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Research Article
A Comparison of Essential Oil and Organic Acid Supplementation on the Live Performance and Intestinal Morphology of Broilers Fed a Diet Containing Palm Kernel Meal

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Abstract

Background and Objective: Non-Starch Polysaccharides (NSPs) are indigestible substances found in plant cell walls. Typically, they trap nutrients, increase the viscosity of intestinal contents and decrease the passage rate of the digesta. That condition is favorable for bacterial growth and can negatively affect bird health. The objective of this study was to compare the antimicrobial effects of commercial Essential Oil (EO) and Organic Acid (OA) supplementation and their effects on the growth performance and intestinal morphology of broilers fed a corn-soybean-based diet containing Palm Kernel Meal (PKM). **Materials and Methods:** The experiment was conducted using a completely randomized design. A total of 1,152, one-day-old broiler chicks (Ross 308) were divided into 6 dietary treatments: C (control), N (negative control), C+EO, C+OA, N+EO and N+OA. Each treatment consisted of 8 replicates with 24 chicks per replicate (12 males and 12 females). All chicks were raised in an evaporative cooling system house with rice husk as the litter material. Feed in mash form and water were provided *ad libitum*. **Results:** Supplementation with EO and OA had no significant effect on the growth performance of the broilers throughout the experimental period (p>0.05). There was no significant difference in the *Escherichia coli* and *Lactobacillus* spp. populations in the ceca of the broilers at 17 and 35 days of age (DOA) (p>0.05). Similar results were observed for ileal crypt depth and villi surface area between the dietary treatments. **Conclusion:** Supplementation with EO and OA at 30 and 2,000 ppm, respectively, had no significant effect on broilers' growth performance, ceca populations of *Escherichia coli* and *Lactobacillus* spp. and gut health.

Key words: Essential oil, organic acid, palm kernel meal, broiler

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cost of poultry production always increases in response to increases in the cost of feed ingredients. Because there is high demand for conventional feed ingredients due to an increase in competition from other livestock species and humans, the use of agro-industrial by-products as alternative poultry feed ingredients is necessary. One of the most promising alternative feed ingredients is Palm Kernel Meal (PKM), which can potentially be incorporated in the diet to reduce feed cost[1].

Palm Kernel Meal (PKM) is aflatoxin-free and palatable and has considerable potential as a carbohydrate and protein source. The PKM has not often been included in the diets of monogastric animals due to its high fiber content. More than 60% of the PKM content is cell wall and the Non-Starch Polysaccharides (NSPs) in the PKM cell wall are mainly composed of insoluble mannose-based polysaccharides[2]. Typically, they trap nutrients, making them unavailable for digestion by endogenous enzymes. Because of their water solubility, these NSPs also increase the viscosity of intestinal contents. Higher viscosity of the digesta reduces the mixing of undigested nutrients and endogenous enzymes, leading to a reduction in the efficacy of endogenous enzymes and ultimately nutrient digestibility. High viscosity also decreases the passage rate of the digesta, which favors bacterial growth and can negatively affect the digestion process and gut health[3].

Feed additives derived from plants, including aromatic plants and Essential Oils (EO), have gained popularity since 2006 when European Union legislations phased out the use of antibiotics as an additive to poultry feed. The EO from plant extracts are an alternative to Antibiotic Growth Promoters (AGPs). They enhance digestive enzyme secretion activity and they also have antimicrobial activity, which benefits the birds' performance[4]. Another potential feed additive is Organic Acid (OA). It has shown positive results in poultry production by reducing intestinal pH, the growth of harmful bacteria and also improved intestinal health so the bird can maximize nutrient absorption. Undissociated forms of OA can penetrate the lipid membranes of bacterial cell walls, causing a reduction in intracellular pH, which leads to cell death[5]. The OA also stimulates pancreatic secretions and provides better intestinal villus integrity[6].

In vitro studies have shown that dietary EO and OA have an inhibitory effect on pathogenic gastrointestinal bacteriain broilers[7]. However, only a few *in vivo* studies have confirmed their growth-promoting effects. The *in vivo* effects of EO and OA on cecal microbial populations and the intestinal morphology of broilers fed a diet containing PKM are not consistent. Therefore, the present experiment was designed to determine the effect of EO (thymol and carvacrol blend) and OA (fumaric and benzoic acid blend) supplementation on live performance, the cecal microbial population and the intestinal morphology of broilers fed a diet containing PKM.

MATERIALS AND METHODS

Animals and management: The experimental protocol used in this study was conducted under the Animal Care and Use for Scientific Research of Kasetsart University (ACKU60-AGK-053). One thousand one hundred and fifty-two broiler chicks (one-day-old, Ross 308) were divided into 6 treatments. Each treatment consisted of 8 replicates of 24 chicks each (12 males and 12 females). The chicks were kept in floor pens (0.09 m^2 floor space per bird) and housed in a controlled temperature room with ventilation and lighting (23 h day^{-1}). Vaccinations were provided according to commercial practice. All broiler chicks were allowed access to water and feed *ad libitum* throughout the experimental period.

Experimental design and diet: The experiment was conducted using a completely randomized design. Chicks were fed diets formulated to meet the requirement recommended by Ross[8]. All birds were fed experimental diets (mash) from 1-35 days of age. There were 2 feeding phases: the starter phase consisting of 22% CP and ME for a total of 3,100 kcal kg^{-1} (1-17 days of age) and the grower phase consisting of 20% CP and ME for a total of 3,150 kcal kg^{-1} (18-35 days of age). There were 6 dietary treatments: (1) corn-soybean-based diet (Control, C); (2) C supplemented with 30 ppm EO (25% thymol and 25% carvacrol) (CEO); (3) C supplemented with 2,000 ppm OA (fumaric acid and benzoic acid 85%) (COA); (4) corn-soybean meal-palm-kernel meal-based diet (Negative, N); (5) N supplemented with 30 ppm EO (NEO) and (6) N supplemented with 2,000 ppm OA (NOA) (Table 1).

Growth performance and carcass quality: The body weight and feed intake of the birds were measured at 1, 17 and 35 days of age. Body Weight Gain (BWG), Feed Intake (FI) and the Feed Conversion Ratio (FCR) were calculated periodically. The number of dead birds was recorded daily formortality rate calculations. At 35 days of age, after an overnight feed deprivation (8 h),192 randomly selected birds (2 males and 2 females from each replicate) were sacrificed by CO_2

Table 1: Calculated nutrient composition of experimental diets

	Starter		Grower		Finisher	
Ingredients (%)	C	N	C	N	C	N
Corn	52.58	47.11	58.97	50.70	64.64	53.61
PKM	-	5.00	-	7.50	-	10.00
Soybean oil	1.75	3.07	2.11	4.12	2.70	5.39
Fullfat soy	20.00	20.00	15.00	15.00	10.00	10.00
SBM 49% CP	21.06	20.24	19.85	18.62	18.85	17.22
L-lysine	0.13	0.15	0.10	0.13	-	0.03
DL-methionine	0.27	0.28	0.25	0.26	0.26	0.27
Choline chloride 60%	0.004	0.005	0.002	0.004	-	0.001
MDCP (P21)	2.13	2.08	1.68	1.66	1.56	1.53
$CaCO_3$	1.41	1.40	1.37	1.34	1.33	1.28
Salt	0.40	0.41	0.41	0.41	0.41	0.42
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient composition						
ME (kcal kg^{-1})	3,100.00	3,100.00	3,150.00	3,150.00	3,200.00	3,200.00
Crude protein (%)	22.00	22.00	20.00	20.00	18.00	18.00
Crude fiber (%)	3.01	3.85	2.88	4.14	2.74	4.41
Calcium (%)	1.00	1.00	0.90	0.90	0.85	0.85
Total P (%)	0.83	0.83	0.71	0.73	0.67	0.68
Available P (%)	0.55	0.54	0.45	0.45	0.42	0.42
Lysine (%)	1.25	1.25	1.10	1.10	0.90	0.90
Methionine (%)	0.61	0.61	0.57	0.58	0.54	0.56

C: Corn-soy-based diet as a positive control group, N: Corn-soy-palm kernel meal-based diet as a negative control group, PKM: Palm kernel meal, CP: Crude protein, SBM: Soyabean meal, MDCP: Mono dicalcium phosphate, ME: Metabolizable energy

asphyxiation. The broiler carcass without blood, feathers and visceral organs was defined as the chilled carcass and expressed as a percentage of the live weight.

Intestinal morphology and microbial enumeration: An assessment of the antimicrobial effects of EO and OA on the intestinal villi was conducted at 17 and 35 days of age. After an overnight feed deprivation (8 h), 96 birds (1 male and 1 female from each replicate) were randomly selected and sacrificed using CO_2 asphyxiation. A section from the end of the ileum (approximately 0.5 cm long) was excised and opened longitudinally at the antimesenteric attachment and gently flushed with NaCl (9 g L^{-1}). The sample was then fixed in a solution of formalin buffer (90 mL L^{-1}) for 12-24 h at 4°C and then rinsed and stored in 70% ethanol at 4°C until analysis was carried out using methods described by De Verdal *et al*.[9]. The cecal content from each bird was collected for microbial enumeration. *Lactobacillus* spp. and *Escherichia coli* populations were counted after their cultivation at 37°C on de Man-Rogosa-Sharpe (MRS) agar in an anaerobic chamber for 48 h and MacConkey agar in an aerobic chamber for 24 h, respectively[10].

Statistical analysis: Analysis of variance of all zootechnical data was conducted using the General Linear Model (GLM). Variables determined to be significant by the F-test were compared using the Duncan's new multiple range test. The treatment effect was assessed by the significant difference test at the 5 and 1% probability levels.

RESULTS

Growth performance and carcass quality: All birds were healthy during the entire experimental period. Mortality was lower than 1.4% with no significant differences among dietary treatments. During the starter period (1-17 days of age), the use of PKM in the diet had no negative effect on overall broiler performance (Table 2). However, during the grower period (18-35 days of age), a negative effect of PKM on body weight gain and the percentage of chilled weight were observed. Birds fed the diet containing PKM (7.5%) had lower body weight gains when compared with the corn-soy-based diet group. This led to a significantly higher FCR ($p<0.05$) and lower percentage of chilled weight ($p<0.01$). Supplementation with commercial EO and OA did not have any effect on the performance of broilers fed a diet containing PKM.

Intestinal morphology: In the present study, the parameters of epithelial morphology crypt depth, villi width and villi surface area at 35 days of age (DOA) were not significantly different between dietary treatments ($p>0.05$) (Table 3).

Table 2: Effect of commercial essential oil and organic acid supplementation on performanceof broilers fed a diet containing PKM

	Treatments							
Parameters	C	CEO	COA	N	NEO	NOA	p-value	Pooled SE
Days 1-17 performance								
Body weight gain (g)	538.37	540.81	543.95	538.09	538.68	537.45	0.907	12.47
Feed intake (g)	681.87	676.42	660.81	670.76	675.24	661.72	0.576	27.16
FCR	1.267	1.251	1.216	1.247	1.253	1.231	0.383	0.05
Days 18-35 performance								
Body weight gain (g)	1477.42	1443.50	1419.07	1407.69	1437.60	1399.79	0.125	59.00
Feed intake (g)	2452.72	2423.73	2433.17	2440.19	2470.84	2426.93	0.795	73.36
FCR	1.661[c]	1.680[bc]	1.717[ab]	1.734[a]	1.720[ab]	1.735[a]	0.013	0.04
Days 1-35 performance								
Body weight gain (g)	2015.78	1984.31	1963.02	1945.78	1976.27	1937.24	0.192	64.39
Feed intake (g)	3134.59	3100.15	3098.65	3119.53	3146.08	3088.65	0.714	83.83
FCR	1.555	1.563	1.580	1.604	1.593	1.595	0.104	0.03
Carcass quality								
Chilled weight[#]	80.27[ab]	81.62[a]	78.75[bc]	78.18[c]	79.05[bc]	79.29[bc]	0.006	0.01

[#]Calculated as percentage of live weight, [a,b,c]Means within each row marked with different superscripts are significantly different (p<0.05), FCR: Feed conversion ratio, PKM: Palm kernel meal

Table 3: Effect of commercial essential oil and organic acid supplementation on the gut morphology of broilers fed a diet containing PKM

	Treatments							
Parameters	C	CEO	COA	N	NEO	NOA	p-value	Pooled SE
17 DOA								
Villi height (μm)	448.89[a]	361.88[b]	359.90[b]	385.15[b]	361.96[b]	384.04[b]	0.002	0.05
Crypt depth (μm)	129.14	128.82	121.07	126.89	121.14	124.59	0.547	0.03
Villi width (μm)	110.47	113.98	114.46	110.53	113.64	111.04	0.914	0.03
Villi surface area (μm^2)	5,017.37	4,160.64	4,185.16	4,298.91	4,146.31	4,269.65	0.076	0.06
35 DOA								
Villi height (μm)	431.63	481.04	448.92	435.42	430.36	500.72	0.144	0.06
Crypt depth (μm)	118.42	118.95	106.43	123.68	116.85	120.26	0.220	0.05
Villi width (μm)	132.34	133.87	136.27	141.56	128.50	141.51	0.112	0.03
Villi surface area (μm^2)	5,754.46	6,526.91	6,188.50	6,215.69	5,611.80	7,156.94	0.060	0.07

[a,b]Means within each row marked with different superscripts are highly significantly different (p<0.01), DOA: Days of age

Table 4: Effect of commercial essential oil and organic acid supplementation on microbial enumeration in the ceca of broilers fed a diet containing PKM

	Treatments							
Parameters	C	CEO	COA	N	NEO	NOA	p-value	Pooled SE
17 DOA								
Escherichia coli (10^8 CFU g^{-1})	3.36	2.53	2.30	2.69	2.59	3.21	0.903	0.19
Lactobacillus spp. (10^8 CFU g^{-1})	7.48	6.01	5.54	5.24	6.29	6.71	0.958	0.33
35 DOA								
Escherichia coli (10^8 CFU g^{-1})	0.34	0.33	0.73	0.31	0.30	0.08	0.152	0.06
Lactobacillus spp. (10^8 CFU g^{-1})	17.71	14.30	8.60	13.69	17.54	12.54	0.389	0.20

DOA: Days of age

Microbial enumeration: The utilization of PKM in the diet did not show any effect on *Escherichia coli* and *Lactobacilli* spp. populations in the cecal contents of broilers measured at 17 and 35 days of age (DOA) (Table 4). Supplementation with commercial essential oil and organic acid also had no significant effect on the *Escherichia coli* and *Lactobacilli* spp. populations (p>0.05).

DISCUSSION

Growth performance and carcass quality: Adequate levels of available amino acids are needed to support optimum growth and carcass yield of fast-growing commercial broilers. Methionine and lysine are considered to be the first and second limiting amino acids in broilers, respectively.

Sundu *et al*.[2] reported that PKM only meets 30 and 50% of the lysine and methionine requirements of young chicks, respectively. Similarly, Esuga[11] reported that the lower nutrient availability in PKM, especially methionine and lysine, could contribute to reduced muscle growth and body weight gainin broilers. Nwokolo *et al*.[12] also reported low levels and digestibility of valine and methionine in PKM. Supplementation with synthetic valine and methionine was needed when using a PKM-based diet. Sundu *et al*.[2] reported that the body weight of birds fed a 30% PKM diet balanced with digestible amino acids and metabolizable energy had increased by 2% over the body weight of birds fed a corn-soy diet. The experimental diets of the present study were formulated based on the total amino acid requirement, not on digestible amino acids. These could have limited amino acid availability for the broilers. Moreover, when PKM was incorporated into the experimental diet, the level of soluble fiber was increased. This could interfere with protein and amino acid digestibility and directly affect muscle protein synthesis[13].

Lee *et al*.[14] reported that the lack of positive effects of EO could be associated with the low inclusion levels of EO and a variety of basal diet compositions. Hashemipour *et al*.[15] reported that supplementation with a 1:1 ratio of the EO thymol:carvacrol at 100-200 mg kg^{-1} improved the average broiler weight gain and feed efficiency. Malayoglu *et al*.[16] also reported that supplementation with 250 mg kg^{-1} of oregano EO significantly increased broiler weight gain during the first 7 days. Similarly, Cross *et al*.[17] found a significant increase in the body weight gain of broilers when supplemented with 1,000 mg kg^{-1} of thyme EO. Although several studies have reported positive effects of EO on broiler performance, the level used in the present study (30 ppm) did not promote beneficial effects.

Intestinal morphology: Intestinal villi are the major site of nutrient absorption and taller villi could increase nutrient absorption. Crypts are the major sites of villi proliferation; deep crypts would indicate rapid tissue turn over and a higher demand for new tissues. A higher level of crude fiber in the PKM-containing diet may affect the villi height in young broiler chickens. Montagne *et al*.[18] suggested that the insoluble fiber in PKM has a more abrasive action, scraping mucin from the mucosa as it passes along the GIT and increasing endogenous losses. Baurhoo *et al*.[19] reported that birds fed a diet containing 2.5% lignin had a significantly lower villi height than birds fed 1.25% lignin at 42 DOA. Sklan *et al*.[20] and Jankowski *et al*.[21] reported that a high crude fiber content in the diet decreased villi height. Similarly, Rahim *et al*.[22] reported that the villi height in the duodenum, jejunum and ileum of broilers decreased with increasing levels of fermented Palm Kernel Expeller (PKE) from 0-36% in their diets. However, reports on the effects of feeding diets containing high levels of crude fiber on intestinal villus height and crypt depth were inconsistent. In contrast, Zulkifli *et al*.[23] reported that birds fed diets containing 25% PKM had taller and wider villi. The authors explained that the higher fiber content of PKM have stimulated development of the mucosal epithelial cells.

Commensal bacteria in the ileum can utilize fiber as an energy source and produce short-chain fatty acids, especially butyric acid, which is an important energy source for epithelial cell growth[24]. Therefore, the morphology and functionality of the different regions of the intestinal tract seem to be a flexible system that is able to adapt to the needs of the organism[25]. In the present study, the villi height of the ileum of birds fed a diet supplemented with essential oils and organic acids was decreased. It did not support earlier reports that higher villi height could be stimulated by supplementation with essential oils or organic acids. However, further investigation is needed to explain this phenomenon.

Microbial enumeration: The antimicrobial potential of essential oils or organic acids might not be the same in *in vivo* and *in vitro* studies. Numerous *in vitro* studies have demonstrated that thymol and carvacrol displayed antimicrobial activity against intestinal microbes such as *Clostridium perfringens*, *Salmonella typhimurium* and *Escherichia coli*[26]. The antimicrobial action of essential oils is mediated by their lipophilic character, which allows them to penetrate the bacterial membrane, releasing membrane components from the cells to the external environment[27]. However, in an *in vivo* study, the effect of essential oils on gastrointestinal microflora was not consistent, possibly because the essential oil antimicrobial activity tests were performed on pure cultures but not on commensal microorganisms found in the gastrointestinal tracts of birds[24]. In addition, at the level of 30 mg kg^{-1}, the essential oil blend might not be at a level high enough to affect microorganisms in the *in vivo* study. Similarly, Jang *et al*.[28] reported that the Lactobacilli population in the ileocecal valve of birds was not influenced by feeding them 25-50 mg kg^{-1} of essential oil (290 g kg^{-1} thymol). However, Jamroz *et al*.[29] reported that the supplementation of 100 mg kg^{-1} of essential oil with 49.5 g kg^{-1} of carvacrol reduced the CFU of *Escherichia coli* to a limited extent and greater inhibition was observed in older birds. Another possible explanation for the inconsistent effects could be the finding of Abdel-Wareth[30] who showed that no improvement in a microbial population is observed if the microflora are already in a state of equilibrium. Therefore, if birds are fed a good quality diet and raised under clean and healthy conditions, the beneficial effects of feed additive supplementation will not be observed.

CONCLUSION

It is concluded that commercial essential oil and organic acid supplementation had no antimicrobial effects on growth performance and intestinal morphology of broilers fed a corn-soybean-based diet containing Palm Kernel Meal (PKM).

SIGNIFICANCE STATEMENT

This study discovered that neither essential oil nor organic acid supplementation could enhance the growth performance and gut health of broilers fed a diet containing low levels of PKM. Many *in vitro* studies have shown that essential oil and organic acid have an excellent inhibitory effect on pathogenic bacteria of broilers. However, the beneficial effects of both additives on cecal microbial populations and the intestinal morphology of broilers fed a PKM diet have not been consistent. This study will help the researcher to better understand that if low levels of PKM have no adverse effect on broiler performance and gut health, supplementation with essential oils or organic acids might not have a beneficial effect on those parameters.

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