' diets alleviated the coccidia challenge by expression of an immune-related gene. Cheng et al. (2017) reported that supplementation of vitamin E in broilers' diet alleviated the immune damage of the bursa of Fabricius in cyclophosphamide immunosuppressed broilers by an increased T-AOC of serum; thus, in the present experiment antibody levels may be increased by an increment in T-AOC of serum.

Data of hematological parameters has been shown in Table 9. Treatments had no significant effect on hematological parameters. The means of lymphocyte values tended to be higher in broilers fed the diet containing BPC in starter period and in starter and grower periods ($P > 0.05$). As Whitehair and Thompson (1956) mentioned, the presence of critical health challenge and stress is essential to disclose the efficacy of and additive on blood hematology, and the current investigation was performed under optimal conditions.

3.4. Total antioxidant capacity of serum

As shown in Table 8, broilers fed the diet containing the excessive level of vitamin E had significantly ($P < 0.001$) higher T-AOC of serum in comparison to those fed the basal diet, or the basal diet supplemented with BPC in starter period and starter and grower periods. In agreement with our results, Cheng et al. (2017) reported that supplementation of vit E to broilers' diet increased T-AOC level, and reduced malondialdehyde in the liver. In the current investigation T-AOC of serum tended to be greater in broilers fed the diet containing BPC in the whole trial; but periodical application of BPC in broilers' diet didn't induce any marked effects on T-AOC of serum. According to Lochs et al. (1990) and Odoom et al. (1990) there is a partial clearance of circulatory peptides from plasma by erythrocytes; thus, the obtained results in the current study are descriptive. Bioactive peptides which indicated antioxidant activities contain hydrophobic amino acids (AA) such as Tyr, Leu, Phe and Ile (Davalos et al., 2004). Since BPC contains high amounts of hydrophobic AA; so continuous application of BPC in broiler diets could improve T-AOC of serum.

4. Conclusion

In conclusion, in the present investigation periodical supplementation of BPC could maximize growth performance of broiler chickens; whereas its periodical application was not shown to improve immune responses and T-AOC of serum. The results indicated that for improving immune responses and T-AOC of serum it is necessary to include BPC in the diet for the entire the experimental period.

Author contributions

Farshid Kheiri, Mostafa Faghani, and Nasir Landy contributed to the conception and design of the study. Nasir Landy performed the experiment. Nasir Landy analyzed data and wrote the paper. All authors read the paper and revised accordingly.

Conflict of interest

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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