Korean Journal for Food Science of Animal Resources

Korean J. Food Sci. An. 2018 April 38(2):403~416
DOI https://doi.org/10.5851/kosfa.2018.38.2.403

pISSN : 1225-8563 eISSN : 2234-246X
www.kosfa.or.kr

ARTICLE

# Influence of Probiotics-Friendly Pig Production on Meat Quality and Physicochemical Characteristics

Sung Yong Chang[1,†], Shah Ahmed Belal[2,†], Da Rae Kang[2], Yang Il Choi[3], Young Hoon Kim[1], Ho Sung Choe[2], Jae Young Heo[4,*], and Kawn Seob Shim[2,*]

[1]Department of Animal Science, Chonbuk National University, Jeonju 54896, Korea
[2]Department of Animal Biotechnology, Chonbuk National University, Jeonju 54896, Korea
[3]Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea
[4]International Agricultural Development and Cooperation Center, Chonbuk National University, Jeonju 54896, Korea

OPEN ACCESS

| Received | February 5, 2018 |
|---|---|
| Revised | March 28, 2018 |
| Accepted | March 29, 2018 |

***Corresponding author** : Kwan Seob Shim
Department of Animal Biotechnology, Chonbuk National University, Jeonju 54896, Korea
Tel: +82-63-270-2609
Fax: +82-63-270-2614
E-mail: ksshim@jbnu.ac.kr

Jae Young Heo
International Agricultural Development and Cooperation Center, Chonbuk National University, Jeonju 54896, Korea
Tel: +82-63-270-5925
Fax: +82-63-270-5927
E-mail: jyheobio@gmail.com

† These authors contributed equally to this work

**Abstract** In this study, the dietary effects of probiotics with a liquid application system on meat quality and physicochemical characteristics of pigs were evaluated. A total of 80 Landrace×Yorkshire×Duroc (LYD) 3-way crossbred pigs (average age 175±5 d) were assigned to a conventional farm and a probiotics farm equipped with a liquid probiotics application system (40 pigs in each farm). The two treatments were: CON (diet without probiotics) and PRO (diet with probiotics). Dietary probiotics decreased shear force in the *longissimus* muscle compared to the control group ($p<0.05$). The treatment diet did not affect backfat thickness, carcass weight, meat color, cooking loss, water holding capacity (WHC), and drip loss. Dietary probiotics significantly reduced ash, salinity, and pH (at 5 and 15 d) ($p<0.05$). There was no significant effect on thiobarbituric acid reactive substance (TBARS) values. Polyunsaturated fatty acid (PUFA) and omega fatty acids (ω3 and ω6) were significantly ($p<0.05$) higher in the PRO group, whereas monounsaturated fatty acid (MUFA) was decreased. The free amino acid composition, serine, lysine, histidine, and arginine levels were significantly lower in the PRO than in the control group. The treatment group exhibited higher nucleotide compounds (hypoxanthine, inosine, GMP, IMP) than the controls. Also, levels of ascorbic acid and thiamin were significantly different ($p<0.05$), while minerals were not significantly different between the groups. In conclusion, feeding of probiotics had effects on shear force, ash, salinity, pH, PUFA, and some amino acids which related to taste and flavor without any negative effects on the pigs' carcass traits.

**Keywords** probiotics, meat quality, chemical composition, crossbred pigs

## Introduction

Increasing awareness about healthy food has led to increasing interests on natural foodstuffs and nutraceuticals such as probiotics (Park et al., 2016). Probiotics are live

© Korean Society for Food Science of Animal Resources. This is an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecom-mons.org/licences/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

microorganisms (cultures of harmless bacteria or yeast species) that equilibrate the intestinal microflora to benefit the host (Ferencik et al., 2000). They have been established to be useful in maintaining the intestinal ecosystem and improving animal health. Also, they have been suggested as the alternative with the highest potential for livestock because antibiotics' negative effects have prominently increased which led to a ban of their use. Furthermore, consumers are increasingly concerned about antibiotic residues in meat (Vondruskova et al., 2010) and it is assumed that the continuous use of antibiotics may increase bacterial resistance, which can threaten both animals' and humans' health (Van der Fels-Klerx et al., 2011).

Among various probiotic bacteria, *Lactobacillus* is the most commonly used probiotic agent (McCony and Gilliland, 2007). *Lactobacilli* are a group of gram-positive, non-motile, non-spore forming, acid-tolerant, non-respiring rod-shaped (bacillus), or spherical (coccus) bacteria with common physiological and metabolic characteristics and they produce lactic acid as the major metabolic end-product of carbohydrate fermentation (Cho et al., 2009). As an alternative, supplementing probiotics gained interest in recent years. The use of probiotics and prebiotics has a positive effect on animals' growth performance (Rai et al., 2013). Additionally, *Lactobacillus* being the most commonly used probiotic agent improves growth performance, feed conversion efficiency, intestinal microbiota, nutrient utilization, gut health, and regulates the pigs' immune system (Dowarah et al., 2017). Some authors have observed advantages of probiotics on meat quality (Alexopoulos et al., 2004; Jukna et al., 2005), while others have reported negative results (Quadros et al., 2001). The results regarding pigs have been inconsistent and the use of probiotics to improve meat quality has been questioned. These disputes may be due to variations in the animals and the probiotics used in the respective research because probiotics are generally host-species-specific (Dunne et al., 1999) and believed to be more effective in their natural habitat i.e., the target species (Kailasapathy and Chin, 2000). Some earlier studies reported that dietary probiotics might improve chicken meat quality attributes (water-holding capacity, lipid oxidation stability, tenderness, and sensory properties) and microbial safety (Aksu et al., 2005; Yang et al., 2010; Zhang et al., 2005), whereas some authors stated that no synergistic effect of probiotics and prebiotics on chicken meat quality exists (Pelícia et al., 2004; Zhang et al., 2012).

To date, the effects of probiotics on pig carcass quality and functional properties of pork have been described in some papers, but the results are not conclusive. Most suggested positive effects of dietary probiotics on the carcass (Alexopoulos et al., 2004; Sudikas et al., 2010) and pork quality (Liu et al., 2013; Suo et al., 2012), and that the probiotic administration could be useful to modify and improve the fatty acid profile and lower cholesterol levels in pig meat (Barowicz et al., 2003; Ross et al., 2012). Considering such studies, the information about feeding probiotics and their effects on pork quality in growing-finishing pigs is very rare. Therefore, the objective of this study was to investigate the efficacy and influence of the probiotic *Lactobacillus plantarum* on meat quality and chemical characteristics in three-way crossbred growing-finishing pigs' *longissimus* muscles.

## Materials and Methods

### Animals, diet, probiotics preparation, and the application system

In order to evaluate the effects of systemic use of probiotics on meat quality, 80 pigs with an average age of 175±5 d were maintained in conventional probiotic farms equipped with a liquid probiotics application system (40 pigs in each farm). All pigs were Landrace×Yorkshire×Duroc (LYD) three-way crossbred animals and commercially feed-fed according to the regimens of FARMSCO Inc. The diets' formula and calculated nutrient composition are shown in Table 1. The probiotic farm supplied a large quantity of probiotics to the pigs during all life stages (sows, piglets, growing pigs, and finishing pigs) via drinking and feeding. High quantities of probiotics were prepared by aero-dynamic fermentation with nutrients during 4 d

**Table 1. Feed ingredients and chemical compositions of diets in growing and finishing pigs**

| Ingredients (%) | Grower | Finisher |
|---|---|---|
| Corn | 54.33 | 54.68 |
| Wheat | 15.00 | 20.00 |
| Soybean meal | 23.05 | 17.86 |
| Beef tallow | 2.45 | 2.48 |
| Molasses | 2.00 | 2.00 |
| Limestone | 0.48 | 1.15 |
| Calcium phosphate | 1.36 | 0.74 |
| Salt | 0.35 | 0.35 |
| Lysine | 0.40 | 0.26 |
| Methionine | 0.20 | 0.13 |
| Threonine | 0.11 | 0.08 |
| Vitamin-mineral mixture[1] | 0.27 | 0.27 |
| Total | 100 | 100 |
| Calculated analysis (%) | | |
| Moisture | 11.55 | 11.69 |
| Crude protein | 17.00 | 15.01 |
| Crude fat | 4.76 | 4.80 |
| Crude fiber | 2.60 | 2.26 |
| Crude ash | 2.69 | 2.46 |
| Calcium | 0.62 | 0.69 |
| Phosphorus | 0.59 | 0.46 |
| Total lysine | 1.14 | 0.90 |
| DE (Mcal/kg) | 3.52 | 3.50 |

[1]The vitamin-mineral premix provided per kilogram of diet: 20,000 IU of vitamin A; 4,000 IU of vitamin $D_3$; 80 IU of vitamin E; 16 mg of vitamin K; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin $B_{12}$; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid; 0.08 mg of biotin; 15 mg of Cu (as copper sulfate); 56 mg of Zn (as zinc oxide); 73 mg of Mn (as manganese oxide); 0.3 mg of I (as potassium iodate); 0.5 mg of Co (as $Co_2O_3 \cdot 7H_2O$); 0.4 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).
DE, digestible energy.

after seeding (3% of the total volume) of *Lactobacillus plantarum* ($2.2\times10^8$ CFU/mL) in polystyrene tanks (2 to 3 ton size). The probiotic preparation ($2.5\times10^7$ CFU/mL) was supplied to pigs by having them drink water directly from the polystyrene tank. The probiotic feed additive was prepared by rotary drum fermentation during 7 d after seeding (2% of the total volume) of the probiotic preparation and mixed with the pig diets (20 kg/ton). The probiotic farm sprayed one to five diluted probiotic preparations inside and outside of the farm facilities instead of chemical disinfectants to prevent the growth of harmful bacteria and pathogens in the immediate environment.

## Carcass measurements and sample collection

At the end of the experiments, 80 pigs (40 per treatment) were transferred to the slaughterhouse. Feeding was terminated 12 h before slaughter. All pigs were conventionally slaughtered at the average market weight of 115 kg. The individual

carcass weight was recorded within 30 min after slaughter and the pig then placed in a chilling room (4℃). The back fat thickness was measured according to the Animal Products Grading Service (2001). After the determination of muscle $pH_{45min}$, samples were vacuum-packed at 4℃ to subsequently measure meat quality, including muscle $pH_{24h}$, color, shear force, WHC, cooking loss, drip loss, and meat composition. The rest of the samples were divided into two parts: one of them was pulverized by liquid nitrogen for nucleotides and free amino-acids and the other one freeze-dried for fatty acids, vitamins, and minerals. The samples of both groups were stored at -70℃ until analysis.

## Evaluation of meat quality

Three-color (L*, a* and b*) coordinate measurements per sample were taken at three different locations on the bloomed cut surfaces of the meat sample blocks using a Minolta chromameter (CR-300, Minolta Camera Co., Ltd., Osaka, Japan), with a 1 cm aperture, illuminant D65 and a 2° viewing angle. According to the Commission International de l'Eclairage (CIE) system, color was expressed as CIE L*, a*, and b* (lightness, redness, and yellowness).

We used two different methods to measure WHC: drip loss and cooking loss. Drip loss was assessed by the gravimetric method of Honikel (1998). The samples were trimmed and weighed, then placed in an inflated plastic bag and hung for 48 h at 4℃. After 48 h, the samples were weighed again. Drip loss was calculated as a percentage based on weight before and after hanging. For cooking loss (Honikel, 1998), the samples were weighed and put in a plastic bag, which was placed in an 80℃ water bath. When the internal temperature of 75℃ was reached, the samples were cooled and weighed again. The difference in weight before and after boiling was expressed as the "percentage cooking loss".

The Warner-Bratzler shear force (WBSF) was determined by taking meat sample blocks of approximately 300 g. Three representative 1.27 cm diameter cores were removed from each steak parallel to the muscle fiber after cooling. Shear force values were determined with a Warner-Bratzler shear attachment using an Instron Universal Testing Machine (Model 3342; Instron Corporation, USA) with the following operating parameters: load cell, 50 kg, cross-head speed, and 200 mm/min. Each core sample was sheared once across the center of the core perpendicular to the muscle fiber. The shear force value was the mean of the maximum forces required to shear each set of core samples and expressed as kg of force (kgf).

## Proximate, pH and 2-thiobarbituric acid reactive substances (TBARS) analyses

The moisture, protein, fat, and ash content of meat were measured according to the AOAC (1995). Moisture was assessed by the dry-oven method. Protein and fat contents were measured by the Kjeldahl method and the Soxhlet apparatus respectively. Ash content was analyzed using a muffle furnace.

Two different pH meters (pK21 pH meter and pH star probe) were used to measure pH accurately in the slaughter house and laboratory respectively within specific time. The pH values of the 10-rib face *longissimus dorsi* muscles were measured at 45 min and 24 h post-mortem in triplicates using a portable pH meter (pK21 pH meter, NWK-Binar GmbH, Germany). Afterwards, the *longissimus dorsi* muscles were excised for further analyses.

The 10-rib face *longissimus* muscle pH values were assessed at 3 d, 5 d, and 15 d in duplicates using a pH star probe (Horiba 6252-10D, USA).

The TBARS as a lipid oxidation value were measured by a modified method of Buege and Aust (1978). The meat samples were aging 0 d and 20 d respectively at 4℃. Completed aging-period samples were stored at -20℃ until analysis. Prior, the samples were thawed at 4℃. A meat sample of 2.5 g was filled into a 50 mL falcon test tube, and homogenized with 7.5 mL deionized distilled water as well as 10 mL of TBA/TCA solution using a Homogenizer (T 25 digital ULTRA-TURRAX®,

Germany) at 12,000 rpm for 20 s. To protect fat oxidation, 25 μL of BHA (butylated hydroxyanisole) was added to each homogenized sample. The homogenate was filled up to 30 mL with distilled water (DW), vortexed, and heated in a 90℃ preheated water bath for 15 min to develop color. The heated homogenized samples were cooled in ice-cold water for 15 min and centrifuged at 3,000×g for 15 min at 4°C by using a Hanil Supra 21K Centrifuge Machine (Hanil Science Industrial Co., Ltd., Korea). After centrifugation, the absorbance of the resultant supernatant solution was taken as a duplicate and averaged by using a UV spectrophotometer (Multiskan Go, Thermo Scientific, USA) at 531 nm compared with a blank containing distilled water and a TBA/TCA solution. TBARS were calculated by multiplying the average absorbance value with 5.88 and the results were expressed as mg malondialdehyde (MDA)/kg of meat.

### Measurement of fatty acids

Fatty acids were measured by gas chromatography (GC). Freeze-dried meat samples (0.5 g) were added to 2 mL of boron-trifluoride in methanol and 2 mL of methanol in glass tubes. The tubes were capped with Teflon-lined caps to prevent loss of volume and the samples were placed on a heating block at 80℃. After 10 min, the tubes were vortexed individually every 5 min for 2 h. After 2 h of repeated vortex mixing, the samples were allowed to cool at room temperature and then after adding 3 mL of distilled water and 3 mL of hexane - the tubes were capped again and mixed by vortexing for 15 s. After centrifugation (2,000 rpm, 5 min) to separate the phases, the supernatant was transferred to GC vials for analysis. GC was performed for 1 μL **s**amples on a Shimadzu GC-2014 instrument (Shimadzu Co., USA) using a FAMEWAX column (30 m×0.32 mm i. d., 0.25 μm; column temperature, 250℃) and nitrogen/air as a carrier gas at 53.8 mL/min (split ratio 30:1). The temperature started at 150℃ and increased to 250℃ with an equilibration time of 3 min.

### Measurement of free amino acids

Meat samples were made into fine powder with liquid $N_2$, a mortar and pestle (0.5 g); afterwards, they were mixed with 1 mL of DW and homogenized two times at 30 s intervals. Then, the samples were centrifuged at 13,000 rpm for 10 min at 4℃. The 100 μL supernatants were transferred into a 2 mL tube, mixed with 900 μL formic acid (4% formic acid in 100% ethanol) by vortexing, and incubated at -20℃ for 1 h. Then they were again centrifuged at 13,000 rpm for 10 min at 4℃ and 100 μL supernatants were mixed with 900 μL formic acid (4% formic acid in 100% ethanol) in a vial. The samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) for which 3 μL aliquots were injected on an UPLC system (Waters Xevo TQ-S, Waters Corporation, Milford, MA, USA). The UPLC column was Imtakt Intrada Amino Acid C18 (50×2 mm, 3 μm). The mobile phase buffer A was ACN:100 mM $CH_5NO_2$=20:80 (v/v) and the buffer B was ACN:THF:25 mM $CH_5NO_2$:FA=9:75:16:0.3 (v/v/v/v). The flow rate was 0.4 mL/min and established a gradient from 0 to 100% for the B buffer for 17 min. The MS was equipped with an ESI interface operating at an ionization voltage of +3,000 V and a source temperature of 380℃. The capillary voltage, con voltage, and source offset were set at 3 kV, 30 kV, and 30 V respectively. Tandem MS analysis was performed using a multi-reaction-monitoring (MRM) mode. The collision energy from the gas flow of desolvation, cone, and nebulizer were set at 650 L/h, 150 L/h and 7 bar, respectively.

### Measurement of nucleotide compounds

The meat samples were pulverized with liquid $N_2$, a mortar and pestle (0.3 g), mixed with 5 mL of 0.5 M perchloric acid, kept in ice for 15 min, and then centrifuged at 9,200×g for 5 min at 4℃ to extract nucleic acids. The extracted 1 mL nucleic acids were transferred into a 2 mL tube, mixed very carefully with 0.25 mL of 2.1 M potassium hydrogen carbonate ($KHCO_3$),

and kept in ice for 15 min. They were then again centrifuged at 9,200×g for 5 min at 4℃ and filtered through a syringe filter (HLB-M, 0.45 μm particle size, 13 mm, Futecs Co., Ltd., Korea). The filtrate was analyzed using high-performance liquid chromatography (HPLC) (Shiseido Nanospace SI-2, Shiseido Co., Ltd. Japan). Regarding the analytical conditions for HPLC, a Imtakt Cadenza CD-C18 reverse phased column (4.6×250 mm, 3 μm, Imtakt Corp., USA) was utilized, with two mobile phases of (1) A, 1,000 mL distilled water+5 mL TBA-OH (tert-butyl ammonium hydroxide) (40%)+1 mL $H_3PO_4$ (phosphoric acid) and (2) B, 1,000 mL methanol+5 mL TBA-OH (40%)+1 mL $H_3PO_4$. The mobile phase flow rate was 0.7 mL/min and the injection volume was 5 μL. The column temperature was maintained at 40℃ and the detection wave length was 254 nm. The peaks of the individual nucleotides were identified using the retention times for the following standards: hypoxanthine, inosine, inosine-5'-phosphate (IMP), adenosine-5'-phosphate (AMP) (Sigma, USA), and the concentration was calculated using the area for each peak.

### Measurement of vitamins and minerals

Vitamin content was analyzed by LC-MS/MS. A total of 10 mg freeze-dried meat powder was sonicated in 100 μL distilled water and then 900 μL methanol was added, mixed by vortexing, and then again sonicated. After centrifugation, the samples were analyzed using a UPLC system (Waters Xevo TQ-S, Waters Corporation, USA). The UPLC column was a Waters ACQUITY UPLC ® BEH C18 (2.1×100 mm, 1.7 μm). For the analysis of water-soluble vitamins, buffer A was 0.1% formic acid in water and buffer B 0.1% formic acid in acetonitrile (ACN). For fat-soluble vitamins, buffer A was 0.1% formic acid in water and buffer B 0.1% formic acid in methanol/ACN (40/60, v/v).

Minerals were measured using inductively coupled plasma mass spectrometry (ICP-MS). A 0.05 g sample in 600 μL of 70% nitric acid in a 15 mL conical tube reacted in a fume hood. After reaction for 2 d, sample tubes were incubated at 80℃ for 5 h. DW was added to adjust the volume to 10 mL and the sample was diluted with 2% nitric acid from $10^1$ to $10^4$ for analysis. Mg, K, Ca, Fe, and Zn were analyzed by ICP-MS (Agilent 7500a, Santa Clara, USA).

### Statistical analysis

Data were analyzed by using the SAS software (SAS Version 9.3). All data were analyzed using an ANOVA (analysis of variance) procedure to test the statistical significance among treatments. The results are presented as the mean±SE and statistical significance was indicated at $p<0.05$, based on *t*-tests.

## Results and Discussion

### Physical characteristics in porcine *longissimus dorsi* muscles fed probiotics

The physical properties in porcine *longissimus dorsi* muscles fed with probiotics are shown in Table 2. The color (CIE L* and a*) did not show any significant difference between the groups, but yellowness (CIE b*) was significantly higher in the PRO group. The shear force of the PRO-treated group was significantly lower compared to the control group. Drip loss of the probiotics treatment group increased at 1 d and 3 d and reduced at 15 d compared to the controls. There was no significant difference in backfat thickness, carcass weight, water holding capacity, and cooking loss between the control and probiotics treatment groups. Liu et al. (2013) reported that the supplementation with probiotics (yeasts, lactic acid-producing bacteria, and *Bacillus subtilis*) reduced the drip loss and cooking loss of pork, but had no effect on pH and shear force. In another

**Table 2. Physical properties in porcine *longissimus dorsi* muscles fed with probiotics**

| Traits | CON (n=40) | PRO (n=40) |
|---|---|---|
| Backfat thickness (mm) | 18.43±0.59 | 16.98±0.53 |
| Carcass weight (kg) | 90.28±0.66 | 87.65±1.16 |
| Color | | |
| CIE L* (Lightness) | 56.08±0.65 | 56.78±0.48 |
| CIE a* (Redness) | 5.57±0.20 | 5.40±0.19 |
| CIE b* (Yellowness) | 8.98±0.15 | 9.48±0.15* |
| Shear force (kgf) | 1.90±0.09 | 1.67±0.06* |
| Water holding capacity (%) | 59.10±0.51 | 59.07±0.46 |
| Cooking loss (%) | 23.10±0.71 | 23.33±0.54 |
| Drip loss (%) | | |
| 1 d | 5.15±0.23 | 5.38±0.24 |
| 3 d | 6.32±0.26 | 6.44±0.30 |
| 15 d | 9.31±0.28 | 9.28±0.32 |

Mean values are presented as the mean±SE.
* $p<0.05$, values in the same row are significantly different.
CON, control (no addition probiotics); PRO, fed with probiotics.

experiment, Yang et al. (2010) stated that probiotic *C. butyricum* with a broiler diet decreased shear force, which indicated improved tenderness. However, experiments reporting the use of *L. plantarum* as a probiotic on shear force in pigs are limited. Also, Sudikas et al. (2010) published that 0.04% probiotic (*Bacillus licheniformis* and *Bacillus subtilis*) supplementation had no influence on the carcass quality but that the carcass weight increased 0.06% compared to controls. Feeding 0.5% probiotics (*Lactobacillus* CAU6001) improved meat quality (Li and Zhang, 2007). Some previous studies have stated the positive effects of probiotics on pigs meat quality (Alexopoulos et al., 2004; Meng et al., 2010) but differ Quadros et al. (2001). These variable results about the effect of probiotics may be due to several aspects such as bacteria strains, level of supplementation, composition of diet and interaction with other dietary additives (Meng et al., 2010). Furthermore, dietary probiotics significantly improved the pork quality, enhanced WHC, reduced drip loss, and produced a more vivid meat color from finishing pigs (Jiang, 2011; Ma, 2011). Therefore, probiotics influenced meat quality traits like color, shear force, and drip loss.

## Proximate, pH and oxidative status (TBARS) analyses in porcine *longissimus dorsi* muscles if fed probiotics

Compared with the control group (Table 3), dietary supplementation with probiotics significantly reduced ash, salinity, and pH (at 5 and 15 d). Probiotics also increased protein, reduced moisture and fat, but did not reach significance levels ($p>0.05$). There was no significant effect on TBARS values, while the treatment group's decreased at 20 d. In a previous study, Sudikas et al. (2010) found that diets with 0.04% and 0.06% probiotic supplementation had no effect on the chemical composition of meat compared to controls. A similar response on pH was observed by Rybarczyk et al. (2016) and they stated that a diet containing bokashi probiotics (*Saccharomyces cerevisiae*, *Lactobacillus casei* and *Lactobacillus plantarum*) decreased at $pH_{24}$ and $pH_{48}$, while they increased at $pH_{120}$. On the basis of this research, pH appears to have a great influence on the quality of food: Freshness, water retention, tenderness, color, and texture are greatly influenced by the food's pH. Commonly, pH is a direct reflection of muscle's acid content and it affects meat color, shear force, and drip loss (Chen et al., 2009). Bendall and

**Table 3. Proximate, pH and oxidative status (TBARS) analyses in porcine *longissimus dorsi* muscles fed with probiotics**

| Items | CON (n=40) | PRO (n=40) |
|---|---|---|
| Moisture (%) | 74.20±0.14 | 74.09±0.18 |
| Fat (%) | 3.16±0.16 | 3.05±0.11 |
| Protein (%) | 21.55±0.12 | 21.74±0.17 |
| Ash (%) | 1.10±0.02 | 1.05±0.01* |
| Salinity (%) | 0.33±0.00 | 0.31±0.00* |
| pH | | |
| 45 min | 6.49±0.03 | 6.52±0.04 |
| 24 h | 6.07±0.02 | 5.96±0.02 |
| 3 d | 5.82±0.03 | 5.77±0.03 |
| 5 d | 5.78±0.03 | 5.66±0.02* |
| 15 d | 5.74±0.03 | 5.65±0.03* |
| TBARS (mg MDA/kg meat) | | |
| 0 d | 0.90±0.06 | 0.97±0.08 |
| 20 d | 1.30±0.18 | 1.15±0.09 |

Mean values are presented as the mean±SE.
* $p<0.05$, values in the same row are significantly different.
CON, control (no addition probiotics); PRO, fed with probiotics.

Swatland (1988) reported that the pH of pork was affected by the rate and extent of post-operative action which decreases from the time of bleeding and then reaches the final pH. Li and Chen (2009) showed that probiotics significantly reduced the MDA content of muscles, inhibited muscle lipid peroxidation, reduced the rate of water loss, stabilized color, and improved the meat quality. Due to reducing drip loss, probiotics lowered lipid peroxidation in the muscles by maintaining the cell membranes' integrity (Liu et al., 2013). As a similar response, Ko et al. (2008) reported that pigs fed diets containing 0.5% green tea probiotic supplementation had lowered meat TBA values compared to finishing pigs fed a 0.5% green tea by-product ($p<0.05$).

### Fatty acid composition in porcine *longissimus dorsi* muscles if fed probiotics

Table 4 showed the results of fatty acid content analysis for porcine fed with probiotics. Palmitic acid (C16:0) and stearic acid (C18:0) were the main triglycerides of saturated fatty acid and they did not show significant differences in the treatments. Oleic acid (C18:l n9) and linoleic acid (C18:2 n6) were the major fatty acids among the unsaturated fatty acids. The content of saturated fatty acid (SFA) and unsaturated fatty acid (USFA) was not significantly different between the treatments. The probiotic group meat showed higher polyunsaturated (PUFA) fatty acid contents, with linoleic acid (C18:2) and concentrations significantly ($p<0.05$) higher compared to the control group, consistent with Ross et al. (2012). However, monounsaturated (MUFA) acids were significantly lower in the probiotic-treated group. The linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) ratio should be approximately 5:1 to promote health and to minimize cardiovascular disease risks. Although the meat of the probiotic group showed linolenic and linoleic acid significantly ($p<0.05$) higher compared to the control group, the n-3 and n-6 in the present study are higher than that recommended values for human health. The intensified activity of lactic bacteria could positively influence the ingestion and absorption processes as well as indirectly influence the meat fatty acids profile.

**Table 4.** **Fatty acid compositions (% of total fatty acids) in porcine *longissimus dorsi* muscles fed with probiotics**

| Fatty acids | CON (n=40) | PRO (n=40) |
|---|---|---|
| Myristic acid (C14:0) | 1.65±0.02 | 1.58±0.02* |
| Palmitic acid (C16:0) | 23.34±0.12 | 23.33±0.16 |
| Palmitoleic acid(C16:l n7) | 2.55±0.03 | 2.31±0.03* |
| Stearic acid (C18:0) | 12.44±0.18 | 12.73±0.19 |
| Oleic acid (C18:l n9) | 42.15±0.24 | 40.63±0.22* |
| Linoleic acid (C18:2 n6) | 15.72±0.22 | 17.27±0.24* |
| γ-Linoleic acid (C18:3 n6) | 0.10±0.01 | 0.08±0.00 |
| Linolenic acid (C18:3 n3) | 0.78±0.01 | 0.83±0.01* |
| Eicosenoic acid (C20:l n9) | 1.02±0.03 | 0.97±0.02 |
| Arachidonic acid (C20:4 n6) | 0.20±0.01 | 0.21±0.01 |
| SFA | 37.44±0.28 | 37.65±0.30 |
| USFA | 62.55±0.28 | 62.34±0.30 |
| MUFA | 45.73±0.26 | 43.93±0.24* |
| PUFA | 16.81±0.24 | 18.41±0.25* |
| ω3 | 0.78±0.01 | 0.83±0.01* |
| ω6 | 16.03±0.23 | 17.57±0.24* |

Mean values are presented as the mean±SE.
* $p<0.05$, values in the same row are significantly different.
CON, control (no addition probiotics); PRO, fed with probiotics; SFA, saturated fatty acid; USFA, unsaturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid.

### Free amino acids and nucleotide compound in porcine *longissimus dorsi* muscles if fed probiotics

Analyses of free amino acid compositions such as serine, lysine, histidine, and arginine revealed significantly lower levels in the PRO group as compared to the CON group (Table 5). Muscles from the probiotic-treated group had higher levels of glycine, alanine, proline, valine, leucine, and isoleucine, whereas glutamic acid, phenylalanine, tyrosine, and threonine were higher in the control group. The treatment group showed a higher nucleotide compound (hypoxanthine, inosine, GMP, IMP) than the controls (Table 6). Glutamic acid, phenylalanine, tyrosine, AMP, IMP, and GMP contribute to meat flavor perceptions and together comprise the umami taste (Kuchiba-Manabe et al., 1991; Lioe et al., 2005; Wood et al., 2004). In contrast, glycine, alanine, lysine, and proline contribute sweet flavors, and other amino acids produce sour or salty tastes (Zhu and Hu, 1993). Remarkably, IMP indirectly contributes to meat flavor through the breakdown of inosine to form hypoxanthine and with free amino acids such as arginine, phenylalanine, valine, leucine, isoleucine, methionine, and histidine, contributes to a bitter taste (Tikk et al., 2006). Cornet and Bousset (1999) stated that an undesirable flavor could result due to accumulation of some free amino acids. However, free amino acids are of great importance in eating quality due to their specific tastes (Nishimura and Kato, 1988).

### Vitamins and minerals in porcine *longissimus dorsi* muscles if fed probiotics

The content of vitamins and minerals in pork meat are shown in Table 7. The levels of thiamin ($B_1$) and ascorbic acid (C) significantly differed in the treatment group ($p<0.05$), whereas riboflavin ($B_2$), niacin ($B_3$), pyridoxine ($B_6$), retinol (A), α-tocopherol (E), and cholecalciferol ($D_3$) showed no significant differences between the groups. Some natural antioxidants can

**Table 5.** **Free amino acid composition (% of free amino acids) in porcine *longissimus dorsi* muscles fed with probiotics**

| Free amino acid | CON (n=40) | PRO (n=40) |
|---|---|---|
| Glycine | 1.14±0.15 | 1.32±0.21 |
| Alanine | 13.25±0.45 | 14.49±0.50 |
| Serine | 3.72±0.16 | 3.27±0.15* |
| Proline | 3.03±0.11 | 3.08±0.16 |
| Valine | 4.17±0.18 | 4.67±0.21 |
| Threonine | 3.66±0.12 | 3.47±0.13 |
| Leucine | 6.06±0.36 | 6.11±0.38 |
| Isoleucine | 2.14±0.20 | 2.15±0.20 |
| Aspartic acid | 1.06±0.67 | 1.03±0.07 |
| Lysine | 4.95±0.22 | 4.03±0.15* |
| Glutamic acid | 7.09±0.31 | 6.26±0.34 |
| Methionine | 2.10±0.15 | 1.87±0.13 |
| Histidine | 2.26±0.09 | 1.93±0.07* |
| Phenylalanine | 4.62±0.31 | 4.26±0.30 |
| Arginine | 4.79±0.23 | 3.88±0.16* |
| Tyrosine | 4.49±0.19 | 4.21±0.18 |
| Cysteine | 0.61±0.02 | 0.61±0.02 |
| Flavor | | |
| Umami[1)] | 8.15±0.67 | 7.29±0.59 |
| Sweet[2)] | 23.06±0.83 | 23.09±0.94 |
| Bitter[3)] | 26.13±0.25 | 24.88±0.26* |

[1)] Aspartic acid+glutamic acid.
[2)] Serine+glycine+alanine+lysine.
[3)] Valine+methionine+isoleucine+leucine+phenylalanine+histidine+arginine.
Mean values are presented as the mean±SE.
* $p<0.05$, values in the same row are significantly different.
CON, control (no addition probiotics); PRO, fed with probiotics.

**Table 6.** **Nucleic acid-related compounds in porcine *longissimus dorsi* muscles fed with probiotics (μmol/g)**

| Nucleotide related compound | CON (n=40) | PRO (n=40) |
|---|---|---|
| Hypoxanthine | 0.22±0.06 | 0.26±0.09 |
| Uridine | 0.07±0.01 | 0.06±0.01 |
| Inosine | 2.09±0.15 | 2.19±0.17 |
| AMP | 0.09±0.03 | 0.07±0.02 |
| GMP | 0.35±0.06 | 0.38±0.07 |
| IMP | 10.95±0.71 | 12.58±0.96 |
| ADP | 1.83±0.14 | 1.77±0.14 |

Mean values are presented as the mean±SE.
CON, control (no addition probiotics); PRO, fed with probiotics; AMP, adenosine monophosphate; GMP, guanosine monophosphate; IMP, inosine monophosphate; ADP, adenosine diphosphate.

**Table 7. Vitamins and minerals content (μg/g) in porcine *longissimus dorsi* muscles fed with probiotics**

| Items | CON (n=40) | PRO (n=40) |
|---|---|---|
| Vitamins | | |
| Retinol (A) | 3.36±0.22 | 3.57±0.21 |
| Cholecalciferol ($D_3$) | 1.31±0.09 | 1.38±0.08 |
| α-Tocopherol (E) | 0.77±0.06 | 0.64±0.05 |
| Ascorbic acid (C) | 3.64±0.02 | 4.22±0.08* |
| Thiamine ($B_1$) | 1.88±0.13 | 1.40±0.14* |
| Riboflavin ($B_2$) | 0.04±0.00 | 0.04±0.00 |
| Niacin ($B_3$) | 0.66±0.04 | 0.62±0.03 |
| Pyridoxine ($B_6$) | 0.18±0.00 | 0.18±0.00 |
| Minerals | | |
| Magnesium (Mg) | 1,161.48±13.27 | 1,174.85±11.76 |
| Potassium (K) | 16,496.00±231.03 | 16,728.00±255.61 |
| Calcium (Ca) | 181.54±2.70 | 179.56±1.93 |
| Iron (Fe) | 11.64±0.26 | 11.52±0.31 |
| Zinc (Zn) | 55.21±1.21 | 53.54±1.07 |

Mean values are presented as the mean±SE.
* $p<0.05$, values in the same row are significantly different.
CON, control (no addition probiotics); PRO, fed with probiotics.

provide biological protection from oxidative processes (Su et al., 2007) and are added in the pre- and post-slaughter stages to enhance meat shelf life and quality. Vitamin C has good antioxidant properties and Wheeler et al. (1996) demonstrated that injecting vitamin C into beef resulted in improved color stability and retail display color of the meat. Magnesium (Mg) and potassium (K) were higher in the treatment group but did not reach significance levels, while calcium (Ca), iron (Fe), and zinc (Zn), decreased compared to the control group. Sherman and Metha (2009) showed that the potassium content increased the quality of uncooked meat and poultry products. Zinc is essential for performance-related functions, collagen synthesis, and immune development (Shankar and Prasad, 1998). Different genetic lines of pigs did not show differences in the mineral content of the meat (Tomović et al., 2011). Our current study showed that probiotics increased antioxidant activity without significant changes of mineral contents in LYD meat.

## Conclusion

The current study provides evidence for the influence of probiotics feeding on shear force, ash, salinity and pH as well as PUFA, some amino acids and nucleotide compounds improved which related to taste and flavor. Probiotics also increased ascorbic acid of meat, which act as a good anti-oxidant. This study suggests that the systemic use of probiotics may produce PUFA-enriched healthy pork via modulating physiochemical properties.

## Acknowledgements

This research was supported by a grant from the Next-Generation BioGreen 21 Program (Project No. PJ01316702), Rural

Development Administration, Republic of Korea and Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the Agri Bioindustry Technology Development Program funded by the Ministry of Agriculture, Food, and Rural Affairs (Project No. 114073-3). The free amino acid, vitamin and mineral samples were analyzed using the LC-MS/MS installed at the Center for University-Wide Research Facilities (CURF) at Chonbuk National University. We thank Ms. Eun-Jin Choi and Dr. Yun-Jo Chung at the CURF of Chonbuk National University.

## References

Aksu MI, Karaoğlu M, Esenbuğa N, Kaya M, Macit M, Ockerman HW. 2005. Effect of a dietary probiotic on some quality characteristics of raw broiler drumsticks and breast meat. J Muscle Foods 16:306-317.

Alexopoulos C, Georgoulakis IE, Tzivara A, Kyriakis CS, Govaris A, Kyriakis SC. 2004. Field evaluation of the effect of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores on the health status, performance, and carcass quality of grower and finisher pigs. Transbound Emerg Dis 51:306-312.

AOAC. 1995. Official methods of analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.

Barowicz T, Migdał W, Pieszka M, Živković B. 2003. The effect of linseed PUFA n-3 and probiotics on fatty acid composition and cholesterol level in *longissimus dorsi* muscle of fatteners. Biotechnol Anim Husb 19:31-36.

Bendall JR, Swatland HJ. 1988. A review of the relationships of pH with physical aspects of pork quality. Meat Sci 24:85-126.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52:302-310.

Chen P, Wang AQ, Shan AS. 2009. Effects of *Ligustrum lucidum* fruits on growth performance, antioxidation and meat quality in arbor acres broilers. Asian-Aust J Anim Sci 22:700-705.

Cho IJ, Lee NK, Hahm YT. 2009. Characterization of *Lactobacillus* spp. isolated from the feces of breast-feeding piglets. J Biosci Bioeng 108:194-198.

Cornet M, Bousset J. 1999. Free amino acid and dipeptides in porcine muscles: Differences between 'red' and 'white' muscles. Meat Sci 51:215-219.

Dowarah R, Verma AK, Agarwal N. 2017. The use of *Lactobacillus* as an alternative of antibiotic growth promoters in pigs: A review. Anim Nutr 3:1-6.

Dunne C, Murphy L, Flynn S, O'Mahony L, O'Halloran S, Feeney M, Morrissey D, Thornton G, Fitzgerald G, Daly C, Kiely B, Quigley EMM, O'Sullivan GC, Shanahan F, Collins JK. 1999. Probiotics: From myth to reality demonstration of functionality in animal models of disease and in human clinical trials. Lactic Acid Bacteria: Genetics, Metabolism and Applications 76:279-292.

Ferencik M, Mikes Z, Seman M, Ebringer L. 2000. Beneficial modification of the human intestinal microflora using orally administered enterococci. In Proceedings of International Probiotic Conference on "The Prospects of Probiotics in Prevention and Therapy of Diseases of Young". pp 11-14.

Honikel KO. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci 49:447-457.

Jiang J. 2011. Effect of ASTA on weight gain and meat quality on finishing pigs. Hunan Feed 5:40-43.

Jukna Č, Jukna V, Šimkus A. 2005. The effect of probiotics and phytobiotics on meat properties and quality in pigs. Vet Zootech 29:80-84.

Kailasapathy P, Chin J. 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 78:80-88.

Ko SY, Bae IH, Yee1 ST, Lee SS, Uuganbayar D, Oh JI, Yang CJ. 2008. Comparison of the effect of green tea by-product and green tea probiotics on the growth performance, meat quality, and immune response of finishing pigs. Asian-Australas J Anim Sci 21:1486-1494.

Kuchiba-Manabe M, Matoba T, Hasegawa K. 1991. Sensory changes in umami taste of inosine 5-monophosphate solution after heating. J Food Sci 56:1429-1432.

Li J, Zhang RJ. 2007. Effect of probiotic on performance, carcass traits and meat quality of broiler chickens. Chinese J Anim Nutri 19:372-378.

Li L, Chen CX. 2009. Effects of probiotics on meat quality of broilers and mechanism. Heilongjiang Anim Sci Vet Medi 11:43-44.

Lioe HN, Apriyantono A, Takara K, Wada K, Yasuda M. 2005. Umami taste enhancement of MSG/NaCl mixtures by subthreshold L-a-aromatic amino acids. J Food Sci 70:S401-S405.

Liu TY, Su BC, Wang JL, Zhang C, Shan AS. 2013. Effects of probiotics on growth, pork quality and serum metabolites in growing-finishing pigs. J Northeast Agric Univ 20:57-63.

Ma QZ. 2011. A test on the effects of application for a multi-strain mixed feed ferment. Feed Indus 32:58-61.

McCony S, Gilliland SE. 2007. Isolation and characterization of *Lactobacillus* species having potential for use as probiotic cultures for dogs. J Food Sci 72:94-97.

Meng QW, Yan L, Ao X, Zhou TX, Wang JP, Lee JH, Kim IH. 2010. Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. J Anim Sci 88:3320-3326.

Nishimura T, Kato H. 1988. Taste of free amino acids and peptides. Food Rev Int 4:175-194.

Park HY, Hamidon F, Rajangan C, Soh KP, Gan CY, Lim TS, Abdullah WNW, Liong MT. 2016. Application of probiotics for the production of safe and high-quality poultry meat. Korean J Food Sci An 36:567-576.

Pelícia K, Mendes AA, Saldanha ESPB, Pizzolante CC, Takahashi SE, Moreira J, Garcia RG, Quinteiro RR, Paz ICLA, Komiyama CM. 2004. Use of prebiotics and probiotics of bacterial and yeast origin for free-range broiler chickens. Braz J Poult Sci 6:163-169.

Quadros ARB, Kiefer C, Ribeiro NLC, Zink LA. 2001. Características qualitativas da carne de suínos alimentados com rações contendo ou não probióticos. In: Anais da 38ª Reunião Anual da Sociedade Brasileira de Zootecnia, Piracicaba, Brazil. pp 794-795.

Rai V, Yadav B, Lakhani GP. 2013. Application of probiotic and prebiotic in animals production: A review. Environ Ecol 31:873-876.

Ross GR, Nieuwenhove CPV, González SN. 2012. Fatty acid profile of pig meat after probiotic administration. J Agric Food Chem 60:5974-5978.

Rybarczyk A, Romanowski M, Karamucki T, Ligocki M. 2016. The effect of Bokashi probiotic on pig carcass characteristics and meat quality. FleischWirtschaft-International 1:74-77.

Shankar AH, Prasad AS. 1998. Zinc and immune function: The biological basis of altered resistance to infection. Am J Clin Nutr 68:447S-463S.

Sherman RA, Mehta O. 2009. Phosphorus and potassium content of enhanced meat and poultry products: Implications for patients who receive dialysis. Clini J Am Soc Nephrol 4:1370-1373.

Sudikas G, Kulpys J, Juškienė V, Juškienė V, Leikus R, Norvilienė J. 2010. The influence of probiotics on carcass, meat and

fat quality in pigs. Vet Med Zoot 52:79-86.

Suo C, Yin Y, Wang X, Lou X, Song D, Wang X, Gu Q. 2012. Effects of *Lactobacillus plantarum* ZJ316 on pig growth and pork quality. BMC Vet Res 8:89-101.

Su L, Yin JJ, Charles D, Zhou K, Moore J, Yu L. 2007. Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. Food Chem 100:990-997.

Tikk M, Tikk K, Tørngren MA, Meinert L, Aaslyng MD, Karlsson AH, Andersen HJ. 2006. Development of inosine monophosphate and its degradation products during aging of pork of different qualities in relation to basic taste and retronasal flavor perception of the meat. J Agric Food Chem 54:7769-7777.

Tomović VM, Petrović LS, Tomović MS, Kevrešan ŽS, Džinić NR. 2011. Determination of mineral contents of semimembranosus muscle and liver from pure and crossbred pigs in Vojvodina (northern Serbia). Food Chem 124:342-348.

Van der Fels-Klerx HJ, Puister-Jansen LF, van Asselt ED, Burgers SLGE. 2011. Farm factors associated with the use of antibiotics in pig production. J Anim Sci 89:1922-1929.

Vondruskova H, Slamova R, Trckova M, Zraly Z, Pavli I. 2010. Alternatives to antibiotic growth promotors in prevention of diarrhea in weaned piglets: A review. Vet Med 55:199-224.

Wheeler TL, Koohmaraie M, Shackelford SD. 1996. Effect of vitamin C concentration and co-injection with calcium chloride on beef retail display color. J Anim Sci 74:1846-1853.

Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR, Enser M. 2004. Effects of fatty acids on meat quality: A review. Meat Sci 66:21-32.

Yang X, Zhang B, Guo Y, Jiao P, Long F. 2010. Effects of dietary lipids and *Clostridium butyricum* on fat deposition and meat quality of broiler chickens. Poult Sci 89:254-260.

Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB, Lee CH. 2005. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poult Sci 84:1015-1021.

Zhang ZF, Zhou TX, Ao X, Kim IH. 2012. Effects of β-glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize soybean meal based diets. Livest Sci 150:419-424.

Zhu SW, Hu JX. 1993. Studies on Jinhua ham tastes and taste substances. J Food Sci 159:8-11.