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Effects of bromelain supplementation on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding, faecal score and faecal noxious gas emission in weanling pigs

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ABSTRACT: A total of 140 weanling pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (b.w.) of 6.75 ± 1.48 kg were used in a six-week trial. Pigs were randomly allotted to one of four experimental treatments according to their initial b.w. (seven pens per treatment with five pigs per pen). Dietary treatments were: CON = control diet, T1 = CON + 0.05% bromelain, T2 = CON + 0.10% bromelain, T3 = CON + 0.20% bromelain. The experiment was divided into two phases (Days 1 to 14 and Days 15 to 42). All diets, in mash form, were formulated to meet or exceed the nutrient requirements (NRC, 2012) for weanling pigs. Feed intake and b.w. were monitored at the end of each phase. T3 treatment had greater (342 vs. 305 g; 409 vs. 387 g; $P < 0.05$) average daily gain (ADG) and average daily feed intake (ADFI) than CON treatment in Phase 1. In Phase 2, the ADG was improved (from T1 to T3: 612, 616, 637 vs. 583 g; $P < 0.05$) in all bromelain treatments compared with CON treatment, ADFI and growth efficiency (G : F) ratio of T3 treatment were higher (833 vs. 803 g; 0.765 vs. 0.726 g; $P < 0.05$) compared with CON treatment. Overall, T3 treatment showed greater (539 vs. 490 g; 691 vs. 664 g; $P < 0.05$) ADG and ADFI than CON. Moreover, pigs fed bromelain diets exhibited increased (0.769, 0.770, 0.780 vs. 0.738; $P < 0.05$) G : F ratios compared with those fed CON diet. Pigs fed bromelain diets exhibited increased (two weeks: 79.06, 79.97, 79.42 vs. 77.98%; 78.51, 78.86, 78.43 vs. 75.69%; six weeks: 74.49, 74.67, 75.02 vs. 72.70 %; 69.43, 70.78, 71.32 vs. 73.39%; $P < 0.05$) apparent total tract digestibility (ATTD) of dry matter and nitrogen compared with those fed CON diet at Week 2 and Week 6. On Day 42, the blood creatinine in the CON group was higher (1.30 vs. 1.04, 0.97, 0.88 mg/dl; $P < 0.05$) compared with the bromelain treatment groups. Faecal *E. coli* counts were decreased (6.22 vs. 6.41 $\log_{10}$ cfu/g; $P < 0.05$) in T2 treatment compared with CON treatment. The faecal ammonia (NH_3) gas emission in T2 and T3 treatments decreased (17.72, 17.33 vs. 22.95 ppm; $P < 0.05$) compared with CON. In conclusion, dietary supplementation with 0.2% bromelain has here been shown to improve the growth performance, apparent total tract digestibility of dry matter and N and to decrease *E. coli* and faecal NH_3 gas emission in weanling pigs.

Keywords: apparent total tract digestibility; blood creatinine; *E. coli*; NH3 gas emission

List of abbreviations

ADFI = average daily feed intake, **ADG** = average daily gain, **ATTD** = apparent total tract digestibility, **DM** = dry matter, **G : F** = growth efficiency, **NH_3** = ammonia

Antibiotic supplementation is well accepted to improve growth and efficiency in swine (Hahn et al. 2006). However, repeated use of antibiotics in animal diets results in severe problems like resistance of pathogens to antibiotics, accumulation of antibiotic residues in animal products and the environment, imbalance of normal microflora, and reduction in beneficial intestinal microflora (Hinton et al. 1986; Barton 2000). This has resulted in a severe restriction or total ban on the use of antibiotics

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in the animal and poultry industry in many countries, which in turn has led to a growing interest in alternatives to antibiotic growth promoters such as enzymes, oligosaccharides, herbs, flavours, minerals and non-starch polysaccharides (Close 2000). Pineapple (*Ananas comosus*) is the leading edible member of the family Bromeliaceae, grown in several tropical and subtropical countries including Philippines, Thailand, Indonesia, Malaysia, Kenya, India and China. It has been used as a medicinal plant in several native cultures (Mondal et al. 2011) and these medicinal qualities of pineapple are attributed to bromelain. Bromelain is an aqueous extract of pineapple that contains a complex mixture of thiol proteases and non-protease components. Proteases constitute the major components of bromelain and include stem bromelain (80%), fruit bromelain (10%) and ananain (5%). Among non-protease components number phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates (Maurer 2001). Assays for the individual protease components of bromelain have recently been established thus raising the possibility of standardising bromelain preparations (Hale et al. 2005).

Bromelain offers a wide range of therapeutic benefits and is increasingly being accepted as a phytotherapeutical drug (Maurer 2001). Similar to the model plant cysteine protease papain, bromelain is remarkably heat stable, retaining proteolytic activity between 40 °C and 60 °C where most enzymes are destroyed or denatured. The optimal temperature for the proteolysis of stem bromelain ranged from 35–50 °C in one study (Greenberg 1955), and up to 60 °C in later studies (Natalucci et al. 1985). Bromelain, unlike most enzymes, has a very wide effective range of activity in both acidic and alkaline conditions that allows it to remain active in a variety of biological environments. It is stable between pH 5.0 and 10.0 and is of broad specificity (Minami et al. 1971). This enzyme is a good alternative to microbial proteases like subtilisins from *Bacillus licheniformis* and *Bacillus amyloliquifaceins* that are enzymes of choice for detergents (Van Beckhoven et al. 1995). However, the effect of bromelain on weanling pigs has not been studied yet. Therefore, the aim of the present study was to evaluate the effects of bromelain supplementation on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding, faecal score and faecal noxious gas emission in weanling pigs.

MATERIAL AND METHODS

The Animal Care and Use Committee of Dankook University approved the experimental procedures used in this study.

Animals and experimental design. A total of 140 weanling pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (b.w.) of 6.75 ± 1.48 kg were used in a six-week experiment. Pigs were randomly allotted to one of four experimental diets according to initial b.w. There were seven replicate pens per treatment with five pigs (two gilts and three borrows) per pen. All pigs were housed in an environmentally-controlled room, which provided 0.26 × 0.53 m^2 for each pig. Each pen was equipped with a one-sided, stainless steel self-feeder and a nipple drinker that allowed access to feed and water *ad libitum*. Dietary treatments were: CON = control diet; T1 = CON + 0.05% bromelain; T2 = CON + 0.10% bromelain; T3 = CON + 0.20% bromelain. The experiment included two phases: Days 1 to 14 and Days 15 to 42 (Table 1). All nutrients in diets were formulated to meet or exceed the recommendations of NRC (2012) for weanling pigs and fed in a mash form. Bromelain (Pinex®) as powder form was supplied by EunjinBio, Cheonan, South Korea and added to the basal diet at percentages of 0.05, 0.1 and 0.2.

Sampling and measurements. Individual pig b.w. and feed disappearance were recorded on Days 14 and 42 to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F). All pigs were fed diets mixed with 0.20% chromium oxide (Cr_2O_3) as an indigestible marker during Days 8 to 14 and Days 36 to 42. Fresh faecal grab samples were collected from two pigs per pen (Days 12 to 14 and Days 40 to 42) via rectal massage. Representative samples were stored in a freezer at –20 °C until analysed (Fenton and Fenton 1979). Before chemical analysis, the faecal samples were thawed and dried at 60 °C for 72 h, after which they were finely ground to a size that could pass through a 1-mm screen. The procedures utilised for the determination of dry matter (DM), nitrogen (N) and energy (E) digestibilities were conducted in accordance with the methods established by the AOAC (1995). Chromium levels were determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan). Nitrogen was determined usiing a Kjectec 2300 Nitrogen Analyser (Foss Tecator AB, Hoeganaes, Sweden). Gross energy was analysed using an oxygen bomb calorimeter (Parr 1600 Instrument Co., Moline, IL, USA).

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Table 1. Feed compositions of control diet (as-fed basis)

Item	Phase 1 (Days 1–14)	Phase 2 (Days 15–42)
Ingredient (%)		
Extruded corn	44.49	61.97
Soybean meal (48% CP)	16.20	25.30
Fermented soybean meal (45% CP)	5.00	2.50
Fish meal (66% CP, Brazil)	3.50	–
Soy oil	2.55	1.05
Lactose	8.30	–
Whey	10.00	5.00
MCP	–	–
DCP	1.5	1.5
Sugar	3.00	–
Plasma powder (AP 920)	3.00	–
L-Lys HCl (78%)	0.39	0.46
DL-Met (50%)	0.30	0.24
L-Thr (89%)	0.19	0.20
Choline chloride (25%)	0.10	0.10
Vitamin premix[1]	0.10	0.10
Trace mineral premix[2]	0.20	0.20
Limestone	0.98	1.13
Salt	0.20	0.25
Total	100.00	100.00
Calculated nutritional content		
ME, kcal/kg	3,540	3,410
CP (%)	20.00	19.00
Lys (%)	1.50	1.35
Met (%)	0.62	0.53
Met + Cys (%)	0.97	0.84
Ca (%)	0.95	0.90
Total P (%)	0.75	0.70
Avail P (%)	0.55	0.43
Crude fat (%)	5.02	3.98
Crude fiber (%)	1.87	2.45

[1]Provided per kg of complete diet: vitamin A, 11 025 IU; vitamin D_3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg

[2]Provided per kg of complete diet: Cu (as $CuSO_4 \times 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \times 5H_2O$), 0.15 mg

For the blood profiles, two pigs from each pen (one gilt and one barrow) were randomly selected and blood samples were collected via anterior vena cava puncture on Days 14 and 42. At the time of collection, blood samples were collected into both non-heparinised tubes and vacuum tubes containing K_3EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ) to obtain serum and whole blood, respectively. After collection, the serum was separated by centrifugation for 15 min at 3000 × g, after which the aliquot was stored at 4 °C until it was analysed for creatinine, blood urea nitrogen (BUN) and IgG using an automatic biochemistry blood analyser (HITACHI 747; Hitachi, Tokyo, Japan). The red blood cell (RBC), white blood cell (WBC) and lymphocyte counts of the whole blood samples were determined using an automatic blood analyser (ADVIA 120; Bayer, Tarrytown, NY, USA).

At Days 14 and 42, faecal samples were collected directly by massaging the rectum of two pigs randomly selected from each pen (one gilt and one barrow). The obtained samples were pooled and placed on ice for transportation to the laboratory, where microbial analyses were immediately carried out according to the method described by Zhao et al. (2013). One gram of the composite faecal sample from each pen was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenised. Viable counts of bacteria in the faecal samples were then established by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillii*, respectively. The *Lactobacilli* medium III agar plates were then incubated for 48 h at 39 °C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37 °C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. Faecal scores were determined at 08:00 and 20:00 using the following faecal scoring system: one hard, dry pellet; two firm, formed faeces; three soft, moist faeces that retains shape; four soft, unformed faeces that assumes the shape of container; five watery liquid that can be poured (Larson et al. 1977). Faecal samples were collected at Days 14 and 42 and then placed in aluminium foil cups. The aluminium foil cups were weighed and placed in a drying oven at 100 °C for 24 h and then reweighed to calculate moisture loss.

Faeces were collected on Day 42 to determine faecal noxious gas emission according to the method described by Yan and Kim (2013). One hundred

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and fifty g fresh faeces and 150 g urine mixture samples were randomly collected from two pigs (one gilt and one barrow) in each pen and stored in 2.6-l plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster for replicates. The samples were fermented for five days at room temperature (28 °C), after which 100 ml of the headspace air was sampled from approximately 2.0 cm above the faecal sample. The concentration of gas was measured within the scope of 5.0–100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0–20.0 ppm (4LK, detector tube; Gastec Corp.). Prior to measurement, the faecal samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the faecal sample and to homogenise the samples.

Statistical analysis. All data were subjected to the statistical analysis as a randomised complete block design using the GLM procedures of SAS (1996) and the pen was designated as the experimental unit. Before carrying out statistical analysis of the microbial counts, logarithmic conversion of the data was performed. Orthogonal polynomials were used to assess the linear effects of increasing dietary concentrations of supplemental bromelain. Differences among treatment means were determined using Duncan's multiple range test with a $P < 0.05$ indicating significance.

RESULTS

Growth performance and ATTD of nutrients

As shown in Table 2, during Days 1 to 14, pigs fed T3 diet had greater ($P < 0.05$) ADG and ADFI than those fed CON diets, but no difference ($P < 0.05$) was observed in G : F ratios among treatments. During Days 15 to 42, the ADG was improved ($P < 0.05$) by all bromelain treatments compared with CON treatment; the ADFI and G : F ratio of pigs fed the T3 diet was higher ($P < 0.05$) compared with pigs fed the CON diet. Overall, pigs fed the T3 diet showed greater ($P < 0.05$) ADG and ADFI than those fed the CON diet. Moreover, pigs fed bromelain diets exhibited increased ($P < 0.05$) G : F ratios compared with those fed the CON diet. As shown in Table 3, pigs fed bromelain diets exhibited increased ($P < 0.05$) ATTD of dry matter and nitrogen compared with animals fed CON diets at Week 2 and Week 6.

Blood profiles

As shown in Table 4, on Day 42, blood creatinine in the CON group was higher ($P < 0.05$) compared than in the bromelain groups, while lymphocyte, RBC, WBC, BUN and IgG counts were not affected ($P > 0.05$) by dietary treatments.

Table 2. The effects of bromelain on growth performance in weanling pigs

Items	CON	T1	T2	T3	SE	Linear effect < 0.05
Phase 1(1–14 days)						
ADG* (g)	305^{c}	317^{bc}	329^{ab}	342^{a}	6	0.010
ADFI* (g)	387^{b}	392^{b}	392^{b}	409^{a}	2	0.001
G/F	0.788	0.809	0.839	0.836	0.016	0.213
Phase 2(14–42 days)						
ADG* (g)	583^{b}	612^{a}	616^{a}	637^{a}	9	0.049
ADFI* (g)	803^{c}	807^{c}	817^{b}	833^{a}	3.00	0.001
G/F	0.726^{b}	0.758^{ab}	0.754^{ab}	0.765^{a}	0.011	0.740
Overall (1–42 days)						
TADG* (g)	490^{c}	514^{b}	520^{b}	539^{a}	6	0.010
TADFI* (g)	664^{c}	668^{c}	675^{b}	691^{a}	2	0.001
TG/F	0.738^{b}	0.769^{a}	0.770^{a}	0.780^{a}	0.009	0.436

CON = control diet; T1 = CON + bromelain 0.05%; T2 = CON + bromelain 0.10%; T3 = CON + bromelain 0.20%; SE = standard error

$^{a–c}$means in the same row with different superscripts differ ($P < 0.05$)

*linear effect ($P < 0.05$)

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Table 3. The effects of bromelain on nutrient digestibility in weanling pigs

Items (%)	CON	T1	T2	T3	SE	Linear effect < 0.05
14 days						
DM*	77.98[b]	79.06[a]	79.97[a]	79.42[a]	0.31	< 0.001
N*	75.69[b]	78.51[a]	78.86[a]	78.43[a]	0.48	< 0.001
Energy	77.47	77.82	78.03	78.81	0.63	0.502
42 days						
DM*	72.70[b]	74.49[a]	74.67[a]	75.02[a]	0.49	0.015
N*	67.07[b]	69.43[a]	70.78[a]	71.32[a]	0.71	0.001
Energy	73.39	75.12	74.10	73.93	0.61	0.436

CON = control diet; T1 = CON + bromelain 0.05%; T2 = CON + bromelain 0.10%; T3 = CON + bromelain 0.20%; SE = standard error

[a,b]means in the same row with different superscripts differ ($P < 0.05$)

*linear effect ($P < 0.05$)

Faecal microflora, faecal scores and faecal noxious gas emission

As presented in Table 5, faecal *E. coli* counts were decreased ($P < 0.05$) by T2 treatment compared with CON treatment. However, no significant difference was observed in faecal scores among the treatments. T2 and T3 treatments decreased ($P < 0.05$) the faecal NH_3 emission compared with CON treatment, but no effect on faecal total mercaptans, H_2S and acetic acid emissions were observed among treatments (Table 6).

Table 4. The effects of bromelain on blood profiles in weanling pigs

Items	CON	T1	T2	T3	SE	Linear effect < 0.05
Lymphocyte (%)						
14 days	44.28	44.12	44.34	44.16	1.27	0.971
42 days	50.78	51.86	48.38	52.92	7.75	0.823
RBC (10^6/µl)						
14 days	6.51	6.76	6.78	6.60	0.16	0.243
42 days	6.70	6.66	6.44	6.40	0.26	0.498
WBC, 10^3/µl						
14 days	29.56	30.08	30.36	30.00	2.37	0.831
42 days	31.16	32.48	32.70	30.92	2.21	0.588
Creatinine (mg/dl)						
14 days	1.15	1.12	1.13	1.15	0.06	0.899
42 days*	1.30[a]	1.04[b]	0.97[b]	0.88[b]	0.08	0.003
BUN (mg/dl)						
14 days	11.28	12.22	11.22	11.48	1.67	0.977
42 days	13.30	12.96	14.20	14.68	0.94	0.567
IgG (mg/dl)						
14 days	269.0	276.0	276.0	271.8	29.5	0.863
42 days	297.8	310.6	305.4	307.2	18.0	0.741

CON = control diet; T1 = CON + bromelain 0.05%; T2 = CON + bromelain 0.10%; T3 = CON + bromelain 0.20%; SE = standard error

[a,b]means in the same row with different superscripts differ ($P < 0.05$)

*linear effect ($P < 0.05$)

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Table 5. The effects of bromelain on faecal microflora in weanling pigs

Items, log_{10}cfu/g	CON	T1	T2	T3	SE	Linear effect < 0.05
Lactobacillus	7.43	7.50	7.51	7.59	0.05	0.270
*E. coli**	6.41[a]	6.36[ab]	6.22[b]	6.35[ab]	0.05	0.029
Fecal score[1]	3.15	3.18	3.14	3.16	0.03	0.670

CON = control diet; T1 = CON + bromelain 0.05%; T2 = CON + bromelain 0.10%; T3 = CON + bromelain 0.20%; SE = standard error

[a,b]means in the same row with different superscripts differ ($P < 0.05$)

*linear effect ($P < 0.05$)

[1]faecal scores were determined at 08:00 and 20:00 using the following faecal scoring system: one hard – dry pellet; two firm – formed stool; three soft – moist stool that retains shape; four soft – unformed stool that assumes shape of container; five watery – liquid that can be poured

DISCUSSION

Studies on bromelain supplementation on swine are limited. However, based on previous studies we could speculate on its effect. In the present study, the T2 treatment (pigs supplied with a level of 0.10% bromelain in diet) exhibited decreased faecal *E. coli* concentrations, in agreement with the study by Chandler and Mynott in 1998. A previous study also confirmed that oral administration of bromelain could inhibit $K88^+$ Enterotoxigenic *Escherichia coli* (ETEC) receptor activity and ETEC attachment to the porcine small intestine (Mynott et al. 1999).

Therefore, as the antibacterial function of bromelain has been confirmed, we expected positive effects on weanling pig growth performance. The weight gain observed in bromelain-treated pigs in this study is consistent with results obtained from field trials conducted on commercial pig farms. A study of 1107 pigs (552 untreated pigs, 555 bromelain-treated) showed an up to 60% reduction in piglet mortality and a 12% increase in weight in bromelain-treated pigs (Chandler et al. 1994). A wide range of therapeutic benefits has been claimed for bromelain, such as reversible inhibition of platelet aggregation, sinusitis, surgical traumas (Livio et al. 1978), thrombophlebitis, pyelonephritis, angina pectoris, bronchitis (Neubauer 1961) and enhanced absorption of drugs, particularly of antibiotics (Renzini and Varego 1972; Maurer 2001). The useful phytomedical applications determined in these earlier can explain the results of the present study, where throughout the whole experimental period, pigs fed with a 0.2% level of bromelain showed greater ADG and ADFI than those fed CON diets. Moreover, all pigs fed bromelain diets exhibited increased G:F ratios compared with those fed CON diets. Bromelain has been used successfully as a digestive enzyme following pancreatectomy, in cases of exocrine pancreas insufficiency and in other intestinal disorders. Because of its wide pH range, bromelain has activity in the stomach as well as the small intestine. It has also been shown to be an adequate replacement for pepsin and trypsin in cases of deficiency (Knill-Jones et al. 1970).

Our data showed that the serum creatinine of pigs fed with bromelain decreased compared with the

Table 6. The effects of bromelain on noxious gas emissions in weanling pigs

Items (ppm)	CON	T1	T2	T3	SE	Linear effect < 0.05
NH_3*	22.95[a]	19.00[ab]	17.72[b]	17.33[b]	1.28	0.001
R.SH	8.83	8.43	7.83	8.38	0.56	0.101
H_2S	8.45	7.60	7.83	7.15	0.56	0.100
Acetic acid	2.63	2.15	2.38	2.45	0.31	0.211

CON = control diet; T1 = CON + bromelain 0.05%; T2 = CON + bromelain 0.10%; T3 = CON + bromelain 0.20%; SE = standard error

[a,b]means in the same row with different superscripts differ ($P < 0.05$)

*linear effect ($P < 0.05$)

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pigs fed the CON diet. Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate) and adenosine triphosphate (ATP, the body's immediate energy supply). In 1995, Zavadova et al. suggested that bromelain increases neutrophil activity, based on a study using healthy volunteers taking oral bromelain. Eckert et al. (1999) performed clinical studies involving breast cancer patients and healthy volunteers and observed the stimulation of immunocytotoxicity of cancer-patient-derived immune cells following oral administration of bromelain.

The faecal *E. coli* concentration of pigs fed 0.2% bromelain was a little higher (not significant) than pigs fed 0.1% bromelain. Although the mechanisms of how bromelain mediates its effects are still not fully understand, we could find some clues from previous studies. According to Taussig et al. (1975) bromelain has very low toxicity with an LD_{50} (lethal dose) greater than 10 g/kg in mice, rats, and rabbits. Toxicity tests on dogs, with increasing levels of bromelain up to 750 mg/kg administered daily, showed no toxic effects after six months. Dosages of 1500 mg/kg per day when administered to rats showed no carcinogenic or teratogenic effects and did not cause any alteration in food intake, histology of the heart, growth rate, spleen, kidney, or haematological parameters (Moss et al. 1963). Eckert et al. (1999) after giving bromelain (3000 FIP unit/day) to humans over a period of ten days found no significant changes in blood coagulation parameters.

Evidence has suggested that bromelain counteracts some of the effects of certain intestinal pathogens like *Vibrio cholerae* and *E. coli*, whose enterotoxin causes diarrhoea in animals. In the current study, weanling pigs fed with bromelain supplementation exhibited reduced *E. coli* counts, whereas no effect on faecal score was observed compared with other diets. In a study designed to examine the effect of bromelain on enterotoxin receptor activity in the porcine small intestine, orally administered bromelain inhibited enterotoxin attachment to pig small intestine in a dose-dependent manner. Attachment was negligible after treatment. Serum biochemical analysis and histopathological examination of treated piglets showed no adverse effects with the bromelain treatment. Administration of bromelain may therefore be useful for preventing enterotoxin-induced diarrhoea (Mynott et al. 1996). Thus, further study is warranted to determine the positive effects of bromelain on faecal score beyond those tested in our study.

Ammonia is a major aerial pollutant originating from livestock and poultry operations (Zhang et al. 2013). High concentrations of ammonia or H_2S can result in hazardous effects for humans and animals (Drummond et al. 1980; Zhang and Kim 2014). It has been suggested that faecal ammonia gas emission is related to nutrient utilisation and the intestinal microbial ecosystem (Ferket et al. 2002; Wang et al. 2009). In our study, NH_3 gas emission was reduced by addition of bromelain to the diet. Bromelain appears to exhibit this effect by interacting with intestinal secretory signalling pathways, including adenosine 3':5'-cyclic monophosphatase, guanosine 3':5'-cyclic monophosphatase and calcium-dependent signalling cascades (Mynott et al. 1999). Other studies suggest a different mechanism of action. In *E. coli* infection, active supplementation with bromelain leads to some anti-adhesion effects which prevent the bacteria from attaching to specific glycoprotein receptors located on the intestinal mucosa by proteolytically modifying the receptor attachment sites (Mynott et al. 1996; Chandler and Mynott 1998). Therefore, the reduction of excreta NH_3 may be considered as a manifestation of improved nutrient digestibility and an improved gut micro-ecological condition.

In conclusion, according to the present study the use of bromelain at a level of 0.1 and/or 0.2% could improve growth performance, nutrient digestibility and decrease faecal *E. coli* concentration and noxious gas emission of weanling pigs. Bromelain had statistically significant effects on average daily gain and average daily feed intake during Phase 2 of this study and also overall.

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