Dietary bromelain improves nutrient digestibility, digesta viscosity and intestinal villus height as well as reduces intestinal *E. coli* population of broiler chickens

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Abstract

The effect of dietary bromelain at doses of 0, 0.05, 0.1, 0.2 and 0.5% on growth performance, nutrient digestibility and faecal nitrogen content of broilers was investigated. Its effect on digesta viscosity, intestinal bacterial populations, intestinal morphology and blood biochemistry were also investigated. A total of 180 one-day old Cobb 500 chicks were randomly allocated to five treatment groups fed with commercial basal diet. At day 21 and 42, birds were slaughtered and ileal digesta was collected for nutrient digestibility, digesta viscosity as well as *Lactobacillus* and *E. coli* population analyses. Intestinal segments were collected to determine villus height and crypt depth under a light microscope. Excreta was collected to determine nitrogen content using the Kjeldahl method. Blood samples were collected at slaughter to determine biochemical parameters. Starter birds fed 0.05 and 0.1% bromelain had higher ($P<0.05$) fat and protein digestibility than the control, respectively. Finisher birds fed 0.05, 0.2 and 0.5% bromelain had higher ($P<0.05$) fat digestibility than the control. As a result of the improved protein digestibility, bromelain showed a trend to decrease faecal nitrogen content ($P=0.096$). However, it seemed that the improved digested protein was not utilized for lean gain as bromelain did not improve the body weight gain and feed conversion ratio ($P<0.05$, respectively). Bromelain improved intestinal villus height and reduced digesta viscosity in the starter birds ($P<0.05$). Bromelain might have reduced the negative effects of dietary anti-nutritional factors that led to improvement of intestinal villus height and digesta viscosity. Bromelain reduced intestinal *E. coli* populations and increased *Lactobacillus* populations ($P<0.05$). Bromelain had no effect on serum alanine aminotransferase (ALT) level ($P>0.05$) and it reduced serum alkaline phosphate (ALP) and aspartate aminotransferase (AST) levels ($P<0.05$, respectively), indicating no adverse effect on liver and kidney functions of broilers. In conclusion, dietary bromelain improved protein and fat digestibility resulting in reduced faecal nitrogen content, with no changes on body weight gain in broilers. Bromelain also increased intestinal villus height, reduced digesta viscosity and reduced intestinal *E. coli* populations.

Keywords: broiler chicken, bromelain, nutrient digestibility, digesta viscosity

Introduction

Bromelain and other cycteine proteases are well known enzymes present in different parts of pineapple (*Ananas comosus*). The stem and fruit bromelains are commercially available and have been used widely in food, medical and pharmaceutical industries. Valuable amounts of protein pass through the gastrointestinal tract of poultry without being completely digested (Lemme et al., 2004). Faecal nitrogen excretion is a result of insufficient protein digestion and there has been an increased public concern regarding its impact on the environment. Studies in the pig demonstrated inclusion of bromelain in the diets improved nutrient digestibility and growth performance (Begum et al., 2015; Hossain et al., 2015). Dietary bromelain complemented the natural endogenous enzymes produced by broilers, but it did not show any beneficial effect on protein digestion (Yu et al., 2002). Exogenous proteases of microbial origin are widely used in the poultry diets as mono-component products or in combination with other enzymes. The general aim for inclusion of protease in poultry diets is to increase the energy and protein digestibility of grain-based diets. Interestingly, the effects of mono-component protease supplementation are beyond hydrolysis of dietary proteins only. For example, proteases were reported to improve digestibility of fat (Fru-Nji et al., 2011; Freitas et al., 2011), starch (Yin et al., 2018) as well as metabolizable energy (Ghazi et al., 2002) in broilers. Consequently, improvement of nutrient digestion by protease supplementation indirectly resulted in alteration of substrates available for microbial growth in the gut (Yin et al., 2018). In addition, inclusion of mono-component protease increased the solubilization of non-starch polysaccharide (NSP) components in broilers fed corn-soybean meal-based diets (Olukosi et al., 2015).

Cysteine proteases have similar mechanism of action to that of serine proteases originating from microbes (Rao et al., 1998). Studies on bromelain supplementation in poultry diets are limited and little is known on its effect on digesta viscosity and intestinal microbial population. The objective of this study was to investigate the effect of bromelain supplementation on growth performance, nutrient digestibility, intestinal morphology and microbial population, faecal nitrogen content and blood biochemistry in broilers.

Materials and Methods

Birds and diets

The experimental protocols used in this research were approved by the Institutional Animal Care and Use Committee of Research Policy at Universiti Putra Malaysia (UPM/IACUC/AUP-R067/2017).

A total of 180 one-day old Cobb 500 chicks were randomly allocated to five treatment groups with six replicates (cages), each containing six birds: i) basal diet (control), ii) basal diet+0.05% bromelain (BR 0.05), iii) basal diet+0.1% bromelain (BR 0.1), iv) basal diet+0.2% bromelain (BR 0.2), and v) basal diet+0.5% bromelain (BR 0.5). All birds were fed commercial corn-soybean meal basal diets in mash form that met the energy and nutrient requirements of broiler chickens (NRC, 1994). The fruit bromelain enzyme used had an enzyme activity of 600 gelatin digestion unit/kg (GDU/kg) (Nutra Choice Sdn. Bhd., Selangor, Malaysia) and was diluted with distilled water to achieve the respective dietary inclusion rate. The experiment was divided into starter (days 0 to 21) and finisher (days 22 to 42) rearing periods. All chickens were vaccinated by routine procedures. The diets and water were provided *ad libitum* throughout the experiment.

Growth performance, abdominal fat and meat quality

Body weight gain of each bird and feed intake were recorded weekly. Subsequently, feed conversion ratio (FCR) was calculated. At day 42, one bird from each replicate was selected randomly and slaughtered. Dressing percentage was calculated from the individual live weight and carcass weight measures. Abdominal fats were collected at the outer region of the proventriculus, the gizzard down to the cloaca. Then, the weight of abdominal fat was divided with individual carcass weight to get abdominal fat yield percentage. The right breast muscle (*Pectoralis major*) was individually packed and stored at 4 °C for 24 h prior to pH, colour, cooking loss and shear force analyses. For drip loss, 40 g of fresh breast muscle sampled on the slaughter day was cut and initial weight was recorded then packed and sealed before being stored at 4 °C. After 24 h post-mortem, the samples were gently patted using tissue paper and then re-weighed. Drip loss was calculated as the percentage of weight loss before and after been stored at 4 °C for 24 h (Honikel, 1998). For meat pH, approximately 0.5 g samples were crushed using liquid nitrogen and homogenised using a homogeniser (Wiggen Hauser® D-500, Germany) for 20 sec in 10 ml ice cold distilled water. Then, the pH meter electrode (Mettler Toledo, AG 8603, Switzerland) was directly introduced into homogenised samples and the readings were recorded. Meat colour was measured using a Colour Flex spectrophotometer (Hunter Lab Reston, VA, USA) to determine lightness (L), redness (a*) and yellowness (b*). Meat samples for cooking loss were individually weighed, vacuum-packaged and cooked in water bath at 80 °C for 20 min. After cooking, samples were immersed in ice cold water for 20 min, gently patted using tissue paper and then re-weighted. Cooking loss was calculated as a percentage of weight loss before and after cooking. After cooking loss analysis, the same samples were stored overnight at 4 °C for subsequent shear force analysis. Each sample was cut into three subsamples, parallel to the muscle fibres with dimension of 1 cm high x 1 cm thick x 2 cm length each. The samples were then placed on the base plate of a TA.HD plus® texture analyser (Stable Micro System, Surrey, UK) fitted with a Volodkovitch bite jaw. The samples were sheared once perpendicular to the longitudinal direction of the fibres at a speed of 0.1 mm/sec and 5 kg load cell was used (Nakyinsige et al., 2014).

Nutrient digestibility, intestinal microbial populations, digesta viscosity and faecal nitrogen content

During the last four days of the starter and finisher periods (days 18 to 21 and days 39 to 42), an indigestible marker, titanium dioxide (TiO_2) at an inclusion level of 3 g/kg diet was added to the diets. At day 21 and day 42, one bird from each replicate was slaughtered. Ileal digesta was collected and stored at -20 ºC until further analyses. The digesta was oven dried at 80 ºC until it reached a constant weight and homogenised. Samples of feed and digesta were analysed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash according to a standard method of Association of Official Analytical Chemist (AOAC, 2005). The TiO_2 contents of the feed and digesta were determined using the method described by Short et al. (1996). Subsequently, apparent ileal digestibility (AID) of DM, CP, EE, CF and ash was estimated using the following formula: AID of nutrient = 100 − [(% TiO_2 in feed/% TiO_2 in ileal digesta) × (% of nutrient in ileal digesta/% of nutrient in feed × 100].

For determination of *Lactobacillus* and *E. coli* population, ileal digesta was collected from one bird from each replicate, slaughtered at day 42. One gram of digesta sample was diluted with 9 mL of 10 g/L sterile peptone

broth, homogenised and then incubated for one hour at room temperature. Viable bacterial count in the ileum samples was determined by plating serial 10-fold dilutions (in 10 g/L peptone solution) on MacConkey agar plates to isolate *E. coli* and MRS agar (de Man, Rogosa, and Sharpe) (Merck, KgaA, Germany) to isolate *Lactobacillus*. The MacConkey agar plates were incubated for 24 h at 37 °C under aerobic conditions. The MRS agar plates were incubated in an anaerobic jar at 37 °C for 48 h. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. Colonies with smooth convex circles, entire edges, and a pink colour were counted as *E. coli*. Results were expressed as $\log_{10}$ of Colony Forming Units (CFU) per gram of digesta (Loh et al., 2014).

For digesta viscosity, ileal digesta was collected from one bird from each replicate, slaughtered at day 42. A total of 2.5 g sample was then diluted to a volume of 25 ml with distilled water. The viscosity was measured using a viscometer (PSL-Rheotek, Poulten Selfe & Lee Ltd., England), expressed in ml/min. The methods used were modified from Choct and Annison (1992).

For faecal nitrogen content, excreta were collected between days 40 and 42 from each cage and stored at -20 ºC until further analysis. Approximately 500 g samples were oven dried at 80 ºC until they reached a constant weight and then ground using mortar and pastel. Nitrogen content of the faecal samples was determined using the Kjeldahl method.

Intestinal morphology

Intestinal morphology was conducted following the methods described by Choe et al. (2012). About 5 cm long segments of duodenum, jejunum and ileum were removed from three birds from each treatment at day 21 and day 42. All intestinal segments were flushed with 0.9% saline, then immersed in 10% neutral buffer formalin solution. After that, the intestinal segments were excised to 3 mm length and transferred into plastic cassettes and kept overnight in neutral buffer formalin solution. The samples were dehydrated and embedded in paraffin wax. After trimming, sections were cut (5 μm), fixed on a glass slide, stained with hematoxylin-eosin and analysed under a light microscope for villus height and crypt depth measurements. Villus height to crypt depth ratio (VH:CD) was subsequently calculated. Measurements of six villi from different segments were recorded and then the average value was calculated for each bird.

Blood biochemistry

Blood samples from the jugular vein were collected from three birds from each treatment to determine biochemical parameters. Serum was obtained by centrifugation at 3000 rpm for 10 min and was kept in Eppendorf tubes at -20 °C until analysis. Serum samples were analysed for alkaline phosphate (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, creatinine, and urea using analytical kits according to the manufacturer's instructions (Fortress Diagnostics, Antrim, UK).

Statistical analysis

The experiment was conducted using a completely randomized design. The data were analysed with one-way analysis of variance (ANOVA) using the generalized linear model (GLM) procedure of SAS version 9.4 (Statistical Analysis System, 2018). Comparisons among significant treatment means were assessed using Duncan's multiple range test. All results were considered statistically significant when $P<0.05$ and trends when $0.05<P<0.10$.

Results

Growth performance

The effect of bromelain supplementation on growth performance is shown in Table 1. At day 21, body weight gain in birds fed bromelain was similar to the control except for the birds fed 0.5% bromelain that had reduced body weight gain (P<0.05). At day 42, body weight gain was similar across treatments. In the overall period, 0.1% and 0.5% bromelain reduced body weight gain compared to the other treatments (P<0.05). Bromelain had no effect on the feed intake in birds at day 21 and day 42. Feed conversion ratio was increased in birds fed bromelain compared to the control at day 21. However, no differences in FCR between treatments were observed at day 42 and the overall period.

Table 1. Effect of dietary bromelain on growth performance of broiler chickens.

Parameter	Treatment[1]					SEM[2]
	Control	BR 0.05	BR 0.1	BR 0.2	BR 0.5	
Body weight gain (kg)						
At day 21	0.83[a]	0.82[a]	0.81[a]	0.82[a]	0.74[b]	0.009
At day 42	1.44	1.49	1.43	1.54	1.37	0.019
Overall period	2.27[a]	2.30[a]	2.23[ab]	2.36[a]	2.12[b]	0.044
Feed intake (kg)						
At day 21	1.07	1.10	1.07	1.11	1.03	0.009
At day 42	2.87	2.93	2.88	2.92	2.64	0.037
Overall period	3.94	4.03	3.96	4.03	3.68	0.024
Feed conversion ratio						
At day 21	1.29[c]	1.35[ab]	1.33[bc]	1.35[ab]	1.39[a]	0.011
At day 42	1.99	1.98	2.02	1.90	1.93	0.023
Overall period	1.74	1.75	1.77	1.71	1.74	0.012

[1]Control basal diet+0 bromelain, BR 0.05 basal diet+0.05% bromelain, BR 0.1 basal diet + 0.1% bromelain, BR 0.2 basal diet + 0.2% bromelain, BR 0.5 basal diet + 0.5% bromelain.
[2]SEM standard error of the Mean.
[a-c] Mean values with different letters in the same row are significantly different (P<0.05).

Carcass and meat quality

Dressing percentage and carcass abdominal fat were not affected by bromelain (Table 2). Colour L values increased in birds fed bromelain compared to the control (P<0.05). Colour b* values were the highest in birds fed 0.05% and 0.1% bromelain (P<0.05). Meat pH was the lowest in birds fed 0.5% bromelain followed by 0.05% bromelain (P<0.05). Bromelain had no effect on colour a* values, drip loss, cooking loss and shear force.

Table 2. Effect of dietary bromelain on carcass and meat quality of broilers.

Parameter	Treatment[1]				
	Control	BR 0.05	BR 0.1	BR 0.2	BR 0.5
Dressing (%)	77.4±0.74	76.8±0.68	75.4±1.69	77.2±0.43	76.7±0.57
Abdominal fat (%)	2.9±0.26	2.6±0.35	3±0.3	2.8±50.32	3.1±0.29
Breast meat colour					
Lightness (L*)	50.9±0.36^{a}	49.0±0.67^{abc}	47.20.81±^{c}	49.8±0.63^{ab}	45.3±1.04^{bc}
Redness (a*)	7.4±0.19	7.5±0.28	7.3±0.49	7.5±0.37	9.0±0.75
Yellowness (b*)	21.0±0.40^{ab}	21.9±0.58^{a}	22.0±0.49^{a}	19.6±0.41^{b}	18.8±0.70^{ab}
Drip loss (%)	1.01±0.175	0.98±0.212	0.96±0.144	1.09±0.180	1.6±0.333
Cooking loss (%)	25.36±1.155	25.83±1.246	24.71±1.005	26.18±0.943	26.44±0.881
Shear force (kg/cm^2)	1.61±0.071	1.84±0.073	1.69±0.097	1.69±0.097	1.93±0.089
Breast pH	5.89±0.047^{a}	5.85±0.036^{ab}	5.75±0.069^{ab}	5.86±0.030^{a}	5.72±0.026^{b}

[1]Control basal diet+0 bromelain, BR 0.05 basal diet+0.05% bromelain, BR 0.1 basal diet + 0.1% bromelain, BR 0.2 basal diet + 0.2% bromelain, BR 0.5 basal diet + 0.5% bromelain.
[a-c] Mean values with different letters in the same row are significantly different (P<0.05).

Nutrient digestibility

The effect of bromelain supplementation on nutrient digestibility is shown in Table 3. At day 21, birds fed 0.05 and 0.1% bromelain had higher fat and protein digestibility than the control (P<0.05, respectively). Dry matter digestibility was the highest in 0.1% bromelain, followed by 0.5% then the control, 0.05 and 0.2% bromelain (P<0.05). Mineral digestibility was the highest in the control, 0.05 and 0.1% bromelain, followed by 0.2% bromelain then 0.5% bromelain. Bromelain had no effect on CF digestibility (P>0.05).

At day 42, birds fed 0.05, 0.2 and 0.5% bromelain had higher fat digestibility than the control and 0.1% bromelain (P<0.05). Protein digestibility was similar to the control in birds fed 0.05% bromelain and improved in birds fed 0.2 and 0.5% bromelain (P<0.05). Bromelain had no effect on DM, CF and mineral digestibility (P>0.05), respectively.

Table 3. Effect of dietary bromelain on nutrient digestibility of broilers.

Parameter	Treatment[1]					SEM[2]
	Control	BR 0.05	BR 0.1	BR 0.2	BR 0.5	
At day 21						
Dry matter	91.3^{c}	91.1^{c}	94.2^{a}	90.2^{c}	92.9^{b}	0.63
Ether extract	65.3^{e}	77.4^{b}	80.5^{a}	71.9^{c}	68.6^{d}	1.44
Crude protein	74.3^{bc}	76.8^{b}	80.9^{a}	73.2^{c}	65.4^{d}	1.75
Crude fiber	84.9	89.3	94.2	85.8	92.0	1.42
Ash	63.4^{a}	61.6^{a}	64.7^{a}	53.7^{b}	44.4^{c}	2.22
At day 42						
Dry matter	91.2	91.8	92.0	92.0	92.7	0.53
Ether extract	68.8^{b}	75.1^{a}	68.6^{b}	75.9^{a}	75.9^{a}	1.71
Crude protein	68.1^{b}	67.6^{b}	63.6^{c}	68.7^{ab}	71.4^{a}	1.59
Crude fiber	84.8	86.0	87.3	88.4	81.8	1.12
Ash	48.1	54.2	42.0	44.4	36.6	4.67

[1]Control basal diet+0 bromelain, BR 0.05 basal diet+0.05% bromelain, BR 0.1 basal diet + 0.1% bromelain, BR 0.2 basal diet + 0.2% bromelain, BR 0.5 basal diet + 0.5% bromelain.
[2]SEM standard error of the Mean.
[a-e] Mean values with different letters in the same row are significantly different (P<0.05).

Intestinal microbial populations, digesta viscosity and faecal nitrogen content

The effect of bromelain supplementation on ileal microbial populations, ileal digesta viscosity and faecal nitrogen content is shown in Table 4. Bromelain reduced the intestinal *E. coli* populations compared to the control (P<0.05). *Lactobacillus* populations in birds fed 0.05% and 0.1% bromelain were similar to the control but higher doses at 0.2% and 0.5% bromelain reduced *Lactobacillus* (P<0.05). Bromelain reduced digesta viscosity at day 21 (P<0.05) but showed no effect at day 42 (P>0.05). Bromelain showed a trend to decrease faecal nitrogen content (P=0.096).

Table 4. Effect of dietary bromelain on faecal nitrogen, digesta viscosity, intestinal microbial population of broilers

Parameter	Treatment[1]					SEM[2]
	Control	BR 0.05	BR 0.1	BR 0.2	BR 0.5	
Faecal nitrogen content (%)	4.54	3.54	4.10	3.67	3.51	0.146
Digesta viscosity (ml/min)						
At day 21	3.41^{a}	2.36^{b}	2.22^{bc}	1.79^{c}	1.73^{c}	0.350
At day 42	1.85^{a}	1.80^{a}	1.73^{a}	1.74^{a}	1.76^{a}	0.100
Microbial population (log cfu/g)						
E. coli	3.72^{a}	3.34^{b}	3.45^{b}	2.51^{d}	2.67^{c}	0.028
Lactobacillus	3.26^{a}	3.42^{a}	3.36^{a}	3.35^{ab}	3.11^{c}	0.044

[1]Control basal diet+0 bromelain, BR 0.05 basal diet+0.05% bromelain, BR 0.1 basal diet + 0.1% bromelain, BR 0.2 basal diet + 0.2% bromelain, BR 0.5 basal diet + 0.5% bromelain.
[2]SEM standard error of the Mean.
[a-d] Mean values with different letters in the same row are significantly different (P<0.05).

Intestinal morphology

The effect of bromelain supplementation on intestinal morphology is shown in Table 5. At day 21, bromelain increased villus height, crypt depth and VH:CD of duodenum compared to the control (P<0.05, respectively). The highest increase for all segments were shown in birds fed 0.05% bromelain. For jejunum, 0.2 and 0.5% bromelain had higher villus height than the control but 0.05 and 0.1% bromelain had lower villus height than the control (P<0.05). Crypt depth was the lowest in the control (P<0.05) giving the highest VH:CD compared to the other treatments (P<0.05). For ileum, bromelain increased villus height, crypt depth and VH:CD compared to the control (P<0.05, respectively). The highest villus height and VH:CD were shown in birds fed 0.2% bromelain.

Table 5. Effect of dietary bromelain on villus height, crypt depth and villus height to crypt depth ratio (VH:CD) of broilers

Parameter (μm)		Treatment[1]					SEM[2]
		Control	BR 0.05	BR 0.1	BR 0.2	BR 0.5	
At day 21							
Duodenum	Villus height	1011^{d}	1537^{a}	1354^{c}	1410^{b}	1395^{b}	13.5
	Crypt depth	168^{e}	223^{a}	208^{d}	210^{c}	215^{b}	1.3
	VH:CD	6.0^{d}	6.9^{a}	6.5^{c}	6.7^{b}	6.5^{c}	0.08
Jejunum	Villus height	813^{b}	812^{bc}	798^{c}	830^{a}	826^{ab}	7.7
	Crypt depth	149^{d}	176^{b}	168^{c}	182^{a}	177^{b}	2.0
	VH:CD	5.5^{a}	4.6^{c}	4.8^{b}	4.6^{c}	4.7^{bc}	0.07
Ileum	Villus height	739^{d}	695^{e}	836^{c}	978^{a}	878^{b}	2.9
	Crypt depth	182^{d}	195^{c}	193^{c}	203^{b}	207^{a}	1.9
	VH:CD	4.1^{d}	3.6^{e}	4.3^{b}	4.8^{a}	4.2^{c}	0.04
At day 42							
Duodenum	Villus height	1270^{d}	1368^{c}	1441^{b}	1725^{a}	1741^{a}	10.6
	Crypt depth	286^{a}	222^{d}	223^{d}	251^{c}	276^{b}	3.0
	VH:CD	4.5^{c}	6.2^{b}	6.5^{ab}	6.7^{a}	6.3^{ab}	0.20
Jejunum	Villus height	942^{e}	959^{d}	1041^{c}	1076^{b}	1094^{a}	5.2
	Crypt depth	264^{a}	187^{c}	196^{b}	196^{b}	198^{b}	2.1
	VH:CD	3.6^{d}	5.1^{c}	5.3^{b}	5.5^{a}	5.5^{a}	0.06
Ileum	Villus height	1319^{a}	917^{d}	950^{c}	951^{c}	1061^{b}	7.5
	Crypt depth	205^{b}	186^{d}	184^{e}	194^{c}	217^{a}	1.0
	VH:CD	6.4^{a}	4.9^{c}	5.2^{b}	4.9^{c}	4.9^{c}	0.06

[1]Control basal diet+0 bromelain, BR 0.05 basal diet+0.05% bromelain, BR 0.1 basal diet + 0.1% bromelain, BR 0.2 basal diet + 0.2% bromelain, BR 0.5 basal diet + 0.5% bromelain.
[2]SEM standard error of the Mean.
[a-e] Mean values with different letters in the same row are significantly different (P<0.05).

At day 42, bromelain increased villus height and VH:CD but decreased crypt depth of duodenum compared to the control (P<0.05, respectively). The highest villus height and VH:CD were shown in birds fed 0.2% bromelain. For jejunum, bromelain

increased villus height and VH:CD but decreased crypt depth compared to the control (P<0.05, respectively). The highest villus height was shown in birds fed 0.5% bromelain and the highest VH:CD was shown in birds fed 0.2 and 0.5% bromelain. For ileum, bromelain decreased villus height and VH:CD compared to the control (P<0.05, respectively). However, crypt depth was the highest in birds fed 0.5% bromelain, followed by the control then the other treatments (P<0.05).

Blood biochemistry

The effect of bromelain supplementation on blood biochemistry is shown in Table 6. Bromelain reduced ALP except for 0.2% bromelain that showed similar values to the control (P<0.05). Bromelain reduced AST compared to the control (P<0.05) but ALT was not affected (P>0.05). Bromelain reduced creatinine and uric acid compared to the control (P<0.05, respectively) but urea was not affected (P>0.05).

Table 6. Effect of dietary bromelain on blood biochemistry of broilers.

Parameter	Treatment[1]					SEM[1]
	Control	BR 0.05	BR 0.1	BR 0.2	BR 0.5	
Alkaline phosphate (ALP) U/L	1514.3[a]	594.0[b]	842.5[b]	1613.0[a]	651.7[b]	121.03
Aspartate aminotransferase (AST) U/L	342.7[a]	239.0[b]	165.5[c]	239.0[b]	148.0[c]	30.54
Alanine aminotransferase (ALT) U/L	4.7[a]	3.3[a]	3.3[a]	4.3[a]	5.0[a]	1.37
Creatinine umol/L	19.3[a]	11.7[b]	13.0[ab]	15.7[ab]	10.3[b]	3.28
Urea mmol/L	0.67[a]	0.57[a]	0.50[a]	0.50[a]	0.50[a]	0.110
Uric acid umol/L	486.0[a]	170.5[d]	269.3[c]	330.5[b]	187.7[d]	23.82

[1]Control basal diet+0 bromelain, BR 0.05 basal diet+0.05% bromelain, BR 0.1 basal diet + 0.1% bromelain, BR 0.2 basal diet + 0.2% bromelain, BR 0.5 basal diet + 0.5% bromelain.
[2]SEM standard error of the Mean.
[a-d] Mean values with different letters in the same row are significantly different (P<0.05).

Discussion

Inclusion of up to 0.1% bromelain improved protein and fat digestibility in the starter birds. However, higher dose of bromelain was required to improve protein and fat digestibility in the finisher birds. Improvement of protein digestibility resulted in the trend for reduced faecal nitrogen content in birds fed with bromelain. Endogenous enzymes such as amylase, trypsin, and lipase are lacking in young chicks because of the underdeveloped pancreas (Nitsan et al., 1991; Kadhim et al., 2014). Therefore, lower dose of bromelain during the starter period was effective in digesting nutrients. In contrast to our study, Yu et al., (2002) reported that bromelain

supplementation did not improve protein digestibility in both the starter and finisher birds. They found that the protein molecular weight in the digesta was not reduced suggesting that bromelain failed to improve digestion of dietary protein. Inclusion of bromelain at 65 CDU/kg diet in the study of Yu et al. (2002) was probably not enough to produce expected responses because of the short transit time of digesta through the small intestines (Doskovic et al., 2013). The variation in results may also be because of the variation in the dietary amino acids, even within the same feed ingredients (Lemme et al., 2004). Studies in the pig showed that bromelain supplementation improved dietary nitrogen digestibility (Begum et al., 2015; Hossain et al., 2015). It should be noted that the differences in the physiology of the stomach and small intestines between animal species could affect exogenous enzyme activity (Hale, 2004; Doskovic et al., 2013).

In addition to improvement of protein digestibility, increased fat digestibility by bromelain suggest that bromelain did not interfere with the functions of other endogenous enzymes, particularly lipase. Bromelain was reported to complement other endogenous enzymes in the gastrointestinal tract of broilers (Yu et al., 2002). For an exogenous protease to work, it is important that the enzyme does not digest other proteins, including endogenous enzymes (Mahagna et al., 1995). Studies using mono-component microbial protease supplementation in broilers also reported improvement of both protein and fat digestibility (Fru-Nji et al., 2011; Freitas et al., 2011). Improved fat digestibility was suggested to be due to a secondary effect of protein digestion that allowed better access of lipase to dietary lipid digestion (Fru-Nji et al., 2011). Other authors suggested that microbial protease may stimulate the digestibility of other nutrients following improvement of energy digestibility along with crude protein and amino acid in broilers (Law et al., 2015; Mahmood et al., 2018).

Corn and soybean meal are the two main ingredients that make up the diet of poultry. However, anti-nutritional factors found in these ingredients such as allergen proteins, protease inhibitors and NSP can impair nutrient digestibility and absorption (Knudsen, 1997; Cowieson et al., 2005; Zheng et al., 2017). Young chicks are more susceptible by the negative effects of anti-nutritional factors and they become more adapted as they grow older (Erdaw et al., 2018). Bromelain addition to soybean meal-based diet successfully removed its allergen protein content that can cause damage to the intestine villi leading to disruption of nutrient absorption (Li, et al., 2014). Furthermore, bromelain was reported to have anti-inflammatory effects within the gastro intestinal tract (Maurer, 2001; Hale, 2004; Hale et al., 2005). In our study, improvement of intestinal villus height suggests that bromelain may reduce the anti-nutritional factors content of soybean meal to some extent.

Furthermore, inclusion of bromelain reduced digesta viscosity in the starter period. Young broilers are particularly more affected by changes in the digesta viscosity. Content of NSP in corn and soybean meal has been associated with increased digesta viscosity in chicken (Choct and Annison, 1992; Meng et al., 2005). Increased digesta viscosity limits the enzyme-substrate interaction resulting in reduced nutrient digestibility (Bedford and Schulze, 1998). It is generally agreed that the negative effect of NSP on digesta viscosity could be overcome by supplementing diets with NSP degrading enzymes through hydrolysis of NSP (Meng et al., 2005; Broz and Ward, 2007; Le et al., 2013). In more recent studies, protease was shown to exhibit effects beyond improving protein digestibility. For example, inclusion of mono-component protease in the diet of broilers

increased solubilisation of NSP components (Olukosi et al., 2015). Hence, it is possible that bromelain may have positive effects on NSP solubilisation that lead to improvement of digesta viscosity.

Mono-component exogenous microbial protease was shown to have positive effects on nutrients and metabolizable energy digestibility resulting in improvement of broiler performance (Ghazi et al., 2002; Fru-Nji et al., 2011). Bromelain supplementation in the pigs also demonstrated improved nutrient digestibility and growth performance (Begum et al., 2015; Hossain et al., 2015). In contrast, body weight gain was not improved by bromelain in the present study. Absorption of digested nutrients occurs at the villi of small intestine. In the current study, bromelain generally increased intestinal villi height in the starter and finisher birds that indicated increased surface area for nutrient absorption. However, it seemed that the absorbed protein was not utilized for lean gain but probably for other metabolic processes. Concurrent with that, bromelain showed no changes in the dressing percentage and carcass abdominal fat. The specific amino acids that become available for absorption due to microbial protease supplementation in broilers could influence body muscle deposition (Freitas et al., 2011). Cowieson et al. (2017) discussed that leucine and arginine play important roles to promote protein deposition in skeletal muscle and energy metabolism that result in enhanced feed efficiency and weight gain. Therefore, it is of interest to find out the effect of bromelain on amino acid digestibility in the future.

In the lactating sows and weanling piglets, inclusion of bromelain in the diets reduced faecal *E. coli* population (Begum et al., 2015; Hossain et al., 2015). Evidence of antibacterial function of bromelain was confirmed by Chandler and Mynott (1998) who found inhibition of *E. coli* receptor activity in piglets fed with bromelain. A more recent study also found that crude bromelain extracted from pineapple fruit was effective in inhibiting *E. coli* using a disc diffusion method (Ali et al., 2015). The current work is the first to document that inclusion of bromelain reduced intestinal digesta *E. coli* population in broilers. *Lactobacillus* population at doses of 0.05 and 0.1% bromelain was not affected. It is known that one of the ways that *Lactobacillus* exhibits its antimicrobial action is through decreasing pH levels from the production of lactic acid (Peng et al., 2016). The unchanged *Lactobacillus* population by bromelain indicates that *E. coli* growth inhibition is not due to *Lactobacillus* but possibly due to an indirect effect of bromelain. Feed enzymes are known to alter gut microbiota diversity and composition (Choct, 2006; Kiarie et al., 2013). Improvement of protein digestibility by bromelain may reduce the indigestible protein substrates available for pathogenic bacteria to grow in the hind gut (Yin et al., 2018). Insights into the mechanism of antimicrobial activity of bromelain and its effect on other gut microbiota warrant further study.

Bromelain supplementation had no effect on serum ALT level and it reduced serum ALP and AST levels. Bromelain also reduced serum creatinine. Similarly, Hossain et al. (2015) reported decreased serum creatinine in pigs fed with bromelain. Based on these findings, the levels of bromelain used in the present study had no adverse effect on liver and kidney functions of broilers. However, bromelain resulted in decreased serum uric acid, which is an important antioxidant in birds (Machin et al., 2004).

Conclusion

Bromelain supplementation in broilers improved protein and fat digestibility that resulted in reduced faecal nitrogen content. Bromelain also improved intestinal villus height and digesta viscosity that could

possibly be due to reduction of dietary anti-nutritional factors. Intestinal *E. coli* population was reduced suggesting the potential antimicrobial effects of bromelain. Although bromelain did not improve the growth performance of broilers, it had no adverse effects on the liver and kidney functions.

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