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Effects of dietary natural and fermented herb combination on growth performance, carcass traits and meat quality in grower-finisher pigs

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ABSTRACT

The effects of an herb combination (pomegranate, *Ginkgo biloba*, licorice) in natural (NPGL) or fermented (FPGL) form administered as 0.4% of the basal diet on the performance and meat quality of grower-finisher pigs were evaluated. Dietary supplementation with NPGL or FPGL reduced the feed intake and back fat thickness of pigs, while increasing lean production. Serum IgG was higher in the FPGL supplemented group. Remarkably, ingestion of NPGL and FPGL reduced the ether extract in the *longissimus dorsi* muscle (LDM) with increased moisture, whereas the cholesterol was lower in the NPGL group. Dietary supplementation of NPGL and FPGL increased the n − 3 fatty acid in LDM with a reduced ratio of n − 6/n − 3. Both NPGL and FPGL significantly reduced the TBARS value of pig meat when fresh and after 2 and 3 weeks of storage. Overall, dietary NPGL and FPGL improved the quality of pig meat by increasing the n − 3 fatty acid levels while reducing the ether extract and TBARS value.
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1. Introduction

Many scientific studies have recently been conducted to improve the composition and nutritional quality of meat. Special attention has been given to fatty acid composition since it is associated with meat quality (including shelf life and flavor) and of human health concerns (especially saturated fatty acid). Feed additives such as vitamins, minerals and antioxidants have been reported to improve pork nutritional characteristics and oxidative stability (Nuernberg et al., 2002). However, consumer concerns over safety and toxicity regarding synthetic antioxidants and other chemical additives in animal feedstuff (Coronado, trout, Dunsea, & Shah, 2002), motivated current nutritional studies on examination and development of different natural feed additives that have functional properties. A number of herbs and medicinal plant by-products have received attention from animal scientists as feed additives for livestock, because of their functional components and functional activities (Zhang et al., 2013; Reddy, Gupta, Jacob, Khan, & Ferreira, 2007). Phytochemical analyses exposed the bioactive components of pomegranate (*Punica granatum* L.) peels [source of the polyphenols and flavonoids], leaves of *Ginkgo biloba* L. [source of flavonoids, polysaccharides and terpenoids], and licorice (*Glycyrrhiza glabra* L.) root [source of saponins, triterpenes (glycyrrhizin) and flavonoids (liquiritin, isoflavonoids)] along with their antioxidant, immunomodulatory, cholesterol lowering and anti-inflammatory properties (Rajan et al., 2011; Ross, Selvasubramanian, & Jayasundar, 2001; Cao, Zhang, Yu, Zhao, & Wang, 2009; Zhou, Wang, Ye, Chen, & Tao, 2015; Fukai et al., 1998; Katamaya et al., 2011; Asan-Ozusaglam & Karakoca, 2014). Several scientific studies have been conducted to evaluate the dietary effects of pomegranate, *Ginkgo biloba* and licorice alone on broilers, pigs and cattle, especially on growth performance and immunity; however, few of these studies have investigated its effects on meat quality (Cao, Zhang, Yu, Zhao, & Wang, 2012; Katamaya et al., 2011; Shabtay et al., 2008). Moreover, the combined effects of these herbs on growth performance and meat quality have not yet been studied. Combination of these herbs are expected to exert their beneficial effects through their combined chemical and pharmaceutical properties.

An alternative approach to sub-therapeutic antibiotics is use of beneficial microorganisms that are capable of modifying gastrointestinal microbial ecosystems and improving the growth performance of pigs (Dierck, 1989). Recently, fermentation of plant materials with different beneficial microorganisms such as *Lactobacillus* spp., *Saccharomyces cerevisiae*, and *Bacillus* spp. has been widely adopted to develop novel functional feed additives for livestock. It is believed that the process of fermentation promotes functional activities such as antioxidant and antimicrobial activity (Lee, Yang, & Mau, 2008; Cao et al., 2012) and increases the vitamins, enzymes and growth factors of fermented products (Ng, Wang, Wang, Tzeng, & Shyu, 2011). Fermented feed contains large numbers of *Lactobacilli* with high concentrations of lactic acid and other volatile fatty acids and has a low pH. Additionally, fermented medicinal plants or herbs could be better than medicinal plants or beneficial bacteria alone, since animals would benefit from the bioactive components of such plants and the presence of beneficial bacteria in their digestive tract. Several scientists have also reported the beneficial

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effects of fermented medicinal plants on growth performance, immune system and meat quality of broilers and pigs (Kim et al., 2012; Jeong & Kim, 2015; Zhou et al., 2015).

In this study, we supplemented pig diets with an herb combination (pomegranate peel extract, *Ginkgo biloba* L. leaves and licorice root) in natural (NPGL) or fermented (FPGL) form. We then investigated the effects of NPGL and FPGL on the carcass characteristics, fatty acid composition and oxidative stability of *Longissimus dorsi* muscle (LDM). We also investigated the growth performance and serum immunoglobulins in grower-finisher pigs.

2. Materials and methods

2.1. Preparation and characterization of natural and fermented herb combination

To prepare the herbal combination, peels of pomegranate (Goheung-gun cultivar, Korea) were collected, cleaned and rinsed in distilled water (1:1 ratio) to prepare the liquid extract according to the method described by Devatkal, Jaiswal, Jha, Bharadwaj, and Viswas (2013). Green leaves of *Ginkgo biloba* L. and roots of licorice were cleaned, air-dried and powdered using a kitchen grinder. The herb combination contained 30% pomegranate peel extract, 4.5% *Ginkgo biloba* leaf power and 0.5% licorice root powder that was mixed with 35% wheat bran and 30% defatted rice bran. After mixing the ingredients, one part of the combination (used as natural) was dried in an air circulatory tray drier at 60 °C for 48 h to reduce the moisture contents. Another portion of the combination was inoculated with 30% (*v*/*w*) *Lactobacillus plantarum* KCTC 3099 and fermented for 2 days at 37 °C while maintaining 40% moisture in a commercial fermenter (W-1000; Wonbalhyo Industry Co., Incheon, South Korea). The fermented medium was then again inoculated with 30% (*w*/*v*) *Saccharomyces cerevisiae* KCTC 7904 and fermented for 3 days at 37 °C. The fermented sample was subsequently dried in a forced air oven (Doori TEC, Doori TEC, FA, Co., Ltd) at 32 °C for 2 days to reduce the moisture levels. Finally, the natural and fermented herb combination were stored in an air-tight plastic bag until mixed with basal diet. The microbial concentration of FPGL was determine after diluting 1 g with 9 mL of double distilled water (DDW). Approximately 1 h later, 1 mL of the dilution was serially diluted 10-fold in 0.85% NaCl solution, cultured in agar media and the number of colonies were counted. Proximate composition of NPGL and FPGL [crude protein (CP), ether extract (EE), moisture, and total ash] were analyzed according to the method described by the Association of Official Analytical Chemists (AOAC, 2000). Trace mineral contents of NPGL and FPGL were determined using an Atomic Absorption Flame Emission Spectrophotometer (Model AA-6200, Shimadzu, Japan). The fatty acid composition was determined by a direct method for fatty acid methyl ester (FAME) synthesis using a gas chromatograph. The pH of natural and fermented herb combination were analyzed using a digital pH meter (Docu-pH + meter, Sartorius, USA). The type and concentration of fermentable sugars [quantified by HPLC using external standards (Supleco, Belafonte, PA)] and organic acids [quantified by Gas Chromatography (Hewlett Packard HP 6890 GC System, Santa Clara, CA)], concentration of total polyphenols, tannic acid and flavonoids contents (quantified by colorimetric analysis) in NPGL and FPGL were analyzed by a commercial analytical company; the Foundation of Agricultural Technology Commercialization and Transfer (FACT, Suwon-si, Gyeonggi-do, Korea). The analytical results are presented in Tables 1 and 2.

2.2. Animal and experimental design

The experimental protocols and care and management of animals were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee, Sunchon National University. A total of ninety-six crossbred (Durox × Landrace × Yorkshire) growing pigs (Barrows, average 39.28 ± 1.04) were randomly allotted to three dietary treatments groups (four replicates with eight pigs per replication) according to initial body weight for a 10 week experiment. The dietary treatments were a control (basal diet), 0.4% natural herb combination (NPGL) with basal diet and 0.4% fermented herb combination (FPGL) with basal diet. Commercially available corn, wheat and soybean meal based grower and finisher diets were used as the basal diet, which contained all nutrients in the levels recommended by NRC (2012). The additives were added at the expense of equal amount of basal diet in a two days interval. The nutrient composition of the experimental diets are shown in Table 3. All pigs were housed in an environmentally controlled, slatted pig house in 12 adjacent pens (3.0 × 3.0 m) and provided with ad libitum access to feed and water.

Table 1
Microbial concentration and nutrient composition of natural and fermented herb combination.

Item[a]	NPGL	FPGL
Microbial stains in FPB, cfu/g		
Lactobacillus plantarum KCTC 3099	–	2.1×10^8
Saccharomyces cerevisiae KCTC 7904	–	1.0×10^7
Chemical composition, % dry matter		
Moisture	7.75	19.36
Crude protein	10.98	12.08
Crude fat	2.63	2.41
Crude fiber	11.90	9.83
Crude ash	2.62	19.39
Trace minerals, g/kg		
Calcium	11.50	13.17
Iron	0.07	0.06
Magnesium	0.89	2.71
Sodium	0.72	1.40
Fatty acids, g/100 g		
∑ Saturated fatty acid	61.78	46.79
∑ Monounsaturated fatty acid	17.00	22.43
∑ Polyunsaturated fatty acid	21.00	30.64
∑ n − 6 fatty acid	29.53	17.11
Linoleic acid (C18:2n − 6)	27.54	14.16
Arachidonic acid (C20:4n − 6)	0.67	1.07
∑ n − 3 fatty acid	1.12	3.89
Alpha-linolenic acid (C18:3n − 3)	0.50	0.69
Eicosapentaenoic acid (C20:5n − 3)	ND	1.45

ND, not detected.
[a] Data are the means of three replicate analysis.

2.3. Measurements and analyses

2.3.1. Growth performance

Individual pig body weights was recorded at the beginning and end of the experiment to calculate the average daily gain (ADG). The feed

Table 2
Concentrations of fermentable sugars, pH, organic acids, phenolics and flavonoids of natural and fermented herb combination.

Item[a]	NPGL	FPGL
Fermentable sugars, %		
Glucose	5.25	2.61
Fructose	5.92	6.09
Sucrose	0.58	0.00
Lactose	Not detected	Not detected
Maltose	Not detected	Not detected
pH	4.92	3.77
Organic acids, mg/kg		
Lactic acid	316.9	2643.8
Acetic acid	210.5	544.9
Propionic acid	Not detected	Not detected
Total polyphenols, mg/kg	6521.9	6010.1
Tannic acid, mg/kg	1876.2	1193.4
Total flavonoids, mg/kg	6301.7	5979.1

[a] Data are the means of three replicate analysis.

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