Probiotic *Bacillus* plus vitamins and minerals enhanced haemoglobin values and relative weight of ileum and improved feed conversion ratio of broilers during brooding period

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Abstract

The study was conducted to investigate the effect of dietary supplement containing probiotic *Bacillus* plus vitamins and minerals on the performance, internal organ development and haematological parameters of broilers post brooding. Two hundred and eighty eight 1-day-old broiler chicks were randomly allotted to four groups, i.e., control birds (birds receiving diet without supplement) and birds receiving supplement either 0.1%, 0.5% or 1% from total diets. The supplement (probiotic *Bacillus* plus vitamins and minerals) contained 12.10 log cfu/g mixture of probiotic *Bacillus*, 0.100 mg vit A, 0.018 mg vit D_3, 0.100 mg vit E, 1200 mg Ca, 750 mg P, 0.08 mg Mg, 0.006 mg Co, 0.045 mg Cu, 0.015 mg Se, 0.180 mg S, 0.010 mg Zn, 0.060 mg KCl, 0.030 mg I, 0.060 mg Fe and 0.100 mg Mn. The chicks were reared for 14 days, and blood and internal organs were collected at day 12 of the experiment. Feed conversion ratio (FCR) was lower ($P \leq 0.05$) in birds fed diet containing 0.5% supplement as compared to control. Body weight and accumulative feed intake of birds at day 14 was not different ($P > 0.05$) among the treatment groups. At day 12, dietary supplementation (0.1% or 1% from total diet) increased ($P \leq 0.05$) the relative weight of ileum of broiler chicks. Moreover, the values of haemoglobin were higher ($P \leq 0.05$) in broiler chicks supplemented with 0.5% probiotic *Bacillus* plus vitamins and minerals, when compared with control. In conclusion, dietary supplementation with probiotic *Bacillus* plus vitamins and minerals was beneficial for the enhanced haemoglobin values and the improved development of ileum and feed efficiency of broiler during brooding period.

Keywords: *broiler chicks, standard commercial feed, performance, probiotics, mineral, vitamin*

Introduction

In Indonesia, broiler farmers mostly use commercial feeds produced by feed mill industries. Commercial feeds have generally been formulated to meet the standard nutrient requirements of broilers. However, feed handling on the farm such as storage conditions and length of time in farm feed bins may adversely change the composition of feeds especially vitamins and minerals (Aviagen 2014). This may to some extent explain why deficiency diseases (particularly vitamin and mineral deficiencies) and the retarded growth rate still often found in broilers fed standard commercial feeds (Islam et al 2004; Rahman et al 2012; Harun-Ar-Rashid et al 2015). For

broiler chicks, brooding period (the first two weeks of the broiler's life) is the critical stage since this period accounts a third of broiler's life (Müller 2013). Indeed, vitamin and mineral deficiencies during the brooding period may have pronounced effect as it depresses the later performance of broilers (Aviagen 2014). To deal with such deficiency diseases (especially during brooding period), broiler farmers randomly supplement vitamins-mineral premix to the feeds. The farmers also supplement growth promoters to have improved growth performance and better feed efficiency of broilers (Rahman et al 2012).

It has been recognized that withholding birds from feed for 24 h after hatching resulted not only in retarded growth and delayed development of the intestine, but also compromised immune responses of broiler chicks (Bhanja et al 2010). Due to logistics and transportation, feed withholding for more than 24 h however often occurs in commercial broiler production in Indonesia (Sugiharto and Lauridsen 2016). Indeed, most of broiler feeds available in the market nowadays do not have enough components/nutrients for compensating the post-hatch feed withholding-induced growth retardation and immune suppression (Sanda et al 2015). Extra supplementation of commercial broiler diets with antibiotic growth promoters (AGPs) may be applied to enhance growth rate and immune responses of broiler chickens (Lee et al 2012; Manafi 2015). However, the global concern about antibiotic resistance both in broilers and human (as consumers) has encouraged nutritionists to reduce or substitute AGPs with natural growth promoters and immune system booster. Of the alternative to AGPs, probiotics have attracted great interest as it may improve growth performance and modulate immune system of broilers (Sugiharto 2016). Taken together, the present study aimed to investigate the effect of dietary supplement containing probiotic *Bacillus* plus vitamins and minerals on the performance, internal organ development and haematological parameters of broilers post brooding.

Materials and methods

Two hundred and eighty eight Lohmann (MB-202) 1-day-old broiler chicks (average body weight= 45.9 ± 0.50 g) were used in the current study. They were withheld from feed and water for 24 h post-hatch to mimic the real condition of broiler chicks handling in Indonesia. In an open-sided naturally ventilated broiler house, the chicks were randomly allotted to four experimental groups of 72 chicks each and 6 replicates of 12 chicks. The groups included control birds (birds receiving diet without probiotic *Bacillus* plus vitamins and minerals) and birds receiving probiotic *Bacillus* plus vitamins and minerals either 0.1%, 0.5% or 1% from total diets. For the entire study period (14 days), the chicks were fed standard commercial diet containing 13% moisture, 21-23% crude protein, 5% crude fat, 5% crude fiber and 7% crude ash (data were obtained from the company). The diets and water were offered *ad libitum* for the entire of experiment. The supplements were added at the expense of the diets. The supplement (probiotic *Bacillus* plus vitamins and minerals) contained 12.10 log cfu/g mixture of *Bacillus* probiotics (i.e., *Bacillus cereus* strain SIIA_Pb_E3, *Bacillus licheniformis* strain FJAT-29133, *Bacillus megaterium* strain F4-2-27 and *Bacillus sp.* 11CM31Y12). In addition to that, the supplement contained 0.100 mg vit A, 0.018 mg vit D_3, 0.100 mg vit E, 1200 mg Ca, 750 mg P, 0.08 mg Mg, 0.006 mg Co, 0.045 mg Cu, 0.015 mg Se, 0.180 mg S, 0.010 mg Zn, 0.060 mg KCl, 0.030 mg I, 0.060 mg Fe and 0.100 mg Mn.

At day 12, blood was collected in Ethylenediaminetetraacetic acid (EDTA)-containing vacutainers for the determination of haematological profile. The same chicks that were blood sampled were then slaughtered by *halal* neck cut (Alshelmani et al 2016). Immediately, the internal organs (heart, liver, spleen, Bursa of Fabricius, duodenum, jejunum and ileum) were obtained and weighed. The intestinal segments were emptied before being weighed. Total

erythrocytes and leukocytes were counted according to the dilution flask method (Bürker chamber was used to count corpuscles). A light microscope with an immersion lens was employed to define the differential leukocytes. Blood smears were prepared based on the coverslip technique. Body weight, accumulative feed intake and feed conversion ratio (FCR) of chicks were recorded at day 14 of the experiment.

Data were analysed according to a completely randomized design by ANOVA using the General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Pen was regarded as the experimental unit during the analysis. Significant differences among experimental groups were later analysed using Duncan's multiple-range test. A significant level of $P \leq 0.05$ wwas implemented.

Results and discussion

Performance and internal organs of broiler chicks

It has been known that optimal growth performance during brooding period is critical, as it determines the final body weight of broiler chickens. Several factors have been recognized to adversely affect the performance of broilers during brooding period, one of which is the delayed access to feed and water (due to logistics and long transport from hatchery to commercial farms) (Müller 2013). In the present study, the body weight of broiler chicks at day 14 was lower as compared to the standard body weight of Lohmann broiler chicks at the same age (Aviagen 2007). This seemed to be the effect of feed and water withholding during the first 24 h applied to chicks in the present study. Indeed, the body weight as well as feed intake of birds at day 14 was not different ($P>0.05$) among the treatment groups. Compared to control, however, FCR was lower ($P \leq 0.05$) in birds fed diet containing 0.5% supplement (Table 1). This result suggested that dietary supplementation with probiotic *Bacillus* plus vitamins and minerals was beneficial in improving the feed efficiency of birds fed standard commercial diets. With regard to probiotics, recent study showed that such additive was beneficial for the improved nutrient metabolism and utilization by broiler chicks (Sugiharto 2016; Zheng et al 2016). Concomitantly, vitamins and minerals are essential for optimal feed utilization and metabolism in poultry (Islam et al 2004). Taken together, supplementation of standard commercial diets with probiotic *Bacillus* plus vitamins and minerals may therefore be beneficial for the improved metabolism and thus feed utilization and efficiency of birds (Islam et al 2004; Rahman et al 2012; Dhama et al 2014).

Table 1. Performances of broiler chicks[†]

Items	Dietary treatments				SE	*P* value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
Body weight (g/bird)	318	324	329	310	8.72	0.44
Feed intake (g/bird)	403	395	386	392	14.7	0.88
FCR	1.47[a]	1.42[ab]	1.36[b]	1.48[a]	0.02	<0.01

[†]Data were collected at day 14

Control= birds receiving diet without supplementation ; Pro-0.1= birds receiving 0.1% probiotic Bacillus plus vitamins and minerals; Pro-0.5= birds receiving 0.5% probiotic Bacillus plus vitamins and minerals; Pro-1.0= birds receiving 1% probiotic Bacillus plus vitamins and minerals; SE= standard error

[a,b] Values with different letters within the same row were significantly different

Result in the present study showed that supplementation of standard commercial diets with 0.1% and 1% probiotic *Bacillus* plus vitamins and minerals increased ($P \leq 0.05$) the relative weight of ileum of broiler chicks at day 12. Concomitant with this, Shabani et al (2012) reported

that feeding probiotic (protexin) was able to increase the relative weight of ileum of broiler chickens. With regard to other internal organs, the current treatments, however, had no significant impact (Table 2). The mechanism through which probiotics increased the relative weight of ileum in the current study was not clear, as the effect of probiotics on the internal organ weight in broilers is equivocal. Indeed, Olnood et al (2015) found no difference in the relative weight of internal organs (liver, spleen, bursa, duodenum, jejunum, ileum) of broilers when feeding probiotic *Lactobacillus johnsonii*.. In addition to probiotic effect, minerals and vitamins may also contribute to the enhanced weight of ileum of broiler chicks in the present study. Islam et al (2004) suggested that supplementation of standard commercial diets with mineral-vitamin premix may enhance digestion, absorption and metabolism, and thus improve the intestinal development of broilers.

Table 2. Internal organs of broiler chicks[†]

Items (% live body weight)	Dietary treatments				SE	*P* value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
Heart	0.82	0.79	0.77	0.89	0.04	0.12
Liver	2.99	2.75	2.84	3.13	0.11	0.09
Spleen	0.08	0.07	0.09	0.09	0.01	0.36
Bursa of Fabricius	0.23	0.23	0.26	0.29	0.03	0.39
Duodenum	1.57	1.59	1.58	1.58	0.08	0.99
Jejunum	2.49	2.53	2.54	2.61	0.12	0.91
Ileum	1.38^{a}	1.69^{b}	1.52^{ab}	1.76^{b}	0.09	0.03

[†] Data were collected at day 12

Control= birds receiving diet without supplementation ; Pro-0.1= birds receiving 0.1% probiotic Bacillus plus vitamins and minerals; Pro-0.5= birds receiving 0.5% probiotic Bacillus plus vitamins and minerals; Pro-1.0= birds receiving 1% probiotic Bacillus plus vitamins and minerals; SE= standard error

[a,b] Values with different letters within the same row were significantly different

Haematological parameters of broiler chicks

In the present study, the values of haemoglobin were higher ($P \leq 0.05$) in broiler chicks supplemented with 0.5% probiotic *Bacillus* plus vitamins and minerals, when compared with control (Table 3). This result was concomitant with that of reported by Çetin et al (2005), in which probiotic (Primalac 454) supplementation resulted in increased levels of haemoglobin in turkeys. In addition to probiotics, vitamins and minerals supplementation in the diets seemed also to contribute to the increased level of haemoglobin of broiler chicks in the current study. Our inference was supported by Bolu and Adedibu (2007) who reported an increase in haemoglobin values with administration of vitamin/mineral premix in broiler diets. Study has shown the positive correlation between haemoglobin level and growth performance and feed efficiency of broilers (Shareef and Al-Dabbagh 2009). Concurrence with this, the higher haemoglobin level in birds supplemented with 0.5% probiotic *Bacillus* plus vitamins and minerals in the current study was in parallel with the lower FCR (Table 1). Considering the function of haemoglobin in transporting oxygen for metabolism, high haemoglobin level may indicate the high metabolic rate and thus improved nutrient utilization by broiler chicks. In the present study, there was no significant difference ($P>0.05$) in the numbers of erythrocytes and haematocrit of broilers among the treatment groups (Table 3).

Table 3. Haematological parameters of broiler chicks [†]

Items	Dietary treatments				SE	*P* value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
Erythrocytes (10^{12}/L)	4.14	4.01	3.37	3.13	0.69	0.64
Haemoglobin (g/dL)	9.05^{a}	10.3^{ab}	11.2^{b}	9.46^{a}	0.54	0.05
Haematocrit (%)	29.2	28.0	28.1	29.1	1.44	0.90
	9.68	8.71	6.87	6.76	1.18	0.25

Leukocytes (10^9/L)						
Heterophil (%)	13.8	17.5	12.5	13.2	3.41	0.73
Basophil (%)	8.00	14.2	12.3	12.0	3.01	0.54
Eosinophil (%)	5.83	8.00	9.17	10.3	2.92	0.73
Lymphocytes (%)	57.5	44.8	55.7	57.7	5.83	0.37
Monosit (%)	14.8	15.5	10.3	6.83	3.35	0.25
H/L ratio	0.31	0.40	0.25	0.25	0.10	0.65

[†]Data were collected at day 12

Control= birds receiving diet without supplementation ; Pro-0.1= birds receiving 0.1% probiotic Bacillus plus vitamins and minerals; Pro-0.5= birds receiving 0.5% probiotic Bacillus plus vitamins and minerals; Pro-1.0= birds receiving 1% probiotic Bacillus plus vitamins and minerals; SE= standard error; H/L ratio= heterophil to lymphocytes ratio

[a,b]Values with different letters within the same row were significantly different

Dietary supplementation with probiotics has been attributed to the increased count of leukocytes in poultry (Paryad and Mahmoudi 2008; Hatab et al 2016). Different from these earlier reports, our present finding showed no significant impact (p>0.05) of dietary probiotics supplementation on the number of leukocytes of broiler chicks during brooding period. Indeed, our present data were in accordance with Çetin et al (2005) showing no difference in leukocytes and the differential leukocyte counts of broilers when feeding probiotics (as compared to control). With regards to vitamins and minerals, the effect of these components on leukocytes and the differential leukocyte counts of broiler chicks seemed to be inconsistent. For instance, dietary supplementation of vitamin C and E increased total leukocytes, lymphocytes and eosinophil (Ipek et al 2007; Ajakaiye et al 2010), while zinc increased peripheral leukocyte counts of broilers (Sajadifar and Miranzadeh 2013). Conversely, Donmez et al (2001) revealed no effect of zinc supplementation on the counts of leukocyte in broiler chickens. Moreover, Bolu and Adedibu (2007) revealed that the number of leukocytes were lower in the birds fed vitamin/mineral premix than that in control birds (fed no vitamin/mineral premix). The differences in types and doses of vitamins and minerals, animals, age and conditions of the trial may be responsible for these above inconsistent data.

Conclusion

- Dietary supplementation with probiotic *Bacillus* plus vitamins and minerals was beneficial for the enhanced haemoglobin values and the improved development of ileum and feed efficiency of broiler during brooding period.

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