Feed efficiency, serum indices and selected intestinal bacteria of the Indonesian indigenous crossbred chickens provided with the blends of butyric acid and *Bacillus subtilis*

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Abstract. Feed efficiency, serum indices and selected intestinal bacterial population were evaluated in the Indonesian indigenous crossbred chickens (IICC) following treatments with the blends of butyric acid and *Bacillus subtilis* in the rations. A total of 200 of the IICC were randomly distributed to four dietary treatments including CNTRL (basal diet without additive), BTRT (basal diet added with 0.1% butyric acid), BACIL (basal diet added with 0.02% *B. subtilis*) and BTRBAC (basal diet added with the blends of 0.1% butyric acid and 0.02% *B. subtilis*). Weight gain, feed intake and feed efficiency of the IICC were recorded weekly. Blood was collected on week 8, and after which the chicks were slaughtered. Immediately, the digesta was obtained from the ileum and caecum of the IICC. Our findings showed that treatments improved ($P<0.05$) feed efficiency, feed cost per gain and income over feed cost of the IICC. However, the dietary treatments had no impact ($P>0.05$) the antioxidative status (serum malondialdehyde and superoxide dismutase), antibody titer toward Newcastle disease vaccine, serum biochemical indices (cholesterol profile, total protein, albumin, globulin, uric acid) and the numbers of lactic acid bacteria and *Enterobacteriaceae* in the ileum and cecum of the IICC. In conclusion, dietary supplementation of butyric acid, *B. subtilis* or the combination of both improved the economic performance of the IICC.

1. Introduction

In-feed antibiotics have traditionally been used to improve the health and growth rate of the Indonesian indigenous crossbred chicken (IICC), which is the crossbreed of the Indonesian indigenous cock and modern laying hen (Isa Brown) [1]. Irrespective of its advantageous, the administration of in-feed antibiotics in poultry has been prohibited throughout Indonesia since January 2018 due to food safety issues. Indeed, the prohibition of antibiotics in diets has been attributed to the impaired feed efficiency and thus economic performances of poultry [2]. In response to the latter problems, nutritionists are now searching for the alternative to in-feed antibiotics particularly for the Indonesian indigenous crossbred chickens. In modern broiler strains, butyric acid has recently been used as feed additive to substitute the role of in-feed antibiotics. Besides reducing the pathogenic bacteria load in the intestine and improving the physiological condition [3], the acid may also support enterocytes development and thereby improving feed utilization by the chicks [4]. The report on the use of butyric acid in the IICC has, however, never been published to date. Probiotic *Bacillus subtilis* is other
example of in-feed antibiotic alternatives that has widely been used in poultry production. Such microbe has been revealed to improve the health and nutrient digestibility of modern broiler strains [5,6] as well as the IICC [1].

Recent study showed that combination of organic acids and other active compounds such as probiotics may improve the efficacy of the additives in improving the health and productivities of modern broiler chickens. Rodjan et al. [7] reported that the combination of organic acids and probiotics resulted in improved intestinal ecology and health as compared to the application of organic acids or probiotics alone. However, different findings were documented by Agboola et al. [8] and Barbieri et al. [9] as they did not find any different effect between the blends of organic acids and probiotics on the production parameters of broilers when compared with the single administration of either organic acids or probiotics. The nature of organic acids and probiotics used and the tolerance of probiotics to acid condition may be responsible for these discrepancies. In our present work, the blends of butyric acid and B. subtilis was used as additive in the IICC diets. With endospores-forming capability, B. subtilis was expected to withstand to extreme condition such as acid condition due to organic acids administration [10]. Overall, feed efficiency, serum indices and selected intestinal bacterial population were evaluated in the IICC following treatments with the blends of butyric acid and B. subtilis in the rations.

2. Materials and Methods
A number of 200 of the IICC were randomly distributed to one of four dietary treatment groups including CNTRL (basal diet without additive), BTRT (basal diet added with 0.1% butyric acid), BACIL (basal diet added with 0.02% B. subtilis) and BTRBAC (basal diet added with the blends of 0.1% butyric acid and 0.02% B. subtilis). Butyric acid (Butipearl) was obtained from Kemin Cavriago, Italy and B. subtilis (Baymix Grobig) from PT. Bayer Indonesia, Jakarta, Indonesia. The additives were supplemented “on top” to the basal feeds. The feeds were formulated in mash form as starter (weeks 1-4) and finisher (weeks 5-8) feeds (Table 1). The feeds and drinking water were offered ad libitum to all IICC for 8 weeks of rearing. At day 4, vaccinations with commercial Newcastle disease vaccine (NDV) were performed through eye drop and subsequently at weeks 4 through drinking water. Weekly, the body weight gain and feed intake was noted, and hence feed efficiency, feed cost per gain and income over feed cost of the IICC were calculated. At week 8, two birds from each replicate (10 birds from each dietary group) were blood sampled via wing vein. The blood was placed in anticoagulant free-tubes and allowed to clot to produce serum. Immediately, one (of two) chick that were blood sampled (five chicks from each dietary group) were slaughtered and eviscerated. For the bacterial enumeration, digesta from the ileum and caecum were collected. The serum biochemistry, antibody titer toward NDV and intestinal populations of lactic acid bacteria (LAB) and Enterobacteriaceae were measured according to the standard methods as mentioned in Sugiharto et al. [1,6]. The serum levels of malondialdehyde (MDA) and serum superoxide dismutase (SOD) were measured spectrophotometrically using kits (Sigma-Aldrich, St. Louis, USA).

A completely randomized design was followed in this present work. The data obtained from this work were treated with analysis of variance (SAS Inst. Inc., Cary, NC, USA). Post-hoc analysis with Duncan’s multiple-range test was performed if the disparity (P<0.05) among the dietary groups were found.
Table 1. Ingredients and nutrient compositions of basal diets.

| Items (%) unless otherwise noted | Starter phase | Finisher phase |
|---------------------------------|--------------|---------------|
| Yellow corn                     | 54.8         | 58.5          |
| Soybean meal                    | 35.7         | 32.8          |
| Meat bone meal                  | 4.75         | 2.00          |
| Soybean oil                     | 1.50         | 3.50          |
| DL-methionine                   | 0.30         | 0.30          |
| L-lysine                        | 0.20         | 0.20          |
| Limestone                       | 0.50         | 0.50          |
| Dicalcium phosphate             | 1.50         | 1.50          |
| Mineral vitamin mix             | 0.50         | 0.50          |
| Salt                            | 0.25         | 0.25          |

Analyzed compositions:
- Crude protein: 21.0
- Crude fiber: 7.00

Calculated compositions:
- Metabolisable energy (kcal/kg): 2,900
- Ca: 1.00
- P (available): 0.60
- Lysine: 1.20
- Methionine: 0.60

1Mineral vitamin mix contained (per kg of diet) of vitamin A 7,750 IU, vitamin D3 1,550 IU, vitamin E 1.88 mg, vitamin B1 1.25 mg, vitamin B2 3.13 mg, vitamin B6 1.88 mg, vitamin B12 0.01 mg, vitamin C 25 mg, folic acid 1.50 mg, Ca-d-pantothenate 7.5 mg, niacin 1.88 mg, biotin 0.13 mg, BHT 25 mg, Co 0.20 mg, Cu 4.35 mg, Fe 54 mg, I 0.45 mg, Mn 130 mg, Zn 86.5 mg, Se 0.25 mg, L-lysine 80 mg, Choline chloride 500 mg, DL-methionine 900 mg, CaCO₃ 641.5 mg, Dicalcium phosphate 1,500 mg

2Metabolizable energy was calculated on the basis of formula as follow: 40.81 \{0.87 \{crude protein + 2.25 crude fat + nitrogen-free extract\} + 2.5\}

3. Results and Discussion

Data on the economic performances of the IICC treated with the blends of butyric acid and *B. subtilis* were presented in Table 2. It was shown that treatments with butyric acid, probiotic *B. subtilis* or the combination of both were capable of reducing \((P<0.05)\) the feed cost per gain and increasing \((P<0.05)\) the feed efficiency and income over feed cost (IOFC). These present findings were in accordance with that of formerly reported by Panda et al. [3], in which dietary supplementation with 0.2% butyric acid improved the feed efficiency of modern broiler strains. The improved intestinal morphology and functions seemed to be attributed to the nutrient digestibility and utilization resulted in improved productivity of the chicks [3,4,11]. In agreement with our current data, the administration of probiotic *B. subtilis* has been revealed to boost the growth rate and improve feed conversion of chickens [1,5,6]. Several studies have demonstrated the potential of probiotic *B. subtilis* in improving the immune functions, intestinal microbial populations and physiological indices of poultry [1,5,6]. These later properties may reasonably promoted the growth rate of poultry. In this study, there was no further improvement with the dietary administration of the blends of butyric acid and *B. subtilis* on the economic performances of the IICC, as compared to the single administration of butyric acid and *B. subtilis* alone. There was no exact reason for the latter condition, but it seemed that the maximum growth potential (genetic potential) of the IICC may hinder the growth-promoting effect of the blends of butyric and *B. subtilis*. In the former work, we found that 10 weeks-old of the Indonesian indigenous crossbred chickens had 830 to 881 g of live body weight [1].
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Data on the current work did not show (P>0.05) any influence of treatments on the antioxidative
status (serum MDA and SOD) and immune response of the IICC (Table 3). In general, the changes in
serum concentrations of SOD and MDA may reflect the stress conditions in poultry [12]. In the
previous study, Tirawattanawanich et al. [13] documented that compared to broiler strains, Thai
indigenous crossbreed chickens exhibited higher tolerance to environmental stress. In agreement to the
latter type of chickens, the high stress tolerance of the IICC seemed to eventuate in less stress
condition. Such circumstance may therefore explain the minimal alterations of SOD and MDA
following the treatments with butyric acid, B. subtilis or the mix of both. It was noticeable in this study
that the antibody titer toward NDV was greatly lower from the safe level (≥3 Log$_2$ GMT). In this
study, NDV vaccination was conducted at day 4 and week 4, while the antibody titer measurement
was performed at week 8. This long time difference between vaccination and the titer antibody
measurement may therefore be attributable to the low antibody titer toward NDV at week 8.

Table 2. Economic performances of the IICC treated with the blends of butyric acid and B. subtilis.

| Experimental groups | CNTRL | BTRT | BACIL | BTRBAC | SE  | P value |
|---------------------|-------|------|-------|--------|-----|---------|
| Feed efficiency (%) | 0.37$^b$ | 0.40$^a$ | 0.42$^a$ | 0.42$^a$ | 0.01 | <0.01   |
| Feed cost per gain ( IDR) | 21,415$^a$ | 19,308$^b$ | 18,641$^b$ | 18,819$^b$ | 376  | <0.01   |
| IOFC ( IDR) | 6,819$^c$ | 8,968$^b$ | 10,555$^a$ | 10,724$^a$ | 391  | <0.01   |

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

CNTRL: basal diet without additive, BTRT: basal diet added with 0.1% butyric acid, BACIL: basal diet added with 0.02% B. subtilis, BTRBAC: basal diet added with the blends of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, BW: body weight, IDR: Indonesian Rupiah (the national currency for the Republic of Indonesia), IOFC: income over feed cost.

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study, NDV vaccination was conducted at day 4 and week 4, while the antibody titer measurement
was performed at week 8. This long time difference between vaccination and the titer antibody
measurement may therefore be attributable to the low antibody titer toward NDV at week 8.

Table 3. Serum malondialdehyde, superoxide dismutase and antibody titer against NDV of the IICC
treated with the blends of butyric acid and B. subtilis.

| Items | Experimental groups | CNTRL | BTRT | BACIL | BTRBAC | SE  | P value |
|-------|---------------------|-------|------|-------|--------|-----|---------|
| MDA (nmol/mL) | 7.56 | 6.97 | 7.85 | 7.54 | 1.04 | 0.94 |
| SOD (U/mL) | 13.6 | 13.6 | 14.6 | 14.7 | 0.67 | 0.45 |
| Antibody titer against NDV (Log$_2$ GMT) | 0.20 | 0.00 | 0.10 | 0.10 | 0.10 | 0.35 |

CNTRL: basal diet without additive, BTRT: basal diet added with 0.1% butyric acid, BACIL: basal diet added with 0.02% B. subtilis, BTRBAC: basal diet added with the blends of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, MDA: malondialdehyde, SOD: serum superoxide dismutase, NDV: Newcastle disease virus, GMT: geometric mean titer.

Data on the serum biochemical parameters of the IICC are listed in Table 4. There was no
substantial effect ( P>0.05) of the dietary treatments on the parameters measured in the present study.
In agreement to our findings, Deepa et al. [4] did not find any significant effect of sodium butyrate on
total protein, albumin, globulin, albumin to globulin ratio, HDL and triglycerides in the serum of
broiler chickens. However, they noticed the significant differences on serum total cholesterol and LDL
between the sodium butyrate-treated broilers and control. With regard to the effect of B. subtilis, our
former study documented that administration of commercial probiotic B. subtilis had no effect on the
total cholesterol, HDL, LDL, total triglycerides, total protein, albumin, globulin and albumin to
globulin ratio in the serum of the IICC [1]. There has no exact explanation for these inconsistent data
above, but the differences in species/strains of the chickens, natures and doses of the additives,
nutritional compositions of the diets as well as the environmental conditions during the study may
account for the different physiological responses of the chicks.

Table 4. Serum biochemical parameters of the IICC treated with the blends of butyric acid and B. subtilis.

| Items | Experimental groups | CNTRL | BTRT | BACIL | BTRBAC | SE  | P value |
|-------|---------------------|-------|------|-------|--------|-----|---------|
| Total cholesterol (mg/dL) | 179.2 | 175.6 | 175.6 | 175.6 | 2.1 | 0.89 |
| HDL (mg/dL) | 45.6 | 45.6 | 45.6 | 45.6 | 2.1 | 0.89 |
| LDL (mg/dL) | 91.2 | 91.2 | 91.2 | 91.2 | 2.1 | 0.89 |
| Total protein (g/dL) | 6.0 | 6.0 | 6.0 | 6.0 | 0.2 | 0.99 |
| Albumin (g/dL) | 3.5 | 3.5 | 3.5 | 3.5 | 0.1 | 0.99 |
| Globulin (g/dL) | 2.5 | 2.5 | 2.5 | 2.5 | 0.1 | 0.99 |
| Albumin to globulin ratio | 1.4 | 1.4 | 1.4 | 1.4 | 0.1 | 0.99 |

CNTRL: basal diet without additive, BTRT: basal diet added with 0.1% butyric acid, BACIL: basal diet added with 0.02% B. subtilis, BTRBAC: basal diet added with the blends of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, MDA: malondialdehyde, SOD: serum superoxide dismutase, NDV: Newcastle disease virus, GMT: geometric mean titer.
Table 4. Serum biochemical indices of the IICC treated with the blends of butyric acid and B. subtilis.

| Items                  | Experimental groups |         |         |         |       |       |
|------------------------|---------------------|---------|---------|---------|-------|-------|
|                        | CNTRL | BTRT | BACIL | BTRBAC | SE    | P value |
| Total triglyceride (g/dL) | 63.7  | 89.7 | 85.1  | 85.7   | 10.7  | 0.24   |
| Total cholesterol (g/dL)  | 125   | 129  | 127   | 123    | 6.21  | 0.89   |
| LDL (g/dL)               | 21.2  | 19.9 | 15.3  | 15.8   | 4.11  | 0.55   |
| HDL (g/dL)               | 95.5  | 98.3 | 90.3  | 106    | 7.99  | 0.59   |
| Total protein (g/dL)     | 4.70  | 4.53 | 4.54  | 4.66   | 0.18  | 0.88   |
| Albumin (g/dL)           | 1.61  | 1.58 | 1.59  | 1.59   | 0.05  | 0.95   |
| Globulin (g/dL)          | 3.09  | 2.95 | 2.95  | 3.08   | 0.15  | 0.86   |
| A/G ratio               | 0.53  | 0.54 | 0.55  | 0.53   | 0.20  | 0.88   |
| Uric acid (g/dL)        | 6.27  | 6.92 | 7.60  | 7.38   | 0.76  | 0.63   |

CNTRL: basal diet without additive, BTRT: basal diet added with 0.1% butyric acid, BACIL: basal diet added with 0.02% B. subtilis, BTRBAC: basal diet added with the blends of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, LDL: low-density lipoprotein, HDL: high-density lipoprotein, A/G: albumin to globulin ratio

It was shown in this current study that treatments with butyric acid, B. subtilis or the combination of both did not affect (P>0.05) the intestinal populations of LAB and Enterobacteriaceae of the IICC (Table 5). In accordance with our findings, earlier study by Chamba et al. [14] reported no effect of sodium butyrate on the number of Escherichia coli in the jejunum of broiler chickens enumerated at days 14, 28 and 42. These data differed from that of reported by Panda et al. [3] and Czerwiński et al. [15] in which feeding sodium butyrate increased and decreased LAB and E. coli counts, respectively, in the intestine of boiler chickens. With regard to the effect of B. subtilis, our former study also did not find any effect of such commercial probiotic on the numbers of LAB and Enterobacteriaceae in the intestine of the IICC [1]. Indeed, the effectiveness of organic acids and probiotics in improving the intestinal microbial ecology of poultry greatly depends on a number of factors including type and acidity of organic acids, inclusion levels, dietary compositions, buffering capacity of the diets, species/strains of probiotics, number of bacterial colonies, species/strains of the chickens used and other experimental factors [2,16].

Table 5. Intestinal populations of lactic acid bacteria and Enterobacteriaceae of the IICC treated with the blends of butyric acid and B. subtilis.

| Items                  | Experimental groups |         |         |         |       |       |
|------------------------|---------------------|---------|---------|---------|-------|-------|
|                        | CNTRL | BTRT | BACIL | BTRBAC | SE    | P value |
| Ileum LAB (log cfu/g)   | 11.7  | 11.6 | 11.7  | 11.7   | 0.06  | 0.33   |
| Enterobacteriaceae (log cfu/g) | 9.67  | 9.48 | 9.28  | 9.68   | 0.21  | 0.50   |
| Caeca LAB (log cfu/g)   | 11.6  | 11.6 | 11.7  | 11.5   | 0.05  | 0.29   |
| Enterobacteriaceae (log cfu/g) | 9.50  | 9.74 | 9.69  | 9.60   | 0.09  | 0.27   |

4. Conclusion
Dietary supplementation of butyric acid, B. subtilis or the mix of both improved the economic performance, without affecting the antioxidative status, serum biochemistry, immune response and intestinal ecology of the IICC.

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