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Original Research

Poultry

Influence of soybean bioactive peptides on growth performance, nutrient utilisation, digestive tract development and intestinal histology in broilers

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Summary

A biologically active peptide derived from soybeans by enzymatic hydrolysis was evaluated for its potential benefits on chicken growth performance, apparent ileal nutrient digestibility and intestinal histology in young broilers. Seven broiler starter diets, based on maize and soybean meal, were formulated to contain 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg of a commercial soybean bioactive peptide (SBP) product (Fortide, Chengdu Mytech Biotech Co. Ltd., Chengdu, Sichuan, China). All diets were equivalent in respect of energy density, and digestible protein, amino acids, and other nutrients. A total of 336, one-day-old male broilers (Ross 308) were allocated to 42 cages (eight birds/cage), which were randomly assigned to the six dietary treatments. There was no significant effect of SBP on weight gain and feed intake of the birds. A significant ($P < 0.01$) effect of SBP was observed for FCR. Inclusion of 1.0, 2.0, 3.0 and 4.0 g SBP/kg of feed resulted in similar FCR values to the diet with no SBP, addition of SBP to the diets at 5.0 and 6.0 g/kg of feed resulted in lower ($P < 0.05$) FCR compared to the diet with no SBP. Inclusion of SBP had no effect ($P > 0.05$) on apparent ileal digestibility of nutrients and energy utilisation. Though not statistically significant, SBP inclusion, regardless of level, resulted in 5.7% and 6.3% increases in digestibility of dry matter and nitrogen, respectively. Birds receiving no SBP had the shortest villi and those fed SBP at 3.0 and 6.0 g/kg of feed tended ($P = 0.075$) to have the greatest villus height. The current findings suggested that including SBP in broiler diets may benefit production through improving feed efficiency, and, to some extent, nutrient digestion and intestinal histology parameters.

Keywords: **Bioactive peptides: broilers: enzymatic hydrolysis: soybeans**

Introduction

Interest in bioactive peptides supplementation in poultry diets has increased over the past decade. Bioactive peptides can be produced from a variety of food ingredients (of animal or plant origin) by microbial fermentation, enzymatic, alkali or acid hydrolysis (Muir *et al.,* 2013). Enzymatic hydrolysis is extensively used in the food industry for detoxification, improvement of nutrient quality, elimination of allergens and production of flavours (Lahl and Braun, 1994; Rolle, 1998; Dust *et al.,* 2005; Sathe *et al.,* 2005). The end products of protein hydrolysis include oligopeptides, small peptides, and free amino acids (Kamnerdpetch *et al.,* 2007). Alashi *et al.* (2013) reported that peptides which are produced during protein hydrolysis have a different amino acid composition and can deliver an array of functional properties. Some proteins or their peptide derivatives manifest biological benefits through direct or indirect effects on physiological functions (Meisel, 2007). Such biological benefits can be delivered through antioxidative, hypocholesterolemic, antimicrobial, antihypertensive, antiobesity, cytomodulatory and immunomodulatory activities (Yang *et al.,* 2009; He *et al.,* 2013; Singh *et al.,* 2014). Hancock and Sahl (2006) stated that bioactive peptides can act as antimicrobial agents by interacting with microbial host invaders or by suppressing or stimulating certain immune responses.

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Bioactive peptides are found in different food sources including eggs, fish, oysters, cereal (barley, maize, rice and wheat), soybean, canola, and meat and bone meal (Matsui *et al.,* 1993; Li *et al.,* 2002; Muir *et al.,* 2013). Of these, peptides from canola (Pan *et al.,* 2011; Aachary and Thiyam, 2012), meat and bone meal (Muir *et al.,* 2013) and soybean (Feng *et al.,* 2007; Karimzadeh *et al.,* 2016) have been evaluated in broiler chicken studies.

Soybeans contain 360–566 g/kg of protein (Singh *et al.,* 2014), and its nutritional value is almost equal to animal protein. Glycinin and ß-conglycinin, which comprise 65–80% of total soy proteins, are the precursors of most of the isolated peptides (Wynstra, 1986). It is well accepted that the consumption of soy protein can reduce the risk of coronary heart disease in humans (Singh *et al.,* 2014). Aglycin, a natural bioactive peptide isolated from soybeans, is stable in digestive enzymes and has anti-diabetic potential. Lu *et al.* (2012) reported that oral administration of aglycin can potentially reduce or prevent hyperglycaemia by increasing insulin receptor signalling pathway in the skeletal muscle of diabetic mice.

Although it is recognised that bioactive peptides can influence physiological functions, information on their use as functional ingredients in broiler nutrition is scarce. The purpose of the present study was to evaluate the effects of different levels of a soybean bioactive peptide (SBP), produced by enzymatic hydrolysis, on performance, nutrient and energy utilisation, development of digestive tract and intestinal histology in broiler starters.

Materials and methods

Seven broiler starter diets, based on maize and soybean meal, were formulated to meet Ross 308 strain recommendations for major nutrients (Ross, 2014) and were equivalent in respect of energy density, and digestible protein, amino acids, and other nutrients. The diets were formulated to contain 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg of a commercial bioactive peptide (Fortide, Chengdu Mytech Biotech Co. Ltd., Chengdu, Sichuan, China; Table 1) in powder form. Titanium dioxide (TiO_2; 3.0 g/kg) was added to all diets as an indigestible dietary marker. Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia) and then steam-conditioned at 70°C for 30 seconds and pelleted using a pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35-mm thickness.

Experimental procedures were conducted in accordance with Massey University Animal Ethics Committee guidelines. A total of 336, one-day-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 42 cages in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the seven dietary treatments was randomly assigned to six cages, each housing eight birds. On d 12, birds were transferred to grower cages and continued on the same diets until d 21. Battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. The temperature was maintained at 31°C on d 1, and was gradually reduced to 22°C by 21 d of age. Diets and water were offered *ad libitum*. Body weight and feed intake were recorded on a cage basis at weekly intervals. Mortality was recorded daily. FCR values were corrected for the body weight of any bird that died or was culled during the experiment. Excreta was scored on days 20 and 21 for consistency on a scale of 1–5, with a score of 1 representing normally formed excreta and 5 representing watery and sticky excreta. The scoring was done by the same individual.

Feed intake and total excreta output of each cage were quantitatively measured from d 18 to 21 post-hatch. Daily collections from each cage were pooled, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4°C pending analysis. The diets and excreta samples were analysed for dry matter (DM), nitrogen (N) and gross energy (GE).

On d 21, six birds per cage were euthanised by intravenous injection (1 ml per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and digesta were collected from the lower half of the ileum, as described by Ravindran *et al.* (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-caecal junction. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction. Digesta from birds within a cage were pooled, lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve and stored at 4°C until laboratory analysis. The diets and digesta samples were analysed

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Table 1. Composition and analysis (g/kg as fed) of the diets.

	Soybean bioactive peptide inclusion (SBP; g/kg)						
Item	0.0	1.0	2.0	3.0	4.0	5.0	6.0
Maize	486.3	487.6	488.7	489.8	491.0	492.1	493.3
Soybean meal, 480 g/kg	316.7	315.7	315.1	314.6	314.0	313.4	312.8
Wheat bran	40.0	38.7	37.2	35.7	34.2	32.8	31.3
Maize gluten meal	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Canola meal	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Soybean oil	22.2	22.2	22.2	22.2	22.2	22.2	22.2
Dicalcium phosphate	17.0	17.1	17.2	17.3	17.3	17.3	17.4
Limestone	8.6	8.5	8.4	8.2	8.1	8.0	7.9
DL-methionine	3.2	3.2	3.2	3.2	3.2	3.2	3.2
L-lysine. HCl	3.4	3.4	3.4	3.4	3.4	3.4	3.3
L-threonine	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Sodium chloride	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	3.7	3.7	3.7	3.7	3.7	3.7	3.7
Trace mineral-vitamin premix[a]	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Titanium dioxide (TiO2)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
SBP[b]	0.0	1.0	2.0	3.0	4.0	5.0	6.0
Calculated analysis[c]							
Apparent metabolisable energy, MJ/kg	12.34	12.34	12.34	12.34	12.34	12.34	12.34
Digestible protein	202	202	202	202	202	202	202
Digestible methionine	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Digestible methionine + cysteine	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Digestible lysine	12.8	12.8	12.8	12.8	12.8	12.8	12.8
Digestible threonine	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Digestible arginine	13.7	13.7	13.7	13.7	13.7	13.7	13.7
Digestible valine	9.8	9.8	9.8	9.8	9.8	9.8	9.8
Digestible isoleucine	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Digestible leucine	20	20	20	20	20	20	20
Crude fat	45.8	45.8	45.7	45.7	45.7	45.7	45.7
Crude fibre	33.9	33.8	33.7	33.6	33.5	33.4	33.2
Calcium	9.8	9.8	9.8	9.8	9.8	9.8	9.8
Non-phytate phosphorus	4.8	4.8	4.8	4.8	4.8	4.8	4.8
Sodium	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Chloride	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Potassium	9.2	9.1	9.1	9.1	9.1	9.1	9.1
Analysed values							
Dry matter	895	893	894	898	895	894	895
Crude protein	246	246	239	246	249	246	254
Starch	313	333	312	325	312	316	325
Fat	45.4	44.6	45.6	46.0	44.1	44.5	44.9

[a]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg. [b]Fortide, Chengdu Mytech Biotech Co. Ltd., Chengdu, Sichuan, China. [c]NRC (1994) feed ingredient tables were used for calculation, except for digestible amino acid values. Bryden *et al.* (2009) feedstuffs digestibility values were used for calculation of digestible amino acids. The following values (g/kg) were used for calculation of digestible amino acids and other components for SBP: protein, 411; arginine, 36.4; cysteine, 5.8; isoleucine, 13.0; leucine, 21.9; lysine, 24.6; methionine, 6.4; threonine, 11.4; valine, 15.6; calcium, 30.9; phosphorus, 9.4; crude fat, 23.4 and crude fibre, 65.0.

for DM, titanium (Ti), N, starch, fat, calcium (Ca), phosphorus (P) and ash.

On day 21, middle toes of birds (six per cage), euthanised for ileal digesta collection, were obtained by severing the toe through the joint between the second and third tarsal bones from the distal end, and pooled per cage. Toe samples were ashed at 550°C for 16 hours and ash content reported as percent of toe.

Dry matter content was determined using standard procedures (Methods 930.15 and 925.10; AOAC, 2005). Ash was determined by standard procedures (method 942.05; AOAC, 2005) using a muffle furnace at 550°C for 16 hours. Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. Samples were assayed for Ti on a UV spectrophotometer following the method of Short *et al.* (1996). Nitrogen content was determined by combustion (Method 968.06; AOAC, 2005) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). Total starch content was determined using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Fat content was determined using the Soxhlet extraction

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procedure (Method 991.36; AOAC, 2005). Calcium and P concentrations were determined by colorimetric methods after ashing the samples at 550°C and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2005).

All data were expressed on a DM basis, and the apparent metabolisable energy (AME) was calculated using the following formula:

$$\mathrm{AME(MJ/\ kg\ diet)} = [(\text{feed intake} \times \mathrm{GE_{diet}}) - (\text{excreta output} \times \mathrm{GE_{excreta}})]/\text{feed intake}$$

Correction for zero nitrogen retention was made as described by Hill and Anderson (1958), using a factor of 36.54 kJ per gram nitrogen retained in the body and N-corrected AME (AMEn) was calculated. Total tract retention (TTR) of nutrients, as a percentage of intakes, was determined as follows:

$$\mathrm{TTR(\%)} = 100 \times [((\text{feed intake} \times \mathrm{nutrient_{diet}}) - (\text{excreta output} \times \mathrm{nutrient_{excreta}}))/(\text{feed intake} \times \mathrm{nutrient_{diet}})]$$

Coefficient of apparent ileal digestibility (CAID) of nutrients was calculated using the following formula:

$$\text{CAID of diet component} = [(\text{diet component/Ti})_{\mathrm{diet}} - (\text{diet component/Ti})_{\mathrm{ileal}}]/(\text{diet component/Ti})_{\mathrm{diet}}$$

Where (diet component/Ti)d = ratio of diet component to Ti in the diet, and (diet component/Ti)i = ratio of diet component to Ti in the ileal digesta.

Cage mean values were considered the experimental unit. Data were analysed by a one-way ANOVA using the General Linear Models procedure of the SAS Institute Inc. (2004) in a completely randomised design. Significant differences between means were separated by the Least Significant Difference test. Differences were considered to be significant at $P < 0.05$.

Results

Mortality during the performance experiment was low. Only eight out of the 336 birds died and the deaths were not related to any specific treatment.

The effects of different inclusion rates of SBP on performance (1–21 d), excreta score and toe ash are summarised in Table 2. There was no significant ($P > 0.05$) effect of SBP on weight gain and feed intake of the birds. A significant ($P < 0.01$) effect of SBP was observed for the feed per gain of birds. While inclusion of 1.0, 2.0, 3.0 and 4.0 g SBP/kg of feed resulted in similar FCR values to the diet with no SBP, addition of SBP to the diets at 5.0 and 6.0 g/kg of feed resulted in lower ($P < 0.05$) FCR compared to the diet with no SBP. Addition of SBP had no effect ($P > 0.05$) on the excreta score and toe ash.

Table 2. Influence of different inclusion rate of soybean bioactive peptide (SBP) on the weight gain (g/bird), feed intake (g/bird), FCR, excreta score and toe ash (%) of broiler starters (d 1 to 21)[a]

SBP inclusion (g/kg of feed)	Weight gain	Feed intake	FCR	Excreta score	Toe ash[b]
No SBP	972	1263	1.301[a]	3.8	12.47
1.0	990	1274	1.287[ab]	3.8	12.30
2.0	993	1269	1.277[ab]	4.1	12.22
3.0	982	1271	1.296[a]	4.1	12.25
4.0	967	1241	1.283[ab]	3.9	12.38
5.0	997	1257	1.264[bc]	4.0	12.50
6.0	987	1230	1.251[c]	3.8	12.45
SEM[c]	17.2	19.1	0.0085	0.12	0.216
Probabilities, P ≤	0.868	0.612	0.002	0.338	0.948

Means in a column not sharing a common letter (a,b,c) are significantly different ($P < 0.05$).
[a] Each value represents the mean of six replicates (eight birds per replicate).
[b] Each value represents the mean of six replicates (six birds per replicate, one toe per bird).
[c] Pooled standard error of mean.

Influence of different inclusion rates of SBP on coefficient of apparent ileal digestibility (CAID) of nutrients, total tract retention (TTR) and energy utilisation is presented in Table 3. There was no significant ($P > 0.05$) effect of SBP on any of these parameters. Though not statistically significant, SBP inclusion, regardless of the level, resulted in an average 5.7% and 6.3% increase in CAID of DM (from 0.611 to 0.646) and N (from 0.716 to 0.761), respectively. Inclusion of SBP at 6.0 g/kg of feed numerically increased TTR of N by 3% from 58.0 to 59.7%.

Influence of different inclusion rates of SBP on the relative weight of the organs and digestive tract, and the morphometry of the duodenum and jejunum of 21-d old broilers is shown in Tables 4 and 5, respectively. There was no significant ($P > 0.05$) effect of SBP on the relative weight of the organs and digestive tract. The effect of SBP on villus height in duodenum tended ($P = 0.075$) to be significant. Birds fed the diet without SBP had the lowest and the birds with SBP inclusion at 3.0 and 6.0 g/kg of feed showed the greatest villus height, with the other treatments being intermediate. The crypt depth, epithelial thickness and goblet cell number in duodenum were not affected ($P > 0.05$) by dietary

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Table 3. Influence of different inclusion rate of soybean bioactive peptide (SBP) on coefficient of apparent ileal digestibility (CAID)[a] of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca), phosphorus (P), gross energy (GE), ash, total tract retention (TTR; %)[b] of DM and N, apparent metabolisable energy (AME, MJ/kg DM) and N-corrected metabolisable energy (AMEn, MJ/kg DM)[b] in broiler starters

SBP inclusion (g/kg of feed)	CAID								TTR		Energy utilisation	
	DM	N	Starch	Fat	Ca	P	GE	Ash	DM	N	AME	AMEn
No SBP	0.611	0.716	0.944	0.910	0.258	0.397	0.673	0.369	68.4	58.0	13.83	12.90
1.0	0.641	0.760	0.952	0.909	0.251	0.403	0.704	0.391	68.3	58.1	13.83	12.89
2.0	0.639	0.759	0.953	0.916	0.296	0.418	0.704	0.409	68.4	57.0	13.83	12.94
3.0	0.644	0.749	0.952	0.903	0.344	0.414	0.702	0.405	68.5	58.0	13.75	12.82
4.0	0.652	0.768	0.950	0.906	0.289	0.433	0.713	0.421	68.4	57.8	13.84	12.90
5.0	0.654	0.769	0.951	0.916	0.329	0.414	0.714	0.418	68.5	57.1	13.80	12.88
6.0	0.644	0.762	0.949	0.893	0.297	0.400	0.704	0.389	68.1	59.7	13.85	12.86
SEM[b]	0.0137	0.0178	0.0049	0.0126	0.0256	0.0196	0.0133	0.0208	0.339	0.682	0.056	0.049
Probabilities, P ≤	0.387	0.397	0.855	0.860	0.137	0.873	0.411	0.591	0.989	0.133	0.911	0.785

[a] Each value represents the mean of six replicates (six birds per replicate).
[b] Each value represents the mean of six replicates (eight birds per replicate), measured from d 18 to 21.
[b] Pooled standard error of mean.

treatments. No significant ($P > 0.05$) effect of SBP was observed for villus height, crypt depth, epithelial thickness and goblet cell number in jejunum.

Discussion

Either an intact protein or small peptide fractions released proteolytically from a parent protein can have various bioactive characteristics (Kitts and Weiler, 2003). Peptides that are released during the hydrolysis practice generally contain from two to 20 amino acid residues. It has been reported that amino acid composition, sequence and size can cause different bioactivities and functionalities in peptides (Dziuba *et al.*, 1999; Pihlanto-Leppälä, 2001).

Results from the current study showed that diet supplementation with different levels of SBP had no effect on weight gain and feed intake but improved FCR. Addition of 5.0 and 6.0 g/kg SBP to the diets resulted in significantly lower FCR values compared to the diet with no SBP. This is consistent with previous studies which demonstrated that dietary supplementation of chickens with bioactive peptides resulted in higher weight gain and better FCR (Wang, 2005; Wang *et al.*, 2011; Mateos *et al.*, 2014). Feng *et al.* (2007) and Karimzadeh *et al.* (2016) stated that bioactive peptides can increase the intestinal enzyme activities, which may have contributed to the improvements observed in feed efficiency.

In the current study, SBP supplementation had no significant effect on any ileal digestibility parameters; but its inclusion, regardless of level, resulted in numerical increases in CAID of DM and N (5.7 and 6.3%, respectively). Furthermore, SBP inclusion at 6.0 g/kg of feed increased TTR of N by 3% from 58.0 to 59.7%. These results are in agreement with the results of Kiers *et al.* (2003), who showed that bioactive peptides in soybean increased nutrient solubility and *in vitro* digestibility. Feng *et al.* (2007) reported that fermented soybean

Table 4. Influence of different inclusion rate of soybean bioactive peptide (SBP) on relative (g/kg body weight) organ weights and empty weight of the digestive tract of 21-d old broilers[a]

SBP inclusion (g/kg of feed)	Proventriculus	Gizzard	Liver	Spleen	Pancreas	Duodenum	Jejunum	Ileum	Caeca	Bursa of Fabricius
No SBP	6.5	11.4	30.9	0.77	2.6	6.1	11.0	7.7	2.6	1.85
1.0	5.0	9.9	30.3	0.86	2.8	5.7	11.1	8.0	2.6	1.97
2.0	5.9	10.6	30.1	0.92	2.9	5.6	10.2	7.3	2.8	1.95
3.0	5.8	10.7	30.1	0.84	2.7	6.3	11.4	8.0	2.4	1.83
4.0	6.5	10.8	28.6	0.81	2.7	5.4	10.2	7.1	2.4	1.99
5.0	4.8	9.9	32.9	0.82	2.6	5.7	10.2	7.3	2.7	1.89
6.0	5.0	10.1	29.3	0.81	3.1	6.0	11.2	7.8	2.9	1.98
SEM[b]	0.52	0.56	1.23	0.054	0.16	0.32	0.46	0.30	0.23	0.143
Probabilities, P ≤	0.109	0.393	0.324	0.594	0.342	0.530	0.236	0.211	0.686	0.971

[a] Each value represents the mean of six replicates (two birds per replicate).
[b] Pooled standard error of mean.

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Table 5. Influence of different inclusion rate of soybean bioactive peptide (SBP) on villus height (μm), crypt depth (μm), epithelial thickness (μm) and goblet cell number (per 100 μm villus height) of the duodenum and jejunum of 21-d old broilers[a]

	Duodenum				Jejunum			
SBP inclusion (g/kg of feed)	Villus height	Crypt depth	Epithelial thickness	Goblet cell number	Villus height	Crypt depth	Epithelial thickness	Goblet cell number
No SBP	1786	167	32.9	9.96	1083	128	23.4	10.1
1.0	1930	171	31.2	9.75	1052	132	23.3	11.4
2.0	1870	164	31.5	9.88	1102	125	24.5	12.0
3.0	1999	177	31.7	10.1	1091	125	24.5	11.0
4.0	1859	174	33.7	8.75	1111	133	24.4	11.0
5.0	1845	178	33.1	9.39	1144	135	23.8	10.6
6.0	1986	177	29.1	8.18	1126	140	22.6	10.4
SEM[b]	55.3	4.4	1.59	0.896	34.5	4.7	1.16	0.65
Probabilities, P ≤	0.075	0.163	0.478	0.698	0.606	0.240	0.899	0.464

[a] Each value represents the mean of six replicates (two birds per replicate, 10 readings per bird).
[b] Pooled standard error of mean.

meal increased the activation of intestinal trypsin, lipase and protease enzymes during the starter period, and enhanced the protease activity during the grower period in broiler chickens. The improvements in nutrient digestibility and retention in broilers fed bioactive peptides have been attributed to the modulation of gut environment, increased digestive enzymes activity, improvement of beneficial intestinal microbial balance, improved small intestinal morphology or stimulation of the mucosal immune system (Jin *et al.,* 2008; Tang *et al.,* 2009; Karimzadeh *et al.,* 2016).

As villus height and crypt depth of duodenum and jejunum are indicative of gut health and development, it is important to measure the intestinal histology parameters to reveal possible mechanisms of bioactive peptides. In the current study, birds fed the diet without SBP had the lowest and the birds with SBP inclusion at 3.0 and 6.0 g/kg of feed showed the greatest villus height. The positive effect of bioactive peptides on small intestinal morphology in broilers is not without precedent and has been reported in several studies (Liu *et al.*, 2008; Bao *et al.*, 2009; Wen and He, 2012). Karimzadeh *et al.* (2016) reported that supplementation of broiler diets with 200 and 250 mg/kg canola bioactive peptides linearly increased villus height, villus height: crypt depth ratio and decreased crypt depth of duodenum, jejunum, and ileum in 42 days old broiler chickens. An increase in the number and size of villi increases the amount of surface area available for nutrient absorption, which in turn can improve FCR (McCalla *et al.,* 2010). This may be, at least in part, explain the better FCR achieved by SBP inclusion in the current study.

The findings of this study support the need for additional experimentation designed to identify the particular bioactive proteins/peptides or other biologic agents in soybean which can influence the gut health and development. Such bioactivity could be the result of a particular constituent protein or protein/peptide combinations.

Conclusions

Identification of bioactive peptides in soybean could be used to benefit the animal production through several mechanisms. In the current study, SBP improved feed efficiency, DM and N digestibility (though not significant) and tended to increase villus height. In the future, it might be possible to further isolate the bioactive peptides and evaluate their targeted feeding to poultry and possibly other intensively raised livestock, with the aim of enhancing production efficiency. Further research is warranted to define the biological characteristics and mode of action of soybean bioactive peptides.

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Declaration of Interest

Yan Gu, Weiwei Xiao and Jing Jia are employees of Chengdu Mytech Biotech Co. Ltd, China.

References

Aachary A.A. and Thiyam U. (2012) A pursuit of the functional nutritional and bioactive properties of canola proteins and peptides. *Critical Reviews in Food Science and Nutrition*, **52**: 965–979.

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Alashi A.M., Blanchard C.L., Mailer R.J. and Agboola S.O. (2013) Technological and bioactive functionalities of canola meal proteins and hydrolysates. *Food Reviews International*, **29**: 231–260.

AOAC. (2005) Official Methods of Analysis, 18th edition. *AOAC International*, Washington DC.

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

Bryden W.L., Li X., Ravindran G., Hew L.I. and Ravindran V. (2009) Ileal Digestible Amino Acid Values in Feedstuffs for Poultry. *Rural Industries Research and Development Corporation*, Canberra, Australia.

Dust J.M., Grieshop C.M., Parsons C.M., Karr-Lilienthal L.K., Schasteen C.S., Quigley J.D., Merchen N.R. and Fahey G.C. (2005) Chemical composition, protein quality, palatability, and digestibility of alternative protein sources for dogs. *Journal of Animal Science*, **83**: 2414–2422.

Dziuba J., Minkiewicz P. and Nalecz D. (1999) Biologically active peptides from plant and animal proteins. *Polish Journal of Food and Nutrition Sciences*, **8**: 3–16.

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

Hancock R.E. and Sahl H.G. (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology*, **24**: 1551–1557

He R., Girgih A.T., Malomo S.A., Ju X. and Aluko R.E. (2013) Antioxidant activities of enzymatic rapeseed protein hydrolysates and the membrane ultrafiltration fractions. *Journal of Functional Foods*, **5**: 219–227.

Hill F.W. and Anderson D.L. (1958) Comparison of metabolisable energy and productive energy determinations with growing chicks. *Journal of Nutrition*, **64**: 587–603.

Jin Z., Yang Y.X., Choi J.Y., Shinde P.L., Yoon S.Y., Hahn T.W., Lim H.T., Park Y., Hahm K.S., Joo J.W. and Chae B.J. (2008) Potato (*Solanum tuberosum L. cv. Golden valley*) protein as a novel antimicrobial agent in weanling pigs. *Journal of Animal Science*, **86**: 1562–1572.

Kamnerdpetch C., Weiss M., Kasper C. and Scheper T. (2007) An improvement of potato pulp protein hydrolyzation process by the combination of protease enzyme systems. *Enzyme and Microbial Technology*, **40**: 508–514.

Karimzadeh S., Rezaei M. and Teimouri Yansari A. (2016) Effects of canola bioactive peptides on performance, digestive enzyme activities, nutrient digestibility, intestinal morphology and gut microflora in broiler chickens. *Poultry Science Journal*, **4**: 27–36.

Kiers J.L., Meijer J.C., Nout M.J.R., Rombouts F.M., Nabuurs M.J. A. and Van der Meulen J. (2003) Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. *Journal of Applied Microbiology*, **95**: 545–552.

Kitts D.D. and Weiler K. (2003) Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design*, **9**: 1309–1323.

Lahl W.J. and Braun S.D. (1994) Enzymatic production of protein hydrolysates for food use. *Food Technology*, **48**: 68–71.

Li C.H., Matsui T., Matsumoto K., Yamasaki R. and Kawasaki T. (2002) Latent production of angiotensin I-converting enzyme inhibitors from buckwheat protein. *Journal of Peptide Science*, **8**: 267–74.

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

Lu J., Zeng Y., Hou W., Zhang S., Li L., Luo X., Xi W., Chen Z. and Xiang M. (2012) The soybean peptide aglycin regulates glucose homeostasis in type 2 diabetic mice via IR/IRS1 pathway. *The Journal of Nutritional Biochemistry*, **23**: 1449–57.

Mateos G.G., Mohiti-Asli M., Borda E., Mirzaie S. and Frikha M. (2014) Effect of inclusion of porcine mucosa hydrolysate in diets varying in lysine content on growth performance and ileal histomorphology of broilers. *Animal Feed Science and Technology*, **187**: 53–60.

Matsui T., Matsufuji H., Seki E., Osajima K., Nakashima M. and Osajima Y. (1993) Inhibition of angiotensin I-converting enzyme by Bacillus licheniformis alkaline pro-tease hydrolyzates derived from sardine muscle. Bioscience, *Biotechnology, and Biochemistry*, **57**: 922–925.

McCalla J., Waugh T. and Lohry E. (2010) Protein hydrolysates/peptides in animal nutrition. In: V.K. Pasupuleti and A. L. Demain (Eds.,), *Protein Hydrolysates in Biotechnology*, pp. 179–190.

Meisel H. (2007) Food-derived bioactive proteins and peptides as potential components of nutraceuticals. *Current Pharmaceutical Design*, **13**: 771–772.

Muir W.I., Lynch G.W., Williamson P. and Cowieson A.J. (2013) The oral administration of meat and bone meal-derived protein fractions improved the performance of young broiler chicks. *Animal Production Science*, **53**: 369–377.

NRC. (1994) *Nutrient Requirements of Poultry*, National Academy Press, Washington, DC.

Pan M., Jiang T.S. and Pan J.L. (2011) Antioxidant activities of rapeseed protein hydrolysates. *Food and Bioprocess Technology*, **4**: 1144–1152.

Pihlanto-Leppälä A. (2001) Bioactive peptides derived from bovine proteins: opioid and ace-inhibitory peptides. *Trends in Food Science and Technology*, **11**: 347–356.

Ravindran V., Hew L.I., Ravindran G. and Bryden W.L. (2005) Apparent ileal digestibility of amino acids in feed ingredients for broiler chickens. *Animal Science*, **81**: 85–97.

Rolle R.S. (1998) Review: Enzyme applications for agro-processing in developing countries: An inventory of current and potential applications. *World Journal of Microbiology and Biotechnology*, **14**: 611–619.

Ross, (2014) Ross 308 Broiler: Nutrition Specification, Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

SAS. (2004) SAS® Qualification Tools User's Guide. Version 9.1.2. SAS Institute Inc., Cary, NC.

Sathe S.K., Teuber S.S. and Roux K.H. (2005) Effects of food processing on the stability of food allergens. *Biotechnology Advances*, **23**: 423–429.

Short F.J.P., Gorton J., Wiseman J. and Boorman K.N. (1996) Determination of titanium oxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology*, **59**: 215–221.

Singh B.P., Vij S. and Hati S. (2014) Functional significance of bioactive peptides derived from soybean. *Peptides*, **54**: 171–179.

Tang Z., Yin Y., Zhang Y., Huang R., Sun Z., Li T., Chu W., Kong X., Li L., Geng M. and Tu Q. (2009) Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin-lactoferrampin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21 d. *British Journal of Nutrition*, **101**: 998–1005.

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

Wang J.P., Liua N., Songa M.Y., Qin C.L. and Ma C.S. (2011) Effect of enzymolytic soybean meal on growth performance, nutrient digestibility and immune function of growing broilers. *Animal Feed Science and Technology*, **169**: 224–229.

Wen L.F. and He J.G. (2012) Dose–response effects of an antimicrobial peptide, a cecropin hybrid, on growth performance, nutrient utilisation, bacterial counts in the digesta and intestinal morphology in broilers. *British Journal of Nutrition*, **108**: 1756–1763.

Wynstra R.J. (1986) Expanding the use of soybeans. Champaign: College of Agriculture, University of Illinois at Urbana. pp. 20.

Yang Z., Gu H., Zhang Y., Wang L. and Xu B. (2009) Small molecule hydrogels based on a class of anti-inflammatory agents. *Chemical Communications*, **2**: 208–209.

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