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Effects of Dietary Supplementation with *Galla Rhois* on Growth Performance and Diarrhea Incidence in Postweaning Piglets

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Abstract : A study investigated the effects of *Galla Rhois* (GR) on growth performance and diarrhea incidence of postweaning piglets. One hundred 28-day-old piglets were randomly assigned into five experimental groups, which were a basal diet alone (NC), chlortetracycline 0.3 g/kg feed (PC) and supplemented with GR 1.0 g/kg feed (GR 1), GR 2.0 g/kg feed (GR 2), and GR 4.0 g/kg feed (GR 3). After 28 days of administration, final body weight (BW) and feed conversion ratio of PC, GR 2 and GR 3 was significantly different compared to those of NC ($p < 0.05$). Additionally, the average daily gain (ADG) and average daily feed intake (ADFI) of PC and all groups treated with GR was significantly different compared to those of NC ($p < 0.05$). Especially, final BW, ADG, ADFI and feed conversion ratio (FCR) of GR 2 and GR 3 were not significant different compared to those of PC. In fecal scores and duration of diarrhea, PC and all groups treated with GR were significantly different compared to NC ($p < 0.05$). In hematogolical and serum biochemical analysis, there were no significant differences in any of the hematogolical and serum biochemical parameters examined in either NC or all groups treated with GR. The results of this study indicated that GR could be a potential candidate as feed additives for the improvement of growth performance and incidence of diarrhea in piglets.

Key words **:** *Galla Rhois*, growth performance, diarrhea scores, diarrhea incidence, piglets.

Introduction

World-widely, antibiotics have been used in the swine industry for the growth promotion, and prevention and treatment of diseases for over fifty years (25). Despite the many advantages of antibiotics to livestock farming, the repeated use of antibiotics swine feed has caused many problems like emergence of antibiotic-resistant bacteria, antibiotic residues in edible animal products, and disturbance in the normal intestinal microflora (5,35,37). Due to the problems of antibiotics, many countries in the world have banned or rigorously limited use of these in animal industry. With the tendency to ban antibiotics in animal feed, the swine industry cannot but get interested in alternative to antibiotics for growth promotion and maintaining health under commercial conditions.

In recent, intensive research has focused on the development of alternative strategies with the aim of maintenance of animal health and performance (8,9). Many researchers have investigated efficient alternatives to antibiotic growth promoters which are probiotics (7,27,33), prebiotics (34,39), organic acids (14,16,30), phytobiotics (12,19,28), clay adsorbents (29,32,36) and chemicals (3,24,31) that can exert beneficial effects on the microflora composition and consequently affect animal health and growth performance.

Galla Rhois (GR) is an outgrowth of plant (*Rhus chinensis* L.) tissue caused by a mite parasite (*Schlechtendalia chinensis* Bell) that is rich in gallotannins, and has been applied in traditional Oriental medicine for the treatment of diarrhea, persistent coughing, and spontaneous perspiration as a result of the effects of astringents, antidiarrheals, hemostatic drugs and other antidotes (18,20).

GR is a natural, non-toxic material that contains a number of tannin-derived components, collectively termed tannic acid, methyl gallate and gallic acid. Notably, the gallotannins are a class of hydrolysable tannin polymers that are formed from gallic acid, which seems to have anti-bacterial, anti-fungal, and anti-viral properties (2,10). Tannic acid, a common form of hydrolysable tannin, has been reported to have inhibitory effects on the growth of some intestinal pathogens, including *Clostridium* spp., *Escherichia coli*, and *Salmonella typhimurium* (11). Methyl gallate is known to inhibit the growth of *Escherichia coli* without negatively influencing the growth of lactic acid-producing bacteria (4).

Although there were some researches for the effects of GR on the growth performance in broiler chicken (21,22), few

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studies exist to investigate the effect of GR for the growth performance and diarrhea incidence on piglets. Then, the present study evaluated the effect of GR for the growth performance and diarrhea incidence on post-weaning piglets.

Materials and Methods

Experimental compound

GR powder was obtained from GS Bio (Jeonju, Korea), which produced the powder from dried plant material and analyzed its components as previously described (1). Briefly, one kg of plant material dried in an oven at 60°C for three days was twice extracted with methanol at room temperature, the residue was removed by filtration (Toyo filter paper no. 2; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and the filtrate was concentrated using vacuum rotary evaporation (Iwai Co., Tokyo, Japan) followed by freezing dry to powder. The composition of the crude extracted powder was analyzed using chromatography on a silica gel column (70-230 mesh; Merck, Darmstadt, Germany) and fractionation on a preparatory high-performance liquid chromatography column (Delta Prep 4000; Waters, Ontario, Canada). Tannins accounted for 45.8% of the total composition of GR, and methyl gallate and gallic acid comprised 7.1% and 4.3% of that, respectively.

Experimental animals and design

This experiment was carried out at the pig farm in the Livestock Farm of Gyeonsang National University, Chinju, Korea. The protocol used for this experiment complied with the guidelines by the Ethical Committee of Gyeonsang National University. This study was used one hundred 28-day-old crossbred piglets (Yorkshire × Landrace × Duroc) (average body weight (BW), 6.85 ± 0.57 kg). The piglets were kept in raised slatted floor pens (1.2 × 1.6 m) at 26 ± 1°C and the humidity was 65 ± 5%. Water and feed (Magic plus No. 2, Furina Feed, Seongnam, Korea) were offered *ad libitum* throughout the experimental period. The piglets were randomly assigned into one of five treatments.

Five treatments were NC (negative control, basal diet without antibiotic), PC (positive control, basal diet + chlortetracycline 0.3 g/kg), GR 1 (basal diet + 1.5 g/kg GR), GR 2 (basal diet + 3.0 g/kg GR) and GR 3 (basal diet + 4.5 g/kg GR). The experiment was carried out for four weeks. BW and feed intake were measured weekly. At the end of the experiment, a 5-ml blood sample was collected in an EDTA tube (BD Vacutainer®, BD, USA) from an anterior vena cava of all piglets. After all collected blood samples were centrifuged (10 min, 3,000 rpm at 4°C), serum samples were stored at −80°C.

Assessment of diarrhea severity

Fecal scoring and duration of diarrhea were conducted daily in the morning. The severity of diarrhea was noted by visually scoring the consistency of the feces on a standardized scale of 0-3 as described by Cox *et al*. (13). Scoring was as follows: for fecal fluidity, 0 = no diarrhea (normal feces), 1 = slight (pasty feces), 2 = moderate (semi-liquid feces), 3 = severe diarrhea (watery feces). The incidence of diarrhea (%) was calculated as the sum of the total number of diarrhea in piglets over the period divided by the number of piglet days in the period multiplied by 100. The rate of diarrhea (%) was calculated as the sum of the total number of diarrhea in piglets over the period by the sum of the total number of piglets multiplied by 100.

Analysis of blood samples

White blood cell (WBC) count, red blood cell (RBC) count, hematocrit, haemoglobin and platelets were analyzed in sampled whole blood using an Advia 120 haematology analyser (Bayer, NY, USA). To identify the toxic effect of GR in liver and kidney of piglets, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, blood urea nitrogen (BUN) and creatinine concentrations were determined in the serum using a Hitachi 911 chemistry analyser (Roche Diagnostics Korea, Seoul, Korea).

Statistical analysis

Values are presented as the mean ± standard deviation (SD). All data were analyzed using one-way ANOVA (SAS Institute, USA), with regard to feed intake and other variables. Duncan's multiple range test was used to compare differences between the treatment groups. Probability values $p < 0.05$ were taken to indicate statistical significance.

Results

Growth performance

The final BW for PC, GR 2 and GR 3 was significantly higher than that of NC ($p < 0.05$), and no significant differences for the final BW were found between PC, and GR 2 and GR 3 (Table 1). The average daily gain (ADG) and ADFI of NC were significantly lower than that of other groups ($p < 0.05$), and there were no significant differences between PC, and GR 2 and GR 3. FCR of NC was significantly higher than that of PC, GR 2 and GR 3 ($p < 0.05$). However, no significant difference was found between NC and GR1.

Assessment of diarrhea severity

During 28-days post-weaning, some piglets in all groups showed diarrhea symptoms (Table 2). In the 28-days trial, all groups treated with GR were significantly decreased fecal scores, rate of diarrhea and incidence of diarrhea compared with NC ($p < 0.05$). In GR 2 and GR 3, all diarrhea parameters were significantly decreased compared with those in PC ($p < 0.05$). In addition, there was no significant difference in all parameters between GR 2 and GR 3.

Analysis of blood samples

The blood cell counts and serum biochemical values are shown in Table 3. The hematological analysis showed no sig-

Table 1. Effect of supplementation with *Galla Rhois* on growth performance of piglets

Items	Treatments				
	NC	PC	GR 1	GR 2	GR 3
Initial BW (kg)	6.8 ± 0.52	6.8 ± 0.65	6.8 ± 0.62	6.8 ± 0.54	6.8 ± 0.57
Final BW (kg)	17.2 ± 2.35[a]	18.9 ± 1.14[b]	17.9 ± 1.77[a]	19.2 ± 1.82[b]	19.0 ± 1.08[b]
ADG (g/pig/day)	371.4 ± 12.3[a]	432.1 ± 10.2[c]	396.4 ± 14.3[b]	442.9 ± 14.1[c]	435.7 ± 15.2[c]
ADFI (g/pig/day)	657.4 ± 19.2[a]	713.0 ± 23.5[c]	681.8 ± 21.4[b]	721.9 ± 24.2[c]	727.6 ± 25.5[c]
FCR	1.77 ± 0.14[a]	1.65 ± 0.12[b]	1.72 ± 0.15[a]	1.63 ± 0.09[b]	1.67 ± 0.11[b]

Values are expressed as mean ± SD.
[a-c]Means within the same row with different letters are significantly different ($p < 0.05$).
NC, negative control; PC, positive control; GR 1, NC + 1.5 g/kg GR; GR 2, NC + 3.0 g/kg GR; GR 3, NC + 4.5 g/kg GR; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Table 2. Fecal scores and duration of diarrhea in piglets fed with dietary supplementation of *Galla Rhois* for 4 weeks

Parameters	Treatments				
	NC	PC	GR 1	GR 2	GR 3
Fecal scores	2.47 ± 0.25[a]	1.86 ± 0.17[b]	2.20 ± 0.23[c]	1.78 ± 0.18[d]	1.69 ± 0.14[e]
Duration of diarrhea (days)	3.58 ± 1.10[a]	2.13 ± 0.68[b]	2.75 ± 0.71[a]	1.74 ± 0.68[c]	1.66 ± 0.64[c]
Rate of diarrhea (%)	25.93 ± 3.46[a]	22.4 ± 3.54[b]	23.2 ± 4.02[b]	18.7 ± 3.24[c]	17.8 ± 3.33[c]
Incidence of diarrhea (%)	3.51 ± 0.32[a]	2.35 ± 0.53[c]	2.56 ± 0.48[b]	2.09 ± 0.52[d]	1.93 ± 0.45[d]

Values are expressed as mean ± SD.
[a-e]Means within the same row with different letters are significantly different ($p < 0.05$).
NC, negative control; PC, positive control; GR 1, NC + 1.5 g/kg GR; GR 2, NC + 3.0 g/kg GR; GR 3, NC + 4.5 g/kg GR; BW, body weight.

Table 3. Blood cell counts and biochemical values in piglets administered with different concentrations of *Galla Rhois* in feed for 28 days

Parameters	Treatment groups				
	NC	PC	GR 1	GR 2	GR 3
WBC (10^6/mm^3)	15.72 ± 1.38	15.61 ± 1.27	14.94 ± 1.73	15.80 ± 1.46	14.95 ± 1.33
RBC (10^3/mm^3)	6.92 ± 0.82	6.80 ± 0.52	6.72 ± 0.79	6.82 ± 0.76	6.91 ± 0.50
Hematocrit (%)	34.65 ± 2.65	34.38 ± 2.35	34.44 ± 2.48	34.36 ± 2.69	33.96 ± 1.91
Hemoglobin (g/dl)	11.68 ± 1.08	11.72 ± 0.89	11.80 ± 0.91	12.09 ± 1.16	11.64 ± 0.94
Platelets (10^3/mm^3)	399.3 ± 47.7	392.4 ± 54.6	386.8 ± 41.4	391.8 ± 51.2	398.0 ± 58.5
AST (U/L)	25.0 ± 1.48	25.5 ± 1.47	24.9 ± 1.36	25.2 ± 1.53	25.4 ± 1.45
ALT (U/L)	29.3 ± 0.98	29.8 ± 1.22	30.2 ± 0.79	29.5 ± 1.24	30.0 ± 1.07
BUN (mg/dl)	20.2 ± 1.80	20.5 ± 1.53	21.0 ± 1.12	20.4 ± 1.70	20.9 ± 1.38
Creatinine (mg/dl)	0.56 ± 0.24	0.58 ± 0.21	0.54 ± 0.25	0.59 ± 0.27	0.57 ± 0.24

Values are expressed as mean ± SD.
[a-c]Means within the same row with different letters are significantly different ($p < 0.05$).
NC, negative control; PC, positive control; GR 1, NC + 1.5 g/kg GR; GR 2, NC + 3.0 g/kg GR; GR 3, NC + 4.5 g/kg GR; BW, body weight; WBC, white blood cell; RBC, red blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

nificant changes of WBC, RBC, hematocrit, hemoglobin and platelets in all groups treated with GR compared to NC. Additionally, there were no significant differences in any of the serum biochemical parameters examined in either NC or all groups treated with GR.

Discussion

In this study, the supplementation of GR was improved BW, ADG, ADFI and FCR in piglets. Better growth performance in animals fed with GR could be due to gallotannin such as penta-m-digalloyl-β-glucoside, gallic acid, and m-digallic acid which have good antibiotic functions (1,2). In a previous study, unlike the supplement of only tannins, the supplement of the complex with tannins and other compounds in GR increased growth performance of broiler chicken (26). In addition, effects of GR were investigated on the growth performance and incidence of diarrhea in growing and finishing

pigs during 130 days, and the feed consumption efficiency of 0.2% GR treated-group was better than that of the control group and diarrhea scores were improved (23). Furthermore, the supplementation of tannins at 4.5 g/kg feed improved feed efficiency and incidence of diarrhea in weaned piglets because tannins reduced intestinal bacterial proteolysis and decreased the depth of ileal crypts (6).

In the present study, the dietary supplement of GR contained the complex with tannins and other compounds could be increased the growth performance and the digestion-absorption rate in piglets due to reducing intestinal bacterial proteolysis by tannins and other components of GR. With the consideration of the dosage and supplement period, anti-diarrheal effect for piglet in this study may be similar to that in the previous study (23). Furthermore, after the supplement of 0.1% direct-fed microbial complex (DFM) for 4 weeks, the incidence rate of diarrhea was 4.17%, which was the same value of 0.1% antibiotic treated-group (38). In this study, the incidence rate of diarrhea in groups treated with GR for four weeks ranged between 1.93% and 2.56%. This result suggested that GR is more effective than the probiotic mixture in reducing the incidence of diarrhea on piglets.

Diarrhea score and incidence of diarrhea in 1% herbal mixture treated-piglets were 1.36 and 1.32% after dietary supplement of the herbal mixture contained seven herbs for 35 days (15). In this study, diarrhea score and incidence of diarrhea in GR 2 were 1.69 and 1.93%, which was slightly higher than those in 1% herbal mixture treated-piglets (15).

The acute LD_{50} of methyl gallate (MG), a major component of GR, in adult Swiss mice was 1.7 g/kg orally, 784 mg/kg intraperitoneally, and 470 mg/kg intravenously, and the chronic LD_{50} of MG in adult Swiss mice was 1.31 g/kg orally and 351 mg/kg intraperitoneally (17). According to the analytical results of GR in this study, the content of MG in GR was about 7.5%. With the consideration of MG content, body weight and ADFI, the results of blood analysis in this study may correspond with those of the previous study.

In conclusion, GR could be proposed as a candidate for the alternatives to antibiotics and recommended as an effect additive for growth performance and reduction of diarrhea in the post-weaning piglet diet. The range between 3.0 and 4.5 kg/ton feed could be the optimal dose for the supplement of GR in the piglet diet.

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이유자돈에 있어서 성장증체 및 설사발생에 미치는 오배자의 급여 효과

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요 약 : 본 연구는 오배자(*Galla Rhois*, GR)가 이유자돈의 성장과 설사발생에 미치는 영향을 조사하였다. 100두의 28일령 이유자돈을 무작위적으로 5개의 실험군(NC, 대조군; PC, chlortetracycline 0.3 g/kg feed; GR 1, GR 1.0 g/kg feed; GR 2, GR 2.0 g/kg feed; GR 3, GR 4.0 g/kg feed3)으로 나누어 실험을 수행하였다. 28일 동안 실험군별로 처리한 후, PC, GR 2 그리고 GR 3의 최종 체중과 사료요구율은 NC와 비교하여 통계적으로 유의성 있게 증가하였다 ($p < 0.05$). 또한, PC와 GR를 급여한 모든 군의 일평균증체량과 일평균사료섭취량도 NC와 비교하여 통계적으로 유의한 차이를 보였다($p < 0.05$). 특히, GR 2와 GR 3의 최종 체중, 일일평균증체량, 일평균사료섭취량 그리고 사료요구율은 PC와 비교하여 유의한 차이를 보이지 않았다. 분변지수와 설사지속시간에 있어서, PC와 GR를 급여한 모든 군이 NC와 비교하여 통계적으로 유의한 차이를 나타내었다($p < 0.05$). 혈액학적 그리고 혈액생화학적 분석 결과, NC와 GR를 급여한 모든 군들 사이에서 혈액학적 그리고 혈액생화학적 지표값들의 통계학적 유의한 차이가 보이지 않았다. 본 연구결과로부터, GR은 자돈에 있어서 설사발생과 성장증체를 개선시키기 위한 강력한 사료첨가제로 사료된다.

주요어 : 오배자, 성장증체, 설사지수, 설사발생, 자돈