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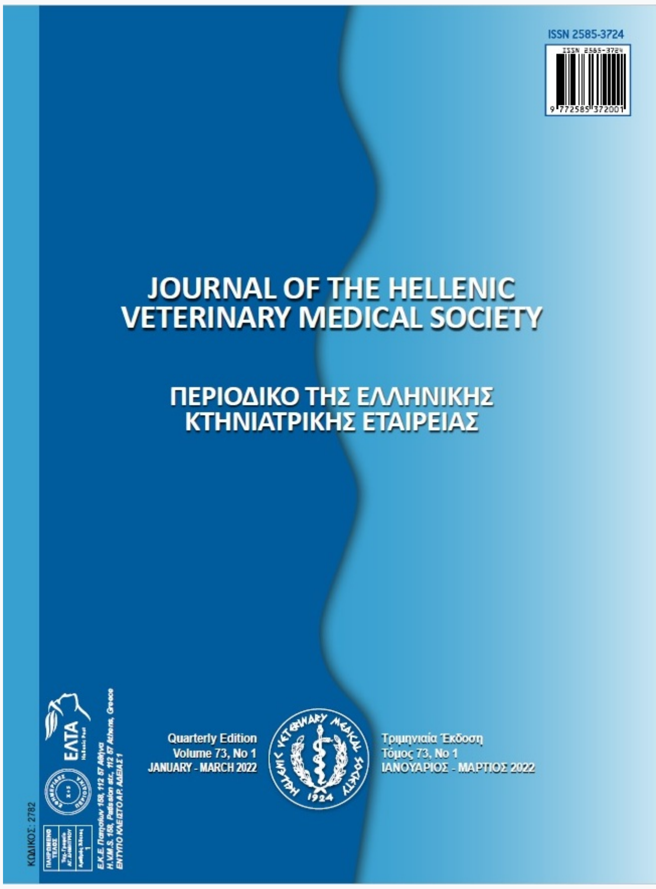

The synergistic effect of probiotic and phytobiotic for improving growth performance and biological indices in broiler chickens

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Research article
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The synergistic effect of probiotic and phytobiotic for improving growth performance and biological indices in broiler chickens

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ABSTRACT: The use of phytobiotics and probiotics show positive effect on the performance in poultry production and can produce healthy protein sources for humans. In this study, the effects of both commercial probiotic and phytobiotic were evaluated on a chicken experimental model. A total of 300 chicks were divided into 4 groups that fed the basal diet, diet containing probiotic (Protexin®), phytobiotic (garlic extract and Tichoke®), and probiotic plus phytobiotic all over the growing period. The growth indices were measured weekly, analyzed at the 21 and 42 days of age. At 42 days of age, blood samples were collected from all chickens. The concentration of liver enzymes, lipid profiles and antioxidant status were measured in blood samples. Results showed that the weight gain was significantly higher and FCR significantly lower in chickens which received probiotic alone or probiotic plus phytobiotic complex in comparison with chickens which received phytobiotic alone or control chickens ($p<0.05$). Furthermore, the addition of phytobiotic plus probiotic showed a significantly increase of blood GPx and TAS level in comparison to chickens that received probiotic or phytobiotic, alone. The level of TG, CHL and LDL were lower and the HDL value was higher in chickens which received phytobiotic or probiotic plus phytobiotic in comparison to chickens fed with probiotic or control chickens ($p<0.05$). The level of AST, ALT and ALP showed a significant decrease in chickens fed with probiotic plus phytobiotic. In conclusion, the continuous administration of the studied probiotic and phytobiotic in broiler diets can induce a synergistic effect on growth improvement, increasing of antioxidant capacity, reducing of serum lipids, and improvement of liver function in broiler chickens.

***Keywords*:** Antioxidant, Chicken, Growth Performance, Phytobiotic, Probiotic.

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INTRODUCTION

Antibiotics are commonly used in the poultry industry to prevent, and treat infectious diseases (Ben *et al.*, 2019). Some antibiotics promote growth of chickens; reduce mortality by reducing the level or activity of gastrointestinal pathogens, ultimately reducing the absorption of bacterial toxins, improving digestion, and absorption of nutrients (Gholami-Ahangaran *et al.*, 2019; Barton, Hart 2001). In addition, some antibiotics, which play a key role in boosting the immune system, are thought to stimulate the immune response (Gholami-Ahangaran *et al.*, 2020).

Probiotics have long been used as a live beneficial microbe in livestock. In poultry diets, it has positive effects improving the microbial balance of the gastrointestinal tract, (Sekhin *et al.*, 2010). According to the WHO (2001), probiotics are living microorganisms that can cause positive effects in the host. Probiotics may contain one or more strains of bacteria or yeast (Sánchez*et al*., 2017). The bacteria species commonly used as probiotics include *Bacillus*, *Bifido bacterium*, *Lactobacillus*, *Enterococcus, Pediococcus, Leuconostoc, Escherichia coli* and *Streptococcus*, whereas the yeast species, mainly include *Saccharomyces cerevisiae* and other *Saccharomyces* species (Pandey *et al.*, 2015; Fijan, 2014, Villena, Kitazawa, 2017). Probiotics benefits include the reduction of the dominance of pathogenic bacteria, balance of microbial populations, and stimulation of immune responses (Sekhon *et al.*, 2010).

Another alternative to the use of antibiotics can be herbs, and their derivatives (Ghoalmi-Ahangaran *et al.*, 2020). The use of medicinal plants in poultry production provides beneficial effects for poultry due to the presence of phenolic, terpenoid and alkaloid compounds (Gheisar, Kim 2018). Phytobiotics include a wide range of plant-derived products which are added to poultry diets to enhance growth performance, improve meat quality, showing immunostimulatory, antimicrobial, anti-inflammatory, and antioxidant properties (Windisch *et al.*, 2008; Kumar *et al.*, 2014). The chemical composition and expected effects of the used phytobiotics depends on the botanical family, part of the plant used (flowers, leaves, seeds, etc.), geographical origin, and harvest season (Bakkali *et al.*, 2008).

Phytobiotics can increase growth by improving taste, increasing the secretion of digestive enzymes (Czech *et al.*, 2009), and modulation of enteric pathogenic bacteria (Ghoalmi-Ahangaran *et al*., 2020). The aim of our study was to evaluate the effectiveness of a commercial standard probiotic (named Protexin®) and a phytobiotic (an artichoke extract named Tichoke® plus garlic extract) on several health indices in broiler chickens.

MATERIAL ANDMETHODS

Study design

The study was undertaken with approval from the Islamic Azad University, Shahrekord Branch ethics committee for care and use of animal for research (Ethical No.: 95-711).

In this study, 300 one-day old broiler chicks (Ross 308) were randomly divided into 4 groups with 5 replicates, so that 15 chickens were allocated in each replicate until 42 days of age. All groups of chickens received freely feed, which was balanced according to Ross 308 production manual (Ross 308 Broiler Nutrition Specification, 2019), *ad libitum* sanitized drinking water, reared under same growing condition comprising continuous lighting program, mechanical ventilation, at least 50% air humidity, and comfortable temperature (Starting from 32 ℃ and gradually decreased to 21 ℃ until end of growing period). All chickens received Newcastle disease (ND) vaccines at 7 (Hiprviar B1, B1 strain), 18 (Nobilis ND Clone 30, Clone 30 strain), 35 (Cevac New L, LaSota strain) days of age.

Chickens of the group one (G1) received a commercial probiotic (Protexin®, Probiotics International Ltd., UK) according to the manufacturer's recommendation. Chickens of the group2 (G2) received phytobiotic (complexing of prepared garlic extract 100 mg/L, and artichoke extract; Tichoke®, Goldaru, Iran, 100mg/L). Chickens of the group 3 (G3) received the mentioned probiotic and mentioned phytobiotic by using both mentioned doses. Finally, chickens of the group4 (G4) did not receive any additives in the basal diet and were considered as negative control. The weight gain (WG), feed intake (FI), feed conversion rate (FCR) were measured weekly, and calculated at the 21and 42 days of age.

At the 42 days of age, chickens of the four groups were weighed, and non-heparinized and heparinized blood samples were taken from wing vein. The serum samples obtained from the non-heparinized were utilized for measuring of humoral antibody against ND vaccine by using the hemagglutination inhibition test according to Allan and Gough (1976). For separation of plasma, the heparinized blood samples were centrifuged at 3, 000 × g for 15 min at 4◦C. All samples were stored at -80◦C until analysis was carried out.

The concentration of plasma total protein (TP), triglyceride (TG), cholesterol (CHL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) levels were determined by commercial kits (Pars-Azmoon Co., Tehran, Iran) in a spectrophotometer (Technicon RA1000, H83014 model, Technicon Industrial Systems, Tarrytown, NY), according to the instructions of the manufacturer. Furthermore, the plasma total antioxidant status (TAS) was determined using Randox total antioxidant capacity test kit (Randox Laboratories Ltd., Crumlin, UK) as described by Miller *et al.* (1993). The blood superoxide dismutase (SOD) activity was measured by Ransod spectrophotometric kit (Ransod, Randox Laboratories Ltd., Crumlin, UK), according to the method previously described by Woolliams *et al.* (1983). The blood Glutathione peroxidase (GPx) activity was assessed by Ransel spectrophotometric kit (Ransel, Randox Laboratories Ltd., Crumlin, UK) as described by Paglia and Valentine (1967).

Statistical analysis

All data were analyzed with the One-way ANOVA method, using SPSS (version 22) statistical package (SPSS Inc., Chicago, IL, US). Significant differences among the treatments were recognized at $P < 0.05$ using Tukey test.

RESULTS

Growth performance

At the growth indices in 21and 42 days of age there was no statistical differences between the chickens of the different groups during the growing period, while the WG and FCR showed statistically significant differences between both ages.

At the 21 days of age, the weight gain was significantly higher in chickens which received the probiotic, or the combination of the probiotic plus phytobiotic in comparison with chickens which only received the phytobiotic or control chickens (P<0.05).

The comparison of WGs in all treated groups at 42 days of age showed the highest WG and it was obtained in chickens which received both probiotic and phytobiotic that it did not show significant differences with chickens fed with probiotic only.

The FCR data in 21 and42 days of age representsthe same pattern as the lowest FCR was noted in chickens which received both probiotic and phytobiotic, and showing no statistically significant differences with those chickens which received probiotic only (Table 1).

Biochemical parameters

The TP in all treated chickens was higher than the TP in the control group. In chickens which received both probiotic and phytobiotic, the TG, CHL, LDL were lower, and the HDL was higher than those parameters observed in chickens only fed with probiotic or the control chickens (p<0.05) (Table 2).

The comparison of the ALT, AST and ALP between the different treated groups showed that chickens that received diet supplemented with phytobioticor probiotic did not have any significant differences with those parameters of the control chickens or chickens which received both probioticand phytobiotic. The minimal value of the ALT, AST and ALP was noted in chickens which received both probiotic and phytobiotic that was significantly lower than the ones of the control group (P<0.05) (Table 2).

The concentration of SOD in all treated groups did not have any significant difference with SOD in control chickens. The GPx and TAS concentration was significantly higher in chickens fed with probiotic alone, phytobiotic alone and probiotic plus phytobiotic. Indeed, the addition of both phytobiotic and probiotic can significantly increase the activity of GPxand TAS in comparison with chickens that received probiotic or phytobiotic alone (P<0.05) (Table 2).

Finally, The IgG antibody titer against ND vaccine in control group was significantly lower than other groups (P<0.05). There was no significant difference in ND titers between chickens which received probiotic alone, phytobiotic alone and probiotic plus phytobiotic (Table 2).

Table 1. The growth parameters in broiler chickens fed with probiotic and phytobiotic at the 21 and 42 days of age

Index/ Groups	Feed intake (gr)		Weight gain (gr)		FCR	
	21 days of age	42 days of age	21 days of age	42 days of age	21 days of age	42 days of age
Probiotic	858±20[a]	3620±150[a]	729±9[a]	2234±81[a]	1.16±0.04[b]	1.59± 0.03[b]
Phytobiotic	863±31[a]	3510±183[a]	701±15[b]	2001±83[b]	1.22± 0.03[a]	1.73±0.02[a]
Probiotic plus Phytobiotic	873±30[a]	3610±186[a]	743±19[a]	2284±90[a]	1.16±0.04[b]	1.58±0.03[b]
Control	882±18[a]	3588±202[a]	710±18[b]	2055±121[b]	1.24±0.02[a]	1.70±0.03[a]

• The different superscript in each column represents significant differences between treatment group (P<0.05).

Table 2. The biochemical parameters in broiler chickens fed with probiotic, phytobiotic and probiotic plus phytobiotic at the 42 days of age

Index/Group	Probiotic	Phytobiotic	Probiotic plus Phytobiotic	Control
TP (gr/dl)	4.14±0.50[a]	3.61±0.42[ab]	4.11±0.45[a]	3.11±0.22[b]
TG (mg/dl)	94±11[b]	72±15[c]	59±19[c]	125±14[a]
CHL (mg/dl)	153±26[a]	130±20[b]	133±22[b]	169±41[a]
HDL (mg/dl)	69±15[b]	75±23[ab]	82±17[a]	65±23[b]
LDL (mg/dl)	63±19[a]	44±14[b]	43±19[b]	78±20[a]
ALT (U/L)	4.50±0.7[ab]	4.30±0.6[ab]	3.90±0.7[b]	5.00±0.8[a]
AST (U/L)	152±26[ab]	135±43[ab]	130±46[b]	166±32[a]
ALP (U/L)	2.90±0.23[a]	2.77±0.34[a]	2.53±0.23[b]	2.95±0.25[a]
SOD (U/mg Hb)	1145±90[a]	1168±103[a]	1175±127[a]	1106±90[a]
GPx (U/mg Hb)	188±26[b]	139±27[b]	200±20[a]	103±24[c]
TAS (mmol/L)	0.74±0.06[b]	0.77±0.06[b]	0.88±0.08[a]	0.49±0.08[c]
HI titer (NDV)	4.04±1.00[a]	4.22±1.01[a]	4.69±1.54[a]	2.30±1.32[b]

• The different superscript in each line represents significant differences between treatment group (P<0.05).

DISCUSSION

In our study, the continuous administration of probiotics (Protexin®) or probiotic (Protexin®) plus phytobiotic (garlic extract and Tichoke®) in poultry diets increases growth indices by improving FCR and WG. The consumption of this phytobiotic has no effect on growth indices when was administrated alone in broiler chickens in our experimental model.

The current literature related to the effect of probiotics on growth indices showed very diverse results of using probiotic in poultry diets from not affecting on growth indices to improvement in all growth indices of treated birds (Gunal *et al.,* 2006; Shargh *et al*., 2012). Gunal *et al.* (2006) and Shargh et al. (2012) reported that the use of probiotic has no effect on growth indices, while Khosravi *et al.* (2010) showed that it has no effect on feed intake and final weight but increases food efficiency. In another report, Murry *et al.* (2006) stated that *Lactobacillus*-based probiotics increased food efficiency and reduced FI. Also, Awad *et al.* (2009) noted the improvement in all growth indices following dietary supplementation with a *Lactobacillus*-based probiotic in chickens. The wide variation in the results of these studies on probiotics seems to be affected by several factors such as growing conditions, diet ingredients, types of probiotics, gastrointestinal pH, presence of stressors, administrated dose, and period of probiotic administration (Wang *et al.*, 2017).

Addition of phytobiotic complex in broiler diets enhanced growth performance at 21 and 42 days of age. The beneficial effect of phytobiotic complex may be partially related to garlic. In a study, Ziarlarimi *et al*. (2011) stated that garlic powder included many organosulfur compounds such as allicin, alliin, ajoene, diallylsulfide, dithiin, and S-allylcysteine, so that garlic as a natural plant-derived feed additive may be successfully used to improve broiler growth performance. The obtained results are in agreement with the findings of Khan *et al.* (2012) who observed that broiler treated with garlic powder had significantly higher live weight gain than control group. The same trend was also found by Suriya *et al.* (2012) who used 0.5% garlic powder in broiler diet. The better effect of garlic as natural feed additives might be due to increased enzymes activity of pancreas, which offer a better environment for digestion and absorption of nutrients (Ismail *et al.,* 2021).

Various studies have been performed to elucidate the effects of the artichoke plant in poultry (Nadia *et al.*, 2007; Fallah *et al.*, 2013; Khoramshahi *et al.*, 2015; Mirderikvandi *et al.*, 2016). The role of this plant was previously studied in liver protection of Japanese quails (Nateghi *et al.*, 2013; Khoramshahi *et al.*, 2015), improving the performance index of laying hens (Nadia *et al.*, 2007; Yildiz *et al.*, 2006), and lowering cholesterol (Fallah *et al.*, 2013; Abdo *et al.*, 2007) in chickens. Several studies evaluated the effect of dried-leaf-powder or extract of artichoke on FI, WG and FCR in broilers, but showing a wide range on the effect of artichoke on growth indices. These results varied from no effect on growth indices (Tajodini *et al.*, 2015 Mirderikvandi *et al.*, 2016) to decrease of the FCR at the end of the growing period (Rouzmehr *et al.*, 2014), increase of the body weight (Lertpatarakomo *et al.*, 2015), increase of the WG and FI (Boroumandnia *et al.*, 2014), increase body weight, FI and decrease of the FCR (Shokri *et al.,* 2018), and decrease of final weight (Abdo *et al.*, 2007). However, the results of our study are consistent with previously reported works showing no effect on growth indices after administration of artichoke (Mirderikvandi *et al.,* 2016; Tajodini *et al.,* 2015).Several studies have been performed in order to elucidate the effects of probiotics on the antioxidant system in birds (Cross *et al.* 2002; Erdogan *et al.* 2010). Amaretti *et al.* (2013) showed that the use of probiotics increases antioxidant capacity, decreases oxygen radicals and reduces oxidative stress. In fact, probiotics produce butyric acid, hydrogen, which may play a stimulating role in the production of antioxidants and free radical scavenging (Zheng *et al.*, 2019).In our study, the use of probiotics had no effect on the SOD but increased the GPx, and TAS, significantly. Cross *et al.* (2002) and Erdogan *et al.* (2010) showed that probiotics had no effect on GPx level. In addition, Aluwong *et al.* (2013) showed that the use of yeast probiotics significantly increased GPx activity without affecting SOD in broilers. In another study, Bai *et al.* (2016) reported a *Bacillus subtilis* based probiotic increased antioxidants in broiler breast muscle. This is associated with increased mRNA expression of antioxidant genes, decreased oxidative damage in the pectoral muscle. Later, the same authors (Bai *et al.*, 2017) also demonstrated that the higher expression of SOD and GPx genes in mitochondria of the hepatic cells was directly related to feeding with probiotics in chickens.

In this study, we demonstrated that the use of artichoke extract alone could increase GPx and the combination of the probiotic and phytobiotic (garlic extract and artichoke extract) also have synergistic activity in increasing GPx and TAS, which even showed significant differences with the groups that received probioticor phytobiotic alone. There is scarcity of clinical research studies on the antioxidant properties of artichoke in poultry because most of the published studies have been performed in laboratory animals (Jimene-Escrig *et al.*, 2003). In rats, Jimene-Escrig *et al.* (2003) studied the antioxidant properties of artichoke and highlighted the in vitro antioxidant properties of this plant detecting increased blood GPx and had no effect on catalase and SOD, which is consistent with the findings of our study. In broilers, Mirderikvandi *et al.* (2016) stated that adding 500 mg/L of artichoke extract in drinking water for 2 weeks had no effect on MDA and reduced GPx. However, we found the increase in antioxidant activity after the use of the artichoke extract is related to the level of scavenging activity of AE. The antioxidant property of artichoke extract could be related to RSA (40% of AE content) and phenolic contents (5% of AE content, in the form of chlorogenic acid). The role of chlorogenic acid as a potent antioxidant has been previously demonstrated under *in vivo* and *in vitro* conditions (Sato *et al.*, 2011).

In the chickens received phytobiotic complex, the levels of TAS and GPx were significantly increased while no effect on SOD. Our results agreed those obtained by Nasiroleslami *et al*. (2018) who showed that broiler fed diet supplemented with guanidinoacetic acid significantly improved GPx activity in broiler liver and decreased serum MDA. Furthermore, Alagawany *et al.* (2007) demonstrated that dietary supplementation of garlic in diet had positive impact on SOD and TAS activities.

Several properties of probiotics in chickens have been previously studied (Makled *et al.*, 2019, Yan *et al.*, 2019; Ashayerizadeh *et al.*, 2011). The positive effects of probiotics in chickens are the improvement on growth indices, specific and non-specific immune responses, gastrointestinal health (Makled *et al.*, 2019), laying performance, bone strength (Yan *et al.*, 2019), but there is little information about the effect of probiotics in improving liver health in chickens. In our study, we found that the continuous consumption of the studied commercial probiotic can improve liver enzymatic activity, reduce total cholesterol and increase total protein. Several studies on the effect of probiotics on serum lipids in poultry were previously published (Ashayerizadeh *et al.* 2011; Abdulrahim *et* *al.*, 1996; Mohan *et al.*, 1996). Ashayerizadeh *et al.* (2011) demonstrated that adding probiotic to broiler diet decrease serum cholesterol level in these animals. In addition, dietary supplementation with probiotic containing *Saccharomyces cerevisiae* was demostrated to decrease cholesterol in both egg yolk (Abdulrahim *et al.*, 1996) andchicken serum (Mohan *et al.*, 1996). Amer and Khan (2012) showed that the supplementation with probiotic containing *Lactobacillus acidophilus*, *B. subtilis*, *S. cerevisiae* and *Aspergillus oryzae* decreases cholesterol in chicken serum after 6 weeks of initiate the addition of probiotics. Further to the ability of probiotic in elimination of lipids, these microorganisms can adsorb, detoxify the microbial toxin in the gastrointestinal tract and prevent the intestinal absorption. Detoxification of poisons in GIT inhibits the effect of toxins on hepatocytes (Markowiak*et al.*, 2019). In our study, the increasing of TP may be related to the influence of probiotic on secretory function of the gastrointestinal tract that leads to increase digestion, adsorption, and subsequently can elevate total protein in plasma. It seems that the positive effects of probiotics on physiological function lead to an improvement in growth indices in chickens.

There are few studies previously reported on the effect of probiotics on liver enzyme profiles (Bityutsky *et al.* 2019; Gholami-Ahangaran *et al.*, 2016). Bityutsky *et al.* (2019) reported that that the use of probiotics in quail could reduce the levels of liver enzymes of ALT and AST. The damage to the liver cell membrane causes these enzymes to be released into the bloodstream (Gholami-Ahangaran *et al.*, 2016). Therefore, the lack of increase in liver enzymes indicates no liver damage following probiotic supplementation.

In this study, AE was able to have effect on serum lipid profile, reducing CHL, TG, LDL and increasing HDL. Regarding the effect of artichoke on lipid metabolism, several studies in humans, mammals and poultry agreed that artichoke can positively affect lipid metabolism. Rouzmehr *et al.* (2014) reported that, although the addition of 200 g per ton of dried artichokes to the diet can increase WG and FI, reduced abdominal fat and blood CHL. Also, Abdo *et al.* (2007) stated that consumption of 6% dried artichoke leaves in the diet causes weight loss. However, AE seems to reduce plasma cholesterol levels by increasing bile secretion and decreasing cholesterol biosynthesis (Edward *et al.*, 2015). In addition, there is evidence that the active ingredients in artichokes have the ability to inhibit HMG-CoA reductase (Gebhardt, 1998). In our study, the decrease of hepatic enzymes including ALT, AST and ALP, and decreased plasma lipid profiles may support this hypothesis because if lipids accumulated in hepatocytes, hepatic complications would manifest as elevated hepatic enzymes in plasma. Certainly, this improve of liver health is in accordance to the increase of antioxidant capacity and it can be due to the increased ability of antioxidants to protect liver cells against toxins and oxidants.

Findings of our study showed that supplementing diets with garlic and artichoke extract decreased blood TG and LDL, and increased TP, and HDL. These results are partially in agreement with Rahimi *et al.* (2011) who found in broilers that blood TG, and LDL concentrations were decreased, while HDL increased when fed enriched-diet with 0.1% garlic powder. At this regard, Issa and Omar (2012) showed that addition of garlic powder at 0.2% and 0.4% reduced significantly concentrations of TG, CHL, LDL in broilers, and increasing the level of HDL compared to control. The potential effect of garlic products may be due to depressing the lipogenic and cholesterogenic activity of liver enzymes such as fatty acid synthase, glucose-6-phosphatase dehydrogenase, malic enzyme, and 3-hydroxy-3-methylglutaryl-CoA reductase, consequently, the mechanism of hypocholesterol and hypolipid syntheses (Mahmoud *et al.,* 2010).

In conclusion, our study demonstrated that the continuous administration of the studied commercial probiotic along with artichoke and garlic extract in broiler chicken diets improved growth indices, increased antioxidant capacity, reduced serum lipids and improved liver function in the experimentally studied broiler chickens.

CONFLICT OF INTEREST

None declared.

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