Wayamba Journal of Animal Science - ISSN :2012-578X; P49-P52, 2010
First Submitted December 08, 2010; Number 1291802285

Effects of herbal extract on growth performance, fecal microbiota, nutrient digestibility, and blood profiles in weanling pigs

Wang J. P. and Kim I. H.

Department of Animal Resource & Science, Dankook University, Cheonan, Choognam, 330-714, Korea

An experiment was conducted to evaluate the effect of dietary supplementation with herbal extracts (HE) on performance, apparent nutrient digestibility, microbial shedding, and blood characteristics in weanling pigs. A total of 120 weaned barrows with average initial BW of 6.93 ± 0.85 kg were included in this 35-d feeding trial. Pigs were randomly distributed into one of four treatment groups on the basis of BW and litter (5 pigs/pen, 6 replicate pens/treatment). Dietary treatments were: 1) negative control (NC), corn-soybean basal diet; 2) antibiotic control (PC), basal diet with antibiotics, 3) basal diets with herbal extract 0.5 g/kg (HE0.5), and 4) basal diets with herbal extract 1 g/kg (HE1). During the experimental period, average daily gain (*ADG*) were higher (linear effect, $P<0.05$) in PC and HE1.0 group, whereas average daily feed intake (*ADFI*) were unaffected by dietary treatment. The pigs fed diet supplemented antibiotics and HE were higher than those fed the NC diet in gain/feed (G/F) during the whole experiment, and a linear effect were be observed in HE treatments ($P<0.05$) as the levels of HE increased. Administration of HE1 and PC increased ($P<0.05$) the digestibility of dry matter (DM) and nitrogen throughout the whole experiment. The number of *E. coli* linearly decreased with increasing levels of HE ($P<0.05$) at d 35. Additionally, HE linearly increased the number of *Lactobacillus* at d 21 ($P<0.05$), while no effect was observed in PC treatment. The fecal pH values were not influenced by the dietary treatment. The white blood cell in the blood was increased ($P<0.05$) in both PC and HE compared with the NC treatment at d 35, with the highest values in HE1 treatment. Moreover, the serum immunoglobulin G (*IgG*) were linearly increased as the HE levels increased ($P<0.05$) at d 7, 21, and 35. And pigs fed the PC diet had higher *IgG* concentration than the control group only at d 35. In conclusion, the addition of HE and PC can improve the performance, inhibition the proliferation of *E.coli,* as well as modulating the immune-related cells in the blood.

Key words: blood profiles/digestibility/ herbal extract/ microbiota/ weaning pigs

INTRODUCTION

Traditionally, herbal extracts (flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots) were adopted in Korea and China for the purpose of promoting health and immune system in humans (Middleton and Kandaswami, 1992; Lee *et al*., 2004). Beneficial effects of herbal extracts on farm animals may arise from activation of feed intake and secretion of digestive secretions, immune stimulation, anti-bacterial, coccidiostatic, anti-viral and antioxidant properties. Xu *et al*. (2003) reported that Chinese herbs could reduce the density of enterotoxigenic *E coli* and increase the density of *Bacillus acidi lactic* or *Bacillus bifidus*.

The indigenous knowledge of the herbs on the nutritional and medicinal properties is useful during the new process searching for antibiotics alternatives.

There are four kinds of medical herbs as *Taraxacum platycarpum*, *Coptis chinensis, Forsythia viridissima* and *Glycyrrhiza uralensis* used in this study. *Taraxacum platycarpum* has antifebrile, antidote, anti-inflammatory, milk promoting effects and is used to treat many kinds of infections in traditional oriental medicine. Alkaloid was the principal ingredients in Coptis chinensis, which was used as antibiotic, anti-inflammatory, anti-cancer, inhibitive action of intestinal actives and styptic (Akhter *et al*., 1977; Makhey 1995, Yamamoto *et al*, 1993; Park *et al*., 2007). Forsythia viridissima has antiphlogistic, drainage, antidote, and diuretic effects (Rim *et al*., 2000; Lee *et al*., 2003). Glycyrrhiza uralensis contained glycyrrhizin of main composition; Glycyrrhiza uralensis has effects of anti-allergy, anti-cancer, anti-inflammatory, anti-virus and enhances steroid substances (Zhao *et al*. 1991; Liu *et al*., 1993). The knowledge of these herb extract is still rather limited regarding their modes of action and aspects of their application.

This study was conducted to evaluate the effects of these herbal extract blends on performance, nutrient digestibility, fecal microbiota, and blood characteristics in weaned pigs.

MATERIALS AND METHODS

Animals and experimental design All experimental procedures conducted in this study were reviewed and approved by the Animal Care and Use Committee of Dankook University. A total of 120 weaned castrated male pigs (Yorkshire × Landrace females mated to Duroc sires) that were weaned at an average age of 21 d and had an average initial BW of 6.93 ± 0.85 kg were included in this 35 d feeding trial. Pigs were randomly distributed into one of four treatment groups on the basis of BW and litter (5 pigs/pen, 6 replicate pens/treatment). Dietary treatments were: 1) negative control (NC), basal diet without antibiotics; 2) positive control (PC), basal diet with antibiotics (40 mg/kg of avilamycin and 100 mg/kg of oxytetracycline during the phase 1 followed by 100 mg/kg of neomycin and 40 mg/kg of chlorotetracycline during the phases 2), 3) basal diet with herbal extract 0.05% (HE0.5), and 4) basal diet with herbal extract 0.10% (HE1). The Herbal extract is composed of 15% Taraxacum platycarpum H. Dahlstedt, Coptis chinensis, Forsythia viridissima Lindley and Glycyrrhiza uralensis Fischer, which were mixed in the dry matter weight ratio of 5: 2: 2: 1. Herbal-extracts was coated liquid paraffin method for enhance preference and added in the diet to replace the same amount of corn at once.

Ingredient, g/kg	Phase 1 (d 0 to 7)	Phase 2 (d 7 to 21)	Phase 3 (d 21 to 35)
Extruded corn	111.5	349.2	451.0
Extruded oat	100.0	-	-
Biscuit meal	-	50.0	90.0
Soybean meal (44% CP)	80.0	200.0	296.5
Fermented soybean meal	78.0	82.0	-
Fish meal	50.0	40.0	25.0
Soy oil	41.5	48.0	30.0
Lactose	100.0	60.0	-
Whey	165.0	100.0	62.5
Milk product[1]	130.0	20.0	20.0
Monocalcium phosphate	12.5	10.0	6.0
Sugar	40.0	20.0	-
L-Lys·HCl (78%)	1.2	2.5	1.6
DL-Met (50%)	2.6	1.5	1.4
L-Thr (89%)	7.7	0.8	-
Choline chloride (25%)	2.0	1.0	1.0
Vitamin premix[2]	1.0	1.0	1.0
Trace mineral premix[3]	2.0	2.0	2.0
Limestone	2.0	2.0	3.0
Salt	3.0	3.0	3.0
Analyzed composition, g/kg			
ME, kcal/kg	3,520	3,515	3,484
CP	223.0	215.2	205.0
Lys	15.1	14.8	13.2
Met	6.0	5.2	4.7
Ca	8.5	7.8	7.4
Total P	7.8	7.7	6.4

1 Primarily contains 210 g/kg fat and 220 g/kg protein.

2 Provided per kg of premix: vitamin A, 11,025 IU; vitamin D3,1,103IU; vitamin E, 44IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic acid, 29 mg; choline, 166 mg; and vitamin B12,33 μg.

3 Provided per kg of complete diet: Fe (as FeSO4·7H2O), 80 mg; Cu (as CuSO4·5H2O), 12 mg; Zn (as ZnSO4), 85 mg; Mn (as MnO2), 8 mg; I (as KI), 0.28 mg; and Se (as Na2SeO3·5H2O), 0.15 mg.

Experimental procedures, Sampling and Assay

Pigs were housed in an environmentally controlled nursery facility with a temperature that was maintained at 29 ℃ and then decreased by 1.2 ℃ weekly until the end of the experiment. Each pen was equipped with a stainless steel feeder and one nipple waterer that allowed for free access to the feed and water throughout the experiment.

For the growth assay, the individual pig BW and pen feed disappearance were recorded weekly and then used to determine the average daily gain (ADG), average daily feed intake (ADFI) and gain/feed (G/F) ratio. On d 0, 7, 21 and 35, 0.2% chromium oxide (Cr2O3) was added to each of the diet as an inert indicator to calculate the apparent total tract digestibility (ATTD) for dry matter (DM), and nitrogen (N) during each dietary phase. After the pigs were fed diet containing the indicator for 4 d, fresh fecal grab samples were obtained from 2 pigs per pen over a 3-d period. All fecal and feed samples from one pen were then pooled and mixed, after which a representative sample was stored in a freezer at -20 ℃ until analysis. Prior to chemical analysis, the fecal samples were thawed and dried at 50 ℃ for 72 h, after which they were finely ground to a size that could pass through a 1 mm sieve. All of the feed and fecal samples were then analyzed for DM and N following the procedures outlined by the AOAC (AOAC, 2000). Chromium was analyzed using UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan) and nitrogen was determined using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden).

Blood samples were collected from 2 pigs that were randomly selected from each pen via anterior vena cava puncture on d 0, 7, 21, and 35. The blood samples were collected into nonheparinized and K3EDTA vacuum tubes (Becton Dickinson Vacutainer System, Franklin Lakes, NJ) to obtain serum and whole blood samples, respectively. Serum samples were then centrifuged (2,000 ×g) for 30 minutes at 4 ℃. Blood samples for immune function analysis were packaged on ice on the day of sampling and then transported to the laboratory immediately. Serum immunoglobulin G (IgG) was analyzed using nephelometry (Dade Behring, Marburg, Germany). The white blood cells (WBC), and lymphocyte counts in the whole blood were determined using an automatic blood analyzer (ADVIA 120, Bayer, NY).

Procedures of Microbial Shedding

Fecal samples were collected directly via massaging the rectum of 2 pigs (1 gilt and 1 barrow) in each pen and then pooled and placed on ice for transportation to the lab, where analysis was immediately carried out. A calibrated, glass-electrode pH meter (WTW pH 340-A, WTH Measurement Systems Inc., Ft. Myers, FL) was used to measure the pH of the fecal samples, which were diluted with deionized water at a ratio of 1:7.5 (wt/wt). One gram of the composite fecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and Lactobacilli medium agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the E. coli and lactobacillus, respectively. The Lactobacilli medium agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The E. coli and lactobacillus colonies were counted immediately after removal from the incubator. For Salmonella, the serially diluted peptone broth tubes were incubated overnight at 37°C, after which 1 mL was transferred to 9 mL of tetratinate broth (Neogen Corporation, Lansing, MI) and then incubated for 48 h at 42°C. From these tubes, 1 mL was used to inoculate 9 mL of Rappaport-Vassiliadis Salmonella Enrichment broth (Neogen Corporation, Lansing, MI) and incubated for 48 h at 42°C. The Rappaport was used to inoculate XLT4 plates for Salmonella isolation, and the Salmonella was, then, identified using LIS (VIDAS Listeria) and TSI(Triple Sugar Iron) agar tubes (Difco Laboratories, Detroit, MI).

Statistical Analysis

Data were analyzed by ANCOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Pen served as the experimental unit for all analyses. When significant interactions were observed, the means were compared using the Duncan's multiple range test, with a $P < 0.05$ indicating significance. The linear and quadratic effect of HE among treatments was determined using a contrast statement. In addition, an analysis of covariance (ANCOVA) was conducted to evaluate the ADG, ADFI, and blood characteristics. For the ANCOVA analyses, the initial data was used as a covariate; therefore, the initial data are not shown in our results.

RESULTS

3.1 Growth performance and Apparent Total Tract Nutrient digestibility (ATTD)

During all three phases, ADG and G/F ratio were linearly improved as the HE levels increased ($P<0.05$), while the ADFI were not effected by any dietary treatment. The PC were also found to improve ($P<0.05$) ADG and G/F compared with NC treatment throughout the whole experiment (Table 2).

Table 2. Effect of dietary herbal extracts supplementation on the performance of weaning pigs1

Item	NC	PC	HE0.5	HE1	SE	P-value Linear	P-value Quadratic
Phase 1 (d 0 to7)							
ADG, g	261[b]	303[a]	278[a]	291[a]	9	0.03	0.21
ADFI, g	401	398	410	409	7	0.24	0.12
G/F	0.651[b]	0.761[a]	0.678[b]	0.711[a]	0.012	0.03	0.09
Phase 2 (d 7 to 21)							
ADG, g	432[b]	468[a]	451[a]	465[a]	11	0.04	0.37
ADFI, g	560	562	543	554	12	0.35	0.54
G/F	0.771[b]	0.833[a]	0.831[a]	0.839[a]	0.022	0.05	0.45
Phase3 (d 21 to35)							
ADG, g	542[b]	594[a]	583[a]	602[a]	10	0.08	0.18
ADFI, g	998	983	973	982	18	0.27	0.47
G/F	0.543[b]	0.604[a]	0.599[a]	0.613[a]	0.020	0.02	0.34
Overall (d o to 35)							
ADG, g	442[b]	485[a]	469[a]	485[a]	13	0.04	0.32
ADFI, g	703	698	688	696	18	0.61	0.32
G/F	0.629[b]	0.695[a]	0.682[a]	0.697[a]	0.012	0.05	0.44

a,b Means in the same row with different superscripts differ ($P < 0.05$).

1 Each mean based on 6 pens (2 pigs from each pen). Abbreviations: NC, corn-soybean basal diet; PC, 40 mg/kg of avilamycin and 100 mg/kg of oxytetracycline during phase 1 followed by 100 mg/kg of neomycin and 40 mg/kg of chlorotetracycline during phase 2 and 3; HE0.5, 0.5 g/kg herbal extract; HE1, 1 g/kg herbal extract; ADG, average daily gain; ADFI, average feed intake; G/F, gain/feed.

2 Pooled standard error

Throughout the entire experiment period, the pigs fed the PC diet or the HE1 had higher dry matter (DM) and nitrogen (N) digestibility than pigs in the NC treatment ($P<0.05$). Moreover, the HE were observed to exert a linear effect on both DM and N digestibility in this trial ($P<0.05$) (Table 3).

Table 3. Effect of dietary herbal extracts supplementation apparent total tract digestibility (ATTD) of DM and N of weaning pigs1

Item	NC	PC	HE0.5	HE1	SE	P-value Linear	Quadratic
Phase 1 (d 0-7)							
DM	85.58^{b}	89.62^{a}	87.81^{ab}	90.14^{a}	0.94	0.04	0.24
N	80.91^{b}	83.40^{a}	82.33^{ab}	84.18^{a}	0.87	0.02	0.05
Phase 2 (d 7-21)							
DM	80.36^{b}	83.99^{a}	81.48^{ab}	83.24^{a}	0.79	0.05	0.08
N	78.37^{b}	81.18^{a}	75.30^{ab}	79.64^{a}	0.84	0.03	0.07
Phase 3 (d 21-35)							
DM	74.62^{b}	77.24^{a}	76.24^{ab}	78.43^{a}	0.80	0.03	0.24
N	74.81^{b}	80.01^{a}	76.51^{ab}	80.04^{a}	0.75	0.04	0.03

a,b Means in the same row with different superscripts differ (P < 0.05).
1 Each mean based on 6 pens (2 pigs from each pen). Abbreviations: NC, corn-soybean basal diet; PC, 40 mg/kg of avilamycin and 100 mg/kg of oxytetracycline during phase 1 followed by 100 mg/kg of neomycin and 40 mg/kg of chlorotetracycline during phase 2 and 3; HE0.5, 0.5 g/kg herbal extract; HE1, 1 g/kg herbal extract; ADG, average daily gain; ADFI, average feed intake; G/F, gain/feed.
2 Pooled standard error.

Fecal Microbiota

HE linearly increased the number of Lactobacillus at d 21 (P<0.05). The number of E. coli linearly decreased with increasing dose of HE (P<0.05) at d 35. Additionally, fecal pH values were not influenced by the dietary treatment (Table 4) during the whole experiment. The Salmonella concentrations were below the detection limit (102 cfu/g) for all treatment groups; therefore, they were not evaluated. Also the inclusion of PC were found to have the same effect as the HE had on the regulation of microbiota.

Table 4. Effect of dietary herbal medicine supplementation on the fecal shedding of microorganism in weaning pigs1

Item	NC	PC	HE0.5	HE1	SE^{2}	P-value Linear	Quadratic
Lactobacillus, $\log_{10}$ cfu/g							
7d	7.57	7.32	7.78	7.12	0.58	0.49	0.21
21d	7.32^{b}	7.98^{b}	8.01^{a}	7.78^{a}	0.13	0.05	0.12
35d	7.45	7.87	7.32	6.79	0.78	0.67	0.39
E. coli, $\log_{10}$ cfu/g							
7d	6.42	6.12	6.30	6.9	0.21	0.78	0.42
21d	6.78	6.21	6.46	6.02	0.19	0.13	0.21
35d	6.52^{a}	5.89^{b}	5.50^{b}	5.12^{b}	0.35	0.04	0.24
pH							
7d	6.45	6.21	6.12	6.31	0.29	0.42	0.35
21d	6.65	6.72	6.61	6.39	0.30	0.19	0.67
35d	6.75	6.67	6.56	6.49	0.21	0.78	0.29

1 Each mean based on 6 pens (2 pigs from each pen). Abbreviations: NC, corn-soybean basal diet; PC, 40 mg/kg of avilamycin and 100 mg/kg of oxytetracycline during phase 1 followed by 100 mg/kg of neomycin and 40 mg/kg of chlorotetracycline during phase 2 and 3; HE0.5, 0.5 g/kg herbal extract; HE1, 1 g/kg herbal extract. 2 Pooled standard error.

Blood Profiles

The effects of herbal extract supplementation on blood characteristics in weanling pigs are presented in Table 5. Both herbal extract and antibiotic treatments had a higher WBC concentration compared with the control treatment at d 35 (P<0.05). The IgG concentrations throughout the experiment were linearly increased by the addition of herbal extract (P<0.01), while the antibiotics also be found to have a higher IgG concentration compared with NC treatment.

Table 5. Effect of dietary herbal medicine supplementation on blood characteristics of weaning pigs1

Item	NC	PC	HE0.5	HE1	SE^{2}	P-values Linear	Quadratic
WBC, 10^3/mm^3							
7d	11.15	10.1	10.9	10.5	3.17	0.33	0.21
21d	9.34	9.62	9.89	10.11	2.29	0.64	0.23
35d	8.51^{b}	9.42^{a}	9.55^{a}	9.91^{a}	1.25	0.21	0.36
Lymphocyte^{3}, %							
7d	59.5	61.4	61.5	60.7	5.07	0.42	0.33
21d	61.7	64.8	64.5	64.1	3.09	0.55	0.22
35d	61.9	62.4	63.1	63.5	2.01	0.67	0.33
IgG, mg/dL							
7d	600.8^{b}	679.2^{ab}	730.5^{a}	745.6^{a}	30.1	<0.01	0.22
21d	610.9^{b}	700.5^{ab}	740.1^{a}	760.2^{a}	24.5	<0.01	0.23
35d	620.1^{b}	720.4^{a}	731.1^{a}	759.8^{a}	28.4	<0.01	0.18

a,b Means in the same row with different superscripts differ (P < 0.05). 1 Each mean based on 6 pens (2 pigs from each pen). Abbreviations: NC, corn-soybean basal diet; PC, 40 mg/kg of avilamycin and 100 mg/kg of oxytetracycline during phase 1 followed by 100 mg/kg of neomycin and 40 mg/kg of chlorotetracycline during phase 2 and 3; HE0.5, 0.5 g/kg herbal extract; HE1, 1 g/kg herbal extract; WBC, white blood cell; IgG, immunoglobulin G. 2 Pooled standard error. 3 Values are presented as a percentage of total white blood cell count.

DISCUSSION

The great stress piglets are under at weanling is becoming even worse due to the early weaning strategy adopted by the modern husbandry (Bosi et al., 2004). In recent years, phytogenic feed additives have been paid increasing attention as an alternative feeding strategy to replace antibiotics growth promoters. The herb used in this study contains many kinds of bioactive compounds such as alkaloids, flavonoids, and tannins. Flavonoid compounds exhibit inhibitory effects against both virus and pathogens. Previous studies have demonstrated the effects of flavonoids against human immunodeficiency virus. Kaul et al. (1985) reported that flavones derivatives were inhibitory to respiratory syncytial virus. Tannins, the bioactive compounds in herbal extracts, may be formed by condensations of flavan derivatives. Dietary supplementation with HE in the current study was found to improve performance that was comparable to equal to the antibiotics inclusion group. Similarly, Park et al (2000) demonstrated that herb extract supplementation improved ADG, ADFI, G/F ratio as the herbal extract level increased.

The active components of the herbal product, cortex mori, semen Plantaginis, Rhizoma Acori tatarinowii and herb Capsellae, are known for their effects of stimulating appetite, anti-inflammatory, for reducing diarrhea, promoting digestive juice secretion and strengthening the stomach, respectively (Lei, 1995). All of the actions aforementioned can result in the better digestibility of DM and N. Similarly, Kong et al. (2009) reported that the Acanthopanax senticosus extract (1 g/kg) can enhance the apparent digestibility of amino acids (AA) of weanling pigs.

Herbs and its extracts are well known to exert antimicrobial actions in vitro against important pathogens, including fungi (Dorman and Deans, 2000; Burt, 2004; Si et al., 2006; O¨zer et al., 2007). Fung et al. (2008) demonstrated that supplementation with herbal extract could increase Lactobacillus amylovorus, Lactobacillus salivarius, Bacillus subtilis, and Clostridium lituseburens, but decreased those co-migrating with Staphylococcus aureus, Salmonella typhimurium, Ruminococcus forques, and E. coli O157:H7, which is in agreement with our current study. Also, Fang, J. et al. (2009) observed that herbal extract can increase the population of Lactobacillus and related lactic acid bacterial by at least 3-fold. The results from the present experiments also suggest the potential of HE in suppressing pathogenic bacteria and enriching beneficial bacteria, such as Lactobacilli.

The gastrointestinal system and its associated lymphoid is the largest immunologically competent organ in the body, and maturation and optimal development of the immune system after birth depend on the development and composition of the indigenous microflora and vice versa (Michael and

Marteau, 2007). The herbal extract exerts the effects to regulate the **m**icrobiota distribution, which are necessary for development of the gut immune system (Blum et al., 2002). This could be related to an increase in the number of Lactobacilli observed in the large intestine of the same animals (Castillo et al., 2006) because lactic acid bacteria, in general, can stimulate the immune system (Schley and Field, 2002). The WBC, lymphocyte, and IgG were positively increased by the herbal extract, while no infection was observed (data not shown). In addition, a reduction in subclinical infections because of antimicrobial effects may contribute to improved nutrient digestibility and a reduction in the demand for nutrients by the gut-associated immune tissue.

In summary, dietary supplementation of herbal extract can regulate the gut microbiota composition, thereby improving the performance, nutrient digestibility, and can modulate immune-related blood cell counts in early weaning piglets.

REFERENCES

1. Akhter M.H., Saoir M. & Bhide N.K. (1977) Anti-inflammatory effect of berberin in rats injected locally with choler toxin. Indian J Med. Res. 65, F133-41.
2. Blum S., Haller. D., Pfeifer A. & E. J. Schiffrin E.J. (2002) Probiotics and immune responses. Clin. Rev. Allergy. Immunol 22, F287-309.
3. Bosi P., Casini L., Finamore A., Cremokolini C., Merialdi G., Trevisi G., Nobili F. & Mengheri E. (2004) Spray-dried plasma improves growth performance and reduces inflammatory status of weaned pigs challenged with enterotoxigenic Escherichia coli K88. J Anim. Sci. 82, F1764-72.
4. Burt S. (2004) Essential oils: Their antibacterial properties and potential applications in food-A review. Int J Food Microbiol 94, F223-53.
5. Castillo M., Martı´n-Oru´e S.M., Roca M., Manzanilla E.G., Badiola I. & Perez J.F. (2006) The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. J Anim. Sci. 84, F2725–34.
6. Dorman H. J. D. & Deans S.G. (2000) Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl. Microbiol 88, F308-16.
7. Gong G., Hai. Y., Ting L., Mei L., Weiduo S., de Lange F.M., Cornelis. & Dewey C. (2008) Characterization of ileal bacterial microbiota in newly-weaned pigs in response to feeding lincomycin, organic acids or herbal extract. Livest. Sci. 116, F318-22
8. Fang J., Yan J.F., Kong X.F., Ruan Z., Liu Z.Q., Huang R.L. & Yin Y.L. (2009) Dietary supplementation with Acanthopanax senticosus extract enhances gut health in weanling piglets. Livest. Sci. 123, F268-75.
9. Kamel C. (2001) Tracing modes of action and the roles of plant extracts in non-ruminants. Recent Adv. Anim. Nutr, 135-50.
10. Kaul T. N., Middleton E. & Ogra P.L. (1985) Antiviral effect of flavonoids on human viruses. J. Med. Virol. 15, F71-79.
11. Kong X. F., Yin F.G., He Q.H., Liu H.J., Li T.J., Hua.ng J.R., Fan M.Z., Liu Y.L, Hou Y.Q., Ruan L Peng Z., Deng Z.Y., Xie M.Y., Xiong H. & Yin Y.L. (2009) Acanthopanax senticosus extract as a dietary additives enhances the apparent ileal digestibility of amino acids in weaned piglets. Livest. Sci. 123, F261-67.
12. Lee J.M., Choe S.W., Jo S.H. & Lee S.J. (2003) Effect of forsythia viridissima extracts on antioxidative system and lipid peroxidation of liver in rats fed high-cholesterol diet. Korean Nutrition Society 36, F990-97.
13. Lei Z.Q. (1995) Expectorant, Antitussive and Antiasthmatic Effects Medicine (Cortex Mori), 244-45; Orifice-OpeningMedicine (Rhizoma Acori Tatarinowii), 273; Li Shui Tong Lin Medicine (Herba Capsellae), 138; Sedative, Hypnotic and Hypoxia-Resisting Effects Medicine (Semen Ziziphi Spinosae), 252-53. In: Herbology. Shanghai.
14. Liu L. M., Yu Z.F. & Wu C.Y. (1993) Studies of the spasm-relieving effect of Glycyrrhiza uralensis Fisch.--Aster tataricus L.F. pulvis mixture on the trachea in guinea pigs. China J Chin. Mat. Med. 18, F556-75.
15. Makhey D., Gatto B., Yu C., Liu A., Liu L.F. & Lavoe E.J. (1995) Protoberverine alkaloid and related compounds as dual inhibitor of mammalian to poisomerase I and II. Med. Chem. Res. 51, 12.
16. Michael de V. & Marteau P.R. (2007) Probiotics and prebiotics: Effects on diarrhea. J Nutr. 137, F803-11.
17. Middleton E., Jr. & Kandaswami C. (1992) Effects of flavonoids on immune and inflammatory cell functions. Biochem. Pharmacol. 43, F1167-79.
18. NRC, Nutrient Requirements of Swine. National Research Council. 10 Edition. National Academy Press. Washington, DC, 1998.
19. O¨zer H., So¨kmen. M., Gu¨ llu¨ ce M., Adigu¨ zel A., Sahi F., So¨kmen A., Kilic H. & O¨. Baris. (2007) Chemical composition and antimicrobial and antioxidant activities of the essential oil and methanol extract of Hippomarathum microcarpum (Bieb.) from Turkey. J Agric. Food Chem. 55, F937-42.
20. Park K. M., Han Y.K. & Park K.W. (2000) Effects of herb-Mix supplementation on the growth performance and serum growth hormone in weaned pigs. Asian-Aust J Anim. Sci. 13, F791-94.
21. Rim Y.S., Park Y.M., Park M.S., Kim K.Y., Kim M.J., Choi. & Y H. (2000) OLE_LINK30OLE_LINK31OLE_LINK32Screening of antioxidant and antimicrobial activity in native plants OLE_LINK30OLE_LINK31OLE_LINK32. Korean J Medicinal Crop Sci. 8, F342-50.
2 2.SAS, SAS User's Guide. Release 6.12 Edition. SAS Institute Inc. Cary, NC, 1996.
23. Schley P. D. & Field C.J. (2002) The immune-enhancing effects of dietary fibres and prebiotics. Br J Nutr. 87, F221–30.
24. Si W., Gong. J., Tsao R., Zhou T., Yu H., Poppe C., Johnson R. & Du Z. (2006) Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. J Appl. Microbiol. 100, F296-305.
25. Xu Z. R., Hu C.H., Xia M.S., Zhan X.A. & Wang M.Q. (2003) Effects of dietary fructo-oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82, F648-54.
26. Zhao S., H. & Cao Y. (1991) Protective effect of Glycyrrhiza uralensis Fisch and its extracts on the chronic desensitization of beta-adrenoceptors in guinea pigs. China J Chin. Mat. Med. 16, F370-84.
27. Zhang Y. Z., Peng Li., Joshua Gong., Wu G.Y., Fan M.Z., Liu Y.L., Hou Y.Q. & Yin Y.L. (2008) Dietary supplementation with Acanthopanax senticosus extract enhances gut health in weanling piglets. Livest. Sci. 123, F268-75.

