Effects of Bioactive Peptides Derived From Feather Keratin On Small Intestinal Function, Meat Quality, And Performance of Broiler Chicks

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Abstract

In this research, the effect of mixed feather bioactive peptides (MFBPs) added in water, on intestinal health, meat quality, and plasma cholesterol level of broiler chickens was evaluated. Eighty-day-old male broiler chick (Ross 308) were randomly divided into two treatments with four replication pens. The dietary treatments were: the drinking water with no additives (control), and drinking water containing 50 ppm MFBPs. Live weight and feed intake were measured at the end of starter (1-10 d), grower (11-24 d), and finisher (25-36 d) periods with calculating average daily gain, and feed conversion ratio. The results indicate that body weight gain was greater ($P<0.05$) in birds received MFBPs in the final period. At 24 days of age, the villus height and muscle layer thickness in different parts of the intestine were higher in birds that received bioactive peptides but epithelial thickness was lower than control birds ($P<0.05$). In addition, the administration of MFBPs decreased ($P<0.01$) serum total cholesterol, triglyceride, and low-density lipoprotein in broilers. Supplementation with MFBPs significantly reduced ($P<0.01$) the malondialdehyde (MDA) amount in the thigh muscle. In conclusion, using the MFBPs in the diet of broilers could improve meat quality, cholesterol concentration in serum, and gut health.

Introduction

In the last years, the nutritional value and health benefits of food products have been paid further attention by consumers. Two of the most important poultry produces are used in the human diet are meat and eggs which oxidative stress in animals can lead to negative changes in the health benefits of meat (Zhang et al., 2011). Hence, increasing the oxidative stability of raw products is an important principle in the food industry (Jiang et al., 2007). A common manner to ameliorate the antioxidant level is dietary manipulation (Zhang et al., 2014). Accordingly, in food and feed industries, unnatural antioxidants such as propyl gallate (PG), butylated hydroxytoluene (BHT), and tertbutylhydroquinone (TBHQ) to prevent peroxidation processes have been used. However, the application of these artificial antioxidants should be severely monitored by reason of potential health concerns (Park et al., 2001). Consequently, using natural antioxidants such as bioactive peptides derived from natural proteins is a considerable achievement in the feed industry (López et al., 2014; Wu et al., 2015).

Feather proteins have been used in a wide range of industrial applications, namely; composites, fibers, tissue engineering scaffolds, and micro, and nanoparticles (Arshad et al., 2016; Barone and Schmidt, 2006; Esparza et al., 2017; Khosa et al., 2013; Khosa and Ullah, 2014; Sangali and Brandelli, 2000). Despite the rapid growth of the biotechnology industry, researchers have paid little attention to recycling chicken feather waste as a source of protein. About more than ninety-two percent of the feather is composed of keratin protein (Papadopoulos, 1985) with an average molar mass of around 10,000 Daltons (Staroń et al., 2014).

Alanine, proline, glycine, serine, glutamic acid, branched-chain amino acids, and cysteine are the most amino acids in keratin structure but lower segments of histidine, lysine, and methionine exist in the keratin protein (Heid et al., 1986; Hillerton, J.E. Berry, 2005). In terms of chemical structure, a large number

of functional groups like -COOH, -SH, -NH₂, and -OH exist on polypeptide chains of keratin lead to biological activities such as lowering blood pressure, antioxidant, and dipeptidyl peptidase-IV-inhibitory (Fakhfakh et al., 2011; Fontoura et al., 2014; Kumar et al., 2012).

It has been confirmed that exogenous antioxidants like N-Acetyl-cysteine (NAC) which have an effective source of sulfhydryl groups may interact with free radicals such as OH[•] and H₂O₂ (Ueno et al., 2011). Have been demonstrated that fish protein hydrolysate (FPH) affected the fatty acid composition in liver, serum, and triacylglycerol rich lipoprotein (Wergedahl et al., 2004). Our recent research indicated that the in vitro antioxidant potency of the hydrolyzed feather was at IC₅₀ values of 5.03 ± 0.215 mg/mL in the DPPH bioassays, and the hydrolyzed product did not inhibit the viability of the fibroblast cells (Alahyaribeik et al., 2021).

To our knowledge, to date, no report is available regarding the influence of the peptides isolated from chicken feather hydrolysate in the in vivo studies. Hence, the objective of the current research was to examine the effects of hydrolyzed feathers on intestinal health, serum cholesterol concentrations, gastrointestinal microflora population, meat quality, and growth performance of broiler chickens.

Materials And Methods

Mixed feather bioactive peptides (MFBPs) synthesis

The mixed feather bioactive peptides (MFBPs) used in the present study was obtained from the biodegradation of chicken feather by *Bacillus licheniformis* (Accession Number: FN678352) isolated from Iranian soil. Feather fermentation was conducted, as reported previously (Alahyaribeik et al., 2020). Briefly, the feather hydrolysates were purified with a membrane of 3 kDa-cutoff at first. The product of filtration was used to reverse-phase high-performance liquid chromatography (model E2995, Waters, Milford, USA, and a C18 column, Agilent 1100, Agilent Technologies Inc., USA) to more purification and the final product labeled as MFBPs. The size exclusion chromatography was carried out on a PerkinElmer series 200 system at 35 °C with a TSK gel PWXL column (7.8 mm × 300 mm) for identifying the molecular mass of MFBPs. And it was shown the range of peptide molecular weights from 4000 to 5000 Daltons.

Animals, diets and management

All of the procedures, animal ethics, and welfare were carried out in accordance with guidelines set out by the faculty of Animal Sciences, University of Tehran, Iran. Eighty-One-day-old male broiler chicks (Ross 308) were randomly allotted to 8 pens (4 replicate pens; 10 birds/ each pen). The dietary treatments were: the drinking water with no additives (control), and drinking water containing 50 ppm MFBPs. The broiler chicks were fed on a pellet basal diet which was formulated to meet nutrient requirement recommendation for Ross 308 broilers by Aviagen (2014) from 1–10, 11-24, and 25–36 d of age for starter, grower, and finisher periods, respectively (Table 1). The birds were housed in suitable pens. A hanging bell drinker and self-feeder for each pen were supplied to free-accessing to water and feed. The

temperature inside the house was initially at 33–35°C for the first 5 d and was decreased progressively by 3°C each week till a temperature of 26 °C was reached. In addition, live weight and feed intake were measured at the end of starter (1-10 d), grower (11-24 d) and finisher (25-36 d) periods with calculating average daily weight gain (ADWG), average daily feed intake (ADFI), feed conversion ratio (FCR), and overall performance parameters from 1 to 36 d of age.

Blood Collection for blood Chemistry

At 24 of age, 4 birds from each diet group (Control and MFBPs) were randomly taken, individually weighed, and slaughtered. Blood samples (3-mL) were accumulated from the wing vein into a heparinized syringe to hamper coagulation. In addition, the amount of serum triglycerides (TG) and total cholesterol (TC) were examined by a blood analyzer (Hitachi, Tokyo, Japan) at 24 days of age. The content of high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were confirmed according to the instructions of the manufacturer (Nanjing, Jiangsu Province, China).

Sampling and morphometry of the small intestine

At days 10 and 24 d of age 4 birds from each diet group (Control and MFBPs) were euthanized. For morphometric analysis, 4 cm of the distal sections of the duodenum, jejunum, and ileum were dissected and submerged in 10% formaldehyde. After 4 days' fixation, cross-sections of the intestine were stained and embedded in paraffin by standard method (Yoon et al., 2013). All morphological variables, namely; villus height (VH), crypt depth (CD), crypt diameter, epithelium thickness, muscle layer thickness, and the ratios of VH: CD were done in 10-µm increments by using a light microscope (Version 6.5 of Optimus software, North Reading, MA).

Microbial counting

At 10 and 24 d in 4 birds per treatment, counting of the bacterial communities in the ileal and cecal contents according to the method of Jin et al. (2009) was conducted (Jin et al., 2009). In the ileum and cecal, total aerobic bacteria (TAB), coliform and *Lactobacillus* counts were analyzed using Nutrient, MacConkey and de Man, Rogosa, Sharpe agar (MRS) after incubating at 37°C for 24, 24 and 48 h respectively. To specify colony-forming units in each gram of collected digest from the cecal and ileum, pour plate method was conducted. Also, before statistical analysis, the microbial populations were log₁₀ converted.

Malondialdehyde assay

One of the most common secondary products of oxidative degradation of lipids is Malondialdehyde (MDA) which was estimated by a spectrophotometric procedure published by Botsoglou *et al* (Botsoglou et al., 1994). Hence, at 24 d, one broiler chicken from each diet group was selected and slaughtered to evaluate meat quality. Briefly, the breast and thigh muscle samples were cut into small samples, and then 4mL trichloroacetic acid (50 g L⁻¹) and 2.5 mL butyrate hydroxytoluene in hexane (8 g L⁻¹) were added,

vortexed, and sonicated for 60 s. The content of the tube was centrifuged at 3000 g for 3 min and then 2.5 mL aliquot from the bottom layer was blended with 3 mL aqueous 2-thiobarbituric acid (8 g L⁻¹). TBA-containing samples were heated for 30 min at 70 °C in a water bath. Following heating, the samples were cooled on ice and provided to common spectrophotometry (UV–visible, Perkin Elmer Lambda25) at an absorbance of 521 nm.

Calculations and Statistical Analysis

Analysis of variance was carried out using pen means. Statistical analyses of data related to performance, microbial population, morphology of the small intestine, serum lipid parameters, and meat quality were performed by PDIFF *T-test* using SAS statistical programs (SAS Institute, 2012). P-values 0.05 were calculated statistically significant and P-values 0.10 as a tendency.

Results And Discussion

Growth performance

The performance of broilers during various phases of raising is displayed in Table 2. No significant differences were noted in ADFI, ADWG, and FCR between two diet groups in the starter (0 to 10 d), and grower (11 to 24 d) periods. In addition, adding MFBA_S in water did not improve ADFI and ADWG; consequently, no betterment was found for FCR in the entire period (0 to 36 d). Similar to the current findings, no effect of dietary supplementation of the potato antimicrobial peptide (AMP) on the performance of pigs has been reported (Jin et al., 2009). However, Ohh et al. (2009) published that adding an increasing level of antimicrobial peptide (AMP) isolated from solanum tuberosum to the diet caused an increase in the overall weight gain (Ohh et al., 2009). The current outcomes may be related to the environmental factors of the trial. It has been shown that the effect of feed additives such as probiotics or plant extracts on performance in a pollution-free environment is less (Gunal et al., 2006; Konjufca et al., 1997). Moreover, this discrepancy in results might be due to the concentration of dietary supplementation of MFBA_S, or the method of action of the MFBA_S.

Interestingly, ADWG was affected in the finisher period that was better in birds that received MFBAS compared to control. In the finisher period, weight gain observed in broilers fed with MFBAS may be due to the improvement in balance and quality of the microflora, small intestinal morphology, and mucosal immune system (Jin et al., 2008; Ohh et al., 2010; Tang et al., 2009). Wang et al. (2009) found that birds fed with antimicrobial peptide (purified from the pig gut) had better weight gain and FCR than birds fed a non-supplemented diet (Bao et al., 2009; Wang et al., 2009).

Ileal and Cecal microbial population

At 10 d of age, the colony-forming units of lactobacillus acidophilus bacteria and total aerobic bacteria in the gut were not considerably influenced by two diet groups (Tables 3). Although ileal of chicks received MFBPs had slightly lower colony-forming units of coliforms than the control chicks, at 10 d. In the second

stage of microflora counting (24 d), experiment supplementation with MFBPs had no significant influence on CFU of total aerobic bacteria in the small intestine and cecum, although there was a tendency for numbers of coliforms to be less in the birds receiving MFBPs. In this experiment, drinking water containing 50 ppm MFBPs had the potential to diminish toxic gut bacteria like coliforms. Recent studies proved that the dietary addition of antimicrobial peptides purified from potato tubers has decreased coliform count in excreta and cecal microbiome of broilers (Ohh et al., 2010, 2009). Inconsistency with present results, Karimzadeh et al. (2016) found that birds fed diets containing canola bioactive peptides (CBP) had lower gram-negative bacteria in ileum and cecal digesta in comparison with control birds (Karimzadeh S, 2016). In addition, Tanmay Paul et al (2015) showed that bioactive peptides isolated from chicken feathers have reduced multiple-antibiotic-resistant (MAR) *Staphylococcus aureus* count in the *in vitro* (Paul et al., 2015). The possible reason for the bactericidal activity of the MFBPs is due to bacterial cell membrane disruption, intracellular leakage, and finally bacterial lysis (Brogden, 2005). Moreover, MFBPs can interact with immune cells and enhance the immune response in the face of inflammation, for example, stimulation of cytokine release, and neutralization of LPS-induced septic effects (Elsbach, 2003). However, the effect of MFBPs on decreasing gram-negative bacteria needs to be further investigated.

Morphology of the small intestine

The effects of mixed feather bioactive peptides on gut morphology in 10 and 24 d are shown in tables 4 and 5 respectively. Generally, an increase in villus height has been defined along with the development of the surface area and the increase in the intestinal capacity for absorption (Samanya and Yamauchi, 2002). In the current study, intestinal villus height of the duodenum, and ileum in chickens that received 50 ppm of MFBPs were higher than ($P < 0.001$) control group at 10 d which indicates an increased absorptive capacity (Table 4).

Moreover, the addition of 50 ppm of MFBPs to the drinking water of chickens increased crypt depth when compared to birds of the control group at 10 days of age. In agreement with the finding of our research, the use of antibacterial peptides isolated from pig's small intestine in the diet of broiler chickens increased villus height of the duodenum and jejunum (Bao et al., 2009). In another research, chickens fed at 90 mg/kg AMP diet had greater villus height in the intestine than chickens fed the diet without supplementation of AMP, while dietary treatments had no significant effects on crypt depth of the intestine. (Choi et al., 2013). In addition, MFBPs had a significant effect ($P < 0.0001$) on the muscle layer thickness in the intestine at 10d. The jejunal epithelium thickness was markedly decreased ($P < 0.001$) using MFBPs in 10d birds, but no effect on the duodenum and ileum. Reduced jejunal epithelium thickness could be due to lower mucin secretion from goblet cells and endogenous protein losses. It has been found that a decrease in the activity of harmful microbes on the surface of the brush border reduces damage to enterocytes in the gut (Hughes, 2003). At 24 d of age, the villus height (duodenum, jejunum, and ileum) and VH: CD (duodenum, jejunum, and ileum) of chickens that received the 50 ppm of MFBPs were not different from control birds. The mixed feather bioactive peptides (MFBPs) supplement at 50 ppm to water significantly decreased ($P < 0.001$) Villus diameter in the duodenum and increased ($P <$

0.0001) crypt depth in the duodenum at 24d. Also, the epithelium thickness (duodenum and ileum) was less ($P<0.05$; Table 5) in chickens that received 50 ppm of MFBPs compared with chickens fed the diet without supplementation of MFBPs at 24d. Silva and Smithard (2002) have been suggested that the decrease in the thickness of the unstirred layer in the small intestine leads to improve nutrient absorption (Silva and Smithard, 2002). In the current research, supplementation of broiler water with 50 ppm of MFBPs increased muscle layer thickness (jejunum) compared to the control group at 24d, suggesting an increased absorptive capacity.

Effect of MFBAS supplemented to diet on serum lipid parameters

The in vivo anticholesterol activity of MFBAS at 10 and 24 days of age was studied. Values of TG, HDL, LDL, VLDL, and experimental groups are shown in Table 6. At d 10 of the experiment, a significant diminish in serum LDL (24.75%), and VLDL (26.9%) levels were noted in the MFBAS group compared to those of the control group. Interestingly, the application of MFBAS for 10 days to MFBAS-fed birds, at a dose of 50 ppm, assisted a significant diminution ($p<0.05$) in serum TG (26.39%), TC (17.89%) level compared to those of the control group. However, there were no significant differences between serum lipid parameters for birds fed MFBAS and control groups at d 10 of the trial. Indeed, the anticholesterol effects of the MFBAS could be caused by some potent bioactive peptides achieved by the enzymatic hydrolysis of the chicken feathers. In fact, the hypothesis of this anticholesterol action may be due to the high bile acid-binding capacity of peptides and cholesterol excretion in the feces or diminish the micellar solubility of cholesterol in the epithelial cells and finally decrease the blood cholesterol level (Nagaoka et al., 1999; Sugano et al., 1990). Another hypothesis on the cholesterol-lowering mechanism is that some peptides can inhibit enzymes that conduct cholesterol biosynthesis such as the HMG-CoA reducta (Soares et al., 2015). In this regard, Liu et al. (2012) also reported that *Rhopilema esculentum* protein hydrolysate reduces the plasma cholesterol and triglyceride level in rats fed with high-fat diet (Liu et al., 2012). More research is needed to illuminate the mechanism of action of mixed feather bioactive peptides (MFBPs) from chicken feathers proteins on the cholesterol-lowering.

Meat Quality

The findings on meat quality of leg and breast are presented in Fig 1. The high extent of polyunsaturated fatty acids in poultry meat make it sensitive to oxidative deterioration. (Jimenez et al., 2002). The level of oxidative degradation of lipid by reactive oxygen species (ROS) can be measured by estimating the MDA rate (Wan et al., 2017). In this study, the application of MBPs reduced the ($p<0.05$) content of MDA in the thigh tissue which displays the effective function of bioactive peptides in the prevention of lipid peroxidation and membrane damage. Therefore, the anti-cholesterol activity of MFBAS could decrease the amount of MDA in the meat; this process resulted in improving the oxidative status and reducing cellular oxidative damage. About 44% of chicken feather protein (keratin) is composed of the amino acid (Dunn, 2004). Cystine has an S-S bond which could play as a natural antioxidant. Organic compounds of sulfhydryl ($-SH$) and sulphenic acids ($-SOH$) may be formed under microbial fermentation of chicken feathers which can be considered as powerful antioxidants. Several studies have been demonstrated that

herbal products rich in polyphenols can reduce lipid peroxidation in the meat due to the absorption of produced free radicals. (Cao et al., 2012; Gouveia and Castilho, 2013). It is proven that the leg muscle is more sensitive to oxidation than breast meat due to its unsaturated fat level (Higgins et al., 1998; Jensen et al., 1997). Hence, breast meat had lower MDA content than leg muscle in this trial. Similarly, Botsoglou et al. have shown that the MDA values in thigh samples were higher than breast samples of broilers (Botsoglou et al., 2002).

Conclusion

Results of the current research showed that MFBPs obtained from microbial hydrolysis of chicken feathers could reduce serum cholesterol levels by 17.89% and improve the oxidative stability of breast and thigh meat. In addition, it is suggested that the MFBPs could use as an excellent additive of antioxidants for poultry in order to prevent some free radical-related diseases and extend the storage life of meat postmortem. Further research is needed to assess the optimal dose of MFBPs that could promote immune stimulatory activity and growth performance.

Declarations

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Conflict of interest

The authors declare no competing financial interest.

Ethics approval

All of the procedures, animal ethics, and welfare were carried out in accordance with guidelines set out by the faculty of Animal Sciences, University of Tehran, Iran.

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All authors confirm that all data and materials as well as software application or custom code support our published claims.

Code availability

Not applicable

Authors' contributions

In this article, Samira Alahyaribeik was responsible for the experimental design and conducting the experiments, data analysis, writing the first draft of the manuscript, corresponding author and submission of the manuscript. Madineh Nazarpour was responsible for the experimental design, data interpretation, and finally, all authors read and approved the manuscript.

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Tables

Table 1. Ingredients and nutrient composition of basal diet

Ingredient	Starter (1-10 d)	Grower (11-24 d)	Finisher (24–36 d)
Corn grain	57.13	59.55	65.42
Soybean meal	34.49	34.35	28.82
Gluten Meal	3.0	-	-
Vegetable oil	1.07	2.08	2.14
Dicalcium phosphate	1.74	1.50	1.28
Limestone	1.16	1.05	0.98
DL-Methionine	0.32	0.29	0.26
Vitamin premix ¹	0.25	0.25	0.25
Mineral premix ¹	0.25	0.25	0.25
Salt	0.3	0.2	0.25
L-Lysine-HCl	0.29	0.18	0.15
L-Threonine	-	0.1	0.1
Colin choloride	-	0.1	-
Sodium bicarbonate	-	0.1	0.1
Chemical composition			
MEn (Kcal/kg)	2,870	2,925	3,000
Crude protein	22.00	20.28	18.27
Methionine	0.645	0.57	0.53
Methionine + Cystine	0.90	0.82	0.75
Lysine	1.19	1.08	0.93
Available phosphorus	0.45	0.41	0.36
Calcium	0.91	0.82	0.73
Sodium	0.14	0.13	0.15

¹Vitamin premix supplied the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 1,800 IU; vitamin E, 11 mg; vitamin K3, 2 mg; vitamin B2, 5.7 mg; vitamin B6, 2 mg; vitamin B12, 0.024 mg; nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg. Mineral premix supplied the following per kilogram of diet: Mn, 100 mg; Zn, 65 mg; Cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; and Co, 0.5 mg.

Table 2 Effect of mixed feather bioactive peptides (MFBPs) on the growth performance of broilers

Item	MFBPs ^a (ppm)		SEM ^b	P-value ^c
Starter (d 1–10)	Control	50		Treat
ADWG ^d	29.735	29.18	1.802	0.818
ADFI ^e	33.67	33.42	1.65	0.891
FCR ^f	1.1393	1.147	0.013	0.879
Grower (d 11–24)				
ADWG ^d	90.58	87.21	2.684	0.279
ADFI ^e	100.09	97.91	1.305	0.291
FCR ^f	1.108	1.122	0.014	0.713
Finisher (d 25–36)				
ADWG ^d	124.37 ^b	132.57 ^a	1.784	0.034
ADFI ^e	200.1	204.84	4.945	0.479
FCR ^f	1.612	1.545	0.064	0.353
Overall (d 1–36)				
ADWG ^d	68.685	70.83	7.686	0.360
ADFI ^e	110.41	110.80	2.128	0.880
FCR ^f	1.613	1.564	0.012	0.520

^a The dietary treatments were: drinking water with no additives (control), and drinking water containing 50 ppm MFBPs.

^b Standard error of the means (data are means of 4 pens of 10 birds eac).

^cMeans within a row without a common superscript are significantly different (P<0.05).

^dADWG: average daily weight gain (g/d per bird); ^eADFI: average daily feed intake (g/d per bird);

^fFCR: feed conversion ratio (g feed/g gain).

Table 3 Effect of MFBPs (mixed feather bioactive peptides) on the Ceca and ileal (d 10 and 24) microbial populations (Log10 CFU/g) in broilers.

Item	MFBPs ^a (ppm)		SEM ^b	P-value ^c
d 10	Control	50		Treat
Ileum				
TAB ^d	5.514	5.096	0.248	0.454
MRS ^e	4.916	5.004	0.275	0.835
Coliforms	3.387	2.41	0.415	0.084
Cecum				
TAB	7.141	6.911	0.175	0.296
MRS	6.449	7.051	0.364	0.36
Coliforms	6.593	6.145	0.276	0.430
d 24				
Ileum				
TAB ^d	7.944	6.304	1.384	0.109
MRS ^e	6.057	7.46	0.610	0.082
Coliforms	4.630	3.672	0.190	0.148
Cecum				
TAB ^d	8.982	7.592	0.654	0.187
MRS ^e	9.0511	10.55	0.966	0.195
Coliforms	9.44	7.49	0.797	0.071

^a The dietary treatments were: drinking water with no additives (control), and drinking water containing 50 ppm MFBPs.

^b Standard error of the means (data are means of 4 pens of 10 birds eac).

^cMeans within a row without a common superscript are significantly different (P<0.05).

^dTAB: Total Aerobic Bacteria

^eMRS: Lactobacillus Acidophilus Bacteria

Table 4 Effect of MFBPs (mixed feather bioactive peptides) on the small intestinal morphology in broiler (d 10).

d 10	MFBPs ^a (ppm)		SEM ^b	P-value ^c
	Control	50		Treat
Villus height, μm				
Duodenum	778.82 ^b	1052.74 ^a	35.820	0.0001
Jejunum	1230.31	1125.18	50.980	0.170
Ileum	563.76 ^b	770.84 ^a	12.590	0.0001
Villus diameter, μm				
Duodenum	85.46	83.79	7.210	0.856
Jejunum	77.86	69.74	7.930	0.451
Ileum	80.54 ^a	42.24 ^b	8.910	0.009
Crypt depth, μm				
Duodenum	134.052 ^b	220.39 ^a	20.763	0.008
Jejunum	228.178 ^b	299.60 ^a	21.737	0.027
Ileum	146.55	149.89	9.5790	0.786
Crypt diameter, μm				
Duodenum	24.33	16.31	1.154	0.122
Jejunum	19.06 ^a	10.18 ^b	1.209	0.004
Ileum	12.70	13.32	1.320	0.819
VH/CD ^d				
Duodenum	6.068 ^a	4.42 ^b	0.310	0.006
Jejunum	5.605 ^a	3.77 ^b	0.461	0.024
Ileum	3.903	5.18	0.423	0.071
Epithelium thickness(μm)				
Duodenum	22.64	19.02	1.087	0.233
Jejunum	34.65 ^a	14.04 ^b	1.528	0.001
Ileum	17.54	19.41	1.346	0.560
Muscle layer thickness(μm)				

Duodenum	52.57 ^b	89.52 ^a	1.152	0.002
Jejunum	81.07 ^b	103.18 ^a	2.987	0.007
Ileum	60.78 ^b	74.77 ^a	1.854	0.001

^a The dietary treatments were: drinking water with no additives (control), and drinking water containing 50 ppm MFBPs.

^b Standard error of the means (data are means of 4 pens of 10 birds eac).

^cMeans within a row without a common superscript are significantly different (P<0.05).

^d VH/CD: villus height to crypt depth ratio.

Table 5 Effect of MFBPs (mixed feather bioactive peptides) on the small intestinal morphology in broiler (d 24).

d 24	MFBPs ^a (ppm)		SEM ^b	P-value ^c
	Control	50		Treat
Villus height, μm				
Duodenum	2060.26	2065.61	41.680	0.927
Jejunum	1828.67	1780.88	47.400	0.430
Ileum	827.43	825.25	37.800	0.960
Villus diameter, μm				
Duodenum	133.418 ^a	80.18 ^b	16.640	0.031
Jejunum	82.33	86.84	8.833	0.679
Ileum	100.00	97.37	10.077	0.844
Crypt depth, μm				
Duodenum	157.41 ^b	309.98 ^a	19.515	0.0001
Jejunum	230.78	270.15	24.827	0.195
Ileum	233.31	221.74	14.321	0.476
Crypt diameter, μm				
Duodenum	15.33	24.12	3.714	0.151
Jejunum	13.17 ^b	22.72 ^a	1.320	0.047
Ileum	32.79	29.33	5.228	0.681
VH/CD ^d				
Duodenum	12.79	9.16	1.193	0.216
Jejunum	8.316	6.39	0.689	0.077
Ileum	3.540	3.66	0.117	0.473
Epithelium thickness (μm)				
Duodenum	48.76 ^a	30.55 ^b	3.768	0.014
Jejunum	50.02 ^b	76.34 ^a	3.562	0.004
Ileum	75.83 ^a	53.79 ^b	2.894	0.002
Muscle layer thickness (μm)				
Duodenum	90.72	97.76	3.206	0.264

Jejunum	140.54 ^b	192.79 ^a	2.277	0.0001
Ileum	194.59 ^a	140.27 ^b	7.904	0.005

^a The dietary treatments were: drinking water with no additives (control), and drinking water containing 50 ppm MFBPs.

^b Standard error of the means (data are means of 4 pens of 10 birds eac).

^cMeans within a row without a common superscript are significantly different (P<0.05).

^d VH/CD: villus height to crypt depth ratio.

Table 6 Effect of MFBPs (mixed feather bioactive peptides) on serum biochemical values in 10 and 24 day-old broilers.

Item	MFBA _S ^a (ppm)		SEM ^b	P-value ^c
d 10 (mg/dl)	Control	50		Treat
HDL ^d	104	91.75	3.275	0.090
LDL ^e	51.30	38.60	2.609	0.006
vLDL ^f	11.025	8.050	0.478	0.030
TG ^g	54.75	40.25	2.393	0.026
TC ^h	166.25	136.5	4.972	0.003
d 24 (mg/dl)				
HDL ^d	82	84.75	5.2817	0.693
LDL ^e	39.4	38.05	3.029	0.8074
vLDL ^f	13.1	9.45	1.099	0.1551
TG ^g	65.5	47.25	5.498	0.1551
TC ^h	127.25	122.33	3.5678	0.2959

^a The dietary treatments were: drinking water with no additives (control), and drinking water containing 50 ppm MFBPs.

^b Standard error of the means (data are means of 4 pens of 10 birds eac).

^cMeans within a row without a common superscript are significantly different (P<0.05).

^d High-Density Lipoprotein; ^e Low-Density Lipoprotein; ^f Very Low-Density Lipoprotein; ^gTriglyceride and ^h Total cholesterol

Figures

Figure 1

Effects of MFBPs on breast muscle and thigh muscle antioxidant ability in 24 d-old broilers. The treatments were: Control group and MFBPs group which received MFBPs in drinking water at a dose of 50 ppm. The data were mean of 4 pens of 10 birds each $\pm$ Standard error of the means. Significant values are indicated with an *.

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