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Abstract

The objective of this study was to determine effects of humic acid (HA) and blueberry leaf powder (BLP) supplementation in pig feed on productivity, blood profiles, and meat quality characteristics of *longissimus* muscle. The experimental design included six treatments: 1) CON, No addition; 2) T1, blueberry leaf powder 0.1%; 3) T2, blueberry leaf powder 0.2%; 4) T3, humic acid 2%; 5) T4: humic acid 2% + blueberry leaf powder 0.1%; and 6) T5: humic acid 2% + blueberry leaf powder 0.2%. HA and BLP supplementation in pig feed significantly increased average daily feed intake (ADFI) values ($p<0.05$). HA supplementation in pig feed had beneficial effects in lipid profiles without altering feed efficiency rate (FER). HA and BLP co-supplementation in pig feed decreased pH in *longissimus thoracis* ($p<0.05$). In addition, sensory characteristics were enhanced when pig feed was supplemented with HA and BLP without causing adverse effects in meat quality. Taken together, addition of HA and BLP in pig feed may produce functional meat products.

Keywords: humic acid, blueberry leaf, organic acid, meat quality characteristics

Introduction

Meat consumption has increased recently in Korea due to increased national income and changes in eating habits. Meat consumption per capita in 2016 was 49.5 kg, of which pork (24.1kg) accounted for 49% of total meat consumption (Key Statistics of Agriculture, Forestry and Livestock Food, 2017). Pork is the mostly consumed meat in Korea, accounting for a considerable portion of the livestock industry. The social demands of functional food are elevated strikingly as consumers seeking well-being lives (Kim et al., 2011). Kwon et al. (2003) have reported that finishing pigs yield higher carcass grade when they are fed an organic material of plant mixtures. Research has been conducted on effects of mugwort, tangerine peel, activated carbon, *Zizyphus vulgaris*, olive oil by-product, grass coal, and rare earth supplementation on growth performance and carcass quality characteristics (Joven et al., 2014).

Both humic acid and blueberry leaf are functional materials. Humic acid has been studied extensively due to its positive effects on meat quality and productivity in the past. The addition of humic acid to feed is known to promote germination by increasing nutrient intake (David et al., 1994). Griban et al. (1988) have also reported that humic acid is a stable organic substance to promote excellent water retention and water holding capacity. In addition, when humic acid is supplied to pig feed, humic acid has roles as antiflogistic, antitoxic, antibacterial, and antiviral agent. Thus, humic acid may be applied for therapy and prevention (Klocking 1994).

Blueberry is known to have positive effects on human health and disease prevention because it contains anthocyanin with antioxidant activities (Brownmiller et al., 2008). Chemical content of blueberry is known to account for about 15% dry materials that has high contents of anthocyanin and flavonoid (Ryszard 2002; Skupien 2006). In addition, blueberry contains sugar, acid, vitamin C, vitamin E, dietary fiber, arbutin, and trace elements such as potassium (K), Ferrum (Fe), zinc (Zn), and manganese (Mn) (Zhang et al., 2011). Among them, anthochthoic acid, a physiologically active substance, is an applicable feed additive and biocompatible biodegradable polysaccharide with excellent film forming ability, antibacterial function, and antioxidant activity (Guiyun et al., 2014).

However, there is a lack of research on effects of blueberry leaf and humic acid supplements on productivity, blood profiles, and meat quality as pig feed additives. Therefore, the objective of this study was to determine effects of blueberry leaf and humic acid supplements on productivity, blood, and meat quality as feed additives in pigs.

Materials and Methods

Animals and Dietary Treatments

All animal studies were approved by Institutional Animal Care and Use Committee (IACUC) of Chungbuk University. Landrace × Yorkshire × Duroc cross hybrid growing pigs (n = 120) were examined. Their initial body weight was ~60 kg. Feeding study was conducted for 7 weeks. The experimental design consisted of six treatments: 1) CON (basic feed); 2) T1, basic feed + 0.1% blueberry leaf powder; 3) T2, basic feed + 0.2% blueberry leaf powder; 4) T3, basic feed + 2% humic acid powder; 5) T4, basic feed + 2% humic acid powder+ 0.1% blueberry leaf powder; and 6) T5, basic feed + 2% humic acid powder + 0.2% blueberry leaf powder. Each treatment was assigned with 20 pigs. Feeding experiments were carried out on Han-Don Union Test Farm in Chungbuk, Korea. Test feeds were prepared with HA and BLP based on requirements of NRC (2012). Water was adjusted to be freely eaten using an automatic water dispenser. Body weight was measured at initiation, 4 weeks, and at the end of the study period (7 weeks) for each treatment group. Weight gain (WG) was calculated at the start of the experiment and 7 weeks after treatment. Feed intake was calculated by subtracting the remaining amount from the feed provided during body weight measurement. Feeding efficiency was calculated by dividing body weight gain by feed intake. Food intake was calculated by subtracting the remaining amount from the feed amount during body weight measurement, and the feed efficiency was calculated by dividing the body weight gain by the feed intake. Hematological analysis was performed by collecting blood from the jugular vein at the end of the study period (7 weeks). The slaughter age is six months, the *longissimus thoracis* between the 6th and 12th rib on the left side of the carcass was resected 24 h after slaughter and analyzed. Procine *longissimus thoracis* were packed into polyethylene bags using vacuum and placed at 4℃ for 14 days. The pH measured during storage 0, 7 and 14 days. The proximate composition, water holding capacity (WHC), meat color, drip loss, cooking loss, sensory evaluation, and subjective evaluation were performed or measured for three replicates at 0 day.

Analysis items

FER (Feeding efficiency rate)

Body weight and food intake were measured daily, respectively. Feeding efficiency was calculated as follows: $\frac{Daily\ weight\ gain}{Daily\ food\ intake}$.

Hematological parameters

White blood cell (WBC), red blood cell (RBC), glutathione, triglyceride, lymphocyte, and total cholesterol levels were analyzed using K3EDTA treated plasma (ADVIA 120, Bayer, USA).

Proximate analysis

Moisture, protein, lipid, and ash contents of *longissimus thoracis* were determined according to previously described A.O.A.C method (2012).

pH

longissimus thoracis (10 g) was homogenized in 100 mL deionized water for 30 seconds at 7,000 rpm with a blender (Bihon seiki, Ace, Japan) and pH was measured with a Mettler. Delta 340 pH meter (Mettler-toledo, Ltd., UK).

Meat color

Surface color of *longissimus thoracis* was measured with a Spectro colormeter (Model JX-777, Color Techno System Co., Japan) standardized with a white plate (L *, 94.04; a *, 0.13; b *, -0.51). a * and b * values of the Hunter Lab color system using a white fluorescent lamp (D65) (L * = brightness, a * = redness, b * = yellowness). The meat color was indicated with L^*, a^*, and b^* value represented lightness of the Hunter lab color coordinates, redness, and yellowness, respectively.

WHC (Water holding capacity)

WHC was used as 0.5 g of crushed *longissimus thoracis*. WHC was calculated as altered weight of *longissimus thoracis* before and after centrifugation (Laakkonen et al., 1970).

Cooking loss

Cooking loss of *longissimus thoracis* was measured using a ~3 cm thick *longissimus thoracis* muscle slice (150 ± 5 g). *longissimus thoracis* slice was vacuum-packed in a polypropylene bag and incubated in a water bath at 70 ° C for 40 min followed by cooling-down at room temperature for 30 min. Cooking loss was determined based on decreased weight expressed as weight percentage (%) of the initial weight.

Drip loss

After *longissimus thoracis* slices (2 cm thick) were shaped into a circular shape (weight 100 ± 5g), they were placed in a vacuumed polypropylene bag at 4 ° C for 24 hr. Drip loss was calculated as weight percentage (%) of the initial sample weight.

Subjective evaluation

Five well-trained in-house tasting panelists evaluated sensory attributes of marbling, texture, meat color, and pork characteristics, using on a 5-point scale for 3 independent trials: 1- extremely low, extremely tough, extremely bright, severe PSE muscle, 5- extremely high, extremely tender, extremely dark, severe DFD muscle.

Sensory characteristics

Sensory characteristics of *longissimus thoracis* were determined with four distinctive sensory tests. Sensory scores were evaluated for four items: flavor, tenderness, juiciness, and total acceptability. Each item was scored in 5-point scale ranging from 1 point (very bad flavor, very tough, very dry, very good total acceptability) to 5 point (very good flavor, very soft, very succulent, very bad total acceptability).

Statistical analysis

Results were analyzed with SAS program (2012) using analysis of variance (ANOVA) and Duncan's multiple test. Statistical significance was set at $p < 0.05$.

Results and Discussion

Effect of HA and BLP consumption on growth rate and feeding efficiency

Consumption of HA and BLP did not significantly alter body weight or average daily gain (ADG) during feeding period. Early phase (0-4 wk) of average daily feed intake (ADFI) was significantly higher in group with HA feeding while late phase (5-7 wk) ADFI was higher in group with BLP feeding (p<0.05). Overall ADFI value (0-7 wk) was higher in T1 (0.1% BLP) than that of the control. Early phase of feeding efficiency rate (FER) was higher in T2 (0.2% BLP only supplemented) than that in T5 (co-supplementation with 2% HA and 0.2% BLP). Late phase FE was significantly higher in HA supplement groups (T4 and T5) than that of the control (p<0.05). However, there was no significant difference in FER among groups during the overall study period. Organic substance of HA may induce protective coating on the mucosal epithelium of the gastrointestinal tract of pig and chicken (Wang et al., 2008). Organic materials of HA can also form an epithelial barrier to provide protection against infections and toxins in animal feed (Huck et al., 1991). In addition, ammonia material of HA as a pig feed additive can increase the efficiency of excretion by increasing digestion absorption rate (Pisarikova et al., 2010). In the present study, HA was also found to be effective in increasing the efficiency of feces excretion as a feed additive for pigs. Ji et al. (2006) have also reported that feed supplemented with HA can increase ADG and FE of pigs.

Effect of HA and BLP consumption on porcine hematological parameters

Consumption of T5 (2% HA with 0.2% BLP) showed a cholesterol lowering effect. Triglyceride levels did not differ among treatment groups. Pigs that consumed 0.2% BLP (T2 and T5) had higher high-density lipoprotein cholesterol (HDL/C) levels than those in other groups. HA and BLP consumption did not alter low-density lipoprotein cholesterol (LDL/C). HDL/C ratio in finishing pig was significantly elevated with 0.2% BLP consumption (p<0.05).

White blood cells (WBC) were reduced by T5 consumption. All treatments showed no significant difference in counts of red blood cells (RBC) or lymphocytes or glutathione level. Catechins in blueberry leaf have potency to improve lipid profiles in blood by reducing total cholesterol while increasing HDL/C (Muramatsu et al., 1986). The peat moss component of HA is a dynamic cation exchanger. Therefore, HA might be able to improve the immune system (Wenk, 2003). Oh et al. (2018) have reported that consumption of HA does not alter WBC in pigs. In the present study, we found that BLP and HA had beneficial effects on blood lipid profiles and immune function, respectively.

Effect of HA and BLP consumption on proximate composition of porcine *longissimus thoracis*

Moisture content of *longissimus thoracis* ranged from 72.52 to 73.89 % when pig feed was supplemented with HA and BLP. T4 treatment elevated moisture contents in porcine *longissimus thoracis*. HA and BLP consumption did not alter porcine protein content in *longissimus thoracis* (ranging from 19.87 to 20.83 %). Moreover, HA and BLP consumption did not significantly change *longissimus thoracis* fat content (6.21 to 6.98 %). Ash contents in porcine *longissimus thoracis* were higher in pigs that consumed 2% HA mixed with BLP (T4 and T5) than those in others. BLP treatment did not alter moisture content, consistent with report of Leusink et al. (2010). However, BLP with extra HA may increase water content in porcine *longissimus thoracis*. Castellini et al. (2002) have also suggested that HA consumption may increase moisture content since HA has potency to improve solubility. In similar setting of other studies, HA and/or BLP consumption is not a limiting factor of porcine fat or protein content (Ozturk et al., 2012).

Effect of HA and BLP consumption on meat quality of porcine *longissimus thoracis*

HA and BLP consumption markedly decreased pH of porcine *longissimus thoracis* during 14 d of storage. Moreover, HA consumption during the storage period showed lower tendency than treatment with BLP consumption. Lightness (L^*) and redness (a^*) values of T5 treatment were significantly lower than those of the control. b^*values were significantly lower in T5 (2% HA+BLP) than those of the control ($p<0.05$). HA and BLP consumption did not alter water holding capacity (WHC) or drip loss value of *longissimus thoracis*. Cooking loss of *longissimus thoracis* in the group with 2% HA consumption (T3) was higher than that with 0.2% BLP consumption. In our study, HA and BLP reduced pH of meat. This might be due to formation of propionic acid and lactic acid (Wanapat et al., 2011). It has been reported that meat color is associated with pH, temperature, light, oxygen, ascorbic acid, enzymes, sugars, degradation products, and ions (Esenbuga et al., 2008). Anthocyanins in blueberry can decrease the a^* and b^* values when it is added to feed (Jimenez-Aguilar et al., 2011; Smith et al., 2000). However, in our study, there was no significant difference in L^*, a^*, or b^*values. This might be due to different anthocyanin contents in blueberries. Consumption of vegetable and organic materials can increase water uptake (Chung et al., 2018). Water retention ability of dietary fiber added with BLP might be higher due to improvement of moisture binding ability of cellulose as component of dietary fiber. However, in our study, WHC was intact. This might be because we added lower amount of BLP into the feed.

Effect of HA and BLP consumption on sensory characteristics and subjective evaluation of porcine *longissimus thoracis*

Either HA or BLP consumption increased tenderness of porcine *longissimus thoracis* significantly than the control ($p<0.05$). Feeding with extra HA alone resulted in more juiciness than T1 and T5 treatments. HA and BLP consumption increased flavor (2.74 to 3.42) than normal chow consumption (CON; 2.26). The group with consumption of 2% HA (T3) had highly acceptability than the control. Regarding subjective evaluation, HA and BLP consumption significantly decreased marbling values of porcine *longissimus thoracis* (p<0.05). HA supplementation significantly increased preference compared to the control (p<0.05). HA and BLP consumption did not significantly impair preference of meat texture or pork characteristics. Anthocyanin of blueberry and citric acid of HA are known to have high water solubility (Chung et al. 2018). In the present study, consumption of HA and BLP might have improved juiciness, flavor, and total acceptability due to increased water solubility. It is known that the L^*value can be decreased by pH reduction characteristic of organic acid (Qiao et al. 2001). Therefore, subjective evaluation of HA and BLP consumption showed low color point and low marbling point.

Conclusions

The purpose of this study was to determine effects of HA and BLP supplementation in pig feed on productivity and meat quality characteristics. HA and BLP consumption did not significantly change FE in the overall study period but improved. HA and BLP consumption reduced cholesterol levels in hematological parameters. In addition, sensory characteristics in groups supplemented with HA and BLP were superior to those of the control. Therefore, HA and BLP might be useful for the development of healthy functional meat processing product (i.e., low-cholesterol meat) as excellent additives to improve productivity and meat quality of finishing pigs.

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Table 1. Changes Growth performances of finishing pigs supplemented with humic acid and blueberry leaf powders.

	day	Treatments[1)]					
		CON	T1	T2	T3	T4	T5
Weight(kg)	0 week	60.4	60.3	60.1	60.2	60.4	60.5
	4 weeks	83.4	83.6	83.1	84.3	84.6	84.3
	7 weeks	102.9	105.5	103.9	105.4	106.2	106.0
[2)]ADG (kg)	0~4 weeks	0.820	0.832	0.822	0.860	0.865	0.849
	5~7 weeks	0.928	1.043	0.993	1.006	1.025	1.039
	0~7 weeks	0.867	0.922	0.895	0.922	0.934	0.930
[3)]ADFI (kg)	0~4 weeks	2.610^{c}	2.674^{c}	2.477^{d}	2.795^{b}	2.807^{b}	3.052^{a}
	5~7 weeks	3.232^{b}	3.498^{a}	3.658^{a}	3.275^{ab}	3.247^{ab}	3.255^{ab}
	0~7 weeks	2.876^{b}	3.012^{a}	2.988^{ab}	2.986^{ab}	2.988^{ab}	2.968^{ab}
[4)]FER (kg)	0~4 weeks	0.341^{ab}	0.314^{ab}	0.336^{a}	0.308^{ab}	0.308^{ab}	0.278^{b}
	5~7 weeks	0.288^{b}	0.306^{ab}	0.278^{b}	0.307^{ab}	0.315^{a}	0.319^{a}
	0~7 weeks	0.301	0.307	0.303	0.309	0.312	0.313

[1)] CON: No addition, T1: blueberry leaf powder 0.1%, T2: blueberry leaf powder 0.2%, T3: humic acid 2%, T4: humic acid 2% + blueberry leaf powder 0.1%, T5: humic acid 2% + blueberry leaf powder 0.2%

[2)] ADG : average daily gain

[3)] ADFI : average daily feed intake

[4)] FER : feed efficiency rate

Means in the same row with different letters (a-d) are significantly different ($p<0.05$)

Table 2. Changes Blood profiles of finishing pigs supplemented with humic acid and blueberry leaf powders

	Treatments[1)]					
	CON	T1	T2	T3	T4	T5
Cholesterol, mg/dL	86.6^{a}	83.3^{ab}	80.8^{ab}	78.0^{ab}	75.8^{ab}	74.8^{b}
Triglyceride, mg/dL	38.5	35.9	37.7	38.7	38.1	34.3
[2)]HDL/C, mg/dL	42.5^{c}	43.4^{bc}	48.7^{ab}	42.6^{c}	43.0^{c}	50.3^{a}
[3)]LDL/C, mg/dL	44.2	39.9	39.1	40.7	40.8	38.4
[4)]WBC, 10^{3}/µl	19.2^{a}	19.0^{a}	18.9^{a}	15.9^{ab}	16.3^{ab}	15.1^{b}
[5)]RBC, 10^{6}/µl	6.5	6.6	6.5	6.8	7.0	6.7
Lymphocyte, %	48.8	44.2	43.4	44.6	43.4	43.7
Glutathion, uM	0.24	0.29	0.35	0.23	0.27	0.31

[1)] CON: No addition, T1: blueberry leaf powder 0.1%, T2: blueberry leaf powder 0.2%, T3: humic acid 2%, T4: humic acid 2% + blueberry leaf powder 0.1%, T5: humic acid 2% + blueberry leaf powder 0.2%

[2)] HDL/C : High-density lipoprotein cholesterol

[3)] LDL/C : Low-density lipoprotein cholesterol

[4)] WBC : White blood cell

[5)] RBC : Red blood cells

Means in the same row with different letters (a-c) are significantly different (p<0.05)

Table 3. Proximate compositons of *longissimus thoracis* from finishing pigs supplemented with humic acid and blueberry leaf powders

	Treatments[1)]					
	CON	T1	T2	T3	T4	T5
Moisture	72.29 ±2.02[b]	72.52 ±2.72[ab]	72.53 ±1.81[ab]	73.06 ±2.31[ab]	73.89 ±3.09[a]	72.85 ±2.51[ab]
Protein	20.58 ±1.39	20.69 ±1.83	20.67 ±0.97	19.87 ±1.77	19.93 ±1.57	20.83 ±0.99
Fat	6.72 ±1.23	6.57 ±2.24	6.43 ±1.36	6.94 ±1.21	6.21 ±0.95	6.98 ±0.57
Ash	1.16 ±0.08[b]	1.12 ±0.13[b]	1.11 ±0.08[b]	1.18 ±0.13[b]	1.27 ±0.11[a]	1.32 ±0.13[a]

[1)] CON: No addition, T1: blueberry leaf powder 0.1%, T2: blueberry leaf powder 0.2%, T3: humic acid 2%, T4: humic acid 2% + blueberry leaf powder 0.1%, T5: humic acid 2% + blueberry leaf powder 0.2%

Means in the same row with different letters (a-b) are significantly different (p<0.05)

Table 4. Meat quality characteristics of *longissimus thoracis* from finishing pigs supplemented with humic acid and blueberry leaf powders

		Treatments[1)]					
		CON	T1	T2	T3	T4	T5
pH	0 day	5.95 ±0.17[a]	5.75 ±0.14[b]	5.70 ±0.09[b]	5.61 ±0.11[c]	5.61 ±0.10[c]	5.59 ±0.12[c]
	7 day	5.77 ±0.19[a]	5.63 ±0.13[b]	5.58 ±0.06[bcd]	5.56 ±0.04[cd]	5.61 ±0.05[bc]	5.52 ±0.11[d]
	14 day	5.92 ±0.20[a]	5.78 ±0.05[b]	5.65 ±0.01[c]	5.55 ±0.06[cd]	5.60 ±0.02[c]	5.43 ±0.08[d]
Hunter color	L	61.01 ±3.95[a]	61.54 ±4.22[a]	60.08 ±4.44[ab]	61.42 ±3.52[a]	61.85 ±4.61[a]	58.94 ±3.99[b]
	a	5.89 ±1.83[a]	5.23 ±1.86[a]	5.16 ±1.48[a]	5.16 ±1.30[a]	5.27 ±1.57[a]	4.11 ±1.39[b]
	b	9.86 ±1.12[a]	9.60 ±1.16[a]	9.41 ±1.04[a]	8.92 ±0.96[bc]	8.51 ±0.86[cd]	8.30 ±0.94[d]
Water holding capacity (%)		63.92 ±9.07	65.99 ±6.35	65.57 ±4.07	62.67 ±9.31	65.29 ±10.24	65.94 ±3.96
Drip loss(%)		2.19 ±0.65	2.26 ±0.92	2.61 ±0.84	2.98 ±0.77	2.43 ±0.7	3.52 ±1.36
cooking loss(%)		22.53 ±2.51[ab]	21.44 ±3.33[ab]	20.36 ±7.51[b]	23.58 ±2.57[a]	23.01 ±3.16[ab]	22.22 ±2.65[ab]

[1)] CON: No addition, T1: blueberry leaf powder 0.1%, T2: blueberry leaf powder 0.2%, T3: humic acid 2%, T4: humic acid 2% + blueberry leaf powder 0.1%, T5: humic acid 2% + blueberry leaf powder 0.2%

Means in the same row with different letters (a-d) are significantly different (p<0.05)

Table 5. Sensory characteristics and subjective evaluation of *longissimus thoracis* from finishing pigs supplemented with humic acid and blueberry leaf powders

		Treatments[1)]					
		CON	T1	T2	T3	T4	T5
Sensory characteristics	Tenderness	2.50 ±1.04[b]	3.05 ±0.89[a]	3.06 ±0.68[a]	3.54 ±0.80[a]	3.20 ±0.83[a]	3.03 ±0.96[a]
	Juiciness	3.09 ±0.88[ab]	2.94 ±1.10[b]	3.18 ±0.81[ab]	3.64 ±0.79[a]	3.05 ±1.09[ab]	2.80 ±1.13[b]
	Flavor	2.26 ±0.75[c]	2.74 ±1.03[bc]	3.10 ±0.85[ab]	3.42 ±0.93[a]	3.03 ±0.97[ab]	3.23 ±1.19[ab]
	Total Acceptability	2.88 ±0.97[b]	3.09 ±0.94[ab]	3.14 ±0.75[ab]	3.57 ±0.85[a]	3.22 ±0.99[ab]	2.98 ±1.17[ab]
Subjective evaluation	Mabling	2.88 ±0.68[a]	2.11 ±0.89[b]	1.97 ±0.65[b]	2.16 ±1.01[b]	2.05 ±0.88[b]	1.80 ±0.66[b]
	Color	3.42 ±0.88[a]	3.00 ±0.87[ab]	2.97 ±0.61[ab]	2.90 ±0.70[b]	2.88 ±0.59[b]	2.91 ±0.83[b]
	Texture	3.45 ±0.49	3.59 ±0.67	3.35 ±0.49	3.50 ±0.67	3.48 ±0.70	3.40 ±0.49
	Pork characteristics	3.35 ±0.83	3.46 ±0.64	3.22 ±0.65	3.23 ±0.68	3.09 ±0.69	3.11 ±0.71

[1)] CON: No addition, T1: blueberry leaf powder 0.1%, T2: blueberry leaf powder 0.2%, T3: humic acid 2%, T4: humic acid 2% + blueberry leaf powder 0.1%, T5: humic acid 2% + blueberry leaf powder 0.2%

Means in the same row with different letters (a-c) are significantly different (p<0.05)