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# Effect of Feeding Metabolites and Acidifier on Growth Performance, Faecal Characteristics and Microflora in Broiler Chickens

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**Abstract:** A study was conducted to study the effects of feeding metabolites which produced from *L. plantarum* and acidifier in the diets of broilers chickens on growth performance, microflora count, digesta and faecal pH, immunoglobulin status and volatile fatty acids. A total of 288 male Cobb randomly assigned to five dietary groups for 42 days, basal diet feed (negative control), basal diet feed+neomycin and oxytetracyline (positive control), basal diet feed+0.1% acidifiers (A), basal diet feed+0.5% metabolite (M), basal diet feed+0.1% acidifiers (A) and 0.5% metabolite (M). Higher final body weight and weight gain, lower daily feed intake and feed conversion ratio were found in metabolites and combination of metabolite and acidifier groups while greater lactic acid bacteria count, low faecal and digesta pH and increase volatile fatty acids were found in 3 treated groups. No significant difference was found for immunoglobulin level.

**Key words:** Metabolites, acidifiers, lactic acid bacteria, broilers, volatile, Malaysia

## INTRODUCTION

The usage of feed antibiotic in modern livestock and poultry farming has been very extensive in order to enhance the productivity in the farm. The major effects of antibiotic in feed are to enhance animal growth performance and prevent infections due to pathogenic bacteria. However, misuse of growth promoting antibiotic causes development of resistance in a number of pathogenic bacteria. Therefore, the need of new antimicrobial drugs to counter the resistance of the microbes is crucial. Furthermore, antibiotic in livestock farming have indirect adverse effect towards human health. It has been shown that *E. coli* isolated from pig fecal was resistant to antibiotics such as neomycin, oxytetracycline, nalidixic acid and chloramphenicol (Loh *et al*., 2006; Van den Bogaard *et al*., 2001). Furthermore, residue of antibiotics is commonly present in the consumer products of animals-based (Van den Bogaard *et al*., 2001). As a result, many countries have started to ban the use of antibiotics in poultry production as growth promoter. In view of the total ban in the use of antibiotics as growth promoters in livestock and poultry production, many parts of the world are experimenting alternative feed additive that may be used to alleviate the problems associated with the withdrawal of antibiotics from feed. Organic Acid Blend (OAB) containing citric acid, fumaric acid, formic acid and propionic acids is one of the alternative feed additives. It has been claimed that acidifier have a positive effect on growth performance (Partanen and Mroz, 1999; Loh *et al*., 2007a; Luckstadt *et al*., 2004). However, the result obtained in poultry is not consistent (Thompson and Hinton, 1997). Metabolites produced from acid lactic bacteria were also known for their natural antibacterial properties. These metabolites contain bacteriocin, organic acids and commonly used as food preservatives. Recently, it has been reported that the metabolites can be used as feed additive to replace in-feed antibiotics in poultry (Thanh *et al*., 2009; Loh *et al*., 2007b, 2009) and pigs (Thu *et al*., 2011). Many enquiries have been raised due to the use of combinations of OAB and metabolites as feed additive in farm. However, the availability of the information on the matter is still very scarce. Thus, a study was conducted to investigate the potential application of OAB and naturally occurring metabolites on the growth performance of broilers, faecal LAB and Enterobacteriaceae (ENT) counts, faecal and digesta pH, faecal volatile fatty acids and immunoglobulin levels.

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## MATERIALS AND METHODS

**Animal and experimental design:** A total of 288 days old Cobb male broiler chicks with initial body weight of 40.30±0.22 g from local hatchery were raised from day old to 42 days of age in block cages. Each cage consisted of 6 chicks and was randomly assigned to 6 replicate of 5 groups of the treatment. The treatment groups were basal diet feed (negative control), basal diet feed+ neomycin and oxytetracycline (positive control), basal diet feed+0.1% Acidifiers (A), basal diet feed+0.5% Metabolite (M), basal diet feed+0.1% acidifiers (A) and 0.5% Metabolite (M). Upon arrival, the chicks were vaccinated against Infectious Bronchitis (IB) and Newcastle Disease (ND) (IB-ND Fort Dodge, Collegeville, USA) by intraocular route on day 1. While the IBD vaccine (UPM93, Myvac, Bangi, Malaysia) against Infectious Bursal Disease (IBD) was applied on day 14 of the rearing period. All the birds were wing banded for identification. Feed and water were given *ad libitium* to the birds until 42 days of age. The starterand finisher diets (Table 1 and 2, respectively) were offered from days 0 until 21 and days 22 until 42 of age, respectively. The metabolites without bacterial cells were produced as described by Loh *et al.* (2010).

**Data and sample collection:** The individual body weight and cage feed intake were recorded weekly. Live weight gain, feed conversion ratio and average daily gain were then calculated from the data obtained. At day 42, twelve of birds with similar body weight were obtained from each treatment group. Blood, faecal and digesta samples from small intestine were collected after slaughtering of the birds. Faecal pH and microflora count were analyzed. Digesta from small intestine was collected and analyze for their microflora count and pH.

**Metabolites and acidifiers:** The stock culture of the four *L. plantarum* strains, RS5, RG11, RG14 and RI11 were prepared from Department of Bioprocess Technology, Universiti Putra Malaysia. The stock culture was sub-cultured in 10 mL Man Rogosa Sharpe (MRS) broth. The cultures revived twice in MRS broth and incubated anaerobically at 30°C before preparing the metabolite. About 1% of overnight culture inoculated into 1 L MRS broth and incubated anaerobically for overnight at 30°C for 12 h. The metabolite was collected by separating the bacterial cells with centrifugation at 12,000 rpm for 15 min. The metabolite was then kept at 4°C (Foo *et al.*, 2003). The acidifier (Orgacids™) was provided by Sunzen Corporation Sdn. Bhd., Malaysia. The acid blends powder consisted of formic (0.58%), phosphoric (44.50%), lactic (0.94%), tartaric (0.83%), citric (0.02%) and malic (0.45%) acids and the inclusion rate of Orgacids™ was 0.1% (w/w).

Table 1: Compositions of starter diet

| | Dietary treatment[1] | | | | |
|---|---|---|---|---|---|
| Ingredients | -ve control | +ve control | 0.1% A | 0.5% M | 0.1% A+0.5% M |
| Corn | 506.00 | 506.00 | 506.50 | 506.50 | 506.00 |
| Soyabean | 293.84 | 293.84 | 294.34 | 293.84 | 293.84 |
| Wheat pollard | 60.71 | 60.61 | 58.71 | 52.21 | 50.71 |
| CPO | 36.00 | 36.00 | 36.00 | 37.00 | 37.50 |
| Fish meal 55% | 76.00 | 76.00 | 76.00 | 78.00 | 78.50 |
| L-Lysine | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| DL-methionine | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Monodicalciumphosphate 21 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Calcium carbonate | 6.80 | 6.80 | 6.80 | 6.80 | 6.80 |
| Choline chloride | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Salt | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Mineral mix[2] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin mix[3] | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Antioxidant | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Toxinbinder | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
| Antibiotic | - | 0.10 | - | - | - |
| Acidifier (Powder) | - | - | 1.00 | - | 1.00 |
| Metabolic (Liquid) | - | - | - | 5.00 | 5.00 |
| Total | 1000.00 | 1000.00 | 1000.00 | 1000.00 | 1000.00 |
| **Calculated analysis** | | | | | |
| Crude protein (%) | 22.50 | 22.48 | 22.47 | 22.47 | 22.48 |
| ME (MJ $kg^{-1}$) | 12.20 | 12.21 | 12.21 | 12.21 | 12.22 |

[1]0.1% A is a acidifier; 0.5% M is a metabolites combination of RS5, RG11, RG14 and RI11; 0.1% A+0.5% M is a mix of acidifier and metabolite combination. [2]Mineral mix that provided per kilogram of the diet: Fe 100 mg; Mn 110 mg; Cu 20 mg; Zn 100 mg; I 2 mg; Se 0.2 mg; Co 0.6 mg. [3]Vitamin mix that provided per kilogram of the diet: Retinol 2.00 mg: Cholecalciferol 0.03 mg; α-tocopherol 0.02 mg; menadione 1.33 mg; cobalamine 0.03 mg; thiamine 0.83 mg; riboflavin 2 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; niacin 23.3 mg; pyridoxine 1.33 mg; [4]A combination of oxytetracylin and neomycin at the concentration of 100 ppm (w/w)

Table 2: Composition of finisher diet

| | Dietary treatment[2] | | | | |
|---|---|---|---|---|---|
| Ingredients | -ve control | +ve control | 0.1% A | 0.5% M | 0.1% A+0.5% M |
| Corn | 549.00 | 549.00 | 550.00 | 549.00 | 549.00 |
| Soyabean | 265.00 | 265.00 | 265.00 | 265.00 | 265.00 |
| Wheat pollard | 78.20 | 78.10 | 76.20 | 70.40 | 68.60 |
| CPO | 36.00 | 36.00 | 36.00 | 37.30 | 37.60 |
| Fish meal 55% | 34.00 | 34.00 | 34.00 | 35.50 | 36.00 |
| L-lysine | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| DL-Methionine | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Monodicalciumphosphate 21 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 |
| Calcium carbonate | 13.00 | 13.00 | 13.00 | 13.00 | 13.00 |
| Choline chloride | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Salt | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Mineral mix[2] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin mix[3] | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Antioxidant | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Toxinbinder | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Antibiotic | - | 0.10 | - | - | - |
| Acidifier (Powder) | - | - | 1.00 | - | 1.00 |
| Metabolic (Liquid) | - | - | - | 5.00 | 5.00 |
| Total | 1000.00 | 1000.00 | 1000.00 | 1000.00 | 1000.00 |
| **Calculated analysis** | | | | | |
| Crude protein (%) | 20.34 | 20.34 | 20.29 | 20.29 | 20.29 |
| ME (MJ $kg^{-1}$) | 12.18 | 12.18 | 12.17 | 12.17 | 12.17 |

[1]0.1% A is a acidifier; 0.5% M is a metabolites combination of RS5, RG11, RG14 and RI11; 0.1% A+0.5% M is a mix of acidifier and metabolite combination. [2]Mineral mix that provided per kilogram of the diet: Fe 100 mg; Mn 110 mg; Cu 20 mg; Zn 100 mg; I 2 mg; Se 0.2 mg; Co 0.6 mg. [3]Vitamin mix that provided per kilogram of the diet: Retinol 2.00 mg: Cholecalciferol 0.03 mg; α-tocopherol 0.02 mg; menadione 1.33 mg; cobalamine 0.03 mg; thiamine 0.83 mg; riboflavin 2 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; niacin 23.3 mg; pyridoxine 1.33 mg; [4]A combination of oxytetracylin and neomycin at the concentration of 100 ppm (w/w)

**Faecal Lactic Acid Bacteria (LAB) and Enterobacteriaceae (ENT) count, faecal and digesta pH:** About 10% of faecal sample was diluted in sterile peptone water, left at room temperature for an hour prior to further 10 fold serial dilutions (v/v). Enumerations of LAB were performed on MRS-agar (Lactobacillus-Agar De Man, ROGOSA and SHAPE) (Merck®, KgaA, Darmstadt). The plates were incubated in anaerobic jar at 30°C for 48 h. ENT were spread and counted on EMB-agar (Eosin-methylene-blue Lactose Sucrose Agar) (Merck®, KgaA, Darmstadt) and incubated aerobically for 24 h at 37°C. The number of Colony Forming Units (CFU) was expressed as logarithm at the base of 10 ($Log_{10}$ CFU) per gram. All samples were repeated in triplicates (Loh *et al*., 2010). About 1 g of the sample was mixed homogenously with 9 mL of deionized distilled water in a universal tube. The pH was measured using Mettler-Toledo pH meter with glass electrode (Mettler-Toledo LTD, England). The meter was calibrated prior to measure the pH of the samples by using buffer solution (Merck, KgaA, Darmstadt) at pH 4 and 7.

**Immunoglobulin level analysis:** The immunoglobulin levels (IgG and IgM) in the blood samples were determined using Rat IgG ELISA kit (Cat. No. 6420; Alpha Diagnostic International). All assay procedure was performed at room temperature (18-30°C). Total 3 replicates per sample were prepared for the analyses. The wells were washed and dried 5 min before addition of samples. In the first incubation, each of pre-determined wells was added with 100 uL of standards, samples (with dilution factor of 1:10,000 for IgM and 1:50,000 for IgG) and control. Reagents were mixed, incubated for 60 min and washed for 4 times before pat dry. In the second incubation, 100 uL of diluted conjugated reagent were added to each well with 30 min of incubation and washed 5 times. About 100 uL TMB substrate was then added to each well and incubated for 15 min in the dark place. Finally, 100 uL of stop solution were added to appropriate wells to stop the reaction. Data was recorded and calculated using a microplate reader at the wavelength of 450 nm.

**Volatile fatty acids:** The Volatile Fatty Acids (VFA) concentration in the faeces was measured using the modified method of Thanh *et al.* (2009). About 1 g of faecal sample (stored at -20°C) from each sample was weighed. About 1 mL of 24% metaphosphoric acid diluted in 1.5 M sulphuric acid (BDH Laboratories, Poole, UK) was added. The mixture was kept at room temperature overnight and centrifuged at 10,000×g for 20 min at 4°C. The collected supernatant was kept in a 2 mL screw capped vial (Kimble Glass Inc., USA). The internal standard 20 mM 4-Methyl-valeric acid (Sigma Chemical Co., St. Louis, MO, USA) was added to the supernatant to achieve 10 mM in the combination and stored at -20°C

until GLC analysis. Volatile fatty acids were separated on a Quadrex 007 Series (Quadrex Corp., New Haven, CT 06525, USA) bounded phase fused silica capillary column (15 m, 0.32 mm ID, 0.25 μM film thickness) in a 6890 N (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector. The purified nitrogen functioned as carrier gas with a flow rate of 60 mL $min^{-1}$. The temperature of the injector and detector was 230°C. The column temperature was set as 200°C. The commercial standards of 20 mM acetic and 10 mM each of propionic butyric, isobutyric, valeric, isovaleric and 4-methyl-valeric acids from Sigma were used as external standards to identify the peaks.

**Statistical analysis:** Results were expressed as mean± Standard of Mean (SEM). The data was analyzed by Analysis of Variance (ANOVA). Duncan Multiple Range test was used to compare the differences of growth performance, Enterobacteriaecea (ENT) and Lactic Acid Bacteria (LAB) count, faecal and digesta pH and immunoglobulin of IgM and IgG among the treatment groups. Differences of $p<0.05$ were considered significant (SAS, 1998).

## RESULTS AND DISCUSSION

**Growth performance:** The growth performance of the broilers fed with metabolites (M) and organic acids (A) is shown in Table 3. There was no significant difference ($p>0.05$) in the final body weight and growth rate between positive control, 0.5% M and combination of 0.1% A and 0.5% M with negative control. Conversely, 0.1% A had the lowest ($p<0.05$) final body weight and growth rate. The 0.5% M and combination of this treatment caused a significantly lower ($p<0.05$) in the average feed consumption than positive control. However, there were no significant difference ($p>0.05$) as compared with negative control and 0.1% A. The 0.5% M and combination treatments had better ($p<0.05$) feed conversion ratio than positive, negative control and 0.1% A.

**Intestinal microflora count, faecal and digesta pH and immunoglobulin level:** Table 4 shows intestinal microflora counts, faecal and digesta pH and immunoglobulin levels. Lower count ($p<0.05$) of digesta and faecal LAB count was found for both control groups compared to treatments supplemented with metabolite and/or acidifiers. Digesta ENT count for 0.5% M, 0.1% A+0.5% M and 0.1% A groups was ranged from 6.33-8.75% lower than positive control. Similar result was shown in faecal ENT where as the difference was ranged from 2.81-9.93% as compared to the positive control groups.

The digesta pH for the 0.5% M was the lowest ($p<0.05$) among the treatment groups. Combination of 0.1% A+0.5% M had no significant difference ($p>0.05$) as compared with positive control and 0.1% A groups. The 0.5% M caused lower faecal pH ($p<0.05$) than positive control group. No significant difference ($p>0.05$) were found between 0.1% A, 0.1% A+0.5% M and control groups. There was no significant difference ($p>0.05$) for IgM and IgG among the treatment groups in the current experiment (Table 4).

Table 3: Growth performance of broilers fed with acidifiers and metabolites diets

| | Dietary treatments | | | | |
|---|---|---|---|---|---|
| Parameter (mM) | -ve control | +ve control | 0.1% A | 0.5% M | 0.1% A+0.5% M |
| Initial weight (g) | 43.89±0.94$^{a}$ | 44.21±1.70$^{a}$ | 44.58±0.53$^{a}$ | 43.56±0.79$^{a}$ | 43.78±0.51$^{a}$ |
| Final weight (kg) | 2.36±0.03$^{ab}$ | 2.47±0.03$^{a}$ | 2.32±0.04$^{b}$ | 2.48±0.06$^{a}$ | 2.47±0.02$^{a}$ |
| Growth rate (g $day^{-1}$) | 55.14±0.80$^{ab}$ | 57.73±0.72$^{a}$ | 54.18±0.94$^{b}$ | 58.01±1.35$^{a}$ | 57.77±0.60$^{a}$ |
| Daily feed intake (g $day^{-1}$) | 98.57±1.43$^{ab}$ | 102.46±0.69$^{a}$ | 98.27±1.46$^{ab}$ | 97.36±1.24$^{b}$ | 95.83±2.45$^{b}$ |
| Feed conversion ratio | 1.79±0.04$^{ab}$ | 1.77±0.03$^{ab}$ | 1.81±0.06$^{a}$ | 1.68±0.03$^{b}$ | 1.66±0.02$^{b}$ |

Table 4: Microflora counts, pH and immunoglobulin level of broiler supplemented with different treatments diets

| | Dietary treatments | | | | |
|---|---|---|---|---|---|
| Parameters | -ve control | +ve control | 0.1% A | 0.5% M | 0.1% A+0.5% M |
| **Microflora count (logCFU $g^{-1}$)** | | | | | |
| Digesta LAB | 8.49±0.010$^{e}$ | 8.66±0.0060$^{d}$ | 8.75±0.0030$^{c}$ | 8.88±0.0030$^{b}$ | 9.18±0.0030$^{a}$ |
| Faecal LAB | 7.45±0.020$^{d}$ | 7.72±0.0100$^{c}$ | 8.88±0.0200$^{a}$ | 8.28±0.0100$^{a}$ | 8.81±0.0090$^{b}$ |
| Digesta ENT | 6.93±0.030$^{a}$ | 6.63±0.0060$^{b}$ | 6.19±0.0700$^{c}$ | 6.05±0.0100$^{d}$ | 6.21±0.0300$^{c}$ |
| Faecal ENT | 5.46±0.010$^{a}$ | 5.34±0.0100$^{b}$ | 5.19±0.0070$^{c}$ | 4.84±0.0600$^{d}$ | 4.81±0.0060$^{e}$ |
| **pH** | | | | | |
| Digesta | 5.89±0.030$^{a}$ | 5.29±0.0370$^{b}$ | 5.18±0.3000$^{b}$ | 3.95±0.0800$^{c}$ | 4.93±0.1700$^{b}$ |
| Faecal | 6.07±0.250$^{ab}$ | 6.23±0.0300$^{a}$ | 5.99±0.0800$^{ab}$ | 5.80±0.0200$^{b}$ | 5.87±0.0200$^{ab}$ |
| **Immunoglobulin** | | | | | |
| IgM (μg $mL^{-1}$) | 663.47±40.36$^{a}$ | 635.92±102.78$^{a}$ | 683.88±214.22$^{a}$ | 965.17±113.98$^{a}$ | 940.68±169.84$^{a}$ |
| IgG (ng $mL^{-1}$) | 9.80±0.800$^{a}$ | 8.73±0.4200$^{a}$ | 8.19±0.8800$^{a}$ | 9.61±0.5300$^{a}$ | 9.02±0.1800$^{a}$ |

The result are presented as mean value±SEM. Value with different subscripts within row differ significantly at $p<0.05$

Table 5: Faecal volatile fatty acids of broilers supplemented with different treatments diets

| | Dietary treatments | | | | |
|---|---|---|---|---|---|
| Parameter (mM) | -ve control | +ve control | 0.1% A | 0.5% M | 0.1%A+0.5% M |
| Acetic | 18.38±0.28$^{c}$ | 24.51±0.44$^{b}$ | 24.17±0.82$^{b}$ | 35.93±0.32$^{a}$ | 35.18±0.74$^{a}$ |
| Propionic | 1.34±0.35$^{a}$ | 1.87±0.30$^{a}$ | 1.34±0.34$^{a}$ | 1.77±0.24$^{a}$ | 2.11±0.39$^{a}$ |
| Iso-Butyric | 0.27±0.03$^{a}$ | 0.26±0.07$^{a}$ | 0.22±0.01$^{a}$ | 0.20±0.01$^{a}$ | 0.36±0.05$^{a}$ |
| Butyric | 1.79±0.19$^{a}$ | 1.79±0.08$^{a}$ | 0.79±0.15$^{b}$ | 1.42±0.22$^{a}$ | 2.72±0.18$^{a}$ |
| Iso-Valeric | 0.42±0.07$^{a}$ | 0.53±0.11$^{a}$ | 0.37±0.08$^{a}$ | 0.37±0.08$^{a}$ | 0.51±0.06$^{a}$ |
| Valeric | 0.68±0.09$^{a}$ | 0.76±0.03$^{a}$ | 0.16±0.03$^{b}$ | 0.19±0.03$^{b}$ | 0.56±0.06$^{a}$ |
| Total | 22.88 | 29.72 | 27.05 | 39.88 | 41.44 |

The result are presented as mean value±SEM. Value with different subscripts within row differ significantly at $p<0.05$

**Volatile fatty acids:** The faecal VFA are shown in Table 5. The main VFA were acetic followed by propionic, iso-butyric, butyric, valeric and iso-valeric but in lower concentration. Higher concentrations ($p<0.05$) of total VFA levels and acetic acid were found in 0.5% M and 0.1% A+0.5% M as compared with the remaining of the treatments. No significant difference ($p>0.05$) was found for propionic, iso-butyric and iso-valeric acid among the treatment groups. The butyric acid was not significant different ($p>0.05$) between control and treated groups except for 0.1% A. However, combination group had higher ($p<0.05$) concentration of butyric acid as compare with 0.1% A. There were significantly different ($p<0.05$) between controls and combination of 0.1% A+0.5% M groups with 0.1% A and 0.5% M groups for valeric acid.

In terms of growth performance, metabolites and combination of metabolites with acidifier that show either metabolite alone or mixed with acidifier were able to improve growth performance that exerted similar or better effect than antibiotic promoter. These could be seen from current study that 0.5% M and 0.5% M+0.1% A had lower feed intake and feed conversion ratio but higher final weight and growth rate compared to control groups. These findings are also support by Thanh *et al.* (2009), the broilers fed with metabolites had better growth performance as compared with antibiotic chickens. These results were agreed with the findings of Ogunbanwo *et al.* (2004) where the bacteriocin from *L. plantarum* improved the growth rate of broilers. These results also verify that metabolites and acidifier could be applied simultaneously in feed to promote growth performance in the broilers without causing detrimental growth effect. In contrast, Foo *et al.* (2003) reported that there was no effect on growth performance of rats after adding *Lactobacillus plantarum* I-UL4 metabolites in drinking water. Trezona (2001) claimed that addition of the organic acids such as acetic acid, citric acid, fumaric acid, lactic acid and formic acid showed a positive effect on growth in pigs. In contrast, feeding organic acid (Izat *et al.*, 1989; Waldroup *et al.*, 1995) or zinc bacitracin (Hamilton and Proudfoot, 1997) to the broilers did not improve growth performance. These results were similar with the current findings, adding 0.1% A have lower final body weight but higher feed intake. This could be explained by acidifier group convert feed less efficiently and directly affects the growth performance. Previous researches (Loh *et al.*, 2007b; Sutton *et al.*, 1991) also show that the addition of LAB metabolites and acidifier can accelerate the development of indigenous LAB in the intestine by providing an optimum pH balance and promoting enzyme secretion through the gastrointestinal tract (Ravindran and Kornegay, 1993).

The current study demonstrated that broilers given metabolites and/or acidifiers had higher digesta and faecal LAB count than control groups. These results indicate that addition of LAB metabolites encouraged and increase the growth and population of indigenous LAB in the intestine and faeces (Foo *et al.*, 2003). Acidic environment produced encouraged lactobacilli rather than pathogen such as Salmonella (Fuller, 1977). Thanh *et al.* (2009) also reported boost of LAB population due to inhibitory effect against intestinal pathogens via competitive exclusion of pathogens by LAB which is one of the most important benefits.

Acidifier and metabolites group had a lower digesta Enterobacteriaceae (ENT) compared to control group. Foo *et al.* (2003) reported that feeding of metabolites produced from *L. plantarum* reduced ENT population in rat. These finding agreed with Loh *et al.* (2003, 2007b) who reported that feeding of fermented product contained LAB reduces ENT population in faeces of pigs, rats and layers. The metabolites able to inhibit the growth of various gram negative bacteria particularly pathogenic *E. coli* and this directly reduces the total Enterobacteriaceae population in the faeces (Loh *et al.*, 2009).

Higher LAB and low ENT counts in metabolites and acidifier groups are closely related to the results of low digesta and faecal pH. These results indicate that metabolite and combination of metabolite plus acidifier group enhance acidic environment in the digestive tract as shown in the low digesta pH. The results were in line with the findings of Shah (2001) as they reported that lowering of pH due to lactic or acetic acid produced by probiotic bacteria in the gut had bactericidal or

bacteriostatic effect. Gheisari *et al.* (2007) also indicates that dietary inclusion of the organic acid reduced pH and produced a suitable environment for lactic acid bacteria proliferation. In contrast, Izat *et al.* (1989) reported that there was no difference in pH intestinal material when commercial broiler fed with various levels of propionic acid indicating that microbial reduction were not due to a decrease in pH. Moran (2001) also showed that there was no significant effect of fermented liquid feed on the pH of the pig lower gastrointestinal tract in contrast. Foo *et al.* (2003) also showed that the rats fed with *L. plantarum* I-UL4 in their drinking water has a lower faecal pH than those of control rats suggesting pH of faeces could be modified by the inclusion of *L. plantarum* metabolites in the drinking water.

The addition of either *L. plantarum* or acidifier to the diet did not influence the immunoglobulin status of the chicken. Similarly, Tejada-Simon *et al.* (1999) reported that oral administration of probiotic to mice did not significantly affect serum immunoglobulin concentrations. In contrast, Cetin *et al.* (2005) reported that the probiotic used in their diet which containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterotococcus faecium*, *Bifidobacterium thermophilus* were able to enhance the immunoglobulin levels and further contributed to the positive effect of growth performance and ability to resist disease in Turkey. Havenaar and Spanhaak (1994) reported that probiotics was able to stimulate the immunity of the chickens in two ways. First, flora from probiotic migrates throughout the gut wall and multiply to a limited extend or second antigen release by the dead organisms are absorbed and thus stimulate the immune system. Panda *et al.* (2000) and Cross (2002) indicated that some probiotics could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogen.

Acetic acid, propionic acid and butyric acid are the main VFA. Current study showed acidifier and metabolites able to give positive effect to the VFA levels. Differences of total VFA levels between acidifier, metabolites and combination of metabolites+acidifier with positive control were about 8.98, 34.19 and 39.43%, respectively. The differences show that VFA in treated groups were highly significant than antibiotic group. These results indicate increase of VFA in metabolites and acidifier groups compared to control due to the greater population of LAB in the treatments (Loh *et al.*, 2009).

The LAB ferments various substrates like lactose, biogenic amines and allergic compounds into short-chain fatty acids and other organics acids and gases (Gibson and Fuller, 2000). The higher acetic acid concentration mainly related to the major end product of the fermentation by the heterofermentative bacteria in the intestine (Van Immerseel *et al.*, 2003).

## CONCLUSION

This study shows higher final body weight, lower daily feed intake and feed conversion ratio in the treatments of metabolites, acidifier and even the combination of 0.5% metabolites and 0.1% acidifier. These results could be explained by better health of gastro intestinal tract due to greater LAB and lower ENT count, lower digesta, faecal pH and higher total VFA. These results indicate that metabolites and acidifier can be used to replace antibiotic as growth promoter without causing unfavourable growth effect.

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