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Research Article
Impact of Locally Isolated Probiotics on Growth Performances, Haemato-Biochemical Profiles and Cecal Microflora of Broiler

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Abstract
Background and Objective: Increasing bacterial resistance to antibiotics has contributed to the urge for the development of alternatives to antibiotics. Accordingly, probiotics have received great attention as an alternative to synthetic antibiotics. Locally isolated probiotics may have beneficial effects on animal health and stand for a better alternative to antibiotics. Therefore, the effects of supplementation of locally isolated *Lactobacillus salivarius* and *Bifidobacterium thermophilum* on growth performances, haemato-biochemical profiles and cecal microbial compositions of broilers were studied in this experiment. **Materials and Methods:** A total of 320 day-old unsexed broiler chicks were allocated to 4 treatment groups each containing 4 replicates of 20 chicks. Groups included basal diet (control); control plus Doxycyclin (AGP); control plus *L. salivarius* (probiotic-I) and control plus *B. thermophilum* (probiotic-II). Experimental birds were raised for 28 days. Growth performances, haemato-biochemical profiles and cecal microbial composition were analyzed following standard protocol. **Results:** Probiotic-I impacted significantly on increased body weight and decreased FCR without significant effect in feed consumption, dressing and survivability rate. Probiotics supplementation resulted in higher liver weight in probiotic-I. Significantly higher Hb, RBC and WBC counts were observed in probiotics supplemented groups compared with AGP and control groups. However, total cholesterol, DLCs, PCV, MCV, MCHC were not affected by probiotics supplementation. Total Coliform and *Salmonella* counts were significantly reduced and *Lactobacilli* were significantly increased with probiotics supplementation. **Conclusion:** Locally isolated *L. salivarius* could be considered as a good potential probiotic for broiler which could be a feasible alternative to antibiotics in broiler diet at finisher stage.

Key words: *Lactobacillus salivarius*, *Bifidobacterium thermophilum*, growth performance, haemato-biochemical profile, cecal microflora

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Growth performance and feed efficiency of poultry are closely interrelated with the qualitative and quantitative microbial load in their gut, the morphological structure of the intestinal wall and the activity of the immune system[1]. Antimicrobial growth promoters (AGPs) have made a tremendous contribution to profitability in intensive husbandry but as a consequence of the increasing concern about the potential for antibiotic resistant strains of bacteria, the European Commission decided to ban all commonly used feed antibiotics[2]. Accordingly, Bangladesh also imposed a complete ban of AGPs in animal & fish feed through the Fish and Animal Feed Act 2010 and Animal Feed Rules 2013. It was reported by Islam *et al.*[3] that 100% of Bangladeshi broiler farms used antibiotics for several reasons including therapy, prophylaxis and growth promotion. Such usages of antibiotics in broiler farming must pose significant health risk to consumers. Therefore, it is obvious that suitable alternatives to antibiotics must be identified. Several studies have proposed the potential feed additives as alternative to AGP that include; probiotic, different herbs or spices and essential oils, acidifiers and organic acids, prebiotic and different dietary enzymes[4,5]. Probiotics have the potential to reduce enteric disease in poultry, therefore, considered as a good alternative to the antibiotics[6]. In broiler nutrition, probiotic species such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Aspergillus* and *Saccharomyces* are widely used to prevent poultry pathogens and diseases and improve broiler's growth performance[7], immunomodulation[8], certain hematological parameters[9] and promoting microbiological meat quality of broilers[10].

Probiotics have become a major focus of lactic acid bacteria research over the past 10 years with special emphasis to the genera *Lactobacillus* and *Bifidobacterium* for improving chicken health in natural way[11]. Probiotics act as competitive exclusion agents; enhance broiler chicken's digestibility and performance parameters by creating the favorable conditions for beneficial bacteria and affect gene expression of carrier proteins responsible for cholesterol absorption[12].

In Bangladesh, the use of probiotics in poultry is gradually being increased. But surprisingly, there is no local probiotic for the huge poultry industry in Bangladesh. The probiotic market in Bangladesh is completely dependent on the importation of probiotic materials and thus, every year the country counts a handsome amount of money for importation of such materials. This dependency on the imported probiotics may be due to the fact that there are no probiotic bacteria isolated yet in Bangladesh even though the potential sources exist[13]. Islam[13] reported that Bangladeshi indigenous chicken poultry naturally possess more beneficial bacteria in their gastrointestinal tract (GIT) than that of the other commercial poultry which might be used for commercial implementation[13]. Therefore, a feeding trial was conducted to investigate the potential probiotic effects of *L. salivarius* and *B. thermophilum* isolated from cecal contents of local chicken on the growth performance, internal organ development, haemato-biochemical traits and cecal microbial population for commercial use in broiler chicken. Their efficacy compared with AGP was also investigated in this study.

MATERIALS AND METHODS

Study location and period: The research was conducted at the Central Poultry Farm and Laboratory of Medicine and Public Health, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from January 2018 to June 2018.

Preparation of probiotic

Bacterial strains: The bacterial strains were obtained from a probiotic development project entitled as "Development of multi species/multi strains probiotic mixture from Bangladeshi local isolates and their validation for potential use in commercial poultry industry (Project ID; LS-1477)" funded by Ministry of Education of Bangladesh and conducted under the Department of Medicine and Public Health, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka from 2016-2018. Previously isolated glycerol stock culture of *L. salivarius* and *B. thermophilum* strains were used to prepare probiotic-I and probiotic-II. The glycerol stocks of isolates were prepared by mixing 0.5 mL of active cultures and 0.5 ml MRS medium including 40% sterile glycerol. The isolated strains were stored at -80°C and further tested for their viability as probiotic before use.

Viability test of stock culture: Fresh working cultures of the selective bacterial isolates were prepared using De Man, Regosa and Sharpe (MRS) agar for Lactobacillus strains and Bifidobacterium selective media, BSC propionate agar base for Bifidobacterial strains. Agar plates were inoculated with previously isolated seed cultures and incubated anaerobically for 48 h at 37°C.

Identification of isolates: Lactobacilli were presumptively identified following the methods described in Bergey's Manual of Systematic Bacteriology[14]. Colony morphologies (color,

shape and size) were examined physically on the selective media for each species. Cell morphology and colony characteristics on selective agar were tested by gram staining. Gram-positive, catalase-negative, non-spore-forming and rod-shaped isolates were examined for *Lactobacilli* confirmation. In contrast, nonmotile, gram-positive, nonsporulating, V-shaped organisms were considered as *Bifidobacteria*. Slide method was used to perform catalase test[15].

Preparation of probiotic mixture: Experimental organisms were inoculated in MRS broth for 48 h at 37°C and the turbidity was checked. The tubes were centrifuged at 5000 rpm for 5 min and the supernatants were discarded. The cells were harvested from 15 mL MRS broth and washed thrice with 1 mL Phosphate Buffered Solution (PBS). An aliquot of 1 mL PBS was added to the pellet in 1.5 mL ependr off tube and dissolved. The tubes were centrifuged at 10000 rpm for 5 min and the supernatant was discarded. An aliquot of 300 µL of 30% glycerol was added to the tube. All tubes were stored at -80°C. Strains were checked for growth and stability, as assessed by viable cell count after 1 week of refrigerated storage, in a liquid fermentation medium.

Experimental protocol and husbandry: A total of 320 one-day-old unsexed "Cobb 500" broiler chicks were randomly allotted to 4 treatment groups with 4 replicate pens (20 birds/replicate pen). The birds were reared for a period of 28 days. The four treatment groups were basal diet (control) control plus Antibiotic Group (Doxycyclin @ 2 g L^{-1} drinking water); control plus Probiotic-I group (1.0×10^8 CFU mL^{-1} of *Lactobacillus salivarius* as probiotic-I) and control plus probiotic-II Group (1.0×10^8 CFU mL^{-1} of *Bifidobacterium thermophilum* as probiotic-II). Throughout the study period the birds were raised in an open sided rice husk-littered floor pens. Continuous lighting with *ad libitum* feed and water was provided throughout the feeding period but no coccidiostat was added in the feed. The initial room temperature was 35°C which was decreased by 3°C each week until 28 days of age. The chicks were vaccinated with the commercial Newcastle disease virus (NDV) and infectious bronchitis (IB) vaccines through eye drops at 4 and 21 days. The Gumboro vaccines were given through drinking water at days 9 and 17 of the experiment, respectively. Chicks were managed according the guidelines suggested by Cobb Broiler Commercial Management Guide[16]. The study was approved by the Ethics Committee of the Sher-e-Bangla Agricultural Research System. The basal diet (antibiotic-free) was formulated to meet the NRC requirements[17] and was fed during the experiment in 2 phases, 0-14 and 15-28 day.

Growth performance and carcass characteristics: Feed intake and body weight were recorded on day 7, 14, 21 and 28. Feed conversion ratio was calculated as the total feed intake to weight gain ratio. At the age of 28 day and after 8 h of fasting for the complete evacuation of the gut, 3 birds from each replicate were subjected to slaughter and evisceration. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. Various parts of the carcasses, i.e., abdominal fat, gizzard (with contents), liver, heart, spleen and bursa were dissected and weighed separately. In addition, the internal organs and abdominal fat were recorded and its relation to the live BW of the bird, in percentage, was calculated.

Sample collection: An aliquot of 5 mL of venous blood was obtained from the wing vein of birds (3 birds $replicate^{-1}$) at 28 day of age. Two ml blood was collected in vacutainers containing ethylene diamine tetra acetic acid (EDTA) whereas the rest 3 mL blood was collected in the vacutainers with no anticoagulant, let to clot at room temperature and centrifuged at 2000 rpm for 15 min to produce serum. Approximately 1 g of cecal content was aseptically collected into a 2 mL self-lock Eppendorf tube and immediately frozen at -40°C to use for the measurement of microflora population.

Analyses of blood samples: The haematological studies were performed within two hours of blood collection. Total cholesterol in serum was measured with Photometer (Model: 5010 VS+, China) according to the manufacturer's instructions. Complete blood counts were determined by using a hematology analyzer (Sysmex×N-450, Japan) as described by Kececi *et al*.[18].

Enumeration of cecal microflora: Cecal contents were analyzed for microbial populations using conventional methods (spread plate method). For the conventional method, the cecal contents were used immediately after collection. One gram of the composite cecal sample from each replicate was diluted with 9 mL of 0.9% saline solution and mixed on a vortex. Viable counts of bacteria in the cecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) into MRS agar plates, Eosin Methylene Blue (EMB) agar media, *Salmonella*-shigella, MacConkey agar plates and Nutrient agar plates to isolate the *Lactobacillus*, *Escherichia coli* and *Salmonella*, total coliform and total viable count, respectively. The Lactobacilli MRS agar plates were then incubated for 48 h at 37°C under anaerobic conditions. The MacConkey agar plates, nutrient agar and

Salmonella shigella agar plates were incubated for 24 h at 37°C under aerobic conditions. After incubation, the colonies were counted and expressed as the numbers of colony forming units (CFU) per gram of cecal content.

Statistical analysis: Data were analyzed using one-way ANOVA followed by Duncan's multiple range test and LSD using the statistical package for social sciences (SPSS) version 16. $p<0.05$ were considered statistically significant.

RESULTS

Production performances of broiler chickens: The effects of probiotics (*L. salivarius* and *B. thermophilum*) and AGP on body weight, body weight gain, feed intake, FCR, dressing and survivability percentage of broiler chickens are summarized in Table 1. Significantly ($p<0.05$) higher body weight (1575.00 g bird^{-1}) was recorded in probiotic-I group compared to that of control group (1499.25 g bird^{-1}) whereas no significant differences were recorded in final body weight of broilers supplemented with probiotics and AGP at the end of the trail period (28 days). Likewise, highest body weight gain was recorded in probiotic-I (1533.50 g bird^{-1}) and lowest in control (1457.00 g bird^{-1}) group, whereas Probiotic-II and AGP groups produced intermediate results.

Our findings indicated the lowest feed intake (FI) in the AGP group and highest FI was in the probiotic-II although no significant difference was observed from other groups ($p>0.05$). Significantly ($p<0.05$) improved FCR (1.35) was also recorded for birds of probiotic-I group followed by the probiotic-II (1.38), AGP (1.38) and control (1.42) groups. However, no significant ($p>0.05$) difference was found between probiotic-II and AGP group (Table 1) for 1-28 days of age. No significant ($p>0.05$) differences in the dressing percentage was obtained in this study. In case of survivability, no mortality as well as no significant difference ($p>0.05$) was found among the treatment groups up to end of the trial (Table 1).

Relative organ and abdominal fat weight: Data in the Table 2 shows significantly increased ($p<0.05$) liver weight in the probiotic-I group as compared to the AGP and control at the end of the experimental period. No significant differences ($p>0.05$) were found in the relative organs weight of heart, gizzard, spleen and bursa (Table 2). Interestingly, the probiotic-supplemented groups showed numerically greater values compared with AGP and control group birds. In addition, treatment of probiotics did not improve the abdominal fat weight (AFW) of broilers, numerically lower values were observed (1.68 and 1.56) in probiotic-I and probiotic-II groups, respectively than that of the control group (1.79).

Table 1: Production performances of broiler chickens supplemented with locally isolated probiotics at 28 day of age

	Treatments[2]					
Components[1]	Control	AGP	Probiotic I	Probiotic II	Mean±SE	$LSD_{(0.05)}$
Initial (g bird^{-1})	42.00±0.41	42.00±0.41	41.50±0.65	41.00±0.71	41.02±0.28	0.791^{NS}
BW (g bird^{-1})	1499.00±18.99^{b}	1530.75±17.66^{ab}	1575.00±16.17^{a}	1553.75±18.19^{ab}	1539.62±10.78	25.151*
BWG (g bird^{-1})	1457.00±18.82^{b}	1488.75±17.39^{ab}	1533.50±15.57^{a}	1512.75±17.66^{ab}	1498.00±10.70	24.614*
FI (g bird^{-1})	2071.25±11.52	2065.00±14.45	2076.25±20.57	2088.00±31.15	2075.12±9.57	29.454^{NS}
FCR (feed gain^{-1})	1.42±0.02^{a}	1.38±0.02^{ab}	1.35±0.01^{b}	1.38±0.024^{ab}	1.38±0.01	0.026*
DP% (skinless)	67.38±1.19	67.68±1.26	70.07±0.55	69.54±0.52	68.76±0.52	1.335^{NS}
Survivability (%)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	0.00^{NS}

^{a,b}Means with different superscripts within a row differ significantly ($p<0.05$). SE: Standard error, LSD: Least significant difference, NS: Non significant, *Means significant at 5% level of significance ($p<0.05$). [1]Components: BW: Body weight, FI: Feed Intake, FCR: Feed conversion ratio, DP: Dressing percentage. [2]Treatments: Control: Basal diet, AGP: Basal diet+Antibiotic (Doxycycline pow. 2 g L^{-1} DW.), Probiotic I: Basal Diet+*L. salivarius*, Probiotic II: Basal diet+*B. thermophilum*

Table 2: Relative giblet and abdominal fat weight (% of live weight) of broiler chickens supplemented with locally isolated probiotics at 28 day of age

	Treatments[2]					
Components[1] (%)	Control	AGP	Probiotic I	Probiotic II	Mean±SE	$LSD_{(0.05)}$
Liver	2.32±0.08^{b}	2.46±0.18^{b}	3.0±0.199^{a}	2.59±0.19^{ab}	2.59±0.10	0.237*
Heart	0.54±0.05	0.54±0.03	0.67±0.08	0.55±0.03	0.58±0.03	0.074^{NS}
Gizzard (filled)	2.63±0.09	2.65±0.05	2.60±0.16	2.71±0.08	2.65±0.05	0.074^{NS}
Spleen	0.17±0.02	0.13±0.02	0.22±0.07	0.18±0.03	0.18±0.02	0.053^{NS}
Bursa	0.15±0.03	0.14±0.03	0.15±0.05	0.25±0.03	0.17±0.02	0.052^{NS}
AFW	1.79±0.32	1.66±0.08	1.68±0.16	1.56±0.15	1.67±0.09	0.279^{NS}

^{a,b}Means with different superscripts within a row differ significantly ($p<0.05$). SE: Standard error, LSD: Least significant difference, NS: Non significant, *Means significant at 5% level of significance ($p<0.05$). [1]Components: AFW: Abdominal fat weight. [2]Treatments; Control: basal diet, AGP: Basal diet+Antibiotic (Doxycycline pow. 2 g L^{-1} DW.), Probiotic I: Basal diet+*L. salivarius*, Probiotic II: Basal diet+*B. thermophilum*

Table 3: Hematological profile of broiler chickens supplemented with locally isolated probiotics at 28 day of age

	Treatments[2]					
Components[1]	Control	AGP	Probiotic I	Probiotic II	Mean±SE	$LSD_{(0.05)}$
Hb (g dL^{-1})	10.34±0.45[b]	10.77±0.25[ab]	11.66±0.13[a]	10.93±0.26[ab]	10.93±0.18	0.416*
RBC (million cum^{-1})	4.00±0.13[b]	4.04±0.06[ab]	4.28±0.04[a]	4.14±0.04[ab]	4.11±0.04	0.108*
WBC (thousands cum^{-1})	6783.30±301.39[b]	8083.33±328.15[ab]	7925.00±647.84[ab]	8708.33±703.74[a]	7875.00±296.44	746.148*
Neutrophils (%)	66.17±3.22	65.83±0.78	58.60±3.38	62.42±3.42	63.23±1.53	4.130[NS]
Lymphocytes (%)	29.08±2.85	28.50±0.78	35.42±3.14	32.33±3.26	31.33±1.40	3.819[NS]
Monocytes (%)	2.17±0.22	2.83±0.44	2.67±0.14	2.50±0.17	2.54±0.14	0.379[NS]
Eosinophil (%)	2.58±0.44	2.83±0.50	3.33±0.24	2.75±0.25	2.88±0.18	0.529[NS]
PCV (%)	39.88±7.64	32.36±0.89	35.14±0.43	41.34±8.61	37.18±2.75	8.170[NS]
MCV (Fl)	30.02±0.08	30.20±0.14	30.10±0.07	30.05±0.08	30.09±0.05	0.136[NS]
MCHC (g dL^{-1})	32.42±0.33	32.25±0.39	32.98±0.19	32.95±0.16	32.65±0.15	0.401[NS]

[a,b]Means with different superscripts within a row differ significantly ($p<0.05$). SE: Standard error, LSD: Least significant difference, NS: Non significant, *Means significant at 5% level of significance ($p<0.05$). [1]Components; Hb: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration. [2]Treatments; Control: Basal diet, AGP: Basal diet+Antibiotic (Doxycycline pow. 2 g L^{-1} DW.), Probiotic I: Basal diet+*L. salivarius*, Probiotic II: Basal diet+*B. thermophilum*

Table 4: Composition of cecal microflora (log10 CFU g^{-1}) of broiler chickens with locally isolated probiotics at 28 d of age

	Treatments[2]					
Components[1] (log_{10} CFU g^{-1})	Control	AGP	Probiotic I	Probiotic II	Mean±SE	$LSD_{(0.05)}$
E. coli	5.38±0.014[a]	5.40±0.025[a]	4.49±0.004[c]	4.55±0.027[b]	4.96±0.113	0.028*
Salmonella spp.	5.33±0.007[a]	5.20±0.021[b]	4.58±0.022[c]	4.51±0.049[c]	4.91±0.095	0.041*
TCC	5.28±0.018[a]	5.20±0.025[b]	4.49±0.007[c]	4.48±0.005[c]	4.86±0.098	0.022*
TVC	5.46±0.004[a]	5.44±0.010[a]	4.89±0.020[b]	4.92±0.027[b]	5.18±0.070	0.025*
Lactobacillus spp.	4.52±0.030[c]	4.50±0.024[c]	5.47±0.005[a]	4.76±0.040[b]	4.81±0.102	0.039*

[a-c]Means with different superscripts within a row differ significantly ($p< 0.05$). SE: Standard error, LSD: Least significant difference, NS: Non significant, *Means significant at 5% level of significance ($p<0.05$). [1]Components; *E. coli*.: *Escherichia coli,* TCC: Total coliform count, TVC: Total viable Count, [2]Treatments; Control: Basal diet, AGP: Basal diet+Antibiotic (Doxycycline pow. 2 g L^{-1} DW.), Probiotic I: Basal diet+*L. salivarius*, Probiotic II: Basal diet+*B. thermophilum*

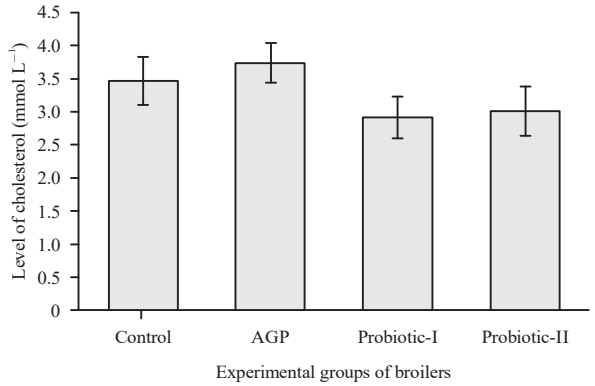

Fig. 1: Total cholesterol in serum of broiler chickens supplemented with locally isolated probiotics at 28 day of age

Control: Basal diet, AGP: Basal diet+Antibiotic (Doxycycline pow. 2 g L^{-1} DW.), Probiotic I: Basal diet+*L. salivarius*, Probiotic II: Basal diet+*B. thermophilum*. The Mean±SEM was plotted

Total cholesterol: Total cholesterol levels in serum of broiler birds at 28 days of age are illustrated in Fig. 1. Lower level (2.92 mmol L^{-1}) of total serum cholesterol was recorded in probiotic-I group compared to that of Probiotic-II (3.02 mmol L^{-1}), control (3.48 mmol L^{-1}) and AGP (3.75 mmol L^{-1}) group. But the findings were not statistically different ($p>0.05$).

Hematological profile: Table 3 shows the hematological profile of broilers. Hemoglobin level was significantly increased in Probiotic-I group (11.66 g dL^{-1}) compared to control (10.34 g dL^{-1}) whereas no significant differences were recorded for hemoglobin level in probiotic-II (10.93 g dL^{-1}) and AGP (10.77 g dL^{-1}) groups. Besides, supplementation of *L. salivarius* in drinking water tended to increase total RBC count significantly ($p>0.05$) in comparison to control. WBC count (thousand/cum) in the probiotic-II (*B. thermophilum*) treated group were significantly ($p<0.05$) increased compared with control group but statistically similar results were found in Probiotic-I (*L. salivarius*) and AGP groups. No significant differences ($p>0.05$) in neutrophils, lymphocytes, monocytes and eosinophils values were detected at day 28 of age (Table 3). No significant effects ($p>0.05$) of different treatments were observed on the PCV values. Similarly, the values of MCV and MCHC were not affected by the local probiotics strains compared with the control and AGP group. But the birds fed locally isolated probiotic strains had numerically the higher values for MCHC compared to the other groups.

Cecal microflora: As is shown in Table 4, the probiotics supplementation significantly ($p<0.05$) influenced both

Lactobacilli and coliform populations in the cecal contents. *Salmonella*, total coliforms and total viable count were significantly ($p<0.05$) lower in the *cecal* contents of probiotic-treated groups than that of the AGP and control groups. However, there were no significant ($p>0.05$) differences in cecal microflora count between two probiotic groups. Additionally, cecal microflora counts were the highest in the control group compared to the other groups (Table 4).

Significantly ($p<0.05$) highest Lactobacilli population was observed in the group of probiotic-I (5.47 CFU g^{-1}) followed by the probiotic-II (4.76 CFU g^{-1}), control (4.52 CFU g^{-1}) and AGP (4.50 CFU g^{-1}). Interestingly, lowest ($p<0.05$) *E. coli* population was observed in the probiotic-I (4.49 CFU g^{-1}) followed by the probiotic-II (4.52 CFU g^{-1}) and control (5.38 CFU g^{-1}) groups.

DISCUSSION

As a part of the digestive ecosystem, microflora in gastrointestinal tract has significant impacts on poultry health and performance[19]. Therefore, probiotics provide a healthy intestinal environment with increased counts of beneficial bacteria and thus can enhance the growth performance of poultry[2]. In this sense, investigating the growth performance, haemato-biochemical parameters and cecal microflora, under influence of two types of locally developed probiotic (*L. salivarius* and *B. thermophilum*) supplementation as alternative to AGP were the main objectives of the present study.

Observed body weights were similar to those reported by Blajman *et al*.[20] who stated *L. salivarius* cultures significantly ($p<0.01$) improved body weight of broilers when compared with the control group during a six-week experimental period. Similarly, Shokryazdan *et al*.[21] reported that *L. salivarius* cultures significantly ($p<0.01$) improved body weight gains when compared with the control chickens. A similar opinion was expressed by Zarei *et al*.[22] who observed the improvements in BW and BWG of broiler chickens fed diets supplemented with a mixture of *Lactobacillus* and *Bifidobacterium* strains. On the other hand, Lee *et al*.[23] recorded that BW gain was not influenced by the addition of direct-fed microbials (DFM) in the broiler diets. The variations in the results of different studies could be due to differences in the strains, sources, viability and concentrations of used bacteria, methods of administration and conditions of chickens. However, it is obvious from the present study that locally isolated probiotic strains, specially the *Lactobacillus salivarius* have good potential to influence body weight gain of broiler chicken. Several studies reported no significant difference ($p>0.05$) in feed intake of broiler fed diet containing mixture of *L. salivarius* throughout the experimental period[20,21]. Similarly, addition of *Bifidobacteria* did not have any significant effect on feed intake of broiler chickens[24]. Other studies also reported that feed intake of chickens was not affected by supplementation of *Lactobacillus*[25] or with the addition of *Lactobacilli* and *Bifidobacteria*[8] in the diet. In contrast, Zulkifli *et al*.[26] found significant variation in feed intake between control and probiotic group. At present, it is not known why supplementation of *Lactobacillus* cultures in the diet of broiler chickens does not affect their feed intake. In layers, it has been reported that supplementation of *Lactobacillus* cultures stimulated their appetite[27]. However, this difference between broilers and layers may be attributed to the fact that broilers have been genetically selected for having high feed intake in comparison to layers and as it has been reported that dietary factors are less important than management and health issues for influencing feed intake in broilers[28]. Therefore, in unstressed broilers, usually it is difficult to see the effects of dietary supplements on feed intake. However, present study have clearly indicated that the locally isolated probiotics have no negative impact on feed intake of broilers. Therefore, the feed intake was not hampered due to the supplementation of probiotics through drinking water as seen in AGP group. In case of FCR, the present findings are strongly in agreement with the findings of Shokryazdan *et al*.[21], who reported that broiler chickens fed cultures of *L. salivarius* showed significantly ($p<0.01$) better FCR than that of the control chickens. In addition, Mountzouris *et al*.[29] reported that broilers treated with probiotic containing *Pediococcus*, *Enterococcus*, *Lactobacillus* and *Bifidobacterium* strains in feed and water had better feed conversion ratio[29]. The results clearly exhibit an impression that the broiler receiving probiotic-I (*L. salivarius*) is the best converter of feed into live weight. Both Lactobacillus spp. and *Bifidobacterium* spp. are reported to improve digestion that brings several health benefits to host[30]. In contrast, Yeo and Kim[31], reported that the diet supplemented with probiotic containing *Lactobacillus* had no positive effect on feed conversion ratio of broilers. The variations in the results of different studies could be due to differences in the strains, sources, viability and concentrations of used bacteria, methods of administration and conditions of chickens. Addition of probiotics in drinking water also maintain the balance of the microflora ecosystem in the digestive tract and provide enzymes that can digest crude fiber, protein, fat and detoxify toxins or their metabolites[32]. However, the significant improvement in FCR of birds fed diets containing the tested probiotic shows that the product is a feasible alternative to antibiotics used as growth promoters. No significant ($p>0.05$)

differences in the dressing percentage was observed in this study which agree with the previous study conducted by Swiatkiewicz *et al.*[33] who found carcass yield was not affected when chickens were fed a probiotic (*L. salivarius*). But contradictory result was observed by Awad *et al.*[5], who reported that carcass yield percentage significantly increased in the probiotic fed broilers as compared with the control. In similar trials with Lactobacillus and *Bifidobacterium* preparations for broilers, Zulkifli *et al.*[26] and O'Dea *et al.*[34] reported no significant differences ($p>0.05$) in mortality between the probiotic and the control groups. However, in the current study, non-significant improvement in dressing and survivability percentage with the supplementation of these probiotics (via drinking water) also displayed growth promoting effect on broiler chickens.

The relative weight of liver observed in this study is in agreement with Hatab *et al.*[35], who reported significantly increased liver weight in the probiotic (*B. subtilis and E. faecium*) treated group as compared to the control group of Hy-line layer chickens. In contrast, Olnood *et al.*[30] reported that the relative weights of the liver were not affected by the probiotic (*L. salivarius* with other three *Lactobacillus* strains) administrated with drinking water. Previous studies on the relative organs weight of heart, gizzard, spleen and bursa presented the similar results and reported that supplementation of lactic acid bacteria in drinking water resulted in non-significant improvements in heart and gizzard weight compared to control group[36,37]. Although, there was no significant difference ($p>0.05$) in the abdominal fat weight (AFW) of broilers in our study but are in strong agreement with Haščík *et al.*[38] who also did not find any effect of probiotics (*Lactobacillus fermentum*) on abdominal fat and carcass characteristics of broiler chicks. Measurement of immune organs weight is a common method for evaluation of immune status in chickens. Such related organs include bursa of fabricius, liver and spleen. Good development of these organs is crucial for optimal immunoglobulin synthesis. Therefore, beneficial effects of *L. salivarius* and *B. thermophilum* supplementation on the gastrointestinal tract can improve the overall health, growth performance and immune response of broiler bird. In fact, the variations in result depend on the uses of different probiotic strains, broiler bird strains, management and climate condition, etc. However, present study findings clearly revealed that, the *L. salivarius* and *B. thermophilum* strains had no adverse effects on the vital organs and the general health of the chickens.

A non-significant cholesterol decreasing effect of native *Lactobacillus* strains was also reported in a study on Japanese quail where native probiotic groups showed lower cholesterol value compared with control birds[39]. However, different results were found in a previous study where serum total cholesterol concentration was significantly ($p<0.05$) reduced with *L. salivarius* culture in broiler chickens when compared to control broilers[21]. The variations in the results of different studies could be due to differences in the strains, sources, viability and concentrations of used bacteria, methods of administrations and trial period of chickens.

Lactobacillus and *Bifidobacteria* could contribute to regulate the serum cholesterol concentrations by de-conjunction of bile acids. Since the excretion of de-conjugated bile acids is enhanced and cholesterol is its precursor, more molecules are spent for recovery of bile acids[40]. As a result of increased synthesis of these acids, it is expected the level of serum cholesterol to be reduced. In general, the effective microorganisms such as *L. salivarius* and *B. thermophilum* strains could be a potential alternative to AGP in broiler diets[41].

The Hb levels observed in the present study were in line with the findings of Beski and Al-Sardary[42], who reported that probiotics significantly increased the concentration of Hb in chicken. The higher Hb concentration in the chicks receiving probiotics may be due to the acidic media of the alimentary tract caused by probiotic fermentation which resulted in better iron salt absorption from the small intestine. Furthermore, probiotic bacteria are known to help the synthesis of vitamin B complex that in turn also aid positively in blood-forming processes[43]. Besides, the improvement in RBC count in the present study could be attributed to improve health status and physiological well-being of the birds administered with probiotic. Our results of WBC count (thousand/cm) were in accordance with the findings of Fathi[44], who obtained significantly higher WBC counts in broilers fed probiotics than those of control birds. The manipulation of intestinal microbiota via the utilization of probiotics influences the development of the immune response. Kabir[45] reported that probiotics stimulate several subsets of immune system cells which in turn play an important role in the regulation of the immune response[45]. Present study recorded increased lymphocytes and monocytes counts in the probiotic supplemented groups compared to that of AGP and control groups. Thus, our findings indicated the potentials of more immunogenic effect on probiotic supplemented groups than the remaining two groups as manifested by increased immune cells. It was reported that dietary supplementation of DFM significantly increased ($p<0.05$) the erythrocyte count, hemoglobin concentration and hematocrit values in turkeys but differential leucocyte counts were not affected by dietary

DFM supplementation[46]. The PCV values found in the present study are in good agreement with previous study conducted by Nyamagonda *et al.*[47] who reported that the addition of probiotic to broiler diet had no significant effects on PCV values compared to control group. However, PCV and the differential leukocytes counts of all groups were within the normal range (32.36-41.34%) for healthy chickens[48]. This implies that supplementation of these two locally isolated probiotics do not have any adverse effects on haematological parameters. Similarly, the values of MCV and MCHC were not affected by the local probiotics strains compared with the control and AGP group. But the birds fed locally isolated probiotic strains had numerically the higher values for MCHC compared to the other groups.

In general, the number of unwanted bacteria was lower and the number of lactobacilli was higher in the probiotic treated groups. This result is supported by previous studies who reported that dietary supplementation of the probiotic increased *Lactobacillus* or beneficial bacteria counts and decreased *E. coli* or pathogenic bacteria counts compared with hens fed the diets without probiotic[49,50]. The results were also in accordance with Deraz *et al.*[36] who concluded that the total coliform and *Salmonella* counts were significantly reduced and/or totally eliminated by supplementation of lactic acid bacteria via drinking water in commercial broiler chicks. One of the prime objectives to use direct-fed microbials in broiler diets is to increase the beneficial organism for the host and to reduce the pathogenic organism which causes infectious diseases[51]. The current study showed that administration of probiotic via the drinking water is a very efficient method to decrease the colonization of *Salmonella* and *E. coli* in poultry intestine and associated with a higher count of *Lactobacillus* spp. It supports the hypothesis that lactobacilli could compete with E. coli for intestinal colonization. The antagonistic abilities of probiotics towards several pathogenic bacteria, such as *E. coli*, *Salmonella* spp. have been well documented[13]. Similarly, Estrada *et al.*[52] observed a tendency to reduce total aerobic bacteria, coliforms and clostridia in broilers receiving *Bifidobacterium bifidum* and reduce the number of carcass condemnation by cellulitis in animals. Higgins *et al.*[53] also stated that lactic acid bacteria played a role in the modulation of intestinal microflora and pathogen inhibition. It was reported that *L. acidophilus* and a mixture of *Lactobacillus* spp. increased the concentration of volatile fatty acids in the ileum and cecum in broiler chickens and reduced the pH value, which may be responsible for a decline of intestinal coliforms[54]. Chicken ceca are heavily populated with microbiota. Therefore, any beneficial dietary modulation of the intestinal environment should be reflected in composition and activities of the cecal microflora. Initial colonization is of great importance to the host because the bacteria can modulate expression of genes in epithelial cells, thus creating a favorable habitat for themselves. The primary colonizers are therefore relevant to the final composition of the permanent flora in full-grown chickens.

CONCLUSION

Locally isolated probiotic bacterial strains (*L. salivarius* and *B. thermophilum*) were non-pathogenic, safe and beneficial to broilers via drinking water, which implies that it could be a promising feed additive as antibiotic substitutes, thus enhance the growth performance of broilers, improves some haematological traits and improve their health. Further research is required to study the underlying mechanisms and to evaluate the economic impact of the use of probiotics in broilers to reveal if the live probiotic is as effective as freeze-dried preparations; those are usually administered with the feed.

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