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Intestinal microbial ecology and serum biochemistry of avian pathogenic *Escherichia coli* infected broilers fed multistrain probiotic preparation

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Abstract

The study aimed to investigate the effect of multistrain probiotic preparation on intestinal microbial ecology and serum biochemistry in broilers infected with avian pathogenic *Escherichia coli* (APEC). A total of 504 one-day-old chicks were grouped to three experimental diets, including CTRL (basal diet without any additive), AGP (basal diet supplemented with 0.04% antibiotic zinc bacitracin) and PROB (basal diet supplemented with 0.5% multistrain probiotic preparation). Within each diet, half of the chicks were infected with APEC (at days 21, 23, 25 and 27) and the other half were not infected. At day 35, blood, ileal segments and digesta were collected for parameter assessment. Irrespective of infection, PROB birds had a higher ($P < 0.05$) ileal villi height than CTRL birds. Coliform population was lower ($P = 0.08$) in AGP-non-infected when compared with CTRL-infected birds. Birds infected with APEC had higher populations of lactose negative-enterobacteria ($P = 0.08$) and enterobacteria ($P = 0.07$) in the ileum, when compared with non-infected birds. Compared to AGP, birds in CTRL group had higher ($P < 0.05$) lactic acid bacteria (LAB) count. The ileal LAB number was higher ($P < 0.05$) in infected than in non-infected birds. In caecum, the number of *Clostridium perfringens* tended ($P = 0.09$) to be higher in infected than in non-infected birds. Birds in PROB tended ($P = 0.08$) to have lower aspartate aminotransferase (AST) level than birds in AGP group. Creatinine level in CTRL chicks increased ($P = 0.07$) following APEC infection. Total triglycerides were higher ($P < 0.05$) in CTRL-infected and AGP-non-infected birds than in other birds. The levels of total cholesterol and low-density lipoprotein (LDL) increased ($P < 0.05$) in CTRL birds after APEC infection, while they decreased in PROB birds following infection. The high-density lipoprotein (HDL) was lower ($P < 0.05$) in PROB-infected than in CTRL-infected and AGP birds. Albumin level was higher ($P < 0.05$) in CTRL-infected than in non-infected birds. In conclusion, feeding multistrain probiotic preparation improved intestinal morphology as indicated by the increased ileal villi height. The probiotic was also potential in alleviating the adverse effect of APEC infection in broilers.

Keywords: ileal villi, infection, poultry

Introduction

Colibacillosis, which is *Escherichia coli* infections, remains a considerable threat to broiler farmers worldwide as it increased the morbidity and mortality of birds (Mbanga and Nyararai 2015). The infection has been associated with the disruption of intestinal morphology (e.g. villous atrophy) and microbial balance of broiler chickens (Cao et al 2013). Moreover, the physiological and biochemical changes have also been attributed to *E. coli* infection in broiler chickens (Sharma et al 2015). These aforementioned conditions may implicate in growth retardation and mortality (Cao et al 2013; Sharma et al 2015). In addition to promoting growth rate, antibiotic growth promoter (AGP) was commonly used to control colibacillosis in broilers in the earlier time (Hampson and Murdoch 2003). Owing to phenomenon of antibiotic resistance, the use of AGP in broiler rations is, however, no longer permitted in most countries (Sugiharto 2016). Indeed, the withdrawal of AGP from broiler diets have been attributed to retarded growth rate and a rise in the incidence of enteric diseases including colibacillosis in broiler chickens (Huyghebaert et al 2011; Sugiharto 2016). Hence, any alternatives to AGP are crucial for the safe and sustainable broiler production worldwide.

Several alternatives have been proposed to substitute the role of AGP in broiler diets, one of which is probiotics. This living microorganism may improve the health and performance by modulating the intestinal microbiology and immunology as well as improving the digestibility and metabolism of broilers (Sugiharto 2016). In the present study, we included multistrain probiotic preparation in the diets as an alternative to AGP 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 2018ab). The potential of such additive in alleviating the adverse effect of *E. coli* infection in broilers has, however, never been documented. The aim of this present study was therefore to investigate the effect of multistrain probiotic preparation on intestinal microbial ecology and serum biochemistry in broilers infected with avian pathogenic *Escherichia coli* (APEC).

Materials and methods

The rearing and treating of chicks were performed according to the standard procedures of rearing and treating of livestock declared in law of the Republic of Indonesia number 18, 2009 concerning animal husbandry and health. A total of 504 one-day-old chicks (Lohmann meat broilers; BW= 45.2 ± 0.37 g; means ± standard deviation) were used in the present study. The experiment was arranged as 3 × 2 factorial design with diets (CTRL [basal diet without any additive], AGP [basal diet supplemented with 0.04% antibiotic zinc bacitracin] and PROB [basal diet supplemented with 0.5% multistrain probiotic preparation]) and infection (not infected or infected with APEC) as main factors. The probiotic was added (“on top”) at the expense of the diets. The chicks were raised in an open-sided broiler house with rice husk as bedding material. Light was available for the entire day, and the feeds and water were provided *ad libitum*. The basal diet was prepared in mash form and formulated (Table 1) according to the Indonesian National Standards for Broiler Feed (SNI 2006). The feed contained no coccidiostat, enzyme and other additives. The multistrain probiotic preparation contained 12.10 log cfu/g multistrains *Bacillus* (i.e., *B. cereus* strain SIIA_Pb_E3, *B. licheniformis* strain FJAT-29133, *B. megaterium* strain F4-2-27 and *Bacillus spp. 11CM31Y12*), 0.100 mg vitamin A, 0.018 mg vitamin D₃, 0.100 mg vitamin 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 2018abc).

Table 1. Ingredients and chemical composition of basal diet

Items (% , unless otherwise noted)	Composition
Maize (crude protein 8.5%)	45.5
Soybean meal (crude protein 46%)	17.0
Wheat flour	10.0
Bread flour	5.00
Rice bran	4.45
Crude 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 ¹	0.50
Calculated composition:	
Metabolizable energy (kcal/kg) ²	3,200
Crude protein	22.0
Crude fat	5.00
Crude fibre	5.00
Ash	7.00

¹Premix 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.²Metabolizable energy was calculated according to formula (Bolton, 1967) as follow: $40.81 \{0.87 [\text{crude protein} + 2.25 \text{ crude fat} + \text{nitrogen-free extract}] + 2.5\}$

The chicks were vaccinated at day 1 with commercial Newcastle disease vaccine (NDV) through spraying. 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 APEC ATCC 8739. The challenge culture of APEC was prepared by retrieving the stock culture (purchased from Microbiologics, MediMark Europe) on nutrient agar at 38°C for 24 h. The grown culture was then regrown for 24 h at 38°C on nutrient agar. The incubated plate was harvested with 10 ml peptone water, and the suspension was diluted 1:25 (v/v) with peptone water to obtain the inoculum of 1×10^8 cfu/ml (Sugiharto et al 2012). The infection was conducted by direct intratracheal instillation of challenge inoculum into the trachea of broiler chicks using a 1.0 ml syringe fitted with a blunt-ended pipette tip (Ask et al 2006; Kaikabo et al 2017). At day 35, blood was collected from the bird's wing veins of chicks (total 42 chicks; 7 chicks from each group). The blood was placed in the anticoagulant-free vacutainers, allowed to clot at room temperature and centrifuged at 2,000 rpm for 15 min. The serum produced was then used for the analyses of antibody titers and serum biochemistry. The same chicks as blood sampled were slaughtered and eviscerated. For the determinations of villi height and goblet cell number, a piece (about 2 cm) of ileal segment was obtained and placed in 10% neutral formalin buffer solution. Further, digesta was collected from ileum and caecum (by gently squeezing) into the sterile sample bottles for the enumeration of bacterial population.

For the histological analysis, 5 µm slices of ileum were prepared and stained with hematoxylin and eosin. The villus height of each ileal segment were measured using an optical microscope fitted with a digital camera. The number of goblet cell per villus was enumerated and the villus area was acquired from 3 villi on each of 2 tissue pieces per bird. The 6 measurements were applied to get a mean goblet cell number per bird. The numbers of selected bacteria in ileum and caecum of broilers were determined based on Sugiharto et al (2017) with few modifications. The numbers of coliform bacteria and lactose-negative enterobacteria were determined on MacConkey agar (Merck KGaA, Darmstadt, Germany) as red and colorless colonies, respectively, following aerobic incubation at 38°C for 24 h. Enterobacteria were determined as the number of coliform and lactose-negative enterobacteria. The colonies of lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe (MRS; Merck KGaA) agar after anaerobic incubation at 38°C for 48 h. The population of *Clostridium perfringens* was enumerated on tryptose sulphite cycloserine (TSC; Merck KGaA) agar plates following anaerobic incubation at 38°C for 48 h. The serum antibody titer against NDV was determined based on the hemagglutination inhibition (HI) test (Villegas1987). The antibody titer is expressed as geometric mean titer (Log2). The serum levels of total triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol, uric acid and creatinine were determined according to the enzymatic colorimetric/colour methods. The determinations of serum total protein, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were conducted based on the spectrophotometric/photometric tests. The value of globulin was obtained from the difference between total protein and albumin in serum. The above mentioned serum biochemical analyses were carried out using kits (DiaSys Diagnostic System GmbH, Holzheim, Germany) following the manufacturer's instructions.

The data were analyzed based on a factorial ANOVA (with two factors) using the General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Interaction between the two factors was initially included in the statistical model, but it was excluded when there was no significant effect. The Duncan's multiple-range test was applied when the significant ($P < 0.05$) differences among groups were observed.

Results

The data of ileal villi height and goblet cell number per villus of broilers are presented in Table 2. Irrespective of infection treatment, PROB birds had a higher ($P < 0.05$) ileal villi height as compared to CTRL birds, but the difference was not significant when compared with AGP birds. Both diets and infection had no effect ($P > 0.05$) on the goblet cell number per villus. There was no interaction ($P > 0.05$) between both experimental factors.

Table 2. The ileal villi height and goblet cell number per villus of broilers

Items	CTRL		AGP		PROB		SE	P value		
	-	+	-	+	-	+		D	I	D*I
Villi height (µm)	645 ^b	585 ^b	665 ^{ab}	637 ^{ab}	726 ^a	757 ^a	50.7	0.04	0.64	0.66
Goblet cells numbers per villus	96.3	112	123	59.5	118	160	35.1	0.42	0.99	0.21

^{a,b} Means in the same row with different letters show significant differences ($P < 0.05$) across experimental diets CTRL: basal diet without any additive; AGP: basal diet supplemented with 0.04% antibiotic zinc bacitracin; PROB: basal diet

supplemented with 0.5% multistrain probiotic preparation; (-): chicks not infected; (+): chicks infected with APEC; SE: standard error; D: diets; I: infection; D*I: interaction between diets and infection

The data on the selected bacterial populations in ileum and caecum of broiler chicks are presented in Table 3. The interaction between diets and infection tended ($P=0.08$) to affect the number of coliform bacteria in the ileum of broilers, in which coliform population was lower in AGP-non-infected when compared with CTRL-infected birds. Irrespective of the diet effect, there was a tendency that birds infected with APEC had higher populations of lactose negative-enterobacteria ($P=0.08$) and enterobacteria ($P=0.07$) in the ileum, when compared with non-infected birds. Compared to AGP birds, the birds in CTRL group had higher ($P<0.05$) LAB population, but the difference was not significant compared to PROB birds. The ileal LAB population was higher ($P<0.05$) in infected than in non-infected birds. In caecum, the number of *C. perfringens* tended ($P=0.09$) to be higher in infected than in non-infected birds.

Table 3. The bacterial populations in ileum and caecum of broilers

Items	CTRL		AGP		PROB		SE	P value		
	-	+	-	+	-	+		D	I	D*I
Ileum (log cfu/g)										
Coliform	9.02	9.45	8.64	9.34	9.29	8.76	0.28	0.65	0.39	0.08
Lactose negative-enterobacteria	10.1	10.3	10.0	10.3	10.2	10.3	0.12	0.93	0.08	0.75
Enterobacteria	10.2	10.4	10.1	10.4	10.3	10.3	0.10	0.76	0.07	0.50
LAB	7.66 ^{a†}	8.26 ^{a‡}	6.18 ^{b†}	7.70 ^{b‡}	6.93 ^{ab†}	7.79 ^{ab‡}	0.35	0.02	0.01	0.40
<i>C. perfringens</i>	3.50	3.64	3.87	3.41	3.76	3.52	0.46	0.98	0.61	0.80
Caecum (log cfu/g)										
Coliform	9.01	8.52	8.84	8.99	9.01	9.60	0.43	0.43	0.81	0.46
Lactose negative-enterobacteria	10.4	10.2	9.96	10.1	10.3	10.1	0.17	0.41	0.64	0.51
Enterobacteria	10.4	10.2	10.0	10.2	10.3	10.3	0.15	0.21	0.99	0.62
LAB	8.27	7.98	8.49	8.62	8.03	8.27	0.33	0.33	0.92	0.70
<i>C. perfringens</i>	5.14	5.53	4.70	6.35	5.08	5.10	0.49	0.68	0.09	0.23

^{a,b} Means in the same row with different letters show significant differences ($P < 0.05$) across experimental diets^{†,‡} Means in the same row with different marks show significant differences ($P < 0.05$) between infection groups CTRL: basal diet without any additive; AGP: basal diet supplemented with 0.04% antibiotic zinc bacitracin; PROB: basal diet supplemented with 0.5% multistrain probiotic preparation; (-): chicks not infected; (+): chicks infected with APEC; LAB: lactic acid bacteria; SE: standard error; D: diets; I: infection; D*I: interaction between diets and infection

The data on serum biochemical parameters and antibody titer against NDV of broilers are presented in Table 4.

Table 4. The serum biochemical parameters and antibody titer against NDV in broilers

Items	CTRL		AGP		PROB		SE	P value		
	-	+	-	+	-	+		D	I	D*I
AST (U/L)	188	208	248	204	192	184	16.7	0.08	0.42	0.17
ALT (U/L)	0.67	1.10	1.41	1.04	1.09	1.18	0.28	0.45	0.83	0.36
Uric acid (g/dL)	5.82	7.52	6.81	6.78	5.78	6.57	0.69	0.63	0.15	0.46
Creatinine (g/dL)	0.07	0.11	0.08	0.06	0.08	0.10	0.01	0.17	0.22	0.07
Total triglyceride (g/dL)	76.2 ^b	101 ^a	102 ^a	78.7 ^b	78.7 ^b	92.0 ^{ab}	7.54	0.79	0.42	0.01
Total cholesterol (g/dL)	134 ^c	168 ^a	153 ^{abc}	146 ^{bc}	158 ^{ab}	133 ^c	7.36	0.73	0.89	<0.01
LDL (g/dL)	55.9 ^b	73.0 ^a	59.7 ^{ab}	58.9 ^{ab}	74.3 ^a	57.1 ^b	6.05	0.54	0.95	0.02
HDL (g/dL)	62.7 ^{ab}	75.1 ^a	73.1 ^a	70.9 ^a	67.4 ^{ab}	57.3 ^b	4.42	0.09	1.00	0.04
Total protein (g/dL)	4.20	4.92	5.29	5.09	4.74	4.63	0.41	0.27	0.69	0.48
Albumin (g/dL)	1.50 ^b	1.68 ^a	1.63 ^{ab}	1.56 ^{ab}	1.59 ^{ab}	1.56 ^{ab}	0.05	0.95	0.59	0.04
Globulin (g/dL)	2.71	3.24	3.66	3.54	3.14	3.07	0.40	0.25	0.73	0.66
A/G ratio	0.57	0.53	0.51	0.48	0.51	0.52	0.04	0.39	0.51	0.83
Antibody titer (Log ₂ GMT)	2.86	2.43	2.00	1.29	1.86	3.43	0.67	0.24	0.80	0.18

^{a,b,c} Means in the same row with different letters show significant differences ($P < 0.05$) among diets and challenge treatments CTRL:

basal diet without any additive; AGP: basal diet supplemented with 0.04% antibiotic zinc bacitracin; PROB: basal diet supplemented with 0.5% multistrain probiotic preparation; (-): chicks not infected; (+): chicks infected with APEC; NDV: Newcastle disease virus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDL: low-density lipoprotein; HDL: high-density lipoprotein; A/G ratio: albumin to globulin ratio; GMT: geometric mean titer; SE: standard error; D: diets; I: infection; D*I: interaction between diets and infection

Irrespective of the challenge treatment, birds in PROB tended ($P=0.08$) to have lower AST level than birds in AGP group. The interaction between diets and infection tended ($P=0.07$) to affect the creatinine level of birds, in which creatinine level in CTRL chicks increased following APEC infection. Total triglyceride level was higher ($P<0.05$) in CTRL-infected and AGP-non-infected birds, when compared with other birds. The levels of total cholesterol and LDL increased ($P<0.05$) in CTRL birds after APEC infection, while they decreased ($P<0.05$) in PROB birds following infection. The level of HDL was lower ($P<0.05$) in PROB-infected than in CTRL-infected and AGP birds. The serum albumin level was higher ($P<0.05$) in CTRL-infected than in CTRL-non-infected birds, but the significant difference was not observed when compared with the other treatment birds. Diets, infections and their interaction had no substantial effects with regards to serum levels of ALT, uric acid, total protein, globulin, albumin to globulin ratio (A/G) ratio and antibody titer against NDV.

Discussion

Dietary inclusion of multistrain probiotic preparation was subjected to ameliorate the negative effects of APEC infection in broiler chickens. Our present findings showed that irrespective of APEC infection, feeding multistrain probiotic preparation resulted in increased ileal villi height in broilers, when compared with control. The increase in villi height across the small intestinal segments (duodenum, jejunum and ileum) has also been reported by Lei et al (2015) when feeding *B. amyloliquefaciens*-based probiotic to broiler chickens. The mechanism through which probiotics increased the ileal villi height in the current study was not exactly known, but the reduced epithelial cell turnover caused by inflammation and toxins from pathogens may result in better villus height (Lei et al 2015). In this study, the number of goblet cell per villus were not different among the treatments, though goblet cell number per villus was numerically higher in PROB than in other chicken groups. Corresponding to this, Shah et al (2018) reported that probiotic mixture increased the number of goblet cell in duodenum of broilers as compared to control. This may implicate in thicker mucus layer which is beneficial in protecting the intestinal mucosa of broilers.

There was a tendency that the number of coliform bacteria was lower in AGP-non-infected when compared with CTRL-infected birds. The exact rationale for the latter condition was not clear, but the antibacterial activity of zinc bacitracin against coliform bacteria may be responsible for lowering the ileal coliform count. This interpretation should, however, be noted with caution as the antibacterial activity of zinc bacitracin was not effective in lowering the ileal coliform count in AGP-infected birds. Irrespective of dietary treatment, the counts of lactose negative-enterobacteria and enterobacteria tended to be higher in APEC infected than in non-infected birds. This result was in accordance with Cao et al (2009) showing a higher *E. coli* count in the intestine of birds challenged with *E. coli* K88 than in unchallenged birds. Also, Engberg et al (2012) showed increased coliform and lactose negative-enterobacteria counts in the ileum of *C. perfringens*-challenged broilers. It is shown in this study that LAB population was lower in AGP than in CTRL chicks, irrespective of the infection treatment. This finding was in agreement with Li et al (2017) reporting that antibiotic zinc bacitracin decreased the population of LAB in the ileum of broilers. The antibacterial activity of zinc bacitracin especially against gram-positive bacteria (including LAB) may be responsible for the lowered ileal LAB population in AGP birds in the present study. Irrespective of diets, infection with APEC resulted in higher count of LAB in the ileum of broilers in the present study. Corresponding to this, Engberg et al (2012) showed higher LAB count in the caeca of *C. perfringens*-challenged broilers when compared with that in non-challenged birds. There was no definite explanation for the above conditions, but the impaired digestive functions of the proximal small intestine (duodenum and jejunum) due to infections may allow more undigested carbohydrates entering ileum (Engberg et al 2012). The latter products may eventually become substrates for the growth of LAB in ileum of broilers. In caecum, the population of *C. perfringens* tended to be higher in APEC infected than in non-infected birds. Concomitantly, Cao et al (2009) reported that 28 days of age broilers infected with *E. coli* K88 had higher population of caecal *C. perfringens* when compared with that in non-infected broilers. The greater amounts of undigested nutrients reaching the caecum (due to disrupted digestive functions of the small intestine related to infections) may increase the available substrates for the growth of *C. perfringens* in the caecum of infected birds (Engberg et al 2012).

Irrespective of challenge treatment, the level of AST was lower in PROB birds than in AGP birds. Sharma et al (2015) noticed that the level of AST increased in birds after infection with *E. coli* O78. However, this may not be the case in our present study as APEC infection did not substantially affect the level of serum AST. Indeed, the increased serum AST level has also been attributed to the stress condition in broilers. Bueno et al (2017) showed that the serum level of AST was significantly higher in broilers raised under cyclic heat stress as compared to those raised under thermal comfort. Owing to the latter report, PROB broilers seemed to be less stressed than AGP broilers, given that *Bacillus*-based probiotics may ameliorate heat-induced behavioural and inflammatory reactions (Wang et al 2018). Note that the birds in our study were raised in an open sided broiler house with the average temperature of $34 \pm 1^\circ\text{C}$ during the day. In CTRL chicks, the serum creatinine level tended to increase following APEC infection, and such increase was not appeared in the other dietary groups. Mahmoud (2015) and Ismail (2017) revealed that the increased level of serum creatinine was associated with the avian influenza viruses and NDV infections in ducks and chickens, respectively. Also, the increase in serum creatinine level was attributed to the challenge with fumonisin B1 (mycotoxin produced by *Fusarium* species) in broiler chicks (Deepthi et al 2017). In these regards, AGP and multistrain probiotic preparation seemed to alleviate the adverse effects of APEC infection in broilers. In CTRL group, serum total triglyceride level increased with APEC infection. This finding was in concomitant with Manafi et al (2017) reporting that *E. coli* challenge increased plasma triglyceride level of broilers. Also, infection with hydropericardium syndrome virus increased the serum triglyceride level of broilers in the study of Nidhi et al (2010). Unlike in CTRL, birds in AGP group had lower serum triglyceride level after APEC infection. This result was in accordance with Manafi et al (2016) revealing that birds fed bacitracin methylene disalicylate and infected with *E. coli* had lower plasma triglyceride level when compared with non-infected birds fed bacitracin. In this present study, serum total cholesterol and LDL levels increased in CTRL birds after APEC infection. Our present findings corresponded to Manafi et al (2017) showing that inoculation with *E. coli* serotype PTCC-1399 resulted in increased cholesterol and LDL levels in blood plasma of broilers. Different from those in CTRL group, the serum levels of cholesterol and LDL in PROB group decreased after APEC infection. Previous study showed that treatment with *B. subtilis* decreased plasma levels of cholesterol and LDL in broiler chickens (Manafi et al 2017). Referring to the latter study, it may be suggested that multistrain probiotic preparation could prevent the increased levels of cholesterol and LDL due to APEC infection. However, this inference should be taken with caution as the serum levels of cholesterol and LDL were significantly higher in non-infected PROB than in non-infected CTRL birds. Indeed, Novak et al (2011) noted that feeding *Bacillus*-based probiotics elevated the serum level of LDL and did not affect the cholesterol level. The different strains of *Bacillus* probiotics and conditions during the experiment may explain these divergent results above. In this study, the level of HDL was lower in PROB-infected than in CTRL-infected and AGP birds. This result was concomitant with Novak et al (2011) reporting that probiotic *Bacillus* lowered HDL cholesterol of broilers, which was unfavorable for broiler chickens. Our present result was, however, different from Manafi et al (2017) showing an increased HDL in broilers fed probiotic *B. subtilis*. The reason for these conflicting data was not known, but the different strains and conditions during experiment may be responsible. Our present data showed that serum albumin level was higher in CTRL-infected than in CTRL-non-infected birds. It has been revealed in the earlier study that *E. coli* infection was associated with the increased plasma level of albumin in broiler chickens (Manafi et al 2017). Conversely, Manafi et al (2016) found that *E. coli* infection resulted in lowered plasma albumin level. In the study of Deepthi et al (2017), it was noticed that challenge with fumonisin B1 increased the level of albumin in serum of broilers. In the case of APEC infection, the challenge bacteria may produce enterotoxin (Nakazato et al 2009) that may exert a similar effect as fumonisin B1 in increasing the serum level of albumin.

Conclusion

- Feeding multistrain probiotic preparation improved intestinal morphology as indicated by the increased ileal villi height.
- The probiotic was also potential in alleviating the adverse effects of APEC infections in broilers.

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