Manuscript Submission to LRRD

Dari: endwidia astuti (endwidia@yahoo.co.id)

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Cc: preston@lrrd.org

Tanggal: Senin, 15 Juli 2019 pukul 10.48 WIB

Dear Prof. Preston,

Attached is the manuscript entitled " Dietary supplementation of butyric acid, probiotic *Bacillus subtilis* or their combination on weight gain, internal organ weight and carcass traits of the Indonesian indigenous crossbred chickens" to be submitted to LRRD for being considered for publication. The paper reported that the combination of butyric acid and *B. subtilis* was beneficial in improving the growth performance and carcass yield of the Indonesian indigenous crossbred chickens.

Thank you very much indeed for your consideration.

Best wishes,
Endang Widiastuti, Ph.D.
Faculty of Animal and Agricultural Sciences, Diponegoro University
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Dari: Reg Preston (reg.preston@gmail.com)

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Tanggal: Selasa, 16 Juli 2019 pukul 06.55 WIB

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TRP

Professor T R Preston, PhD, DSc

Investigador Emérito
Centro para la Investigación en Sistemas Sostenibles
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Carrera 25 No 6-62 Cali, Colombia

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> On Sun, Jul 14, 2019 at 10:48 PM endwidia astuti <endwidia@yahoo.co.id> wrote:

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Dari: Reg Preston (reg.preston@gmail.com)

Kepada: endwidia@yahoo.co.id

Tanggal: Jumat, 26 Juli 2019 pukul 00.01 WIB

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Remember that the HTML version is a mirror image of the edited file in Word. To facilitate our work please ensure that the final version follows closely the style of recent papers in LRRD.

Regards

TRP

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Dietary supplementation of butyric acid, probiotic *Bacillus subtilis* or their combination on weight gain, internal organ weight and carcass traits of the Indonesian indigenous crossbred chickens

Endang Widiastuti, Isroli Isroli, Retno Murwani, Tri A. Sartono, Hanny I. Wahyuni, Turrini Yudiarti and Sugiharto Sugiharto

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia

endwidia@yahoo.co.id

Abstract

The study investigated the effect of dietary supplementation of butyric acid, probiotic *Bacillus subtilis* or their combination on the weight gain, internal organ relative weight and carcass traits of the Indonesian indigenous crossbred chicken (IICC). Two hundred day-old of the IICC were randomly distributed to CNTRL (basal diet supplemented with no additive), BTRC (basal diet supplemented with 0.1% butyric acid), BACIL (basal diet supplemented with 0.02% *B. subtilis*) and BTRBAC (basal diet supplemented with the blend of 0.1% butyric acid and 0.02% *B. subtilis*).Weight gain, feed intake and feed conversion ratio (FCR) were weekly gathered. At week 8, the chicks were killed, and from which the internal organs weight and carcass yield were determined. Feed consumption was less ($p<0.05$) in BTRBAC than in other groups at week 4. At week 8, weight gain and feed intake were higher ($p<0.05$), while FCR was lower ($p<0.05$) in the treated than in the control IICC.The relative weight of duodenum was lower ($p<0.05$) in BTRBAC than in BTRC chicks. The blend of butyric acid and *B. subtilis* resulted in greater ($p<0.05$) proportion of the eviscerated carcass. In conclusion, the combination of butyric acid and *B. subtilis* was essential in improving the growth performance and carcass yield of the IICC.

Keywords: antibiotic alternative, crossbred chicken, organic acid, probiotics

Introduction

Recently, the meat from the Indonesian indigenous crossbred chicken (IICC), which is the hybrid of the Indonesian indigenous roosters and modern laying hen (Isa Brown), has gained increasing interest from the consumers due to its unique taste and texture compared to meat from the modern broilers (Pramono 2006). The increasing demand for such product may consequently encourage the farmers' effort to increase population as well as productivity of the IICC. For the latter purpose, farmers have traditionally used antibiotics in the IICC diets. In response to the consumers'concern regarding to the phenomenon of antimicrobial resistance, the application of in-feed antibiotics in poultry diets have, however, been prohibited in Indonesia start from 2018. Indeed, the negative effect of antibiotic withdrawal from chicken diets has widely been suffered by poultry farmers. The in-feed antibiotic retraction may lead to many problems related to infections and retarded growth rate in chickens (Sugiharto 2016; Sugiharto and Ranjitkar 2019).For the sustainable and profitable production, it is therefore essential to find the effective alternative to in-feed antibiotics for the IICC. Among the alternatives to in-feed antibiotics, organic acids particularly butyric acid has long been used as feed additive in broiler production. The acid has been shown to protect the chicks from the attack of pathogenic bacteria (Panda et al 2009). Unlike other organic

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Commented [U1]: Shorten name of treatments: Cont; BUA, BAS, BUBA acids, butyric acid is a substrate or energy source that is very important for enterocytes or intestinal epithelial cells (Deepa et al 2018). For this reason, administration of butyric acid could improve the morphology and functions of the small intestine and thus growth performance of chicks (Salmanzadeh 2013; Kaczmarek et al 2016; Deepa et al 2018). Other than organic acids, probiotics have been used by farmers to help maintaining health and improve the production performances of chicks. Of the probiotics,*Bacillus subtilis*has widely been applied in poultry production (Sugiharto et al 2018a).

To maximize the health- and growth-promoting effects on chickens, organic acids may be combined with probiotics. Earlier study by Rodjan et al (2018) revealed that the blend of organic acids and probiotics was attributed to better intestinal morphology and microbial ecosystem when compared with the single use of either organic acids or probiotics. In contrast, Agboola et al (2015) and Barbieri et al (2015) did not observe any synergistic effect between organic acids and probiotics on the growth performance of broilers.It seemed that the nature of organic acids and probiotics combined as well as the acid tolerance of probiotics to organic acidsmay account for the above divergent results. In this current study, butyric acid was combined with *B. subtilis* to maximize the production performance of the IICC fed antibiotics-free diets. Probiotic *B. subtilis* was selected given its ability to form endospores enabling the bacteria to tolerate many extreme conditions (Ulrich et al 2018), including acidic properties of butyric acid. The present study aimed to investigate the effect of dietary supplementation of butyric acid, probiotic *Bacillus subtilis* or their combination on the weight gain, internal organ relative weight and carcass traits of the IICC.

Materials and methods

Two hundred day-old of the IICC were employed in the present trial. At arrival at the chicken house, the initial body weight (BW; 38.1±0.37 g) of chicks were recorded and distributed to four dietary treatment groups, each consisting of five replicates with 10 chicks in each. These dietary groups were CNTRL (chicks provided with basal diet supplemented with no additive), BTRC (chicks provided with basal diet supplemented with 0.1% butyric acid), BACIL (chicks provided with basal diet supplemented with 0.02% *B. subtilis*) and BTRBAC (chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% *B. subtilis*). Butyric acid (Butipearl, Kemin Cavriago, Italy) and *B. subtilis* (Baymix Grobig, PT. Bayer Indonesia, Jakarta, Indonesia) were incorporated into the basal feeds at the ultimate of the mixing process. The basal feeds were prepared in mash form and formulated as starter and finisher feeds (Table 1). The basal feedscontained no antibiotics,enzymes, antiprotozoal and antifungal agents. The feeds and water were served *ad libitum*to all chicks for the entire period of trial.

Table 1. Ingredients and nutrient compositions of diets

Items (%, unless otherwise noted)	Starter (1-4 weeks)	Finisher (5-8 weeks)
Maize	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, 990 g	0.30	0.30
L-Lysine, 780 g	0.20	0.20
Limestone	0.50	0.50
Dicalcium phosphate	1.50	1.50
Premix[1]	0.50	0.50

Salt	0.25	0.25
Calculated composition:		
Metabolisable energy[2] (kcal/kg)	2,900	3,080
Crude protein	22.0	20.0
Crude fiber	5.50	5.50
Ca	1.00	1.00
P (available)	0.60	0.60
Lysine	1.20	1.20
Methionine	0.60	0.60

[1]Premix contained (per kg of diet) of vit A 7,750 IU, vit D3 1,550 IU, vit E 1.88 mg, vit B1 1.25 mg, vit B2 3.13 mg, vit B6 1.88 mg, vit B12 0.01 mg, vit 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, OTC 37.5 mg, CaCO3 641.5 mg, dicalcium phosphate 1500 mg
[2]Metabolizable energy was calculated on the basis of formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

Vaccinations using commercial Newcastle disease vaccine (NDV) were conducted at day 4 and week 4 through eye drop and drinking water, respectively. The data on weight gain, feed intake and feed conversion ratio (FCR) were weekly gathered throughout the experiment. At the ultimate of experiment (week 8), five chicks per treatment group (one chicks from each replicate) were killed (by neck-cutting) and de-feathered. The chicks were then eviscerated, and the internal organs were quickly obtained, emptied and weighed. The carcass yield and commercial cuts of chicks were also determined.

The present in vivo study was designed according to a completely randomized design. The data collected were subjected to analysis of variance (SAS Inst. Inc., Cary, NC, USA). The Duncan's multiple-range test was further carried out if the differences ($p<0.05$) were seen among the treatment groups.

Results and discussion

Data on the performances of the IICC are presented in Table 2. Accumulative feed intake was less ($p<0.05$) in BTRBAC than in other treatment groups at week 4. At week 8, weight gain and accumulative feed intake were higher ($p<0.05$), while the FCR was lower ($p<0.05$) in the treated IICC when compared with the control IICC. These present findings were in agreement with that of formerly reported in broiler chicken studies. Panda et al (2009) documented that feeding diets supplemented with 0.2% butyric acid improved the growth rate and FCR of broiler chicks. It seemed that butyric acid supplementation was able to improve the morphology of the intestine resulting in better growth performance and nutrient utilization by the chicks (Panda et al 2009; Kaczmarek et al 2016; Sugiharto 2016; Deepa et al 2018). With regard to the effect of probiotic *B. subtilis*, such dietary supplementation has also been reported to improve the growth and feed efficiency both in modern broiler chickens (Sugiharto et al 2018a) as well as in the IICC (Sugiharto et al 2018b). The capability of *B. subtilis* in improving the physiological conditions, immune system and the intestinal ecology of the IICC may explain the growth-promoting effect of above mentioned additive (Sugiharto et al 2018ab). In this study, the combination of butyric and *B. Subtilis* did not further improve the growth performance of the IICC. The reason for the latter condition was not exactly known, but the maximum growth potential (genetic potential) of the IICC perhaps limit the

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growth-promoting potential of the blends of butyric and *B. subtilis*. Previously, we reported that the IICC reached the live BW of 830 to 881 g at 10 weeks of age (Sugiharto et al 2018b).

Table 2. Performances of the Indonesian indigenous crossbred chickens

Items	Experimental groups				SE	p value
	CNTRL	BTRC	BACIL	BTRBAC		
Week 4						
Weight gain(g)	208	233	229	227	12.5	0.50
Accumulative FI(g)	425[a]	415[a]	420[a]	405[b]	3.36	<0.01
FCR	2.01	1.82	1.86	1.81	0.11	0.35
Week 8						
Weight gain(g)	586[c]	664[b]	759[a]	782[a]	15.3	<0.01
Accumulative FI(g)	1604[b]	1640[b]	1814[a]	1884[a]	24.1	<0.01
FCR	2.75[a]	2.48[b]	2.39[b]	2.41[b]	0.05	<0.01

CNTRL: chicks provided with basal diet supplemented with no additive, BTRC: chicks provided with basal diet supplemented with 0.1% butyric acid, BACIL: chicks provided with basal diet supplemented with 0.02% B. subtilis, BTRBAC, chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, FI: feed intake, FCR: feed conversion ratio

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It was apparent in this current study that the relative weight of duodenum was lower ($p<0.05$) in BTRBAC as compared particularly with that in BTRC chicks (Table 3). In the latter case, it was difficult to infer that the lower duodenum relative weight was attributed to the negative effect of the blend of butyric acid and *B. subtilis*on the IICC, as the weight gain of the BTRBAC was greater than BTRC chicks. The lower duodenum relative weight in the BTRBAC seemed due to the higher live BW of the chicks in the BTRBAC group that had been used as the denominator in the calculation (Sugiharto et al 2018a).

Table 3. Internal organs of the Indonesian indigenous crossbred chickens

Items (% live BW)	Experimental groups				SE	p value
	CNTRL	BTRC	BACIL	BTRBAC		
Heart	0.48	0.49	0.55	0.50	0.02	0.08
Liver	2.41	2.27	2.31	2.27	0.08	0.55
Proventriculus	0.71	0.74	0.71	0.68	0.06	0.92
Gizzard	3.95	3.50	3.44	3.18	0.20	0.09
Spleen	0.26	0.20	0.27	0.21	0.03	0.41
Thymus	0.34	0.38	0.39	0.44	0.06	0.72
Bursa of Fabricius	0.12	0.11	0.10	0.09	0.02	0.70
Duodenum	0.69[ab]	0.89[a]	0.67[ab]	0.46[b]	0.08	0.01
Jejunum	1.19	1.21	1.16	0.84	0.18	0.44
Ileum	0.95	0.86	0.75	0.95	0.12	0.62
Pancreas	0.31	0.32	0.34	0.29	0.03	0.63

CNTRL: chicks provided with basal diet supplemented with no additive, BTRC: chicks provided with basal diet supplemented with 0.1% butyric acid, BACIL: chicks provided with basal diet supplemented with 0.02% B. subtilis, BTRBAC, chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, BW: body weight

In this study, the administration of butyric acid or *B. subtilis* alone did not exert any effect on the eviscerated carcass of the IICC. Interestingly, the combination of butyric acid and *B.*

subtilis resulted in greater (*p<0.05*) proportion of the eviscerated carcass (Table 4). This may suggest the beneficial effect of the blend of butyric acid and *B. subtilis* in increasing the carcass yield of the IICC. The mechanism by which the blend of butyric acid and *B. subtilis* affecting the carcass yield of the IICC remains unclear, but the attribution of such blend in increasing the final BW of the IICC seemed to be responsible. Our latter inference was supported by Marapana et al (2016) previously reporting that eviscerated carcass of chicks tended to increase as the slaughter weight increased.

Table 4. Carcass traits of the Indonesian indigenous crossbred chickens

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Items	**Experimental groups**				**SE**	**p value**
	CNTRL	**BTRC**	**BACIL**	**BTRBAC**		
	(% live BW)					
Eviscerated carcass	54.2^{b}	56.8^{b}	58.0^{ab}	61.2^{a}	1.33	0.02
	(% eviscerated carcass)					
Breast	23.3	24.4	21.5	21.5	1.53	0.46
Thigh	16.4	17.1	16.4	17.0	0.39	0.49
Drumstick	17.7	17.2	17.6	17.4	0.48	0.88
Wings	16.9	16.0	16.3	16.0	0.37	0.27
Back	25.6	25.3	28.1	28.2	1.53	0.39

CNTRL: chicks provided with basal diet supplemented with no additive, BTRC: chicks provided with basal diet supplemented with 0.1% butyric acid, BACIL: chicks provided with basal diet supplemented with 0.02% B. subtilis, BTRBAC, chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, BW: body weight

Conclusion

The combination of butyric acid and *B. subtilis* was essential in improving the growth performance and carcass yield of the IICC.

Acknowledgement

The study was financially supported by the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java Indonesia.

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review manuscript

Dari: endwidia astuti (endwidia@yahoo.co.id)

Kepada: reg.preston@gmail.com

Tanggal: Sabtu, 27 Juli 2019 pukul 23.23 WIB

Dear Prof. Preston,

Thank you very much for the opportunity given to us to revise our submitted manuscript to LRRD. We have revised the manuscript according to the suggestions and comments given by the reviewers (in red color in the manuscript). We wish the revision could fulfil the request from the reviewers.

Once again, thank you very much

Best wishes,

Endang Widiastuti

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Dietary supplementation of butyric acid, probiotic *Bacillus subtilis* or their combination on weight gain, internal organ weight and carcass traits of the Indonesian indigenous crossbred chickens

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Abstract

The study investigated the effect of dietary supplementation of butyric acid, probiotic ~~*Bacil*~~*Bacillus subtilis* or their combination on the weight gain, internal organ relative weight and carcass traits of the Indonesian indigenous crossbred chicken (IICC). Two hundred day-old of the IICC were randomly distributed to ~~CNTRL~~CONT (basal diet supplemented with no additive), ~~BTRC~~BUA (basal diet supplemented with 0.1% butyric acid), ~~BACIL~~BAS (basal diet supplemented with 0.02% *B. subtilis*) and ~~BTRBAC~~BUBA (basal diet supplemented with the blend of 0.1% butyric acid and 0.02% *B. subtilis*). Weight gain, feed intake and feed conversion ratio (FCR) were weekly gathered. At week 8, the chicks were killed, and from which the internal organs weight and carcass yield were determined. Feed consumption was less (*$p<0.05$*) in ~~BTRBAC~~BUBA than in other groups at week 4. At week 8, weight gain and feed intake were higher (*$p<0.05$*), while FCR was lower (*$p<0.05$*) in the treated than in the control IICC. The relative weight of duodenum was lower (*$p<0.05$*) in ~~BTRBAC~~BUBA than in ~~BTRC~~BUA chicks. The blends of butyric acid and *B. subtilis* resulted in greater (*$p<0.05$*) proportion of the eviscerated carcass. In conclusion, the combination of butyric acid and *B. subtilis* was essential in improving the growth performance and carcass yield of the IICC.

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Keywords: antibiotic alternative, crossbred chicken, organic acid, probiotics

Introduction

Recently, the meat from the Indonesian indigenous crossbred chicken (IICC), which is the hybrid of the Indonesian indigenous roosters and modern laying hen (Isa Brown), has gained increasing interest from the consumers due to its unique taste and texture compared to meat from the modern broilers (Pramono 2006). The increasing demand for such product may consequently encourage the farmers' effort to increase population as well as productivity of the IICC. For the latter purpose, farmers have traditionally used antibiotics in the IICC diets. In response to the consumers' concern regarding to the phenomenon of antimicrobial resistance, the application of in-feed antibiotics in poultry diets have, however, been prohibited in Indonesia start from 2018. Indeed, the negative effect of antibiotic withdrawal from chicken diets has widely been suffered by poultry farmers. The in-feed antibiotic retraction may lead to many problems related to infections and retarded growth rate in chickens (Sugiharto 2016; Sugiharto and Ranjitkar 2019).For the sustainable and profitable production, it is therefore essential to find the effective alternative to in-feed antibiotics for the IICC. Among the alternatives to in-feed antibiotics, organic acids particularly butyric acid has long been used as feed additive in broiler production. The acid has been shown to protect the chicks from the attack of pathogenic bacteria (Panda et al 2009). Unlike other organic acids, butyric acid is a substrate or energy source that is very important for enterocytes or intestinal epithelial cells (Deepa et al 2018). For this reason, administration of butyric acid could improve the morphology and functions of the small intestine and thus growth performance of chicks (Salmanzadeh 2013; Kaczmarek et al 2016; Deepa et al 2018). Other than organic acids, probiotics have been used by farmers to help maintaining health and improve the production performances of chicks. Of the probiotics, ~~*Bacil*~~*Bacillus subtilis* has widely been applied in poultry production (Sugiharto et al 2018a).

To maximize the health- and growth-promoting effects on chickens, organic acids may be combined with probiotics. Earlier study by Rodjan et al (2018) revealed that the blend of organic acids and probiotics was attributed to better intestinal morphology and microbial ecosystem when compared with the single use of either organic acids or probiotics. In contrast, Agboola et al (2015) and Barbieri et al (2015) did not observe any synergistic effect between organic acids and probiotics on the growth performance of broilers. It seemed that the nature of organic acids and probiotics combined as well as the acid tolerance of probiotics to organic acids may account for the above divergent results. In this current study, butyric acid was combined with *B. subtilis* to maximize the production performance of the IICC fed antibiotics-free diets. Probiotic *B. subtilis* was selected given its ability to form endospores enabling the bacteria to tolerate many extreme conditions (Ulrich et al 2018), including acidic properties of butyric acid. The present study aimed to investigate the effect of dietary supplementation of butyric acid, probiotic ~~*Bacil*~~*B. subtilis* or their combination on the weight gain, internal organ relative weight and carcass traits of the IICC.

Materials and methods

Two hundred day-old of the IICC were employed in the present trial. At arrival at the chicken house, the initial body weight (BW; 38.1±0.37 g) of chicks were recorded and distributed to four dietary treatment groups, each consisting of five replicates with 10 chicks in each. These dietary groups were ~~CNTRL~~CONT (chicks provided with basal diet supplemented with no additive), ~~BTRC~~BUA (chicks provided with basal diet supplemented with 0.1% butyric acid), ~~BACIL~~BAS (chicks provided with basal diet supplemented with 0.02% *B. subtilis*) and ~~BTRBAC~~BUBA (chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% *B. subtilis*). Butyric acid (Butipearl, Kemin Cavriago, Italy) and *B. subtilis* (Baymix Grobig, PT. Bayer Indonesia, Jakarta, Indonesia) were incorporated into the basal feeds at the ultimate of the mixing process. The basal feeds were prepared in mash form and formulated as starter and finisher feeds (Table 1). The basal feeds contained no antibiotics, enzymes, antiprotozoal and antifungal agents. The feeds and water were served *ad libitum* to all chicks for the entire period of trial.

Table 1. Ingredients and nutrient compositions of diets

Items (%, unless otherwise noted)	Starter (days 1-28)	Finisher (days 29-56)
Maize	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, 990 g	0.30	0.30
L-Lysine, 780 g	0.20	0.20
Limestone	0.50	0.50
Dicalcium phosphate	1.50	1.50

Premix[1]	0.50	0.50
Salt	0.25	0.25
Calculated composition:		
Metabolisable energy[2] (kcal/kg)	2,900	3,080
Crude protein	22.0	20.0
Crude fiber	5.50	5.50
Ca	1.00	1.00
P (available)	0.60	0.60
Lysine	1.20	1.20
Methionine	0.60	0.60

[1]Premix contained (per kg of diet) of vit A 7,750 IU, vit D3 1,550 IU, vit E 1.88 mg, vit B1 1.25 mg, vit B2 3.13 mg, vit B6 1.88 mg, vit B12 0.01 mg, vit 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, ~~OTC 37.5 mg,~~ CaCO3 641.5 mg, Dicalcium phosphate 1500 mg

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

Vaccinations using commercial Newcastle disease vaccine (NDV) were conducted at day 4 and week 4 through eye drop and drinking water, respectively. The data on weight gain, feed intake and feed conversion ratio (FCR) were weekly gathered throughout the experiment. At the ultimate of experiment (week 8), five chicks per treatment group (one chicks from each replicate) were killed (by neck-cutting) and de-feathered. The chicks were then eviscerated, and the internal organs were quickly obtained, emptied and weighed. The carcass yield and commercial cuts of chicks were also determined.

The present in vivo study was designed according to a completely randomized design. The data collected were subjected to analysis of variance (SAS Inst. Inc., Cary, NC, USA). The Duncan's multiple-range test was further carried out if the differences ($p<0.05$) were seen among the treatment groups.

Results and discussion

Data on the performances of the IICC are presented in Table 2. Accumulative feed intake was less ($p<0.05$) in ~~BTRBAC~~BUBA than in other treatment groups at week 4. At week 8, weight gain and accumulative feed intake were higher ($p<0.05$), while the FCR was lower ($p<0.05$) in the treated IICC when compared with the control IICC. These present findings were in agreement with that of formerly reported in broiler chicken studies. Panda et al (2009) documented that feeding diets supplemented with 0.2% butyric acid improved the growth rate and FCR of broiler chicks. It seemed that butyric acid supplementation was able to improve the morphology of the intestine resulting in better growth performance and nutrient utilization by the chicks (Panda et al 2009; Kaczmarek et al 2016; Sugiharto 2016; Deepa et al 2018). With regard to the effect of probiotic *B. subtilis*, such dietary supplementation has also been reported to improve the growth and feed efficiency both in modern broiler chickens (Sugiharto et al 2018a) as well as in the IICC (Sugiharto et al 2018b). The capability of *B. subtilis* in improving the physiological conditions, immune system and the intestinal ecology of the IICC may explain the growth-promoting effect of above mentioned additive (Sugiharto et al 2018ab). In this study, accumulative feed intake was notably higher ($p<0.05$) in BAS and BUBA than in CONT and BUA chicks. In the case of feed intake in BAS group, the corresponding results were formerly documented by Abdel-Hafeez et al (2017) and Gao et al (2017), at which feeding probiotic *B. subtilis* resulted in increased feed consumption in modern broiler strains. These authors suggested that the increase in the appetite and the improved intestinal morphology and functions accounted for the substantial increased feed utilization and, thereby, feed intake in broiler chicks provided with *B. subtilis*. With regard to the high feed intake in BUBA, the effect of probiotic *B. subtilis* seemed to be more dominant than that of butyric acid, as we did not find any increasing effect of butyric acid on the feed consumption of chicks as compared to the IICC in control group. In this study, the combination of butyric and *B.* ~~*Subtilis*~~ *subtilis* did not further improve the growth performance of the IICC. The reason for the latter condition was not exactly known, but the maximum growth potential (genetic potential) of the IICC perhaps limit the growth-promoting potential of the blends of butyric and *B. subtilis*. Previously, we reported that the IICC reached the live BW of 830 to 881 g at 10 weeks of age (Sugiharto et al 2018b).

Table 2. Performances of the Indonesian indigenous crossbred chickens

Items	Experimental groups				SE	p value
	~~CNTRL~~ CONT	~~BTRC~~ BUA	~~BACIL~~ BAS	~~BTRBAC~~ BUBA		
~~Week 4~~ Days 1-28						
BW (g)	246	271	267	265	6.17	0.32
Weight gain (g/d)	~~208~~ 7.42	~~233~~ 8.34	~~229~~ 8.18	~~227~~ 8.11	~~12.5~~ 0.22	0.50
Accumulative FI (g/d)	~~425^{a}~~ 15.2^{a}	~~415^{a}~~ 14.8^{a}	~~420^{a}~~ 15.0^{a}	~~405^{b}~~ 14.5^{b}	~~3.36~~ 0.08	<0.01
FCR	2.01	1.82	1.86	1.81	0.11	0.35
~~Week 8~~ Days 1-56						
BW (g)	624^{c}	702^{b}	797^{a}	820^{a}	15.3	<0.01
Weight gain (g/d)	~~586^{c}~~ 10.5^{c}	~~664^{b}~~ 11.9^{b}	~~759^{a}~~ 13.6^{a}	~~782^{a}~~ 14.0^{a}	~~15.3~~ 0.34	<0.01
Accumulative FI (g/d)	~~1604^{b}~~ 28.6^{b}	~~1640^{b}~~ 29.3^{b}	~~1814^{a}~~ 32.4^{a}	~~1884^{a}~~ 33.6^{a}	~~24.1~~ 0.52	<0.01
FCR	2.75^{a}	2.48^{b}	2.39^{b}	2.41^{b}	0.05	<0.01

~~CNTRL~~ CONT: chicks provided with basal diet supplemented with no additive, ~~BTRC~~ BUA: chicks provided with basal diet supplemented with 0.1% butyric acid, ~~BACIL~~ BAS: chicks provided with basal diet supplemented with 0.02% B. subtilis, ~~BTRBAC~~ BUBA, chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, BW: body weight, FI: feed intake, FCR: feed conversion ratio

It was apparent in this current study that the relative weight of duodenum was lower ($p<0.05$) in ~~BTRBAC~~ BUBA as compared particularly with that in ~~BTRC~~ BUA chicks (Table 3). In the latter case, it was difficult to infer that the lower duodenum relative weight was attributed to the negative effect of the blend of butyric acid and *B. subtilis*on the IICC, as the weight gain of the ~~BTRBAC~~ BUBA was greater than ~~BTRC~~ BUA chicks. The lower duodenum relative weight in the ~~BTRBAC~~ BUBA seemed due to the higher live BW of the chicks in the ~~BTRBAC~~ BUBA group that had been used as the denominator in the calculation (Sugiharto et al 2018a).

Table 3. Internal organs of the Indonesian indigenous crossbred chickens

Items (% live	Experimental groups	SE	p value

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BW)	~~CNTRL~~ CONT	~~BTRC~~BUA	~~BACIL~~BAS	~~BTRBAC~~ BUBA		
Heart	0.48	0.49	0.55	0.50	0.02	0.08
Liver	2.41	2.27	2.31	2.27	0.08	0.55
Proventriculus	0.71	0.74	0.71	0.68	0.06	0.92
Gizzard	3.95	3.50	3.44	3.18	0.20	0.09
Spleen	0.26	0.20	0.27	0.21	0.03	0.41
Thymus	0.34	0.38	0.39	0.44	0.06	0.72
Bursa of Fabricius	0.12	0.11	0.10	0.09	0.02	0.70
Duodenum	0.69^{ab}	0.89^{a}	0.67^{ab}	0.46^{b}	0.08	0.01
Jejunum	1.19	1.21	1.16	0.84	0.18	0.44
Ileum	0.95	0.86	0.75	0.95	0.12	0.62
Pancreas	0.31	0.32	0.34	0.29	0.03	0.63

~~CNTRL~~CONT: chicks provided with basal diet supplemented with no additive, ~~BTRC~~BUA: chicks provided with basal diet supplemented with 0.1% butyric acid, ~~BACIL~~BAS: chicks provided with basal diet supplemented with 0.02% B. subtilis, ~~BTRBAC~~BUBA, chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, BW: body weight

In this study, the administration of butyric acid or *B. subtilis* alone did not exert any effect on the eviscerated carcass of the IICC. Interestingly, the combination of butyric acid and *B. subtilis* resulted in greater ($p<0.05$) proportion of the eviscerated carcass (Table 4). This may suggest the beneficial effect of the blend of butyric acid and *B. subtilis* in increasing the carcass yield of the IICC. The mechanism by which the blend of butyric acid and *B. subtilis* affecting the carcass yield of the IICC remains unclear, but the attribution of such blend in increasing the final BW of the IICC seemed to be responsible. Our latter inference was supported by Marapana et al (2016) previously reporting that eviscerated carcass of chicks tended to increase as the slaughter weight increased.

Table 4. Carcass traits of the Indonesian indigenous crossbred chickens

Items	**Experimental groups**				**SE**	**p value**
	~~CNTRL~~ CONT	**~~BTRC~~BUA**	**~~BACIL~~BAS**	**~~BTRBAC~~BUBA**		
BW at slaughtering (g)	626^{c}	701^{b}	800^{a}	822^{a}	19.3	<0.01
			(% live BW)			
Eviscerated carcass	54.2^{b}	56.8^{b}	58.0^{ab}	61.2^{a}	1.33	0.02
			(% eviscerated carcass)			
Breast	23.3	24.4	21.5	21.5	1.53	0.46
Thigh	16.4	17.1	16.4	17.0	0.39	0.49
Drumstick	17.7	17.2	17.6	17.4	0.48	0.88
Wings	16.9	16.0	16.3	16.0	0.37	0.27
Back	25.6	25.3	28.1	28.2	1.53	0.39

~~CNTRL~~CONT: chicks provided with basal diet supplemented with no additive, ~~BTRC~~BUA: chicks provided with basal diet supplemented with 0.1% butyric acid, ~~BACIL~~BAS: chicks provided with basal diet supplemented with 0.02% B. subtilis, ~~BTRBAC~~BUBA, chicks provided with basal diet supplemented with the blend of 0.1% butyric acid and 0.02% B. subtilis, SE: standard error, BW: body weight

Conclusion

The combination of butyric acid and *B. subtilis* was essential in improving the growth performance and carcass yield of the IICC.

Acknowledgement

The study was financially supported by the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java Indonesia.

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