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Fecal Microbial Population and Growth in Broiler Fed Organic Acids and Palm Fat-Composed Diet

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ABSTRACT

Organic acids (OA) are natural constituents of plant and animal tissues and their uses as feed additives are now being studied worldwide. Organic acids consisted of different acids and mixtures of several acids have an antimicrobial function and promote the growth performance of animals. The current experiment was designed to study the inhibitory activity of the organic acids, palm fat (PF), a combination of organic acids-palm fat (OAPF) against various pathogens and investigate the performance in dietary inclusion of OAPF in broilers. A feeding trial was conducted to determine the growth performance and microbial population in the dietary inclusion of OAPF in broilers. A total number of 96 one-day-old chickens (Cobb 500) were used in this study and divided into two treatment groups with six replicates per treatment. The treatment group was T1 (diet without OAPF) and T1OA (diet with OAPF). The differences among treatment means were tested using an independent t-test. The results showed that T1OA had approximately two-fold inhibitory activity against *Escherichia coli* **E-30 compared to T1. Broilers fed diet supplemented with OAPF had higher ($p<0.05$) final body weight (BW) and total weight gain (WG) compared to broilers fed T1. Broiler fed with T1OA had lower ($p<0.05$) feed conversion ratio (FCR) than T1. Inclusion of OAPF in the diet also increased ($p<0.05$) the lactic acid bacteria (LAB) and reduced ($p<0.05$)** *Enterobacteriaceae* **(ENT) cell population. The inclusion of OAPF in the diet showed significantly improved nutrient digestibility and had a beneficial effect on the growth performances of the broiler chickens with a positive effect on the bacterial population in GIT.**

Keywords: food pathogens; inhibitory activity; growth performance; organic acids

INTRODUCTION

Organic acids (OA) have been used for decades in the animal industry due to its antimicrobial function and feed preservation (Mirza *et al*., 2016; Hajati, 2018). Organic acids are weak acids and are only partially dissociated. Organic acids with short-chain acids (C1-C7) are associated with antimicrobial activity. Organic acids with antimicrobial function mostly have a pKa between 3 to 5. They are less acidic and more soluble in water (Huyghebaert *et al.,* 2011). Therefore, many organic acids are used as drinking water supplements or feed additives. Besides, organic acids are normally used as an acidifier in poultry feeds and have been considered as an alternative way to improve nutrient digestibility. Organic acids could improve gastric proteolysis and increased digestibility of dietary crude protein (CP) and dietary amino acids (AA) (Samanta *et al.,* 2010). There were also many reports stated that organic acids were able to improve the growth and feed efficiency, enhance nutrient utilization (Lückstädt & Mellor, 2011), and inhibit pathogen bacteria (Dittoe *et al.,* 2018).

Palm fat (PF) is an edible oil derived from the fruits of the oil palm *Elaeis guineensis*. Palm fat is commonly used in broiler feed because it is rich in saturated fatty acids (Smink *et al.,* 2008). Energy sources such as palm fat and oils can supply dietary essential fatty acids that cannot be produced by animals such as linoleic and linolenic acids. These fatty acids can help the animals in the absorption of fat-soluble vitamins and provide specific fatty acids. Nowadays, there are many reports regarding the use of organic acids only in animal feeding trials. Organic acids could be used as feed additives to improve the growth performance, nutrient digestibility, and health status of livestock and broilers (Ghazala, 2011). Organic acids also could prevent the spreading of pathogenic bacteria and improve the gastrointestinal tract (GIT) health of animals (Broom, 2015). Therefore, the mixing of palm fat and organic acids might be able to improve the performance of the broiler chickens. However, information on nutrient retention by using the combination of OAPF has not been reported yet, and when it is used, it should be translated into values in order to formulate a more precise feed formulation in

broiler chickens. For this reason, the current experiment was aimed to study the inhibitory activity of the organic acids and combination of organic acids and palm fat (OAPF) based on the modified inhibitory activity (MAU/mL) against pathogenic bacteria. A feeding trial was also carried out to investigate the growth performance and microbial population in dietary supplementation of OAPF in broiler chickens.

MATERIALS AND METHODS

Organic Acids-Palm Fat Preparations

The mixture of organic acids (OA) consisted of 14% formic acids, 36% lactic acids, 25% citric acids, 13% malic acids, 10% tartaric acids, and 2% phosphoric acids. The palm fat powder was extracted from palm oil by using a dryer machine (Sunzen Feedtech Sdn Bhd., Malaysia). The gross energy content in the palm-fat powder was 8182 kcal/kg. The mixture of organic acids was added with 0.6% of palm-fat powder (OAPF) that was used in this study. The pH of the mixture of organic acids and palm fat (OAPF) was 0.65.

Experimental Microorganism and Pathogenic Bacteria

Indicator microorganism used in this study was *Pediococcus acidilactici* 4–46 (*P. acidilactici*), which was a common food spoilage bacterium that could be found in food products for both animals and humans (Waite *et al.*, 2009). Firstly, the stock cultures of *P. acidilactici* were revived twice in MRS broth (Lactobacillus-broth De Man, ROGOSA, and SHARPE, Merck, KgaA, Darmstadt). Then, the stock cultures were incubated at 30°C for 24 hours and 48 hours in a stationary condition. After spreading the plate with revived cultures in MRS media, the plates were incubated for another 48 hours at 30°C. After that, a single colony of the revived cultures was picked from the plate and put in 10 mL of MRS broth and incubated for 24 hours. This culture was then sub-culturing into another 10 mL MRS broth and further incubated for another 24 hours. Then, the cultures were ready to be used for the inhibitory test.

The pathogenic bacteria used in the study were purchased from the American Type Culture Collection (ATCC). The reviving cultures steps of *Salmonella enterica* S-1000 (ATCC6961), *Escherichia coli* E-30 (ATCC35349), *Listeria monocytogenes* L-MS (ATCCBAA2659), and Vancomycin Resistant *Enterococci* (VRE) (ATCC49610) were done as described in the steps in reviving indicator microorganism for the inhibitory test. However, the cultivation of VRE and *S. enterica* were used in nutrient media and incubated at 37°C and 30°C, respectively. The *E. coli* was cultivated in LB broth at 37°C while *L. monocytogenes* was cultured at 30°C in *Listeria* Enrichment media. All the cultivations were completed under the agitation speed of 150 rpm for 48 h.

Experimental Design

This study was approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia under the reference of UPM/IACUC/AUP-R049/2018. A completely randomized design (CRD) was used in this experiment. A total number of 96 one-day-old chickens (Cobb 500) were used in this study and randomly divided into two treatment groups with six replicates per treatment.

Animal Rearing System

A total number of 96 one-day-old broilers (Cobb 500) were used in this experiment. This experiment was conducted in the Poultry Research Unit, Universiti Putra Malaysia. The broilers were wing banded and weighed individually. After that, the broilers were randomly allocated into two treatment groups, with six replicates per treatment. Each experimental unit contained eight chickens. The experimental chickens were allocated to two treatment groups, which were T1 (diet without organic acids and palm fat) and T1OA (diet with supplementation of organic acids and palm fat).

Feed and water were offered *ad libitum* until 42 days of age. Starter diets were offered for experimental chickens from 0 to 21 days of age, and grower diets from 22 to 42 days of age. The calculated nutrients of the diets fed to the experimental chickens were shown in Table 1.

Variables Measured

Agar well diffusion assay. Agar well diffusion method (Tagg & McGiven, 1971) was used in the inhibitory activity test. Treatments of organic acids, palm fat, and OAPF were used to test against the indicator microorganism and pathogenic bacteria. Firstly, treatments with two-fold-serial dilution from 2^0 to 2^{-5} were carried out by using 0.85% (w/v) NaCl solution. After that, each treatment was inoculated at 20 μL into the corresponding well on the pre-punched agar plate. For example MRS-agar plate for *P. acidilactici*, *Listeria,* Enrichment agar plate for *L. monocytogenes*, and nutrient agar plate for *S. enterica* and *E. coli* respectively. In addition, 100 μL of treatment was inoculated into the pre-punched Brain Heart Infusion agar plate for VRE. Each well had a diameter of 5.5 mm. A volume of 3 mL corresponding soft agar inoculated with 1% (v/v) of *P. acidilactici*, *L. monocytogenes*, *S. enterica*, VRE, and *E. coli*, respectively, and adjusted to OD_{600mm} to 1.0 were overlaid in the agar plate. Organic acids (OA), OAPF, and PF were allowed to diffuse completely in the agar for 1 hour at room temperature. After that, the agar plates were then incubated at 30°C for 24 hours. The initial diameter size was recorded with the observation of the highest dilution factor and the diameter of the inhibitory zone size greater than 0.1 cm in the plates. The measurement of the inhibitory zone's diameter (mm) was recorded, and the calculation of modified inhibitory activity was based on the equation below:

Modified inhibitory activity =
[(1/Higest dilution zone) / Volume of supernatant (μL)] x diameter of inhibitory zone (mm)

Optical density and pH determination. Optical density (OD) was used to measure the concentration of bacteria

Table 1. Compositions and nutrient contents of starter and finisher diets with and without organic acids and palm fat

Ingredients	Dietary treatment			
	Starter		Finisher	
	T1	T1OA	T1	T1OA
Corn, (%)	51.24	51.49	53.68	53.83
Soybean, (%)	33.44	33.34	28.75	28.75
Wheat Pollard, (%)	5.8	5.5	6.9	6.6
OAPF, (%)	0	0.15	0	0.15
Palm oil, (%)	3.6	3.6	5.5	5.5
DL-Methionine, (%)	0.3	0.3	0.26	0.26
L- Lysine-HCl, (%)	0.45	0.45	0.29	0.29
L-Threonine, (%)	0.2	0.2	0.15	0.15
Di-calcium phosphate 21, (%)	1.9	1.9	1.8	1.8
Calcium carbonate, (%)	1.9	1.9	1.8	1.8
Choline Chloride, (%)	0.1	0.1	0.1	0.1
Salt, (%)	0.4	0.4	0.3	0.3
Mineral Mix[1], (%)	0.25	0.25	0.15	0.15
Vitamin Mix[2], (%)	0.25	0.25	0.15	0.15
Antioxidant[3], (%)	0.025	0.025	0.02	0.02
Toxin binder[4], (%)	0.15	0.15	0.15	0.15
Total, (%)	100	100	100	100
Calculated analysis				
Energy (kcal ME/kg)	3,035	3,036	3,182	3,181
Protein (%)	21	21	19	19
Methionine (%)	0.46	0.46	0.4	0.4
Lysine (%)	1.2	1.19	0.97	0.97

Note: T1= diet without organic acids and palm fat, T1OA= diet with supplementation of organic acids and palm fat.
[1]Mineral mix contains Fe 100 mg/kg, Mn 110 mg/kg, Cu 20 mg/kg, Zn 100 mg/kg, I 2mg/kg, Se 0.2 mg/kg, Co 0.6 mg/kg.
[2]Vitamin premix contains retinol 2 mg/kg, cholecalciferol 0.03 mg/kg, α-tocopherol 0.02 mg/kg, menadione 1.33 mg/kg, cobalamin 0.03 mg/kg, thiamine 0.83 mg/kg, riboflavin 2 mg/kg, folic acid 0.33 mg/kg, biotin 0.03 mg/kg, pantothenic acid 3.75 mg/kg, niacin 23.3 mg/kg, pyridoxine 1.33 mg/kg.
[3]Antioxidant contains butylated hydroxy anisole (BHA).
[4]Toxin binder contains natural hydrated sodium calcium aluminum silicates.

in a suspension. Centrifugation was carried out at 10,000 × g for 15 min by one mL of culture from each treatment group. Then, the cell pellet was washed once with 0.85% (w/v). The spectrophotometer (Novaspec III, Biochrom, Cambridge, UK) was used to determine the OD at 600 nm. Lastly, the pH of organic acids, OAPF, and palm fat was determined by using a pH meter (Mettle-Toledo., England).

Fecal lactic acid bacteria (LAB) and *Enterobacteriacaeae* (ENT) populations. The fecal population of LAB and ENT were determined using a method described by Foo *et al.* (2003) and Thanh *et al.* (2009). Firstly, fecal samples were kept at 25ºC for 1 hour at once when the 10-fold dilution (w/v) was completed in sterile peptone water. After the 1 hour of soaking time, 10-fold serial dilutions (v/v) were carried out. MRS-agar (Lactobacillus-Agar De Man, ROGOSA and SHARPE, Merck, KgaA, Darmstadt) was used to do the LAB counts. Plates were incubated in anaerobic jars at 30°C for 48 hours. The incubation of ENT has performed aerobically at 37°C for 24 hours after spreading and counting them on EMB-Agar (Eosin-methylene blue Lactose Sucrose Agar, Merck, KgaA, and Darmstadt). The expression of the number of CFU was applied by base 10 logarithm of CFU (log CFU) per gram. The whole samples were prepared in triplicates.

Nutrient digestibility. Titanium dioxide (TiO_2) added at 3 g/1000 g into the feed and digesta acted as an indigestible marker. The TiO_2 was measured according to the method described by Short *et al.* (1996). The collected ileal digesta from the chickens after slaughtering was kept in a pill box and stored at -20ºC until further analyses. Approximately 0.1 g of each sample was weighed and transferred into porcelain crucibles. Then the samples were placed in the furnace and set at 580°C for 13 h. A total of 10 mL 7.4 M sulfuric acid (H_2SO_4) was added into each crucible. The mixture was then gently boiled for 1 h or until being dissolved. The solution was subsequently transferred into a beaker containing 25 mL of distilled water and followed by a 100 mL volumetric flask using Whatman filter paper No. 541. A total of 20 mL 30% hydrogen peroxide (H_2O_2) was added to each flask and made up to 100 mL with distilled water.

The standard of TiO_2 solution was prepared as 0.3 mg/mL. Approximately 150 mg of pure TiO_2 was added to 100 mL of concentrated H_2SO_4 and boiled as the standard solution. The content was carefully transferred into a 500 mL volumetric flask by rinsing with 200 mL distilled water. A 100 mL concentrated H_2SO_4 was added to the solution and made up to 500 mL with distilled water. The standard curve was prepared at a range between 0 to 10 mL. Approximately 0.3 mg/mL of TiO_2 was added into individual 100 mL volumetric flasks. A 10 mL of 7.4 M H_2SO_4 was added in order to obtain a combined volume of 10 mL. A 20 mL of H_2O_2 (30%) was added to each volumetric flask, and the contents were made up to 100 mL with distilled water. The sample without TiO_2 was used as a blank. All samples and standard solutions were measured by using a spectrophotometer set at a wavelength of 410 nm, and the relation between optical density and concentration was linear up to the highest concentration at 0.03 mg/mL.

Proximate analysis of nutrients was applied to feed and digesta in order to calculate nutrient digestibility (AOAC, 1995). An auto analyzer (Lachat Instruments QuikChem 8000 Series FIA + System) was used to determine the overall phosphorus contents. The calcium was measured by atomic absorption spectrometry (Perkin Elmer Analyst 400). Apparent ileal digestibility (AID) of dry matter (DM), crude protein (CP), and ether extract were calculated using titanium marker ratios in the diet and digesta according to Tancharoenrat *et al.* (2014) using the formula below:

AID= 100 - [100 x (% TiO2 in feed / % TiO2 in digesta) x (% Nutrient digesta / % nutrient feed)

Statistical Analysis

All the collected data were analyzed statistically by using the Statistical Analysis System package (SAS)

Version 9.4 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA). Statements of statistical significance were based on $p<0.05$. The differences among treatment means were tested using an independent t-test.

RESULTS

Inhibitory Activity

The modified inhibitory activity (MAU/mL) against *Pediococcus acidilactici* 4–46, VRE, *Listeria monocytogenes* L-MS, and *Salmonella enterica* S-10 were presented in Table 2. The results showed that palm fat (PF) did not show any inhibitory activity against all the tested foodborne pathogens (data were not shown in Table 2). However, organic acids and OAPF demonstrated inhibitory effects against 4 foodborne pathogens i.e., *Listeria monocytogenes* L-MS, Vancomycin Resistant VRE, *Escherichia coli* E-30, and *Salmonella enterica* S-1000. However, inhibitory activity was not observed in *Pediococcus acidilactici* 4–46. A combination of organic acids and palm fat (OAPF) showed a significantly higher inhibitory effect against *Salmonella enterica* S-1000, *Listeria monocytogenes* L-MS, and *Escherichia coli* E-30. Also, OAPF had approximately two folds inhibitory activity against *Escherichia coli* E-30 compared to OA.

Microbial Population

Table 3 shows the microbial population in experimental broilers fed ration without organic acids and palm fat supplementation (T1) and a diet supplemented with a combination of organic acids and palm fat (T1OA) at week 6. The results showed that broilers fed T1OA showed a significantly higher ($p<0.05$) LAB population than control broilers fed T1. However, the lower ENT population was found in broiler fed T1OA compared to that fed T1.

Nutrient Digestibility

Table 4 shows the nutrient digestibility of broilers fed T1OA at different levels of nutrients during the starter stage and grower stage. The dry matter, crude protein, and crude fat digestibility in the broilers fed OAPF were significantly higher ($p<0.05$) compared to that fed T1.

Growth Performance

Table 5 shows the growth performances of experimental broilers for 0-42 days fed T1 supplementation. There was no significant difference ($p>0.05$) among the treatment groups in the initial body weight. However, broiler fed T1OA had a significantly higher ($p<0.05$) final body weight compared to the control broiler fed T1. Moreover, broilers fed T1OA also showed a significantly higher ($p<0.05$) feed intake compared to broiler fed T1. However, broilers fed a ration with T1OA had significantly lower FCR ($p<0.05$) compared to control broilers fed T1.

Table 2. Modified inhibitory activity (MAU/mL) and pH of different treatments against pathogenic bacteria

Treatment[1]	pH	Inhibitory activity (MAU/mL)				
		P. acidilactici	VRE	*L. monocytogenes*	*S. enterica*	*E. coli*
Organic acids + palm fat (OAPF)	0.65 ± 0.01	ND	16.67 ± 0.33[a]	190.00 ± 5.77[a]	253.33 ± 8.82[a]	150.00 ± 5.77[a]
Organic acids	0.62 ± 0.01	ND	13.67 ± 0.33[a]	161.67 ± 4.41[b]	206.67 ± 3.33[b]	61.67 ± 4.41[b]

Note: ND= Not detected. [1]The results are presented as mean values. Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 3. Fecal microbial counts of broiler chickens fed ration with and without organic acids and palm fat supplementation

Variables	Dietary treatment[1]	
	T1	T1OA
Microbial count week 6, Log CFU/g		
Lactic acid bacteria	7.66 ± 0.01[b]	8.12 ± 0.09[a]
Enterobacteriacaeae	5.82 ± 0.05[a]	4.82 ± 0.09[b]

Note: T1= Dietary starter and grower without organic acids-palm fat (OAPF), T1OA= Dietary starter and grower with 0.15% OAPF. [1]The results are presented as mean values. Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Nutrients digestibility of broiler chicken fed ration with and without organic acids and palm fat supplementation from 0-42 days

Variables	Dietary treatment[1]	
	T1	T1OA
Digestibility coefficient (%) – Starter diet		
Dry matter	90.63 ± 0.63[b]	91.21 ± 0.36[a]
Crude protein	67.45 ± 0.77[b]	72.22 ± 0.52[a]
Ether extract	69.10 ± 1.47[b]	74.09 ± 1.13[a]
Digestibility coefficient (%) – Finisher diet		
Dry matter	88.30 ± 0.42[b]	89.16 ± 0.33[a]
Crude protein	67.08 ± 1.29[b]	71.78 ± 0.79[a]
Ether extract	68.85 ± 1.71[b]	74.28 ± 1.78[a]

Note: T1= Dietary starter and grower without organic acids-palm fat (OAPF), T1OA= Dietary starter and grower with 0.15% OAPF. [1]The results are presented as mean values. Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 5. Growth performance broiler chicken fed ration with and without organic acids and palm fat supplementation from 0-42 days

Growth performance (0-42 days)[1]	Treatment[1]		
	T1	T1OA	SEM
Initial body weight, (g/bird)	45.19	45.27	0.02
Final body weight, (g/bird)	2739.74[b]	3008.27[a]	21.08
Cumulative weight gain, (g/bird)	2694.55[b]	2963.00[a]	21.07
Cumulative feed consumption, (g/bird)	4429.94[b]	4782.63[a]	30.53
Feed conversion ratio	1.64[a]	1.61[b]	0.01
Average daily weight gain, (g/bird)	64.16[b]	70.55[a]	0.50

Note: T1= Dietary starter and grower without organic acids-palm fat (OAPF), T1OA= Dietary starter and grower with 0.15% OAPF. [1]The results are presented as mean values. Means in the same row with different superscripts differ significantly ($p<0.05$).

DISCUSSION

Inhibitory Activity

The absence of inhibitory activity in *Pediococcus acidilactici* 4–46 might be due to the tolerance of the LAB towards the acidic condition of the organic acids. Acidifying agents such as organic acids and OAPF can reduce the pH around the environment and the survivability of pathogenic bacteria. The presence of organic acids and OAPF can lower the pH, which can stop the spreading of pathogenic bacteria and spoilage microorganisms. This condition caused the reduced population of *Salmonella* sp. and *E. coli* (Naseri *et al.,* 2012). From this study, the use combination of two antimicrobials (organic acids and palm fat) is foreseen to have a synergistic effect against pathogenic bacteria. This conclusion can be observed from the OAPF showed approximately two folds inhibitory activity against *Escherichia coli* E-30 compared to organic acids alone. The increased modified inhibitory activity observed after the combination of organic acids and palm fat may be due to the loss of cell membrane integrity and the changes in cell permeability. Fatty acids in palm fat also show a lot of inhibitory activities and have antimicrobial properties against a large number of microbial species (Desbois *et al.,* 2010). The mechanism for these synergistic antimicrobial effects might be due to the capacities of organic acids or palm fat to destroy the integrity of the cell by damaging the cell membrane. This process causes the disruption of the cell membrane and hastens the entry of the other antimicrobial compounds (fatty acids, organic acids, and hydrogen (H) ions) into the cell. The increased antimicrobial activity may also be caused by the invasion by the other antimicrobial compounds, especially H ions, which penetrate into the cell and enter the cytoplasm (Wu *et al.,* 2016). Therefore, the H ions present in palm fat and organic acids alone in current treatment groups cannot penetrate into the cells due to their polarities. However, the combination of organic acids and palm fat may cause damage to the cell membrane, subsequently allowing H ion to enter into the cell. Therefore, the antimicrobial activity is much greater observed in a combination with organic acids and palm fat treatment than individual palm fat or organic acids treatment.

Microbial Population

This study highlights the effects of OAPF on the fecal microbial population of broilers. The improved performance of the birds might due to the reduction of pathogenic bacteria and the increased number of beneficial bacteria by supplementation of OAPF in the diet. The decreased ENT population might due to OAFP that can enter the cell wall and interrupt the cellular functions of the microbial population (Davidson, 2001). The microbial compounds (organic acids and H ions) of OAPF can enter the cell wall and destroy the activity of some pathogens, including *Salmonella* spp, *E. coli*, *Listeria* spp., and others. Hence, the decreased numbers of some pathogenic bacteria can happen in animals fed a ration with OAPF. A combination of organic acids and palm fat (OAPF) can reduce the pH of the surrounding environment in the gastrointestinal tract. The pH reduction in the gastrointestinal tract might cause the increased LAB count and decreased ENT count (Loh *et al.,* 2008; Shazali *et al.,* 2014; Kareem *et al.,* 2016). The increased LAB count resulted in the enzyme activity improvement in the gut, which helps the digestion and absorption of nutrients in the experimental chickens. Besides that, the reduction of the ENT population also causes a decrease in pathogenic bacteria in the gut, which allows a lesser microbial competition for nutrients with the host and leads to better nutrient utilization in the experimental chickens (Rosyidah *et al.,* 2011).

Nutrient Digestibility

The treatment group supplemented with OAPF showed the improved digestibility of dry matter (DM), crude protein (CP), and ether extract (EE) compared with the treatment group without OAPF. The improved digestibility of EE might due to the value-added of palm fat in the organic acids. The combination of palm fat and organic acids caused more efficient energy utilization. This condition was caused by the low pH of organic acids enhances the improvement of energy digestion in palm fat powder. The results are similar to those reported by Samanta *et al.* (2010) that organic acids enhanced gastric proteolysis and increased the digestibility of crude protein. A combination of organic acids and palm fat can reduce the pH around the GIT. The increased utilization of the nutrients when the enzyme activity increased was caused by the reduction of pH in the GIT. Low pH also decreased the pathogenic bacteria in the GIT, which allows a lesser microbial competition for nutrients with the host. Therefore, there is more nutrients available for the chickens to absorb and digest. This condition leads to an increase of nutrient digestibility in chickens fed ration supplemented with organic acids and palm fat compared to control chickens without organic acids and palm fat supplementation.

Growth Performance

In this study, the diet supplemented with OAPF improved the growth performance of experimental broilers. This observation is similar to the previous finding by Brzóska *et al.* (2013). It is concluded that the inclusion of OAPF in the diet will support the promotion of body weight gains. It is highly likely that the increased body weight gain is due to the positive effects of supplementations of organic acids and palm fat on the population of flora in the gut. This result is caused by the properties of organic acids and palm fat supplementation that may have affected the integrity of bacterial cell membranes or interfered with the nutrient's transportation, energy metabolism leading to better utilization of nutrients (Ricke *et al.,* 2003). The higher feed intake in the experimental chickens fed ration supplemented with organic acids and palm fat might due to the higher body weight gain and more feed intake to support the growth of the broilers. In this study, the feed conversion ratio of the experimental chickens also showed an improvement by providing the organic acids and palm fat in the feed. The improvement of FCR might be due to the improved nutrient digestibility resulting in the increased BWG in the chickens fed ration supplemented with organic acids and palm fat (Adil *et al.,* 2011).

CONCLUSION

The combination of organic acids and palm fat (OAPF) have the antimicrobial ability to inhibit the growth of various pathogenic bacteria. Supplementation of OAPF had a positive effect on the LAB population, decreasing the ENT population by lowering the pH. Therefore, the supplementation of OAPF is more effective compared to organic acids alone. Moreover, OAPF can be used as feed additives to improve the gut health and growth performance of broilers and livestock animals.

CONFLICT OF INTEREST

Loh Teck Chwen serves as an editor of the Tropical Animal Science Journal, but has no role in the decision to publish this article. Except for that, there is no conflict of interest among the authors of this article.

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