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Body weight, haematological indices and intestinal morphometric of broilers provided with diets containing formic acid, butyric acid or their blends

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ABSTRAK

Penelitian ini bertujuan mengkaji pengaruh pemberian asam format, asam butirat atau kombinasi keduanya terhadap pertumbuhan, profil darah dan morfometrik usus ayam broiler. Sebanyak 240 ayam broiler (Lohmann MB-202) dikelompokkan ke empat perlakuan, yakni CONT (ayam diberi pakan basal tanpa aditif), BTRT (ayam diberi pakan basal dengan asam butirat 0,03%), FRMT (ayam diberi pakan basal dengan 0,1% asam format) dan BTRT + FRMT (ayam diberi pakan basal yang mengandung 0,03% asam butirat dan asam format 0,1%). Bobot badan dan konsumsi pakan dicatat setiap minggu, sementara darah dikoleksi pada hari ke-21 dan 35. Pada hari ke-35, ayam disembelih dan segmen usus halus dan digesta dikoleksi. Pada hari ke-21, bobot badan ayam BTRT+FRMT lebih tinggi (P<0,05) daripada kelompok lain. Kombinasi asam format dan butirat menghasilkan nilai MCHC (P<0,05) yang lebih tinggi dalam darah ayam pada hari ke-21. Asam organik menghasilkan konsentrasi trombosit yang lebih rendah (P<0,05) pada hari ke 35. Suplementasi asam butirat meningkatkan (P<0,05) konsentrasi albumin, trigliserida dan LDL pada hari ke-35. Kombinasi asam format dan butirat menghasilkan titer antibodi yang lebih tinggi (P<0,05) terhadap vaksin Newcastle disease pada hari ke-21. Asam format menurunkan (P<0,05) jumlah bakteri laktosa negatif enterobacteria dalam ileum ayam pada hari ke-35. Sebagai kesimpulan, kombinasi asam format dan butirat memperbaiki performa pertumbuhan dan kekebalan ayam broiler.

Kata kunci: asam organik, ayam broiler, kesehatan, pertumbuhan

ABSTRACT

The study investigated the influence of dietary incorporation of formic acid, butyric acid or their blends on growth rate, haematological indices and intestinal morphometric of broilers. A number of 240 Lohmann MB-202 chicks were pass rounded to four dietary groups, including CONT (chicks taking in basal feed with no additive), BTRT (chicks receiving basal feed with 0.03% butyric acid), FRMT (chicks receiving basal feed with 0.1% formic acid) and BTRT+FRMT (chicks receiving basal feed containing 0.03% butyric acid and 0.1% formic acid). Weight of chicks and intake were measured every week, while blood sample was collected at day 21 and 35. At day 35, birds were slaughtered and small intestinal segments and digesta were collected. At day 21, body weight was bigger (P<0.05) in BTRT+FRMT than in other birds. Feeding the blends of formic and butyric acids resulted in higher (P<0.05) value of mean corpuscular haemoglobin concentration (MCHC) at day 21. Feeding organic acids was associated with the lower (P<0.05) proportion of thrombocytes in broilers at day 35. Supplementation of butyric acid resulted in higher (P<0.05) serum albumin concentration at day 35. Butyric acid produced higher (P<0.05) levels of triglyceride as well as low-density lipoprotein (LDL) in the serum. The mixture of formic and butyric acids resulted in higher (P<0.05) titer of antibody against Newcastle disease vaccine (NDV) at day 21. Feeding formic acid decreased (P<0.05) ileal population of lactose negative enterobacteria at day 35. Overall, dietary supplementation of the blends of formic and butyric acids resulted in improved growth and immune defence of broiler chicks.

Keywords: broiler, health, organic acid, performance

INTRODUCTION

After being used for more than five decades, antibiotics growth promoters (AGP) have eventually been banned in Indonesia start from January 1, 2018. Regardless of consumer health issues (the phenomenon of resistant antibiotics), the ban on AGP has had an impact on decreasing productivity and increasing mortality of broiler chickens (Sugiharto, 2016). Based on these conditions, alternative replacements for AGP are needed for the continuity of the broiler livestock industry in Indonesia and global.

Organic acids are among the alternative substitutes for AGP, which has been widely applied to maintain health and maximize the growth performance of broiler chickens (Khan and Iqbal, 2016). Formic acid is a type of organic acid with the highest acidity. Formic acid has been widely used as an antibacterial agent so that it can keep chickens away from infections (Pathak *et al.*, 2016). This type of acid is also able to improve digestibility and growth of broiler chickens (Hernández *et al.*, 2006; Pathak *et al.* 2016). Yet, the use of formic acid at certain levels can have a negative impact on chickens, because formic acid has a very strong odor that can affect the palatability of feed. Formic acid can also be deleterious for chickens as it can damage the intestinal mucosa (Ragaa and Korany, 2016). Another type of organic acid that may be potential for replacing the role of AGP in broilers is butyric acid. Butyric acid is reported to prevent broiler chickens from the invasion by pathogenic bacteria (Panda *et al.*, 2009). Butyric acid is also able to improve digestibility and absorption of nutrients so that it has a beneficial impact on the growth rate of broiler chicks (Kaczmarek *et al.*, 2016). Unlike other organic acids, butyric acid is a substrate or energy source that is very important for enterocyte or intestinal epithelial cells (Deepa *et al.*, 2018). In this regard, administration of butyric acid may improve the intestinal development and functions in terms of digestive and absorptive capacity of birds (Salmanzadeh, 2013; Deepa *et al.*, 2018).

Apart from the various benefits of formic and butyric acid in broilers, the effectiveness of using these additives as the substitutes for AGP is still a debate. Several factors are very likely to influence the efficacy of organic acids in broiler chickens, including levels, types and nature or sources of organic acids used, differences in chemical composition of feed (especially buffering capacity of feed), palatability of feed (if organic acids are mixed in feed) and conditions of broiler house especially the hygiene of broiler house (Kim *et al.*, 2015). To maximize the effects of acids, several types of organic acids are often applied together (a combination/blend of organic acids). Dibner and Buttin (2002) reported that the use of a combination of several organic acids can produce a synergistic effect so that it can provide more benefits for broilers. Referring to the role of formic acid as an antibacterial agent and butyric acid as an energy source for enterocyte, the use of blends of formic and butyric acids is expected to maximize the role of these acids as the alternative to AGP for modern broiler chicks. The present study investigated the influence of dietary incorporation of formic acid, butyric acid or their blends on growth rate, haematological indices and intestinal morphometric of broilers.

MATERIALS AND METHODS

A number of 240 chicks (Lohmann MB-202; body weight of 36.7 ± 1.56 g) were employed in the present study. The birds were allotted to four treatments, each consisting of 60 birds (six replicates with 10 birds in each). These experimental groups were CONT (chicks taking in basal feed with no additive), BTRT (chicks taking in basal feed containing 0.03% butyric acid), FRMT (chicks receiving basal feed with 0.1% formic acid) and BTRT+FRMT (chicks receiving basal feed containing 0.03% butyric acid and 0.1% formic acid). The organic acids were incorporated "on top" to basal feed. The doses of formic acid (Baymix Latibon®Plus ME, Dr. Eckel GmbH, Niederzissen, Germany) and butyric acid (Butipearl, Kemin Cavriago, Italy) were applied based on the manufacturer's recommendations. The feeds (in mash form) were prepared (Tables 1 and 2) to comply the Indonesian National Standards for Broiler Feed (SNI, 2006). The basal diet was free from antibiotics, enzymes, coccidiostat and anti-fungal agents. Throughout the trial, the diets and water were given *ad libitum* to all birds.

At day 0, all chicks were vaccinated with commercial avian influenza vaccine (AIV) and Newcastle disease vaccine (NDV) by means of aerosol spraying. The live weight of birds, feed intake and feed conversion ratio (FCR, ratio between feed consumed and gain of birds) were recorded on weekly basis throughout the experimental period. At day 21 and 35, six chicks per treatment group (one chick from each pen/replicate) was blood sampled (from wing veins). The blood was partly put in ethylenediamine tetraacetic acid (EDTA)-tubes and the other part was placed in anticoagulant free-tubes. Blood from the EDTA-tubes was analyzed for the full blood indices, while the blood without anticoagulant was processed into serum. In the latter case, the blood was left for 2 h (at room temperature) to clot. Centrifugation was conducted at 3,000 rpm for 15 min until the serum was produced.

At the ultimate of experiment (day 35), one chick from each pen was randomly obtained and blood sampled, and the blood was treated similar to that of previously did at day 21. The chicks were then killed (slaughtered by neck-cut), defeathered and eviscerated. Immediately, the visceral organs were collected, emptied and weighed. To measure the height of intestinal villi, about 2 cm segments of duodenum, jejunum and ileum were carefully collected and placed in 10% neutral formalin buffer solution (Leica Biosystems Richmond, Inc., Richmond, USA). Concomitantly, the contents of ileum and caecum were obtained and placed in the sample tubes for the bacterial determination in the intestine.

Table 1. Ingredients and Chemical Compositions of Starter Diet (Day 0–21)

Items (%, unless otherwise noted)	Composition
Yellow corn	45.5
SBM	17.0
Wheat flour	10.0
Bread flour	5.00
Rice bran	4.45
CPO	3.50
CGM	3.60
DDGS	3.00
MBM	2.80
Chicken feather meal	2.00
Bone meal	1.50
Lysine	0.55
Methionine	0.37
L-threonine	0.08
Salt	0.15
Premix[1]	0.50
Nutrient contents:	
ME (kcal/kg)[2]	3,000
CP	22.0
Crude fat	5.00
Crude fibre	5.00
Ash	7.00

[1]Premix contained (per kg of diet) of Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, vitamin A 375 IU, vitamin D 150 IU, vitamin E 0.080 mg

[2]Metabolizable energy was calculated according to formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

SBM: soybean meal, CPO: crude palm oil, CGM: corn gluten meal, DDGS: distiller dried grains with solubles, MBM: meat bone meal, ME: metabolizable energy, CP: crude protein

Table 2. Ingredients and Chemical Composition of Finisher Diet (Day 22–35)

Items (%, unless otherwise noted)	Composition
Yellow corn	64.0
SBM	20.0
PMM	10.9
Rice	2.30
Coconut oil	1.50
Methionine	0.30
Lysine	0.20
Dicalcium phosphate	0.30
Premix[1]	0.50
Nutrient contents:	
ME (kcal/kg)[2]	3,064
CP	20.0
Crude fat	5.17
Crude fibre	5.13
Ash	7.00

[1]Premix contained (per kg of diet) of Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, vitamin A 375 IU, vitamin D 150 IU, vitamin E 0.080 mg

[2]Metabolizable energy was calculated according to formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

SBM: soybean meal, PMM: poultry meat meal, ME: metabolizable energy, CP: crude protein

The number of blood cells were measured by means of a hematology analyzer (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). The titers of antibody against NDV were determined in serum according to hemagglutination inhibition test. The total serum triglyceride was assessed on the basis of an enzymatic colorimetric method using glycerol-3-phosphate oxidase. The latter method with cholesterol oxidase/p-aminophenazone was employed to determine the levels of total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The concentrations of total protein in serum was assessed by photometric test according to the biuret method. The serum level of albumin was determined by photometric test with bromocresol green. The globulin concentration were recorded from the difference between serum total protein and albumin concentration. The serum concentrations of uric acid and creatinine were measured based on the enzymatic colour test. The serum analyses were carried out with kits from DiaSys Diagnostic Systems GmbH, Holzheim, Germany.

For the histological analyses, the slices of duodenum, jejunum or ileum (5 µm each) were stained using hematoxylin as well as eosin. The villi height of each intestinal piece was determined by means of an optical microscope connected to a digital camera (Leica Microsystems GmbH, Wetzlar, Germany). The number of bacteria in the ileum and caecum were assessed according to Sugiharto *et al.* (2017). Total coliform bacteria and lactose-negative enterobacteria were enumerated as red and colourless colonies, respectively, on MacConkey agar (Merck KGaA, Darmstadt, Germany) after overnight aerobic incubation at 38°C. The population of lactic acid bacteria (LAB) was counted on de Man, Rogosa and Sharpe agar (MRS; Merck KGaA) following anaerobic incubation at 38°C for 2 days.

The trial was designed on the basis of a completely randomized design. The data obtained from the present trial were analysed according to analysis of variance (Steel and Torrie, 1997). Duncan's multiple-range test was carried out if substantial differences ($P<0.05$) were observed across the treatment groups. The data are listed as means and standard deviation.

RESULTS AND DISCUSSION

Results in this present study showed that at day 21 BW of broiler was bigger ($P<0.05$) in BTRT+FRMT than in other birds. The higher BW was also observed in BTRT+FRMT group at day 35, although the value did not reach the substantial level ($P>0.05$; Table 3). This finding was in agreement with Dibner and Buttin (2002) who previously exhibited that combination of organic acids produced better growth performance in broiler chicks when compared with the single organic acid. Recent report by Polycarpo *et al.* (2017) also revealed that the combination of organic acids generated better performance in broilers compared to the use of single organic acid. In the latter case, the synergistic effect of blends of organic acids may occur, and thereby elicit more benefits for broiler performance (Sugiharto, 2016; Polycarpo *et al.*, 2017). It was apparent in this study that dietary supplementation of organic acids did not affect the internal organ weight of broilers at day 35 (Table 4). This result was in line with Cengiz *et al.* (2012) documenting

Table 3. Growth Performance of Broiler Chicks

Days	CONT	FRMT	BTRT	BTRT+FRMT
Day 1 to 21				
BW (g)	478 ± 15.4[b]	493 ± 13.8[b]	495 ± 12.6[b]	503 ± 16.1[a]
Accumulative FI (g)	967 ± 11.7[ab]	1040 ± 7.50[ab]	953 ± 10.0[b]	1068 ± 4.01[a]
FCR	2.21 ± 0.38	2.32 ± 0.15	2.08 ± 0.30	2.43 ± 0.16
Day 1 to 35				
BW (g)	1,216 ± 120	1,265 ± 170	1,256 ± 143	1,313 ± 75.9
Accumulative FI (g)	2,115 ± 352	2,180 ± 363	2211 ± 368	2244 ± 374
FCR	2.44 ± 0.07	2.44 ± 0.08	2.41 ± 0.14	2.41 ± 0.04

[a,b]Means in the same row with different letters show significant differences (P<0.05)
CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid, BW: body weight, FCR: feed conversion ratio, FI: feed intake

Table 4. Relative Weight of Internal Organs of Broiler Chicks at Day 35

Items (% live BW)	CONT	FRMT	BTRT	BTRT+FRMT
Heart	0.37 ± 0.03	0.36 ± 0.06	0.31 ± 0.46	3.33 ± 0.04
Liver	2.36 ± 0.24	2.25 ± 0.27	2.45 ± 0.21	2.75 ± 0.98
Pancreas	0.34 ± 0.03	0.36 ± 0.06	0.31 ± 0.05	0.33 ± 0.04
Proventriculus	0.48 ± 0.07	0.50 ± 0.08	0.48 ± 0.04	0.49 ± 0.07
Gizzard	1.87 ± 0.12	1.82 ± 0.40	1.87 ± 0.13	1.89 ± 0.20
Duodenum	0.63 ± 0.07	0.61 ± 0.10	0.54 ± 0.12	0.52 ± 0.07
Jejunum	1.23 ± 0.15	1.16 ± 0.20	1.07 ± 0.18	1.09 ± 0.82
Ileum	1.03 ± 0.26	1.01 ± 0.23	0.92 ± 0.10	0.86 ± 0,12
Caeca	0.58 ± 0.08	0.57 ± 0.08	0.48 ± 0.12	0.56 ± 0.13
Bursa of Fabricius	0.16 ± 0.06	0.15 ± 0.09	0.10 ± 0.04	0.12 ± 0.07
Spleen	0.13 ± 0.03	0.13 ± 0.04	0.16 ± 0.04	0.15 ± 0.07
Thymus	0.30 ± 0.06	0.35 ± 0.05	0.31 ± 0.07	0.36 ± 0.06

CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid, BW: body weight no difference in the internal organ relative weight in broilers supplemented with organic acid blends. Also, Wang *et al.* (2010) observed no substantial influence of feeding organic acids on the internal organs weight of chickens.

The data on the blood indices of broilers are highlighted in Table 5. It was shown in this present trial that feeding the blends of formic and butyric acids resulted in higher (P<0.05) value of MCHC in the blood of broilers at day 21. This finding differed from Abudabos *et al.* (2015) showing no notable effect of feeding organic acids on the MCHC of birds. Considering that MCHC reflects the haemoglobin content of erythrocytes, the increased MCHC value may imply in the elevated haemoglobin production to increase the oxygen transport capacity of erythrocytes. This latter inference was supported by Ulupi *et al.* (2018) revealing that during the hypoxic state broiler chicks tend to produce large quantities of

Table 5. Blood Profiles of Broiler Chicks at Days 21 and 35

Days	CONT	FRMT	BTRT	BTRT+FRMT
Day 21				
Erythrocytes (10^6/µL)	2.27 ± 0.31	2.21 ± 0.21	2.07 ± 0.27	2.16 ± 0.30
Haemoglobin (g/dL)	9.75 ± 1.37	9.42 ± 0.86	9.25 ± 0.88	9.25 ± 1.25
Haematocrit (%)	22.1 ± 7.93	25.2 ± 2.16	23.4 ± 0.54	24.7 ± 3.25
MCV (fL)	117 ± 5.16	116 ± 5.86	114 ± 3.63	115 ± 2.80
MCH (pg)	43.0 ± 1.70	42.7 ± 2.62	44.9 ± 3.19	41.2 ± 4.61
MCHC (%)	37.0 ± 1.31^b	37.4 ± 1.96^b	39.8 ± 3.39^b	49.2 ± 7.37^a
Leukocytes (10^3/µL)	49.5 ± 9.14	59.2 ± 10.5	59.3 ± 7.37	54.8 ± 9.55
Heterophils (10^3/µL)	3.83 ± 1.63	4.58 ± 1.69	4.75 ± 2.07	4.75 ± 1.33
Lymphocytes (10^3/ µL)	43.2 ± 6.98	51.4 ± 10.2	50.9 ± 8.03	47.3 ± 9.40
Eosinophils (10^3/µL)	8.17 ± 2.40	8.50 ± 2.07	9.17 ± 2.04	9.67 ± 1.97
Thrombocytes (10^3/µL)	2.50 ± 0.95	3.17 ± 0.52	3.00 ± 0.77	2.83 ± 0.41
Day 35				
Erythrocytes (10^6/µL)	2.12 ± 0.53	2.13 ± 0.32	2.49 ± 0.29	2.42 ± 0.24
Haemoglobin (g/dL)	8.92 ± 2.44	8.92 ± 1.56	10.3 ± 1.13	9.75 ± 1.04
Haematocrit (%)	21.7 ± 5.31	22.0 ± 3.41	25.6 ± 3.22	24.3 ± 2.66
MCV (fL)	104 ± 2.99	105 ± 3.85	104 ± 2.90	102 ± 1.58
MCH (pg)	41.8 ± 2.68	41.9 ± 2.58	41.5 ± 1.30	40.6 ± 2.05
MCHC (%)	40.8 ± 2.61	40.4 ± 1.47	40.5 ± 1.68	40.1 ± 2.09
Leukocytes (10^3/µL)	60.9 ± 9.59	65.3 ± 11.7	64.9 ± 11.5	60.3 ± 9.36
Heterophils (10^3/µL)	10.6 ± 2.31	10.7 ± 4.82	8.92 ± 3.85	7.75 ± 3.14
Lymphocytes (10^3/ µL)	45.6 ± 9.37	49.1 ± 11.0	51.5 ± 7.73	48.0 ± 9.72
Eosinophils (10^3/µL)	4.75 ± 1.04	4.92 ± 1.66	4.50 ± 1.52	4.08 ± 0.10
Thrombocytes (10^3/µL)	16.70 ± 7.31^a	9.83 ± 1.72^b	9.67 ± 1.86^b	7.67 ± 1.21^b

^{a,b}Means in the same row with different letters show significant differences (P<0.05)

CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration erythrocytes in the form of reticulocytes. This, consequently, increases the production of haemoglobin and thereby increases the MCHC values. In the present study, the change in MCHC value was not accompanied by the changes in the numbers of erythrocytes and haemoglobin, which was similarly seen in the study of Ulupi *et al.* (2018). The higher MCHC value in BTRT+FRMT chicks may also be related to the higher metabolic activity resulting in higher BW in these respective chicks at day 21. It was interesting to mention in this study that treatment with organic acids was attributed to the lower (P<0.05) level of thrombocytes in broilers at day 35. Given that higher level of thrombocytes may indicate the inflammatory condition (due to infection) in chicks (Ferdous *et al.*, 2008), the lower value of thrombocytes in the organic acids fed broilers may therefore suggest the lower threat of infections in the respective chicks.

Data in the current study showed that feeding butyric acid resulted in higher (P<0.05) level of serum albumin of broilers at day 35 (Table 6). Concomitant to our result, Deepa *et al.* (2018) noticed that feeding butyric acid glycerides at the level of 0.4% increased the concentration of serum albumin in broiler chicks. It was most likely that feeding butyric acid improved protein

Table 6. Serum Biochemical Parameters of Broiler Chicks at Days 21 and 35

Days	CONT	FRMT	BTRT	BTRT+FRMT
Day 21				
Albumin (g/dL)	1.08 ± 0.11	1.15 ± 0.16	1.15 ± 0.05	1.20 ± 0.12
Total protein (g/dL)	2.27 ± 0.34	2.50 ± 10.4	2.47 ± 0.13	2.62 ± 0.23
Globulin (g/dL)	1.19 ± 0.23	1.35 ± 0.22	1.32 ± 0.10	1.41 ± 0.12
Uric acid (mg/dL)	6.01 ± 1.27	4.88 ± 1.83	5.04 ± 1.49	5.03 ± 1.37
Creatinine (mg/dL)	0.05 ± 0.01	0.05 ± 0.03	0.04 ± 0.01	0.11 ± 0.09
Triglyceride (g/dL)	73.7 ± 14.6	65.0 ± 10.8	66.2 ± 12.5	70.8 ± 7.69
Total cholesterol (g/dL)	112 ± 15.5	138 ± 23.9	130 ± 8.24	132 ± 16.8
HDL (g/dL)	71.8 ± 9.02	80.0 ± 7.07	78.3 ± 4.59	75.2 ± 9.54
LDL (g/dL)	26.2 ± 13.8	44.7 ± 21.9	38.7 ± 11.9	42.8 ± 13.4
Day 35				
Albumin (g/dL)	0.98 ± 0.10^{b}	1.07 ± 0.43^{b}	1.30 ± 0.16^{a}	0.87 ± 0.19^{b}
Total protein (g/dL)	2.61 ± 0.47	2.82 ± 1.12	3.37 ± 0.65	2.27 ± 0.50
Globulin (g/dL)	1.62 ± 0.46	1.57 ± 0.47	2.02 ± 0.53	1.40 ± 0.34
Uric acid (mg/dL)	5.23 ± 0.69	6.18 ± 2.78	5.13 ± 1.35	4.73 ± 1.83
Creatinine (mg/dL)	0.23 ± 0.38	0.65 ± 0.70	0.07 ± 0.03	0.09 ± 0.04
Triglyceride (g/dL)	62.6 ± 19.2^{b}	62.3 ± 12.9^{b}	76.2 ± 16.9^{a}	47.2 ± 9.71^{b}
Total cholesterol (g/dL)	91.2 ± 8.13	104 ± 36.8	121 ± 30.3	83.7 ± 13.44
HDL (g/dL)	35.3 ± 5.85	35.6 ± 10.1	47.5 ± 11.2	36.0 ± 6.12
LDL (g/dL)	43.4 ± 5.72^{b}	57.8 ± 18.9^{b}	65.7 ± 20.5^{a}	41.0 ± 11.6^{b}

^{a,b}Means in the same row with different letters show significant differences (P<0.05)
CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid, HDL: high-density lipoprotein, LDL: low-density lipoprotein

digestibility (Imran *et al.*, 2018) as well as intestinal villi morphology leading to improved protein absorption in broilers (Sugiharto, 2016). Other possibility could be that butyric acid improved liver function resulting in higher production of albumin in the liver (Leeson *et al.*, 2005). Similar to serum albumin, dietary treatment of butyric acid was associated with the higher (P<0.05) levels of triglyceride and LDL in the serum of broilers. These findings differed from Mansoub (2011) showing the lowering impact of butyric acid on the serum concentrations of triglyceride, cholesterol and LDL of broilers. Also, Pouraziz *et al.* (2013) reporting no effect of butyric acid glycerides on the serum levels of triglyceride, LDL and HDL of broilers. The reason for the increased levels of serum triglyceride and LDL in this study remained unclear, but the improved absorptive capacity of the intestinal villi for triglyceride due to butyric acid treatment may be the reason. It was shown in this current study that feeding the blends of formic and butyric acids resulted in higher (P<0.05) antibody titer against NDV in broiler chickens at days 21 (Table 7), especially when compared with that of fed butyric acid alone. This finding further supported Dibner and Buttin (2002) and Sugiharto (2016) who confirmed that the blends of organic acids elicit better health effect in broilers compared to the use of single organic acid.

Data regarding the height of intestinal villi of broiler chicks are shown in Table 8. Compared to other birds, the birds receiving diet containing butyric acid had higher (P<0.05) intestinal villi height. In accordance with our result, Sikandar *et al.* (2017) also showed the higher intestinal villi height in broiler chicks at day 21 and 35 with feeding butyric acid. This condition could be understood as butyric acid is the preferable nutrient for enterocytes (as a source of energy) for promoting the development and functions of broiler intestine (Wu *et al.*, 2018). With regard to the selected intestinal bacterial populations, data in our present study showed that feeding diet containing formic acid decreased (P<0.05) the

Table 7. Antibody Titer against NDV at Days 21 and 35

Days (Log_2 GMT)	CONT	FRMT	BTRT	BTRT+FRMT
Day 21	2.33 ± 0.82[ab]	3.00 ± 0.00[ab]	2.50 ± 0.84[b]	3.83 ± 2.50[a]
Day 35	2.50 ± 1.38	1.66 ± 1.96	2.83 ± 1.97	2.50 ± 1.76

[a,b]Means in the same row with different letters show significant differences (P<0.05)
CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid, NDV: Newcastle disease vaccine, GMT: geometric mean titer

Table 8. Intestinal Villi Height of Broiler Chicks at Day 35

Segments (μm)	CONT	FRMT	BTRT	BTRT+FRMT
Duodenum	944 ± 273[c]	1199 ± 123[b]	1355 ± 191[a]	964 ± 103[c]
Jejunum	1073 ± 196	1088 ± 318	1169 ± 340	1268 ± 355
Ileum	653 ± 192	641 ± 111	657 ± 150	611 ± 203

[a,b,c]Means in the same row with different letters show significant differences (P<0.05)
CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid

Table 9. Selected Intestinal Bacterial Populations of Broiler Chicks at Day 35

Intestinal Segments	CONT	FRMT	BTRT	BTRT+FRMT
Ileum (log cfu/g)				
Coliform	6.82 ± 1.47	7.24 ± 1.24	7.23 ± 1.16	7.68 ± 0.13
Lactose negative enterobacteria	8.41 ± 0.38[a]	7.43 ± 1.32[b]	8.45 ± 0.59[a]	8.59 ± 0.32[a]
LAB	10.30 ± 0.79	9.64 ± 2.33	10.11 ± 0.53	10.62 ± 0.56
Caecum (log cfu/g)				
Coliform	8.42 ± 1.08	8.22 ± 0.56	8.65 ± 0.89	8.39 ± 0.33
Lactose negative enterobacteria	7.76 ± 0.70	8.53 ± 0.78	8.40 ± 0.60	8.48 ± 0.57
LAB	10.52 ± 1.35	11.31 ± 0.32	11.33 ± 0.23	11.44 ± 0.39

[a,b]Means in the same row with different letters show significant differences (P<0.05)
CONT: chicks receiving basal feed without additive, BTRT: chicks receiving basal feed with 0.1% butyric acid, FRMT: chicks receiving basal feed with 0.1% formic acid, BTRT+FRMT: chicks receiving basal feed containing 0.1% butyric acid and 0.1% formic acid, cfu: colony forming unit

number of lactose negative enterobacteria in the ileal content of broilers at day 35 (Table 9). Similar to our observation, Al-Natour and Alshawabkeh (2005) noted the effectiveness of formic acid in reducing the population of *Salmonella gallinarum* (example of lactose negative enterobacteria) in the intestine of broilers. Accordingly, Pathak *et al.* (2016) has used formic acid as an antibacterial agent to protect the chickens from bacterial infections. The nature of acid molecule in formic acid and the capacity of such acid in lowering the pH of the intestine may be responsible for the decreased ileal population of lactose negative enterobacteria in the present study (Al-Natour and Alshawabkeh, 2005).

CONCLUSION

Dietary supplementation of the blends of formic and butyric acids improved body weight and immune defence of broilers.

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