Indian Journal of Animal Sciences **85** (2): 195–201, February 2015/Article

Evaluation of lactulose on growth performance, nutrient digestibility, hematology, fecal microbial shedding and fecal noxious gas emission in the growing-finishing pigs

M M HOSSAIN[1], M BEGUM[2] and I H KIM[3]

Dankook University, No.29 Anseodong, Cheonan, Choongnam, South Korea 330 714

Received: 22 June 2014; Accepted: 22 November 2014

ABSTRACT

This study was conducted to evaluate the effects of lactulose on growth performance, nutrient digestibility, blood characteristics, fecal microbial shedding and fecal noxious gas emission in the growing- finishing pigs. Pigs (80) with the average BW of 20.82 (SD = 3.27 kg) and age of 10 weeks were allotted to 4 dietary treatments with 4 replicate pens / treatment and 5 pigs (3 gilts and 2 borrows) / pen. Dietary treatments included: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. Pigs fed L10 and L15 diets had greater average daily gain (ADG) throughout the overall period when compared with CON diet. The N and DM digestibility were greater for L10 and L15 treatments than CON treatment at wk12 and 18, respectively. Pigs on L15 supplementation treatment had increased lymphocytes and IgG than CON treatment at 12 wk. WBC and RBC counts were improved in L15 treatment at the end of study. Pigs fed with L10 and L15 diets increased fecal *Lactobacillus* and reduced *E. coli* count compared with CON diet. Fecal NH_3 gas emission was lower in the lactulose supplemented treatments compared with CON treatment on d 3 and 5, respectively. Moreover, fecal H_2S was reduced in L10 and L15 diets compared with CON diet on d 5. In conclusion, L10 and L15 supplementation improved growth performance, nutrient digestibility, blood parameters and fecal *Lactobacillus* count but reduced fecal *E. coli*, NH_3 and H_2S gas emission in growing-finishing pigs.

Key words: Blood parameters, *Escherichia coli*, Growing-finishing pigs, Growth performance, Lactulose

Indiscriminate use of antibiotics in animal diets resulted in severe problems like resistance of pathogen to antibiotics, accumulation of antibiotic residues in animal products and environment, imbalance of normal microflora, and reduction in beneficial intestinal microflora (Hinton *et al.* 1986, Barton 2000).This results into severe restriction or total ban on the use of antibiotics in animal industry in many countries which leads to a growing interest in the use of one of the alternative to antibiotics, is non-digestible oligosaccharides such as xylo-oligosaccharides, lactulose, isomalto-oligosaccharides, gluco-oligosaccharides, galacto-oligosaccharides, fructo-oligosaccharides, oligofructose (Andrieux 2001, Banner *et al.* 2004). Lactulose (4-O-ß -D-galactopyranosyl-D-fructose) is metabolized in the colon by the saccharolytic microflora (Bird *et al.* 1990). It can be used to affect the intestinal microflora by stimulating the growth of *Lactobacillus* in the gut and could be fermented by *lactobacilli*, *bifidobacteria* and other bacteria species (*Clostridium perfringens*, *E. coli* or *Bacteroides*) (Mitsuoka *et al.*1987, Fleige *et al.* 2007).

Previous studies stated that lactulose elicits a prebiotic effect (increased counts of *Bifidobacteria* and *Lactobacillus*) in pigs (Konstantinov *et al.* 2004). Dietary supplementation of prebiotics to the diets of animal led to improved performance through improving gut microbiota and stimulation of immune system (Spring *et al.* 2000, Xu *et al.* 2003, Pelicano *et al.*2004). Sutton and Patterson (1996) confirmed the effect of lactulose on reducing fermentative diarrhea by reducing *E. coli* during the pig rearing period. Furthermore, lactulose could improve body weight gain, apparent metabolizable nitrogen as well as reduced ammonia and acetic acid gas emission in broilers (Cho and Kim 2013). To the best of our knowledge, effects of lactulose on growing-finishing pigs have not been investigated yet. Thus, objective of the experiment was to evaluate the hypothesis that performance, immunity, fecal microbial shedding and fecal noxious gas emission would be positively influenced by the lactulose supplementation to basal diets in growing-finishing pigs.

MATERIALS AND METHODS

Animals and experimental design: A total of 80 (Landrace × Yorkshire) × Duroc pigs with an initial body

Present address: [1,2]PhD Research Fellow (mhanbau @gmail.com, mostarina_bizlee@yahoo.com), [3]Professor (inhokim@dankook.ac.kr), Department of Animal Resource and Science, Dankook University, No.29 Anseodong, Cheonan, Choongnam.

weight (BW) of 20.82±3.27 kg (age of 10 weeks) were utilized in 18-week experiment. The pigs were allotted to 1 of 4 treatments in a randomized complete block design. There were 4 replicated pens / treatment with 5 pigs (3 gilts and 2 borrows) / pen. Dietary treatments included: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. The diets were formulated to meet or exceed the nutrient requirement recommendations of the NRC (2012) (Table 1). Lactulose as powder form was procured and added to the basal diet at a percentage of 0.1 and 0.15. The pigs were housed in an environmentally controlled nursery facility with slatted plastic flooring and a mechanical ventilation system. Each pen was equipped with a one-sided self-feeder and a nipple drinker to allow the pigs *ad lib.* access to feed and water throughout the experimental period. All animals received human care as outlined in the Guide for the Care and Use of Experimental Animals (Dankook University, South Korea, Animal Care Committee).

Chemical analysis: Feed samples were ground to pass through a 1–mm screen, and then analyzed for DM (method 934.01; AOAC 2000), N (method 968.06; AOAC 2000), Ca (method 984.01; AOAC 1995), and P (method 965.17; AOAC 1995). Individual AA composition was measured using an AA analyzer after a 24 h hydrolysis in HCl (Spackman *et al.* 1958). Nitrogen was determined and CP was calculated as N × 6.25. Gross energy was analyzed by oxygen bomb calorimeter.

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

Items	Growing (0–6 weeks)	Finishing (6–18 weeks)
Ingredients, %		
Corn	70.22	76.50
Soybean meal, 46% CP	22.07	16.40
Corn gluten meal	3.00	3.00
Tallow	2.76	2.31
Limestone	0.70	0.74
Salt	0.20	0.20
Tricalcium phosphate	0.65	0.49
Lysine	0.05	0.01
Ethoxyquin	0.05	0.05
Vitamin premix[1]	0.20	0.20
Mineral premix[2]	0.10	0.10
Calculated composition, %		
ME, kcal/kg	3,400	3,400
CP	17.0	15.0
Lys	0.90	0.70
Ca	0.55	0.50
Total P	0.50	0.45
Analyzed composition, %		
CP	16.99	15.02
Lys	0.90	0.71
Ca	0.56	0.48
Total P	0.51	0.45

[1]Provided per kg diet: 20 000 IU of vitamin A; 4000 IU of vitamin D3; 80 IU of vitamin E; 16 mg of vitamin K3; 4mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B12; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin. [2]Provided per kg diet: 140 mg of Cu; 179 mg of Zn; 12.5 mg of Mn; 0.5 mg of I; 0.25 mg of Co and 0.4 mg of Se.

Sampling and measurements: Individual pig BW and feed refusals were recorded on day 42, 84 and 126 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 (Fenton and Fenton 1979) during day 36 to 42, day 78 to 84 and day 120 to 126. Fresh fecal grab samples were randomly collected from 1 gilt and 1 barrow in each pen via rectal massage. Representative samples were stored at –20°C until analyzed. Before chemical analysis, the fecal samples were thawed and dried at 60°C for 72 h in a forced-air oven,after which they were finely ground to a size that could pass through a 1-mm screen . All feed and fecal samples were then analyzed for DM, N and GE as described earlier. Chromium was analyzed using UV absorption spectrophotometry. The apparent total tract digestibility (ATTD) was then calculated using the following formula: digestibility (%) = $\{1 - [(Nf \times Cd)/(Nd \times Cf)]\} \times 100$, where, Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM) and Cf = chromium concentration in feces (% DM).

For the blood profiles, 2 pigs from each pen (1 gilt and 1 barrow) were randomly selected and blood samples were collected via anterior vena cava puncture on day 42, 84 and 126. Half of the sample was transferred into either a vacuum (clot activator with gel) or a 5 ml K_3EDTA vacuum tube and stored at –4°C. The white blood cell (WBC), red blood cell (RBC) and lymphocyte counts in the whole blood were determined using an automatic blood analyser. The serum was separated by centrifugation for 30 min at 2000×*g* at 4°C, the aliquot was stored at –4°C (within 24 h) until determination of the serum immunoglobulin G (IgG) concentration using an automatic biochemistry blood analyzer.

At the end of experiment, fecal samples were collected directly by massaging the rectum of 2 pigs randomly selected from each pen (1 gilt and 1 barrow) and pooled and placed on ice for transportation to the laboratory, where microbial analysis were immediately carried out according to Zhao *et al.* (2013). Composite fecal sample (1 g) was diluted with 9 mL of 1% peptone broth and homogenized. Viable counts of bacteria in the fecal samples were conducted by plating serial 10–fold dilutions (in 1% peptone solution) onto lactobacilli medium III agar plates (Medium 638), MacConkey agar plates, Perfringens agar base, and Wilkins-Chalgrenagar supplemented with glacial acetic acid (1 mL/L) and mupirocin (100 mg/L) extracted from antimicrobial discs (Rada *et al.*1999) to isolate the *Lactobacillus*, *E. coli*, *C. perfringens*, and *Bifidobacteria*, respectively. The lactobacilli medium III agar plates were incubated for 48 h at 37°C under anaerobic conditions. The MacConkey and Perfringens agar plates were incubated for

24 h at 37°C and the Wilkins-Chalgren agar plates were incubated for 72 h at 37°C. The microflora colonies were counted immediately after removal from the incubator. Concentration of microflora was finally expressed as $\log_{10}$ colony-forming units per gram of intestinal content.

For analysis of the fecal NH_3, H_2S and total mercaptan, 300g of fresh feces were randomly collected from 2 pigs (1 gilt and 1 barrow) in each pen on the last 2 days of the experiment according to the method described by Yan and Kim (2013). The total fresh sampled feces were then stored in 2.6-L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were allowed to ferment for 1 day at room temperature (25°C). After the fermentation, a gas sampling pump was utilized for gas ($NH_3$and H_2S) detection. After collection, the boxes were re-sealed with adhesive plaster to measure the fecal noxious content at days 3 and 5 as aforementioned. Prior to measurement, the fecal samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the fecal sample and to homogenize the samples.

Statistical analysis: In the current study, all data were subjected to statistical analysis via a randomized complete block design using the GLM procedures of SAS/STAT®9.2(SAS Institute 2008), with a pen serving as the experimental unit. Duncan's multiple test was used to compare the means of the treatments. Before conducting statistical analysis of the *Lactobacillus, E. coli, Clostridium perfringens* and *Bifidobacteria* counts, the value was transformed logarithmically. Variability in the data was expressed as the standard error of means (SEM) and a $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Growth performance: Growth rate of the pig is one of the key indicators affecting the profitability of pig meat production. Pigs fed LAC15 and LAC10 diets had greater (801g, 793g vs. 778g; $P<0.05$) ADG than those fed CON diet during the whole experiment (Table 2). No significant difference was observed in other parameters throughout the whole experiment. Currently, there is little information on the influence of feeding lactulose to growing-finishing pigs. However, there have been some studies investigating other non-digestible oligosaccharides. In an experiment, growth performance was improved when young pigs were fed diets supplemented with fructooligosaccharides (FOS) (Mul and Perry 1994). Krueger *et al.* (2002) stated that lactulose supplementation in diets for periparturient sows and weaning pigs improves ADG. Moreover, Cho and Kim (2013) demonstrated that supplementation of diets with 2 g/kg lactulose improved ADG in broiler chickens. Zhao *et al.* (2013) also stated that fructan supplementation improved ADG in finishing pigs. Likewise, Hu and Wang (2001) demonstrated that supplementation with 0.50 and 0.75% FOS improved ADG (9.7 and 10.7%, respectively) in finishing pigs. It was suggested that the improved growth performance observed in response to dietary lactulose supplementation occurs through an increased nutrient digestibility and improved fecal microbiota (Tang *et al.* 2005, Cho and Kim 2013, Li and Kim 2013). On the other hand, several studies on FOS showed little or no effect on growth performance of pigs (Farnworth *et al.* 1992, Orban *et al.* 1997, Houdijk *et al.* 1998). These equivocal results may be a consequence of different types of prebiotics and the doses used, differences in the genetic composition, age or sex of pigs and other environmental or management factors.

Table 2. Effect of lactulose supplementation on growth performance in growing-finishing pigs[1]

Items	CON	L05	L10	L15	SEM[2]
0 – 6 week					
ADG, g	704	730	734	749	14.00
ADFI, g	1,648	1,655	1,623	1,640	31.00
G:F	0.428	0.442	0.452	0.456	0.011
6 – 12 week					
ADG, g	815	819	818	819	3.00
ADFI, g	2,136	2,146	2,218	2,219	46.00
G:F	0.382	0.382	0.369	0.370	0.008
12 – 18 week					
ADG, g	816	822	828	835	7.00
ADFI, g	2,868	2,868	2,855	2,824	25.00
G:F	0.285	0.287	0.290	0.296	0.004
Overall					
ADG, g	778[b]	790[ab]	793[a]	801[a]	4.00
ADFI, g	2,217	2,223	2,232	2,228	22.00
G:F	0.351	0.355	0.356	0.360	0.004

[1]Abbreviation: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. [2]Standard error of mean. [a,b]Means in the same row with different superscripts differ ($P<0.05$).

Apparent total tract digestibility of nutrients: The benefits of short chain fatty acids to the host organism include the use of acetic acid as an energy source for peripheral tissues, utilization of propionic acid by the liver for gluconeogenesis, and utilization of butyric acid by colonocytes as a primary energy fuel (Choct and Kocher 2000, Montagne *et al.*2003), which would increase nutrient digestibility. Prebiotic can be fermented by gut microbiota, especially *Lactobacillus* and *Bifidobacterium*, which, in turn, increased mineral absorption (Abrams *et al.* 2005) and height of intestinal villi (Crittenden and Playne 1996). Also, they can produce and release enzymes, thus increasing the activity of intestine digestive enzyme in pigs and broilers (Ghadban 2002, Boguslawska-Tryk *et al.* 2012). In this study, pigs fed with L10 and L15 diets showed higher (75.86, 77.36 vs 72.32; $P<0.05$) N digestibility (%) compared to those fed with CON at 12 weeks (Table 3). Moreover, pigs fed L10 and L15 diets led to a greater ($P<0.05$) DM digestibility compared to CON diet at 18 week. Previous studies have indicated that lactulose enhanced the performance of broliers (Cho and Kim 2013). Similarly, FOS has stimulatory effect on intestinal microbiota, which results in an increase in villi height and

Table 3. Effect of lactulose supplementation on nutrient digestibility in growing-finishing pigs[1]

Items, %	CON	L05	L10	L15	SEM[2]
6 week					
Dry matter	77.28	78.54	79.32	81.10	1.27
Nitrogen	72.60	73.88	74.82	74.88	1.26
Energy	78.18	79.32	80.10	80.68	1.44
12 week					
Dry matter	80.16	80.64	81.16	81.66	1.39
Nitrogen	72.32^{b}	75.34^{ab}	75.86^{a}	77.36^{a}	1.05
Energy	77.68	78.44	78.98	81.24	1.78
18 week					
Dry matter	76.34^{b}	79.62^{ab}	80.72^{a}	81.32^{a}	1.25
Nitrogen	73.24	73.54	75.72	76.26	1.29
Energy	76.14	78.68	78.78	80.82	1.57

[1]Abbreviation: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. [2]Standard error of mean. [a,b]Means in the same row with different superscripts differ (P<0.05).

Table 4. Effect of lactulose supplementation on blood profiles in growing-finishing pigs[1]

Items	CON	L05	L10	L15	SEM[2]
6 week					
WBC, 10^3/µl	15.03	15.01	13.73	15.88	0.95
RBC, 10^6/µl	6.59	6.81	6.56	6.90	0.11
Lymphocyte, %	55.2	56.6	55.4	57.8	1.41
IgG, mg/dL	773	763	712	698	32.00
12 week					
WBC, 10^3/µl	14.94	15.62	15.27	15.84	0.94
RBC, 10^6/µl	6.67	6.71	6.94	6.87	0.08
Lymphocyte, %	53.5^{b}	55.2^{ab}	57.6^{a}	57.3^{a}	0.91
IgG, mg/dL	688^{b}	770^{a}	690^{b}	783^{a}	25.00
18 week					
WBC, 10^3/µl	13.76^{b}	15.30^{ab}	15.31^{ab}	17.12^{a}	0.82
RBC, 10^6/µl	6.57^{b}	6.54^{b}	6.97^{ab}	7.08^{a}	0.14
Lymphocyte, %	57.2	54.4	57.6	57.4	1.50
IgG, mg/dL	720	727	676	717	32.00

[1]Abbreviation: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. [2]Standard error of mean.[a,b]Means in the same row with different superscripts differ (P<0.05).

crypt depth ratio at the mucosa of distal jejuna in growing pigs (Spencer *et al.*1997). Besides, FOS stimulates the activity of digestive enzymes (total protease, trypsin, and amylase) in the small intestinal contents of growing pigs (Xu *et al.* 2002) which corroborates the findings obtained in the present study.

Blood profiles: As blood profiles are routinely monitored to evaluate the sensitive response to various antibiotic alternatives, some blood measurements were evaluated to assess the effect of lactulose supplementation. Pigs fed with L15 and L10 diets showed higher lymphocyte value (57.3%, 57.6% vs. 53.5%; P<0.05) than that in CON treatments, whereas, L05 and L15 diets showed increased IgG than CON and L10 diets at 12 week (Table 4). Moreover, WBC

Table 5. Effect of lactulose supplementation on fecal microbiota in growing-finishing pigs[1]

Items, $^{log}_{10}$cfu/g	CON	L05	L10	L15	SEM[2]
Lactobacillus	6.78^{b}	6.84^{b}	7.52^{a}	7.61^{a}	0.01
E. coli	6.65^{a}	6.51^{ab}	5.86^{b}	5.85^{b}	0.05
Clostridium perfringens	7.71	7.65	7.65	7.60	0.28
Bifidobacteria	8.45	8.42	8.50	8.52	0.15

[1]Abbreviation: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. [2]Standard error of mean.[a,b]Means in the same row with different superscripts differ (P<0.05).

Table 6. Effect of lactulose supplementation on fecal noxious gas emission in growing-finishing pigs[1]

Items, ppm	CON	L05	L10	L15	SEM[2]
NH_3					
D 1	11.21	10.12	10.25	9.87	1.07
D 3	17.55^{a}	15.31^{b}	15.11^{b}	14.90^{b}	0.18
D 5	19.92^{a}	18.77^{b}	18.43^{b}	18.44^{b}	0.15
H_2S					
D 1	1.14	1.00	1.12	0.91	0.22
D 3	16.71	16.42	16.17	16.01	0.31
D 5	25.23^{a}	25.00^{a}	24.14^{b}	23.89^{b}	0.26
Total mercaptans					
D 1	12.15	12.34	12.15	12.01	0.23
D 3	22.54	22.11	22.05	21.74	0.47
D 5	29.14	29.15	29.03	29.20	0.31

[1]Abbreviation: CON, basal diet; L05, CON + 0.05% lactulose; L10, CON + 0.10% lactulose; L15, CON + 0.15% lactulose. [2]Standard error of mean. [a,b]Means in the same row with different superscripts differ (P<0.05).

and RBC count were higher in L15 compared with CON treatment (P<0.05). This finding indicates that lactulose may affect the intestinal ecosystem, thereby influencing the immunity of the body. According to Schumann (2002) most dietary lactulose is absorbed into the cardiovascular system and cause an immune response. Our previous study observed that prebiotic supplementation increased WBC and lymphocyte in growing pigs (Li and Kim 2013) whereas Zhou *et al.* (2011) stated that chito-oligosaccharides decreased the lymphocyte concentration in the weanling pigs. The results varied may be due to the different type of prebiotics, animal and environmental conditions employed during the experiment.

Fecal microbial shedding: Pigs fed L15 and L10 supplemental diets showed higher (7.61, 7.52 vs. 6.78, 6.84; P<0.05) *Lactobacillus* (log_{10}cfu/g) than CON and L05 treatments and lower *E. coli* (log_{10}cfu/g) count (5.85, 5.86 vs. 6.65; P<0.05) than that in CON treatments at the end of experiment (Table 5). Lactulose is a NDO which cannot be hydrolyzed by digestive enzymes but fermented to short chain fatty acids, such as acetate, propionate and butyrate in the lower gut (Klaschik *et al.* 2003). The process of VFA formation leads to acidification and reduced pH of the ileal environment, and promotes growth of beneficial types of

bacteria, including *Bifidobacterium, Eubacterium*, and *Lactobacillus* and suppressed *E. coli* counting the large bowel (Boguslawska-Tryk *et al.* 2012), which could be used to explain the increased *Lactobacillus* and decreased *E. coli* in this study. Cho and Kim (2013) have suggested that lactulose inclusion in the basal diet can reduce the *E. coli* and improve the *Lactobacillus* count in the broilers. Moreover, recently some researchers also reported that addition of NDO enhanced the growth of *Lactobacillus* but inhibited growth of *E. coli* in the small intestine and proximal colon of growing and finishing pigs (Xu *et al*. 2002; Zhao *et al.* 2013, Li and Kim 2013). In our study, fecal *E. coli* concentration was reduced, while *Lactobacillus* concentration was increased by lactulose supplementation in the growing-finishing pig diet. As we know, lactulose can enhance the intestinal *Lactobacillus* and reduce *E.coli* population, and the fecal microbial flora population is similar with that in the intestine (Zhao *et al.* 2013). In our study, no significant differences were observed in *Clostridium perfringens* and *Bifidobacteria* counts. This may be due to the different doses applied, sensitivity towards additives, species and environmental factors.

Fecal noxious gas emission: Zahn *et al.* (1997) suggested that NH_3 and H_2S are the main components of pig manure that contribute to the air pollution. High concentrations of NH_3 or H_2S can cause hazard effects to humans and animals (Drummond *et al.* 1980, Zhang and Kim 2014). It was suggested that fecal ammonia gas emission is related to nutrient utilization and the intestinal microbial ecosystem (Ferket *et al.* 2002). Therefore, a great emphasis was placed on the strategies to reduce the emission of those and other pollutants in pigs. In this study, pigs fed lactulose supplementation had decreased ($P<0.05$) NH_3 emission in the feces at days 3 and 5 (Table 6). The inclusion of L10 and L15 treatments also decreased ($P<0.05$) H_2S concentration compared with CON and L05 treatments at day 5. In agreement with our results, Cho and Kim (2013) stated that lactulose supplementation reduced NH_3 and H_2S gas emission in broilers. Moreover, fructo oligosaccharides supplementation in finishing pigs also confirmed the similar results in our previous study (Zhao *et al.* 2013). It is well suggested that the N from ammonia is generally derived from fermentation of unabsorbed N entering the large intestine (Canh *et al.* 1998, Song *et al.* 2012). Moreover, Panesar and Kumari (2011) stated that lactulose is metabolized by lactic acid bacteria that live in the hind gut. These bacteria produce short chain fatty acids and carbon dioxide which leads to a low pH in gut contents. It has been shown that fecal pH greatly affects NH_3 emission (Hoeksma *et al.* 1993) and that lower pH results in decreased NH_3 and H_2S emissions. Therefore, in the present study, the reason for the decreased NH_3 and H_2S emission from pigs fed lactulose is likely to be caused by the apparently increased nutrient digestibility and improved microbial ecology compared with control group.

In conclusion, our results stated that lactulose supplementation at a level of 0.1% and/or 0.15% can increase growth performance, apparent total tract digestibility of N and DM, blood parameters, fecal *Lactobacillus* content and decrease fecal *E.coli*, fecal NH_3 and H_2S gas emission, when compared with the CON treatment. Future investigations are necessary to confirm the effect of lactulose on those parameters in growing-finishing pigs.

REFERENCES

Abrams S A, Griffin I J, Hawthorne K M, Liang L, Gunn S K, Darlington G and Ellis K J. 2005. A combination of prebiotic short-and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *American Journal of Clinical Nutrition* **82**: 471–76.

Andrieux C. 2001. Prebiotics and Health. *Post-Antibiotics Era of Animal Nutrition*. The 3rd Techno World Meeting, February 23, 2001, Published by CTC-BIO Co, Korea.

AOAC. 1995. *Official method of analysis*. 16th edn. Association of Official Analytical Chemists, Washington, DC.

AOAC. 2000. *Official method of analysis*. 17th edn. Association of Official Analytical Chemists, Gaithersburg, MD.

Banner G R, Bohmer B M and Julia W E. 2004. Investigation on the precaecal and faecal digestibility of lactulose and inulin and their influence on nutrient digestibility and microbial characteristics. *Archives of Animal Nutrition* **58**: 353–66.

Barton M D. 2000. Antibiotic use in animal feed and its impact on human health. *Nutrition Research Reviews* **13**: 279–99.

Bird S P, Hewitt D, Ratcliffe B and Gurr M I. 1990. Effects of lactulose and lactitol on protein digestion and metabolism in conventional and germ free animal models: relevance of the results to their use in the treatment of portosystemic encephalopathy. *Gut* **31**: 1403–06.

Boguslawska-Tryk M, Piotrowska A and Burlikowska K. 2012. Dietary fructans and their potential beneficial influence on health and performance parameters in broiler chickens. *Journal of Central European Agriculture* **13**: 272–91.

Canh T T, Sutton A L, Aarnink A J, Verstegen M W, Schrama J W and Bakker G C. 1998. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. *Journal of Animal Science* **76**: 1887–95.

Cho J H and Kim I H. 2014. Effects of lactulose supplementation on performance, blood profiles, excreta microbial shedding of *Lactobacillus* and *Escherichia coli*, relative organ weight and excreta noxious gas contents in broilers. *Journal of Animal Physiology and Animal Nutrition* **98**: 424–30.

Choct M and Kocher A. 2000. Non-starch carbohydrates: Digestion and its secondary effects in monogastric. *Proceedings of the Nutrition Society of Australia* **24**: 31–38.

Crittenden R G and Playne M J. 1996. Production, properties, and applications of food-grade oligosaccharides. *Trends in Food Science and Technology* **7**: 353–61.

Drummond J G, Curtis S E, Simon J and Norton H W. 1980. Effects of aerial ammonia on growth and health of young pigs. *Journal of Animal Science* **50**: 1085–91.

Farnworth E R, Modler H W, Jones D J, Cave N, Yamazaki H and Rao A V. 1992. Feeding Jerusalemartichoke flour rich in fructooligosaccharides to weanling pigs. *Canadian Journal of Animal Science* **72**: 977–80.

Fenton T W and Fenton M. 1979. An improved method for chromic oxide determination in feed and feces. *Canadian Journal of Animal Science* **59**: 631–34.

Ferket P R, van Heugten E, van Kempen T A T G and Angel R.

2002. Nutritional strategies to reduce environmental emissions from nonruminants. *Journal of Animal Science* **80**: E168– 328 E182.

Fleige S, Preißinger W, Meyer H H D and Pfaffl M W. 2007. Effect of lactulose on growth performance and intestinal morphology of pre-ruminant calves using a milk replacer containing *Enterococcus faecium*. *Animal* **1**: 367–73.

Ghadban G S. 2002. Probiotics in broiler production- A review. *Archiv Fur Geflugelkunde* **66**: 49–58.

Hinton M, Kaukas A and Linton A H. 1986. The ecology of drug resistance in enteric bacteria. *Journal of Applied Bacteriology Symposium Supplement* **15**: 77–92.

Hoeksma P, Verdoes N and Monteny G J. 1993. Two options for manure treatment to reduce ammonia emission from pig housing. *Nitrogen Flow in Pig Production and Environmental Consequences.* pp. 301–306. (Eds) Verstegen M W A, den Hartog L A, van Kempen G J M and Metz J H M. EAAP Publ, Wageningen, the Netherlands.

Houdijk J, Bosch M, Verstegen M and Tamminga S. 1997. Type and level of dietary oligosaccharides change volatile fatty acid composition in ilealchyme of young pigs. *Proceedings of 7th International Symposium of Digestibility and Physiology of Pigs*. pp. 475–78. Saint Malo, France.

Hu C H and Wang Y M. 2001. Effects of supplemental fructo oligosaccharide on growth performance, intestinal microflora and digestive enzymes of finishing pigs. *Journal of Wuxi University of Light Industry* **20**: 568–72.

Klaschik E, Nauck F and Ostgate C. 2003. Constipation - modern laxative therapy. *Supportive Care in Cancer* 11: 679–85.

Konstantinov S R, Awati A, Smidt H, Williams B A, Akkermans A D and de Vos W M. 2004. Specific response of a novel and abundant Lactobacillus amylovorus- like phylotype to dietary prebiotics in the guts of weaning piglets. *Applied and Environmental Microbiology* **70**: 3821–30.

Krueger M, Schroedl W, Isik K, Lange W and Hagemann L. 2002. Effects of lactulose on the intestinal microflora of periparturient sows and their piglets. *European Journal of Nutrition* **41**: 26– 31.

Li J and Kim I H. 2013. Effects of levan-type fructan supplementation on growth performance, digestibility,blood profile, fecal microbiota, and immune responses after lipopolysaccharide challenge in growing pigs. *Journal of Animal Science* **91**: 5336–43.

Mitsuoka T, Hidaka H and Eida T. 1987. Effect of fructo-oligosaccharides on intestinal microflora. *Die Nahrung* **31**: 426–36.

Montagne L, Pluske J R and Hampson D J. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology* **108**: 95–117.

Mul A J and Perry F G. 1994. The role of fructo-oligosaccharides in animal nutrition. In: P. C. Garnsworthy, J. H. Pemberton, and R. G. Cole, editors, Recent advances in animal nutrition.Nottingham University Press, Loughborough, UK. pp. 57–79.

NRC. 2012. *Nutrient Requirement of Swine*. 11th edn. National Research Council, Academy Press, Washington, DC.

Orban J I, Patterson J A, Adeola O, Sutton A L and Richards G N. 1997. Growth performance and intestinal microbial populations of growing pigs fed diets containing sucrose thermal oligosaccharide caramel. *Journal of Animal Science* **75**: 170–75.

Panesar P S andKumari S. 2011. Lactulose: Production, purification and potential applications. *Biotechnology Advances* **29**: 940–48.

Pelicano E R L, De Souza P A, De Souza H B A, Leonel F R, Zeola N M B L and Boiago M M. 2004. *Productive Traits of Broiler Chickens Fed Diets Containing Different Growth Promoters*. Asian Technology Conference, Brazil.p. 18.

Rada V, Sirotek K and Petr J. 1999. Evaluation of selective media for *bifidobacteria*in poultry and rabbit caecal samples. *Journal of Veterinary Medicine Series B* **46**: 369–73.

SAS 2008.SAS User's Guide. SAS Institute Inc., Cary, NC, USA.

Schumann C. 2002. Medical, nutritional and technological properties of lactulose. An update. *European Journal of Nutrition* **41**: 17–25.

Song Z T, Dong X F, Tong J M and Wang Z H. 2012. Effects of waste vinegar residue on nutrient digestibility and nitrogen balance in laying hens. *Livestock Science* **150**: 67–73.

Spackman D H, Stein W H and Moore S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Analytic Chemistry* **30**: 1190–96.

Spencer J D, Touchette K J, Liu H, Allee G L, Newcomb M D, Kerley M S and Pace L W. 1997. Effect of spray-dried plasma and fructooligosaccharide on nursery performance and small intestinal morphology of weaned pigs. *Journal of Animal Science* **75**: 99.

Spring P, Wenk C, Dawson K A and Newman K E. 2000. The effects of dietary mannan oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the caeca of Salmonella- challenged broiler chicks. *Poultry Science* **79**: 205–11.

Sutton A L and Patterson J A. 1996. Effects of dietary carbohydrates and organic acid additions on pathogenic *E. coli* and other microorganisms in the weanling pig. *Proceedings of the 5th International Symposium on Animal Nutrition,* Kaposvár, Hungary, 31–61. Kaposvár, Hungary: Pannon Agricultural University.

Tang Z R, Yin Y L, Nyachoti C M, Huang R L, Li T J, Yang C B, Yang X J, Peng J, Qi D S, Xing J J, Sun Z H and Fan M Z. 2005. Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. *Domestic Animal Endocrinology* **28**: 430–41.

Xu Z R, Hu C H, Xia M S, Zhan X A and Wang M Q. 2003. Effects of dietary fructo oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Journal of Animal Science* **82**: 1030–36.

Xu Z R, Zou X T, Hu C H, Xia M S, Zhan X A and Wang M Q. 2002. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of growing pigs. *Asian-Australasian Journal of Animal Science* **15**: 1784–89.

Yan L and Kim I H. 2013. Effect of probiotics supplementation in diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, faecal microbial population and faecal noxious gas content in growing pigs. *Journal of Applied Animal Research* **41**: 23–28.

Zahn J A, Hatfield J L, Do Y S, Dispirito A A, Laird D A and Pfeiffer R L. 1997. Characterization of volatile organic emissions and wastes from a swine production facility. *Journal of Environmental Quality* **26**: 1687–96.

Zhang Z F and Kim I H. 2014. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta

odor contents in broilers. *Poultry Science* **93**: 364–70.
Zhao P Y, Wang J P and Kim I H. 2013. Evaluation of dietary fructan supplementation on growth performance, nutrient digestibility, meat quality, fecal microbial flora, and fecal noxious gas emission in finishing pigs. *Journal of Animal Science* **91**: 5280–86.
Zhou T X, Cho J H and Kim I H. 2011. Effects of supplementation of chito-oligosaccharide on the growth performance, nutrient digestibility, blood characteristics and appearance of diarrhea in weanling pigs. *Livestock Science* **144**: 263–68.