Dietary probiotics as a strategy for improving growth performance, intestinal efficacy, immunity, and antioxidant capacity of white Pekin ducks fed with different levels of CP

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ABSTRACT The potential impacts of probiotics on the performance and health status of white Pekin ducks fed with optimal or suboptimal dietary CP were evaluated during the growing period. A total of 180 male white Pekin ducks (14-day-old ducks with an initial weight of 415.65 ± 2.20 g) were randomly divided into 4 experimental groups (45 in each group of 5 replicates) in a 2 × 2 factorial design. The main factors included 2 dietary CP levels (18 or 14%) and dietary probiotic addition (with or without probiotics). The probiotic source was supplemented at 0.2 g per kilogram of diet from a blend of *Lactobacillus acidophilus* and *Lactobacillus casei*. The results showed that the diet containing 18% CP and probiotics significantly increases the final and total weight gain. Activities of intestinal enzymes (amylase, lipase, and protease), morphometrics (villus length, goblet cell count, and cryptal depth), and carcass percentage were also increased significantly. Total protein content, lysozyme activity, bactericidal activity, nitro blue tetrazolium levels, alternative complement pathway, superoxide dismutase activity, and catalase activity were significantly increased, whereas glucose, cortisol, and total cholesterol levels were decreased when treated with diet containing 18% CP and probiotics. Conversely, the group treated with diet containing 14% CP without probiotics showed the poorest performance, carcass properties, immune response, and antioxidant potential. In conclusion, probiotic addition to the 14% CP diet improved the performance of white Pekin ducks caused by reduced CP diet to performance due to the 18% CP diet without probiotic supplementation.

Key words: growth performance, intestinal efficacy, blood health, immune response, oxidative status

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INTRODUCTION

Poultry is one of the most profitable, rapid growing, vital human agricultural economic investments that contribute to providing high-quality animal protein, lipids, vitamins, and minerals (FAO, 2013). Several attempts have been made to increase the return of poultry investment including intensification, hybridization, and selection. These approaches may lead to some undesirable consequences such as disease outbreaks, increased stress, fat accumulation, leg problems, and metabolic disorder. From a nutritional point of view, dietary modulation represents a remarkable way of modifying animals' performance and well-being (Abou El-Ghar and Abd El-Karim, 2016; Akbari et al., 2016, 2018; Fathi et al., 2016; El-Senousey et al., 2018).

Animal-desired growth requires a balanced diet that contains all the necessary nutrients (Das et al., 2014; Beski et al., 2015; Sheoran, 2017; Uniyal et al., 2017; Sebola et al., 2018). The source of protein is a vital component of the diet owing to its high percentage in the diet, its cost, and its biological role (Wijtten et al., 2004; Beski et al., 2015).

Soybean meal is the most widely used plant-based protein source for poultry owing to its high nutritional value, especially the high content of protein with a proper essential amino acid profile (Frempong et al., 2019). Adequate dietary CP is nutritionally and economically base in all feeding systems. The appropriate use of low-protein diets has become common to solve the problem of protein cost, solve the problem of environmental problems related to excreted nitrogen,

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and allow alternative feedstuffs (Ravangard et al., 2017). However, excessive protein deficiency in the diet may impair poultry performance (Aftab et al., 2006; Namroud et al., 2008; Houshmand et al., 2012; Jiang et al., 2018). In such a case, growth promoters may offset the effects of dietary protein deficiency.

For decades, antibiotics have been used as promoters for growth and well-being; but with growing concern about the results of their implementation (e.g., suppression of the immune system, resistant pathogen strain development, toxic residue accumulation, and environmental hazards), they have been banned in many countries (Castanon, 2007; Mehdi et al., 2018; Roth et al., 2019). Hence, natural strategies such as functional feed additives (medicinal herbs, probiotics, prebiotics, and synbiotics) are efficacious promising alternatives to antibiotics (Bozkurt et al., 2014; Dhama et al., 2015). The inclusion of probiotics in broiler diets boosts the performance, utilization of feed protein, and immunity (Navidshad et al., 2009; Kim et al., 2011; Salim et al., 2013).

Considering the positive impacts of probiotics, it is expected that dietary inclusion of probiotics reduces the negative effects of low dietary protein. Therefore, the present work is designed to assess the use of a probiotic mixture on the performance, digestive enzyme activity, intestinal histomorphology, antioxidant capacity, and immune responses of white Pekin ducks fed with low- or high-protein diets for 42 d.

MATERIALS AND METHODS

Ethical Approval

The research was conducted in accordance with the standards of animal care and use for scientific purposes. The present study was approved by the Ethics Committee of Animal Production Department, Faculty of Agriculture, Tanta University, Egypt (approval no. $AY_{2019\text{-}2020}$/Session 6/2020.01.13), and updated by the Supreme Council of Egyptian Universities (2020.03.21).

Birds and Management

One-day-old white Pekin ducks were purchased from a commercial hatchery in Tanta city, Gharbia Governorate, Egypt, and transferred to a private farm under the supervision of Animal Production Department, Faculty of Agriculture, Tanta University, Egypt. Ducks were cared for on the floor under the same managerial and hygienic conditions by feeding on a commercial diet containing 23% CP for 2 wk (starter period). The birds were housed in plastic mesh pens (4.5 m × 4.5 m × 60 cm) that were placed 0.4 m above the floor in a naturally ventilated room. In the housing facility, the photoperiod was 10-hour light: 14-hour dark with a light intensity of 10 to 20 lux/m^2. During the experiment period, the temperature range was 20°C to 26°C, and the birds were allowed ad libitum access to fresh water and pellet feed for 42 d.

After the starter period, a total of one hundred eighty 14-day-old male ducks, with an average initial BW of 415.65 ± 2.20 g, were randomly divided into 4 experimental groups (45 in each group) in 5 replicates (n = 9). The designated area of each replicate was a 3 × 3 m^2 floor pen.

Experimental Design

Ducks were fed on a diet containing 18% CP (high dietary protein) vs. 14% CP (low dietary protein). In addition, ducks were fed on an 18 or 14% CP diet supplemented with 0.2 g of probiotics per kilogram of final feed. The probiotic source used in this study includes a blend of *Lactobacillus acidophilus* (5.40×10^{12}/g) and *Lactobacillus casei* (5.25×10^{12}/g).

Experimental diets were manufactured at room temperature after the ingredients were well mixed for 15 min and pelleted using a California mill machine with 4-mm diameters and air-dried at room temperature. All diets were maintained at −20°C in a freezer until use to prevent any alteration in the feed owing to microbial activity. Feeds and water were provided ad libitum throughout an experimental period for 42 d. Experimental diets were analyzed following the standard analysis methods (AOAC, 2007). In brief, moisture was measured by oven drying at 110°C until weight stability. CP and crude lipid were estimated using the Kjeldahl method, and solvent extraction was carried out using a Soxhlet method. Dietary fiber content was measured using an enzymatic–gravimetric method. Ash was analyzed by combustion at 550°C for 4 h in a muffle furnace. Calcium and phosphorus levels were determined by flame atomic absorption spectroscopy after the digestion of dietary samples in nitric perchloric and fluorhydric acid. ME was mathematically calculated by multiplying the combustion coefficient of protein, lipid, and carbohydrate in their ratio in diets. Amino acid concentrations were analyzed by high-performance liquid chromatography (HPLC-Agilent HP 1200 series apparatus, Waldbronn, Germany) using a Nova-Pak C18 column (4 μm, 3.9 × 4.6 mm) according to Salah et al. (2019). Ingredients and proximate chemical analysis are shown in Table 1.

Performance Variables

Averages of BW and total feed intake of each replicate in each group were recorded weekly. In addition, total weight gain (**TWG**) and feed conversion ratio (**FCR**) were calculated taking mortality into account.

Sampling Schedule and Analytical Procedures

At the end of the trial period (42 d), feeding was prevented overnight before slaughter and final sampling (histology samples) to reduce the handling stress and ensure the emptiness of the intestine for the histological assessment.

Table 1. Experimental diet ingredients and proximate chemical analysis (%).

Ingredients, %	18% CP	14% CP
Yellow corn	67.8	72
Soybean meal, 44%	26.78	15
Wheat bran	1.62	9.2
Calcium carbonate	1.48	1.5
Dicalcium phosphate	1.62	1.6
DL-Methionine	0.05	0.05
Salt (NaCl)	0.35	0.35
Mineral and vitamin mixture[1]	0.3	0.3
Total	100	100
[2]Proximate analyses		
CP	18.04	14.37
Ether extract (EE)	2.71	3.04
Fiber	3.98	4.05
Nitrogen-free extract (NFE)	60.36	63.41
Ash	2.73	2.57
ME, Kcal/kg	2,907	2,906
Calcium (%)	0.80	0.80
Available phosphorus (%)	0.30	0.30
Lysine (%)	1.30	1.30
Methionine (%)	0.50	0.50
Cysteine (%)	0.40	0.37
Threonine (%)	1.03	2.04
Methionine + cysteine (%)	0.90	0.86

[1]Mineral and vitamin mixture (3 kg): vitamin A (12000000 IU), vitamin D3 (2000000 IU), vitamin E (10,000 mg), vitamin K3 (2000 mg), vitamin B1 (1,000 mg), vitamin B2 (5,000 mg), vitamin B6 (1,500 mg), vitamin B12 (10 mg), biotin (50 mg), choline chloride (250000 mg), pantothenic acid (10,000 mg), nicotinic acid (30,000 mg), folic acid (1,000 mg), manganese (60,000 mg), zinc (50,000 mg), iron (30,000 mg), copper (10,000 mg), iodine (1,000 mg), selenium (100 mg), and cobalt (100 mg).
[2]Proximate analyses were performed in triplicates.

Carcass Characteristics

Five birds randomly taken from each group (one from each replicate) in the morning were weighed, rapidly slaughtered in the absence of other birds, fully bled, and defeathered after death confirmation. The viscera and the internal fat were carefully eviscerated. Weights of the fresh carcass, liver, gizzard, intestine, and fat relative to the live BW were determined for calculations of percentages of the carcass, liver, gizzard, intestine, and fat, respectively.

The small intestine was isolated, and then, empty large samples (the median part) were taken for histological morphometry and estimation of digestive enzyme activity. Samples from the liver were taken to estimate antioxidant enzymes.

Intestinal Enzyme Activities

The intestinal samples were opened and gently washed with puffer saline solution (PBS: 0.90%; pH 7.5), and samples from the internal surface (brush border) were carefully collected, homogenized, and centrifuged for 5 min at 8,000 rpm. The supernatant was maintained at −80°C for further analyses. Amylase and lipase activities were quantified spectrophotometrically at A_{714} and A_{540} (Wang et al., 2019). Protease activity was assessed following the protocol of Sigma's nonspecific protease activity with casein as a substrate (Cupp-Enyard, 2008).

Intestinal Histomorphometry Assessment

Samples from the intestinal tissues were fixed in 10% formalin for 72 h, dehydrated in graded concentrations of ethanol alcohol (60–100%), embedded in paraffin, and then sectioned at a thickness of 5–7 µm (Rotary Microtome 2145; Leica Microsystems, Microsystems Inc., Buffalo Grove, IL). The sections were mounted on clean glass slides and stained with hematoxylin and eosin for general morphometry and with periodic acid–Schiff for analysis of goblet cell count (**GCC**) (Levison, 1997). Intestinal histomorphometry assessment was performed for 5 villi using image analysis software (NIH, Bethesda, MD) in terms of villus length (Vl = the tip of villi to the villus–crypt junction) and cryptal depth (Cd = the crypt–villus junction to the base of the crypt).

Table 2. Performance variables of white Pekin ducks fed with test diets for 42 d.

	Parameters					
Main effect	Initial BW (g)	Final BW (g)	Total weight gain (g)	Total feed intake (g)	Feed conversion (g/g gain)	Survival rate (%)
CP level (CP %)						
18% CP	430.11 ± 2.23	2,533.65 ± 29.16^{a}	2,103.54 ± 30.98^{a}	6,946.90 ± 45.11	3.31 ± 0.03^{b}	100.00 ± 0.00
14% CP	430.76 ± 405	2,339.69 ± 49.66^{b}	1,908.93 ± 49.48^{b}	6,788.42 ± 63.37	3.57 ± 0.08^{a}	100.00 ± 0.00
Probiotic supplementation						
(−)	430.84 ± 4.25	2,363.02 ± 35.81^{b}	1,932.18 ± 36.46^{b}	6,833.85 ± 54.39	3.55 ± 0.03^{a}	100.00 ± 0.00
(+)	430.03 ± 1.81	2,510.31 ± 60.23^{a}	2,080.28 ± 60.60^{a}	6,901.47 ± 71.74	3.32 ± 0.09^{b}	100.00 ± 0.00
Experimental diets						
18% CP (−)	430.54 ± 2.67	2,491.25 ± 31.58^{a,b}	2,060.71 ± 34.25^{a,b}	6,916.05 ± 66.18	3.36 ± 0.02^{b}	100.00 ± 0.00
14% CP (−)	431.14 ± 3.02	2,234.79 ± 26.36^{c}	1,803.65 ± 25.84^{c}	6,751.65 ± 120.80	3.74 ± 0.01^{a}	100.00 ± 0.00
18% CP (+)	429.68 ± 4.18	2,576.04 ± 38.17^{a}	2,146.36 ± 42.34^{a}	6,977.75 ± 69.59	3.25 ± 0.03^{c}	100.00 ± 0.00
14% CP (+)	430.37 ± 8.45	2,444.58 ± 25.15^{b}	2,014.21 ± 22.10^{b}	6,825.19 ± 64.29	3.39 ± 0.02^{b}	100.00 ± 0.00
Two-way ANOVA (*P*-values)						
CP %	0.903	<0.001	<0.001	0.095	<0.001	-
Probiotics	0.879	0.001	0.002	0.442	<0.001	-
Interaction	0.993	0.077	0.088	0.945	0.001	-

[a-c]Means in the same column bearing uncommon superscript letters are statistically different ($P < 0.05$).
Probiotics: a blend of *Lactobacillus acidophilus* (5.40×10^{12}/g) and *L. casei* (5.25×10^{12}/g). The (−) symbol refers to no probiotic supplementation. The (+) symbol refers to a probiotic supplementation.
The results are represented as means ± SEM ($n = 5$).

Table 3. Carcass characteristics of white Pekin ducks fed with test diets for 42 d.

	Parameters					
Main effect	BW, g	Carcass (%)	Liver (%)	Gizzard (%)	GIT (%)	Fat (%)
CP level (CP %)						
18% CP	2,530.31 ± 29.85^{a}	74.14 ± 0.68^{a}	2.28 ± 0.03^{b}	3.15 ± 0.04	5.22 ± 0.15^{a}	1.00 ± 0.01^{b}
14% CP	2,336.35 ± 49.54^{b}	71.45 ± 0.76^{b}	2.54 ± 0.10^{a}	3.09 ± 0.06	4.40 ± 0.14^{b}	1.37 ± 0.16^{a}
Probiotic supplementation						
(−)	2,359.69 ± 60.42^{b}	72.14 ± 1.04	2.54 ± 0.09^{a}	3.08 ± 0.05	4.54 ± 0.18^{b}	1.36 ± 0.16^{a}
(+)	2,506.98 ± 35.87^{a}	73.45 ± 0.73	2.28 ± 0.03^{b}	3.17 ± 0.05	5.07 ± 0.22^{a}	1.02 ± 0.03^{b}
Experimental diets						
18% CP (−)	2,487.92 ± 34.50^{a,b}	73.64 ± 1.14^{a,b}	2.34 ± 0.04^{b}	3.12 ± 0.06	4.94 ± 0.04^{b}	1.01 ± 0.02^{b}
14% CP (−)	2,231.45 ± 24.93^{c}	70.64 ± 1.34^{b}	2.74 ± 0.05^{a}	3.04 ± 0.09	4.14 ± 0.04^{c}	1.70 ± 0.12^{a}
18% CP (+)	2,572.70 ± 38.31^{a}	74.64 ± 0.90^{a}	2.23 ± 0.03^{b}	3.18 ± 0.04	5.49 ± 0.18^{a}	0.99 ± 0.01^{b}
14% CP (+)	2,441.24 ± 25.37^{b}	72.26 ± 0.66^{a,b}	2.33 ± 0.03^{b}	3.15 ± 0.10	4.65 ± 0.17^{b}	1.04 ± 0.05^{b}
Two-way ANOVA (*P*-values)						
CP %	<0.001	0.033	<0.001	0.443	<0.001	0.001
Probiotics	0.002	0.244	<0.001	0.277	0.003	0.001
Interaction	0.081	0.771	0.006	0.729	0.898	0.001

Values are expressed as means ± SEM (n = 5).

[a,b]Means in the same column that lack common superscripts are differ significantly ($P < 0.05$).

Probiotics: a blend of *Lactobacillus acidophilus* (5.40×10^{12}/g) and *L. casei* (5.25×10^{12}/g). The (−) symbol refers to no probiotic supplementation. The (+) symbol refers to a probiotic supplementation;

Abbreviation: GIT, gastrointestinal tract.

Blood Samples

Blood samples (5 mL) were taken before morning feeding from 5 birds in each group. Blood was gathered from the brachial wing vein using 2 sets of sterile tubes. The first tubes containing anticoagulants (20 IU heparin/mL) were used for evaluation of hematological parameters in whole blood, whereas the second tubes without anticoagulants were used for serum separation by centrifugation under cooling (4°C) at 3,000 rpm for 10 min for biochemical analysis.

In the whole blood samples, the hematocrit value was quantified using a microhematocrit tube technique by centrifugation for 5 min at 13,000 rpm (Goldenfarb et al., 1971). Hemoglobin, red blood cells, and white blood cells were determined. Serum biochemical components in terms of glucose, cortisol (a sensitive responder to nonchronic changes than

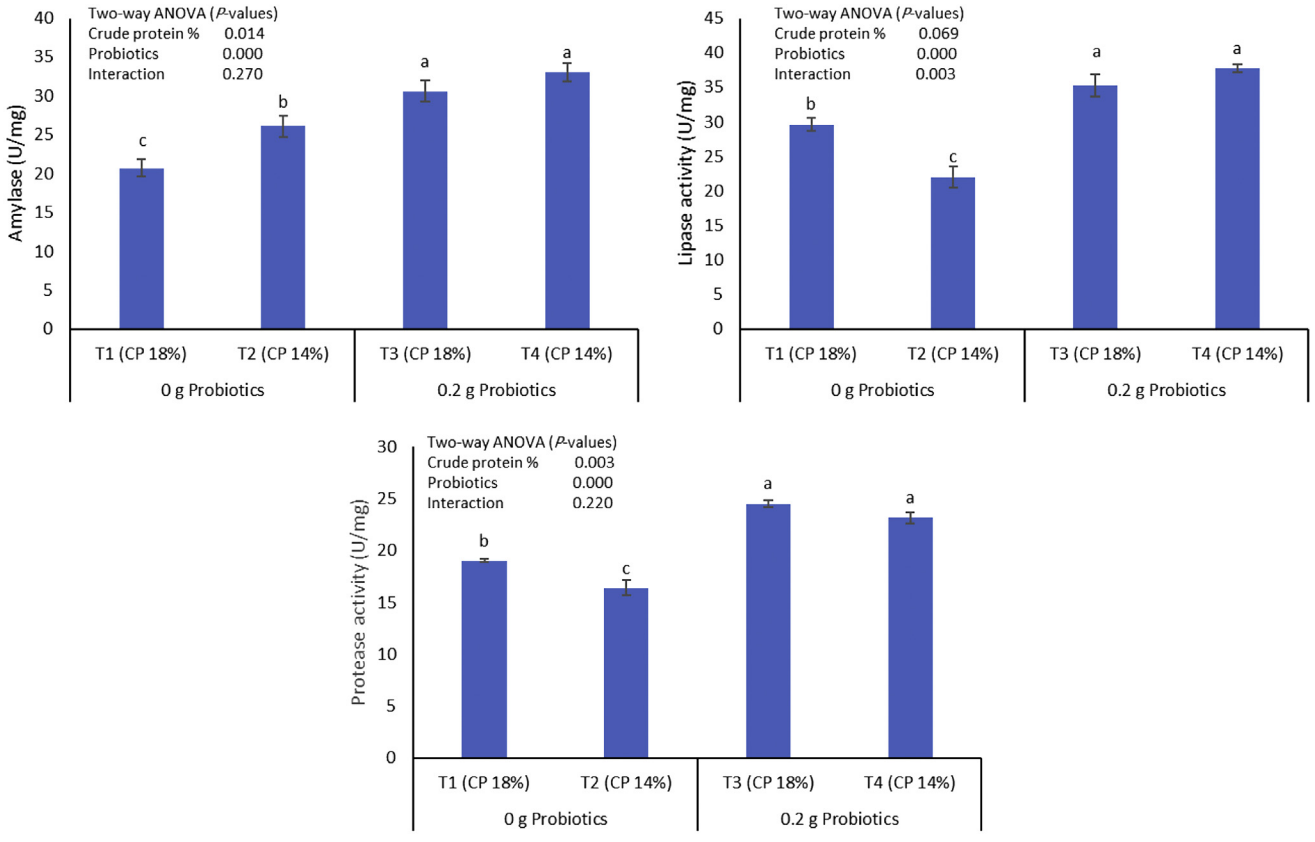

Figure 1. Intestinal digestive enzyme activities (amylase, lipase, and protease) of white Pekin ducks fed with test diets for 42 d.

Table 4. Anterior intestinal morphometrics of white Pekin ducks fed with test diets for 42 d.

	Parameters			
Main effect	Villus length (Vl), μm	Cryptal depth (Cd), μm	Vl/Cd ratio	Goblet cell count per villus
CP level (CP %)				
18% CP	295.66 ± 9.73[a]	151.71 ± 7.70	1.96 ± 0.05[a]	18.39 ± 1.94[a]
14% CP	238.78 ± 14.28[b]	139.29 ± 5.97	1.71 ± 0.10[b]	14.93 ± 2.37[b]
Probiotic supplementation				
(−)	242.68 ± 15.37[b]	139.15 ± 3.80[b]	1.75 ± 0.12	12.04 ± 1.20[b]
(+)	291.75 ± 12.05[a]	152.02 ± 5.64[a]	1.92 ± 0.05	21.28 ± 0.76[a]
Experimental diets				
18% CP (−)	275.59 ± 6.48[b]	140.81 ± 3.49[b]	1.96 ± 0.09[a]	14.33 ± 1.13[b]
14% CP (−)	209.77 ± 12.97[c]	137.49 ± 7.56[b]	1.53 ± 0.12[b]	9.74 ± 0.84[c]
18% CP (+)	315.72 ± 5.38[a]	162.61 ± 5.78[a]	1.95 ± 0.04[a]	22.45 ± 1.00[a]
14% CP (+)	267.78 ± 19.18[b]	141.61 ± 4.17[b]	1.89 ± 0.07[a]	20.12 ± 0.74[a]
Two-way ANOVA (*P*-values)				
CP %	<0.001	0.059	0.021	0.006
Probiotics	<0.001	0.047	0.080	<0.001
Interaction	0.291	0.149	0.059	0.263

Values are expressed as means ± SEM (n = 5).
[a,b]Means in the same column that lack common superscripts are differ significantly ($P < 0.05$).
Probiotics: a blend of *Lactobacillus acidophilus* (5.40×10^{12}/g) and *L. casei* (5.25×10^{12}/g). The (−) symbol refers to no probiotic supplementation. The (+) symbol refers to a probiotic supplementation.

corticosterone), total protein (**TP**), total cholesterol, and triglyceride concentrations and activity of alanine transaminase and aspartate transaminase were analyzed using the EMEG automatic analyzer Model 2000 Evolution with Bayer Diagnostics Reagents strips (Spinreact Co., Esteve de Bas, Girona, Spain) according to the manufacturer's guidelines.

Nonspecific Immune Responses

Serum lysosomal activity was diagnosed via a 96-well microplate turbidity assay as described by Lygren et al. (1999). At room temperature, 10 μL of serum was placed in each of the 96-well microplate tubes, and 190 μL of a mixture (0.2 mg of *Micrococcus lysodeikticus* per milliliter of PSB, pH 7.4) was added by gentle shaking. Changes in the turbidity values were recorded spectrophotometrically using a microplate reader (UVM 340; Biochrom, Cambridge, UK) at 450 nm after incubation for 1 and 5 min. One unit of lysozyme activity is equal to the amount of enzyme that causes a reduction in the absorption of 0.001/min.

Serum bactericidal activity was detected colorimetrically at a wavelength of 570 nm (Wang et al., 2018). In brief, 50 μL of serum samples was mixed with 50 μL of the bacterial suspension (*Streptococcus agalactiae*: 1.78×10^7) at 25°C for 2.5 h by slow rotation at 110 rpm (microtube rotator, Wavex-Tube Rotator E11270, Abdos Labtech Pvt. Ltd., Uttrakhand, India). The resultant mixtures were placed in 96-well microplate tubes containing 15 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (5,000 ppm; M5655, Sigma-Aldrich, Al-Gomhoria Co., Tanta, Egypt) by soft shaking at 25°C for 15 min, and the formed formazan was liquefied with 50 μL of dimethyl sulfoxide. The bacterial suspension in PBS without the serum sample was considered to be the positive control. The bactericidal activity expressed as a percentage of *S. agalactiae* inhibition relative to the positive control is as follows:

$$S.agalactiae\text{inhibition}\% = \frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}} \times 100$$

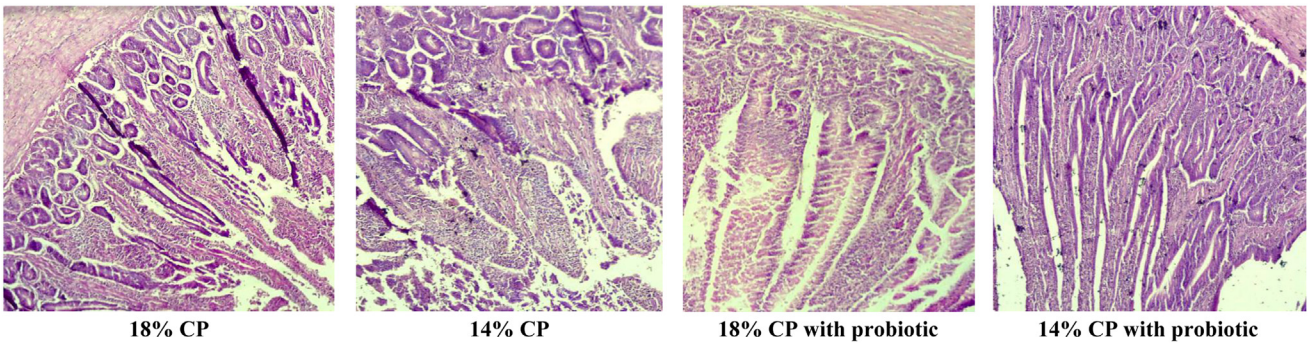

Figure 2. Cross-sectional view of the small intestine of white Pekin ducks fed with experimental diets showing the longest and wider villi in ducks fed with the 18% CP diet with probiotic supplementation and the shortest and widest villi in ducks fed with the 14% CP diet. (H&E staining, 100×). Abbreviation: H&E, hematoxylin and eosin.

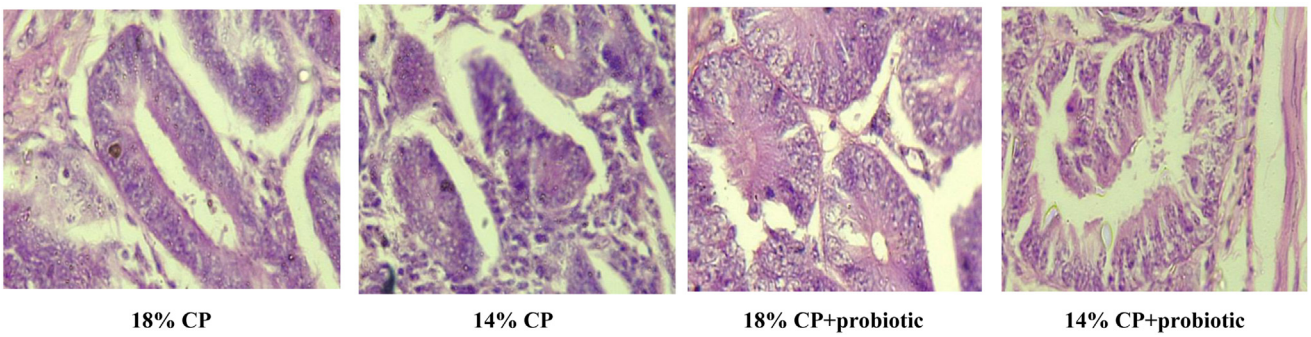

Figure 3. Cross-sectional view of the small intestine of white Pekin ducks fed with experimental diets showing an increasing number of goblet cells in the intestinal tissue of ducks fed with the 18% CP diet supplemented with probiotics and of those fed with the 14% CP diet supplemented with probiotics. (PAS, 400×). Abbreviation: PAS, periodic acid–Schiff.

Whole-blood respiratory burst activities were measured colorimetrically at 630 nm by performing nitro blue tetrazolium (**NBT**) assay according to Secombes (1990). Alternative complement pathway activities of serum samples (**ACP**) were estimated following a previously described method of Van Doan et al. (2016).

Antioxidant Assessment in Liver Tissues

Liver samples were homogenized in ice-cold NaCl (0.86%) and centrifuged at 12,000 rpm for 10 min at 4°C. The resultant supernatants were collected for superoxide dismutase (**SOD**) and catalase (**CAT**) colorimetric detection at 550 and 280 nm, respectively, using a microplate Spectro reader with a detection kit (Jian-Cheng, Nanjing, China) following the manufacturer's procedures.

Statistical Analysis

Data that resulted from 5 birds in each replicate per treatment were analyzed using SPSS version 22 (SPSS Inc., IL) for Windows at a 5% probability level following factorial (2 × 2) design of ANOVA to test the effects of the dietary CP level, probiotic supplementation, and their interactions. The homogeneity and normality of variance were examined by the Shapiro–Wilk and Levene tests, and means of interaction were tested for the significant differences using the least significant difference test. The results were presented as a mean of 5 replicate values ± SEM.

RESULTS

Performance Variables

Performance (Table 2) in terms of final BW and TWG was increased ($P < 0.05$) when the birds were fed with the 18% CP diet compared with birds fed with the 14% CP diet. Addition of probiotics to the 14% CP diet ($P < 0.05$) improved final BW and TWG compared with the 14% CP diet, and the final BW and TWG did not differ significantly from those in the group fed with the 18% CP diet. The FCR was lower ($P < 0.05$) in ducks fed 18% CP than those fed 14% CP diet. The dietary probiotic supplementation decreased ($P < 0.05$) FCR, regardless of CP levels.

The significant interaction between the CP level and probiotic addition reflected the best FCR in the group treated with the 18% CP diet with probiotics, followed by the group fed with the 18% CP diet without probiotics and the 14% CP diet with probiotics, whereas the group fed with the 14% CP diet showed the poorest FCR ($P < 0.05$). It is of interest to note that the total feed intake of ducks was not affected by dietary CP levels, probiotic addition, and their interaction. The survival rate was similar (100% in all groups).

Carcass Characteristics

Carcass characteristics of white Pekin ducks fed with experimental diets are shown in Table 3. Percentages

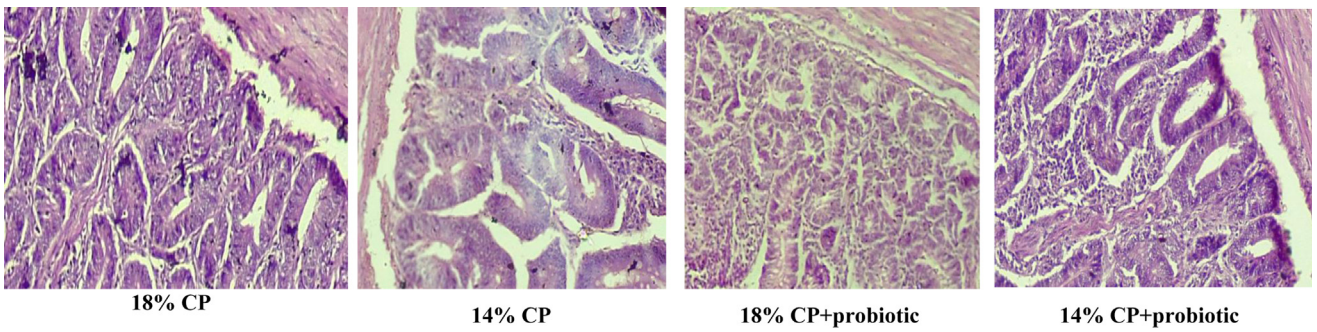

Figure 4. Cross-sectional view of the small intestine of white Pekin ducks fed with experimental diets showing the highest intestinal cryptal depth in ducks fed with the 18% CP diet supplemented with probiotics and the lowest cryptal depth in those fed with the 14% CP diet (H&E staining, 200×). Abbreviation: H&E, hematoxylin and eosin.

Table 5. Blood hematological indices of white Pekin ducks fed with test diets for 42 d.

	Parameters			
Main effect	Hematocrit (Ht, %)	Hemoglobin (Hb, g/dL)	Red blood cells (RBC, 106/μL)	White blood cells (WBC, 103/μL)
CP level (CP %)				
18% CP	30.86 ± 0.63	9.53 ± 0.22	2.38 ± 0.13	90.29 ± 1.31
14% CP	30.42 ± 0.49	9.82 ± 0.10	2.42 ± 0.14	90.82 ± 1.82
Probiotic supplementation				
(−)	31.17 ± 0.28	9.60 ± 0.18	2.31 ± 0.08	89.95 ± 1.87
(+)	30.10 ± 0.68	9.75 ± 0.19	2.50 ± 0.17	91.16 ± 1.18
Experimental diets				
18% CP (−)	30.96 ± 0.54	9.53 ± 0.37	2.23 ± 0.12	89.73 ± 2.33
14% CP (−)	31.37 ± 0.26	9.68 ± 0.16	2.38 ± 0.10	90.16 ± 3.46
18% CP (+)	30.75 ± 1.29	9.53 ± 0.34	2.53 ± 0.22	90.85 ± 1.67
14% CP (+)	29.46 ± 0.46	9.96 ± 0.09	2.46 ± 0.30	91.47 ± 2.03
Two-way ANOVA (*P*-values)				
CP %	0.572	0.319	0.835	0.835
Probiotics	0.191	0.612	0.380	0.636
Interaction	0.288	0.62	0.610	0.970

Values are expressed as means ± SEM (n = 5).

Probiotics: a blend of *Lactobacillus acidophilus* (5.40×10^{12}/g) and *L. casei* (5.25×10^{12}/g). The (−) symbol refers to no probiotic supplementation. The (+) symbol refers to a probiotic supplementation.

of carcass yield and the gastrointestinal tract increased ($P < 0.05$), whereas percentages of the liver and fat decreased ($P < 0.05$) by increasing CP content to 18% and with probiotic addition. However, the group fed with the diet containing the lowest CP level (14%) without probiotics showed an opposite trend for the liver, fat, and intestine percentages compared with the other groups.

Intestinal Digestive Enzymes

Activities of intestinal enzymes (amylase, lipase, and protease) of white Pekin ducks are illustrated in Figure 1. The activity of amylase was lower ($P < 0.05$), whereas activities of lipase and protease were higher ($P < 0.05$) in ducks fed with the 18% CP diet than in those fed with the 14% CP diet. The dietary probiotic supplementation increased ($P < 0.05$) the activity of amylase, lipase, and protease, regardless of CP levels.

Intestinal Histomorphometrics

Table 4 shows intestinal morphometrics in terms of villus length (**Vl**), cryptal depth (**Cd**), Vl-to-Cd ratio, and GCC of white Pekin ducks fed with the test diets. An amelioration ($P < 0.05$) in Vl, Vl-to-Cd ratio, and GCC was noticed by increasing the dietary CP level. The dietary probiotic supplementation increased ($P < 0.05$) Vl, Cd, and GCC. Generally, birds fed with the 14% CP diet without probiotics showed the lowest intestinal histomorphometric values (Vl, Cd, Vl/Cd, and GCC). These findings may suggest a pronounced effect of the CP level or probiotic addition on Vl (Figure 2) and GCC (Figure 3) in ducks. However, Cd (Figure 4) was affected significantly only by probiotic addition with increase in the level of CP to 18%.

Blood Parameters

Tables 5 and 6 represent the blood hematological and biochemical parameters of white Pekin ducks fed with the tested diets. Levels of serum glucose, cortisol, and total cholesterol decreased ($P < 0.05$), whereas levels of TP increased ($P < 0.05$) in those fed with the 18% CP diet than in those fed with the 14% CP diet. Probiotic addition reduced ($P < 0.05$) glucose, cortisol, and total cholesterol levels, whereas it increased ($P < 0.05$) TP levels in blood serum of ducks fed with 14 and 18% CP diets (Table 6).

Nonspecific Immune Responses

Nonspecific immune responses (lysozyme activity, bactericidal activity, NBT percentage, and ACP) of white Pekin ducks fed with the tested diets are shown in Figure 5. Levels of lysozyme, bactericidal activity, NBT percentage, and ACP were higher ($P < 0.05$) in duck groups fed with the 18% CP diet than in those fed with the 14% CP diet. Probiotic addition increased ($P < 0.05$) levels of lysozyme, NBT percentage, and ACP in duck groups fed with 18% CP and 14% CP diets. Meanwhile, probiotic addition increased ($P < 0.05$) bactericidal activity in the duck group fed with the 14% CP diet. Probiotic addition to diet with low CP (14%) content improved levels of immunity in ducks to reach values of those fed with diet containing high CP (18%) content without probiotic addition.

Antioxidant Potential

Activities of SOD and CAT in liver tissue increased ($P < 0.05$) in ducks fed with the 18% diet than in those fed with the 14% CP diet. Supplementation of probiotics increased SOD and CAT activities ($P < 0.05$), regardless of CP levels. This addition to diet containing low CP (14%) content improved the level of SOD activity in

Table 6. Blood biochemical indices of white Pekin ducks fed with test diets for 42 d.

Main effect	Parameters						
	Glucose (mg/dL)	Cortisol (ng/mL)	Total protein (TP, g/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Alanine transaminase (ALT, IU/l)	Aspartate transaminase (AST, IU/l)
CP level (CP %)							
18% CP	110.94 ± 2.52^{b}	46.44 ± 0.76^{b}	6.76 ± 0.21^{a}	177.41 ± 3.01^{b}	119.83 ± 1.05	76.69 ± 1.16	243.71 ± 3.59
14% CP	126.34 ± 8.34^{a}	49.59 ± 2.42^{a}	6.10 ± 0.28^{b}	199.88 ± 9.23^{a}	118.24 ± 1.36	75.24 ± 1.58	244.84 ± 2.46
Probiotic supplementation							
(−)	130.11 ± 6.55^{a}	51.44 ± 1.63^{a}	5.96 ± 0.22^{b}	201.77 ± 8.37^{a}	118.58 ± 1.07	76.69 ± 1.23	246.37 ± 3.03
(+)	107.17 ± 1.99^{b}	44.58 ± 0.22^{b}	6.90 ± 0.17^{a}	175.52 ± 2.40^{b}	119.49 ± 1.41	75.24 ± 1.52	242.18 ± 2.86
Experimental diets							
18% CP (−)	116.02 ± 0.62^{b}	48.10 ± 0.17^{b}	6.34 ± 0.18^{b}	183.27 ± 2.43^{b}	119.80 ± 1.40	76.79 ± 1.47	247.34 ± 4.94
14% CP (−)	144.20 ± 3.97^{a}	54.78 ± 1.45^{a}	5.57 ± 0.26^{c}	220.27 ± 1.26^{a}	117.37 ± 1.50	76.59 ± 2.33	245.39 ± 4.52
18% CP (+)	105.86 ± 2.34^{c}	44.77 ± 0.25^{c}	7.17 ± 0.14^{a}	171.56 ± 2.23^{c}	119.87 ± 1.88	76.60 ± 2.14	240.08 ± 5.20
14% CP (+)	108.47 ± 3.56^{b,c}	44.39 ± 0.39^{c}	6.63 ± 0.21^{a,b}	179.48 ± 2.84^{b}	119.11 ± 2.51	73.89 ± 2.28	244.28 ± 3.09
Two-way ANOVA (P-values)							
CP %	0.001	0.003	0.012	<0.001	0.420	0.506	0.809
Probiotics	<0.001	<0.001	0.002	<0.001	0.640	0.507	0.380
Interaction	0.002	0.002	0.597	<0.001	0.667	0.563	0.515

Values are expressed as means ± SEM (n = 5).

[a–c]Means in the same column bearing different superscript are significantly different at ($P < 0.05$).

Probiotics: a blend of *Lactobacillus acidophilus* (5.40×10^{12}/g) and *L. casei* (5.25×10^{12}/g). The (−) symbol refers to no probiotic supplementation. The (+) symbol refers to a probiotic supplementation.

ducks to reach the values of those fed with diet containing high CP (18%) content without probiotic addition (Figure 6).

DISCUSSION

Supporting high-production animals demands precise strategies, such as exogenous enzymes, immunostimulant agents, antioxidants, acidifiers, probiotics, prebiotics, and synbiotics, to maintain quantity and quality of animal products and health (Gao et al., 2007; Klasing, 2007; Gong et al., 2013; Munir and Maqsood, 2013; Puvača et al., 2019; El Basuini et al., 2020). The use of natural alternatives to antibiotics as growth promoters and immunostimulants of poultry has become widespread (Castanon, 2007; Papatsiros et al., 2013; Stanton, 2013; Murugesan et al., 2015; Calik et al., 2019). Probiotics have become popular among commercial poultry producers to ameliorate overall poultry health and performance (Roto et al., 2015). Using the correct probiotic cocktail (synergistic species) is more beneficial than using each type separately as a result of combining the roles of each species in the mixture (Best et al., 2017).

The results of growth performance showed clear reductions when white Pekin ducks were fed on a low-protein diet (14% CP). Enhancement in growth performance parameters was observed when the probiotic was added to the optimal diet (18% CP). The improved FCR may be one of the reasons for the promoted growth in groups fed on probiotic diets. In this regard, several studies demonstrated an improvement in the growth and nutrient utilization by the addition of probiotics under the optimal or suboptimal nutritional levels (Torres-Rodriguez et al., 2005; Kim et al., 2011; Salim et al., 2013; Bozkurt et al., 2014; Ravangard et al., 2017). The observed decrease in FCR may be associated with the improved intestinal status owing to modulating the intestine structure, secretion, microflora, or probiotics' anti-inflammatory impacts (Fuller, 2001; Lutful Kabir, 2009; Olnood et al., 2015; Yadav and Jha, 2019).

Improved growth performance is associated with increasing carcass yield percentage and decreasing fat percentage of ducks fed the 18% CP diet supplemented with probiotics. This finding may be linked to the improvement in feed utilization as a result of the increased feed intake and activity of digestive enzymes. Moreover, the accumulation of nutrients in tissues is dependent on feed intake, intestinal absorption, and metabolism (Pavlidis et al., 2007; Wideman et al., 2013). In line with the obtained results, some authors indicated that carcass weight showed a positive linear trend with the probiotic level (Azadegan Mehr et al., 2007; Pourakbari et al., 2016). In addition, previous reports showed that probiotic supplements reduce the percentage of abdominal fat (Anjum et al., 2005; Azadegan Mehr et al., 2007; Ravangard et al., 2017). The detected increased percentage of liver weight in ducks fed with a low protein level (14% CP) may indicate the presence of fatty liver or the occurrence of inflammation as a

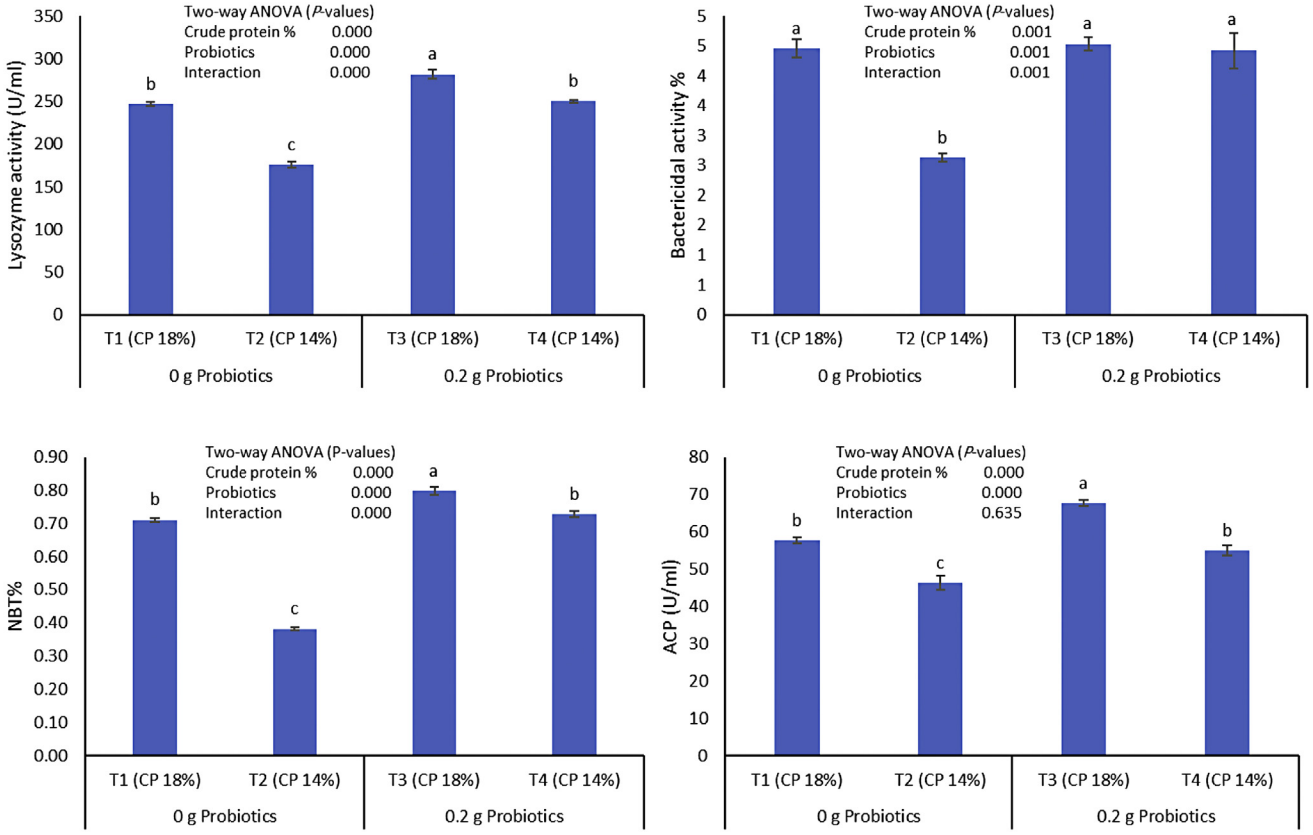

Figure 5. Nonspecific immune responses (lysozyme, bactericidal activity, nitro blue tetrazolium (NBT%), and alternative complement pathway [ACP]) of white Pekin ducks fed with test diets for 42 d.

result of an imbalance in energy-to-protein ratio in the diet and the consequent hormonal and metabolism imbalance (Rozenboim et al., 2016).

It is well known that diet affects the intestine and its biological role (Yegani and Korver, 2008; Lilburn and Loeffler, 2015). The internal villus function is to absorb nutrients, whereas the blind end allows for an increased retention time for dietary content within the digestive tract. Goblet cells that secrete mucus have antimicrobial impacts, simplify transport through the intestinal epithelium, maintain integrity of the intestinal epithelium, and prevent pathogens from entering the intestine (Forder et al., 2007; Reynolds et al., 2020). The results of intestinal enzyme activity showed improvement with supplementation of probiotics, which explains the improvement in FCR and growth. This improvement may be linked to a change in the intestinal structure such as the depth of the intestinal crypts. The histological examination revealed an increase in the absorption area in terms of the heights of intestinal Vl in the anterior section, the greatest number of goblet cells, and Cd in the probiotic-treated groups. In addition, the positive aspects of probiotics on intestinal histometric parameters in the present study can be linked to the

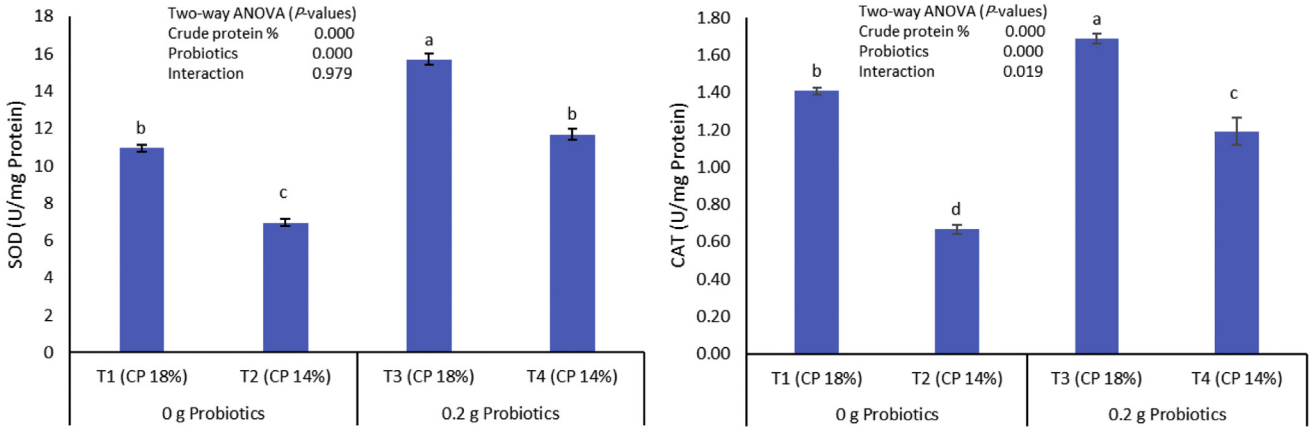

Figure 6. Antioxidant status of enzymes such as superoxide dismutase (SOD) and catalase (CAT) of white Pekin ducks fed with test diets for 42 d.

modulation of the gut microbiota as healthy gut microbiota play effective roles in the performance of the host body such as nutrient utilization, digestibility, metabolism, immune responses, and prevention of intestinal disorders and homeostasis imbalances (Balcázar et al., 2006; Laparra and Sanz, 2010; Rombout Jan et al., 2011; Best et al., 2017). Affirmative impacts of the use of probiotics on the tunica mucosa of the intestine have been reported in terms of a remarkable increase in villus height and Cd in the intestine of ducks fed with diets supplemented with probiotics (Elhassan et al., 2019). Similarly, De Souza et al. (2018) described an increase in the intestinal Cd for chicks receiving diets with probiotics.

Blood metabolites are accurate indicators that monitor an animal's health status, response to internal and external stimuli, and stressors (Amaral et al., 2017). The results of blood analysis that showed no abnormal values for all measured parameters were recorded. In addition, no remarkable alterations were noticed in the activity of liver metabolic enzymes (alanine transaminase and aspartate transaminase), reflecting the absence of any toxicity or impairment of liver function. Glucose, cortisol, and cholesterol values were higher in ducks fed with the 14% CP diet without probiotic supplementation, yet they were still within the normal range in ducks. The normal level of glucose and cortisol reflects the absence of stressors in growing ducks in all groups, and this is consistent with previous reports (Akşit et al., 2006; Li et al., 2009; Scanes, 2016; Weimer et al., 2018). Reductions in glucose and cortisol levels with probiotic-based diets may be attributed to either hypoglycemic hormone stimulation (insulin) or reduction in glucose absorption. The decreased level of cholesterol with probiotic supplementation may be linked to the hypocholesterolemic impact of probiotics. However, the elevation of the TP level by increasing the CP level or probiotic addition may indicate an amelioration in duck health (Hatab et al., 2016; Kim et al., 2017). Similar results were reported for probiotic addition on blood serum parameters of broilers (Ashayerizadeh et al., 2011; Haque et al., 2017; Yazhini et al., 2018) and Japanese quails (Siadati et al., 2017).

From an immunological point of view, probiotics are a novel approach in modulating animal immune responses, directly or indirectly, through different pathways (e.g., metabolic, neurological, or endocrine) (Huang et al., 2004; Haghighi et al., 2005; Selvaraj, 2012). Phagocytosis is a major defense component in animals on which the mode of action of lysozymes, bactericidal activity, respiratory burst (NBT), and ACP are dependent (Stuart and Ezekowitz, 2005; El Basuini et al., 2020). Probiotics resulting in raising duck immunity can be linked to the improved intestine at the structural or microbial levels that induce nutrient digestion, absorption, and utilization as well as stimulate cells of the immune system, produce antimicrobial substances, reduce toxic metabolic substances, and exclude pathogens (Rescigno et al., 2001; Haghighi et al., 2005; Awad et al., 2006; Apata, 2008; Kizerwetter-Swida and Binek, 2009).

The oxidative state is positively linked to the animal's immunity and well-being (Akbarian et al., 2016). An imbalance between the level of reactive oxygen species production and disposal leads to oxidative stress (Lee et al., 2019). The oxidative system includes several enzymes such as SOD and CAT that help eliminate excess reactive oxygen species and sustain cell homeostasis (Aruoma, 1998). In the present study, probiotic implementation mediates a significant increase in SOD and CAT activities with optimal and suboptimal dietary CP, suggesting an enhanced antioxidant potential. Several reports have detected boosted antioxidant enzyme (SOD and CAT) levels in poultry provided with different probiotics (Aluwong et al., 2013; Abudabos et al., 2016; Bai et al., 2017; Ogbuagu et al., 2018; Deraz et al., 2019).

In conclusion, feeding ducklings with reduced dietary CP (18 *vs.* 14%) content resulted in reduced performance and affected physiological markers. The addition of probiotics to the diets improved the performance of birds fed with either diet. Feeding probiotics to birds fed with the 14% CP diet resulted in return of their performance to the level of that in birds fed with the 18% CP diet not supplemented with probiotics.

DISCLOSURES

The authors declare no conflict of interest.

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