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ORIGINAL ARTICLE

Effects of Microbial Additive Supplementation on Meat Quality and Fatty Acid Profiles of Growing-Finishing Pigs

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Abstract

The objective of this study was to investigate the effects of microbial additive on the meat quality and fatty acid (FA) profiles of growing-finishing pigs. A total of 180 growing-finishing pigs (Landrace x Yorkshire x Duroc; mixed sex; 14 weeks of age; 58.0 ± 1.00 kg) were randomly distributed into three treatments with three pens consisting of 20 growing-finishing pigs per pen for 60 days. The experimental treatments were as follows: 0, 0.5, and 1.0% microbial additive. The crude protein, cooking loss, drip loss, water holding capacity, and shear force in loin muscle were no significant differences among treatments (p>0.05), except for the moisture and crude fat contents. The pH and TBARS of loin muscle shown no significant differences among treatments (p>0.05). However, the L^* and a^* values of loin muscle were the highest in the 1.0% supplementation group compared with the other treatments (p<0.05). The b^* value of loin muscle was the highest in the control group compared with the other treatments (p<0.05). Linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, and n-3 FAs contents of loin muscle were the highest in 1.0% supplementation group compared with the other treatments (p<0.05). In conclusion, using 1.0% microbial additive supplementation can improve meat quality by increasing polyunsaturated FA concentration and meat color in pig loins.

Key words: Fatty acid profiles, Meat quality, Microbial additive, Growing-finishing pigs

1. Introduction

Antibiotics were used to prevent diseases and promote the growth of animals (Phillips et al., 2004). However, repeated use of antibiotics has led to the emergence of antibiotic-resistant bacteria and antibiotic residues in meat (Cha et al., 2015). As a result, many countries have banned the use of antibiotics in the livestock industry and have recognized the need for the development of suitable alternatives. Such effective antibiotic alternatives include bacteriophages, plant extracts, probiotics, prebiotics, phytobiotics, organic acids, and feed enzymes (Junka et al., 2005; Millet and Maertens, 2011). Microbial additives are live microbial feed additives that beneficially affect the animal by maintaining their intestinal microbial balance (Fuller, 1989). The positive effects of probiotics on pigs include improved barrier function, stimulation of innate immune response,

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Table 1. Effects of microbial additive supplementation on chemical and physicochemical characteristics of loin

Item	Supplement, %[1]			SEM	Contrast[2]	
	0	0.5	1.0		L	Q
Chemical characteristics (%)						
Moisture	74.4[a]	73.5[ab]	73.1[b]	1.308	0.014	0.093
Crude protein	24.8	24.7	24.8	0.084	0.112	0.641
Crude fat	1.52[b]	2.22[a]	2.22[a]	1.220	0.002	0.105
Physicochemical characteristics						
Cooking loss (%)	39.5	39.3	38.7	1.931	0.079	0.771
Drip loss (%)	3.49	3.32	2.69	1.220	0.018	0.255
Water holding capacity (%)	50.8	51.9	52.4	1.011	0.024	0.341
Shear force (kg/cm^2)	2.22	2.32	2.12	0.361	0.541	0.112

[1]Supplemented microbial additive at 0, 0.5, and 1.0% of basal diet.
[2]L: linear effect, Q: quadratic effect.
[a,b]Means in the same row with different superscripts differ significantly ($P<0.05$).

and improvement in growth performance (Fuller, 1992; O'Toole and Conney, 2008). Furthermore, microbial additives significantly reduce drip loss, enhance Water Holding Capacity (WHC), and improve meat quality from finishing pigs (Jiang, 2011; Ma, 2011). Microbial additive supplementation in finishing pigs showed higher carcass yield and weight (Junka et al., 2005; Kumar et al., 2009). Junka et al.(2005) reported that the supplementation of microbial additives in finishing pigs decreased cooking loss, meat hardness, and increased WHC. However, the use of probiotics to improve meat quality has been questioned, and the results in pigs have been inconsistent (Junka et al., 2005; Meng et al., 2010; Tufarelli et al., 2017). In addition, few studies have been conducted to investigate the effect of multi-species microbial additives on the meat quality of finishing pigs. In our previous study, newly isolated microbial additives used in the present study improved feed efficiency and fecal microflora in weaning pigs (Lee et al., 2021).

Therefore, this study aimed to investigate the effect of microbial additives on the meat quality and Fatty Acid (FA) profiles of growing-finishing pigs.

2. Materials and Methods

2.1. Slaughter procedure

The animal experimental protocols were conducted at Booheong pig farm (Changyeong, South Korea) and approved by the Animal Care and Use Committee of Gyeongsang National University (Jinju, South Korea). A total of 180 growing-finishing pigs (Landrace x Yorkshire x Duroc; mixed sex; 14 weeks of age; 58.0 ± 1.00 kg) were randomly distributed into three treatments with three pens consisting of 20 growing-finishing pigs per pen for 60 days of adaptation and 7 days of collection period. The microbial additive used in the present experiment consisted of *Lactobacillus plantarum* SK3121 (9.0 log10 cfu/g), *Bacillus subtilis* SK877 (9.0 log10 cfu/g), *Bacillus subtilis* BBG-B5 (9.0 log10 cfu/g), and *Saccharomyces cerevisiae* SK3587 (9.0 log10 cfu/g), which was tested in our previous study (Lee et al., 2021). This product was purchased from Bigbiogen (Anseong, South Korea). The experimental treatments were as follows: 0% (basal diet), 0.5% (basal diet+0.5% microbial additive), and 1.0% (basal diet+1.0% microbial additive). The basal diet consisted of 18.8% crude protein (CP) and 3,100 ME (kcal/kg).

Table 2. Effects of microbial additive supplementation on the pH, TBARS and meat color of loin

Item	Supplement, %[1]			SEM	Contrast[2]	
	0	0.5	1.0		L	Q
pH	5.67	5.64	5.51	0.104	0.454	0.212
TBARS[3] (mg MDA/kg)	0.13	0.11	0.10	0.075	0.791	0.415
Meat color						
L* (Lightness)	51.9[b]	51.6[b]	54.8[a]	1.499	0.001	0.004
a* (Redness)	6.41[b]	6.45[b]	6.68[a]	0.583	0.033	0.673
b* (Yellowness)	2.75[a]	2.17[b]	2.26[b]	0.418	0.083	0.011

[1]Supplemented microbial additive at 0, 0.5, and 1.0% of basal diet.
[2]L: linear effect, Q: quadratic effect.
[3]TBARS: thiobarbituric acid reactive substances.
[a,b]Means in the same row with different superscripts differ significantly ($P<0.05$).

The pigs were weaned and housed in a pen with automatically controlled light and temperature conditions and fully slatted floors with concrete panels. Each pen was equipped with a one-hole feeder and nipple waterer to provide diets and water that available *ad libitum*. Pigs were fed twice a day at 0900 and 1700 h. At the end of the feeding trial, all animals (n=180) were slaughtered in the manner approved by the Ministry of Agriculture, Food and Rural Affairs, South Korea. After plucking and eviscerating, all carcasses were chilled at 2°C for 24 h, after which the loin was obtained from each using a perpendicular cut to the backbone between the seventh and eighth ribs.

2.2. Analysis

The loin moisture, CP, and crude fat contents were analyzed according to the AOAC methods (1990). The cooking loss was determined by calculating the weight loss during cooking. The sample in a plastic bag was boiled in a water bath at 90°C for 30 min, and then, the cooking loss was calculated as the percentage weight loss based on the initial sample weight. Drip loss was determined according to the methods of Jama et al. (2008) and was measured as the change in percent weight after 24 h storage at 4°C. For WHC, loin samples (10 g) were placed into a propylene centrifugal vial and heated in a water bath for 30 min at 70°C. After cooling to room temperature, the samples were centrifuged at 1000 ×g for 10 min at 4°C to determine the amount of gravy. Shear force (kg/cm^2) was measured using an Instron Universal Testing Machine (Model 4443, Instron, USA) with a V-shaped shear blade. From each sample, 1.3 cm diameter cores were obtained from the samples cooked to 70℃ internal temperature for 30 min. The pH was measured on homogenates of 3 g muscle in 27 ml of deionized water using a pH meter (MP 230, Mettler Toledo Co., Switzerland). The thiobarbituric acid reactive substances (TBARS) were assessed according to the procedure described by Buege and Aust(1978). A 5 g loin sample was weighed into a 50 mL test tube and homogenized with 15 mL of deionized distilled water using a Plytron homogenizer for 10 sec at the highest speed (T25 basic, IKA, Selangor, Malaysia). The sample homogenate (1 mL) was transferred to a disposable test tube, and butylated hydroxyanisole (10%, 50 uL) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) solutions (2 mL) were added. The sample was mixed using a vortex mixer and then incubated in a boiling water bath for 15 min to develop color. The sample was cooled down, and then the ab-

Table 3. Effects of microbial additive supplementation on fatty acid profiles of loin (% of total FA)

Item	Supplement, %[1]			SEM	Contrast[2]	
	0	0.5	1.0		L	Q
C14:0	1.16	1.16	1.18	0.018	0.869	0.457
C16:0	26.1	26.3	26.0	2.071	0.336	0.184
C16:1n-7	3.20	3.02	2.93	0.501	0.144	0.834
C17:1	0.33	0.32	0.32	0.009	0.939	0.927
C18:0	14.1	14.6	14.3	2.806	0.153	0.717
C18:1n-9	38.2	38.5	38.7	2.259	0.087	0.553
C18:2n-6	13.3	12.3	12.5	1.708	0.275	0.513
C20:0	0.21	0.20	0.20	0.062	0.641	0.794
C18:3n-3	0.78^{b}	0.87^{a}	0.88^{a}	0.136	0.115	0.552
C20:4n-6	2.28	2.44	2.38	0.738	0.775	0.740
C20:5n-3	0.12^{b}	0.12^{b}	0.15^{a}	0.018	0.005	0.380
C22:5n-3	0.19^{b}	0.14^{b}	0.30^{a}	0.087	0.119	0.015
C22:6n-3	0.07^{b}	0.05^{b}	0.17^{a}	0.054	0.080	0.006
SFA[3]	41.6	42.3	41.7	4.021	0.440	0.056
MUFA[4]	41.4	41.9	42.0	3.613	0.352	0.078
PUFA[5]	16.7	15.9	16.4	2.026	0.346	0.105
PUFA/SFA	0.40	0.38	0.39	0.072	0.261	0.592
n-6	15.6	14.7	14.9	1.947	0.089	0.653
n-3	1.16^{b}	1.18^{b}	1.50^{a}	0.153	<0.001	0.295
n-6/n-3	13.4^{a}	12.5^{ab}	9.92^{b}	2.077	0.002	0.428

[1]Supplemented microbial additive at 0, 0.5, and 1.0% of basal diet.
[2]L: linear effect, Q: quadratic effect.
[3]Saturated fatty acids.
[4]Mono-unsaturated fatty acids.
[5]Poly-unsaturated fatty acids.
[a,b]Means in the same row with different superscripts differ significantly ($P<0.05$).

sorbance was read at 531 nm with a blank containing 1 mL of double-distilled water and 2 mL of TBA/TCA solution. The amount of TBARS was expressed as mg of malondialdehyde (MDA) per kg of sample. Meat color was measured on the muscle surface from each sample after exposing the meat surface to the air for 30 min for blooming, using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Tokyo, Japan) standardized with a white calibration plate (Y=93.5; x=0.3132, y=0.3198). For FA analysis, the loin sample (1 g) was freeze-dried (LABCONCO, FreeZone 12plus) and methylated using the direct methylation method described by Jenkins et al.(2001). The extracted FA methyl ester was analyzed with a gas chromatograph (Varian 450-GC, Varian) equipped with an auto-sampler (CP-8400; Varian), a flame ionization detector, and a Varian capillary column (CP-Sil 88 for FA Methyl Esters, 100 m × 0.25 mm × 0.2 μm). The carrier gas was nitrogen. The injector and detector were maintained at 230°C. The oven temperature was initially set at 120°C for 1 min, increased by 5°C/min up to 190°C, held at 190°C for 30 min, increased again by 2°C/min up to 220°C, and held at 220°C for 40 min. The peaks of samples were identified, and concen-

trations were calculated based on the retention time and peak area of known standards.

2.3. Statistical analysis

The data were analyzed by the analysis of variance (ANOVA) using the generalized linear model (GLM) procedure of SAS (Statistical Analysis System, version 8.2). Tukey test was used to identify differences among treatments. Its model was $Yij = m + Ti + eij$, where Yij = response variable, m = overall mean, T = effect of treatment, and eij = error effect. Polynomial contrasts (linear and quadratic effects) were also used to evaluate the effects of increasing microbial additive supplementation levels. Significance was determined at $P \leq 0.05$, while tendency was considered at $0.05 < P \leq 0.10$.

3. Results and Discussion

3.1. Chemical and physicochemical characteristics of pork loin

The results of the chemical and physicochemical characteristics of pork loin are shown in Table 1. The moisture and crude fat contents were lower and higher in microbial additive groups compared with the control group ($P < 0.05$), respectively. However, CP, cooking loss, drip loss, WHC, and shear force in loin muscle showed no significant differences among treatments ($P > 0.05$). Jin et al.(2006) and Kim et al.(2007) reported that supplementation with microbial additives increased the crude fat content. In Korea, it is generally reported that meat with high crude fat content has good flavor, aroma, and tenderness in meat(Kim et al., 2007). In the present study, crude fat was increased linearly (P=0.002) with increasing levels of microbial additive, which is thought to provide excellent palatability to the meat. Our results showed that drip loss decreased linearly (P=0.018) with increasing levels of microbial additive, but WHC increased linearly (P=0.024) with increasing levels of microbial additive. Furthermore, the cooking loss tended to decrease linearly (P=0.079) with increasing levels of microbial additive. Drip loss and WHC are commonly assessed as indicative of meat quality, with lower drip loss value indicating better meat quality (Balasubramanian et al., 2016; Liu et al., 2013). The current study showed that drip loss was lower in microbial additive supplementation groups compared with the control group, indicating that probiotics reduced lipid peroxidation in the muscles by maintaining cell membrane integrity and reducing water loss rate, thereby affecting WHC (Balasubramanian et al., 2016). Junka et al.(2005) reported that supplementation with microbial additives decreased the cooking loss, but increased WHC. In addition, Liu et al.(2013) showed that supplementation with multi-probiotics reduces the drip loss and cooking loss of the loin muscle.

3.2. pH, TBARS, and meat color of pork loin

The results of the pH, TBARS, and meat color of pork loin are shown in Table 2. The pH and TBARS in loin muscle showed no significant differences among treatments ($P > 0.05$). However, the lightness (L^*) and redness (a^*) were the highest in the 1.0% supplementation group compared with the other treatments ($P < 0.05$). The yellowness (b^*) was highest in the control group compared with the other treatments ($P < 0.05$). In general, meat pH is a direct reflection of the muscle acid content, which affects meat shear force, drip loss, WHC, and meat color(Honikel, 1987; Swan and Boles, 2002; Chen et al., 2009; Yoo et al., 2018). Meat with a high pH has a more compact muscle structure, which limits oxygen diffusion and light absorption (Swan and Boles, 2002). However, the present study showed that pH of the loin muscle showed no significant differences among treatments. In the present study, TBARS showed no significant difference among treatments, but Li and Chen

(2009) reported that microbial additives significantly decreased the MDA content of the muscles, inhibited muscle lipid peroxidation, and improved meat quality. Meanwhile, meat color is important because it affects the first impressions of meat by consumers. In the current study, the L^* and a^* values increased by microbial additive supplementations. Jiang (2011) showed that supplementation with microbial additives increased the L^* and a^* values, slightly improving the overall meat quality. Cho et al.(2005) observed an increase in the a^* value when pig diets were supplemented with microbial additives.

3.3. Fatty acid profiles of pork loin

The results of FA profiles of pork loin are shown in Table 3. Palmitic acid (C16:0) and stearic acid (C18:0) were the main triglycerides of saturated FAs (SFAs), and they did not show significant differences among the treatments. The contents of SFAs, mono-unsaturated FAs (MUFA), and poly-unsaturated FAs (PUFAs) were no significant difference among the treatments. Linolenic acid (C18:3n-3), eicosapentaenoic acid (C20:5n-3), docosapentaenoic acid (C22:5n-3), and docosahexaenoic acid (C22:6n-3) contents were highest in 1.0% supplementation group compared with the other treatments ($P<0.05$). In addition, oleic acid (C18:1n-9) content tended to increase ($P=0.087$) linearly with increasing levels of microbial additive. Although there was no significant difference, n−6 FAs content tended to decrease ($P=0.089$) linearly with increasing levels of microbial additive. In contrast, n−3 FAs content was increased ($P<0.001$) linearly with increasing levels of microbial additive. The n-6/n-3 ratio significantly decreased ($P=0.002$) linearly with increasing levels of microbial additive. The composition of FAs strongly influences meat quality because FA profiles differ in the hardness or cohesiveness of fats. In addition, it influenced the storage properties of meat according to the ratio of unsaturated FAs (UFAs) and SFAs (Wood et al., 2008). In general, high SFAs content has been reported to improve lipid oxidation and color stability (Du et al., 2000). However, Shantha and Decker (1994) reported that consuming meat with a high ratio of essential FAs and UFAs with low SFAs has a positive health benefit for humans, mainly in protecting against cardiovascular disease. In the present study, the SFAs and PUFAs in loin muscle showed no significant differences among treatments; thus, our observations did not support these findings. Kalavathy et al.(2006) also showed that feeding broiler chickens with microbial additives had no effect on individual PUFAs of the muscle when compared with no microbial additives. Meanwhile, our results showed that the content of linolenic acid (C18:3 n-3) was higher in microbial additive supplementation groups compared with the control group, which was thought to be due to the inclusion of probiotics such as *Saccharomyces,* which can increase the linolenic acid content and UFA/SFA ratio in pectoral meat through a positive effect on the intestinal flora (Endo et al., 1999). Connor (2000) reported that high concentrations of n-3 FAs in meat with low concentrations of blood cholesterol and triglycerides could prevent cardiovascular diseases. In addition, eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) play important role in avoiding hyperlipidemia and type 2 diabetes (Mori et al., 2000; Woodman et al., 2002). In the present study, the concentrations of EPA, DHA, and n-3 FAs were the highest in 1.0% microbial additive supplementation, which was thought to improve meat quality and human health.

4. Conclusion

This study indicates that drip loss decreased linearly with increasing levels of microbial additive, but WHC increased linearly with increasing levels

of microbial additive. The L^* and a^* values were highest in 1.0% supplementation group compared with the other treatments. The b^* value was highest in the control group compared with the other treatments. The concentrations of EPA, DHA, and n-3 FAs were highest in 1.0% microbial additive supplementation, which was thought to improve meat quality and human health. Therefore, it could be recommended at 1% supplementation of microbial additives to improve the meat quality of pigs.

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