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Research article

Impact of dietary supplementation with soybean bioactive peptides and vitamin E on certain gastrointestinal traits, nutrient digestibility, and digestive enzymes activity in broiler chickens

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Abstract: In this study, we aimed to evaluate the effects of supplementing broilers' diets with soybean bioactive peptides (SBP) and vitamin E (VE) on jejunum morphology, gastrointestinal traits, nutrient digestibility, and the activities of digestive enzymes. Day-old broiler chicks (n=180; Ross 308) were randomly assigned to five groups, with three replicates containing twelve chicks each. The group that served as the control received a standard basal diet (BD), while the experimental treatments (2, 3, 4) were fed the same basal diet (BD) supplemented with 2.5, 5, or 7.5 g/kg of SBP, respectively, and G5 received a BD with 250 mg of VE/kg. The results revealed that SBP supplementation significantly ($P \leq 0.05$) enhanced the digestion coefficient of protein, fat, and crude fiber (except 2.5 g/kg SBP). SBP supplementation had a significant ($P \leq 0.05$) effect on the small intestine, improving trypsin enzyme activity, protein efficiency ratio (PER), and feed passage rate (FPR). It also enhanced gut structure by increasing villi height and crypt depth in the jejunum compared to the control. The results showed that VE supplementation significantly ($P \leq 0.05$) improved the protein coefficient and PER compared to the control. Moreover, compared to the control, incorporating SBP or VE had a non-significant effect on the relative weight, relative length, and weight-to-length ratios of the gastrointestinal tract, small intestine, cecum, and amount of protein intake of broilers. Thus, supplementation of SBP at 2.5 g/kg positively affects trypsin activity, PER, FPR, and gut morphology. Moreover, levels of 5.0 and 7.5 (g/kg) not only improve these qualities but also enhance nutrient digestibility in the broiler gut.

Keywords: Ross 308; feed passage rate; SBP; jejunum morphology

1. Introduction

Bioactive peptides (BIPs) are specific protein fragments comprising 2–20 amino acid residues. These peptides are released from original proteins through processes such as chemical or enzymatic hydrolysis, and bacterial fermentation [1]. Peptides that are isolated from various protein sources and offer specific physiological and biological effects are referred to as BIPs [2]. According to Abdollahi et al. in 2017 [3], BIPs are distinct fragments of proteins that positively affect bodily functions and overall health. In the last decade, there has been an increasing interest in incorporating these peptides into poultry diets, as they were developed as functional foods for poultry [4,5].

Soybeans can enhance production efficiency as a feed source for broiler chickens due to their high nutritional content and well-balanced amino acid composition [6]. Additionally, several BIPs found in soybeans possess physiological functions, including lipid-lowering effects, anti-inflammatory and antioxidant properties, and immunomodulatory effects. Specific soybean peptides, such as Lunasin and Soymorphins, demonstrate multiple benefits and may help prevent several chronic diseases [7]. Studies have shown that soybean bioactive peptides (SBPs) exhibit significant antibacterial potency, antioxidant activity, and immunomodulatory properties [8–10]. According to Wei et al. [11], the addition of SBP improved immune and antioxidant levels, boosted the integrity of the small intestinal physiological structure and barrier function and the diversity of cecum microbes, and decreased the apoptotic ratio of small intestinal epithelial cells. Studies have also demonstrated that incorporating bioactive soybean peptides, extracted through enzymatic hydrolysis, into bird feed significantly improves feed efficiency, nutrient digestibility, and intestinal morphology [3,8,12]. Moreover, Peng et al. [13] demonstrated that SBP has a positive effect on the swift development of broilers by enhancing their immune system performance, improving ileal morphology, conserving the integrity of the intestinal barrier, modulating the composition of intestinal microbiome, and enhancing metabolic function. These benefits collectively enhance intestinal health and overall bird performance.

Vitamin E (VE) is a powerful dietary supplement known for its health benefits and potent antioxidant properties. It is commonly added to poultry feed to improve productive performance, the general physiological condition of the birds [14], enhance antioxidant levels, and boost the immune system [12,15]. Rahawi et al. [16] reported that the antioxidant defense system, which is represented by the antioxidant vitamins A, E, and C, has a significant impact on most of the physiological, productive, and immune properties of birds. Additionally, Abdul-Majeed et al. [17] confirmed that antioxidants have a vital function in preserving animal health and have a special role in animal physiological, reproductive, and productive performance. Furthermore, VE and selenium supplementation could be beneficial in enhancing the welfare and productivity of poultry under stress [18]. It is reported that the inclusion of VE supplements (0, 100, 200, or 400 IU/kg), especially the higher dose of 400 IU/kg, presented beneficial effects on feed efficiency, intestinal structure, and absorptive area in growing broilers subjected to hypoxic conditions [19]. Additionally, VE supplementation has been shown to improve intestinal structure and reduce intestinal inflammation in chickens [20]. In this study, VE, a commercially available antioxidant, was compared with BIPs (soya peptides) to investigate their antioxidant activities and assess their properties under a single set of environmental conditions. According to the National Research Council (NRC), chickens require 10 IU of VE per kg of feed [21].

There is limited information that juxtaposes the bioactivity of soybean peptides and VE in relation

to their effects on the morphological and histological traits of the gastrointestinal tract in broiler chickens. Thus, we aimed to assess the impact of supplementing SBP and VE on gastrointestinal measurements, nutrient digestibility, digestive enzyme activity, and the morphology of the jejunum in broilers.

2. Materials and methods

This experiment was conducted in the poultry field of the College of Agriculture, University of Basrah, from 1/19/2022 to 2/23/2022. The duration of the experiment was 35 days, followed by an additional four days for a digestive experiment. The SBP used in this study was sourced from Vanavaran Novin Gostar Biotechnology Co., Tehran, Iran, as a yellow-brown powder. Additionally, VE was obtained from the American company SOLGAR, based in Manhattan.

2.1. Ethical approval

All animal procedures complied with national and international animal welfare guidelines. The experimental protocol was reviewed and approved by the Research Ethics Committee of the College of Veterinary Medicine, University of Basrah (Approval No. 76/37/2025, 18 May 2025).

2.2. Birds and treatments

One hundred and eighty one-day-old Ross 308 broiler chicks were obtained from a commercial hatchery and randomly assigned to five dietary treatments, each consisting of three replicate cages housing 12 chicks per replicate. All birds were reared in individual wire cages under uniform environmental conditions from 1 to 35 days of age. A corn–soybean meal starter diet was supplied during days 1–21, followed by a grower diet from days 22–35; the ingredient composition and calculated nutrient profiles of the basal diets are presented in Table 1.

The five treatments were as follows: a control group fed the unsupplemented basal diet; three experimental groups receiving basal diets supplemented with 2.5, 5.0 or 7.5 g/kg soybean bioactive peptides (SBP), respectively; and a fifth group offered the basal diet plus 250 mg/kg vitamin E (equivalent to 373 IU DL-α-tocopherol/kg).

All basal diets were formulated to meet the nutrient requirements of broiler chickens outlined by the National Research Council (NRC, 1994). The vitamin–mineral premix incorporated into the basal formula supplied an inherent 40 mg/kg vitamin E. The extra 250 mg/kg vitamin E was added externally on top of the complete basal diet without modifying the inclusion rate of any basal raw materials. This supra-nutritional supplemental dose was selected to investigate the extra antioxidant and intestinal protective functions of vitamin E. Feed and fresh water were provided ad libitum throughout the trial.

2.3. Gastrointestinal tract and cecum: Relative length and weight

At the end of the 35th day of the trial, three birds with similar body weights from each treatment group were selected to analyze the gastrointestinal and cecum traits. After weighing and slaughtering the birds, the intestines were separated from the gizzard. Measurements of the alimentary canal, small intestine, and cecum were taken, and their relative lengths were calculated based on live weight as

per [22]. The parts were then cleaned of any remaining materials, weighed, and their ratios to live body weight were determined.

Table 1. Ingredients and composition of basal diet (g/Kg).

Item	Starter	Grower
Maize, Yellow	530.0	540.0
Wheat	60.00	90.00
Soybean meal (480g CP/kg)	320.0	290.0
Broiler concentrate*	53.00	40.00
Sunflower oil	10.00	15.00
Dicalcium phosphate	5.00	5.00
Limestone fine	5.00	5.00
DL-Methionine	1.70	1.10
L-Lysine	2.30	0. 90
Vitamin-mineral premix**	10.00	10.00
Salt	3.00	3.00
	Calculated analysis***	
ME (kcal/kg)	2988.49	3054.29
Crude protein (g)	230.30	215.50
Crude fiber (g)	37.80	36.40
Calcium (g)	12.40	11.00
Avl. phosphorus(g)	4.60	4.00
Lysine (g)	13.40	11.10
Methionine (g)	5.00	4.20
Methionine + Cystine (g)	7.80	7.20

*Super concentrate includes the following ingredients: Metabolizable energy 2800 kcal/kg, 40% CP, 3.8% CF, 10% calcium, 4.5% available phosphorus, 0.14% Sodium, 1.8% lysine, 0.55% methionine, 2.89% methionine + cysteine. **Provides per kilogram of diet: Vitamin A, 6 mg; vitamin D_3, 0.15 mg; vitamin E, 40 mg; vitamin K_3, 4 mg; B_1, 3 mg; B_2, 12 mg; B_6, 10mg; vitamin B_{12}, 0.04 mg; niacin, 60 mg; Choline chloride, 700 mg; Calcium D-pantothenate, 20 mg; Folic acid, 2mg; Biotin, 0.2mg; Iron, 90 mg; Copper, 30 mg; Manganese, 120 mg; Zinc, 140 mg; Iodine, 4 mg; Selenium, 0.8 mg.***Calculated from NRC values (1994).

2.4. Nutrient digestibility

A digestion trial was conducted on birds at the age of 36–39 days, where 6 birds (3 males + 3 females) were kept from each treatment in individual cages. All birds were given the same grower diet for 3 days, mixed with 0.2% of chromic oxide (Cr_2O_3)/kg as an inert indicator. To determine the quantity of the indicator in the feed and excreta, the marker was thoroughly mixed with the bird feed. Daily feed consumption and excreta collection were measured quantitatively. The excreta were dried at 65°C in a drying oven for 72 hours [23], before being ground, and then placed in sealed plastic containers for laboratory testing to determine the percentages of protein, fat, and crude fiber using the methodology as described in [24].

After the birds were slaughtered, the digested materials were collected from the jejunum and ileum, placed in plastic containers, and stored in a refrigerator at 4°C until the nutrient levels in the dry

digested samples could be assessed. The amount of chromium was measured using Flame Atomic Absorption Spectroscopy, and the digestibility of nutrients was calculated using the equation provided in [25].

$$\text{Digestion coefficient of a nutrient} = 100\text{-}(100\times \frac{\%\ \text{Indicator in feed} \times \%\ \text{Nutrient in feces}}{\%\ \text{Indicator in feces} \times \%\ \text{Nutrient in feed}})$$

2.5. Determination of digestive enzyme activities

After the birds were slaughtered, 10 cm sections of the duodenum, along with their contents, were collected and stored at -20°C. The samples were prepared following the method described in [26] with some modifications. To isolate the duodenal contents, duodenal digesta was gently extruded manually. They were then diluted in a solution containing sodium chloride (159 mmol/L), albumin (2 g/L), and calcium chloride (20 mmol/L) to achieve a pH of 7. The mixture was thoroughly mixed for 60 seconds. After mixing, ice-cold physiological saline (Ringer's Solution) was added to the samples, and the centrifugation was performed at a speed of 6000 RPM for 20 minutes at a temperature of 4°C. The filtrate (supernatant) was retained for later analysis of enzymatic activity. The activities of the digestive enzymes trypsin, lipase, and amylase in the duodenum were measured using a ready-made kit from the American company Sigma. The tests were conducted at a temperature of 37°C, following the manufacturer's instructions. Finally, the absorbance of the clear blue supernatant was recorded at 620 nm.

2.6. Protein efficiency ratio (PER)

After calculating the total protein consumed for each replicate, the PER was determined using the equation provided in [27]:

$$\text{Protein efficiency ratio} = \frac{\text{Body weight gain (g)}}{\text{Protein intake (g)}}$$

2.7. Determination of feed passage rate (FPR)

To study the rate of feed passage (RFP), an experiment was performed on 6 birds from each treatment from 36-39 days of age. The birds were fasted for 12 hours before their specialized diets were provided with 2 g of chromium oxide per kg of feed, as an indigestible marker. Marked feed was supplied for 5 hours. All birds were then fed the original, unmarked diet. The time of first marking in the droppings, as observed by visual observation, was recorded for each bird. Finally, the feed passage was calculated using the equation described in [28].

2.8. Histological examination

After slaughtering the birds (35 days), extracting their internal entrails, and separating their intestines, sections of the jejunum area, no longer than two centimeters, were taken for a histological study. The specimen was cleaned with normal saline and then fixed in 10% buffered formalin. Paraffin blocks were prepared using standard histological laboratory techniques, including dehydration,

filtering, and paraffin embedding. The samples were sectioned into thin slices of 5 μm using a rotary microtome. The sections were then stained using the conventional Hematoxylin and eosin technique. The slides' villus height and crypt depth of each section were measured using a light microscope (LEICA ICC50HD, Germany) at 10 × magnification under normal illumination conditions. The measurements were taken using the ocular micrometer lens. The average was calculated from five measurements from each sample in triplicate. Finally, the ratio of villus height to crypt depth was calculated.

2.9. Statistical analyses

The data were analyzed using a one-way ANOVA in SPSS (2022). Means were compared with Duncan's Multiple Range Test [29] at $P \leq 0.05$.

3. Results and discussion

3.1. Gastrointestinal tract and cecum measurements

The data presented in Table 2 show that there were no significant differences ($P \geq 0.05$) among the treatments in terms of the weight or length of the gastrointestinal tract (GIT), small intestine (Si), or cecum, in addition to the weight-to-length ratios of the GIT and Si or cecum at 35 days of age.

Table 2. Mean ± SE for the effect of dietary treatments on gastrointestinal traits and cecum in broilers.

Item	Control	SBP (g/kg)			VE (mg/kg)
	(0.0)	2.5	5.0	7.5	250
GITW (%)	14.66±0.66	12.55±0.37	12.62±0.98	12.77±0.55	13.64±0.37
SiW (%)	8.21±0.73	6.92±0.17	7.60±0.74	7.23±0.26	7.37±0.59
CeW (%)	1.05±0.03	0.88±0.09	0.91±0.05	0.90±0.11	1.00±0.08
GITL (%)	12.28±0.45	10.77 ±0.42	11.39±0.58	11.16±0.26	12.29±0.68
SiL (%)	9.78±0.36	8.95±0.30	9.56±0.53	8.99±0.23	9.73±0.28
CeL(%)	1.66±0.14	1.26±0.08	1.37±0.04	1.33±0.16	1.59±0.17
GITW: GITL	1.15±0.01	1.17±0.04	1.10±0.03	1.14±0.04	1.11±0.05
SiW: SiL	0.77±0.03	0.77±0.01	0.79±0.06	0.80±0.01	0.69±0.07
CeW: CeL	0.64±0.03	0.69 ±0.02	0.67±0.05	0.67 ±0.01	0.63 ±0.01

SBP: Soybean bioactive peptides, VE: Vitamin E, W: weight, L: length, GIT: gastrointestinal tract, Si: small intestine Ce: cecum. The means of the groups without superscripts in the same row are statistically insignificant ($P \geq 0.05$).

3.2. Nutrient digestibility

Table 3 shows the effects of bioactive soybean peptides (SBP) and VE (VE) on the digestibility coefficient (DC) of nutrients for broiler chickens. The results revealed a significant improvement ($P \leq 0.05$) in protein digestibility for the treatment groups that received SBP and VE. Additionally, the groups that received SBP (G3 and G4) demonstrated improved ($P \leq 0.05$) digestibility coefficients for fat and crude fiber compared to the control group. However, there were no significant differences in the fat and crude fiber DC between the VE group and the control group. The improved digestion

coefficient and increased nutrient utilization in soybean peptide may be attributed to the increased villi length in birds' intestines. The extended length of intestinal villi contributes to longer digestion and absorption times. Evidence indicates a rising trend in the actions of intestinal absorption, which is supported by the increased length of villi. This adaptation slows the passage of food, minimizes the moistening of food particles, and enhances feed efficiency [30]. The protein digestion process improved at the acidity level in the digestive system due to its effect on the protease enzyme, which showed an increase in lactic acid in the contents of the small intestine. The increase in lactic acid levels enhances and facilitates enzyme activity besides its active involvement in feed decomposition [31]. Additionally, lactic acid bacteria help decompose fats with their secreted enzymes, improving the fat digestibility [32]. Liu et al. [12] found that feeding laying hens a diet supplemented with enzymolytic soybean meal results in improved protein and fat availability. Furthermore, the results of Osho et al. [8] confirmed that SBP significantly improve the dry matter digestibility and digestible energy in broilers. Abdollahi et al. [3] confirmed that BIPs from soybean seeds can enhance digestion and nutrient absorption in poultry. The significant improvement in protein digestibility in broiler chickens in the VE-supplemented group may be due to the antioxidant effect of VE, which may delay cell death and improve the viability of intestinal cells [33], which in turn may improve intestinal secretions. Hassanpour et al. [19] confirmed that the VE-supplemented group improve villus dimensions and increase surface area, indicating enhanced nutrient absorption in the intestines.

3.3. Digestive enzyme activities

Table 3 shows the results of digestive enzyme activity in the duodenum. The SBP group showed a significant increase in trypsin activity compared to the control ($P \leq 0.05$), while the VE group showed results similar to those of the control group. The results indicated that supplementing the diet of broiler chickens with biologically active peptides at a level of 2.5 g/kg is sufficient to achieve maximum trypsin activity in their digestive tract. No further improvement was observed with higher inclusion levels. This can be attributed to the saturation of physiological mechanisms that regulate digestive enzyme secretion, meaning the system reaches its peak response at this optimal dosage. Additionally, these peptides improve gut function and digestive efficiency at relatively low concentrations. In contrast, higher levels may trigger negative feedback mechanisms that limit further enzyme secretion. The accumulation of digestion end products could also suppress additional trypsin release. As a result, the lack of significant differences among treatments reflects a regulated physiological response aimed at maintaining homeostasis, making the lowest dose the most efficient option biologically and economically.

SBPs stimulate protease activity while having minimal or inhibitory effects on lipase and amylase. This selectivity is due to the unique interaction between SBPs and the body's protein digestion feedback loop. SBPs occupy regulatory receptors, maintaining high levels of trypsin and other proteases, without completely halting pancreatic enzyme secretion. In this context, Ding et al. [34] noted that enzyme-treated soy protein (ETSP) enhances the activity of intestinal digestive enzymes, such as trypsin, which facilitates the breakdown of proteins and improves nutrient digestion. On the other hand, there was very little interaction between the SBP and amylase. The structure of these specific bioactive soybean peptide chains does not match the active sites of carbohydrate-digesting enzymes, such as amylase. Regarding lipase inhibition, while the bioactive soybean peptides increase the activity of proteolytic enzymes, they may reduce fat digestion due to their binding to bile acids.

Moreover, soybean-specific pepsin hydrolases are known to bind to bile acids such as sodium taurocholate. Given the ability of basic and hydrophobic peptides to bind strongly to bile acids such as taurocholic acid, Ishii et al. [35] identified 9 pancreatic lipase inhibitory peptides from hydrolyzed pepsin solution in soybeans, most of which inhibit pancreatic lipase by binding to bile acids (bile acids are necessary for fat digestion).

There were no significant differences in the activities of amylase and lipase among the groups ($P \geq 0.05$), and research on the use of peptides in poultry feed and their impact on digestive enzyme activity is limited. However, a study conducted by Feng et al. [36] showed that fermenting soybean meal using *Aspergillus oryzae* fungus resulted in increased activity of trypsin, lipase, and protease enzymes in the intestinal contents of broiler chickens. Ding et al. [34] found that adding enzyme-treated soy protein to laying hens' diets increases trypsin enzyme activity in their duodenum, but has no significant effect on amylase and lipase enzyme activity in either the duodenum or jejunum of their small intestine. The increase in trypsin activity observed in the groups with added soybean peptides can be attributed to their low molecular weight. This property facilitates their transport to essential organs and enhances the pancreas's biological activity, leading to increased enzyme secretion. Moreover, the improvement may result from peptides enhancing immunity, activating endocrine glands, and producing physiological and regulatory processes that positively affect the body [37]. The findings support the results of Ding et al. [34], which indicated an increase in trypsin enzyme activity in the duodenum when enzyme-treated soy protein is added to laying hens' diet.

Table 3. Mean ± SE for the effect of dietary treatments on nutrient digestibility, digestive enzyme activities, protein efficiency, and passage rate in broilers.

Item	Control	SBP (g/kg)			VE (mg/kg)
	(0.0)	2.5	5.0	7.5	250
		Nutrient digestibility (%)			
CP	75.95^{b}±2.52	80.73^{ab}±2.43	87.09^{a}±2.27	85.14^{a}±2.39	83.54^{a}±0.92
CF	66.22^{c}±3.31	73.09^{bc}±2.37	79.88^{ab}±1.72	82.61^{a}±2.39	69.29^{c}±2.07
C. fiber	64.25^{b}±3.26	72.01^{ab}±2.13	78.65^{a}±1.04	76.82^{a}±2.25	68.25^{b}±2.51
		Digestive enzymes activities (U/mL)			
Amylase	8.91±1.02	9.35±1.52	9.28±1.07	10.57±1.77	7.53±0.27
Lipase	14.97±1.07	19.46±1.29	16.08±2.46	17.16±0.75	18.37±0.71
Trypsin	25.54^{b}±0.77	32.20^{a}±0.85	30.34^{a}±2.09	32.26^{a}±0.89	28.75^{ab}±0.68
		Protein parameters			
PI(g/bird)	567.11±45.92	573.25±12.20	537.79±21.52	560.23±17.42	525.51±14.17
PER	2.43^{b}±0.13	2.99^{a}±0.05	2.95^{a}±0.02	2.96^{a}±0.02	2.73^{a}±0.08
FPR(min)	1.63^{a}±0.06	1.46^{b}±0.03	1.47^{b}±0.02	1.42^{b}±0.04	1.56^{ab}±0.03

^{a,b,c} Means with different superscript letters within a row are significantly different ($P \leq 0.05$). SBP: Soybean bioactive peptides, VE: Vitamin E, CP: crude protein, CF: crude Fat, PI: protein intake, PER: Protein efficiency ratio, FPR: feed passage rate.

3.4. Protein efficiency and feed passage rate

The PER and feed passage rate (FPR) are summarized in Table 3. Compared to the control group, the PER in the SBP and VE groups showed a significant increase ($P \leq 0.05$), while the FPR decreased

significantly ($P \leq 0.05$) in all groups except for the VE group. Additionally, the protein intake remained unaffected by the additive groups. The improvement in PER in bioactive soybean peptides groups may be attributed to the efficiency of small peptide absorption compared to the same amount of free amino acids [37]. The improvement in PER and FPR in the SBP groups may be attributed to enhanced nutrient absorption rates in the birds' intestines. This improvement results from an increase in the surface area for absorption due to an increase in villi height in the ileum and duodenum of the small intestine [30]. Research has demonstrated that small peptides can be actively absorbed by intestinal cells through specific transporters. Once inside the cells, these peptides are converted into single amino acids, which are then either transported via the portal vein or utilized by the digestive tract [38]. The enhancement in the PER may be due to improved absorption of small peptides compared to the same level of free acids in the diet [37]. Furthermore, increasing the population of beneficial microorganisms in the intestine can help reduce the transit time of food substances, thus improving their digestibility and absorption [39].

3.5. Jejunum morphology

The morphological analysis results are shown in Table 4. In comparison to the control group, the SBP and VE groups exhibited a significant ($P \leq 0.05$) increase in villus height (VH) and crypt depth (CD) in the jejunum. There were no significant effects observed in the ratio of villus height to crypt depth among the experimental groups. Moreover, the addition of BIPs to bird feed has been shown to positively affect intestinal morphology in birds. Specifically, studies have shown that increasing villi height in the small intestine improves nutrient absorption by increasing the surface area available for absorption [40]. The significant increase in VH and CD in the jejuna part of the intestine of broiler chickens within the SBP group can be attributed to the improvement of intestinal conditions by lactic acid bacteria. These bacteria play a crucial role in restoring and producing mucin fiber networks, which act as an essential barrier to maintain intestinal integrity [41]. A study also indicated that soy-derived BIPs and soy protein isolates can promote the growth of certain probiotic bacteria, suggesting their potential as prebiotics in food and feed. For example, *Lacticaseibacillus rhamnosus* has been shown to utilize hydrophobic peptides (containing 3 to 5 amino acid residues) and hydrophilic peptides (containing more than 5 amino acid residues) [42]. Additionally, soy peptides and proteins have been reported to enhance the growth of *L. rhamnosus*, giving it a competitive advantage over *Escherichia coli* [43,44].

L. rhamnosus in the gut mucosa (the gooey layer that lines your digestive tract) creates abarrier when it grows that tries to prevent pathogens (bad microbes) from getting through. *L. rhamnosus* can produce a biofilm that protects the mucosa (moist inner lining of organs, such as the intestines and vagina). The biofilm can be described as a complex cellular structure with pathways through which messages are transmitted, facilitating the transport of molecular signals and nutrients, thus enhancing the protection of microbes against external influences. Moreover, build biofilms (or communities) to feed and protect themselves. So, *L. rhamnosus* has been deemed a probiotic because of its resistance to acid and bile as well as its good growth characteristics that enable it to survive and persist within the gastrointestinal tract [45]. It has also been reported to be highly resistant to technological processes and has a great adhesion capacity to the intestinal epithelial layer to subsequently inhibit the growth and adherence of several pathogens [46,47]. The stimulation of intestinal regions with biologically active soybean peptides can lead to increased villus length and crypt depth, potentially enhancing

nutrient absorption. These peptides stimulate reflex responses that boost nutrient uptake.

Although the exact mechanism by which these peptides enhance villus length is not fully understood, Karimzadeh and Yansari [48] suggested that small peptides may increase the number and size of villi in the small intestine compared to whole proteins. Osho et al. [8] reported that broiler chickens fed diets supplemented with SBPs experience an increase in the height of the villi and an improved ratio of villi height to crypt depth in the jejunum. The study also noted an increase in villi height in the ileum. Furthermore, the broilers that consumed these supplemented diets showed enhanced resistance to Coccidiosis, helped prevent villi atrophy, and reduced the weakness in digestive enzymes activity. Moreover, Ren et al. [49] found that stimbiotic can enhance gut microbiota, improve intestinal function, and potentially increase the utilization of dietary fiber in the cecum. Additionally, Makki et al. [50] noted that fiber-degrading bacteria ferment and utilize fiber, producing short-chain fatty acids (SCFAs). These SCFAs can help reduce intestinal inflammation and improve the integrity of the intestinal barrier. Moreover, the negative impact of the necrotic enteritis (NE) challenge was found to affect nutrient digestibility, immune response, and gut health. Peng et al in 2025 [13] observed that SBPs significantly enhance gut health in chickens. This improvement is characterized by an increase in intestinal villus height, a reduction in pro-inflammatory factors such as IL-1β and interferon-γ, and an upregulation of tight junction proteins like Zonula occludens protein 1 (ZO-1), and occludin. A study conducted by Liu et al. [12] showed that laying hens fed a diet containing SBP at a dose of 5 grams per kilogram experience an increase in villus height in the duodenum and jejunum. This suggests that BIPs may play an important role in maintaining healthy intestinal morphology and function in birds.

Table 4. Mean ± SE for dietary groups regarding jejunum morphology in broilers.

Item	Control	SBP (g/kg)			VE (mg/kg)
	(0.0)	2.5	5.0	7.5	250
Villus height (μm)	498.71^{b}±28.81	645.88^{a}±36.45	657.85^{a}±48.34	628.72^{a}±27.74	559.45^{ab}±21.46
Crypt depth (μm)	82.22^{b}±3.47	120.83^{a}±11.30	120.36^{a}±7.94	118.56^{a}±11.69	98.76^{ab}±5.11
V/C	6.10±0.56	5.46±0.71	5.52±0.63	5.36±0.30	5.69±0.36

^{a,b} Means in the same row with no common superscript are different significantly ($P \leq 0.05$). SBP: Soybean bioactive peptides, VE: Vitamin E, V/C: villus height/crypt depth in jejunum.

Our findings showed that the group of broilers receiving SBPs exhibited more uniform intestinal villi and a greater villus height. This suggests an improved ability to absorb nutrients, and these results are consistent with those reported in [11,13], who confirmed that SBP is a feed additive that can improve intestinal morphology.

4. Conclusion

In conclusion, dietary supplementation of bioactive soybean peptides up to 7.5 (g/kg) improved the activity of trypsin enzyme, nutrient digestibility, PER, FPR, and gut morphology in broilers. Additionally, including VE at 250 mg/kg improved protein digestibility and the PER in broiler chickens.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Rabia J. Abbas conceived, supervised the study and wrote the manuscript. Waleed H. Sa'adoon conducted experiments and analyzed data. Rabia J. Abbas and Waleed H. Sa'adoon revised the content. All authors read and approved the final version.

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