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The advantages and synergistic effects of Gunnera (*Gundelia tournefortii* L.) extract and protexin in chicken production

Majid Gholami-Ahangaran[1] | **Maziar Haj-Salehi**[2] | **Asiye Ahmadi-Dastgerdi**[3] | **Maryam Zokaei**[4]

[1] Department of Poultry Diseases, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

[2] Grarduated of Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

[3] Department of Food Science and Technology, Ardestan Branch, Islamic Azad University, Ardestan, Iran

[4] Department of Food Science and Technology, Faculty of Nutrition Science, Food Science and Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Correspondence
Majid Gholami-Ahangaran, Department of Poultry Diseases, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, P.O. Box 166, Shahrekord, Iran.
Email: mgholami6@gmail.com

Funding information
Shahrekord Branch, Islamic Azad University

Abstract

Background: Probiotics and phytogenics in the poultry diet have many positive effects on productivity. The combination of these feed additives has not been studied.

Objective: This study was designed for evaluation of synergistic effects of protexin (P) and Gunnera (Gundelia tournefortii L.) extract (GX) on growth, biochemical, hematological and antioxidant status of broiler chickens.

Methods: Totally, 300 chicks were divided into 4 groups that fed the basal diet, diet containing P, GX, and GX plus P (GX-P) in all over the growing period. At 42 days of age, blood samples were collected from all chickens. The concentration of aspartate transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglyceride (TG), cholesterol (CHL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), superoxide dismutase (SOD), total antioxidant capacity (TAC), Glutathione peroxidase (GPx), haematological parameters, and humeral antibody against Newcastle disease vaccine was measured.

Results: The Results showed that the feed conversion ratio in chickens fed GX-P was significantly lower than others. Also, in chickens fed GX or GX-P, the TG and CHL concentration was significantly lower and GPx and TAC concentration was significantly higher than others, while chickens that received P or GX-P showed higher haemoglobin and TP concentration. The antibody response was significantly higher in chickens fed P. The ALT, AST, ALP, and SOD concentration did not show any significant difference in all chickens.

Conclusion: Continuous utilization of P along with GX in broiler diets can induce synergist effect on feed efficacy and antioxidant status, lowering lipid profiles with no effect on liver function in chickens.

KEYWORDS
antioxidant, chicken, Gunnera, performance, probiotic

1 | INTRODUCTION

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 (Sekhon et al., 2010). According to the definition provided by the Food and Agriculture Organization and the World Health Organization (2001), probiotics are living microorganisms that can cause positive effects in the host. 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 (Pandey et al., 2015). 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 (Sekhon et al., 2010).

The use of medicinal plants in poultry production provides beneficial effects for poultry due to the presence of valuable compounds in plants (Farkhondeh et al., 2011; Attia et al., 2011, 2014; Gheisar & Kim 2018). 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 (Attia et al., 2017; Windisch et al., 2008). Active substance and chemical composition of phytobiotics depend on the part of the plant used (flowers, leaves, seeds, etc.), geographical origin, and harvest season. Due to the active ingredients in different plants belonging to different families, different effects of plants are expected (Gholami-Ahangaran et al., 2021; Bakkali et al., 2008). However, these phytobiotics can have immunostimulatory, antimicrobial, anti-inflammatory, and antioxidant properties (Kumar et al., 2014).

Based on the literature, there is evidence of the effect of phytobiotics on the modulation of pathogenic bacteria and inhibition of their attachment to the intestinal wall (Gholami-Ahangaran et al., 2020). Also, phytobiotics can increase secretion of digestive tract and excrete the pathogenic bacteria and fermentation of their toxic metabolites (Farkhondeh et al., 2011; Shargh et al., 2012). It seems that all mentioned effects are involved in improving growth performance following phytobiotic supplementation in the poultry diet (Gheisar & Kim, 2018).

Gundelia tournefortii L. (Gunnera) belonging to Asteraceae (Compositae) family is a medicinal plant, native to Western Asia, for example, Iran, Turkey, Azerbaijan, and Turkmenistan. Gunnera is one of the most abundant plants in the steppe and mountainous regions of Iran, which is easily propagated and grows in all mountainous regions of Iran (Carapetian & Zarei, 2005). The flowers, leaves, seeds, and stems of Gunnera are used as food sources. This plant is considered a useful food and traditional medicinal plant due to its abundance of vitamins A, B, C, and minerals (Ertug, 2000). Artichoke-like properties have been mentioned for the stem of this plant, and it is believed that it is useful for lowering blood lipids, especially cholesterol. Dry seeds of Gunnera are known to be effective for the treatment of vitiligo disease in Eastern Anatolia folk medicine. Fresh seeds of Gunnera are used in pickles and are also effective diuretics. The seed oil of this plant is mainly linoleic and oleic acid, which has a high nutritional value (Coruh et al., 2007).

Previously, the antioxidant activities of Gunnera extracts were demonstrated for their radical scavenging and lipid peroxidation inhibition capacities. Furthermore, the effects of Gunnera extracts on glutathione-S-transferase (GST) activities for detoxification of lipid peroxidation by-products were revealed. This medicinal plant contains phenolic compounds. Phenolic content of the seed extracts of Gunnera was higher (105.1 lg/mg extract) than the aerial parts of this medicinal plant (64.4 lg/mg extract). Plant extracts with high polyphenols are known to have important inhibitory effects on GST (Coruh et al., 2007).

Therefore, in a recent study, the performance of a commercial standard probiotic (protexin) with a phytobiotic (Gunnera extract) was compared to evaluate the effectiveness of each product in increasing performance and health indices in broiler chickens.

2 | MATERIALS AND METHODS

2.1 | Experimental design

In this study, 300 broiler chicks (Ross 308) were randomly divided into four groups with five replicates, so that 15 chickens were allocated in each replicate (1 × 1.5 m) until 42 days of age. All chickens received freely feed and water (ad libitum) and reared under the same growing condition in closed system comprising continuous 24 h lighting programme, mechanical ventilation, at least 50% air relative humidity, and comfortable temperature (start from 32°C and gradually decreased weekly to 20°C). All chickens received Newcastle disease (ND) vaccine at 7, 18, and 35 days of age. The basal diet of all groups was balanced according to NRC (NRC, 1994) (Table 1). Chickens in the first group received a commercial probiotic (Protexin, Probiotics International Ltd.) according to the manufacturer's recommendation: one gram per litre of drinking water in the first week, 150 g per ton in the starter, 100 g per ton in the growing, and 50 g per ton in the final diet. The chickens in the second group received phytobiotic (Gunnera extract) and 100 mg/L drinking water. Chickens in the third group received mentioned probiotic and phytobiotic (protexin and Gunnera extract), and chickens in the fourth group, as control, did not receive any additives in basal diet. The weight gain (WG), feed intake (FI), and feed conversion rate (FCR) were measured weekly and calculated in 42 days of age.

At 42 days of age, all chickens were weighed, and non-heparinized and heparinized blood samples were taken from wing vein. The non-heparinized samples were used for the preparation of serum samples. The serum samples were utilized for measuring humoral antibody against ND vaccine according to Allan and Gough (1976) based on four haemagglutinin units. The blood samples were divided into two same parts to measure the biochemical parameters in whole blood and plasma. For the separation of plasma, the heparinized blood samples were centrifuged at 3000 × *g* for 15 min at 4°C. All samples were stored at −80°C until analysis was carried out.

Haematology examinations were performed on blood samples containing anticoagulant and covered haematocrit percentage, white and

TABLE 1 The diet ingredients and nutrients value

Ingredients	Starter diet (1–3 weeks)	Finisher diet (4–6 weeks)
Corn	57.00	57.50
Soybean meal	37.00	34.00
Vegetable oil	2.50	4.00
Salt (sodium chloride)	0.30	0.30
Dicalcium phosphate	1.30	1.70
Shell	1.00	1.50
Methionine	0.25	0.30
Lysine	0.15	0.20
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	1.20
Available phosphate	0.45	0.55
Methionine + cysteine	0.80	0.70
Lysine	1.20	1.10

red blood cells count, neutrophile, lymphocyte, monocyte, eosinophile, and basophile percentage. In addition, mean cell volume (MCV), mean cell haemoglobin (MCH), and mean concentration of haemoglobin cell (MCHC) were measured.

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 was determined using commercial kits, spectrophotometrically (Technicon RA1000, H83014 model, Technicon Industrial Systems) according to the manufacture's instruction (Pars-Azmoon Co.). Furthermore, plasma total antioxidant capacity (TAC) was determined using Randox total antioxidant capacity test kit (Randox Laboratories Ltd.) as described by Milleret et al. (1993). Blood superoxide dismutase (SOD) activity was measured by Ransod spectrophotometric kit (Ransod, Randox Laboratories Ltd.) according to the method of Woolliams et al. (1983). Blood Glutathione peroxidase (GPx) activity was assessed by Ransel spectrophotometric kit (Ransel, Randox Laboratories Ltd.) as described by Paglia and Valentine (1967).

2.2 | Protexin

This commercial probiotic presented at a concentration of 2×10^9 CFU/g containing *Streptococcus faecium*, *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.

TABLE 2 The growth parameters in broiler chickens fed probiotic and/or Gunnera extract at 42 days of age

Index/groups	FI	WG	FCR
Probiotic	3700 ± 188^{a}	2200 ± 190^{a}	1.68 ± 0.04^{ab}
Gunnera extract	3600 ± 250^{a}	2150 ± 150^{a}	1.67 ± 0.05^{ab}
Probiotic plus Gunnera extract	3500 ± 190^{a}	2300 ± 182^{a}	1.53 ± 0.08^{b}
Control	3600 ± 210^{a}	2100 ± 193^{a}	1.72 ± 0.03^{a}

Abbreviations: FCR, feed conversion rate; FI, feed intake; WG, weight gain.
Note: The different superscript letters in each column represent significant differences between treatment group ($p < 0.05$).

2.3 | Gunnera extract

In spring of 2018, Gunnera leaves were collected from Lorestan province (western area of Iran) and dried in the shade. Then, the powder grind and suspended in water and ethanol (50:50) for 48 h. The extract was prepared by the maceration method according to Gholami-Ahangaran et al. (2019). The extract was filtered with Whatman paper 2.0 and concentrated in the oven for 72 h.

2.4 | Statistical analysis

The data was statistically analyzed based on the one-way analysis of variance (ANOVA) method, using SPSS (version 22) statistical package (SPSS Inc.). Significant differences among the treatments were recognized at $p < 0.05$, using Tukey's test.

3 | RESULTS

3.1 | Growth performance

The growth indices in 42 days of age represent that there was no statistical differences in the WG and FI of chickens in different groups, while the FCR was changed by adding probiotic, Gunnera extract, or probiotic plus Gunnera extract to diet. At 42 days of age, the lowest FCR was seen in chickens received probiotic plus Gunnera, while there was no significant difference in FCR between chickens Gunnera extract or probiotic with chickens fed probiotic plus Gunnera extract (Table 2).

3.2 | Biochemical parameters

In chickens received protexin or protexin plus Gunnera extract, the TP was higher than control chickens. Also, TG and CHL were significantly lower in chickens fed Gunnera extract or probiotic plus Gunnera extract ($p < 0.05$) (Table 3).

The comparison of ALT, AST, and ALP in different treatment groups represent that there is no significant difference between different groups (Table 3).

TABLE 3 The biochemical parameters in broiler chickens fed probiotic and/or Gunnera extract at 42 days of age

Index/Group	Probiotic	Gunnera extract	Probiotic plus Gunnera extract	Control
TP (gr/dl)	4.24 ± 0.20^{a}	3.52 ± 0.62^{ab}	4.00 ± 0.35^{a}	3.05 ± 0.32^{b}
TG (mg/dl)	104 ± 20^{b}	80 ± 20^{c}	60 ± 25^{c}	135 ± 23^{a}
CHL (mg/dl)	150 ± 23^{a}	118 ± 29^{b}	126 ± 20^{b}	160 ± 25^{a}
HDL (mg/dl)	80 ± 19^{a}	70 ± 21^{a}	82 ± 12^{a}	70 ± 20^{a}
LDL (mg/dl)	50 ± 25^{a}	35 ± 18^{a}	42 ± 15^{a}	60 ± 15^{a}
ALT (U/L)	4.62 ± 0.9^{a}	4.70 ± 0.5^{a}	4.80 ± 0.5^{a}	4.90 ± 0.8^{a}
AST (U/L)	150 ± 35^{a}	149 ± 45^{a}	144 ± 40^{a}	145 ± 39^{a}
ALP (U/L)	2.80 ± 0.21^{a}	2.80 ± 0.29^{a}	2.70 ± 0.30^{a}	2.95 ± 0.20^{a}
SOD (U/mg Hb)	1106 ± 109^{a}	1150 ± 145^{a}	1140 ± 132^{a}	1106 ± 115^{a}
GPx (U/mg Hb)	160 ± 35^{ab}	190 ± 24^{a}	210 ± 25^{a}	126 ± 30^{b}
TAC (mmol/L)	0.59 ± 0.11^{ab}	0.77 ± 0.09^{a}	0.86 ± 0.10^{a}	0.45 ± 0.14^{b}
HI titre (NDV)	5.25 ± 0.60^{a}	4.2 ± 0.9^{ab}	4.5 ± 0.6^{ab}	3.77 ± 0.80^{b}

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transferase; CHL, cholesterol; GPx, glutathione peroxidase; HDL, high-density lipoprotein; HI, haemagglutination inhibition; LDL, low-density lipoprotein; SOD, superoxide dismutase; TAC, total antioxidant status; TG, triglyceride; TP, total protein.
Note: The different superscript letters in each line represent significant differences between treatment group ($p < 0.05$).

TABLE 4 The haematological indices in broiler chickens fed probiotic and/or Gunnera extract at 42 days of age

Index/groups	Control	Probiotic plus Gunnera extract	Gunnera extract	Probiotic
PCV (%)	35 ± 3.6	37 ± 3.8	35 ± 4.5	34 ± 3.6
RBC (10^6/mm^3)	3.05 ± 0.40	3.13 ± 0.46	3.11 ± 0.58	3.22 ± 0.50
WBC (10^6/mm^3)	2.70 ± 0.30	2.75 ± 0.40	2.80 ± 0.35	2.63 ± 0.30
Neutrophils (%)	36.10 ± 2.70	35.76 ± 2.53	37.97 ± 3.17	39.15 ± 2.20
Lymphocytes (%)	61.49 ± 2.94	58.65 ± 2.60	56.70 ± 2.60	56.05 ± 2.105
Monocytes (%)	3.80 ± 0.99	4.33 ± 0.40	4.30 ± 0.30	3.64 ± 0.46
Eosinophils (%)	1.11 ± 0.60	1.78 ± 0.75	2.40 ± 0.27	1.85 ± 0.59
Basophils (%)	0.48 ± 0.13	0.39 ± 0.208	0.50 ± 0.19	0.70 ± 0.16
Hb (g/dl)	10.00 ± 0.32^{b}	12.33 ± 0.39^{a}	10.30 ± 0.50^{b}	11.81 ± 0.46^{a}
MCV (fl)	90.23 ± 0.25	90.04 ± 0.33	90.10 ± 0.40	90.50 ± 0.40
MCH (pg)	30.56 ± 0.19	33.33 ± 0.12	32.12 ± 0.18	31.45 ± 0.15
MCHC (%)	30.19 ± 0.31	35.36 ± 0.31	34.35 ± 0.29	32.50 ± 0.39

Abbreviations: MCH, mean cell haemoglobin; MCV, mean cell volume; MCHC, mean concentration of haemoglobin cell.
Note: The different superscript letters in each row represent significant differences between treatments ($p < 0.05$).

The concentration of SOD in all treatment groups did not have any significant difference. The blood GPx and TAC concentration were significantly higher in chickens fed Gunnera extract or probiotic plus Gunnera extract ($p < 0.05$), while the addition of probiotic did not have any significant difference with other groups (Table 3).

The IgG antibody titre against ND vaccine in control group was significantly lower than other groups ($p < 0.05$). Also, ND titre in chickens received probiotic was higher than other chickens ($p < 0.05$) (Table 3).

3.3 | Haematology

As the results showed, haematocrit, white blood cell (WBC) and red blood cell (RBC) count, neutrophil, lymphocyte, monocyte, basophil percentage, haemoglobin, MCV, MCH, and MCHC were not significantly different among the chickens in all groups. Only Hb level in the chickens received probiotic or probiotic plus Gunnera extract was significantly higher than control chickens ($p < 0.05$) (Table 4).

4 | DISCUSSION

The results of the present study have shown that continuous utilization of probiotic plus Gunnera extract in chicken diets improve FCR, but consumption of probiotic or Gunnera extract has no effect on growth indices alone. This finding can explain synergistic effect of Gunnera extract with probiotics in growing indices. A review of previous studies on the effect of probiotics on growth indices showed that the results of using probiotic in the diet are very diverse, and the range of results varies from not affecting growth indices to improvement in all growth indices (Gunal et al., 2006; Shargh et al. 2012). 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 (Wang et al., 2017).

Although there is no report related to Gunnera application in chicken diet, various studies have been performed on the effects of the artichoke plant in poultry. Artichoke has a close homology in respect of phytobiological characteristics by Gunnera. These plants belonging to *Asteraceae* family, and Gunnera is a traditional species in Iran. Previously, the effect of artichoke in liver protection of Japanese quails (Khoramshahi et al., 2015; Nateghi et al., 2013), improving the performance index of laying hens (Nadia et al., 2007; Yildiz et al., 2006) and lowering cholesterol (Abdo et al., 2007; Fallah et al., 2013) have been studied. The results of these studies varied from no effect on growth indices (Mirderikvandi et al., 2016; Tajodini et al., 2015) to decrease FCR at the end of the growing period (Rouzmehr et al., 2014), increase body weight (Lertpatarakomo et al., 2015), increase WG and FI (Boroumandnia et al., 2014), increase body weight and FI, decrease FCR (Shokri et al., 2018), and even decrease in final weight (Abdo et al., 2007). However, our results on Gunnera extract effect on chicken growth represented that administration of Gunnera extract had no effect on growth indices. Certainly, the geographical area of the cultivation, plant chemical composition, harvest season, dose, duration of administration, and phytobiotic type can affect the results.

Various studies have been performed on the effects of probiotics on the antioxidant system. In this study, the use of probiotics had no effect on the SOD but increased the GPx and TAC significantly. Cross et al. (2002) and Erdoğan 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. It seems that the strains of probiotics can be effective on antioxidant results.

In this study, the use of Gunnera extract could increase GPx and TAC, and it even seems that the combination of probiotic and Gunnera extract has synergistic activity in increasing GPx and TAC so that the combination significantly increased GPx and TAC, which showed significant differences with the probiotic received groups. There is no clinical research on the antioxidant properties of Gunnera in poultry. However, the increase in antioxidant activity in the present study after the use of Gunnera is related to the level of scavenging activity of Gunnera. The radical scavenging activity and phenolic contents (in the form of chlorogenic acid) of *Asteraceae* family mainly contribute to the effect of Gunnera on antioxidant capacity. The role of chlorogenic acid as a potent antioxidant has been previously demonstrated in vivo and in vitro (Sato et al., 2011). The nutrients and antioxidant capacity of Gunnera extract can supply essential nutrient for metabolism of RBC and improve haematological profiles in chickens that received Gunnera extract or Gunnera extract with probiotic. The increasing Hb in chickens fed Gunnera extract with or without probiotic is in line with this hypothesis.

This study shows that continuous consumption of Gunnera extract can reduce lipid serum but probiotic no effect on lipid level. The synergistic effect of Gunnera extract and protexin showed lowering of lipid profile including CHL and TG in serum. There are several studies on the effect of probiotics on serum lipids in poultry. A study by Amer and Khan (2012) showed that the supplementation with probiotic contained *Lactobacillus acidophilus*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae* significantly decreases cholesterol in serum after 6 weeks. Further to the ability of probiotic in elimination of lipids, these microorganisms can adsorb and detoxify the microbial toxin in the gastrointestinal tract and prevent the intestinal absorption. Detoxification of poisons in gastrointestinal tract (GIT) inhibits the effect of toxins on hepatocytes (Markowiak et al., 2019). The increase of TP in the present study may be related to the influence of probiotic on secretory function of GIT that leads to increase digestion and adsorption and subsequently can elevate total protein in plasma. It seems that the positive effects of probiotics on physiological function lead to an increase in health status and increase immunity defence in chickens. Increasing total protein following administration of protexin in this study can positively affect on the synthesis of antibodies. So, the chickens that received probiotics have best response to ND vaccine. Previously the effect of useful microorganisms on immunity was demonstrated and our finding agrees with previous reports (Awad et al., 2009).

There are few studies on the effect of probiotics on liver enzymes. Bityutskyy et al. (2019) 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 (Gholami-Ahangaran et al., 2016). Therefore, lack of increase in liver enzymes indicates no liver damage following probiotic supplementation.

In this study, Gunnera was able to affect serum lipid profile, reduce CHL and TG. Regarding the effect of *Asteraceae* family on lipid metabolism, various studies have been performed in humans, animals, and poultry. In most of these studies, there is an agreement that artichoke belonging to *Asteraceae* family can have potent effect on lipid metabolism. There is no study related to Gunnera effect on lipid metabolism in chickens, but there are some reports related to artichoke. Rouzmehr et al. (2014) reported that addition of 200 g per ton of dried artichokes to the diet can reduce abdominal fat and blood CHL. Also, Abdo et al. (2007) stated that consumption of 6% dried artichoke leaves in the diet causes a reduction in the amount of abdominal fat. However, *Asteraceae* family seems to reduce plasma cholesterol levels by increasing bile secretion and decreasing cholesterol biosynthesis (Edwards et al., 2015). In addition, there is evidence that the active ingredients in this phytobiotic have the ability to inhibit

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Gebhardt, 1998). No effect on hepatic enzymes including ALT, AST, ALP, and decreased plasma lipid profiles may support this hypothesis that lipids accumulated in hepatocytes can destroy hepatocyte and elevate hepatic enzymes in plasma. No effect on liver enzymes following the use of Gunnera extract or the simultaneous use of probiotics and Gunnera extract can be a marker of liver health and no hepatotoxicity. Certainly, no effect on liver function 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) (recommended dose in each growing period) along with Gunnera extract (100 mg/L) in broiler diets can improve FCR, increase antioxidant status, decrease serum lipids, and has no adverse effect on liver function in broiler chickens.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Majid Gholami-Ahangaran: Methodology, Project administration, Supervision, Writing-original draft; Maziar Haj Salehi: Investigation, Resources, Software, Visualization; Asiye Ahmadi-Dastgerdi: Methodology, Project administration, Writing-review & editing; Maryam Zokaei; Methodology, Validation.

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ETHICS STATEMENT

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

DATA AVAILABILITY STATEMENT

All of the data is in access to the corresponding author.

PEER REVIEW

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ORCID

Majid Gholami-Ahangaran https://orcid.org/0000-0002-2725-1091
Asiye Ahmadi-Dastgerdi https://orcid.org/0000-0002-3986-1866
Maryam Zokaei https://orcid.org/0000-0002-0473-7267

REFERENCES

Abdo, M. A., Radwan, N. L., & Selim, N. A. (2007). The effect of artichoke leaves meal on the utilization of dietary energy for broiler chickens. *International Journal of Poultry Science*, *12*, 973–982.

Allan, W. H., & Gough, R. E. (1976). A comparison between the haemagglutination inhibition and complement fixation tests for Newcastle disease. *Research in Veterinary Science*, *20*, 101–103.

Aluwong, T., Kawu, M., Raji, M., Dzenda, T., Govwang, F., Sinkalu, V., & Ayo, J. (2013). Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. *Antioxidants*, *2*, 326–339.

Amer, M. Y., & Khan, S. H. (2012). A comparison between the effects of a probiotic, an antibiotic on the performance of Desi chickens. *Veterinary World*, *5*, 160.

Attia, Y. A., Al-Harthi, M. A., & Hassan, S. S. (2017). Turmeric (*Curcuma longa* Linn.) as a phytogenic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks. *Revista Mexicana De Ciencias Pecuarias*, *8*(1), 11–21.

Attia, Y. A., El-Hanoun, A. M., Bovera, F., Monastra, G., El-Tahawy, W. S., & Habiba, H. I. (2014). Growth performance, carcass quality, biochemical and haematological traits and immune response of growing rabbits as affected by different growth promoters. *Journal of Animal Physiology and Animal Nutrition*, *98*(1), 128–139.

Attia, Y. A., Zeweil, H. S., Alsaffar, A. A., & El-Shafy, A. S. (2011). Effect of non-antibiotic feed additives as an alternative to flavomycin on productivity, meat quality and blood parameters in broilers. *Archiv für Geflügelkunde*, *75*, 40–48.

Awad, W. A., Ghareeb, K., Abdel-Raheem, S. M., & Böhm, J. (2009). Effects of dietary inclusion of probiotic, synbiotic on growth performance, organ weights, intestinal histomorphology of broiler chickens. *Poultry Science*, *88*, 49–56.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—A review. *Food and Chemical Toxicology*, *46*, 446–475.

Bityutskyy, V., Tsekhmistrenko, S., Tsekhmistrenko, O., Melnychenko, O., & Kharchyshyn, V. (2019). Effects of different dietary selenium sources including probiotics mixture on growth performance, feed utilization, serum biochemical profile of quails. In V. Nadykto (Ed.), Modern development paths of agricultural production (. 623–632). Springer.

Boroumandnia, Z., Mohammadzadeh, S., Azizi, M., & Khaldari, M. (2014). The effect of adding artichoke extract to drinking water on performance, carcass characteristics, blood lipoprotein concentration in broiler chickens. *Iranian Journal of Applied Animal Science*, *3*, 71–79.

Carapetian, J., & Zarei, G. (2005). Variation in protein, oil and fatty acid contents in three wild species of safflower (*Carthamus*) from west Azerbaijn, Iran. *International Journal of Botany*, *1*(2), 133–137.

Coruh, N., Celep, A. S., Özgökçe, F., & İşcan, M. (2007). Antioxidant capacities of *Gundelia tournefortii* L. extracts and inhibition on glutathione-S-transferase activity. *Food Chemistry*, *100*(3), 1249–1253.

Cross, M. L. (2002). Microbes versus microbes: Immune signals generated by probiotic lactobacilli, their role in protection against microbial pathogens. *FEMS Immunology and Medical Microbiology*, *34*, 245–253.

Edwards, S. E., da Costa Rocha, I., Williamson, E. M., & Heinrich, M. (2015). *Phytopharmacy: An evidence-based guide to herbal medicinal products*. John Wiley, Sons.

Erdoğan, Z., Erdoğan, S., Aslantaş, Ö., & Çelik, S. (2010). Effects of dietary supplementation of synbiotics, phytobiotics on performance, caecal coliform population, some oxidant/antioxidant parameters of broilers. *Journal of Animal Physiology and Animal Nutrition*, *94*, e40–e48.

Ertug, F. (2000). An ethnobotanical study in central Anatolia (Turkey). *Economic Botany*, *54*(2), 155–182.

Fallah, R., Kiani, A., & Azarfar, A. (2013). Effect of artichoke leaves meal, mentha extract (*Mentha piperita*) on immune cells, blood biochemical parameters of broilers. *Global Veterinary*, *10*, 99–102.

FAO/WHO. (2001). *Report of a joint FAO/WHO expert consultation on evaluation of health, nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. FAO/WHO Publishing.

Farkhondeh, T., Sadighara, P., Bahmani, E., Gholami-Ahangaran, M., & Moghtadaei, E. (2011). The anti-parasite effect of garlet tablets on Limnatis nilotica. *Journal of Herbal Drugs*, *2*, 69–71.

Gebhardt, R. (1998). Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (*Cynara scolymus* L.) extracts. *Journal of Pharmacology and Experimental Therapeutics*, *286*, 1122–1128.

Gheisar, M. M., & Kim, I. H. (2018). Phytobiotics in poultry, swine nutrition—A review. *Italian Journal of Animal Science*, *17*, 92–99

Gholami-Ahangaran, M., Rangsaz, N., & Azizi, S. (2016). Evaluation of turmeric (*Curcuma longa*) effect on biochemical, pathological parameters of liver, kidney in chicken aflatoxicosis. *Pharmaceutical Biology*, *5*, 780–787.

Gholami-Ahangaran, M., Peimani, N., & Dastgerdi, A. A. (2019). The effect of thyme (*Thymus daenensis*) supplement on growth, hygienic parameters of broilers meat. *Iraqi Journal of Veterinary Sciences*, *33*, 87–92.

Gholami-Ahangaran, M., Ahmadi-Dastgerdi, A., & Karimi-Dehkordi, M. (2020). Thymol, carvacrol, as antibiotic alternative in green healthy poultry production. *Plant Biotechnology Persa*, *2*, 22–25.

Gholami-Ahangaran, M., Karimi-Dehkordi, M., Akbari Javar, A., Haj Salehi, M., & Ostadpoor, M. (2021). A systematic review on the effect of Ginger (Zingiber officinale) on improvement of biological and fertility indices of sperm in laboratory animals, poultry and humans. *Veterinary Medicine and Science*, https://doi.org/10.1002/vms3.538

Gunal, M., Yayli, G., Kaya, O., Karahan, N., & Sulak, O. (2006). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora, tissue of broilers. *International Journal of Poultry Science*, *5*, 149–155.

Khoramshahi, M., Samadi, F., Ganji, F., Zerehdaran, S., & Samadi, S. (2015). The effect of artichoke leaf powder on performance, internal organs weight, some blood parameters of Japanese quail poisoned with carbon tetrachloride. *Animal Science Research*, *25*, 57–67.

Kumar, M., Kumar, V., Roy, D., Kushwaha, R., & Vaiswani, S. (2014). Application of herbal feed additives in animal nutrition—A review. *International Journal of Livestock Research*, *4*, 1–8.

Lertpatarakomo, R., Jaipeng, P., Sirimongkolworaku, S., Paditporn, K., & Mitchaothai, J. (2015, August). Effect of artichoke (*Cynara scolymus* L.) extract supplementation in drinking water on growth performance of broiler chickens. *20th European Symposium on Poultry Nutrition*. Prague, Czech, Republic.

Markowiak, P., Śliżewska, K., Nowak, A., Chlebicz, A., Żbikowski, A., Pawłowski, K., & Szeleszczuk, P. (2019). Probiotic microorganisms detoxify ochratoxin A in both a chicken liver cell line, chickens. *Journal of the Science of Food and Agriculture*, *99*, 4309–4318.

Miller, J. K., Brzezinska-Slebodzinska, E., & Madsen, F. C. (1993). Oxidative stress, antioxidants, animal function. *Journal of Dairy Science*, *76*, 2812–2823.

Mirderikvandi, M., Kiani, A., Khaldari, M., & Alirezaei, M. (2016). Effects of artichoke (*Cynara scolymus* L.) extract on antioxidant status in chicken thigh meat. *Iranian Journal of Veterinary Medicine*, *10*, 73–81.

Nadia, L. R., Zeinab, M., Abdo, A., & Hassan, R. A. (2007). Effect of feeding artichoke leaves meal on productive, reproductive performance of Mandarah hens. *International Journal of Poultry Science*, *6*, 826–834.

Nateghi, R., Samadi, F., Ganji, F., & Zerehdaran, S. (2013). Hepatoprotective effects of *Cynara scolymus* L. extract on CCl4 induced liver injury in broiler chickens. *International Journal of Agricultural Science Research*, *3*, 678–688.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative, qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, *70*, 158–169.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics, synbiotics—A review. *Journal of Food Science and Technology*, *52*, 7577–7587.

Rouzmehr, F., Mohit, A., KoshSekeh, M., & Hassanzade, M. (2014). The effect of the additive containing artichoke extract (APC) on growth, blood cholesterol level, carcass characteristics, immune system of broiler chickens. *Iranian Journal of Veterinary Clinical Pathology*, *8*(1), 357–366.

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., & Iseki, K. (2011). In vitro, in vivo antioxidant properties of chlorogenic acid, caffeic acid. *International Journal of Pharmaceutics*, *403*, 136–138.

Sekhon, B. S., & Jairath, S. (2010). Prebiotics probiotics, synbiotics: An overview. *Journal of Pharmaceutical Education and Research*, *1*, 13.

Shargh, M. S., Dastar, B., Zerehdaran, B., Khomeiri, M., & Moradi, A. (2012). Effects of using plant extracts, a probiotic on performance, intestinal morphology, microflora population in broilers. *Journal of Applied Poultry Research*, *21*, 201–208.

Shokri, A., Akbari Gharaei, M., Varmaghani, S., Taherpour, K., Khatibjoo, A., & Soltani, M. (2018). Effect of *Cynara scolymus* powder on performance, ascites syndrome in broiler chickens. *Journal of Animal Production*, *20*, 55–67.

Tajodini, M., Samadi, F., Hasani, S., Hashemi, S. R., & Samadi, S. (2015). Influence of artichoke (*Cynara scolymus*) leaf powder on growth performance, carcass traits, blood parameters in broiler chickens. *Iranian Journal of Applied Animal Science*, *5*, 141–146.

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., & Li, W. (2017). Antioxidant properties of probiotic bacteria. *Nutrients*, *9*, 521.

Windisch, W., Schedle, K., Plitzner, C., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine, poultry. *Journal of Animal Science*, *86*, 140–148.

Woolliams, J. A., Wiener, G., Gerson, P. H., & Mcmurray, C. H. (1983). Variation in the activities of glutathioneperoxidase, superoxide-dismutase, in the concentration of copper in the blood in various breed crosses of sheep. *Respiratory Journal of Veterinary Science*, *34*, 253–256.

Yildiz, G., Sacakli, P., & Gungor, T. (2006). The effect of dietary Jerusalem artichoke (*Helianthus tuberosus* L.) on performance, egg quality characteristics, egg cholesterol content in laying hens. *Czech Journal of Animal Science*, *51*, 349–354.

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