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Short Communication

Dietary bromelain-C.3.4.22.32 supplementation improves performance and gut health in sows and piglets ☆

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ABSTRACT

Ninety six multiparous sows (Landrace × Yorkshire, average parity = 1.50 ± 0.03) and/or their litters were used to investigate the effects of bromelain-C.3.4.22.32 (BR) on reproductive and growth performance, diarrhea score (DS), apparent total tract digestibility (ATTD) of nutrients, blood profiles, fecal microbiota and colostrum and milk contents. The pigs were randomly allotted to 1 of 4 treatments to give 24 replicates per treatment. Dietary treatments were as follows: (1) CON (basal diet), (2) BR1 (CON + 0.5 g/kg BR), (3) BR2 (CON + 1 g/kg BR) and (4) BR3 (CON + 2 g/kg BR). Fecal samples (1 g) from each pen were diluted with 9 mL of 10 g/L peptone broth for evaluation of fecal microbiota. The piglets suckling sows fed the BR3 diet had linearly increased average daily gain (ADG) and weaning weight (P = 0.02 and 0.01, respectively). Lactating sows fed the CON diet had less fecal *Lactobacillus* and more fecal *Escherichia coli* counts (linear P = 0.03 and linear and quadratic P = 0.01 and 0.04, respectively). At weaning, sows fed the BR3 diet had linearly higher apparent total tract digestibility (ATTD) of nitrogen (N; P = 0.04). Lactating sows fed the BR3 diet had linearly lower blood urea nitrogen (BUN) and higher lymphocyte counts (P = 0.02 and 0.04, respectively). Consequently, piglets suckling sows fed the BR3 diet had higher IgG counts and lower blood urea nitrogen (BUN; linear, P = 0.03 and 0.04, respectively). Sows fed the CON diet had linearly higher colostrum and milk somatic cell counts (SCC; P = 0.01 and < 0.01, respectively). Milk protein was linearly higher in sows fed the BR3 diet (P = 0.04). In conclusion, the results indicated that dietary supplementation of BR in late gestation and lactation improved performance in sows and suckling piglets.

1. Introduction

Antibiotic supplementation has been well accepted to boost growth and efficiency in swine (Hahn et al., 2006). However, repeated use of antibiotics in animal diets has resulted in severe issues, such as the resistance of infective agents to antibiotics, accumulation of antibiotic residues in animal merchandise and setting, imbalance of regular microflora and reduction in the amount of useful enteric microflora (Barton, 2000; Zhao et al., 2013). Such problems have caused severe restriction or total ban on using antibiotics in animal and poultry trade in several countries, which has led to a rise in the search for alternatives to antibiotic growth promoters (AGP) such as enzymes, various plant extracts, prebiotics, probiotics, herbs, herbal products and spices (Zhao et al., 2013; Cho and Kim, 2014; Zhang and Kim, 2014).

Pineapple is the common name of *Ananas comosus* (syns. *A. sativus, Ananassa sativa, Bromelia ananas, B. comosa*). It has been used as a medicinal plant in several native cultures (Mondal et al., 2011; Pavan et al., 2012), due to the medicinal qualities inferred by bromelain. Bromelain belongs to a group of protein digesting enzymes extracted commercially from the stem (EC.3.4.22.32) or fruit (EC.3.4.22.33) of pineapple (Pavan et al., 2012). Bromelain has been claimed to provide a wide range of therapeutic benefits, such as reversible inhibition of platelet aggregation, sinusitis, surgical traumas (Hale et al., 2005; Pavan et al., 2012), thrombophlebitis, pyelonephriti angina pectoris and bronchitis (Engwerda et al., 2001; Hale et al., 2005; Pavan et al., 2012), anti-inflammatory activity and modulation of immunological response via apoptotic response of lymphocyte caused by cyst cysteine protease (Engwerda et al., 2001; Hale et al., 2005; Sikasunge et al., 2008). Evidence has suggested that bromelain counteracts some of the effects of certain intestinal pathogens like *Escherichia coli,* whose enterotoxin causes diarrhea in pigs (Pavan et al., 2012). These effects may be attributed to the sulfhydryl proteolytic fraction, the

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main chemical compounds in bromelain, which also contains phosphatase, glucosidase, peroxidase, cellulase, escharase and several protease inhibitors (Kelly, 1996; Pavan et al., 2012).

Previous studies on bromelain and medicinal plant extracts indicated that fecal microbiota and immune system could influence the intestinal nutrient utilization and subsequently increase colostrum and milk contents in animals (Wang et al., 2008; Zhao and Lacasse, 2008). Although the effect of BR transferred to the suckling piglets which had transfer of the compounds into milk were not evaluated herein, according to Castell et al. (1997) and Chobotova et al. (2010), bromelain and its compounds can be absorbed and transferred to milk without degradation or loss of biological activity. Therefore, based on the previous studies, we hypothesized that BR supplementation in sow gestation and lactation diets could improve sow and litter performance. However, very few studies have examined the impact of bromelain in laboratory animals and humans, and the effect of BR on sows and piglets has not yet been studied. Thus, the objective of the present study was to evaluate the effects of BR on growth performance, DS, nutrient digestibility, blood profiles, fecal microflora and colostrum and milk contents in sows and piglets.

2. Materials and methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University.

2.1. Experimental design, animals and housing

A total of 96 sows (Landrace × Yorkshire, average parity is 1.5, SD=0.3) and their litters were used in a 28-d experiment. On day 108 of gestation, sows were assigned to four dietary treatments to give 24 replicates per treatment based on parity number according to a randomized complete block design. During gestation, sows were housed individually in stalls of 2.00 × 0.60 m^2. The stall had partly slatted floors that consisted of a 0.80 m concrete solid floor and a 1.05 m concrete slatted floor. Approximately 10 d before the expected time of parturition, sows were moved to farrowing rooms, each with 2.20 × 1.60 m^2. The concrete solid floor was equipped with floor heating. Piglets were weaned at d 21. Cross-fostering of piglets took place within 2 d of parturition and occurred only among sows of the same experimental treatment. Each litter was standardized to 10 piglets. All rooms were equipped with auto-controlled heating and mechanical ventilation systems. The care and treatment of the sows were according to animal welfare legislation.

2.2. Diets and feeding

The pigs were fed with corn-soybean based diets (Table 1). The dietary treatments were as follows: CON, basal diet; BR1, CON+0.5 g/kg BR; BR2, CON+1 g/kg BR; BR3, CON+2 g/kg BR. Bromelain-C.3.4.22.32 used in our study was provided by EunjinBio, Cheonan, South Korea. From d 108 of gestation to parturition, sows were fed 2.5 kg/day of their respective experimental lactation diets. On the day of parturition, the sows were not offered feed. After farrowing, sows were fed their respective experimental lactation diets until weaning. During lactation, daily feed allowance was increased gradually until sows had ad libitum access to feed by wk 2. All diets were provided in meal form twice daily and sows had free access to drinking water throughout the experimental period. Diets were formulated (Table 1) to meet or exceed the nutrient requirements of gestating and lactating sows (NRC, 2012). Piglets were allowed free access to a commercial creep diet (CJ, Seoul, Korea; 9.03 MJ of NE/kg; 220 g of CP/kg; 11.2 g of ileal digestible lysine/kg; 2.8 g of digestible P/kg) from day 10 after birth until weaning.

Table 1
Sow diet composition (as-fed basis).

Items	Gestation diet	Lactation diet
Ingredients, %		
Corn	57.10	51.12
Soybean meal, 46% CP	10.65	24.61
Wheat bran	12.00	4.00
Rapeseed meal	3.70	2.50
Rice bran	6.00	5.00
Tallow	3.59	6.05
Molasses	3.60	3.50
Dicalcium phosphate	1.52	1.64
Limstone	0.99	0.76
Salt	0.60	0.50
L-lysine HCl, 98%	0.05	0.12
Vitamin premix[1]	0.10	0.10
Mineral premix[2]	0.10	0.10
Calculated composition, %		
ME (MJ/kg)	3.19	3.44
Analyzed composition, %		
CP	13.09	17.10
EE	6.88	9.09
Lys	0.65	1.00
Ca	0.88	0.84
P	0.76	0.72

[1] Provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D3, 2000 IU; vitamin E, 48 IU; vitamin K3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; d-pantothenic, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B6, 2 mg; and vitamin B12, 28 μg.

[2] Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 90 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 15 mg; Zn (as $ZnSO_4$), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.25 mg.

2.3. Chemical analysis

Feed samples were ground to pass through a 1 mm screen, after which they were analyzed for DM (method 934.01; AOAC, 2000), CP (method 990.03; AOAC, 2000), ether extract (method 920.39; AOAC, 2000), Ca (method 984.01; AOAC, 1995) and P (method 965.17; AOAC, 1995). Individual AA composition was measured using an AA analyzer (Beckman 6300; Beckman Coulter Inc., Fullerton, CA) after a 24 h hydrolysis in HCl. Nitrogen was determined (Kjeltec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes Sweden) and CP was calculated as N × 6.25. Gross energy was analyzed by oxygen bomb calorimeter (Parr 1600 Instrument Co., Moline, IL, USA).

2.4. Experimental procedures and laboratory analysis

The BW and backfat of sows were checked on d 108 of gestation, immediately after farrowing (d 0), and at weaning (d 21) to calculate the body weight loss and the backfat loss during that period. The backfat thickness of the sows (6 cm off the midline at the 10th rib) was measured using a real-time altrasound instrument (Piglog 105, SFK Technology, Herlev, Denmark). Values from the two measurements were averaged to obtain a single backfat measurement, which method according to the report of Wang et al. (2008). The feed consume was recorded during gestation and lactation periods to calculate the ADFI. Detection of estrus was conducted twice per day from weaning onwards, at 8 am and 4 pm every day. A sow was considered to be in estrus when exhibiting a standing response induced by a back pressure test when in the

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presence of a boar. Number of piglets borne and weaned pigs was also recorded to calculate the survival rate. Individual piglet BW and litter weights were assessed on d 0 and 21 to calculate ADG. The incidence of diarrhea in piglets was observed and recorded 3 times per day throughout the study. In order to assess the severity of diarrhea, faeces from each pen were scored according to the method of Zhou et al. (2012). In brief, the scores were as follows: (1) (hard, dry pellets in a small, hard mass). (2) (hard, formed stool that remains firm and soft) (3) (soft, formed and moist stool that retains its shape), (4) (soft, unformed stool that assumes the shape of the container), (5) (watery, liquid stool that can be poured). A cumulative DS per diet and day was then assessed (Smiricky et al., 2002).

On weaning day, fecal samples were randomly collected from 12 sows per treatment by rectal palpation and pooled and placed on ice for transportation to the laboratory, where microbial analysis were immediately carried out according to the method described by Zhao et al. (2013). The obtained fecal sample (1 g) from each pen was diluted with 9 mL of 10 g/L peptone broth (Becton, Dickinson and Co., Rutherford, NJ, USA) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 10 g/L peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, 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.

To determine the apparent total tract digestibility (ATTD) for dry matter, nitrogen and gross energy, sows were fed diets containing chromic oxide (2 g/kg) as an indigestible marker for 5 d (d 38–42) followed by fecal grab sampling from 12 randomly selected sows per treatment via rectal palpation on weaning day. Fecal and feed samples were stored at −20 °C until required for analysis. Fecal samples were thawed and dried in an oven at 70 °C for 72 h and then ground along with feed samples to pass through a 1 mm screen before being analyzed for DM, GE and N as described before. Chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) according to the methods of Williams et al. (1962). The ATTD was then calculated using the following formula: digestibility = $\{1-[(Nf \times Cd)/(Nd \times Cf)]\} \times 100\%$, where, Nf = nutrient concentration in faeces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM) and Cf = chromium concentration in faeces (% DM).

Blood samples were collected via jugular venipuncture into K_3EDTA vacuum tubes and clot activator vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) from 12 randomly selected sows per treatment at weaning and from two randomly selected piglets (1 barrow and 1 gilt) per sow at weaning. After collection, the serum was separated by centrifugation for 15 min at 3000 × *g*, after which the aliquot was stored at −20 °C until it was analyzed for creatinine, blood urea nitrogen (BUN) and IgG using an automatic biochemistry blood analyzer (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 analyzer (ADVIA 120; Bayer, Tarrytown, NY, USA) according to the method described by Li and Kim (2014).

Approximately 30–40 ml of colostrum and milk was collected from 12 randomly selected sows per treatment from functional glands within 2 h from termination of farrowing and on d 21 of lactation, respectively. Milk and colostrum fat, protein, lactose and solids were analyzed by a commercial laboratory using a Milkoscan System 4000 (Foss North America, Eden Prairie, MN; AOAC, 2000) and somatic cell counts (SCC) were analyzed by DeLaval (cell counter DCC).

2.5. Statistical analysis

Both sow and piglet performance data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for a randomized complete block design. Sow BW and backfat data were analyzed using a repeated measurement method. The model included diet as a fixed effect whereas sow and period were included as random effects. The sow was considered as the experimental unit. Parity effect was not significant, so it was not included in the model. Piglet birth weight was used as covariates for weaning weights during lactation. Lactation length was used as a covariate for number of piglets survivability, sows and piglets weaning weight, sows BW loss, ADFI and backfat thickness loss, and piglets ADG. Before conducting statistical analysis of the fecal microflora counts, we performed a logarithmic conversion of the data. Orthogonal polynomial contrasts were used to assess the linear and quadratic effects of increasing dietary concentrations of supplemental BR. Variability in the data was expressed as the pooled standard error of mean (SEM) and probability level of $P < 0.05$ was considered significant.

3. Results

No differences ($P > 0.05$) were observed on reproductive performance and BW change in sows among treatments, and the values averaged 10.33 (SEM = 0.21) and 9.78 (SEM = 1.10) for litter size of born alive and weaned piglets, respectively; 227.01 (SEM = 6.81), 206.95 (SEM = 6.53) and 189.93 (SEM = 6.58) for BW (kg) on d 108 of gestation, immediately after farrowing (d 0) and at weaning (d 21), respectively; 20.05 (SEM = 1.12), and 17.03 (SEM = 2.17) for BW loss (kg) from d 108 of gestation to d 0 and from d 0 to d 21, respectively; 1.86 (SEM = 0.30) and 8.01 (SEM = 0.21) for ADFI (kg) during gestation and lactation, respectively; 22.42 (SEM = 0.63), 22.11 (SEM = 0.67) and 17.51 (SEM = 0.94) for backfat thickness (mm) on d 108 of gestation, d 0 and d 21, respectively; 4.60 (SEM = 0.57) for backfat thickness loss (mm) from d 0 to d 21 and 4.55 (SEM = 0.33) for estrus interval (data not shown). Supplementation with the BR3 diet linearly increased the ADG and weaning body weight in suckling pigs ($P = 0.02$ and 0.01, respectively; Table, 2). No differences ($P > 0.05$) in DS were observed among suckling piglets. In addition, there were no differences in either piglet survival rate or stillbirth rate among treatments ($P > 0.05$). Sows fed with the BR

Table 2
Effect of bromelain-C.3.4.22.32 supplementation on growth performance in piglets suckling sows[1].

Items	CON	BR1	BR2	BR3	SEM[2]	*P* value	
						Linear	*Quadratic*
Piglet survival, %	94.12	94.23	95.15	95.19	3.30	0.66	0.61
BW, kg							
Birth Weight	1.34	1.41	1.51	1.49	0.09	0.23	0.22
Weaning	6.073	6.682	6.856	6.953	0.26	0.01	0.34
ADG, g	225	251	254	260	4.14	0.02	0.16
Stillbirth, %	2.14	1.68	1.73	1.87	4.78	0.36	0.45
Diarrhea Score	3.30	3.30	3.20	3.20	0.24	0.11	0.36

[1] Ninety six sows with an average parity of 1.5 (SD = 0.3), CON, basal diet; BR1, CON + 0.5 g/kg BR; BR2, CON + 1 g/kg BR; BR3, CON + 2 g/kg BR.
[2] Pooled standard error of mean.

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Table 3
Effect of bromelain-C.3.4.22.32 supplementation on fecal microflora in lactating sows[1].

Items, log_{10} cfu/g	CON	BR1	BR2	BR3	SEM[2]	*P* value	
						Linear	*Quadratic*
Lactobacillus	7.28	7.37	7.39	7.40	0.03	0.03	0.75
E. coli	6.57	6.39	6.26	6.28	0.07	0.01	0.04

[1] Fecal samples were randomly collected from 12 sows per treatment, CON, basal diet; BR1, CON+0.5 g/kg BR; BR2, CON+1 g/kg BR; BR3, CON+2 g/kg BR.
[2] Pooled standard error of mean.

Table 4
Effect of bromelain-C.3.4.22.32 supplementation on nutrient digestibility in lactating sows[1].

Items, %	CON	BR1	BR2	BR3	SEM[2]	*P* value	
						Linear	*Quadratic*
DM	64.02	66.33	67.79	66.06	1.27	0.16	0.61
N	63.96	64.55	66.80	68.95	1.34	0.04	0.42
Energy	63.38	64.72	65.88	65.54	1.27	0.65	0.35

[1] Fecal samples were randomly collected from 12 sows per treatment, CON, basal diet; BR1, CON+0.5 g/kg BR; BR2, CON+1 g/kg BR; BR3, CON+2 g/kg BR.
[2] Pooled standard error of mean.

supplementation diets had increased *Lactobacillus* population, while the fecal *E. coli* population decreased in the BR supplementation diets (linear, $P=0.03$ and linear and quadratic, $P=0.01$ and 0.04, respectively; Table 3). At weaning, the ATTD of N was greater in sows fed the BR3 diet (linear, $P=0.04$; Table 4). The ATTD of energy (*E*) and dry matter (DM) showed no differences among treatments ($P>0.05$).

The BUN in lactating sows of the BR3 group was lower (linear, $P=0.02$; Table 5). Lactating sows fed the BR3 diet had linearly higher lymphocyte counts ($P=0.04$). Piglets suckling sows fed with the BR3 diet also had linearly lower blood BUN ($P=0.04$); however, piglets suckling sows fed the BR2 and BR3 diets had

Table 5
Effect of bromelain-C.3.4.22.32 supplementation on blood profiles in lactating sows and suckling piglets[1].

Items	CON	BR1	BR2	BR3	SEM[2]	*P* value	
						Linear	*Quadratic*
Lactating sows							
WBC, $10^3/\mu L$	17.74	15.16	15.46	11.05	2.08	0.36	0.52
RBC, $10^6/\mu L$	6.13	6.05	5.40	4.93	0.65	0.64	0.64
Lymphocyte, %	12.51	12.60	14.73	19.61	1.90	0.04	0.82
IgG, mg/dL	476	486	466	464	24.11	0.36	0.84
Creatinine, mg/dL	1.85	1.86	1.74	1.58	0.09	0.65	0.36
BUN, mg/dL	15.71	15.39	14.10	12.01	1.12	0.02	0.74
Suckling piglets							
WBC, $10^3/\mu L$	15.22	14.81	16.49	16.34	1.26	0.65	0.27
RBC, $10^6/\mu L$	6.30	6.61	6.32	6.79	0.26	0.74	0.37
Lymphocyte, %	23.49	27.00	36.89	27.90	7.09	0.85	0.54
IgG, mg/dL	551	562	571	581	4.99	0.03	0.44
Creatinine, mg/dL	1.86	1.80	1.76	1.75	0.08	0.85	0.63
BUN, mg/dL	16.79	15.91	13.50	12.60	1.08	0.04	0.13

[1] Blood samples were randomly collected from 12 sows per treatment and two piglets per sow, CON, basal diet; BR1, CON+0.5 g/kg BR; BR2, CON+1 g/kg BR; BR3, CON+2 g/kg BR.
[2] Pooled standard error of mean.

Table 6
Effect of bromelain-C.3.4.22.32 supplementation on colostrum and milk contents in lactating sows[1].

Items	CON	BR1	BR2	BR3	SEM[2]	*P* value	
						Linear	*Quadratic*
Colostrum							
Lactose, %	3.22	3.28	3.21	3.23	0.06	0.86	0.73
Protein, %	14.33	14.25	14.16	14.81	0.43	0.48	0.46
Fat, %	5.71	5.73	5.93	5.92	0.09	0.04	0.66
Solid, %	24.50	23.95	24.75	23.88	0.61	0.71	0.15
SCC	342,864	281,611	263,243	260,260	22,502	0.01	0.19
Milk							
Lactose, %	4.97	4.85	4.94	4.93	0.07	0.88	0.70
Protein, %	5.01	5.31	5.32	5.47	0.11	0.04	0.15
Fat, %	7.13	7.19	7.04	6.91	0.25	0.45	0.65
Solid, %	19.18	18.64	20.25	18.85	0.52	0.83	0.36
SCC	133,318	119,336	98,293	94 189	8028	< 0.01	0.17

[1] Milk and colostrum samples were randomly collected from 12 sows per treatment, CON, basal diet; BR1, CON+0.5 g/kg BR; BR2, CON+1 g/kg BR; BR3, CON+2 g/kg BR.
[2] Pooled standard error of mean.

linearly higher serum IgG ($P=0.03$). No significant differences were observed in the other characteristics (WBC, RBC and creatinine) among treatments in sows and piglets ($P>0.05$). Sows fed the CON diet had linearly increased colostrum and milk SCC ($P=0.01$ and <0.01, respectively; Table 6). In contrast, milk protein was increased in sows fed the BR3 diet (linear, $P=0.04$).

4. Discussion

In the present study, the reproductive performance and BW change of sows were not affected by bromelain supplementation. However, the piglets suckling sows fed with BR had linearly improved growth performance. This observation is possibly related to the increased serum IgG concentrations in piglets suckling sows fed the BR diets, because the improved immunity may have positively effects on growth performance (Wheeler et al., 2001). Moreover, ADG and immunity of suckling piglets can also be affected by higher milk production, milk constituents, age, conformation, management, feeding, health status and other factors (Johansen et al., 2004). Indeed, using the equation by Noblet and Etienne (1989) to estimate milk production showed that the sows fed the CON, BR1, BR2 and BR3 diets produced on average 710, 763,762 and 739 g/piglet/d of milk over the entire lactation period, respectively. In addition, colostrum and milk protein from sows fed the BR-containing diets had linearly increased, suggesting the more muscle development and body protein synthesis in suckling piglets (Zhao and Lacasse, 2008; Etienne and Noblet, 1993).

The gastrointestinal and lymphoid systems are the largest immunologically competent organs in the body, and it has been suggested that the development and composition of the indigenous microflora are the principal factors influencing maturation and optimal development of these systems (Cho and Kim, 2014). Moreover, *E. coli* are shed by clinically healthy animals and may cause a drop in the growth performance and diarrhea in domesticated animals (Nagy and Fekete, 1999). Therefore, fecal *E. coli* and *Lactobacillus* counts were investigated herein and we observed that lactating sows fed BR supplemented diets had both linearly and quadratically reduced *E. coli* populations. Evidence has suggested that bromelain counteracts some of the effects of certain intestinal pathogens such as *Vibrio cholera* and *E. coli* (Mynott et al., 1996; Spitzer et al., 2014) by interacting with intestinal

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secretory signaling pathways (Mynott et al., 1996, 1997; Spitzer et al., 2014), leading to anti-adhesion effects which prevent the bacteria from attaching to specific glycoprotein receptors located on the intestinal mucosa through proteolytic modification of the receptor attachment sites (Chandler and Mynott, 1998; Mynott et al., 1999). In addition, feeding lactating sows a diet supplemented with BR linearly increased *Lactobacillus* counts in sows. *Lactobacillus* produces broad-spectrum bacteriocins which also allow the elimination of various enteropathogens such as *Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica*, *Salmonella* and *E. coli,* resulting in dramatic alteration of the gut microbiota (Jacobsen et al., 1999). To the best of our knowledge, no research has been done to evaluate the effects of bromelain and/or its chemical compounds on fecal microflora. Thus, more research is needed to understand the mechanism of BR influence on the fecal microflora in lactating sows.

In the present study, supplementation with BR3 linearly increased the apparent total tract digestibility of N in sows, which is consistent with results of others showing increased digestibility of nutrients due to feeding diets containing BR to rats, chickens and pigs (Chandler and Spicer, 1991; Maurer, 2001). Bromelain is known to digest protein because it contains different kind of enzymes (Pavan et al., 2012), which may have been the reason for the higher N digestibility in the current study. Moreover, treatment with bromelain showed the ability to modulate the intestinal environment, which may be associated with the apparent improvement of nutrient digestibility observed herein.

Blood profiles are regularly checked to evaluate the physiological condition of animals. BUN is a known indicator of kidney health. Lactating sows fed the BR3 diet, as well as their suckling piglets, were observed to have reduced BUN, because of the improved N digestibility (Whang and Easter, 2000; Pavan et al., 2012) in the current study. When considering the immune system, lymphocytes and IgG are very important. Blood lymphocyte and IgG concentrations can be used as a parameter to reflect the humoral immune status of animals, due to their important roles in immune function (Zhao et al., 2013; Cho and Kim, 2014). Bromelain could modulate immune system function (Maurer, 2001), which may directly affect swine growth performance. Treatment of cells with bromelain causes decreased activation of CD4 (+) T cells and reduced expression of CD25 (Secor et al., 2009), which can explain the increased lymphocyte counts and immunity observed in lactating sows in the present study. Moreover, lactating sows fed the BR3 diet had increased milk protein, which could improve the IgG in the suckling piglets. Thus, BR supplementation can improve immunity in lactating sows and suckling piglets. Further research can be done to evaluate the effects on lymphocyte sub populations in addition to the present findings.

Regarding colostrum and milk content in the lactating sows, BR supplementation linearly reduced SCC while increasing milk protein, depending on the dosage levels. The mammary gland is made up of a remarkably sensitive tissue which has the capability of producing large volumes of secretion, milk, under normal or healthy conditions (Etienne and Noblet, 1993; Harrell et al., 1993). When bacteria enter the gland and establish an infection, inflammation is initiated, which is accompanied by an influx of white cells from the bloodstream, altered secretory function and changes in the volume and composition of secretion (Etienne and Noblet, 1993; Wang et al., 2008). Since cell numbers in milk are known to be closely associated with inflammation and udder health, the SCC was accepted as the international standard measurement of milk quality (Harrell et al., 1993; Wang et al., 2008). Jones et al. (1984) reported that SCC is directly related to milk yield, with low levels of SCC improving the yield (Lucey and Rowlands (1984)). Moreover, higher N digestibility can improve the synthesis of milk protein and consequent milk yield (Zhao and Lacasse, 2008), which can be illustrated by the present findings. Though no research has been done to determine the effects of BR on milk and colostrum contents, the present results suggest that treatment with bromelain can improve the protein in milk.

5. Conclusion

In conclusion, supplementation of BR in the diet of lactating sows had no effects on the indicators of reproductive performance, but improved the body weight, average daily gain and serum IgG concentration in suckling piglets. The feeding of diets with BR supplementation caused increase of the apparent total tract digestibility of nitrogen, the blood lymphocyte counts, and milk protein in the sows. In contrast, BR supplementation reduced blood BUN and SCC in the sows and suckling piglets.

6. Implication

Our studies suggested that the addition of BR in gestation and lactation diets could positively affect the performance of sows because of the increased nutrient digestibility, gut health and improved immune system. We also hypothesized the BR could be used as a promoter to improve the performance of suckling piglets through increased colostrum and milk compositions during lactation. Moreover, these results imply that BR may have a role to play in antibiotic-free feeding programs in sows and piglets farming.

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, 'Dietary bromelain-C.3.4.22.32 supplementation improves performance and gut health in sows and piglets'.

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