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The Effect of Evening Primrose Extract (*Oenotherabiennis*) on Growth Performance, Nutrient Digestibility, Blood Characteristics, Faecal Microbiota and Fecal Scorein Weaned Pigs

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***Abstract* – A 6-week experiment using 100commercial cross-bred piglets, (Duroc × Yorkshire) × Landrace, weaned at 21 d with a bodyweight (BW) of 6.21 ± 0.62 kg was conducted to evaluate the effects of evening primrose extract (EPE) on performance, nutrient digestibility, blood characteristics, fecal microbial concentration and fecal score. Piglets were randomly allocated to five treatments consisting of a positive control containing an antibiotic (33 ppm Tiamulin; PC), a negative control (NC, without antibiotic) and the same diet supplemented with 0.05, 0.1 and 0.2% EPE. In the period 3-6 weeks and overall (0-6 weeks) pigs offered the diets supplemented with EPE exhibited similar performance as those offered the PC diet and tended to grow faster and were significantly more feed efficient than those offered the NC diet. During 3 to 6 weeks, piglets fed the EPE diets had greater ADG (P=0.04) and G/F (P=0.04) than the piglets fed the control diet. Compared with NC, the supplementation of EPE significantly decreased the fecal score of piglets in the first 2 weeks after weaning. Compared with the NC, the supplementation of EPE increased (P =0.02) the DM digestibility at 6 week. The pigs fed the EPE diets had a lower (P=0.01) LDL-cholesterol than the pigs fed the NC diet. Compared with NC, pigs fed EPE diets had a higher *Lactobacillus* number (P=0.01) and lower *E. coli*number (P=0.01) in feces. None of these parameters differed significantly between pigs offered the EPE and PC diets. In conclusion, the results indicate that evening primrose extract supplementation can increase the growth performance, DM digestibility, the population of fecal *Lactobacillusspp.* and decrease the serum LDL-cholesterol concentration and the population of fecal *E. coli.* Meanwhile, there is no difference for the growth performance, blood profiles and the fecal micro flora of the piglet between evening primrose extract and antibiotic. As a result, EPE could be a potential candidate of antibiotic alternative.**

***Keywords* – Blood Characteristics, Evening Primrose Extract, Fecal Microbial, Growth Performance, Nutrient Digestibility.**

INTRODUCTION

With the withdrawal of antibiotics at prophylactic levels in the feed of farmed livestock from 2006 in Europe, much interest focused on antibiotics alternative which can improve the health and growth performance in livestock.

In recent years, many types of herbal extract shave been used in livestock production as alternatives to antibiotic growth promoters (Liu et al., 2008; Yan et al., 2011a) [1][2].Many previous studies showed that plant extracts contain different molecules and could been considered as antibiotics alternative because they could improve growth performance and nutrient digestibility, decreased noxious gas emission, and control post weanling syndrome (Hernandez el al., 2004; Wenk, 2003; Hong et al., 2004; Cho et al., 2006; Yan et al., 2011a)[3][4][5][6][2]. Therefore, many herbal extracts have been used in swine production industry as feed additives which can stimulate the animal growth performance and keep the animal healthy (Wang et al., 2008; Huang et al., 2010; Yan et al., 2011b) [7][8][9].

Evening primrose is a biennial herb that has a long history as an alternative medicine. It has been reported to be used in the treatment of diseases in humans, such as significant anti-ulcer and cytoprotective effect (Al-shabanah, 1997) [10].This may be due to its high content of poly phenols (Matsumoto-Nakano et al., 2011) [11]and abundant γ-linolenic acid(Hudson, 1984) [12]in extracts of evening primrose. Polyphenolsexhibit a wide range of biological effects such asantioxidation, antitumor and antibacterial properties (Salah et al., 1995; Gladine et al., 2007) [13][14]. Previous studies from our laboratory showed that polyphenol-rich plant extracts can enhance the growth, antioxidant status, nutrient metabolism and immune activity of pigs(Ao, et al., 2011; Yan et al., 2011b) [15][9]. However, Bioavailability differs greatly from one polyphenol to another (Manach et al., 2005) [16].Gamma-linolenicacid was also found to have excellent efficacy in reducing the incidence and severity of inflammatory/hyper proliferative diseases, without compromising host defenses (Kleijnen, 1994; Johnson, et al., 1997; Fan and Chapkin, 1998) [17][18][19]. To date, studies on the utilization of evening primrose extract in weaned piglets have not been carried out. The current experiment was designed to investigate the effects of evening primrose extract supplementation on growth performance, nutrient digestibility, and blood characteristics fecal micro biota and fecal score in weanling pigs.

II. MATERIAL AND METHODS

All pigs used in this trial were handled in accordance with the guidelines set forth by the Animal Care and Use Committee of Dankook University (Cheonan City, South Korea).

Table I: Compositions and chemical composition of diet during phase 1[1]

Item	PC	EPE (%)			
Ingredient, g/kg		0.0	0.05	0.1	0.2
Extruded corn	35.52	35.62	35.57	35.52	35.42
Extruded oat	5.00	5.00	5.00	5.00	5.00
Soybean meal, 44% CP	20.20	20.20	20.20	20.20	20.20
Fermented soybean meal	8.00	8.00	8.00	8.00	8.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Soy oil	4.80	4.80	4.80	4.80	4.80
Lactose	6.00	6.00	6.00	6.00	6.00
Whey powder	9.80	9.80	9.80	9.80	9.80
Milk product[2]	2.20	2.20	2.20	2.20	2.20
Monocalcium phosphate	1.00	1.00	1.00	1.00	1.00
Sugar[3]	2.00	2.00	2.00	2.00	2.00
L-Lys·HCl, 78%	0.25	0.25	0.25	0.25	0.25
DL-Met, 50%	0.15	0.15	0.15	0.15	0.15
L-Thr, 89%	0.08	0.08	0.08	0.08	0.08
Choline chloride, 25%	0.10	0.10	0.10	0.10	0.10
Vitamin premix[4]	0.10	0.10	0.10	0.10	0.10
Mineral premix[5]	0.20	0.20	0.20	0.20	0.20
Limestone	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30
Tiamulin(CTC, Korea)	0.10	-	-	-	-
EPE	-	-	0.05	0.10	0.20
Calculated composition					
ME, kcal/kg	3,526	3,550	3,543	3,535	3,510
Analyzed composition, g/kg					
CP	21.05	21.09	21.13	20.98	21.01
Lys	1.39	1.38	1.39	1.39	1.38
Met	0.50	0.49	0.51	0.50	0.49
Ca	0.80	0.80	0.80	0.80	0.80
Total P	0.76	0.76	0.76	0.76	0.76

[1]Abbreviation: NC= basal diet; PC=basal diet supplemented with 0.1% antibiotics (Tiamulin, CTC); EPE0.05, EPE0.01, EPE0.2= basal diets supplemented with 0.05, 0.1, 0.2% EPE.
[2]Whole milk product that contains 210 g of crude fat and 220 g of CP/kg.
[3]Sugar contain 89% sucrose.
[4]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.
[5]Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 80 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

A. Preparation of Evening Primrose Extract

Dried evening primrose was chopped and pulverized to pass a 2-mm screen, then extracted with 2 volumes of 70% methanol in a large-scaleextractor (CoBiotechk, Seoul, Korea) at room temperature for 24 h. The methanolic extract was filtered 2 to 3 times over cheesecloth, and the filtrate was evaporated under vacuum, lyophilized and crushed into a powder (EPE).The content of total phenolics was measured colorimetrically (Swain and Hillis, 1959; Naczk and Shahidi, 1989) [20][21] and expressed as (+)catechin-equivalents. The EPE used in the present study contained 83.4 mg/g (+) catechin-equivalents of total phenolics.

B. Experimental Design, Animals, and Facilities

A total of 100 newly-weaned Yorkshire ×Landrace × Duroc pigs, d 21 ± 1, average BW6.21 ±0.62 kg were allotted to 5 dietary treatments and fed for 6 wk.Piglets were randomly allocated to five treatments consisting of a positive control containing an antibiotic (33 ppm Tiamulin; PC), a negative control (NC, without antibiotic) and the same diet supplemented with 0.05, 0.1 and 0.2% EPE, substituted for equal weights of corn. The diets were formulated to meet or slightly exceed NRC (1998)[22] requirements (Tables 1, 2).Diets were separated into those fed from d 0to 14 (Phase 1) and from d 15 to 42 (Phase 2). Each treatment was applied to 4 replicate pens, each of which contained 3 barrows and 2 gilts, but otherwise assigned at random.

The piglets were housed in an environmentally-controlled nursery facility with 0.6×2.0 m slatted plastic floor pens (0.6×2.0 m, height 0.5 m). Room temperature was maintained at 30 ± 1°C for the first week, then gradually reduced by 1°C per week to 25°C. Pens were provided with a stainless steel feeder and one nipple waterer, which allowed *adlibitum* access to feed and water

throughout the experiment. The diets were presented in meal form.

C. Samples and measurements

Individual BW were taken and recorded on d 14 and 42 of the experiment to calculate average daily gain (ADG). Feed consumption was recorded every day on a pen basis to calculate average daily feed intake (ADFI) and gain/feed ratio (G/F).

From d 8 to 14 and d 36 to 42, chromic oxide was added to the diets at 0.2% as an indigestible marker(Fenton and Fenton, 1979) [23] for the determining of thecoefficient of total tract apparent digestibility (CTTAD) of dry matter (DM), nitrogen (N) and gross energy (GE). Fecal samples were obtained from all pigs in the afternoon by rectal massage and pooled on a within-penbasison d 12, 13, 14 and d 40, 41, 42, feed samples were obtained on the same day from each pen. Allfecal samples (4samples per treatment) and feed samples (4 samples per treatment) were stored in a freezer at –20°C until further analysis. Fecal and feed samples were lyophilized and finely ground to pass through a 1 mm screen, after which they were analyzed for DM (Method 930.15; AOAC, 1995) [24], Ca (Method 984.01; AOAC, 1995) [24], and P (Method 965.17; AOAC, 1995) [24]. Lysine and methionine were measured using an AA analyzer (Beckman 6300; Beckman Coulter, Inc., Fillerton, CA). Nitrogen was determined by a nitrogen analyzer (Kjectec 2300; Foss Tecator AB, Hoeganaes, Sweden), and GE by an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The CTTAD of nutrients was calculated using the formula according to Stein et al. (2006) after which they were analyzed for DM (Method 930.15; AOAC, 1995) [24], Ca (Method 984.01; AOAC, 1995) [24], and P (Method 965.17; AOAC, 1995) [24]. Lysine and methionine were measured using an AA analyzer (Beckman 6300; Beckman Coulter, Inc., Fillerton, CA). Nitrogen was determined by a nitrogen analyzer (Kjectec 2300; Foss Tecator AB, Hoeganaes, Sweden), and GE by an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The CTTAD of nutrients was calculated using the formula according to Stein et al. (2006) [25].

Table 2: Compositions and chemical composition of diet during phase 2[1]

Item Ingredient, g/kg	PC	NC	EPE0.05	EPE0.1	EPE0.2
Extruded corn	454.0	455.0	454.5	454.0	453.0
Extruded oat	92.0	92.0	92.0	92.0	92.0
Soybean meal, 44% CP	296.5	296.5	296.5	296.5	296.5
Fish meal	25.0	25.0	25.0	25.0	25.0
Soy oil	30.0	30.0	30.0	30.0	30.0
Whey powder	61.5	61.5	61.5	61.5	61.5
Milk product[2]	21.0	21.0	21.0	21.0	21.0
Monocalcium phosphate	6.0	6.0	6.0	6.0	6.0
L-Lys·HCl, 78%	1.5	1.5	1.5	1.5	1.5
DL-Met, 50%	1.5	1.5	1.5	1.5	1.5
Choline chloride, 25%	1.0	1.0	1.0	1.0	1.0
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0
Mineral premix[4]	2.0	2.0	2.0	2.0	2.0
Limestone	3.0	3.0	3.0	3.0	3.0
Salt	3.0	3.0	3.0	3.0	3.0
Tiamulin(CTC, Korea)	1.0	-	-	-	-
EPE	-	-	0.5	1.0	2.0
Calculated composition					
ME, kcal/kg	3,412	3,450	3,432	3,428	3,418
Analyzed composition, g/kg					
CP	205.0	205.5	206.0	204.8	206.1
Lys	13.1	13.0	13.2	13.1	13.0
Met	4.5	4.4	4.5	4.4	4.5
Ca	7.4	7.2	7.4	7.4	7.3
Total P	6.4	6.4	6.4	6.3	6.4

[1]Abbreviation: NC= basal diet; PC=basal diet supplemented with 0.1% antibiotics (Tiamulin, CTC); EPE0.05, EPE0.01, EPE0.2= basal diets supplemented with 0.05, 0.1, 0.2% EPE.

[2]Whole milk product that contains 210 g of crude fat and 220 g of CP/kg.

[3]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.

[4]Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 80 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

Table III: The effects of evening primrose extract on growth performance and fecal score in weanling pigs[1]

Items	PC[1]	NC	EPE0.05	EPE0.1	EPE0.2	SEM[2]	P-value	Contrast[3]				
								NC vs EPE	PC vs EPE	NC vs PC	Linear	Quadratic
Phase1 (0-2 w)												
ADG, g	340	328	336	339	330	9	0.45	0.58	0.72	0.23	0.47	0.35
ADFI, g	428	417	430	419	411	11	0.16	0.90	0.67	0.02	0.35	0.41
G/F	0.79	0.79	0.78	0.81	0.80	0.023	0.82	0.69	0.84	0.39	0.25	0.90
Phase2 (3-6 w)												
ADG, g	547	517	560	549	542	12	0.04	0.04	0.87	0.41	0.06	0.17
ADFI, g	753	762	761	771	764	20	0.53	0.89	0.64	0.57	0.55	0.85
G/F	0.73	0.68	0.76	0.71	0.71	0.015	0.03	0.04	0.72	0.05	0.09	0.28
Overall (0-6 w)												
ADG, g	478	454	485	479	471	8	0.01	0.08	0.98	0.32	0.25	0.19
ADFI, g	645	647	650	653	646	14	0.157	0.88	0.79	0.22	0.59	0.60
G/F	0.74	0.70	0.75	0.73	0.73	0.012	0.082	0.04	0.69	0.05	0.32	0.09
Fecal score[4]	3.15	3.17	3.10	3.09	3.09	0.03	0.246	0.03	0.12	0.94	0.18	0.08

[1]Abbreviation: NC= basal diet; PC=basal diet supplemented with 0.1% antibiotics (Tiamulin, CTC); EPE0.05, EPE0.01, EPE0.2= basal diets supplemented with 0.05, 0.1, 0.2% EPE;
[2]Standard error of the mean.
[3]NC vs EPE means NC compared with the average value of all 3 EPE diets. PC vs EPE means PC compared with the average value of all 3 EPE diets.
[4]Fecal scores were determined at 08:00 and 20:00 daily in phase 1 using the following fecal scoring system: 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery liquid that can be poured.

At the end of wk2 and wk 6 of the experiment, 2 pigs (1 gilt and 1 barrow) were randomly selected from each pen and bled by jugular venipuncture using both uncoated and K_3EDTA-coated vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ)to obtain serum and whole blood. After allowing 60 min for clotting, samples were centrifuged for 15 min at 3000 ×g at 4°C and the serum was harvested and then used to determine the metabolic variables. The number of white blood cells (WBC), red blood cells (RBC) and percent lymphocytes concentrations in the whole blood samples were determined using an Automatic Blood Analyzer (ADVIA 120; Bayer, New York, NY). The concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in the serum samples were analyzed with an automatic biochemical analyzer (RA-1000; Bayer, New York, NY) using colorimetric methods.

Table IV: The effects of an antibiotic and dose of evening primrose extract (EPE) on the coefficient of apparent total tract nutrient digestibility at two time periods in weanerpigs[1]

Items,%	PC1	NC	EPE0.05	EPE0.1	EPE0.2	SEM[2]	P-value	Contrast[3]				
								NC vs EPE	PC vs EPE	NC vs PC	Linear	Quadratic
2 week												
Dry matter	84.5	83.9	84.4	84.3	84.2	0.7	0.57	0.83	0.35	0.66	0.24	0.69
Nitrogen	82.5	82.2	82.7	83.2	83.3	1.00	0.12	0.72	0.63	0.53	0.56	0.85
Energy	85.6	84.5	85.8	85.1	85.1	0.78	0.66	0.32	0.45	0.39	0.32	0.90
6 week												
Dry matter	80.4	79.9	82.3	81.2	80.4	0.80	0.03	0.02	0.72	0.57	0.14	0.15
Nitrogen	78.8	78.7	80.1	79.5	79.2	1.32	0.58	0.43	0.68	0.72	0.21	0.84
Energy	79.8	79.6	81.9	80.6	80.0	0.80	0.43	0.17	0.51	0.41	0.14	0.49

[1]Abbreviation: NC= basal diet; PC=basal diet supplemented with 0.1% antibiotics (Tiamulin, CTC); EPE0.05, EPE0.01, EPE0.2= basal diets supplemented with 0.05, 0.1, 0.2% EPE;
[2]Standard errorof the mean.
[3]NC vs EPE means NC compared with the average value of all 3 EPE diets. PC vs EPE means PC compared with the average value of all 3 EPE diets.

At the end of the experiment, fecal samples were collected from all pigs, pooled on a within pen basis, and held on ice for no more than 2 hr before processing. Pooled feces (1 g) from each pen were homogenized in 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ), 10-fold serial dilutions (in 1% peptone solution) were plated on MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* spp. medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany). The *Lactobacilli* plates were incubated anaerobically for 48 h at 39°C and MacConkey plates were incubated anaerobically for 24 h at 37°C, then the colony numbers of *Lactobacillus*or *E. coli*, respectively, were recorded.

During the first 2 wk, fecal consistency scoreswere determined twice daily at 08:00 and 20:00using the

following scoring system from 1 to 5 (Hu et al., 2012) [26]: 1 = hard feces, 2 = firm well formed, 3 = soft and partially formed feces, 4 = loose, semi-liquid feces, and 5 = watery feces.Scores were recorded on a pen basis after observations of individual pigs and the appearance of feces in the pen.

D. Statistical analyses

For all analyses, replicate (pen) served as the experimental unit. Data was analyzed by ANOVA using the General Linear Models (GLM) procedure of SAS (SAS Institute, 2001) [27]. When significant differences among the five groups were detected, orthogonal contrasts were used to the effect of treatments: NC vs. EPE and PC vs. EPE. Linear and quadratic contrasts were used to compare effects of increasing dietary EPE levels (0.05 - 0.2%). Variability in the data was expressed as the pooled SEM. $P< 0.05$ was considered significant difference.

Table V**:** The effects of evening primrose extract on blood profiles in weanling pigs[1]

Items	PC	NC	EPE0.05	EPE0.1	EPE0.2	SEM[2]	P-value	Contrast[3]				
								NC vs EPE	PC vs EPE	NC vs PC	Linear	Quadratic
Lymphocyte, %												
2 wk	45.82	44.92	45.26	46.14	45.66	4.57	0.48	0.90	0.98	0.03	0.33	0.94
6 wk	52.28	52.92	55.78	54.88	51.48	3.62	0.81	0.79	0.67	0.71	0.25	0.37
RBC, 10^6/µl												
2 wk	6.21	6.31	6.32	6.25	6.18	0.09	0.46	0.57	0.76	0.80	0.19	0.71
6 wk	6.22	6.32	6.16	6.27	6.28	0.12	0.99	0.51	0.92	0.92	0.43	0.47
WBC,												
2 wk	12.40	13.08	13.64	12.56	12.21	0.86	0.95	0.77	0.66	0.98	0.26	0.58
6 wk	15.03	14.97	14.80	15.59	15.16	1.63	1.00	0.90	0.93	0.89	0.35	0.93
Total cholesterol, mg/dL												
2 wk	136.4	137.8	137.4	138.8	135.8	10.4	0.60	0.94	0.89	0.57	0.48	0.82
6 wk	122.2	123.0	120.6	120.8	121.6	9.8	0.97	0.51	0.69	0.74	0.19	0.57
LDL-cholesterol, mg/dL												
2 wk	55.2	59.0	54.4	58.6	53.0	5.9	0.46	0.62	0.99	0.59	0.36	0.93
6 wk	39.2	43.4	34.4	33.0	40.2	2.5	0.20	0.01	0.22	0.62	0.14	0.29
HDL-cholesterol, mg/dL												
2 wk	59.4	60.6	57.6	57.2	59.6	6.2	0.65	0.75	0.87	0.62	0.18	0.69
6 wk	37.6	39.6	42.0	41.2	40.2	2.1	0.87	0.49	0.12	0.81	0.15	0.46

[1]Abbreviation: NC= basal diet; PC=basal diet supplemented with 0.1% antibiotics (Tiamulin, CTC); EPE0.05, EPE0.01, EPE0.2= basal diets supplemented with 0.05, 0.1, 0.2% EPE;
[2]Standard errorof the mean.
[3]NC vs EPE means NC compared with the average value of all 3 EPE diets; PC vs EPE means PC compared with the average value of all 3 EPE diets.

III. RESULTS

A. Growth Performance and Nutrient Digestibility

During phase 1 (0-14 days), pigs fed with PC diet had higher (P = 0.02) ADFI than those fed with NC diet. In phase 2 (23-42 days) and overall pigs in the PC and EPE treatments exhibited similar performance. In both periods, pigs offered the EPE diets grew significantly faster and exhibited a significantly higher G/F ratio than those in the NC treatment (Table 3). In phase 2 (23-42 days) and overall pigs on the PC treatment had higher (P=0.05) G/F ratio than those in the NC treatment. The fecal score during the first two weeks of the study was also significantly lower (P=0.03) for pigs on the EPE treatment compared with their NC counterparts, but similar between PC and NC treatment.

Table VI: The effects of of an antibiotic and dose of evening primrose extract (EPE) on the faecalmicroflora of pigs six weeks after weaning[1]

Items, log_{10}cfu/g	PC	NC	EPE0.05	EPE0.1	EPE0.2	SEM[2]	P-value	Contrast[3]				
								NC vs EPE	PC vs EPE	NC vs PC	Linear	Quadratic
Lactobacillus	7.32	7.46	7.58	7.63	7.51	0.04	0.154	0.01	0.57	0.06	0.12	0.21
E. coli	6.25	6.37	6.17	6.14	6.13	0.06	0.229	0.01	0.29	0.46	0.35	0.17

[1]Abbreviation: NC= basal diet; PC=basal diet supplemented with 0.1% antibiotics (Tiamulin, CTC); EPE0.05, EPE0.01, EPE0.2= basal diets supplemented with 0.05, 0.1, 0.2% EPE;
[2]Standard errorof the mean.
[3]NC vs EPE means NC compared with the average value of all 3 EPE diets. PC vs EPE means PC compared with the average value of all 3 EPE diets.

B. Nutrient Digestibility

The results for apparent tract digestibility are given in Table 5.There were no treatment effects at two weeks. At 6 weeks the apparent tract digestibility of DM did not differ between the EPE and PC treatments but was significantly higher (P=0.02) for the EPE than for the NC treatment. The apparent tract digestibility of N and GE was unaffected by treatments.

C. Blood Variables

Addition of the antibiotic or EPE did not significantly affect (P>0.05) the numbers of RBC, or WBC, or serum concentrations of total and HDL-cholesterol(Table 5).At wk 2, the lymphocyte of pigs fed with PC diet was higher (P=0.03) than those fed with NC diet. At wk 6, LDL cholesterol averaged (mean value) for pigs on the NC treatment and was reduced by 17% by EPE supplementation (P =0.01). There was no significant effect (P>0.05) was observed between PC and EPE diets.

D. Fecal Micro flora Population

Compared with NC, pigs fed EPE diets had a higher *Lactobacillus* number (P=0.01) and lower *E. coli* number (P=0.01) in feces (Table 6). There was no significant effect between the PC and the EPE groups.

IV. Discussion

A. Growth Performance and Nutrient Digestibility

In the present study, piglets fed with the EPE diets had a greater ADG and G/F ratio than those fed with thebasal diet. There was no effect on the ADFI, which is in agreement with Greiner et al. (2001) [28] who reported that weaner pigs fed with 200to 400 mg/kg *Houttuyniacor data* could improve growth performance. Cho et al. (2012) [29] also showed that Laquer tree extracts did improve the ADG and G: F. The main bioactive compounds used in these experiments were polyphenolics. Polyphenolic compounds are of great importance in the expression of antioxidant and antitumor properties (Knekt et al., 2002) [30]. Several previous studies have reported that a wide range of herbal species and extracts expressed beneficial properties in the digestive tract by balancing microbiota, an optimal precondition for an effective protection against pathogenic micro-organisms and an intact immune system (Wenk, 2003; Srinivasan et al., 2004; Czech et al., 2009) [4][31][32].This may be why, in the present study, the supplementation ofEPE enhanced the DM digestibility compared with the basal diet. However, Grela (2000) reported that the supplementation with herbal extracts increased ADG and ADFI, but had no effect on G:F of finishing pigs [33]. This discrepancy may be due to the herbal composition. Since the quality of herbal products can differ greatly due to the different herbal materials, selection of particular herbs and the forms of their administration which may include many different kinds of bioactive compounds (Windisch et al., 2008) [34]. Another reason for this inconsistency may be attributed to the age of the pig used. As pigs age, their digestive ssystems change profoundly (Nousiainen and Setala, 1993) [35].

In the current study, EPE enhanced the performance of pigs and there was no difference between pigs on the antibiotic and EPE treatment(s).The results suggest EPE may be an effective alternative to antibiotics for improving the performance of weaner pigs. However, the present is the first study on the effects of evening primrose extract in swine. Further studies are still required to verify and substantiate its effect on young growing pigs.

B. Blood Characteristics

In the current study, we found that EPE supplementation decreased the concentration of LDL cholesterol, even though it had no effect on the concentration of HDL cholesterol and total cholesterol compared with the basal diet. As all we know, LDL cholesterol collects in the walls of blood vessels, causing atherosclerosis in humans. Higher LDL cholesterol levels lead to a greater risk of heart attack in human from a sudden blood clot in an artery narrowed by atherosclerosis(Kim et al., 2006; Sniderman et al., 1980) [36][37]. Evening primrose seeds contain an oil characterized by its high content of γ-linolenic acid (all cis-6,9,12-octadecatrienoic acid) which is a type of PUFA(Hudson, 1984) [12].Many studies haveindicated that γ-linolenic acid can lower plasma cholesterol levels (Horrobin and Manku,1983, Sugano et al.,1986; Lee et al., 2007) [38][39][40]. The reason for this phenomenon may be due to that the elaborated product of GLA , dihomo-γ-linolenic acid (DGLA), that can be converted by inflammatory cells to 15-(S)-hydroxy-8,11,13-eicosatrie-noic acid and prostaglandin E_1which possess both anti-inflammatory and anti- proliferative properties (Fan and Chapkin,1998) [19].

C. Fecal Microbial Population and Fecal Score

Fecal changes in young growing animals are frequent and can be signs of infection by digestive pathogens (bacteria, viruses, parasites) and indicators of nutritional and environmental stress (Grellet et al., 2012) [41]. Some herbs have been found to have anti-microbial activity and anti-viral properties (Hammer et al. 1999) [42]. In the current study, feeding pigs withEPE enhanced *Lactobacillus* and reduced populations of *E. coli* organisms compared with control group. This is in agreement with the Namkung et al.(2004) who reported that herbal supplementation (0.75% inclusion; containing cinnamon, thyme and oregano extract) reduced the proliferation of potentially harmful *coliform* bacteria only [43]. It should be noted that different types of antibiotic would affect the gut microflora in different ways.The ability of the herbal extract to kill bacteria depends on their chemical structure (Si et al., 2006) [44]. Evening primroses extracts have a polyphenol present as a component in it, which possesses anti-pathogenic properties (Salah et al., 1995; Gladine et al., 2007) [13][14]. In our study, there was no difference observed for the fecal microflora between EPE diet and the PC diet

In conclusion, the results indicate that evening primrose extract supplementation can increase the growth performance, DM digestibility, the population of the *Lactobacillus* and decrease the LDL-cholesterol concentration and the population of *E. coli.* Meanwhile, there is no difference for the growth performance, blood

profiles and the fecal microflora of the piglet between evening primrose extract supplementation and antibiotic supplementation. As a result, EPE could be a potential candidate of antibiotic alternative.

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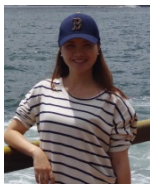

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