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Effect of plant extracts on growth performance and insulin-like growth factor 1 secretion in growing pigs

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ABSTRACT - The objective of the present study is to evaluate the effects of plant extracts on growth performance, nutrient digestibility, and immune blood characteristics in growing pigs. A total of 80 [(Landrace × Yorkshire) × Duroc] pigs with an initial body weight (BW) of 27.31±2.15 kg were used in a 6-wk experiment. Pigs were allotted to one of four treatments (1 - Control (CON) (basal diet); 2 - PE1 (CON + 0.05% plant extracts); 3 - PE2 (CON + 0.10% plant extracts); and 4 - PE3 (CON + 0.15% plant extracts)) in a randomized complete block design according to sex and initial BW. The PE1 and PE2 treatments provided a greater average daily gain than the CON treatment. From weeks 0 to 6 the pigs fed diets PE2 and PE3 showed greater apparent total tract digestibility of dry matter than those fed the CON diet. Pigs fed the plant-extract treatments had a reduction in total mercaptan emission on day 3 compared with control treatment. On day 5, fecal acetic acid content was decreased with increased blood WBC (white blood cells) and lymphocyte counts and serum IGF-1concentration by plant extract supplementation compared with the control treatment. All the results showed a 95% significance level. Supplementation of plant extracts can improve growth performance and nutrient digestibility in growing pigs, decrease fecal gas emission, and increase immune components such as WBC and lymphocytes, and serum IGF-1 concentration in growing pigs.

Key Words: IGF-1, plant extract

Introduction

The insulin-like growth factor I (IGF-1) is secreted by the liver and stimulated by GH, which in turn facilitates cartilage ossification and growth promotion (Suzuki et al., 2004). Owens et al. (1999) reported that plasma IGF-1 was positively associated with growth rate and feed efficiency in pigs. It was suggested that IGF-1 promotes lean tissue growth, whereas circulating IGF-1 is originated from the liver. The muscle and fat may act via endocrine mechanisms to stimulate lean tissue growth or may reflect somatic tissue production of IGF-1, which in turn acts as a paracrine growth factor. IGF-1 is used as a physiological criterion for genetic animal improvement, as the IGF-1 concentration increases steadily during animal growth, in contrast to the large circadian variation of GH (Scanes et al., 1987). IGF-1 is an essential growth factor for cell growth and development (Liu and LeRoith, 1999).

Four plant extracts of *Phlomis umbrosa Turcz*, *Cynanchum wilfordii Hemsley*, *Zingiber officinale Rosc*, and *Platycodi Radix* were specifically selected from natural pharmaceutical herbs that promote the secretion of IGF-1 (Kim, 2006). Choi et al. (2002) and Kim et al. (2002) reported that these plant extracts are very effective promoters of IGF-1 secretion, in experiments on humans and rats. A clinical test evaluating the effects of plant extracts on humans revealed that serum IGF-1 concentration showed a statistically significant increase in the groups treated with plant extract. Liu et al. (2008) reported that herbal extract supplementation led to an increase in average daily gain (ADG), serum IGF-1 level, and IGF-1 receptor mRNA in the tissue (stomach, duodenum, muscle) of pigs.

The enhanced growth performance and tissue-specific regulation of the IGF-1R mRNA level is due to suggestive herbal extract supplementation and controls IGF system in animals. Therefore, the objective of this study was to evaluate the effects of plant extracts on the IGF-1 on growth performance, nutrient digestibility, and blood immune characteristics of growing pigs.

Material and Methods

The experimental protocols describing the management and care of animals was reviewed and approved by the Animal Care and Use Committee of Dankook University, South Korea.

The plant extracts were processed with hot water at a temperature ranging from 60 to 95 °C. Extraction at lower temperatures yields a product that poorly induces secretion

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of IGF-1, while extraction at temperatures exceeding 95 °C is harsh and can destroy or disrupt the extraction of molecules. The crude extract was cooled, the precipitates were removed by centrifugation, and the components were separated on the basis of molecular weight, to obtain the desired extract containing the active ingredients with relatively low molecular weight (Kim, 2006).

A total of 80 [(Landrace × Yorkshire) × Duroc] pigs with an initial body weight (BW) of 27.31±2.15 kg were used in a 6-wk experiment. Pigs aged 70 days were allotted to one of four treatments in a randomized complete block design according to sex and initial BW. There were four replicate pens per treatment, with five pigs per pen, in a total of 16 pens. The dietary treatments (1 - CON (basal diet) (Table 1); 2 - PE1 (CON + 0.05% plant extracts); 3 - PE2 (CON + 0.10% plant extracts); and 4 - PE3 (CON + 0.15% plant extracts)) were formulated to meet or exceed the nutrient requirements recommended by NRC (1998). The plant extracts included the extracts from *Phlomis umbrosa Turcz*, *Cynanchum wilfordii Hemsley*, *Zingiber officinale Rosc*, and *Platycodi Radix*. Pigs were housed in an environmentally controlled nursery facility with slatted plastic flooring and a mechanical ventilation system. Each pen was equipped with a one-sided self-feeder and a nipple drinker to allow the pigs *ad libitum* access to feed and water throughout the experimental period.

The individual pig weight was measured at the beginning and end of the 6-wk experimental period by recording feed intake and the gain/feed ratio on a pen basis for analyzing ADG. The backfat thickness of the pigs was measured over the shoulder (first rib), on the midback (last rib), and on the rump (last lumbar vertebra) using a real-time ultra sound instrument (Piglog 105, SFK Technology, Herlev, Denmark). For the analysis of the fecal NH_3, H_2S, total mercaptan, and acetic acid, fresh feces were collected from two pigs in each pen on the last two days of the experiment. The total sampled feces were thawed and homogenized, before use. The feces were placed in 2.6-L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were allowed to ferment for one day at room temperature (25 °C), after which 100 mL of the headspace air was sampled from approximately 2.0 cm above the fecal sample. The concentrations of NH_3, H_2S, mercaptan, and acetic acid were measured within the scope of 5.0-100.0 ppm (No.3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0-20.0 ppm (4LK, detector tube; Gastec Corp.). After collection, the boxes were re-sealed with adhesive plaster to measure the fecal noxious content at days 3 and 5 as aforementioned. Prior to measurement, the fecal samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the fecal sample, and homogenized.

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

Ingredient (g/kg)	Basal diet
Ground maize	674.5
Soybean meal	181.4
Rice bran	50.0
Molasses	50.0
Animal fat	20.0
Defluorinated phosphate	11.2
Calcium carbonate	6.8
L-lysine•HCL	2.0
Salt	1.5
Vitamin premix[1]	0.5
Mineral premix[2]	1.5
Choline chloride	0.4
L-threonine	0.2
Chemical composition[3]	
Digestible energy (MJ/kg)	14.1
Crude protein (g/kg)	148.0
Lysine (g/kg)	8.9
Ca (g/kg)	7.4
P (g/kg)	5.4

[1] Provided per kg of complete diet: vitamin A - 4,000 IU; vitamin D3 - 800 IU; vitamin E - 17 IU; vitamin K - 2 mg; vitamin B2 - 4 mg; vitamin B6 - 1 mg; vitamin B12 - 16 µg; pantothenic acid - 11 mg; niacin - 20 mg; biotin - 0.02 mg.
[2] Provided per kg of complete diet: Cu - 220 mg; Fe - 175 mg; Zn - 191 mg; Mn - 89 mg; I - 0.3 mg; Co - 0.5 mg; Se - 0.15 mg.
[3] All calculated values are based on NRC (1998) tabular values.

Chromium oxide (Cr_2O_3) was added to the diet as an indigestible marker at 0.20% seven days prior to fecal collection at the 6th wk for calculation of dry matter (DM) and nitrogen (N) digestibility. Fecal grab samples were collected at random from at least two pigs in each pen at the 6th wk of the experiment. All feed and feces samples were stored immediately at –20 °C until analysis. Fecal samples were dried at 70 °C for 72 h and finely ground to pass through a 1-mm screen. The procedures utilized for the determination of DM and N digestibility were conducted in accordance with the methods established by the AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the apparent total digestibility (ATTD) of DM and N were calculated using indirect methods.

At the end of the experimentation period, two pigs were randomly chosen from each pen and blood samples taken by jugular venipuncture were collected in vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). White blood cells (WBC), red blood cells (RBC) and lymphocytes were determined using an automatic blood analyzer (ADVIA 120, Bayer, USA). For the analysis of serum biochemistry characteristics, samples were centrifuged at 3,000 rpm at 4 °C for 15 min and the serum was separated. One half of the blood samples was subsequently centrifuged at 3,000 g for 15 min at 4 °C, and the plasma was harvested. Thereafter,

the samples were frozen and stored at –20 °C until further analysis. Concentrations of IGF-1 were evaluated using commercially available ELISA kits (USCNK, China) and analyzed using a Microplate reader (Versmax, Molecular device, USA).

In the current study, all data were subjected to statistical analysis in a randomized complete block design using the GLM procedures of SAS (Statistical Analysis System, version 9.2), with the pen serving as the experimental unit and the initial BW used as a covariate for average daily feed intake (ADFI) and ADG. Duncan's multiple range test was used to compare the means of the treatments. Variability in the data was expressed as the pooled standard error (SE) and P<0.05 was considered to be statistically significant.

Results

The PE1 and PE2 treatments showed higher ADG (P<0.05) than the CON treatment (Table 2). However, there were no significant differences observed in ADFI or Gain/Feed (P>0.05). At the end of the 6-wk period, the backfat thickness showed no significant difference (P>0.05) between the treatments.

During the 0 to 6 weeks of experiment, the pigs fed the PE3 diet showed greater DM digestibility (P<0.05) than those fed the CON diet (Table 3). The pigs fed the PE2 diet had a higher N digestibility than those fed the CON treatment (P<0.05). However, there was no significantly different result observed for energy digestibility. Pigs fed the plant extract showed a decrease (P<0.05) in the total mercaptan emission, by the third day, compared with those receiving the control treatment (Table 4). On day 5, acetic acid was decreased in the plant extract-supplemented diets, compared with the control treatment (P<0.05). The NH_3 gas emission was found to have been decreased (P<0.05) on day 7 and no difference was observed in H_2S gas emission.

Supplementation with plant extract showed significant results in WBC and lymphocytes, when compared with the CON treatment (P<0.05), with no differences observed in RBC profile. However, the serum IGF-1concentration was significantly different in the treatment with plant extracts compared with the control treatment (P<0.05). Among the treatments with plant extracts, PE3 showed a significantly higher numerical value compared with the other groups (Table 5). The IGF-1 level in the animals of the control group was 150.1 ng/mL.

Discussion

Liu et al. (2008) reported that supplementation with herbal extract increased average daily gain, serum IGF-1 level, muscle IGF-1 mRNA, and muscle IGF-1 receptor mRNA in pigs. Ra et al. (2003) reported that the plant extracts-administered group showed higher (49.3% and 52.3%) growth promotion than the control group (46.2%), by the increase in the GH and IGF-1 levels in rats. Anti-diarrheal herbs can improve growth performance and prevent diarrhea in pigs (Cho et al., 2012).

Table 2 - Effect of plant extracts on growth performance of growing pigs

Item	CON	PE1	PE2	PE3	SE	P-value
0 to 6 wk						
Average daily gain (g)	646b	683a	678a	662ab	10	0.016
Average daily feed intake (g)	1,523	1,575	1,599	1,567	27	0.102
Gain/feed	0.424	0.434	0.424	0.422	0.006	0.081
Backfat thickness (6 wk) (mm)						
Fore	10.2	10.6	10.6	10.4	0.2	0.132
Middle	8.6	8.7	8.6	8.7	0.2	0.090
Back	10.2	10.4	9.8	10.0	0.3	1.000
Average	9.7	9.9	9.7	9.7	0.2	1.000

CON - basal diet; PE1 - CON + 0.05% plant extracts; PE2 - CON + 0.1% plant extracts; PE3 - CON + 0.15% plant extracts.
SE - standard error.
a,b - means in the same row with followed by different letters differ (P<0.05).

Table 3 - Effects of plant extracts on nutrient digestibility in growing pigs

Item (%)	CON	PE1	PE2	PE3	SE	P-value
Dry matter	76.89b	77.22ab	77.48ab	78.03a	0.31	0.023
N	75.40b	75.95ab	76.63a	75.61ab	0.38	0.041
Energy	75.48	76.21	75.68	75.19	0.41	0.120

CON - basal diet; PE1 - CON + 0.05% plant extracts; PE2 - CON + 0.1% plant extracts; PE3 - CON + 0.15% plant extracts.
SE - standard error.
a,b - means in the same row with followed by different letters differ (P<0.05).

Table 4 - Effects of plant extracts on fecal gas emission in growing pigs

Item (ppm)	CON	PE1	PE2	PE3	SE	P-value
NH_3						
1 day	13.4	12.5	12.5	11.8	0.73	0.652
3 day	16.1	15.1	15.6	15.3	1.03	0.118
5 day	17.0	16.6	16.5	16.5	1.12	0.104
7 day	18.5a	16.3b	16.1b	15.8b	0.40	0.002
H_2S						
1 day	0.015	0.013	0.010	0.014	0.01	1.000
3 day	9.1	8.8	8.8	8.5	0.74	0.512
5 day	23.3	22.7	22.0	20.7	1.08	0.110
7 day	18.0	18.6	16.6	15.8	1.23	0.100
Total mercaptan						
1 day	8.3	8.1	8.2	7.8	0.43	1.000
3 day	21.8a	19.9b	19.7b	19.6b	0.51	0.001
5 day	24.9	23.4	22.7	21.4	1.24	0.082
7 day	21.1	20.5	20.2	19.2	0.76	0.120
Acetic acid						
1 day	12.4	11.6	11.4	10.7	1.15	0.112
3 day	16.4	14.7	14.6	13.6	1.64	0.748
5 day	24.0a	19.6b	18.5b	18.6b	1.10	<0.001
7 day	20.2	19.2	18.7	18.5	0.88	0.106

CON - basal diet; PE1 - CON + 0.05% plant extracts; PE2 - CON + 0.1% plant extracts; PE3 - CON + 0.15% plant extracts.
SE - standard error.
a,b - means in the same row with followed by different letters differ (P<0.05).

Table 5 - Effects of plant extracts on blood characteristics of growing pigs

Item	CON	PE1	PE2	PE3	SE	P-value
6 wk						
IGF-1 (ng/mL)	150.1c	193.6b	200.4b	220.6a	5.29	0.007
RBC (10^6/mL)	6.12	6.77	6.85	6.98	0.45	0.321
WBC (10^3/mL)	24.99b	26.56a	27.89a	28.12a	1.20	0.043
Lymphocytes (%)	61.0b	67.1a	72.4a	70.9a	2.03	0.014

CON - basal diet; PE1 - CON + 0.05% plant extracts; PE2 - CON + 0.1% plant extracts; PE3 - CON + 0.15% plant extracts.
SE - standard error.
a,b - means in the same row with followed by different letters differ (P<0.05).

Klindt et al. (1998) reported that the injected pigs showed greater BW gain than those on the control treatment, and backfat deposition rate was not influenced by the administration of IGF-1. Greater concentrations of IGF-1 were observed in pigs with 90 kg BW from a line selected for rapid growth, compared with the pigs from a line selected for slow growth (Lamberson et al., 1995). Owens et al. (1994) suggested that circulating IGF-1 serves as a reporter of growth performance, rather than a mediator of growth performance. Circulating IGF-1 concentrations were significantly correlated with the body size in three types of swine, which differed in growth rate and mature body weight, suggesting that IGF-1 is involved with the *in vivo* regulation of swine growth (Buonomo et al., 1987). However, Cameron et al. (2003) reported that serum IGF-1 was useful to predict ADFI physiologically, but not ADG and backfat depth, at six weeks of age. Studies demonstrated the relationships between plasma IGF-1 and growth performances, showing positive and negative correlation of plasma IGF-1 concentration at 25 kg BW, with estimated lean percentage at 90 kg. On the other hand, the IGF-1 concentration at 60 kg was positively correlated with ADG, in Landrace and Yorkshire female pigs, although the IGF-1 concentration was correlated with ADG at approximately 75 kg of BW in finishing barrows (Lee et al., 2002; 2005). Eigenmann et al. (1984) studied the relationship between IGF-1 and GH, in which the circulating IGF-1 levels and body size were found to be highly significant, and body weight was correlated with IGF-1 levels rather than with the GH secretory capacity. In this study, supplementation of plant extracts increased the growth performance with an increase in IGF-1.

Oral administration of IGF-1 to unsuckled neonatal pigs increased small intestinal weight, protein and DNA content, and jejunal and ileal villus height, which means that oral infusion of IGF-1 increases the intestinal mucosal growth in pigs (Burrin et al., 1996). The plasma IGF-1 level was increased to the maximum, approximately 12 h after feeding, and plasma IGF-1 was probably considered to have an effect of energy balance under an abnormal

temperature in pigs (Morovat et al., 1994). In a study on sepsis rats, IGF-1 improved the gut mucosal weight in the duodenum, jejunum, ileum, and colon (Chen et al., 1995). According to Lemmey et al. (1994), administration of IGF-l improved gastrointestinal absorptive function by partial gut resection, which most likely reflects an increase in the gut absorptive surface area. In this study, the levels of IGF-1 increased with the addition of plant extracts, which is considered a positive factor that enhances gastrointestinal activities, thus decreasing the fecal gases. It is known that NH_3 and H_2S are the main gaseous components of pig manure (Zahn et al., 1997). Cho et al. (2006) and Huang et al. (2010) suggested that extract of dietary herbs could reduce the fecal noxious gas content in pig manure. One reason for the reduction might be the increase in nutrient digestibility (Yan et al., 2010). In the present study, DM and N digestibility was increased by the supplementation of plant extracts, with a reduced NH_3 gas emission, indicating that the plant extract could develop the digestive organ, increase nutrient digestibility, and decrease fecal gas emission. According to Yan et al. (2011b), dietary *Houttuynia cordata* and *Taraxacum officinale* extracts can increase growth performance, digestibility, and WBC concentration in finishing pigs. Administration of *Saururus chinensis* extract improves the serum lipid protein profile and decreases the emission of noxious gases in finishing pigs (Ao et al., 2011).

In *in vivo* models using mice, IGF-1 stimulates erythroid colony formation, and is the first defined mitogen that stimulates the late stages of erythroid differentiation (Kurtz et al., 1982). With the presence of erythropoietin (RBC-controlling hormone) in the culture, physiological concentrations of IGF-1 (0.5-1 ng/mL) stimulated erythroid cell growth and differentiation from bone marrow or peripheral blood (Claustres et al., 1987). Supplementation of herbs increases growth performance, lymphocyte count, RBC, and WBC concentrations, and decreases the fecal noxious gas content in growing pigs (Yan et al., 2011a) and weaning pigs (Yan et al., 2012). Zhou et al. (2013) reported that dietary supplementation of the herb extract *Coptis chinensis* could increase blood erythrocytes in growing-finishing pigs. IGF-1 is a growth factor for the immune system that increases the total lymphocyte number and the number of CD4+8+ T cells in thymus and spleen in rats (Hinton et al., 1997). IGF-1 was infused into mice to evaluate its effects on bone marrow B lymphopoiesis, and resulted in a significant increase in the total number of bone marrow B lineage cells and splenic B cells, 2 wk post-treatment (Stuart et al., 1991). Addition of plant extracts may be beneficial due to the increase in WBC, lymphocyte, and IgG levels, which in turn increases immunity. Also, the administration of plant extracts may induce positive effects on animals at early stages of their development. Kim et al. (2002) reported that the plant extracts-treated group showed an increase in serum IGF-1 concentration from 245.6 to 275.6 ng/mL, in two months. Also, Choi et al. (2002) reported that the concentration of IGF-1 in blood was 1,140 ng/mL 8 h after the oral administration of plant extracts. Therefore, the present study postulates that plant extract supplementation may have increased the immune function by increasing IGF-1 and various actions of immunity. The IGF-1 level in the animals of the control group was 150.1 ng/mL.

Conclusions

Plant extract supplementation can improve the growth performance and nutrient digestibility of growing pigs. Thus, the inclusion of plant extract has a positive impact on nutrient digestibility and stimulates intestinal enzyme activities. Plant extract treatments reduce fecal gas emission and increase immune components such as white blood cells and lymphocyte. Also, serum IGF-1 concentration is increased by the supplementation of plant extracts.

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