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Assessment of probiotic-supplementation on growth performance, lipid peroxidation, antioxidant capacity, and cecal microflora in broiler chickens

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ABSTRACT

The aim of this work was to investigate the efficacy of a couple of probiotic lactic acid bacterial isolates, *Lactococcus lactis* ssp. *lactis* and *Lactobacillus plantarum* added separately or in combination to broiler diets. The experimental treatments received a basal diet with 22.4% protein and 3,160 kcal/kg. Two hundred and ten 1-day-old Hubbard broilers were allocated in seven experimental groups as follows: Control group and six groups treated by both probiotic strains in drinking water with intended final concentration of 10^9 cfu/ml and/or 10^{12} cfu/ml separately or in combination for a period of 42 days and tested on scheduled intervals. Treatment effects on performance of broilers (organs weights) as well as certain serum constituents were determined. The composition of cecal microflora was also evaluated. Probiotic supplementation had no significant effect but some organs had relative weights slightly higher compared to control. However, the relative weight of the thyroid, spleen, and pancreas was significantly increased. Broilers that received both types of probiotic strains separately or in combination had significant decreases ($p < 0.05$) in both serum alanine aminotransaminase level and malondialdehyde along with a significant increase ($p < 0.05$) in total antioxidant capacity compared to control. The microbiological analysis indicated that the lactic acid bacterial population boosted predominantly. The total coliform and *Salmonella* counts were significantly reduced and/or totally eliminated in broiler groups supplemented with probiotics. In conclusion, this study showed that both probiotic lactic acid bacterial strains can be considered as a nutritional source for broiler chickens.

INTRODUCTION

The current world trend is to either eliminate or reduce the use of antibiotics in poultry feeds to avoid the appearance of antibiotic resistant bacterial populations with special concern of antibiotics used in human diseases treatments (Menten, 2001; Dale, 1992; Pelicano *et al*., 2003). According to the United States Department of Agriculture, feed-borne antibiotic growth promoters have been fed 100% of the broilers and turkeys in the USA during the rearing period. In Brazil, with the exception of naturally grown, probably almost all broilers are given growth promoters as additives in ration (Menten, 2002). The great scrutiny on the use of antibiotic growth promoters by some scientists, consumers, activists, politicians, and bureaucrats in many countries has resulted in ban or severe restriction on the use of antibiotics as growth-promoting agents for poultry and starting a search for new and safer alternatives (Russell and Grimes, 2009; Menten, 2002). Recently, alternatives for substituting these traditional growth promoters have been evaluated and two alternatives proposed, among others, are prebiotics and probiotics (Rodrıguez *et al*., 2012; Pelicano *et al*., 2003).

The first attempt at using living bacteria to replace antibiotics in poultry was by Tortuero (1973), and probiotic use

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has gained widespread interest since. Probiotics are live microflora that is fed to animals and beneficially affect the host animal by colonizing the intestinal environment and improving its microbial balance (Fuller, 1989). The probiotics have influencing enterocyte turnover, producing bacteriostatic compounds that limit the growth of pathogenic bacteria, and competing with pathogenic bacteria for binding sites and nutrients (Farthing, 2004). Besides, these microorganisms are responsible for production of metabolites such as vitamins of the B complex and digestive enzymes, stimulation of the immune system by influencing enterocyte turnover, detoxifying colonic contents, promoting lactose tolerance, and lowering serum cholesterol concentrations (Li *et al*., 2009; Salma *et al*., 2007; Willis *et al*., 2007; Walter *et al*., 2008).

The most common utilized probiotic strains in animals are including, lactic acid bacteria (LAB) (*L. plantarum*, *L. bulgaricus*, *L. helveticus*, *L. acidophilus*, *L. lactis*, *L. casei*, *L. salivarius*, and *Bacillus subtilis*), *Enterococcus* (*E. faecium and E. faecalis*), *Bifidobacterium* spp., yeast and fungi (*Aspergillusoryzae* and *Saccharomyces cerevisiae*) (Huang *et al*., 2004). So far, assortments of microbial species such as *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Streptococcus*, and *Saccharomyces* have been used as probiotics in poultry (Owings *et al*., 1990; Jin *et al*., 1998; Ghadban, 2002; Kalavathy *et al*., 2003; Patterson and Burkholder, 2003; Gil De Los Santos *et al*., 2005). The ability of LAB to exclude foodborne pathogens such as *Salmonella* spp. has been intensively investigated with diverging degrees of prosperity (Patterson and Burkholder, 2003). When administered alone to commercial poults with idiopathic diarrhea, the LAB-based probiotics have been shown to exert a marginal beneficial effect on turkey performance that is comparable to that of antibiotics (Higgins *et al*., 2005).

Lactococcus lactis ssp. *lactis* (*Lact. lactis*) and *Lactobacillus plantarum* (*L. plantarum*) are among a wide variety of microbial species that have been isolated and fully characterized in our lab that showed significant activities against *Salmonella enteric* ATCC (American Type Culture Collection) 25566 and *Yersinia enterocolitica* ATCC 23715. Furthermore, our previous works demonstrated that these LAB isolates are probiotic candidates tolerated to simulated gastric juice, bile salt resistance, the hydrophobicity of the cell surface, resistance to low phenol concentration, autoaggregation, coaggregation, and reduction of cholesterol (Deraz, 2017; khalil *et al*., 2012).

The aim of this work was to evaluate the efficacy of both species probiotic *Lact. lactis* and *L. plantarum* separately or in combination on broiler nutrition along an experimental period of 42 days. Broiler performance, aspartate aminotransferase (AST) enzyme activity, malondialdehyde (MDA) content, and total antioxidant capacity (TAC) in serum were determined. Because chicken ceca are the most heavily populated gastrointestinal (GI) tract region (Mead, 2000), it was hypothesized that any beneficial dietary modulation of the intestinal environment should reflect in composition and activities of the cecal microflora. Therefore, certain cecal microflora at ages of 14, 28, and 42 days was also determined.

MATERIALS AND METHODS

Bacterial strains

Probiotic strains and bacteriocin-producing *Lact. lactis* and *L. plantarum* (Deraz, 2017; Khalil *et al*., 2012) were used for probiotic preparations. Stock cultures of both strains were stored at −80°C in De Man, Regosa and Sharpe (MRS) medium containing 25% (v/v) glycerol as a cryoprotectant. To produce fresh working cultures, strains were propagated twice in MRS at 37°C for 16–18 hours before experimental use.

Broiler chicks and husbandry

Hubbard commercial broiler chicks were purchased from Poultry Research Center, Faculty of Agriculture, Alexandria University. The animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health.

The husbandry was conducted at the Poultry Research Center, Faculty of Agriculture, Alexandria University. Two hundreds and ten of 1-day age broiler chicks were randomly divided into seven groups, 30 chicks each. Chicks were caged in wire floor batteries under controlled environmental house along an experimental period of 42 days. Experimental diets were formulated to provide chicks with 22.4% protein and 3,160 kcal/kg. Feed and water were provided *ad libitum*. Fresh water was provided on a daily basis during the experiment period to all the pens to ensure the viability of the probiotic culture. Remaining water from the previous day was discarded before adding fresh water, including that from pens receiving the probiotic in drinking water. To reach the target application rate, expected water consumption was estimated based on the age of broilers receiving probiotic.

Experimental design and probiotic treatments

The randomly divided groups were treated as follows: The first group was provided diets and water *ad libitum* with no addition and considered as a control group. The remaining was supplemented with probiotic strains at various concentrations. Groups 2 and 3 (T1 and T2) were provided with *Lact. lactis* (10^9 cfu/ml and 10^{12} cfu/ml, respectively). Groups 4 and 5 (T3 and T4) were provided with *L. plantarum* (10^9 cfu/ml and 10^{12} cfu/ml, respectively). Finally, groups 6 and 7 (T5 and T6) were provided with a combination of both probiotic strains at different concentrations. T5 received *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml). T6 received *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml). The intended LAB concentrations per ml drinking water were either 10^9 or 10^{12} cfu of each strain. To check for actual probiotic concentrations in water throughout the experimental period, 10-fold dilutions of drinking water samples were plated on MRS agar plates in duplicate then incubated overnight at 37°C.

Slaughtering and organ weighting

Chicks were fasted over-night then individually weighted. Three broilers per treatment (T) at the age of 14, 28, and 42 days were slaughtered by severing the jugular vein. After scalding, feather picking, and evisceration carcass, organs (intestinal weight and length, pancreas, spleen, liver, kidney, fabrishia, thymus, thyroid gland, and adrenal gland) were weighted. Percentages of organs were calculated based on live body weights. Relative weight of each organ was calculated according to Almeida *et al*. (1979) as follows: Relative weight = (organ weight/live body weight) × 100

Blood sampling

While slaughtering, blood samples were collected from each treatment group into dry clean centrifuge tubes, blood samples, were then centrifuged for 15 minutes at 3,500 rpm to obtain serum, and stored at −20°C for later analysis.

Serum analysis

Aspartate aminotransferase (AST) enzyme activity measured according to the method of Reitman and Frankel (1957). The MDA content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS) by the method of Wills (1965). TAC was assayed by the method described by Koracevic *et al*. (2001). Serum calcium (Ca) and phosphorus (P) concentration as (mg/dl) were measured according to the method of Tietz (1986) and ammonium molybdate methods by Gomorri (1942), respectively.

Cecal microflora

The carcasses were subsequently opened and the entire intestinal tract was removed aseptically. The tract was then divided into sections that were ligated with light twine before separating the ceca from the small intestine and then stored in sterile bags at −80°C. For the bacterial enumeration, cecal digesta was thawed and aseptically emptied in a new sterile bag. Immediately cecal digesta was diluted to 10-fold (i.e., 10% w/v) with sterile ice-cold anoxic phosphate-buffered saline (PBS) (0.1 M; pH 7.0) and subsequently homogenized for 3 minutes in a stomacher. Each cecal homogenate was serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently plated in duplicate on selective agar media for target bacterial groups and the enumeration results were expressed as colony-forming units (cfu) log 10 per ml. In particular, total viable count using plate count agar, MRS agar for LAB, MacConkey agar media for coliforms, *Salmonella* & *Shigella* agar plates for *Salmonella* counts were used. Plates were then incubated at 37°C for 24 to 72 hours.

Statistical analysis

Data were analyzed by analysis of variance using the general linear model procedure (Statistical Analysis System (SAS), 2001). Differences among means were determined using Duncan test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of probiotic supplementation on relative organ weights

One of the widespread methods used for immune status evaluation in chicken is the measurement of immune organ weight (Heckert *et al*., 2002). Such relative organs comprise liver, spleen, bursa of fabricius, and thymus. For optimal *I*g synthesis, adequate expansion of these organs is crucial (Glick, 1977). All birds were in sound health during the experimental period of 42 days. The slaughtered birds were randomly selected from a straight-run broiler chick group. In all cases, each value represents an average of three readings. The effects of addition of *Lact. lactis* and *L. plantarum*, alone or in combination, on relative weights of various organs are shown in Table 1. Supplementation with probiotics resulted in numerically high improvements compared to control group. Relative weights of the major digestive and immune organs of broilers after 42 days of experimental period were not statistically significantly affected by types or doses of both probiotic strains tested. Although the intestinal length of broilers at 42 days was insignificantly influenced ($p < 0.05$) by type or doses of probiotic strains used, addition of probiotics at concentration of 10^{12} cfu/ml (T2 and T4) highly improved the intestinal length. *Lact. lactis* at concentration of 10^{12} cfu/ml (T2) showed the longest intestine (52.51 cm), followed by *L. plantarum* at the same concentration (T4) with the intestinal length of 47.78 cm. These results are in agreement with Denli *et al*. (2003) who reported that probiotics did not influence significantly ($p > 0.05$) the intestinal length of broilers after 42 days and suggested that refinement of feed efficacy, enhancing nutrient availability, and increasing of the feed digestion and absorption caused by probiotic containing treatments led to shorter intestine length.

In our experiments, the probiotics did not affect the relative weights of intestinal tracts of broilers after 42 days. Similar results were observed by Jin *et al*. (1998), Huang *et al*. (2004), and Olnood *et al*. (2015) who demonstrated that the probiotic supplement *Lactobacillus*, *Lactobacillus johnsonii*, *L. casei* or *L. acidophilus* did not have an effect on organ weights and intestinal weight. Interestingly, the treated groups received the probiotic preparations either individually or combined and had relatively higher intestine weights compared to control group (Table 1), suggesting that mode of action of probiotic strains would be alike.

On the other hand, probiotic supplementation significantly increased the relative weights of thyroid, spleen, and pancreas. These results totally coincided with the observations of Hatab *et al*. (2016) who reported that the thymus and spleen relative weight were significantly increased in the probiotic-fed broilers as compared to the control. The increase in the relative weight of spleen is also in agreement with the findings of Willis *et al*. (2007) who found that feeding broilers on probiotic caused increases in the relative weights of the spleen in the treated group. The increase in the relative weight of pancreas was also in agreement with the findings of Olnood *et al*. (2015) who found that feeding broilers on probiotic caused increases in the relative weights of the pancreas of the treatment group. Subsequently, valuable effects of *Lact. lactis* and *L. plantarum* supplementation in the gastrointestinal tract could result in amelioration of immune response leading to improvement of overall health and performance of chicks.

AST activity, MDA content, and total antioxidant capacity (TAC)

Table 2, 3 and 4 show values of serum aspartate aminotransaminase (AST) level level (U/l), lipid peroxidation determinedas the concentration of MDA mg/dl, and TAC (mmol/l) of tested broilers aged 14, 28, and 42 days.

AST activity has been known as precise serological indicators in the deterioration of the hepatic tissues (Abdel-Wahhab and Aly, 2005). We found out that serum AST levels decreased significantly ($p < 0.05$) in all experimental groups treated with probiotic strains when compared to control (Table 2). Furthermore, there were significant differences ($p < 0.05$) in serum AST level of experimental groups treated with probiotics. AST primarily situated in the cytoplasm and sent out into the blood system only when hepatic structural integrity is influenced

Table 1. Values of internal organs ($X \pm SE$)* of broiler chickens given *Lact. lactis* and *L. plantarum* alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6) after 42 days.

Organs	Treatments**							*p* value
	Control	T1	T2	T3	T4	T5	T6	
Gizzard	3.10 ± 0.09	3.25 ± 0.31	3.46 ± 0.18	3.25 ± 0.27	3.70 ± 3.32	3.01 ± 0.20	3.08 ± 0.39	0.572
Somatic	0.83 ± 0.06	0.75 ± 0.02	0.91 ± 0.09	0.77 ± 0.05	0.79 ± 0.12	0.75 ± 0.05	0.79 ± 0.02	0.625
Intestinal weight	9.39 ± 1.98^{Ns}	11.37 ± 1.28^{Ns}	14.33 ± 2.19^{Ns}	11.31 ± 0.85^{Ns}	9.73 ± 1.49^{Ns}	10.99 ± 0.79^{Ns}	11.27 ± 1.26^{Ns}	0.384
Intestinal length	39.92 ± 4.06^{Ns}	43.57 ± 1.08^{Ns}	52.51 ± 8.57^{Ns}	43.30 ± 7.48^{Ns}	47.78 ± 5.86^{Ns}	42.84 ± 0.77^{Ns}	44.65 ± 4.02^{Ns}	0.736
Thyroid	0.015 ± 0.001^{b}	0.025 ± 0.007^{ab}	0.023 ± 0.002^{ab}	0.022 ± 0.002^{ab}	0.019 ± 0.003^{b}	0.024 ± 0.003^{ab}	0.036 ± 0.008^{a}	0.131
Adrenal	0.026 ± 0.006^{Ns}	0.023 ± 0.004^{Ns}	0.027 ± 0.003^{Ns}	0.015 ± 0.000^{Ns}	0.024 ± 0.006^{Ns}	0.019 ± 0.001^{Ns}	0.020 ± 0.003^{Ns}	0.332
Kidney	0.94 ± 0.02^{Ns}	1.09 ± 0.09^{Ns}	1.06 ± 0.05^{Ns}	0.96 ± 0.01^{Ns}	1.11 ± 0.44^{Ns}	1.01 ± 0.04^{Ns}	0.99 ± 0.13^{Ns}	0.988
Thymus	0.29 ± 0.07^{Ns}	0.39 ± 0.04^{Ns}	0.39 ± 0.09^{Ns}	0.36 ± 0.10^{Ns}	0.35 ± 0.03^{Ns}	0.40 ± 0.06^{Ns}	0.42 ± 0.03^{Ns}	0.834
Fabrishia	0.16 ± 0.04^{Ns}	0.18 ± 0.02^{Ns}	0.17 ± 0.01^{Ns}	0.24 ± 0.05^{Ns}	0.16 ± 0.04^{Ns}	0.19 ± 0.04^{Ns}	0.21 ± 0.02^{Ns}	0.598
Liver	3.46 ± 0.06^{Ns}	4.35 ± 0.76^{Ns}	3.62 ± 0.03^{Ns}	3.72 ± 0.21^{Ns}	5.91 ± 1.76^{Ns}	3.65 ± 0.18^{Ns}	3.82 ±0.12^{Ns}	0.296
Spleen	0.08 ± 0.00^{b}	0.10 ± 0.02^{ab}	0.11 ± 0.02^{ab}	0.11 ± 0.01^{ab}	0.16 ± 0.02^{a}	0.10 ± 0.01^{ab}	0.11 ±0.02^{ab}	0.180
Pancreas	0.43 ± 0.02^{c}	0.55 ± 0.03^{abc}	0.47 ± 0.04^{bc}	0.63 ± 0.01^{a}	0.50 ± 0.07^{abc}	0.50 ± 0.02^{abc}	0.59 ±0.05^{ab}	0.035
Heart	0.64 ± 0.02	0.74 ± 0.07	0.68 ± 0.06	0.65 ± 0.03	0.75 ± 0.09	0.62 ± 0.03	0.70 ± 0.06	0.630

^{abcd}Means with different superscripts are significantly different ($p < 0.05$).*Each value represents the mean for three replicates.

**Chickens treated groups: T1, *Lact. lactis* (10^{9} cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^{9} cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^{9} cfu/ml); and T6, *Lact. lactis* (10^{9} cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

Table 2. Values ($X \pm SE$)* of AST liver enzyme in serum (U/l) of broiler chickens given *Lact. lactis* and *L. plantarum* alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6).

Period	Treatments**							*p* value
	Control	T1	T2	T3	T4	T5	T6	
14 days	171.34 ± 10.5^{a}	141.5 ± 3.1^{b}	133.4 ± 2.9^{bc}	131.7 ± 2.7^{bc}	111.5 ± 8.9^{cd}	134.1 ± 14.1^{bc}	99.0 ± 5.1^{d}	0.001
28 days	161.1 ± 11.3^{a}	105.4 ± 6.7^{cd}	127.3 ± 10.2^{ab}	114.8 ± 6.0^{c}	111.7 ± 12.0^{c}	78.2 ± 14.0^{d}	120.8 ± 0.9^{bc}	0.001
42 days	140.6 ± 7.8^{ab}	129.0 ± 14.2^{abc}	107.2 ± 4.6^{c}	135.2 ± 6.9^{abc}	115.0 ± 10.8^{a}	146.2 ± 10.1^{a}	113.6 ± 7.6^{bc}	0.019
Overall means	157.7 ± 6.7^{a}	125.3 ± 7.0^{b}	122.6 ± 6.8^{b}	127.2 ± 4.2^{b}	112.7 ± 9.5^{b}	119.48 ± 12.2^{b}	111.1 ± 4.2^{b}	0.005

^{abcd}Means with different superscripts are significantly different ($p < 0.05$).*Each value represents the mean for three replicates.

**Chickens treated groups: T1, *Lact. lactis* (10^{9} cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^{9} cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^{9} cfu/ml); and T6, *Lact. lactis* (10^{9} cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

(Fan *et al*., 2015). Therefore, in our study, the increased serum AST activity observed in the control groups of chickens evidence that at least certain damage occurred in the liver and the decreased levels of AST may be associated with hepato-protective effects of the probiotic strains used. Among the six doses of applied probiotic treatments, T6 group, co-administration of *Lact. lactis* (10^{9} cfu/ml) and *L. plantarum* (10^{12} cfu/ml) showed the lowest AST activities (99.0, 120.8, and 113.6 U/l) along the whole experimental period followed by T4 which administered by *L. plantarum* at a final concentration of 10^{12} cfu/ml with AST activities of 111.5, 111.7, and 115.0 (U/l) at 14, 28, and 42 days, respectively.

Our results were coincided with Santoso *et al*. (1995) who recorded that the probiotics had a lower level of AST. While Hussein (2014) reported that there were no effect on serum AST activities, after the addition of probiotic (*Saccharomyces cerevisiae*) as compared to control. However, in another study, addition of *Saccharomyces cerevisiae* caused significant increase in serum AST activity (Mannaa *et al*., 2005). The decrease in AST activity acquired in the current study harmonized comparable results of studies on rats provided with *B. infantis* and *L. plantarum* to which decreased AST activity (Osman *et al*., 2007). The variations in the enzymatic activities may be due to animal species and probiotic interferences (Aluwong *et al*., 2013). We proposed that decreased blood AST activities within the normal range in treated groups suggested normal status of liver function as a result of biological supplementation with *L. plantarum* and *Lact. lactis*.

TAC is the contrivance used to determine the level of free radicals scavenged in test sample (Ghiselli *et al*., 2000) which utilized to assess the antioxidant capacity of biological samples (Marques *et al*., 2014; Pinchuk *et al*., 2012; Bartosz, 2010). Free radicals could be produced in tissues and cells from outer sources (such as pollution, drugs, and food), internal (such as inflammation, diseases, or metabolism) or as a result of diminished protective capacity (Rice-Evans *et al*., 1991) and any excess in free radicals production can result in oxidative damage (Ghiselli *et al*., 2000; Rubio *et al*., 2016). Two known mechanisms have generated by organisms as an antioxidant defense system, one of them is based on the activity of antioxidant enzymes which neutralize free radicals and the other build on the subsistence of low-molecular-weight antioxidants which directly interact with oxidant molecules leading to terminate the free radical chain reaction (Ognik *et al*., 2016, 2017).

MDA is the direct product of lipid peroxidation developed after radical attack on unsaturated fatty acids which can react with biomolecules and do cytotoxic, genotoxic effects and also could cause mutagenic lesions implicated in various diseases. Therefore, MDA content has an important role as an indicator of the lipid peroxidation level and as an indirect

Table 3. Values ($X \pm SE$)* of MDA content (mg/dl) in the serum of broiler chickens given *Lact. lactis* and *L. plantarum* alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6).

Period	Treatments**							*p* value
	Control	T1	T2	T3	T4	T5	T6	
14 days	26.07 ± 3.03^{b}	18.60 ± 0.96^{bc}	17.57 ± 1.60^{bc}	11.20 ± 0.64^{c}	21.30 ± 1.67^{a}	19.67 ± 2.22^{bc}	14.37 ± 5.74^{c}	0.001
28 days	19.27 ± 2.96^{c}	15.20 ± 1.92^{ab}	7.70 ± 0.69^{c}	11.77 ± 0.70^{bc}	8.47 ± 0.59^{c}	16.70 ± 0.59^{a}	11.37 ± 0.30^{bc}	0.002
42 days	48.50 ± 2.66^{a}	8.83 ± 0.49^{d}	27.17 ± 3.24^{b}	13.93 ± 2.86^{cd}	11.30 ± 0.50^{d}	19.50 ± 1.76^{c}	14.57 ± 2.75^{cd}	0.001
Overall means	31.28 ± 6.13	14.21 ± 1.57	17.48 ± 4.46	12.30 ± 0.96	13.69 ± 5.28	18.62 ± 0.96	13.43 ± 1.91	0.068

[abcd]Means with different superscripts are significantly different ($p < 0.05$).*Each value represents the mean for three replicates.

**Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

Table 4. Values ($X \pm SE$)* of TAC mmol/l in the serum of broiler chickens given *Lact. lactis* and *L. plantarum* alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6).

Period	Treatments**							*p* value
	Control	T1	T2	T3	T4	T5	T6	
14 days	1.80 ± 0.13^{bc}	2.04 ± 0.13^{ab}	2.36 ± 0.11^{a}	1.52 ± 0.23^{c}	2.11 ± 0.06^{ab}	2.11 ± 0.03^{ab}	2.18 ± 0.06^{ab}	0.006
28 days	1.95 ± 0.09	1.89 ± 0.10	2.26 ± 0.09	1.93 ± 0.13	2.12 ± 0.10	1.99 ± 0.18	2.05 ± 0.06	0.333
42 days	2.01 ± 0.10	2.28 ± 0.11	1.93 ± 0.05	2.20 ± 0.18	2.19 ± 0.14	2.28 ± 0.08	2.44 ± 0.03	0.071
Overall means	1.92 ± 0.06^{bc}	2.07 ± 0.08^{abc}	2.18 ± 0.08^{a}	1.88 ± 0.13^{c}	2.14 ± 0.05^{ab}	2.13 ± 0.07^{abc}	2.22 ± 0.06^{a}	0.031

[abcd]Means with different superscripts are significantly different ($p < 0.05$).*Each value represents the mean for three replicates.

**Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

reflection of the extent of cell damage and aging in an organism (Spiteller, 2001; Puvača *et al*., 2015).

In our study, administration of probiotic preparations of *Lact. lactis* and/or *L. plantarum* at concentrations of 10^9 or 10^{12} cfu/ml to chickens during their entire rearing period caused a significant reduction in MDA content and significant increase in TAC in blood serum compared to control groups (Tables 3 and 4). At 28 days old, probiotic-treated groups T2 (*Lact. lactis*, 10^{12} cfu/ml) and T4 (*L. plantarum*, 10^{12} cfu/ml) were recorded the lowest values of MDA contents of 7.70 and 8.47 mg/dl, respectively. T3 (*L. plantarum*, 10^9 cfu/ml) and T6 (*Lact. lactis*, 10^9 cfu/ml) plus (*L. plantarum*, 10^{12} cfu/ml) recorded MDA values of 11.77 and 11.37 mg/dl, respectively in relationship to other treated groups (Table 3). However, at 42 days, the lowest MDA values were recorded with groups T1 (*Lact. lactis*, 10^9 cfu/ml) followed by T4 (*L. plantarum*, 10^{12} cfu/ml) with MDA values of 8.83 and 11.30 mg/dl, respectively (Table 3). The significant reduction in MDA could be attributed to the probiotic ability to confer sufficient antioxidant protection against lipid peroxidation during the entire rearing period.

At 14 and 42 days of age, the levels of TAC were significantly increased ($p < 0.05$) in almost all treated groups in comparison to control. The more prominent significantly increase ($p < 0.05$) was in group T6 (*Lact. lactis*, 10^9 cfu/ml) plus (*L. plantarum*, 10^{12} cfu/ml) at 42 days old (Table 4). The current data coincide with the conclusion of Rajput *et al*. (2013) and Ognik and Krauze (2016) who stated that probiotics increase the activities of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase and reduce the concentration of MDA and uric acid. Zheng *et al*. (2016) elucidate that the probiotic *Enterococcus faecium* bacteria promote resistance of biological macromolecules oxidation and take off hydroxyl radicals, herewith increasing the body's antioxidant capacity. Similar findings were also reported in broilers following probiotic administration by Rajput *et al*. (2013) and Shen *et al*. (2014) with supplementation of *Saccharomyces boulardii*, *Bacillus subtilis*, and *L. plantarum*, respectively.

Moreover, broilers receiving both types of probiotic strains separately or in combination had observed increase in serum calcium concentrations (Table 5). Calcium concentrations were obviously increased in almost all treated groups in comparison to control along the rearing period and the prominent increases were in group T2 (*Lact. lactis*, 10^{12} cfu/ml) and T5 (*Lact. Lactis,* 10^{12} cfu/ml) plus (*L. plantarum*, 10^9 cfu/ml) with mean values of 12.15 and 12.29 mg/dl, respectively, compare to control with a mean value of 8.94 mg/dl (Table 5). However, the mean values of inorganic phosphorous concentrations were almost similar to control group except for groups T2 (*Lact. lactis*, 10^{12} cfu/ml), T4 (*L. plantarum*, 10^{12} cfu/ml), and T6 (*Lact. lactis,* 10^9 cfu/ml) plus (*L. plantarum*, 10^{12} cfu/ml) with values of 11.64, 11.46, and 11.85 mg/dl, respectively, compared to control with a mean value of 12.33 mg/dl (Table 6).

The observed increase in serum calcium and slight decrease in inorganic phosphorous concentrations in the treated groups as compared to the control group are in coincidence with the findings of Strompfova *et al*. (2006) who recorded a significant raise in serum calcium level of treated groups with *E. faecium*. However, the results were in contrast with the results obtained by Hashemzadeh *et al*. (2013) who stated no significant influence of probiotic on serum calcium and phosphorous levels in broiler chicks. Gilman and Gashman (2006) and Scholz *et al*. (2007) accounted that probiotics can promote the calcium absorption from intestinal tract. Furthermore, effectuation of probiotics resulted in beneficial influences of added probiotic on the damaged egg ratio through increased calcium retention in layers (Nahashon *et al*., 1996).

Table 5. Values of ($X \pm SE$)* of calcium concentrations (mg/dl) in of broiler chickens given *Lact. lactis* and *L. plantarum* alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6).

Period	Treatments**							*p* value
	Control	T1	T2	T3	T4	T5	T6	
14 days	9.02 ± 0.60	11.50 ± 0.36	11.05 ± 0.55	11.62 ± 0.59	12.12 ± 1.44	11.76 ± 0.57	12.15 ± 0.02	0.921
28 days	9.20 ± 0.91	10.84 ± 0.77	12.91 ± 0.88	11.39 ± 0.07	11.24 ± 0.78	12.27 ± 1.14	11.25 ± 0.17	0.548
42 days	8.6 ± 0.91	12.31 ± 0.50	12.50 ± 0.80	10.79 ± 1.22	11.64 ± 0.88	12.85 ± 1.32	10.84 ± 0.83	0.658
Overall means	8.94 ± 0.61	11.55 ± 0.36	12.15 ± 0.47	11.27 ± 0.41	11.67 ± 0.55	12.29 ± 0.55	11.41 ± 0.31	0.630

*Each value represents the mean for three replicates.

**Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

Table 6. Values ($X \pm SE$)*of phosphor concentrations (mg/dl) of broiler chickens given *Lact. lactis* and *L. plantarum* alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6).

Period	Treatments**							*P* Value
	Control	T1	T2	T3	T4	T5	T6	
14 days	12.84 ± 1.45	9.98 ± 2.24	11.30 ± 1.14	12.92 ± 1.03	12.09 ± 0.16	13.09 ± 0.47	11.41 ± 1.08	0.408
28 days	12.41 ± 1.74	15.05 ± 3.55	12.98 ± 0.73	13.80 ± 1.21	9.85 ± 1.64	12.52 ± 0.78	12.25 ± 0.25	0.556
42 days	11.74 ± 2.36	11.61 ± 0.75	10.64 ± 1.68	11.69 ± 2.65	12.43 ± 0.78	11.53 ± 1.17	11.87 ± 1.98	0.996
Overall means	12.33 ± 1.0	12.21 ± 1.44	11.64 ± 0.71	12.80 ± 0.94	11.46 ± 0.66	12.38 ± 0.49	11.85 ± 0.67	0.918

*Each value represents the mean for three replicates.

**Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

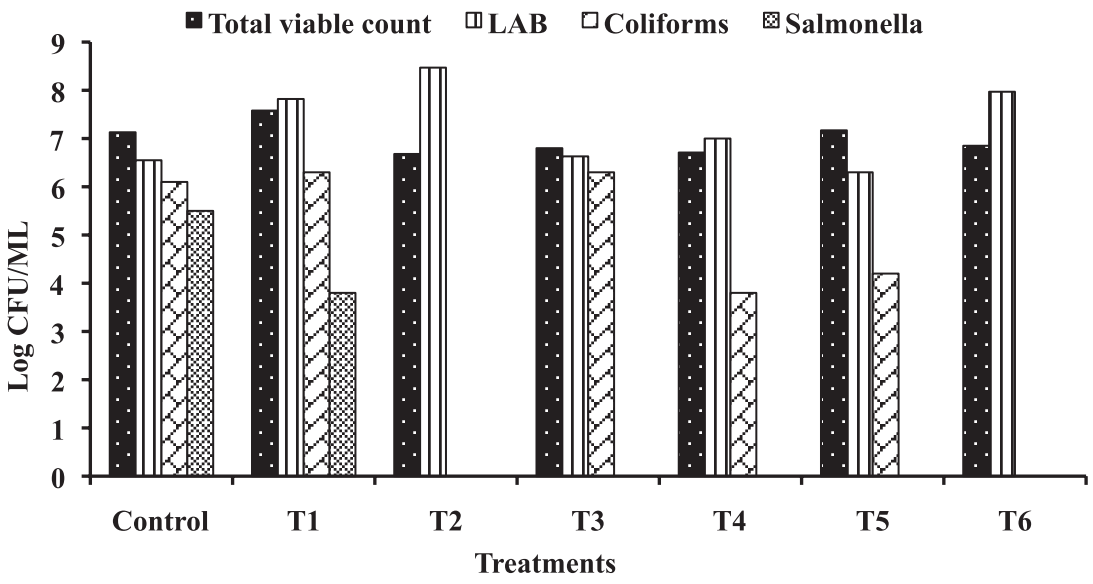

Figure 1. Cecal microflora composition of broiler chickens at the age of 14 days old given probiotic strains. Each bar represents the mean for three birds per treatment. Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

Cecal microflora composition

Figure 1, 2 and 3 show effect of *Lact. lactis* and *L. plantarum* either separately or combined at different inclusion levels on the composition of cecal microflora at 14, 28, and 42 days of age. The represented data revealed that the total viable bacterial count, total coliform counts, and *Salmonella* counts were significantly reduced in some broilers groups supplemented with probiotics as compared to control depending on probiotic concentrations and/or sampling periods. However, it was also noted an increase in total viable bacterial count in birds supplemented with *Lact. lactis* at level of 10^9 cfu/ml compared to control group. The obtained results of the microbiological analysis indicated that the lactic acid bacterial population boosted predominantly and were the most numerous

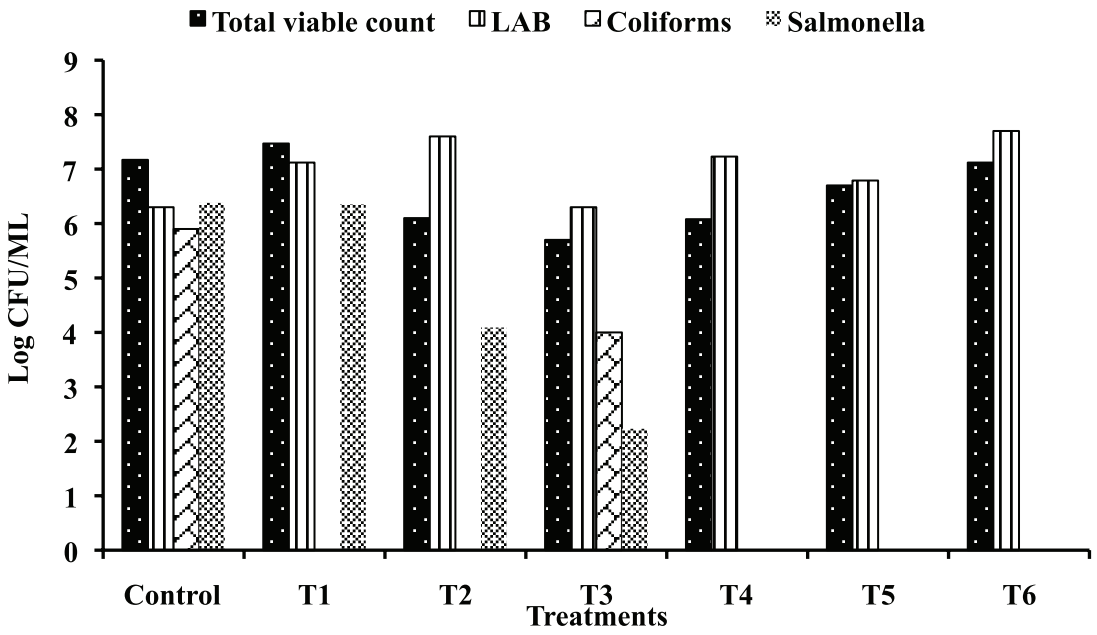

Figure 2. Cecal microflora composition of broiler chickens at the age of 28 days old given probiotic strains. Each bar represents the mean for three birds per treatment. Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

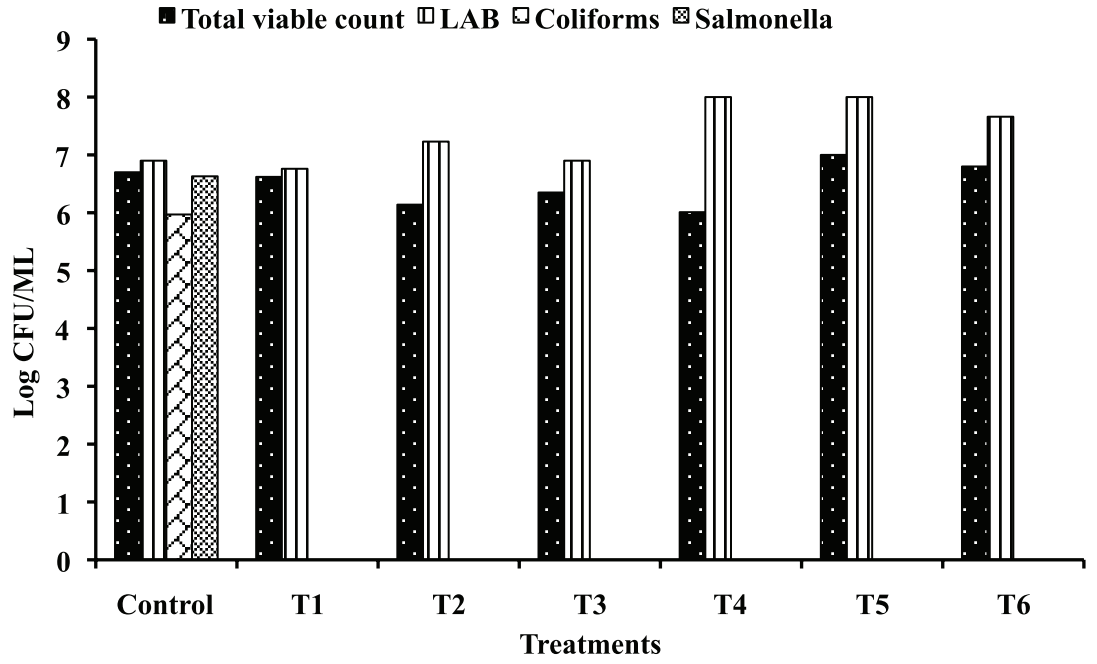

Figure 3. Cecal microflora composition of broiler chickens at the age of 42 days old given probiotic strains. Each bar represents the mean for three birds per treatment. Chickens treated groups: T1, *Lact. lactis* (10^9 cfu/ml); T2, *Lact. lactis* (10^{12} cfu/ml); T3, *L. plantarum* (10^9 cfu/ml); T4, *L. plantarum* (10^{12} cfu/ml); T5, *Lact. lactis* (10^{12} cfu/ml) plus *L. plantarum* (10^9 cfu/ml); and T6, *Lact. lactis* (10^9 cfu/ml) plus *L. plantarum* (10^{12} cfu/ml).

microorganisms present in the cecum of broiler chicks that consumed a basal diet with microbial supplement of *Lact. lactis* and *L. plantarum* either separately and/or in combinations using different inclusion levels in comparison with control group (Fig. 1, 2 and 3). Lactic acid bacterial counts reached a maximum concentration in T2 group subjected to *Lact. lactis* (10^{12} cfu/ml) and T6 (*Lact. lactis*, 10^9 cfu/ml) plus (*L. plantarum*, 10^{12} cfu/ml) after 14 days; afterwards these values declined but remained significantly high. These results are in agreement with those of Mountzouris *et al*. (2007, 2010) who reported that probiotics-

supplemented diets of broilers gave higher *Lactobacilli*, *Bifidobacterium,* and gram-positive cocci concentrations of the cecal microflora compared to controls. These observations were also stated by other researchers (AbuTarboush *et al*., 1996; Jenny *et al*., 1991; Ellinger *et al*., 1980). In addition, considerable number of investigations confirmed that probiotic addition in broiler feed could regulate the intestinal microflora and enhance the beneficial bacteria concentration such as LAB, and at the same time, inhibit the proliferation of harmful bacteria (Line *et al*., 1998; Li *et al*., 2008). On the contrary, these results are in partial disagreement with those of Giannenas *et al*. (2012) and Pourakbari *et al*. (2016) who did not detect differences in *Lactobacilli* and *Enterococci* counts, in the cecum of broilers fed a probiotic supplemented diet compared to control.

Total coliform counts and S*almonella* counts in the ceca were highly diminished or totally eliminated throughout the assay, with almost no variance of values within the individual treated groups. Variations observed only in counts of a couple of groups, namely, T1 (*Lact. lactis*, 10^9 cfu/ml) and T3 (*L. plantarum*, 10^9 cfu/ml) compared to control group (Fig. 1, 2 and 3). The proliferation of both total coliform counts and *Salmonella* was prevented in favor of LAB in almost all treatments compared to control. A similar potential of the particular probiotic to modulate the composition of cecal microflora and suppress potentially pathogenic bacteria such as coliforms and *Salmonella* was previously evidenced (Koenen *et al*., 2004; Teo and Tan, 2007; Higgins *et al*., 2008; Vicente *et al*., 2008; Mountzouris *et al*., 2010). Continual probiotic supplementation to animals feed has been found to enhance the proliferation of beneficial intestinal microflora in two routes, first by competitive insularity and second through antagonistic activity towards pathogenic bacteria (Jin *et al*.,1997; Riddell *et al*., 2010). In this way, probiotics can leverage the intestinal microbiota as well as host health, also increasing nutrient utilization, producing antimicrobial compounds, and stimulating the immune system (Corcionivoschi *et al*., 2010). The bactericidal effect of probiotic was probably due to production of different antimicrobial compounds by the probiotic strains such as antimicrobial peptides (bacteriocins), organic acids, diacetyl, hydrogen peroxide, and carbon peroxide. Some bacteriocins produced by specific probiotic strains can fulfill a role in the inhibition of common broiler pathogens (Ali, 2010). Both probiotic strains used in the current study, *Lact. lactis* and *L. plantarum* have a confirmed bacteriocin production activity against *Salmonella enteric* ATCC 25566, *Yersinia enterocolitica* ATCC 2371, and *Bacillus cereus* ATCC 49064 (Deraz, 2017; Khalil *et al*., 2012).

In conclusion, this study showed beneficial effects of dietary inclusion of both bacteriocins producing and probiotic strains *Lact. lactis* and *L. plantarum* and can be considered as a wealthy source of chicken nutritional supplement.

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