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 10⁹ cfu/ml and/or 10¹² 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 (Rodriguez 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...
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 bacteriocin 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, Enterococcos, 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 lactic 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 enterococotica 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 (10⁶ cfu/ml and 10³ cfu/ml, respectively). Groups 4 and 5 (T3 and T4) were provided with L. plantarum (10⁶ cfu/ml and 10³ 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 (10² cfu/ml) plus L. plantarum (10⁶ cfu/ml). T6 received Lact. lactis (10³ cfu/ml) plus L. plantarum (10² cfu/ml). The intended LAB concentrations per ml drinking water were either 10⁶ or 10³ 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 weighing

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 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 Ig 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 determined as 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 significantly 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>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>ρ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gizzard</td>
<td>3.10 ± 0.09</td>
<td>3.25 ± 0.31</td>
<td>3.46 ± 0.18</td>
<td>3.25 ± 0.27</td>
<td>3.70 ± 3.32</td>
<td>3.01 ± 0.20</td>
<td>3.08 ± 0.39</td>
<td>0.572</td>
</tr>
<tr>
<td>Somatic</td>
<td>0.83 ± 0.06</td>
<td>0.75 ± 0.02</td>
<td>0.91 ± 0.09</td>
<td>0.77 ± 0.05</td>
<td>0.79 ± 0.12</td>
<td>0.75 ± 0.05</td>
<td>0.79 ± 0.02</td>
<td>0.625</td>
</tr>
<tr>
<td>Intestinal weight</td>
<td>9.39 ± 1.98</td>
<td>11.37 ± 1.28</td>
<td>14.33 ± 2.19</td>
<td>11.31 ± 0.85</td>
<td>9.73 ± 1.49</td>
<td>10.99 ± 0.79</td>
<td>11.27 ± 1.26</td>
<td>0.384</td>
</tr>
<tr>
<td>Intestinal length</td>
<td>39.92 ± 4.06</td>
<td>43.57 ± 1.08</td>
<td>52.51 ± 8.57</td>
<td>43.30 ± 7.48</td>
<td>47.78 ± 5.86</td>
<td>42.84 ± 0.77</td>
<td>44.65 ± 4.02</td>
<td>0.736</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.015 ± 0.001</td>
<td>0.025 ± 0.007</td>
<td>0.023 ± 0.002</td>
<td>0.022 ± 0.002</td>
<td>0.019 ± 0.003</td>
<td>0.024 ± 0.003</td>
<td>0.036 ± 0.008</td>
<td>0.131</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.026 ± 0.006</td>
<td>0.023 ± 0.004</td>
<td>0.027 ± 0.003</td>
<td>0.015 ± 0.009</td>
<td>0.024 ± 0.006</td>
<td>0.019 ± 0.001</td>
<td>0.020 ± 0.003</td>
<td>0.332</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.94 ± 0.02</td>
<td>1.09 ± 0.09</td>
<td>1.06 ± 0.05</td>
<td>0.96 ± 0.01</td>
<td>1.11 ± 0.44</td>
<td>1.01 ± 0.04</td>
<td>0.99 ± 0.13</td>
<td>0.988</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.29 ± 0.07</td>
<td>0.39 ± 0.04</td>
<td>0.39 ± 0.09</td>
<td>0.36 ± 0.10</td>
<td>0.35 ± 0.03</td>
<td>0.40 ± 0.06</td>
<td>0.42 ± 0.03</td>
<td>0.834</td>
</tr>
<tr>
<td>Fabricia</td>
<td>0.16 ± 0.04</td>
<td>0.18 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.24 ± 0.05</td>
<td>0.16 ± 0.04</td>
<td>0.19 ± 0.04</td>
<td>0.21 ± 0.02</td>
<td>0.598</td>
</tr>
<tr>
<td>Liver</td>
<td>3.46 ± 0.06</td>
<td>4.35 ± 0.76</td>
<td>3.62 ± 0.03</td>
<td>3.72 ± 0.21</td>
<td>5.91 ± 1.76</td>
<td>3.65 ± 0.18</td>
<td>3.82 ± 0.12</td>
<td>0.296</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.08 ± 0.00</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.16 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>0.180</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.43 ± 0.02</td>
<td>0.55 ± 0.03</td>
<td>0.47 ± 0.04</td>
<td>0.63 ± 0.01</td>
<td>0.50 ± 0.07</td>
<td>0.50 ± 0.02</td>
<td>0.59 ± 0.05</td>
<td>0.035</td>
</tr>
<tr>
<td>Heart</td>
<td>0.64 ± 0.02</td>
<td>0.74 ± 0.07</td>
<td>0.68 ± 0.06</td>
<td>0.65 ± 0.03</td>
<td>0.75 ± 0.09</td>
<td>0.62 ± 0.03</td>
<td>0.70 ± 0.06</td>
<td>0.630</td>
</tr>
</tbody>
</table>

Table 1. Values of internal organs (X ± 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.

Table 2. Values (X ± 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).

(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 (10^6 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 administrated by L. plantarum at a final concentration of 10^3 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 Santos 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 (Manna, 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, 2007). The variations in the enzymatic activities may be due to animal species and probiotic interferences (Alouong, 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 (Ogink 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
reflection of the extent of cell damage and aging in an organism (Spiteller, 2001; Puvaca et al., 2015).

In our study, administration of probiotic preparations of Lact. lactis and/or L. plantarum at concentrations of 10⁸ or 10¹⁰ 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, 10⁸ cfu/ml) and T4 (L. plantarum, 10¹² cfu/ml) were recorded the lowest values of MDA contents of 7.70 and 8.47 mg/dl, respectively. T3 (L. plantarum, 10⁰ cfu/ml) and T6 (Lact. lactis, 10⁶ cfu/ml) plus (L. plantarum, 10¹² cfu/ml) recorded MDA values of 11.77 and 11.37 mg/dl, respectively in relationship to control along the rearing period and the prominent increases of probiotic on serum calcium and phosphorous levels in broiler chickens given Lact. lactis and L. plantarum alone (Treatments 1, 2, 3, and 4) or in combination (Treatments 5 and 6). Calcium concentrations 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, 10¹² cfu/ml) and T5 (Lact. Lactis, 10¹² cfu/ml) plus (L. plantarum, 10⁰ 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, 10¹² cfu/ml), T4 (L. plantarum, 10¹² cfu/ml), and T6 (Lact. lactis, 10⁰ cfu/ml) plus (L. plantarum, 10¹² 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).
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

Table 5. Values of (X ± 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).

<table>
<thead>
<tr>
<th>Period</th>
<th>Treatments**</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>14 days</td>
<td>12.84 ± 1.45</td>
<td>9.98 ± 2.24</td>
</tr>
<tr>
<td>28 days</td>
<td>12.41 ± 1.74</td>
<td>15.05 ± 3.55</td>
</tr>
<tr>
<td>42 days</td>
<td>11.74 ± 2.36</td>
<td>11.61 ± 0.75</td>
</tr>
<tr>
<td>Overall means</td>
<td>12.33 ± 1.0</td>
<td>12.21 ± 1.44</td>
</tr>
</tbody>
</table>

*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 ± 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).

<table>
<thead>
<tr>
<th>Period</th>
<th>Treatments**</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>14 days</td>
<td>9.02 ± 0.60</td>
<td>11.50 ± 0.36</td>
</tr>
<tr>
<td>28 days</td>
<td>9.20 ± 0.91</td>
<td>10.84 ± 0.77</td>
</tr>
<tr>
<td>42 days</td>
<td>8.6 ± 0.91</td>
<td>12.31 ± 0.50</td>
</tr>
<tr>
<td>Overall means</td>
<td>8.94 ± 0.61</td>
<td>11.55 ± 0.36</td>
</tr>
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</table>

*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).
microorganisms present in the cecum of broiler chicks that consumed a basal diet with microbial supplement of \textit{Lact. lactis} and \textit{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 \textit{Lact. lactis} \((10^{12} \text{ cfu/ml})\) and T6 (\textit{Lact. lactis}, \(10^{9} \text{ cfu/ml}\) plus \textit{L. plantarum}, \(10^{12} \text{ cfu/ml}\) after 14 days; afterwards these values declined but remained significantly high. These results are in agreement with those of Mountzouris \textit{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 Salmonella 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, 10^6 cfu/ml) and T3 (L. plantarum, 10^6 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 (Corigioniovisci 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, Versinia 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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