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Effect of Essential Oils and Feed Enzymes on Performance and Nutrient Utilization in Broilers Fed a Corn/Soy-based Diet

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Abstract: The objective of this study was to assess the effect of an encapsulated blend of Essential Oils (EOS) and a cocktail of various Enzymes (ES) including phytase and xylanase on the performance and nutrient utilization in broilers fed a corn/soy-based diet reduced in calcium, phosphorus and AME. Total 640 one day-old male chickens were allocated to 4 dietary treatments, following a 2 x 2 factorial design, 2 inclusion levels of EOS (0 or 100 g/t of feed) and 2 inclusion levels of ES (0 or 350 g/t of feed). The results indicated that there was no interaction in performance of broilers between EOS and ES. During the first three weeks, the mortality of broilers fed the diet containing EOS was lower ($p<0.05$) and the addition of EOS to the diet improved utilization of nitrogen and ileal digestibility of energy ($p<0.05$). In addition, EOS appeared to have affected gut microflora and the concentration of microbial nitrogen and Volatile Fatty Acids (VFA). On day 43, EOS supplementation increased the content of microbial nitrogen in ileum and caecum digesta and the concentration of acetate and butyrate in caecum ($p<0.05$), but it reduced the content of propionate in caecum. Supplementation of enzyme preparation at 350g/t to the basal diet improved the ileal digestibility of calcium and phosphorus as well as apparent metabolisability of energy and nitrogen ($p<0.05$). Supplementing enzyme preparation increased weight gain of broilers from 1 to 21 days. These results demonstrated that the addition of EOS and feed enzyme preparation in a corn/soy-based diet reduced in calcium, phosphorus and AME provided significant benefits in terms of nutrient utilization. The positive effect of EOS was more pronounced during the starter phase.

Key words: Essential oils, xylanase, phytase, broilers, growth performance, digestibility, microbial nitrogen, volatile fatty acids

INTRODUCTION

The application of antibiotics in animal feeds has resulted in great concerns by the consumers due to cross-resistance against pathogens and residues in tissues (Li and Li, 2003). Such concerns caused the ban of antibiotics growth promoters in EU. Some countries, especially in Asia, are now expected to follow the same path. It is also apparent that the ban of antibiotics in animal feeds significantly reduced the profit of broiler producers due to the depression of growth rate and feed conversion ratio. In order to improve the economic return of the broiler industry and ensure the production of healthy and safe food for human consumption, the replacement of antibiotics, especially plant extracts and essential oils, has been widely studied (Botsoglou *et al.*, 2003; Jamroz and Kamel, 2002; Botsoglou *et al.*, 2002; Alcicek *et al.*, 2003; Mitsch *et al.*, 2004). Some essential oils such as thymol and cinnamaldehyde have generally been recognized as safe (GRAS), which is endorsed by the Flavor and Extract Manufacturers' Association and Food and Drug Administration of the USA (Lee *et al.*, 2003). In addition to their antimicrobial activity (Dorman and Deans, 2000), essential oils exhibit antioxidants activities (Botsoglou *et al.*, 2002) and can stimulate animal digestive systems (Jamroz and Kamel, 2002; Ramakrishna *et al.*, 2003) by increasing digestive enzymes secretion and improving the utilization of digestive products through enhanced liver functions (Hernandez *et al.*, 2004).

The application of feed enzymes is widely adopted by broiler producers. It has long been accepted that enzymes can increase the utilization of energy, protein, calcium and phosphorus (Graham *et al.*, 1989; Bedford and Classen, 1992). More importantly, the application of enzymes can also reduce microbial proliferation in the hindgut due to the improvement in nutrient utilization in the foregut (Engberg *et al.*, 2004). As a consequence, the amount of nutrients available for bacteria usage is significantly decreased. However, very little research has been carried out to assess the interaction between

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essential oils and enzymes in broilers. The objectives of the current study were to 1) assess the effect of feed enzymes and essential oils on the performance and nutrient digestion of broilers and 2) investigate the effect of enzymes and essential oils on broiler gut microflora.

MATERIALS AND METHODS

Experimental design and birds: The experiment was a 2 x 2 factorial design with the main factors being Essential Oils (EOS) (no EOS; EOS, 100g/t feed) and Enzymes Supplementation (ES) (no enzyme; ES, 350g/t feed). There were four treatments: 1, control diet (C); 2, C+100g/t EOS; 3, C+350g/t ES; 4, C+100 g/t EOS+350g/t ES. A total of 640 one-day-old male Arbor Acres broilers were weighed and randomly allocated to 4 treatments, with each treatment having 8 replicates of 20 birds. The essential oils product (Enviva™ EO, Danisco Animal Nutrition, UK) contains 2 active substances: cinnamaldehyde and thymol. The enzyme preparation (Porzyme® and Phyzyme® XP, Danisco Animal Nutrition) included 250 g/t xylanase and 100 g/t phytase, supplied 2,000 U xylanase and 500 FTU phytase /kg feed. The essential oils and enzyme preparation were stored in 4°C until mixing of the basal diet.

Diets, feeding management and sample collection: The experiment was carried out over 2 growing periods: 1 to 21 and 22 to 42 days of age. The control diet was corn/soybean meal based diet without any antibiotic, but had reduced the levels of calcium (Ca by 0.12%), phosphorus (P by 0.14%) and AME (by 0.63 MJ/kg) (Table 1). This reduction allows for EOS and ES responses. A single batch of base diet was prepared and then aliquot parts were mixed with the essential oils and enzyme preparation before fed to broilers. Celite was added as an external marker for estimation of the coefficient of total tract apparent nutrient retention and ileal digestibility of nutrients.

The study was conducted under poor hygienic conditions. During the experiment, no antibiotics were used and the housing or facility was not disinfected. The birds were raised in 3-layered cages in a room with continuous lighting and controlled ventilation, where temperature was maintained at 33-36°C for the first week and then gradually reduced according to normal management practices. All procedures were approved by the Gansu Agricultural University Institutional Animal Care and Use Committee. Diets were provided *ad libitum* as mash form and feed consumption per replicate was recorded daily. Water was freely accessible and water consumption per replicate was also recorded daily using a measuring graduate. Birds at 1, 21 and 42 days of age were weighed for the calculation of Feed Conversion Ratio (FCR). The mortality was recorded daily and used for correcting FCR.

Table 1: Ingredients and chemical composition of the experimental starter and finisher diets (as fed)

Ingredients	1-21 days	22-42 days
Corn	57.67	64.40
Soybean meal	28.55	15.74
Corn protein powder	0.00	3.00
Cottonseed meal	4.00	6.00
Rapeseed meal	5.00	6.00
Rapeseed oil	0.00	0.54
L-lysine	0.23	0.31
DL-methionine	0.19	0.15
Dicalcium phosphate	0.90	0.30
Limestone	1.20	1.30
Premix[a]	0.50	0.50
Salt	0.36	0.36
Celite	1.40	1.40
Total	100.00	100.00
Nutrient level[b]		
AME (MJ/kg)	11.81	12.34
CP	21.28	18.15
CF	3.62	3.36
Ca	0.83	0.69
AP	0.28	0.18
TP	0.60	0.46
D-Met	0.46	0.41
D-Lys	1.06	0.90

[a]Premix (per kg diet): Vitamin A, 15,000 IU; Vitamin D_3, 5,100 IU; Vitamin E, 25.5 mg; Vitamin K, 2.5 mg; Vitamin B_1, 3.6 mg; Vitamin B_2, 6.9 mg; Vitamin B_6, 4.5 mg; Vitamin B_{12}, 0.036 mg; Vitamin B_3, 66.3 mg; Calcium-D-pantothenate, 18 mg; D-biotin, 0.18 mg; Folic acid, 1.4 mg; Fe, 90 mg; Zn, 65 mg; Cu, 9 mg; Mn, 110 mg; I ($Ca(IO_3)_2$), 0.7 mg; Se(Na_2SeO_3), 0.35 mg; antioxidant, 2 mg. (Cu, Mn, Fe and Zn were added in form of sulfate). [b]The content of CP, Ca and TP are analysed, others are calculated. AME: Apparent metabolisable energy, CP: Crude protein, CF: Crude fiber, Ca: Calcium, AP: Available phosphorus, TP: Total phosphorus, D-Met: Digestible methionine, D-Lys: Digestible lysine. The Ca, P and AME were reduced by 0.12%, 0.14% and 0.63 MJ/kg respectively from a commercial diet specification

From 11 to 14 and 32 to 35 days of age, excreta from each replicate were collected daily and mixed thoroughly. After the collection period, subsamples were taken and dried at 60°C in an oven to a constant weight. All samples were ground to 1 mm prior to analysis.

On day 22 and day 43, 267 birds were sacrificed under commercial conditions respectively and ileal digesta from caecum to yolk sac were flushed out using a syringe and mixed and pooled for each replicate. Caecal contents were also collected using the same method. About 55 g ileal digesta or 30g caecal content was used to measure Microbial Nitrogen (MN) and Volatile Fatty Acids (VFA) and the rest was freeze-dried and stored at -20°C prior to analysis.

Chemical analysis: Diets, excreta and ileal digesta samples were analysed for Dry Matter (DM), Crude Protein (CP), Gross Energy (GE), Calcium (Ca), Phosphorus (P) and Acid Insoluble Ash (AIA) to estimate nutrient digestion and retention. DM, CP, Ca and P were

determined according to AOAC (1980) while GE was measured using WZR-IA Auto-calorimeter (Changsha Bente Apparatus Ltd., Hunan, China).

Acid insoluble ash was determined according to the method described by Hao *et al*. (1980). In brief, 0.7 g feed or digesta sample with 100 ml 4 mol HCL was added into 400 ml glass breaker and boiled for 30 min. The solution was filtered with two speed quantitative papers, followed by washing the residue until no acid remained. The residue and filter paper were then transferred into a pre-weighed crucible and ashed at 600°C. The residue after ashing was cooled and weighed.

The Microbial Nitrogen (MN) concentration in ileum and caecum digesta contents was assayed according to Obispo and Dehority (1999) and Lu and Xie (1991). The Fig. 1 was the standard curve of different purine content

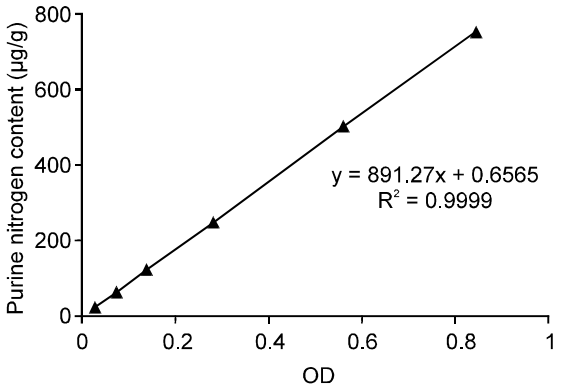

Fig. 1: Standard curve for nitrogen content of purine. y: Purine content (μg). x: Absorbance or Optic Density (OD)

vs. corresponding optical density. Substitute the Optical Density (OD) value of samples into the above regression equation allowed for the calculation of the corresponding purine content (μg/g), which was used to calculate the microbial nitrogen concentration of digesta samples according to the ratio 10.8% of purine nitrogen accounted for microbial nitrogen (Duncan *et al.*, 2004). The results were expressed in mg MN/g dry matter or mg MN/g nitrogen.

The Volatile Fatty Acids (VFA) concentration in ileum and caecum digesta was measured according to the method described by Fu (2001). 2-ethyl butyric acid (2EB) was used as an internal standard.

Statistical analysis: Data was analyzed by a two-way analysis of variance using the statistical package SPSS 11.5 (2002). The main factors were EOS (2 levels) and ES (2 levels). A general linear model was used to detect the effect of essential oils, enzymes and the interactions. The values presented in the tables are means and pooled SEM.

RESULTS

Growth performance: There was no interaction between EOS and ES in performance of broilers in the entire experiment period ($p>0.05$, Table 2). The addition of EOS to the basal diet decreased mortality by over 6% unit from week 1-3 ($p<0.05$). However, no effect was noted over week 4-6. Generally, the mortality in this experiment was higher than commercial situation due to the poor the hygiene conditions, the absence of antibiotics and the low diet specifications (reduced Ca, P and AME) which the young birds are very sensitive. Adding EOS tended to decrease water to feed ratio from 4-6 weeks ($p = 0.082$), but no difference in weight gain,

Table 2: Effect of essential oils and enzyme preparation on the performance of broilers fed diet containing essential oils and enzymes

	Treatment[a]					P-value		
	C	C+EOS	ES	EOS+ES	SEM	EOS	ES	EOS x ES
1-21 day								
Weight gain (g)	522.1	517.7	536.5	553.3	5.43	0.548	0.020	0.304
Feed intake (g)	796.9	803.0	817.7	828.2	6.93	0.554	0.108	0.873
Feed conversion ratio (g feed/g gain)	1.52	1.55	1.52	1.49	0.01	0.956	0.072	0.091
Water/feed (ml/g)	2.37	2.38	2.35	2.36	0.01	0.905	0.435	0.935
Mortality (%)	15.6	9.4	17.5	11.84	1.46	0.045	0.449	0.917
22-42 day								
Weight gain (g)	1290.3	1298.0	1388.3	1366.1	18.23	0.838	0.024	0.671
Feed intake (g/)	2532.1	2565.2	2650.9	2635.1	26.77	0.872	0.087	0.649
Feed conversion ratio (g feed/g gain)	1.96	1.97	1.92	1.93	0.02	0.794	0.298	0.988
Water/feed (ml/g)	2.42	2.35	2.38	2.27	0.03	0.082	0.211	0.750
Mortality (%)	5.9	8.2	8.7	3.9	1.35	0.649	0.788	0.206
1-42 day								
Weight gain (g)	1812.4	1815.7	1924.8	1919.4	20.64	0.979	0.009	0.911
Feed intake (g)	3328.9	3368.2	3468.6	3463.3	30.92	0.783	0.064	0.718
Feed conversion ratio (g feed/g gain)	1.83	1.85	1.80	1.80	0.01	0.807	0.183	0.756
Water/feed (ml/g)	2.41	2.36	2.37	2.29	0.02	0.122	0.214	0.762

[a]C: control diet, EOS: essential oils (100 g/t feed), ES: enzymes (including 250 g/t xylanase and 100 g/t phytase)

feed intake and FCR of broilers were observed. Enzyme supplementation increased weight gain of broilers ($p<0.05$) and tended to improve FCR from 1-3 weeks of age ($p = 0.072$). Feed to water ratio was not affected by enzymes supplementation ($p>0.05$).

Nutrient digestion: There was no interaction between EOS and ES in the ileal digestibility of nutrients measured ($p>0.05$, Table 3). On day 21, EOS improved ($p<0.05$) ileal digestibility of dry matter and energy by 6.5% and 6.9% unit, respectively compared with control. On day 42, ES improved ($p<0.05$) Ca and P digestibility and tended to increase ($p = 0.076$) dry matter digestibility, but EOS had no effect on ileal digestibility of energy, protein, Ca and P at day 42 ($p>0.05$).

The results on apparent nutrient retention and AME revealed that there was no interaction between EOS and ES at day 11-14 ($p>0.05$, Table 4). The inclusion of EOS increased dry matter, energy and protein apparent digestibility by 3.6, 4.1 and 7.5% unit respectively compared to the control ($p<0.05$). As a consequence, diet AME was improved by 0.8 MJ/kg ($p<0.05$). The ES treatment increased ($p<0.05$) the apparent digestibility of dry matter and energy and AME ($p<0.05$), with the combination of EOS and ES resulting in the highest AME among treatments. Over 32-35 day, the addition of ES resulted in higher ($p<0.05$) apparent digestibility of dry matter, energy and protein, but adding EOS to basal diets had no effects on the nutritional digestibility. For apparent energy digestibility and diet AME, a significant interaction between the EOS and enzyme preparation factors was observed ($p<0.05$). The addition of EOS improved dietary AME only when the enzyme preparation was also included in the feed.

Microbial Nitrogen (MN) and Volatile Fatty Acids (VFA) concentration in ileal and caecal digesta: At 22 days of age, EOS and ES treatments did not affect MN and VFA concentration in the ileal digesta ($p>0.05$, Table 5). On day 43, the interaction between EOS and ES treatment in MN was indicated by a significant increase in MN when both EOS and ES were added in a combination ($p<0.05$). The supplementation of EOS also decreased propionate content in the ileum ($p<0.05$).

On day 22, MN was not measured due to the limited amount of samples. EOS and enzyme preparation did not affect VFA concentration in caecum contents ($p>0.05$, Table 6). On day 43, the addition of EOS increased MN

Table 3: Effect of essential oils and enzyme preparation on ileal digestibility of nutrients in broilers fed diet containing essential oils and enzymes

	Treatment[a]					P-value		
	C	C+EOS	ES	EOS+ES	SEM	EOS	ES	EOS x ES
21 days								
Dry matter (%)	49.86	56.35	51.18	56.07	1.36	0.047	0.843	0.761
Energy (%)	50.08	56.93	50.90	56.73	1.48	0.039	0.910	0.856
Crude protein (%)	68.73	72.44	69.82	72.11	1.02	0.180	0.859	0.743
DE (MJ/kg)	8.38	9.62	8.54	9.58	0.25	0.029	0.896	0.834
42 days								
Dry matter (%)	61.12	62.80	63.30	63.60	0.42	0.220	0.076	0.382
Energy (%)	61.87	63.84	63.27	63.76	0.47	0.221	0.500	0.453
Crude protein (%)	72.90	73.14	72.04	70.55	0.56	0.586	0.147	0.452
Calcium (%)	50.60	55.26	59.16	59.43	1.34	0.280	0.013	0.333
Total phosphorus (%)	28.53	29.97	50.71	55.95	3.30	0.158	0.000	0.408
DE (MJ/kg)	10.51	10.88	10.78	10.92	0.08	0.148	0.363	0.507

[a]C: control diet, EOS: essential oils (100 g/t feed), ES: enzymes (including 250 g/t xylanase and 100 g/t phytase)

Table 4: Apparent nutrient retention and diet AME coefficient of boilers fed diet containing essential oils and enzymes

	Treatment*					P-value		
	C	C+EOS	ES	EOS+ES	SEM	EOS	ES	EOS x ES
11-14 day								
Dry matter (%)	60.85	64.40	62.45	65.94	0.42	0.000	0.003	0.941
Energy (%)	63.15	67.20	64.37	68.37	0.45	0.000	0.025	0.962
Crude protein (%)	47.16	54.63	47.89	52.93	0.70	0.000	0.909	0.264
AME (MJ/kg)	10.56	11.35	10.80	11.54	0.08	0.000	0.020	0.805
32-35 day								
Dry matter (%)	68.59	68.00	70.12	70.94	0.29	0.789	0.000	0.101
Energy (%)	70.17[ab]	69.75[b]	71.53[ab]	73.21[a]	0.33	0.171	0.000	0.028
Crude protein (%)	52.88	52.93	54.15	56.74	0.49	0.123	0.005	0.138
AME (MJ/kg)	11.93[ab]	11.88[b]	12.19[ab]	12.54[a]	0.06	0.058	0.000	0.018

[a,b]Means within rows with no common superscript differ significantly ($p<0.05$).

*C: control diet, EOS: essential oils (100 g/t feed), ES: enzymes (including 250 g/t xylanase and 100 g/t phytase)

Table 5: Effect of essential oils and enzyme preparation on Microbial Nitrogen (MN) and Volatile Fatty Acids (VFA) concentration in ileal digesta in broilers fed diet containing essential oils and enzymes

	Treatment*					P-value		
	C	C+EOS	ES	EOS+ES	SEM	EOS	ES	EOS x ES
22 days								
MN, mg/g dry matter	0.480	0.720	0.590	0.718	0.173	0.159	0.668	0.654
mg/g nitrogen	21.70	31.70	26.91	30.97	7.70	0.221	0.688	0.595
VFA (mg/g)								
Total VFA[c]	0.424	0.438	0.430	0.433	0.054	0.832	0.987	0.883
Acetate	0.401	0.424	0.417	0.421	0.049	0.708	0.844	0.789
Other VFA	0.023	0.014	0.013	0.012	-	-	-	-
43 days								
MN, mg/g dry matter	0.433[b]	0.418[b]	0.453[b]	0.667[a]	0.057	0.031	0.007	0.016
mg/g nitrogen	19.86[ab]	18.67[b]	19.31[ab]	26.12[a]	2.44	0.131	0.070	0.041
VFA (mg/g)								
Total VFA	0.738	0.699	0.719	0.648	0.173	0.657	0.777	0.896
Acetate	0.403	0.425	0.426	0.421	0.043	0.773	0.742	0.651
Propionate	0.217	0.200	0.213	0.197	0.009	0.033	0.620	0.999
Butyrate	0.290	0.315	0.295	0.303	0.015	0.144	0.760	0.449
Other VFA	0.145	0.083	0.108	0.103	0.038	-	-	-

[a,b]Means within rows with no common superscript differ significantly ($p \leq 0.05$).

[c]Total VFA includes Acetate, Propionate, Butyrate, Isobutyrate, Isovalerate and Valerate. *C: control diet, EOS: essential oils (100 g/t feed), ES: enzymes (including 250 g/t xylanase and 100 g/t phytase)

Table 6: Effect of essential oils and enzyme preparation on Microbial Nitrogen (MN) and Volatile Fatty Acids (VFA) concentration in caecum content in broilers fed diet containing essential oils and enzymes

	Treatment*					P-value		
	C	C+EOS	ES	EOS+ES	SEM	EOS	ES	EOS x ES
22 days								
VFA (mg/g)								
Total VFA[c]	2.553	2.685	2.708	2.778	0.257	0.588	0.509	0.867
Acetate	1.815	1.962	1.878	1.923	0.118	0.273	0.895	0.552
Propionate	0.507	0.437	0.443	0.425	0.030	0.064	0.101	0.240
Butyrate	0.318	0.375	0.363	0.385	0.033	0.107	0.255	0.461
Other VFA	0.083	0.048	0.052	0.080	-	-	-	-
43 days								
MN, mg/g dry matter	6.640	9.320	7.048	8.263	0.960	0.014	0.641	0.302
VFA (mg/g)								
Total VFA	3.16	3.50	3.19	3.46	0.241	0.087	0.974	0.836
Acetate	2.343	2.669	2.488	2.609	0.153	0.047	0.697	0.350
Propionate	0.561	0.438	0.458	0.431	0.045	0.024	0.092	0.133
Butyrate	0.240[c]	0.399[a]	0.316[bc]	0.363[ab]	0.030	0.000	0.365	0.016
Other VFA	0.065	0.057	0.072	0.078	0.017	-	-	-

[a,b]Means within rows with no common superscript differ significantly ($p \leq 0.05$).

[c]Total VFA include Acetate, Propionate, Butyrate, Isobutyrate, Isovalerate and Valerate. *C: control diet, EOS: essential oils (100 g/t feed), ES: enzymes (including 250 g/t xylanase and 100 g/t phytase)

content regardless the presence of the enzyme preparation in the feed ($p<0.05$). Among the VFA, acetate and butyrate contents were increased ($p<0.05$), whereas propionate content was decreased by EOS ($p<0.05$).

DISCUSSION

EOS and mortality: Many studies have shown that EOS exhibit antimicrobial activity (Dorman and Deans, 2000; Singh *et al*., 2002; Elgayyar *et al*., 2001; Valero and Salmeron, 2003), which can reduce the microbial load in broilers digestive system and consequently improve performance and/or liveability of birds. However, this effect depends on the environmental conditions in the broiler house. When birds are kept under good sanitary conditions, it is less likely to observe any significant effect of EOS supplementation on performance (Botsoglou *et al*., 2002). In the current study, no antibiotics were added to the feed or water and the housing or broiler facility was not disinfected during the experiment, providing a more convenient way to assess the antimicrobial activity of EOS. Results showed that the reduction in mortality when EOS was fed was significant from 1 to 21 days, indicating a beneficial effect of the antibiotic alternative product under the conditions of this trial. However, it has to be noted that overall mortality was higher than that in commercial practice. This was probably related to the experimental conditions described above. According to veterinary reports and dissected broilers, the main disease affecting the birds during the experiment was pullorum caused by

Colibacillosis and *Salmonella*. Therefore, it could be suggested that the actives substances contained in the EOS product (cinnamaldehyde and thymol) might have had a positive effect on gut microflora by reducing the amount of pathogenic bacteria. Vicente *et al*. (2007) reported that the natural capsaicin, extracted from paprika seeds at 36 mg/kg the diet, had a prophylactic effect on experimental *Salmonella enteritidis* infection in Dekalb laying hens. It has also been shown that essential oil molecules from nutmeg, peppermint, clove, cinnamon and thyme have a positive effect against *Bacillus* cereus (Valero and Salmeron, 2003).

The effect of EOS and ES on growth performance and nutrient digestibility: The benefits of essential oils and plant extract products on broiler performance were not consistent, especially when birds were reared under highly controlled conditions without specific challenge for the animal. Botsoglou *et al*. (2002) reported that the addition of the oregano oil to the basal diet had little influence on weight and FCR. Allen *et al*. (1997) reported that essential oils could only increase body weight when broilers were infected with *coccidium*, but no benefits could be observed when the birds were healthy. In the current study, broilers were fed with a low Ca, P and AME diet which should be the main reason for the absence of positive effect on growth when EOS is used alone and cannot provide the Ca and P for birds. The results showed that digestibility of dry matter and crude protein and dietary AME content were increased remarkably by the EOS supplementation during the first 3 weeks. The improvement in nutrient utilization may be associated with better secretion of digestive enzymes (Platel *et al*., 2002) as Lee *et al*. (2003) reported that adding 100g/t thymol and cinnamaldehyde to the broiler diet can promote the secretion of pancreatic digestive enzymes which was not measured in the current study. The lack of effect of EOS on performance of broilers might associate with the high mortality and larger variation in performance between replicates. For example, the coefficient of variance for body weight at 42 days of age was 19.5, 16.2, 19.4 and 17.1% for control, C+EOS, ES and EOS+ES treatments, respectively.

Supplementing enzyme preparation can increase the utilization of energy, protein, calcium and phosphorus (Graham *et al*., 1989; Bedford and Classen, 1992) and consequently improve the growth performance of broilers. The present study showed that the addition of xylanase and phytase in a corn/soy-based diet reduced in P and Ca improved energy, Ca and P utilization and consequently increased weight gain of broilers. This is not surprising because the major anti-nutritional factors in this type of diet are dietary fibre and phytate which are the substrate of xylanase and phytase respectively. Based on the NSP contents in common feed ingredients published by China Feed Industry Information (Anon, 2009), the content of xylan in early and late stage in the current diet are 52.7 g/kg and 50.5 g/kg respectively. The addition of 250 g/t xylanase can significantly improve the nutrient digestion by breaking down the matrix of dietary fibre.

The effect of EOS and ES on VFA in intestinal contents: The amount of VFA in the digesta can reflect quantity of bacteria in the intestine. Acetic acid, propionate and butyric acid can not only improve multiplication of mucosal cells as energy source but also decrease intestinal pH and enhance disease resistance (e.g. restrain harmful germ as *Escherichia coli*). This study showed that adding EOS to the basal diet at day 22 did not affect the VFA content in both caecum and ileal content, but on day 43, decreased propionate in both ileal and caecum content and increased the content of acetate and butyrate in caecum content. The change of VFA concentration may be affected by the species and quantity of bacteria in the gut which might be influenced by the EOS and/or ES. However, the bacteria population in the gut was not monitored in this study.

Butyric acid is the major energy source of colon mucous membrane cell. It can promote mucous membrane cell multiplication to maintain Intestine Tract (IT) healthy. This might suggest that EOS can promote IT epithelium growth and maintain IT healthy. The data in the current study also confirmed that acetic acid can be transformed into butyric acid by butyryl CoA and acetyl CoA transferase (Louis *et al*., 2004). The lower propanoic acid content obtained in the current study may be due to the shorter IT in broilers where the lactic produced in forepart of IT cannot be abundantly transformed into propanoic acid. The reason for lower propanoic acid content and higher acetic acid may also relate to lactobacillus and bifidobacteriums because bifidobacteriums can promote lactic acid transforming into butyric acid (Duncan *et al*., 2004). While the EOS used in the current study can promote multiplication of beneficial germs in IT, resulting transformation of lactic acid into butyric acid and a reduction of propanoic acid content, the effect of EOS on microbial population is not studied in the current work.

In summary, the present study confirmed the benefits of using exogenous enzymes in broiler feeds. The addition of xylanase and phytase enzyme activities in a corn/soy-based diet reduced in P, Ca and AME significantly improved nutrient utilization and feed AME. These positive effects were associated with greater performance. Furthermore, the encapsulated blend of essential oils (cinnamaldehyde and thymol) provided additional benefits, resulting in a significant reduction of bird mortality during the first 3 weeks of age and better nutrient utilization and higher feed AME. The addition of a combination of enzyme and the essential oils not only resulted in the lowest bird mortality throughout the study, but also increased ileal and overall energy and protein digestibility. More detailed studies are still needed to

elucidate the effect of a combination of essential oils and feed enzymes on antimicrobial and microbial population under various circumstances.

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