

Palembang, 14 - 15 November 2017

The 9<sup>th</sup>

# ISISM

(International Seminar of  
Indonesian Society for  
Microbiology)

## WORK BOOK

*"Optimising Microbe  
Utilization for Human  
Welfare"*

HOTEL HORISON ULTIMA, PALEMBANG  
SOUTH SUMATERA PROVINCE, INDONESIA

## **Performance, Haematological Parameters Of Broilers Fed Commercial Diets Supplemented With Novel Probiotic *Bacillus* plus Vitamins And Minerals**

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### **ABSTRACT**

*The aim of this study was to investigate the effect of supplementation with novel probiotic Bacillus plus vitamins and minerals on growth performance, haematological parameters and carcass traits of broilers fed standard commercial feeds. Two hundred and eighty eight 1-day-old broiler chicks were randomly allocated to four groups, including control birds (birds receiving standard commercial feed without additional supplement) and birds receiving standard commercial feeds supplemented with probiotic Bacillus plus vitamins and minerals either 0.1%, 0.5% or 1%. Supplementation did not affect ( $p>0.05$ ) the final body weight, feed intake and feed conversion ratio (FCR) of broilers. Dietary supplementation of 0.1 and 0.5% probiotic Bacillus plus vitamins and minerals reduced ( $p\leq 0.05$ ) heart relative weight of broilers. Supplementation tended ( $p=0.07$ ) to increase the relative weight of ileum and pancreas of broilers. Supplemented birds had lower ( $p\leq 0.05$ ) numbers of leukocytes and eosinophils compared to unsupplemented birds. There were tendencies that supplementation with probiotic Bacillus plus vitamins and minerals resulted in lower ( $p=0.07$ ) counts of lymphocytes and heterophils when compared with no supplementation. Supplementation of 0.5% probiotic Bacillus plus vitamins and minerals resulted in lower ( $p\leq 0.05$ ) serum concentration of uric acid when compared with control. There was no significant effect of supplementation on carcass traits, pH and drip loss of broiler meats. In conclusion, supplementation of standard commercial broiler feeds especially with 0.5% probiotic Bacillus plus vitamins and minerals increased the relative weight of ileum and pancreas, lowered counts of leukocytes, eosinophils, heterophils and lymphocytes and reduced serum concentration of uric acid.*

**Keywords:** broilers, commercial feeds, minerals, probiotics, vitamins

### **1. Introduction**

For several decades, Indonesian broiler farmers rely on the commercial feeds produced by feed mill industries. Although commercial broiler feeds have been formulated to meet the standard nutritional requirements of the birds, most feeds often failed to comply with the goal of broiler farmers in term of target final body weight as compared to genetic potential for growth of the modern broiler chicks. The change in the composition of feeds especially vitamins and minerals due to bad feed handling on the farm (such as storage conditions and length of time in farm feed bins) may be responsible for the latter condition (Aviagen, 2014). Apart from feed problem, the retarded growth during brooding period due to the delayed feed and water access post hatch (because of logistic operations and transportation from hatchery to commercial farms) may also limit the growth potential of modern broiler strains (Bhanjaet al., 2010; Wang et al., 2014). To optimize the growth potential of modern broilers in the commercial farms, supplementing the standard commercial broiler feeds with growth promoters (Rahman et al., 2012), probiotics (Anjumet al., 2005), minerals and vitamins (Islam et al., 2004; Ajuwon et al., 2011; Harun-Ar-Rashid et

al., 2015), green feeds (Etelaet al., 2007), enzymes (Fernandes et al., 2015) or certain amino acids (Murakami et al., 2012) has been conducted.

With regard to probiotics, vitamins and minerals, these components have been known to exert immune-enhancing effect on poultry (Sanda, 2015; Sugiharto, 2016). Hence, supplementation of standard commercial broiler feeds with probiotics simultaneously with vitamins and minerals seemed to be beneficial not only for better growth, but also for health of broiler chicks on the commercial farms. Commercial broiler chickens have been attributed to the excessive fat deposition, especially in the abdomen area (Fouad and El-Senousey, 2014). This abdominal fat content may reduce carcass yield of broilers, and any attempt to decrease the abdominal fat pad in broiler chicks will therefore be valuable. Feeding probiotics has been reported able to reduce fat content (Fouad and El-Senousey, 2014) as well as improve carcass traits of broiler chickens (Shabaniet al., 2012). Certain vitamins, for example vitamin E (Zaboliet al., 2013; Albuquerque et al., 2017) and vitamin A (Kucuket al., 2003) have also been reported to decrease the abdominal fat content and enhance carcass mass of broilers. Concomitantly, dietary supplementation with mineral such as Mn (Lu et al., 2006; Ghosh et al., 2016), Zn (Kucuket al., 2003) and S (Nikoofard et al., 2016) was beneficial to decrease the abdominal fat deposition in broiler chickens. Moreover, supplementation of broiler feeds with Cu, Fe, Zn, and Mn improved meat quality of broilers (Yang et al., 2011). On this basis, feeding probiotics together with certain vitamins and minerals may be expected to reduce the fatness and improve carcass characteristics of broilers.

It has been shown in our previous study that supplementation of standard commercial broiler diets with novel probiotic *Bacillus* plus vitamins and minerals enhanced haemoglobin levels, promoted the development of ileum and improved feed efficiency of broiler during brooding period (Isroliet al., 2017). Hence, it would be interesting to investigate the effect of such dietary treatment on the haematological profile and performances of broiler chickens at later age. Taken together, the present study aimed to investigate the effect of supplementation with novel probiotic *Bacillus* plus vitamins and minerals on growth performance, haematological parameters and carcass traits of broiler chicks fed standard commercial feeds.

## 2. Materials and methods

A total of 288 unsexed Lohmann (MB-202) 1-day-old broiler chicks (body weight =  $45.9 \pm 0.50$  g; means  $\pm$  standard deviation) were employed in the present study. To simulate the real condition of commercial broiler chicks handling in Indonesia (exerting growth retardation effect on broiler chicks), all chicks were deprived from feed and water for 24 h after hatching. In an open-sided broiler house, broiler chicks were randomly allocated to one of four treatment groups of 72 chicks each (6 replicates of 12 chicks). The groups were control birds (birds receiving standard commercial feed without additional supplement) and birds receiving standard commercial feeds supplemented with probiotic *Bacillus* plus vitamins and minerals either 0.1%, 0.5% or 1%. The feeds were obtained from the local feed mill company. The chemical compositions of feed are presented in Table 1. The feeds and water were provided *ad libitum* throughout the study. The supplement was added at the expense of the feeds. The supplement (novel 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), 0.100 mg vit A, 0.018 mg vit D<sub>3</sub>, 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 *Bacillus* strains were isolated from the rumen content of cow and showed probiotic potentials based on their antimicrobial activities (against *Escherichia coli* and *Staphylococcus aureus*) and tolerance to gastrointestinal conditions (Isroli et al., 2017).

At day 4 and 21 of the experiment, the chicks were vaccinated with commercial Newcastle disease virus (NDV) vaccine through eyedrops and drinking water, respectively. Body weight and feed consumption of broilers were recorded weekly. At day 28, blood was obtained from the bird's wing veins and collected in ethylenediaminetetraacetic acid (EDTA)-containing vacutainers for the determination of haematological profile. The rest of the blood was collected in the vacutainers with no anticoagulant. The latter blood was let to clot at room temperature, and centrifuged at 2,000 rpm for 15 min to obtain serum. The serum was frozen until the determination of antibody titers and biochemical analyses. At day 42, the chicks were slaughtered, de-feathered and eviscerated. Directly, the internal organs were removed and weighed. Samples of breast muscle were collected for the determination of pH and drip loss.

The haematological profile (complete blood counts) was determined with a hematology analyzer (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). Antibody titers to NDV vaccine were determined in serum based on haemagglutination inhibition (HI) assay (Villegas, 1987). The titers were expressed as geometric mean titers ( $\log_2$ ). Total triglyceride in the serum was determined based on enzymatic colorimetric method using glycerol-3-phosphate oxidase (DiaSys Diagnostic System GmbH, Holzheim, Germany). Total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were determined according to enzymatic colorimetric method with cholesterol oxidase/p-aminophenazone (DiaSys Diagnostic System GmbH, Holzheim, Germany). The enzymes of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured spectrophotometrically with a Reflotron system (Roche Diagnostics Corporation, Indianapolis, IN, USA). Serum total protein was determined by photometric test based on the biuret method with the kit (total protein kit, DiaSys Diagnostic System GmbH, Holzheim, Germany) according to the manufacturer's instructions. Albumin in serum was measured by photometric test using bromocresol green (DiaSys Diagnostic System GmbH, Holzheim, Germany). Data of globulin was obtained by subtracting albumin values from total protein in serum. Uric acid in the serum was determined according to the enzymatic color test. pH and drip loss of broiler meats were measured based on Sugiharto et al. (2017) with little modifications. The meats were weighed and the pH was determined around 45 min following slaughter. The meats were then put in a Whirl-pak bag, stored in a refrigerator (5°C) and reweighed after 24 h. The pH of meat was also measured at that time. The meat drip loss was calculated based on the weight loss and presented as percentage.

Data obtained from the experiment were analysed based on a completely randomized design by ANOVA using the General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Pen was treated as the experimental unit during the analysis. Significant differences among treatment groups were further analysed using Duncan's multiple-range test. A significant level of  $p \leq 0.05$  was implemented.

### 3. Results

#### 3.1. Performance of broiler chicks

Supplementation of standard commercial broiler feeds with probiotic *Bacillus* plus vitamins and minerals did not affect ( $p > 0.05$ ) the final body weight, accumulative feed intake and FCR of broiler chicks (Table 2).

#### 3.2. Internal organs of broiler chicks

The data on internal organs of broiler chicks are presented in Table 3. Supplementation of standard commercial diets especially with 0.1 or 0.5% of probiotic *Bacillus* plus vitamins and minerals reduced ( $p \leq 0.05$ ) the relative weight of heart of broiler chicks. Dietary supplementation tended ( $p = 0.07$ ) to increase the relative weight of ileum and pancreas of broilers. No noticeable difference ( $p > 0.05$ ) was observed with regards to other internal organs of broiler chicks.

#### 3.3. Haematological parameters of broiler chicks

The data on haematological profile of broilers are presented in Table 4. Broiler chicks offered standard commercial feed supplemented with probiotic *Bacillus* plus vitamins and minerals had lower ( $p \leq 0.05$ ) numbers of leukocytes and eosinophils compared to unsupplemented chicks. There were tendencies that supplementation with probiotic *Bacillus* plus vitamins and minerals resulted in lower ( $p = 0.07$ ) counts of lymphocytes and heterophils when compared with no supplementation. The effect of dietary supplementation was not significant with regards to erythrocyte profiles of broilers.

Supplementation of 0.5% probiotic *Bacillus* plus vitamins and minerals in the diets resulted in lower ( $p \leq 0.05$ ) concentration of uric acid in the serum of birds when compared with that in unsupplemented birds. The other biochemical parameters in the serum of birds were not affected ( $p > 0.05$ ) by the dietary supplementations.

#### 3.4. Carcass characteristics of broiler chicks

The data on the effect of dietary supplementation with probiotic *Bacillus* plus vitamins and minerals on the carcass characteristics of broilers are presented in Table 5. In general, there was no significant effect of supplementation on the carcass traits, pH and drip loss of broiler meats.

### 4. Discussion

Extra supplementations of standard commercial broiler feeds with certain components have generally been expected to optimize the genetic potentials of modern broiler strains in term of growth performance. In the present study, final body weight of broilers, however, did not differ among the treatment groups (though final body weight was numerically higher in

supplemented than in unsupplemented birds). Our present data were therefore different from those of previously reported (Islam et al., 2004; Anjumet al., 2005; Ajuwonet al., 2011; Rahman et al., 2012; Harun-Ar-Rashid et al., 2015) showing higher final body weight in broilers when feeding supplemented-commercial feeds. The differences in nature and qualities of supplements and commercial feeds, feed handling on the farm as well as the condition of trial seemed to be responsible for the above divergent results.

Our present data showed that supplementation with probiotic *Bacillus* plus vitamins and minerals decreased the relative weight of heart of broiler chicks. This result was different from Anjumet al. (2005) reporting no effect of probiotic (protexin) treatment and Hatabet al. (2016) showing an increased heart relative weight when feeding probiotics *Bacillus subtilis* and *Enterococcus faecium* to broilers. The definite reason for the lower relative heart weight in the supplemented birds was not known, but it might be due to the higher live body weight of the supplemented chicks as compared to unsupplemented ones (note that there was no difference in the absolute weight of heart among treatment groups in the present study). Apart from the dietary treatment, genetic study revealed that improvement of growth rate has been accompanied by the decrease in absolute and relative heart weight of modern broiler strains (Gaya et al., 2007). In the present study, supplementation of standard commercial feeds with 0.5% probiotic *Bacillus* plus vitamins and minerals resulted in relatively higher weight of ileum when compared especially with control. This finding was consistent with our earlier study, in which dietary supplementation with 0.5% probiotic *Bacillus* plus vitamins and minerals increased relative weight of ileum of broiler chicks at day 12 of age (Isroliet al., 2017). Each component in the supplements (i.e., probiotics, vitamins and minerals) seemed to contribute for the development of ileum of broilers. Olnoodet al. (2015a) have recently reported an increased weight of ileum in broilers at day 21 and 42 of age when feeding probiotic *Lactobacillus*. In this case, probiotic microorganisms may improve the ileal microbial community and, hence, positively influence the development of gastrointestinal tract especially ileum of broiler chickens (Sugiharto, 2016). With regard to vitamins and minerals, Islam et al. (2004) suggested that supplementation of standard commercial diets using mineral-vitamin premix improved digestion, absorption and metabolism, and thus promoted the intestinal development of chicks. Indeed, the higher relative weight of ileum in supplemented birds was in parallel with the higher final body weight of birds (though the values were not statistically significant). There was a tendency in this study that supplementation with probiotic *Bacillus* plus vitamins and minerals increased the relative weight of pancreas. This result was in accordance with Olnoodet al. (2015b) showing an increased relative weight of pancreas with feeding probiotic *Lactobacillus johnsonii* to broilers at 21 days of age. Similar to the latter authors, the reason for the increase weight of pancreas was not known in the current study. Taken together, dietary supplementation may improve the development and functionality of ileum and pancreas resulting in a better digestive process and thus growth performance of broiler chickens.

Results in the present study showed that broiler chicks supplemented with probiotic *Bacillus* plus vitamins and minerals had lower counts of leukocytes, heterophils, eosinophils and lymphocytes when compared with the unsupplemented birds. It was apparent in the studies of Shah et al. (2011) and Akhtar et al. (2015) that infected broilers had higher numbers of leukocytes and differential leukocytes relative to non-infected birds. This

increased leukocytes and differential leukocytes was attributed to the induction of immune responses of the infected broilers against infectious agents (Akhtar et al., 2015). Owing to these earlier data, it may be suggested that supplementation with probiotic *Bacillus* plus vitamins and minerals was able to alleviate the potential infections (from the field), resulting in less induction of immune responses of broilers. Our inference was also supported by the fact in the present study that supplemented birds had relatively higher antibody titers against NDV (when compared with the unsupplemented birds), and are thus more protective especially against NDV. Note that from clinical point of view, a HI titers of less than 3 log<sub>2</sub> is generally considered as negative for specific immunity against ND (Allan and Gough, 1974), and this condition was observed in the unsupplemented birds in the current trial. Study by Sandaet al. (2015) revealed that most of feeds available in the market today don't have enough components to stimulate immune responses of broiler chicks. Owing to this fact, supplementation of standard broiler commercial feeds with probiotic *Bacillus* plus vitamins and minerals seems to be beneficial.

In the present study, the concentration of uric acids in serum was lower in birds supplemented with 0.5% probiotic *Bacillus* plus vitamins and minerals when compared especially with control. El-Katchaet al. (2014) reported a negative correlation between the levels of plasma uric acid and protein retention in broiler chickens. Owing to this, the lower level of serum uric acid in supplemented birds may be associated with the higher retention of protein in the body of birds, as indicated by the relatively higher final body weight of the respective chicks. However, this inference should be interpreted with caution as the levels of protein in serum did not show any difference among the treatment groups. Besides the indicator of protein metabolism, serum uric acids concentration has been used to indicate infections and toxicosis in farm animals. Lin et al. (2015) reported that serum uric acid concentration was found higher in infected (with nephropathogenic infectious bronchitis virus) growing layers as compared to non-infected ones. Concomitantly, serum concentration of uric acid elevated in broiler chickens after feeding mycotoxins (Kaloreyet al., 2005). Ridi and Tallima (2017) in their review pointed out that uric acid is the major antioxidant molecule in the blood, which is essential to counteract oxidative damage due to infections and toxicosis. Uric acid has also been reported able to stimulate the immune responses of animals against the invasion of pathogenic microorganisms (Nery et al., 2015). Overall, supplementation of standard commercial broiler feeds with probiotic *Bacillus* plus vitamins and minerals seemed beneficial to reduce the invasion of infectious agents, resulting in less production of uric acids (as part of defence mechanisms against infections). Concomitant with our result, Kaloreyet al. (2005) reported that polyherbal preparation could inhibit the rise in serum uric acid concentration in aflatoxin fed broiler chicks.

In the current study, extra supplementation of standard commercial diets with probiotic *Bacillus* plus vitamins and minerals had no significant effect on fat deposition and carcass characteristics of broiler chickens. With regard to probiotics, previous studies revealed the role of probiotics in reducing the fatness (Fouad and El-Senousey, 2014) and improving the carcass traits (Shabaniet al., 2012) of modern broiler strains. Conversely, some studies failed to show such effect, for instance Toghyaniet al. (2011) reported no effect of probiotic (Protoxin™) on abdominal fat content and carcass yield of broilers.

Similarly, Haščiket *al.*(2016) did not find any effect of probiotics (*Lactobacillus fermentum*) on abdominal fat and carcass characteristics of broiler chicks. The different types of probiotic microorganisms, strains of broiler chicks and conditions of trials seemed to be responsible for the above divergent results. Similar to probiotics, the role of vitamins and minerals in affecting fat deposition and carcass characteristics of broiler chickens was minimum in the current study. Our result was therefore different from the data mentioned in the previous section. The different kinds and levels of vitamins and minerals used in the experiment as well as the conditions of the trials seemed to be responsible for the divergent results.

## 5. Conclusion

In conclusion, supplementation of standard commercial broiler feeds especially with 0.5% probiotic *Bacillus* plus vitamins and minerals increased the relative weight of ileum and pancreas, lowered counts of leukocytes, eosinophils, heterophils and lymphocytes and reduced serum concentration of uric acid.

## Acknowledgements

The study was funded by Directorate of Research and Community Service, the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through “Penelitian Unggulan Perguruan Tinggi” No. 007/SP2H/LT/DRPM/2017, 5 May 2017.

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**Table 1**  
**Chemical compositions of feeds**

Items (% dry matter)	Composition based on feed label <sup>2</sup>	Composition based on proximate analysis <sup>3</sup>
Moisture	13.0	10.1
Crude protein	21.0–23.0	19.8
Crude fat	5.00	3.39
Crude fiber	5.00	2.09
Crude ash	7.00	3.32

<sup>1</sup>Feed was composed of corn, rice bran, fish meal, soybean meal, coconut meal, meat and bone meal, broken wheat, peanut cake, canola, leaf meal, vitamins, calcium, phosphate and trace minerals.

<sup>2</sup>Data were provided by feed mill company.

<sup>3</sup>Analysis was conducted at the Faculty of Animal and Agricultural Sciences, Diponegoro University. Analysis was conducted in duplicate.

**Table 2**  
**Performances of broiler chicks**

Items	Dietary supplementations				SE	p value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
Body weight (g/bird)	1,989	2,030	2,054	2,011	43.3	0.75
Feed intake (g/bird)	3,725	3,922	3,893	3,689	86.8	0.17
FCR	1.92	1.98	1.94	1.88	0.03	0.22

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

**Table 3**  
**Internal organs of broiler chicks**

Items (% live body weight)	Dietary supplementations				SE	p value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
Heart	0.45 <sup>a</sup>	0.39 <sup>b</sup>	0.38 <sup>b</sup>	0.44 <sup>ab</sup>	0.02	0.05
Liver	2.34	2.33	2.41	2.30	0.11	0.90
Bile	0.04	0.05	0.03	0.04	0.01	0.23
Proventriculus	0.56	0.57	0.54	0.61	0.04	0.60
Gizzard	1.10	1.27	1.09	1.21	0.10	0.50
Spleen	0.11	0.12	0.11	0.13	0.01	0.80
Thymus	0.27	0.29	0.25	0.26	0.03	0.87
Bursa of Fabricius	0.05	0.07	0.05	0.06	0.01	0.22
Duodenum	0.45	0.51	0.49	0.54	0.03	0.18
Jejunum	1.01	1.08	1.03	1.13	0.06	0.54
Ileum	0.81	0.93	1.05	0.95	0.06	0.07
Caecum	0.32	0.34	0.40	0.40	0.03	0.21
Pancreas	0.21	0.26	0.22	0.26	0.01	0.07

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

<sup>a,b</sup>Values with different letters within the same row were significantly different

**Table 4**  
**Haematological haematological parameters of broiler chicks**

Items	Dietary supplementations				SE	p value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
<b>Haematological parameters</b>						
Hemoglobin (g/dL)	9.65	9.00	8.37	8.88	0.43	0.16
Erythrocytes (10 <sup>6</sup> /μL)	2.06	1.99	1.81	1.94	0.10	0.34
Hematocrit (%)	29.0	28.2	25.9	26.5	1.32	0.35
MCV (fl)	141	142	144	140	2.61	0.76
MCH (pg)	46.8	45.5	46.6	45.8	0.72	0.58
MCHC (g/dL)	33.2	32.0	32.4	32.8	0.63	0.61
Leukocytes (10 <sup>3</sup> /μL)	26.3 <sup>a</sup>	18.8 <sup>b</sup>	20.2 <sup>b</sup>	19.6 <sup>b</sup>	1.96	0.05
Heterophils (10 <sup>3</sup> /μL)	1.12	0.73	0.82	0.67	0.12	0.07
Eosinophils (10 <sup>3</sup> /μL)	1.57 <sup>a</sup>	1.05 <sup>b</sup>	1.18 <sup>b</sup>	0.98 <sup>b</sup>	0.14	0.03
Lymphocytes (10 <sup>3</sup> /μL)	23.6	17.05	18.2	17.9	1.80	0.07
Thrombocytes (10 <sup>3</sup> /μL)	17.8	13.7	15.0	15.0	1.99	0.52
H/L ratio	0.05	0.04	0.05	0.04	0.01	0.62
<b>Serum biochemical parameters</b>						
AST, U/L	314	355	332	294	66.2	0.93
ALT, U/L	7.72	6.82	5.40	5.92	1.17	0.53
Uric acid (g/dL)	5.75 <sup>a</sup>	6.52 <sup>a</sup>	3.40 <sup>b</sup>	4.97 <sup>ab</sup>	0.66	0.01
Total triglyceride (g/dL)	82.8	88.5	79.4	83.7	9.39	0.91
Total cholesterol (g/dL)	107	114	115	114	5.37	0.67
LDL (g/dL)	25.4	38.2	41.2	28.1	6.28	0.19
HDL(g/dL)	64.5	53.5	57.8	69.3	6.48	0.35
Total protein (g/dL)	2.67	2.65	2.90	2.77	0.11	0.40
Albumin (g/dL)	1.09	1.09	1.14	1.09	0.04	0.73
Globulin (g/dL)	1.60	1.62	1.76	1.70	0.08	0.47
Antibody titer (Log <sub>2</sub> GMT)	2.67	3.17	3.67	3.33	0.37	0.32

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

<sup>a,b</sup>Values with different letters within the same row were significantly different

**Table 5**  
**Carcass characteristics of broiler chicks**

Items	Dietary supplementations				SE	p value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
% live weight						
Eviscerated carcass	71.7	68.4	68.6	67.6	1.20	0.13
Giblets <sup>1</sup>	3.88	3.99	3.88	3.95	0.17	0.96
% Eviscerated carcass						
Breast	37.1	38.3	38.7	37.2	1.01	0.61
Thigh	15.7	15.8	15.9	16.1	0.59	0.98
Drumstick	13.0	13.6	13.1	13.8	0.39	0.52
Wing	10.4	10.7	10.4	10.9	0.27	0.36
Abdominal fat	2.24	1.92	2.36	2.12	0.22	0.49

Items	Dietary supplementations				SE	p value
	Control	Pro-0.1	Pro-0.5	Pro-1.0		
pH <sub>45min</sub>	5.97	5.87	5.97	5.92	0.10	0.86
pH <sub>24 h</sub>	5.72	5.72	5.78	5.73	0.05	0.79
Drip loss (%)	1.38	2.00	1.18	1.58	0.34	0.39

<sup>1</sup>Giblets: heart, gizzard and liver.

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