Livestock_Research_for_Rural_Development_30_11_2018.1_Nop.18.pdf

by T Yuiarti

Effect of *Bacillus* probiotics on internal organs and carcass characteristics of broiler chicks infected with avian pathogenic *Escherichia coli*

I Isroli, T Yudiarti, E Widiastuti, H I Wahyuni, T A Sartono and S Sugiharto

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia (50275)
sgh_undip@yahoo.co.id

Abstract

The present study was conducted to investigate the effect of feeding *Bacillus* probiotics on internal organs relative weight and carcass characteristics of broiler chicks infected with avian pathogenic *E. coli* (APEC). Five hundred and four one-day-old broiler chicks were allocated either to CNTRL (chicks receiving basal diet as control), AGPS (chicks receiving basal diet supplemented with 0.04% zinc bacitracin) or PROBIO (chicks receiving basal diet supplemented with 0.5% *Bacillus* probiotics). Half of the chicks in each diet were assigned to infection and the rest were not infected with APEC. At day 35, one bird from each experimental unit was slaughtered, eviscerated and cut into commercial portions. Internal organs were obtained and weighed, and samples of breast meat were subsequently collected for the determinations of pH and drip loss of meat. The liver relative weight was higher ($P<0.05$) in PROBIO and infected birds, as compared to other dietary treatments and non-infected birds, respectively. Irrespective of infection, birds in AGPS group had a higher ($P<0.05$) proportion of thigh compared to PROBIO birds. Meat from CNTRL birds had lower ($P<0.05$) pH values at 45 min and 24 hours after slaughtered when compared with AGPS and PROBIO birds. Meat from CNTRL birds also had higher ($P<0.05$) drip loss. There was no substantial interaction between dietary and infection treatments on the parameters measured. In conclusion, dietary inclusion of *Bacillus* probiotics increased the liver relative weight, prevented the decrease in meat pH and reduced the drip loss of broiler meats.

Keywords: *broiler, carcass, E. coli infection, meat, organs, probiotics*

Introduction

As the supply organs, internal organs have crucial roles in the life and production of broilers. Hence, the development of these organs should be monitored to ensure the optimal health and growth of chicks. Indeed, bacterial infection such as *E. coli* infection may negatively affect the development of internal organs, and eventually the production and health performances of broilers (Guabiraba and Schouler 2015). Today, consumers are more aware of the nutritional qualities of meats, and therefore production of the high quality and nutritious broiler meats is of importance. In general, there are several factors affecting the qualities of carcass and meat in broiler chickens, including genotype, age, sex, dietary ingredients, density, environment, exercise and stress. Besides causing production problems as mentioned above, *E. coli* infection in general may adversely impact on the quality of broiler meat (Istiqomah et al 2013). In the earlier time, in-feed antibiotic was extensively used to control bacterial infections in broilers (Hampson and Murdoch 2003). Owing to phenomenon of antibiotic resistance, the use of in-feed antibiotic in broiler rations is, however, no longer permitted in most countries (Sugiharto 2016). Hence, any alternatives to in-feed antibiotic are crucial for the safe and sustainable broiler production worldwide.

Other than improving growth performance and controlling bacterial infections, probiotics have been shown to ameliorate the detrimental changes in broiler meat composition due to infections (Wang et al 2017). In the present study, we included *Bacillus* probiotics in diets as an alternative to in-feed antibiotic for broilers.

The additive has been shown able to improve the digestive functions and physiological conditions and increase populations of beneficial bacteria in the intestine of broiler chicks (Isroli et al 2017; Sugiharto et al 2018a,b). The effect of such additive on internal organs and carcass characteristics from the infected broilers has, however, never been documented. The aim of this present study was therefore to investigate the effect of *Bacillus* probiotics on internal organs relative weight and carcass characteristics of broilers infected with avian pathogenic *E. coli*.

Materials and methods

Five hundred and four one-day-old broiler chicks (Lohmann strain) were used in the 3 × 2 factorial design experiment with diets and infection as main factors. Upon arrival at the broiler house, the chicks (body weight: 45.2 ± 0.37 g; means ± standard deviation) were randomly allocated to three dietary groups: CNTRL (chicks receiving basal diet as control), AGPS (chicks receiving basal diet supplemented with 0.04% zinc bacitracin) and PROBIO (chicks receiving basal diet supplemented with 0.5% *Bacillus* probiotic). Half of the chicks in each experimental diet were assigned to infection and the rest of the chicks were not infected. Hence, there were 6 groups of 84 chicks each (7 replicates of 12 chicks). The additives were added at the expense of the diets. The feeds and water were provided *ad libitum* throughout the study period (days 0 to 35). The basal diet was prepared (Table 1) to comply the national standard for broiler feed in Indonesia (SNI 2006). The basal feed was provided in mash form and contained no antibiotics, coccidiostat, enzymes or other feed additives/supplements. The probiotic preparation contained approximately 12.10 log cfu/g of four strains of *Bacillus* (i.e.,*B. cereus* SIIA_Pb_E3, *B. licheniformis* FJAT-29133, *B. megaterium F4-2-27and Bacillus spp. 11CM31Y12),* 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 (Isroli et al 2017; Sugiharto et al 2018a,b).

Table 1. Ingredients and nutrient composition (DM basis) of basal feed[1]

Items	Composition (%, unless otherwise noted)
Maize (CP 8.5%)	45.5
Soybean meal (CP 46%)	17.0
Wheat flour	10.0
Bread flour	5.00
Rice bran	4.45
Palm oil	3.50
Corn gluten meal	3.60
Distiller dried grains	3.00
Meat bone meal	2.80
Hydrolyzed chicken feather meal	2.00
Bone meal	1.50
Lysine	0.55
Methionine	0.37
L-threonine	0.08
Salt	0.15
Premix[2]	0.50
Calculated composition:	
Metabolizable energy (kcal/kg)[3]	3,200
Crude protein	22.0
Crude fat	5.00
Crude fiber	5.00
Ash	7.00

[1]Ingredients and nutrient composition of basal feed used in the current study was similar with that of used by Sugiharto et al (2018c)

[2]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, vit A 375 IU, vit D 150 IU, vit E 0.080mg

[3] Metabolizable energy was calculated based on formula (Bolton 1967) as follosw: 40.81 {0.87 [CP + 2.25 crude fat + nitrogen -free extract] + 2.5}

CP: crude protein

The chicks were vaccinated at day 0 with commercial Newcastle disease (ND) and avian influenza (AI) vaccines through intramuscular injections. At days 21, 23, 25 and 27, half of the birds within each diet were challenged with 0.5 mL of a culture containing 10^8 cfu/mL avian pathogenic *E. coli* (APEC) ATCC 8739. The infection was conducted by direct intra-tracheal instillation of challenge inoculum into the trachea of broiler chicks using a 1.0 mL syringe fitted with a blunt-ended pipette tip (Kaikabo et al 2017). At day 35, live body weight (BW) was recorded and a total of 42 birds (7 birds from each group/one bird from each replicate) were slaughtered. The chicks were eviscerated and cut into commercial portions. Internal organs were obtained and weighed, and for the determinations of pH and drip loss, breast meats were collected. The determinations of pH and drip loss of broiler meat were conducted according to Wang et al (2015). The meat was weighed and the pH was measured (with Eutech EcoTestr pH 1, Thermo Fisher, Singapore) at about 45 min after slaughter. The breast meat was then placed in a Whirl-Pak bag. After being stored in a refrigerator (4-5°C) for 24 h, the meat was reweighed and the pH was again determined. The drip loss was determined as the weight loss of meat after 24 h storing at the refrigerator. The drip loss are presented as percentage. The parameters measured were internal organs relative weight, commercial cuts, pH and drip loss of broiler meat. The data were analyzed according to a completely randomized design by analysis of variance using the General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). The pen was considered as the experimental unit. The differences among groups were further analyzed using Duncan's multiple-range test.

Results and discussion

Data in this study showed that liver relative weight was higher in PROBIO than in other treatment groups (Table 2). Our finding is in accordance with Awad et al (2009) and El-Faramaw et al (2016) showing the increased liver relative weight when feeding probiotic *Lactobacillus* sp. and mixed probiotics containing *L. casei, L. plantarum* and *Enterococcus faecium*, respectively, to broiler chickens. The reason for the increased liver relative weight is not exactly known, but the increase in liver glycogen due to probiotic treatment may be one possibility. In the study of Sharma et al (2016), it was apparent that treatment with probiotics *L. casei* and *Bifidobacterium bifidum* increased liver glycogen level and thus liver weight in Wistar rats. In the current study, the relative weight of liver was higher in the infected than that in non-infected broilers. Similar result was reported by Abalaka et al (2017), in which *E. coli* infection resulted in enlarged liver in broiler chickens. Moreover, Rocha et al (2013) showed an increased liver relative weight in broiler chicks challenged with *Salmonella* Typhimurium. The inflammation and necrotic lesions in the liver due to *E. coli* infections seemed to be responsible for the increased relative weight of liver in the present study (Abalaka et al 2017). There was a clear tendency (*P=0.06*) that APEC infection increased the relative weight of gizzard in this study. No definite explanation for the latter condition, but APEC infection was most likely to elicit stress that can induce gizzard erosions and ulcerations, and eventually increase the gizzard weight of broilers (Grabarević et al 1993). The substantial increase in the relative weight of gizzard has also been reported by Elaroussi et al (2006) when feeding mycotoxin ochratoxin A to broiler chickens. It has been shown in the present study that thymus relative weight tended (*P=0.09*) to be lower in PROBIO than in CNTRL and AGPS groups. This finding was consistent with Sugiharto et al (2018b) showing a lower thymus relative weight in broilers supplemented with *Bacillus* mixture as probiotics. The latter authors inferred that the lower relative weight of thymus may be associated with the less infections in PROBIO broilers. There was a tendency (*P=0.06*) that the relative weight of Bursa of Fabricius decreased in PROBIO chicks after challenge with APEC. Such decrease did not occur in other treatment groups. The decrease in Bursa of Fabricius was previously reported by Nakamura et al (1986) in broiler chicks after inoculation with *E. coli*. Considering its immune functions, the decreased relative weight of Bursa of Fabricius may be attributed to the impaired immune competences in broilers. In this study, the decreased relative weight of Bursa of Fabricius seemed not to harm the birds, as the relative weight of Bursa of Fabricius was still within the normal range. Olnood et al (2015) reported that the relative weight of Bursa of Fabricius in broiler chicks ranged from 0.12 to 0.19 %BW. There was a tendency (*P=0.09*) that infected birds had a lower proportion of abdominal fat pad as compared to non-infected birds. As an energy reserve, abdominal fat may be oxidized to produce energy. During infections, the birds need more energy for maintenance (recovery), while feed intake may be lowered (Kostadinović et al 2011). The latter conditions may increase lipolysis from the lipid depots and consequently lower the relative weight of abdominal fat in broilers.

Table 2. Internal organs relative weight of broilers

% live BW	CNTRL		AGPS		PROBIO		SEM	*p*		
	–	+	–	+	–	+		D	I	D*I
Heart	0.42	0.42	0.43	0.44	0.44	0.43	0.02	0.84	0.86	0.93
Liver	2.39^{by}	2.56^{bx}	2.43^{by}	2.63^{bx}	2.64^{ay}	2.85^{ax}	0.11	0.03	0.03	0.99
Proventriculus	0.52	0.53	0.46	0.51	0.51	0.49	0.03	0.41	0.60	0.46
Gizzard	1.53	1.64	1.42	1.59	1.62	1.87	0.12	0.11	0.06	0.82
Small intestine	2.64	2.38	2.14	2.58	2.46	2.63	0.18	0.51	0.39	0.12
Pancreas	0.32	0.31	0.28	0.30	0.30	0.29	0.02	0.56	0.98	0.61
Spleen	0.10	0.09	0.08	0.10	0.10	0.09	0.01	0.91	0.86	0.34
Thymus	0.38	0.36	0.39	0.39	0.31	0.31	0.04	0.09	0.81	0.99
Bursa of Fabricius	0.14	0.16	0.13	0.14	0.17	0.13	0.02	0.42	0.98	0.06
Abdominal fat	1.39	1.27	1.49	1.27	1.42	1.25	0.13	0.91	0.09	0.92

[a,b] *Means in the row with different letters show significant differences ($P<0.05$) among dietary treatments*
[x,y] *Means in the row with different letters show significant differences ($P<0.05$) between infected and non-infected birds*
CNTRL: chicks receiving basal diet as control; AGPS: chicks receiving basal diet supplemented with 0.04% zinc bacitracin; PROBIO: chicks receiving basal diet supplemented with 0.5% Bacillus probiotics; BW: body weight; (–): chicks not infected; (+): chicks infected with avian pathogenic E. coli; D: diets; I: infection

Irrespective of infection, birds in AGPS group had a higher proportion of thigh compared with PROBIO birds (Table 3). Izat et al (1990) reported that antibiotics (Bacitracin methylene disalicylate and virginiamycin) treatment increased dressing percentage and parts yield of broiler chickens. In the case of our study, the eviscerated carcass relative weight did not substantially differ among the treatment groups. Hence, the lowered relative weight of thigh in PROBIO birds seemed to be compensated by the increased relative weight of other parts such as breast and back (though the values did not reach significant levels).

Table 3. Commercial cuts of broilers

	CNTRL		AGPS		PROBIO		SEM	*p*		
	–	+	–	+	–	+		D	I	D*I
	% live weight									
Eviscerated carcass	64.9	64.4	65.1	61.0	65.2	67.1	1.68	0.20	0.53	0.22
	% Eviscerated carcass									
Breast	32.6	33.8	32.8	29.5	34.2	32.2	1.89	0.46	0.39	0.47
Thigh	17.6^{ab}	16.9^{ab}	18.0^{a}	18.3^{a}	16.3^{b}	17.1^{b}	0.58	0.04	0.84	0.46
Drumstick	14.5	14.4	14.6	15.4	14.3	13.9	0.50	0.21	0.84	0.50
Wing	11.7	11.6	11.5	12.6	11.7	11.3	0.43	0.42	0.59	0.20
Back	23.7	23.3	23.0	24.2	23.4	25.5	0.90	0.52	0.20	0.39

[a,b] *Means in the row with different letters show significant differences ($P<0.05$) among dietary treatments CNTRL: chicks receiving basal diet as control; AGPS: chicks receiving basal diet supplemented with 0.04% zinc bacitracin; PROBIO: chicks receiving basal diet supplemented with 0.5% Bacillus probiotics; (–): chicks not infected; (+): chicks infected with avian pathogenic E. coli; D: diets; I: infection*

Data in our present study showed that, irrespective of infection, meat from CNTRL birds had lower ($P<0.01$) pH values at 45 min and 24 hours after slaughter compared with AGPS and PROBIO birds. Meat from CNTRL birds also had higher ($P<0.05$) drip loss, when compared with other birds (Table 4). pH of meat has been used as an indicator of meat quality, in which the low pH may implicate in negative effect on the water holding capacity (WHC) and processing characteristics of meat (Petracci et al 2015).Note that low WHC implies in the abnormalities of meat such as pale-soft-and-exudative (PSE)-like meat. It has been shown that low pH value was associated with protein degradation and lower shear force in meat (Huang et al 2016). In this regard, breast meat from AGPS and PROBIO chicks may be supposed to have better nutritional characteristics especially with regard to protein content in meat, as compared to CNTRL meat. In this study, the lower pH value in CNTRL meat was in parallel with the higher drip loss in CNTRL meat.

The latter condition seemed to be related to the low WHC in meat as Bowker and Zhuang (2015) reported that drip loss was greater in broiler meat with low WHC.

Table 4. pH and drip loss of broiler meats

Items	CNTRL		AGPS		PROBIO		SEM	*p*		
	–	+	–	+	–	+		D	I	D*I
pH 45 min	5.97^{b}	6.13^{b}	6.14^{a}	6.23^{a}	6.29^{a}	6.27^{a}	0.06	<0.01	0.11	0.32
pH 24 h	5.77^{b}	5.80^{b}	5.93^{a}	5.90^{a}	5.96^{a}	5.94^{a}	0.05	<0.01	0.90	0.83
Drip loss (%)	5.68^{a}	5.71^{a}	4.03^{ab}	5.61^{ab}	3.96^{b}	3.88^{b}	0.72	0.04	0.33	0.40

^{a,b} Means in the row with different letters show significant differences ($P<0.05$) among dietary treatments
CNTRL: chicks receiving basal diet as control; AGPS: chicks receiving basal diet supplemented with 0.04% zinc bacitracin; PROBIO: chicks receiving basal diet supplemented with 0.5% Bacillus probiotics; (–): chicks not infected; (+): chicks infected with avian pathogenic E. coli; D: diets; I: infection

Conclusions

- Feeding *Bacillus* probiotic increased the liver relative weight, prevented the decrease in meat pH and reduced the drip loss of broiler meats.
- Hence, *Bacillus* probiotic has potential to improve the meat quality of broilers.

Acknowledgements

The experiment was funded by the Directorate of Research and Community Service, the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through "Penelitian Terapan Unggulan Perguruan Tinggi" No. 101-110/UN7.P4.3/PP/2018, 05 February 2018.

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Received 29 August 2018; Accepted 14 September 2018; Published 1 November 2018

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