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Dietary supplementation of protexin and artichoke extract for modulating growth performance and oxidative stress in broilers

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Abstract: In this study, the effects of Protexin and artichoke extract (AE) were evaluated on the performance and oxidative stress of chickens. Totally, 300 chicks were divided into 4 groups that were fed a basal diet, a diet containing Protexin, AE, and Protexin plus AE all over the growing period. The growth indices were measured weekly and analyzed at 21 and 42 days of age. At 42 days of age, blood samples were collected from all chickens. The concentrations of liver enzymes, lipid profiles, and antioxidant status were measured in blood samples. Results showed that the weight gain (WG) was significantly higher and the feed conversion ratio (FCR) significantly lower in chickens that received Protexin, or Protexin plus AE, in comparison with chickens that received AE and control chickens ($P<0.05$). Furthermore, the addition of AE plus Protexin can significantly increase the activity of blood Glutathione peroxidase (GPx) and total antioxidant status (TAS) with respect to chickens that were fed Protexin and AE alone. The triglyceride (TG), cholesterol (CHL), and low-density lipoprotein (LDL) was lower and high-density lipoprotein (HDL) were higher in chickens that received AE or Protexin plus AE, with a comparison of chickens fed Protexin and control chickens ($P<0.05$). The levels of aspartate transferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) show a significant decrease in chickens that get Protexin plus AE. In conclusion, continuous utilization of Protexin along with artichoke extract in broiler diets can promote growth performance and modulate oxidative stress in broilers.
Keywords: chicken, performance, artichoke extract, protexin.

Introduction

Formerly, antibiotics were commonly used in the poultry industry to prevent and treat infectious diseases. Some antibiotics promote growth and reduce mortality by decreasing gastrointestinal pathogens and ultimately declining the absorption of bacterial toxins, improving digestion, and absorption of nutrients (24). In addition, some of which also play a role in boosting the immune system and are thought to stimulate the immune response. Continued use of antibiotics in livestock production induces antibiotic resistance in microbial strains which the possibility of transferring via food to humans can increase (13). Furthermore, antibiotic residues in meat and by-products can be transferred to humans via the food chain, which can cause many side effects for humans. Antibiotics are linked to an increase in allergies, genomic mutations, and cancer (12). Humans have always thought of a suitable alternative to minimize the need for antibiotics, and today, those are less popular in livestock diets (25).

Probiotics have long been used as a live beneficial microbe in livestock and poultry diets that improve the microbial balance of the gastrointestinal tract and have positive effects (47). According to the definition made by FAO/WHO, probiotics are living microorganisms that can positively affect the host (21). Probiotics may contain one or more strains of bacteria or yeast. The types of bacteria used as probiotics include *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, and yeasts, mainly *Saccharomyces cerevisiae* and *Saccharomyces* species (42). Probiotics alter the gastrointestinal tract by reducing the dominance of pathogenic bacteria and microbial populations, and stimulate the immune response, lower lipids, and increase vital organ health (47).

Another alternative to antibiotics can be found in herbs and their derivatives. The use of medicinal plants in poultry production provides beneficial effects for poultry due to the presence of valuable compounds in plants (25). Phytobiotics include a wide range of plant-derived products, including herbs, essential oils, spices, and extracts, which are added to livestock and poultry diets to enhance performance and increase product quality (52). The active substances and chemical composition of phytobiotics depend on the part of the plant used (flowers, leaves, seeds, etc.), geographical origin, and harvest season. The active ingredients in various plants belonging to distinct families are also different, and therefore their effects are expected to be different (11). However, these phytobiotics can have immunostimulatory, antimicrobial, anti-inflammatory, and antioxidant properties (31).

Due to the antimicrobial properties of medicinal plants, herbal medicine has recently become popular for poultry production without antibiotics.The use of medicinal plants in poultry production eliminates the antibiotic residual concerns in the meat and eggs. Phytobiotics can even increase the total antioxidant capacity of meat and have positive effects on consumer health (25). Antioxidant compounds in plants help the antioxidant system to remove free radicals, preventing the oxidants from damaging the vital organs and improving the performance of poultry (11).

Based on literature, phytobiotics can increase growth by improving taste and enhancing the secretion of digestive enzymes (17). In addition, there is evidence of the effect of phytobiotics on the modulation of pathogenic bacteria and inhibition of their attachment to the intestinal wall. Also, phytobiotics can increase secretion of the digestive tract and excrete the pathogenic bacteria and their toxic metabolites. It seems that all the mechanisms are involved in improving performance following phytobiotic supplementation in the poultry diet (25). Therefore, in this recent study, the performance of a commercial standard probiotic (Protexin) with a phytobiotic (artichoke extract) was compared to evaluate the effectiveness of each product in increasing performance and health indices in broiler chickens.

Materials and Methods

Study design: In this study, 300 broiler chicks (Ross 308) were randomly divided into 4 groups with 5 replicates each, so that 15 chickens were allocated to each replicate until 42 days of age. All chickens were fed and watered freely (ad libitum) and raised under the same growing conditions, which included a continuous lighting program, mechanical ventilation, at least 50% air humidity, and a comfortable temperature. All chickens were immunized with the Newcastle disease (ND) vaccine at 7, 18, and 35 days of age. The basal diet of all groups was balanced according to the Ross 308 production manual (Table 1) (44). Chickens in the first group received a commercial probiotic (Protexin, Probiotics International Ltd., UK) according to the manufacturer's recommendation; one gram per liter of drinking water in the first week, 150 grams per ton in the starter, 100 grams per ton in the growing, and 50 grams per ton in the final diet. The chickens in the second group were fed phytobiotic (artichoke extract, Tichoke, Goldaru, Iran), 100 mg/L. Chickens in the third group were given probiotic and phytobiotic supplements (Protexin and Tichoke), while chickens in the fourth group were given no supplements at all. The weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were measured weekly and calculated in two steps (at 21 and 42 days of age).

Table 1. The diet ingredients and nutrients value.

Ingredients	Starter diet (1-3 weeks)	Finisher diet (4-6 weeks)
Corn	57.00	57.65
Soybean meal	36.80	34.00
Vegetable oil	2.70	4.10
Salt (Sodium chloride)	0.30	0.30
Dicalcium Phosphate	1.40	1.80
Shell	1.20	1.60
Methionine	0.10	0.05
Commercial Premix*	0.50	0.50
Total	100	100
Calculated values	**Starter diet (1-3 weeks)**	**Finisher diet (4-6 weeks)**
Metabolic Energy (Kcal/Kg)	2970	3050
Protein (%)	21.30	20.00
Calcium	1.00	1.10
Available phosphate	0.45	0.55
Methionine+Cysteine	0.80	0.70
Lysine	1.20	1.10

At 42 days of age, all chickens were weighed and non-heparinized and heparinized blood samples were taken from the wing vein. The non-heparinized ones were used for the preparation of serum samples. The serum samples were utilized for the measuring of humoral antibodies against ND vaccine according to Allan and Gough (3) based on 4 haemagglutinin units. The blood samples were divided into two identical parts to measure the biochemical parameters in whole blood and plasma. 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), and alkaline phosphatase (ALP) levels were determined spectrophotometrically, using commercial kits (Technicon RA1000, H83014 model, Technicon Industrial Systems, Tarrytown, NY), according to the instructions of manufacture (Pars-Azmoon Co., Tehran, Iran). Furthermore, plasma total antioxidant status (TAS) was assayed using the Randox total antioxidant capacity test kit (Randox Laboratories Ltd., Crumlin, UK) as described by Miller et al. (35). Blood superoxide dismutase (SOD) activity was measured by the Ransod spectrophotometric kit (Ransod, Randox Laboratories Ltd., Crumlin, UK), according to the Woolliams et al. (53) method. Blood Glutathione peroxidase (GPx) activity was assessed by the Ransel spectrophotometric kit (Ransel, Randox Laboratories Ltd., Crumlin, UK) as described by Paglia and Valentine (41).

Protexin®: This commercial probiotic is presented at a concentration of 2 × 10^9 CFU/g and contains *Streptococcus feacium*, *Streptococcus termophilus*, *Lactobacillus plantarum*, *Lactobacillus johnsonii*, *Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium bifidum, Aspergillus ourozai,* and *Candida pentolopsy*. This probiotic is a product of Probiotics International Ltd., UK.

Tichoke ®: Tichoke is a commercial dried artichoke extract (AE) prepared from *Cynara scolymus* that contains 5% caffeoylquinic acid as chlorogenic acid. Based on the DPPH method (43), the radial scavenging activity (RSA) in the samples was on average 40%. This phytobiotic is a product of Goldaru, IRAN.

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 the Tukey test.

Results

Growth performance: The growth index at 21 and 42 days of age indicates that there were no statistical differences in the FI of chickens in different groups at all over the growing period, while the WG and FCR were influenced by adding probiotic, phytobiotic, and probiotic plus phytobiotic to the diet.

At 21 days of age, the WG was significantly higher in chickens fed probiotics, or probiotic plus phytobiotic in comparison with chickens receiving phytobiotic or control diets (P<0.05).

The comparison of WG in all treatment groups at 42 days of age showed the highest WG was obtained in chickens receiving probiotic plus phytobiotic, while it did not show significant differences with chickens fed probiotic.

The FCR data at 21 and 42 days of age represent same pattern and the lowest FCR was illustrated in chickens get probiotic plus phytobiotic that possess no significant difference with chickens fed probiotic (Table 2).

Biochemical parameters: The TP was statistically higher than the control group in treated chickens, except for the group that received phytobiotics. In chickens that received probiotic plus phytobiotic, the TG, CHL, and LDL were lower, but HDL was higher than in chickens fed probiotic or control chickens, significantly (P<0.05) (Table 3).

The comparison of ALT and AST in different treatment groups reveals that chickens that received diets supplemented with phytobiotics or probiotics did not have any significant differences with control chickens or chickens that received probiotic plus phytobiotic. The comparison of ALP in different treatment groups shows that chickens that were fed a diet supplemented with phytobiotics or probiotics did not have any significant differences with control chickens, but there was a significant difference when chickens received probiotic plus phytobiotic. In terms of ALP parameters, the value obtained in the probiotic plus phytobiotic group is lower than in all other groups. The chickens that received probiotic plus phytobiotic had significantly lower ALT, AST, and ALP levels than the control group (P<0.05) (Table 3).

Table 2. The growth parameters in broiler chickens fed probiotic and phytobiotic at 21 and 42 days of age.

Index/ Groups	Feed intake (g)		Weight gain (g)		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
Protexin	862±22[a]	3610±163[a]	731±11[a]	2230±80[a]	1.17±0.03[b]	1.61±0.02[b]
Artichoke extract	860±32[a]	3500±190[a]	700±27[b]	2000±81[b]	1.23±0.02[a]	1.75±0.02[a]
Protexin plus Artichoke extract	870±35[a]	3600±181[a]	740±24[a]	2280±92[a]	1.17±0.03[b]	1.57±0.02[b]
Control	880±28[a]	3580±200[a]	703±20[b]	2050±123[b]	1.25±0.02[a]	1.74±0.02[a]
P value	0.75	0.78	0.12	0.02	0.01	0.01

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

Table 3. The biochemical parameters in broiler chickens fed probiotic, phytobiotic and probiotic plus phytobiotic at 42 days of age.

Index/Group	Protexin	Artichoke extract	Protexin plus Artichoke extract	Control	P value
TP (g/dl)	4.24±0.50[a]	3.52±0.42[ab]	4.00±0.45[a]	3.05±0.22[b]	0.022
TG (mg/dl)	96±11[b]	70±15[c]	56±19[c]	128±14[a]	0.0014
CHL (mg/dl)	150±26[a]	129±20[b]	130±22[b]	167±41[a]	0.034
HDL (mg/dl)	68±19[b]	73±21[ab]	80±12[a]	63±20[b]	0.025
LDL (mg/dl)	62±18[a]	42±12[b]	40±11[b]	77±19[a]	0.012
ALT (U/L)	4.62±0.8[ab]	4.22±0.7[ab]	3.95±0.6[b]	5.04±0.7[a]	0.046
AST (U/L)	150±27[ab]	139±40[ab]	135±48[b]	160±36[a]	0.032
ALP (U/L)	2.98±0.26[a]	2.70±0.39[a]	2.50±0.20[b]	2.85±0.22[a]	0.038
SOD (U/mg Hb)	1135±99[a]	1178±133[a]	1190±107[a]	1096±95[a]	0.671
GPx (U/mg Hb)	181±16[b]	189±17[b]	220±19[a]	100±21[c]	<0.001
TAS (mmol/L)	0.64±0.05[b]	0.67±0.07[b]	0.79±0.09[a]	0.45±0.07[c]	0.003
HI titer (NDV)	4.55±1.01[a]	4.2±1.22[a]	4.8±1.35[a]	2.77±1.30[b]	0.046

- The different superscript in each line represents significant differences between treatment group (P<0.05).
- TP: Total Protein, TG: Triglyceride, CHL: Cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, AST: Aspartate transferase, ALT: Alanine aminotransferase, ALP: alkaline phosphatase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, TAS: Total antioxidant Staus, HI: Haemaglutination inhibition.

The concentration of SOD in all treatment groups did not have any significant difference. The blood GPx and TAS concentrations were significantly higher in chickens fed probiotic, phytobiotic, and probiotic plus phytobiotic, while the addition of phytobiotic plus probiotic can significantly increase the activity of blood GPx and TAS with respect to chickens that received probiotic or phytobiotic alone (P<0.05) (Table 3).

The IgG antibody titer against ND vaccine in the control group was significantly lower than in other groups (P<0.05). There is no significant difference in ND titer in chickens that received probiotic, phytobiotic, or probiotic plus phytobiotic (Table 3).

Discussion and Conclusion

The results of the study have shown that continuous utilization of probiotics (protexin) or probiotics plus phytobiotics (protexin plus Tichoke) in poultry diets increases growth by improving FCR and WG. Consumption of Tichoke as a phytobiotic has no effect on growth indices, alone. Therefore, it seems that the phytobiotic of Tichoke has no effect on growth indices in broiler chickens. A review of previous studies on the effect of probiotics on growth indices showed that the results of using probiotics in the diet are very diverse and the range of results varies from not affected on growth indices to improvement in all growth indices (27, 30, 48). Gunal et al. (27) and Shargh et al. (48) reported that the use of probiotics has no effect on growth indices, while Khosravi et al. (30) showed that it has no effect on FI and final weight but increases food efficiency. In another report, Murry et al. (38) stated that *lactobacillus*-based probiotics increase food efficiency but reduce FI. Also, Awad et al. (8) showed improvement in all growth indices following dietary supplementation with a *lactobacillus*-based probiotic in chickens. Variation in the results of studies on probiotics seems to be affected by growing conditions, diet ingredients, type of probiotic, gastrointestinal pH, stress, dose, and period of probiotic administration (51).

Various studies have been performed on the effects of the artichoke plant on poultry. Previously, the role of this plant in liver protection of Japanese quails (29, 40), improving the performance index of laying hens (39, 55), and lowering cholesterol (1, 20) has been studied. There are many studies that evaluated the effects of artichoke in the form of dried leaf powder or extract on FI, WG, and FCR in broilers, although the results on the effect of artichoke on growth indices are very diverse. These results varied from no effect on growth indices (36, 50) to decreased FCR (45, 49), increased WG (1, 15, 32, 49), and elevation of FI (15, 49). However, the results of the present study are consistent with some studies that indicated the administration of artichoke had no effect on growth indices (36, 50). Certainly, the geographical area of cultivation, plant chemical composition, harvest season, dose, duration of administration, and phytobiotic type can influence the results.

Various studies have been performed on the effects of probiotics on the antioxidant system. Amaretti et al. (5) showed that the use of probiotics increases antioxidant capacity and decreases oxygen radicals. In addition, several studies have shown that the use of probiotics reduces oxidative stress. In fact, probiotics produce butyric acid and hydrogen, which may play a stimulating role in the production of antioxidants and free radical scavenging (56). In the recent study, the use of probiotics had no effect on the SOD but increased the GPx and TAS significantly. Cross et al. (16), and Erdogan et al. (19) observed that probiotics had no effect on GPx levels. In addition, Aluwong et al. (4) demonstrated that the use of yeast probiotics significantly increased GPx activity without affecting SOD in broilers. In another study, a *Bacillus subtilis* based probiotic increased antioxidants in broiler pectoral muscle. This is associated with increased mRNA expression of antioxidant genes and decreased oxidative damage in the pectoral muscle (9). Also, in another study, Bai et al. (10) showed that the higher expression of SOD and GPx genes in the mitochondria of the liver was related to feeding with probiotics in chickens. It seems that the strains of probiotics can be effective in achievingantioxidant results.

In the recent study, the use of artichoke extract could increase GPx and total antioxidant capacity, and it even seems that the combined use of probiotics and phytobiotics of artichoke extract has synergistic activity in increasing GPx and TAS. There is a significant difference in GPx and TAS values in chickens fed probiotic or phytobiotic compared with chickens receiving symbiotic. There is limited clinical research on the antioxidant properties of artichoke in poultry, and most studies have been in laboratory animals. Previously, Jimene-Escrig et al. (28) studied the antioxidant properties of artichoke and showed that this plant has acceptable antioxidant properties in vitro. Also, feeding of this plant for 3 weeks at 14% of feed in normal rats increased blood GPx levels and had no effect on catalase and SOD, which is consistent with the findings of the present study. In broilers, Mirderikvandi et al. (36) stated that adding 500 mg/L of artichoke extract in drinking water for 2 weeks did not influence malondialdehyde and reduced GPx. However, the increase in antioxidant activity in the present study after the use of AE is related to the level of scavenging activity of AE. The RSA of about 40% and phenolic content of about 5% (in the form of chlorogenic acid) are mainly contributed to by the effect of AE on antioxidant capacity. The role of chlorogenic acid as a potent antioxidant has been previously demonstrated in vivo and in vitro (46).

Until now, the various properties of probiotics in chickens have been studied. Positive effects on growth indices, increasing the level of specific and non-specific immunity, gastrointestinal health (33), increasing performance in layers and bone strength (54) are some of the cases that have been reported in various studies, but there is little information about the effect of probiotics in improving metabolic function, especially liver health in chickens. The results of the recent study show that continuous consumption of a commercial probiotic (Protexin) can improve liver enzymatic activity, reduce total cholesterol, and increase total protein. There are several studies on the effect of probiotics on serum lipids in poultry. Ashayerizadeh et al. (7) demonstrated that adding probiotics to the broiler diet decreased cholesterol levels when compared with control chickens or chickens fed prebiotics or antibiotics. Also, dietary supplementation with probiotics containing *Saccharomyces cerevisiae* reduced cholesterol in egg yolk (2) and serum in chickens (37). In a study performed by Amer and Khan (6), it was illustrated that the supplementation with probiotics containing *Lactobacillus acidophilus*, *Bacillus subtilis*, *Saccharomyces cerevisiae,* and *Aspergillus oryzae* significantly decreased cholesterol in serum after 6 weeks. Previously, it was explained that *L. acidophilus* absorbs cholesterol and decreases the cholesterol level in medium (26). Apart from their ability to eliminate lipids, probiotic microorganisms can also adsorb and detoxify microbial toxin in the gastrointestinal tract (GIT) and prevent its intestinal adsorption. Detoxification of poisons in the GIT inhibits the effect of toxins on hepatocytes (34). The increase of TP in the present study may be related to the influence of probiotics on secretory function of the GIT that leads to increased digestion and absorption and, subsequently, can elevate total protein in plasma. It seems that the positive effects of probiotics on physiological function lead to an increase in growth indices in chickens.

There are few studies on the effect of probiotics on liver enzymes. Bityutsky et al. (14) showed that the use of probiotics in quail could reduce the levels of liver enzymes of ALT and AST. Damage to the liver cell membrane causes these enzymes to be released into the bloodstream (23). Therefore, no changes in liver enzymes indicates no liver damage following probiotic supplementation.

In this study, AE was able to affect to serum lipid profile, reduce CHL, TG, and LDL, and increase HDL. Regarding the effect of artichoke on lipid metabolism, various studies have been performed in humans, animals, and poultry, and in most of these studies, there is an agreement that artichoke can affect lipid metabolism. Rouzmehr et al. (45) reported that although the addition of 200 g per ton of dried artichokes to the diet can increase WG and FI, it reduces abdominal fat and blood CHL. Also, Abdo et al. (1) stated that consumption of 6% dried artichoke leaves in the diet causes weight loss, and this finding is consistent with reducing the amount of abdominal fat. However, AE seems to reduce plasma cholesterol levels by increasing bile secretion and decreasing cholesterol biosynthesis (18). In addition, there is evidence that the active ingredients in artichokes have the ability to inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (22). Decreased 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. Due to the fact that in liver problems, liver enzymes usually leak out of liver cells and increase in the blood (23), a decrease in liver enzymes following the use of phytobiotics or the simultaneous use of probiotics and phytobiotics can be a marker of liver health. Certainly, this increase in the level of liver health is in line with the increase in antioxidant capacity and can be due to the increased ability of antioxidants to protect liver cells against toxins and oxidants.

In conclusion, continuous use of probiotic (Protexin) along with phytobiotic (Artichoke extract) in broiler diets can improve growth indices, increase antioxidant capacity, reduce serum lipids and improve liver function in broiler chickens.

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Ethical Statement

This study was confirmed and approved by Ethics Committee of Sharekord Branch, Islamic Azad University for care and use of animal for research (IR. Shk. 99. 128).

Conflict of Interest

The authors declare no conflict of interest.

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