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Effects of Dietary Supplementation with Blended Essential Oils on Growth Performance, Nutrient Digestibility, Blood Profiles and Fecal Characteristics in Weanling Pigs

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ABSTRACT : The influence of dietary supplementation with blended essential oil on growth performance, nutrient digestibility, blood profiles and fecal characteristics was evaluated in 125 crossed ((Duroc×Yorkshire)×Landrace) pigs (6.21±0.20 kg initial body weight and 21 d average age). The pigs were allotted to the following treatments: i) NC (antibiotic free diet), ii) PC (NC diet+44 ppm tylosin), iii) T1 (NC diet+0.1% essential oil), iv) T2 (NC diet+0.1% essential oil (with 0.3% Benzoic acid)) and v) T3 (NC+22 ppm tylosin and 0.05% essential oil). Average daily gain (ADG) was improved in the T2 group on d 14 ($p<0.05$). In addition, nutrient digestibility was partially affected (both positively and negatively) by the treatments. Furthermore, the immune system was stimulated and the fecal pH and fecal noxious gases were improved in pigs that received the diets supplemented with essential oil ($p<0.05$). The appearance and score of diarrhea also tended to be lower in pigs that were subjected to the essential oil treatments. Collectively, the results of this study indicate that supplementation of the diet with blended essential oils could replace treatment with antibiotics to improve growth performance and fecal characteristics. (**Key Words :** Essential Oil, Fecal Characteristic, Growth Performance, Weanling Pig)

INTRODUCTION

Antibiotics are commonly fed to animals in livestock production systems to prevent disease and metabolic disorders, as well as to improve feed efficiency. However, in recent years, public concern over the routine use of antibiotics in livestock has increased due to the emergence of antibiotic resistant bacteria that may represent a risk to human health. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Plant extracts have been shown to offer a unique opportunity in this regard (Wallace, 2004) because many plants produce secondary metabolites, such as saponins and tannins, which have antimicrobial properties. The beneficial effects of essential oils on farm animals include the activation of feed intake and secretion of digestive juices, immune stimulation, and anti-bacterial, coccidiostatic, antiviral and antioxidant properties (Wenk, 2003). Contrary to their name, essential oils are not true oils (*i.e.*, lipids), but are commonly derived from the components responsible for fragrance, or *Quinta essentia*, of plants. Essential oils are considered safe for human and animal consumption, and are categorized as generally recognized as safe (GRAS; FDA, 2004) in the USA.

The antibiotic effect of essential oils (0.03% supplementation) has been confirmed by several studies (Hong et al., 2004; Cho et al., 2006), which found that they could be used to replace antibiotic supplements in feed provided to weanling pigs with no negative effects on growth performance. In addition, a study conducted by Cho et al. (2006) also found that essential oils appeared to improve nitrogen digestibility and reduce the production of noxious gas. The volatile components of many plants are responsible for the characteristic aroma and antibacterial activity of spices that have historically been used to inhibit bacterial growth (Hirasa and Takemasa, 1998). However, these essential oils appear to be selective in their antibacterial action, with the spectrum of antibacterial activity varying with the compounds tested (Janssen et al., 1986; Demetzos et al., 1997; Lis-Balchin and Deans, 1997).

Several experiments have investigated the reduction of CP concentration in the diet of growing-finishing pigs to determine if it reduced N excretion or ammonia emission

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from manure. Until recently, the emission of manure odors by nursery pigs has been largely ignored (Whitney et al., 1999; van Zeeland et al., 2000), most likely because the output of feces and urine is relatively low. However, nursery pigs require diets with high amino acid concentrations (NRC, 1998), and any excess protein will contribute to urea formation, which may be hydrolyzed to odors, such as ammonia, by urease present in feed and manure (Aarnink et al., 1998). In addition, the concentration of odors in nursery rooms may be high because ventilation rates are often reduced to conserve heat when outside temperatures are low.

This study was conducted to investigate the effect of dietary supplementation with blended essential oils on growth performance, nutrient digestibility, blood profiles and fecal characteristics in weanling pigs.

MATERIALS AND METHODS

Animals and diets

One hundred and twenty five crossed ((Duroc× Yorkshire)×Landrace) pigs (6.21±0.20 kg initial body weight and 21 d average age) were used in this experiment. The experimental period lasted for 6 weeks and was conducted using a randomized complete block design with pigs being assigned to categories based on their body weight and sex. There were five pigs per pen and five pens per treatment. The treatment groups included: i) NC (antibiotic free diet), ii) PC (NC diet+44 ppm tylosin), iii) T1 (NC diet+0.1% essential oil), iv) T2 (NC diet+0.1% essential oil (with 0.3% Benzoic acid)) and v) T3 (NC+ 22 ppm tylosin and 0.05% essential oil). The diets were formulated to meet or exceed the nutrient requirements recommended by the National Research Council (1998).

Each group of pigs was housed in an environmentally well controlled nursery facility with slatted plastic flooring and a mechanical ventilation system. The environmental temperature was controlled and maintained at 30°C for the first week of the experiment and was then reduced by 1°C per week for the next three weeks. Each pen contained a stainless steel feeder and one nipple waterer that allowed for *ad libitum* access to feed and water throughout the experiment.

The blended essential oil was formulated by abstracts of *Cinnamomum verum*, *Origanum vulgare* spp., *Syzygium aromaticum*, *Thymus vulgaris* and *Rosmarinus*, and the main antimicrobial components were cinnamaldehyde, carvacrol, eugenol, thymol and eugenol.

Performance and fecal consistency score measurements

The ADG and average daily feed intake (ADFI) were measured on d 14, 28 and 42, at which time the gain/feed ratio was also calculated. Chromic oxide (0.2%) was added to all of the diets as an indigestible marker to allow the digestibility to be evaluated. On d 14, 28 and 42, fecal samples were collected from at least two pigs per pen by rectal massage and then pooled within the pen. Each of the fecal samples, together with the feed samples, was stored in a refrigerator at -20°C until further analysis was performed. Concentrations of the dry matter (DM), nitrogen (N) and energy in the feed and feces were analyzed according to the AOAC (1995) procedures. Chromium was analyzed by UV

Table 1. Compositions of experimental diets (as-fed basis)

Ingredients (%)	Phase 1 (d 0-14)	Phase 2 (d 15- 42)
Expanded corn	5.35	34.62
Expanded oat	10.00	-
Biscuit meal[a]	-	5.00
Soybean meal	8.00	20.00
Fermented soybean meal	7.80	8.20
Fish meal	5.00	4.00
Soy oil	4.15	4.80
Lactose	10.00	6.00
Whey	16.50	10.00
Milk product[1]	13.00	2.00
Lecithin	0.50	-
Monocalcium phosphate	1.25	1.00
Organic acid	1.00	0.80
Glucose	5.00	-
Sugar	4.00	2.00
Plasma powder	6.50	-
L-lysine-HCl	0.12	0.25
DL-methinine	0.26	0.15
L-threonine	0.77	0.08
Zinc oxide	0.30	0.30
Choline chloride	0.20	0.10
Vitamin premix[b]	0.10	0.10
Mineral premix[c]	0.20	0.20
Limestone	-	0.20
Salt	-	0.20
Chemical composition[d]		
ME (Kcal/kg)	3,540	3,545
Crude protein (%)	22.00	21.00
Lysine (%)	1.57	1.41
Methionine (%)	0.60	0.49
Calcium (%)	0.80	0.78
Phosphorus (%)	0.76	0.76

[a] Mainly contains 21% fat and 22% protein.
[b] 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, d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.
[c] Provided per kg of complete diet: Fe, 200 mg; Cu, 12 mg; Zn, 200 mg; Mn, 8 mg; I, 0.28 mg; Se, 0.15 mg.
[d] Calculated values.

absorption spectrophotometry (Shimadzu, UV-1201, Japan), N was measured using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden) and the gross energy content was measured using an adiabatic bomb calorimeter (model 1281, Parr,Moline, IL). The DM, N and energy digestibility was then calculated using the indicator method.

Fresh feces and urine samples were collected randomly from at least two pigs in each pen every afternoon between d 40 and 42. Samples were collected at 2-h intervals in containers with sealed lids and then immediately stored at 4°C for the duration of the period. After the collection period, the fresh feces and urine collected from each pen were mixed well (100 g+500 ml) and then placed in two 2.6-L plastic boxes that each contained a small hole in one side. All samples were prepared in duplicate. The slurry pH was measured immediately after mixing at room temperature (25°C) using a glass electrode (NWKbinar pH, K-21, Landsberg, Germany) that was directly submerged into the slurry samples following the manufacturer's protocols. The pH was measured at three different sites in the middle layer (approximately 1 cm beneath the surface) of the samples and the mean pH was then used for statistical analyses. After the pH was measured, the small hole that was present in each box was sealed with adhesive plaster and the samples were allowed to ferment for a period of 30 days at room temperature (25°C). After the fermentation period, the gases that formed were analyzed using a Gastec (model GV-100) gas sampling pump (Gastec Corp., Gastec detector tube No. 3L and 3La for NH_3; No. 4LL and 4LK for H_2S; No. 70 and 70L for RSH, Gastec Corp, detector tube, Japan). Prior to measurement, the slurry samples were manually shaken for approximately 30 s to disrupt any crust formation on the surface of the slurry sample and to homogenize them. The adhesive plaster was then punctured and 100 ml of headspace air was sampled from approximately 2.0 cm above the slurry surface. Following sampling of the gas, each box was re-sealed with adhesive plaster.

The appearance of diarrhea and the diarrhea score were determined during the first 7 days of the experiment. Fecal consistency scoring was based on the following index described by Sherman et al. (1983): 0, normal (feces firm and well formed); 1, soft consistency (feces soft and formed); 2, mild diarrhea (fluid feces, usually yellowish); and 3, severe diarrhea (feces watery and projectile).

Determination of blood constituents

The concentrations of red blood cells (RBC), white blood cells (WBC), lymphocytes, neutrophils and monocytes in the blood were measured to investigate the effects of plant extract supplementation in weanling pigs. At the beginning of the experiment, two pigs that looked healthy were selected at random from each pen (n = 50) and blood samples were collected via jugular venipuncture .The same pigs were bled again on d 14, 28 and 42. At each collection time, the blood samples were collected into both a nonheparinized and a K3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) to enable evaluation of the serum and whole blood, respectively. The white blood cell (WBC), red blood cell (RBC) and lymphocyte counts were analyzed using an automatic blood analyzer (ADVIA 120, Bayer, NY). The serum samples were then centrifuged (2,000×g) for 30 minutes at 4°C, after which the serum immunoglobin G (IgG) concentration was determined using an automatic biochemistry analyzer (HITACHI 747, Japan).

Statistical analyses

All data were evaluated by one-way ANOVA using the GLM procedure (SAS). Significant differences in the mean values among dietary treatments were analyzed by repeated measures and Duncan's tests using the GLM procedure. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

Table 2 shows the effects of dietary supplementation with blended essential oil on growth performance in weanling pigs. From d 0 to 14, the ADG of pigs fed the T2 diet was greater than that of pigs fed the NC and T1 diets ($p<0.05$). However, no differences in the ADG were observed among groups for the remainder of the experiment. In addition, no differences in the ADFI and the gain/feed ratio were observed among the treatments throughout the experiment. These results are in partial agreement with those of Hong et al. (2004), who indicated that the addition of dietary plant extract to weanling pigs affected the ADG from d 10 to 20 after weaning. In addition, Holden and McKean (2002) reported that the ADG and the feed/gain ratio of pigs fed 2 or 3% botanical items were similar to those of pigs that were provided with antibiotic treatments and were greater than those of pigs that did not receive antibiotic. Furthermore, Kamel (2001) and Silvia and Asensio (2002) demonstrated that pigs fed a complex containing essential oils and organic acid had an increased ADG and ADFI.

Nutrient digestibility

The nutrient digestibility is presented in Table 3. On d 14, DM digestibility in the T2 group showed the highest level, whereas that of the NC treatment showed the lowest level ($p<0.05$). Overall, the highest value of N digestibility was observed in pigs in the T2 group ($p<0.05$), whereas the lowest N digestibility was observed in pigs in the NC group ($p<0.05$). The energy digestibility of pigs in the PC, T2 and

Table 2. Effects of dietary supplementation with blended essential oils on growth performance in weanling pigs

Items	NC[1]	PC[1]	T1[1]	T2[1]	T3[1]	SE[2]
0-14 days						
ADG (g)	266[b]	286[ab]	269[b]	295[a]	276[ab]	7
ADFI (g)	410	425	417	436	420	22
Gain/feed	0.648	0.673	0.644	0.676	0.655	0.017
14-28 days						
ADG (g)	503	527	514	530	504	15
ADFI (g)	740	737	723	723	738	31
Gain/feed	0.683	0.716	0.711	0.733	0.690	0.018
28-42 days						
ADG (g)	662	634	680	660	681	15
ADFI (g)	1,106	1,088	1,102	1,102	1,128	28
Gain/feed	0.599	0.584	0.617	0.600	0.604	0.010
Overall						
ADG (g)	476	483	487	495	487	13
ADFI (g)	749	750	747	753	762	23
Gain/feed	0.636	0.644	0.652	0.657	0.641	0.009

[1] NC = Negative control; PC = Positive control including 44 ppm tylosin; T1 = NC diet including 0.1% essential oil, T2 = NC diet including 0.1% essential oil (with 0.3% benzoic acid), T3 = NC diet including 0.05% essential oil and 22 ppm tylosin.
[2] Pooled standard error.
[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).

T3 groups was significantly higher than that of pigs in the NC and T1 treatments ($p<0.05$), with the N digestibility on d 28 being significantly greater in pigs in the T1 group than in those in the NC, PC and T2 group. DM and energy digestibility in the T3 group were also lower than in the T1 group, however, at the end of experiment, DM and digestibility was highest in the T3 group ($p<0.05$), and the lowest level was observed in the T1 group ($p<0.05$). N and energy digestibility were not affect by any of the treatments.

Our results were consistent with those of Köhler (1997), who reported that an observed improvement in the digestibility of nitrogen may have occurred due to treatment with a blended essential oil (essential oil HC 737) inducing the production of digestion enzymes in the intestine. However, Cho et al. (2006) found that essential oils had a negative effect on nitrogen digestibility. In this study, the digestibility of DM in the essential oil treatment groups was alterative. However, the gut microflora of weanling pigs in the period immediately following weaning is very weak, therefore, the antibiotic effects of essential oil supplementation may influence the formation of intestinal bacterial colonies, which could influence the DM

Table 3. Effects of dietary supplementation with blended essential oils on nutrient digestibility in weanling pigs

Items (%)	NC[1]	PC[1]	T1[1]	T2[1]	T3[1]	SE[2]
D 14						
Dry matter	81.16[c]	85.63[ab]	84.79[b]	87.46[a]	86.27[ab]	0.60
Nitrogen	81.10[c]	86.37[ab]	84.24[b]	86.56[a]	87.50[a]	0.74
Energy	81.80[b]	89.00[a]	83.20[b]	89.06[a]	87.38[a]	0.74
D 28						
Dry matter	78.04[b]	78.18[b]	81.99[a]	78.69[b]	79.54[b]	0.62
Nitrogen	79.06[b]	79.99[b]	83.01[a]	79.39[b]	81.29[ab]	0.69
Energy	81.72[b]	81.50[b]	85.46[a]	82.07[b]	82.23[b]	0.74
D 42						
Dry matter	77.99[abc]	77.17[bc]	77.09[c]	78.44[ab]	78.67[a]	0.42
Nitrogen	78.82	77.64	77.09	78.28	78.97	0.82
Energy	79.16	79.62	78.61	79.46	78.85	0.56

[1] NC = Negative control; PC = Positive control including 44 ppm tylosin; T1 = NC diet including 0.1% essential oil, T2 = NC diet including 0.1% essential oil (with 0.3% benzoic acid), T3 = NC diet including 0.05% essential oil and 22 ppm tylosin.
[2] Pooled standard error.
[a-c] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Effects of dietary supplementation blended essential oils on blood biochemical profiles in weanling pigs

Items	NC[1]	PC[1]	T1[1]	T2[1]	T3[1]	SE[2]
RBC ($\times 10^6$/μl)						
Initial	6.26	5.91	5.83	5.99	6.22	0.26
14 days	6.25	6.59	6.22	6.41	6.31	0.27
28 days	5.81	6.56	6.38	6.05	6.17	0.38
Final	6.77	6.75	6.64	6.74	6.57	0.30
WBC ($\times 10^3$/μl)						
Initial	8.59	6.41	8.30	10.43	9.28	1.89
14 days	14.42^{ab}	13.04^{b}	19.11^{a}	15.20^{b}	18.01^{a}	1.51
28 days	12.82	13.91	17.42	16.21	13.85	2.11
Final	12.31	16.46	13.83	16.41	13.68	1.61
Lymphocyte (%)						
Initial	50.54	42.14	35.62	45.48	44.44	5.14
14 days	53.08^{a}	43.48^{ab}	40.74^{b}	50.38^{ab}	45.66^{ab}	3.12
28 days	56.42	60.96	48.10	56.66	55.72	5.18
Final	50.14^{b}	52.42^{ab}	50.70^{ab}	51.40^{ab}	64.78^{a}	4.41
IgG (mg/dl)						
Initial	389.80	391.00	347.00	361.40	322.60	32.96
14 days	305.60^{a}	239.00^{ab}	291.00^{ab}	245.68^{ab}	226.80^{b}	22.51
28 days	228.40	253.20	257.80	217.80	272.20	19.57
Final	286.80	296.40	362.00	391.20	322.40	35.72

[1] NC = Negative control; PC = Positive control including 44 ppm of tylosin; T1 = NC diet including 0.1% essential oil, T2 = NC diet including 0.1% essential oil (with 0.3% benzoic acid), T3 = NC diet including 0.05% essential oil and 22 ppm tylosin.
[2] Pooled standard error.
[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).

digestibility. Further studies are required to elucidate the effects of essential oils on energy digestibility.

Blood biochemical profiles

No differences in RBC concentration were observed among the treatments during the study (Table 4), however, the WBC concentration was significantly higher in the T1 and T3 group than in the PC and T2 group on d 14 ($p<0.05$). In addition, the lymphocyte profile was greater in the NC group than in the T1 group ($p<0.05$), however, it was significantly lower in the NC group than in the T3 group ($p<0.05$). Immunoglobin G was also only affected on d 14, with the T3 group showing a negative effect when compared to the NC group ($p<0.05$). Previous studies have shown that plant extract supplementation did not affect RBC concentration, but that WBC levels are greater in groups treated with plant supplements than those treated with antibiotic supplementation (Hong et al., 2004; Lien et al., 2006).

Fecal pH and fecal noxious gas emission compounds

Table 5 presents the effects of dietary supplementation with blended essential oils on fecal pH and fecal noxious gas emission compounds in weanling pigs. The pH value was lower in the T2 and T3 groups than in the other groups ($p<0.05$), however, the pH of the PC treatment was the highest among all the treatments ($p<0.05$). In addition, the NH_3 emission was decreased in the T1, T2 and T3 groups when compared with the PC group ($p<0.05$). The RSH did not differ among treatments, and H_2S and acetic acid

Table 5. Effects of dietary supplementation with blended essential oils on fecal pH and fecal noxious gas emission compounds in weanling pigs

Items	NC[1]	PC[1]	T1[1]	T2[1]	T3[1]	SE[2]
pH	8.07^{b}	8.36^{a}	8.06^{b}	7.61^{c}	7.58^{c}	0.08
NH_3 (ppm)	170.00^{ab}	273.33^{a}	146.67^{b}	70.00^{b}	74.67^{b}	32.25
RSH (ppm)	5.33	8.00	16.00	6.67	4.00	6.37

[1] NC = Negative control; PC = Positive control including 44 ppm tylosin; T1 = NC diet including 0.1% essential oil, T2 = NC diet including 0.1% essential oil (with 0.3% benzoic acid), T3 = NC diet including 0.05% essential oil and 22ppm tylosin.
[2] Pooled standard error.
[a-c] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 6. Effects of dietary supplementation with blended essential oils on the appearance of diarrhea and diarrhea score in weanling pigs

Items (ppm)	NC[1]	PC[1]	T1[1]	T2[1]	T3[1]	SE[2]
No. of pigs	25	25	25	25	25	-
1-3 days (Score)[3]	4 (0.53)	2 (0.40)	2 (0.30)	3 (0.33)	4 (0.58)	-
4-7 days (Score)[3]	0 (0.05)	0 (0.05)	2 (0.18)	0 (0.00)	0 (0.00)	-
Overall (Score)[3]	4 (0.29)	2 (0.23)	4 (0.24)	3 (0.16)	4 (0.29)	-

[1] NC = Negative control; PC = Positive control including 44 ppm tylosin; T1 = NC diet including 0.1% essential oil, T2 = NC diet including 0.1% essential oil (with 0.3% benzoic acid), T3 = NC diet including 0.05% essential oil and 22 ppm tylosin.
[2] Pooled standard error. [3] Diarrhea score.

emissions were not detected.

NH_3 emission tended to decrease in groups treated with essential oil blends A and B, as well as in the group treated with a combination of antibiotics and essential oil blend A. In this study, no significant difference was observed when the pH of the T1, T2 and T3 groups were compared to that of the NC group. However, NH_3 emission is not only influenced by fecal pH, but also by other factors, such as ammonium concentration. Cho et al. (2006) suggested that providing weanling pigs food that was supplemented with essential oil could reduce the fecal noxious gas concentration, which was confirmed by the results of our study. The carrier, benzoic acid, in the T2 treatment is a type of organic acid, which may have caused the pH to be lower in the T2 group than in the T1 group. However, this lower pH in T2 did not cause a lower NH_3 emission.

Variations in the concentrations of sulfuric odorous compounds in animal feces occur primarily as a result of differences in the sulfur composition of the diets and the metabolism of sulfur-containing amino acids (methionine, cystine and cysteine), which generate sulfuric odorous compounds such as H_2S and RSH (Kiene and Hines, 1995). In this study, differences in RSH were not detected, however, this may have occurred because of the similar dietary treatments among groups.

Kiarie et al. (2007) reported that inclusion of FS in the diet of piglets shifted microbial activity from the ileum to the hindgut, which resulted in greater DM digestibility and lower fecal pH and ammonia concentration, which was confirmed by the results of the present experiment.

Diarrhea appearance and diarrhea score

The appearance and score of diarrhea in the weanling pigs is presented in Table 3. From d 1 to d 3, the number of pigs with diarrhea in the NC, PC, T1, T2 and T3 treatment groups was 4, 2, 2, 3, and 4, respectively, and the diarrhea scores were 0.53, 0.40, 0.30, 0.33 and 0.58, respectively. However, with the exception of T1, the amount of pigs with diarrhea and the diarrhea scores were decreased in all of the treatment groups when compared to the control group. Kyriakis et al. (1998) suggested that Origanum essential was effective at controlling post weaning diarrhea syndrome, which is consistent of the results of this study.

CONCLUSION

Dietary supplementation with blended essential oils could be used to replace antibiotics in the diets of weanling pigs to improve early post-weaning ADG without negative effects on growth performance. This is supported by obvious positive effects on the characteristics of feces observed in the treatment groups, as well as enhanced nutrient digestibility that was observed in response to treatment with various ratios of essential oil supplementation. However, the unstable effect on the immune system requires additional study.

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