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The effects of antibiotic, probiotic, organic acid, vitamin C, and *Echinacea purpurea* extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens

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Primary Audience: Broiler Researchers, Nutritionists, Veterinarians

SUMMARY

The present context investigated the comparative study on the supplementation of antibiotic, probiotic, organic acid, vitamin C, and herbal extract after vaccination into drinking water and their effects on performance, carcass quality, blood biochemical parameters, immune system, and intestinal flora in broiler chicks for 42 days. A total of 420 one-day-old male broiler chicks (Ross 308) were randomly assigned into 7 treatments with 3 replicates (pens) per treatment and 20 male chicks for each replicate (pen). The experimental treatments consisted of drinking water (control, without additive); drinking water + antibiotic sulfamet; drinking water + C-Vet-50; drinking water + antibiotic sulfamet + C-Vet-50; drinking water + probiotic Primalac; drinking water + butyric acid; and drinking water + extract of *Echinacea purpurea* Moench (coneflower). There were no differences observed among the treatments for feed intake, but during the whole experimental period, the highest body weight gain was found in the chicks fed with drinking water + antibiotic sulfamet + 50 cc vitamin C ($P < 0.05$). There were no differences ($P > 0.05$) observed among the treatments for feed conversion ratio ($P > 0.05$). Moreover, there were no differences reported among treatments for carcass characteristics at the end of the experiment. Among the treatments, drinking water + 50 cc vitamin C, and drinking water + extract of *E. purpurea* reduced ($P < 0.05$) the levels of cholesterol, triglycerides, and low-density lipoproteins. Drinking water + 50 cc vitamin C, drinking water + Primalac, and drinking water + extract of *E. purpurea* increased ($P < 0.05$) the lymphocytes count and decreased the heterophils count and heterophil:lymphocyte ratio. The highest *Escherichia coli* count and lowest *Lactobacillus* count in ileal content of the broilers were observed in the control group ($P < 0.05$). The additives used in this study may be incorporated in the drinking water of broiler chickens as growth promoters and for improved performance. A further, wider supplementation study is required to understand the performance, immune system, variation in

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the intestinal microbial counts, and any other possible alteration in the intestinal biota of the broilers.

Key words: antibiotics, broiler, *E. purpurea*, organic acid, vitamin C

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DESCRIPTION OF PROBLEM

Poultry production is one of the most important economic activities in Iran. Traditionally, native breeds of poultry were reared in villages under extensive and mixed systems. However, about 60 yr ago, the exotic breeds were imported to the country. Over the yr, the poultry industry has grown from the setting of the backyard to become a multibillion-dollar business creating thousands of jobs for young professionals. For example, in 1392 — the Iranian year equivalent to 21 March 2013 to 20 March 2014 — Iran produced 2.15 million metric tons of poultry, a rise of about 12% on a year-to-year basis [1], and it currently holds the 10th place in the world in terms of poultry production. In recent yr, the risk of transmission of certain transboundary poultry diseases to previously unaffected areas has increased as a result of globalization and the possible persistence and spread of disease agents through domestic and wild reservoirs. The widespread distribution of Newcastle disease (**ND**) and the epidemics of avian influenza (**AI)** that have occurred over the last 10 yr provide examples of the negative impact of such diseases on the poultry producing sector and on society as a whole [2–4]. Different strategies can be implemented to effectively prevent and control the spread of animal diseases at international, national, and farm levels, and poultry disease control plans often include the use of vaccination [5].

Vaccines are widely used to prevent and control contagious diseases in poultry but vaccination causes stress [5]. It has been shown that there are some compounds present in coneflower (*Echinacea purpurea*) that can decrease stress and its side effects [6, 7]. *E. purpurea* is a perennial herb with a tough caudex. The plant grows in rocky prairie sites in open wooded regions. This medicinal plant was exploited as an anti-inflammatory agent and painkiller, and for a variety of ailments, including toothache, coughs, colds, sore throats, and snakebite due to its vast therapeutic properties. *E. purpurea* improves immunity [8] and its effects have been studied in poultry [9, 10], mice [11], and pigs [12]. However, there is no research evaluating the performance of commercial broilers that have received the additive in drinking water after vaccination. In view of this, the objective of this study was to evaluate the effect of supplementing antibiotic, probiotic, organic acid, vitamin C, and extract of *E. purpurea* in drinking water after vaccination on performance, carcass quality, blood biochemical parameters, microbiota, and immunity of broiler chickens.

MATERIALS AND METHODS

Experimental Place and Date

This experiment was performed in a commercial poultry house during 2015 (August-September 2015 in Abkenar, Iran). The handling and treatments of broiler chickens were approved by the Ethic Committee of Sanandaj Branch, Islamic Azad University, Sanandaj, Iran, and care was taken to minimize the number of animals used.

Broiler Chickens, Feeding, and Management

The experimental design was completely randomized, containing 7 treatments in 3 replicates (pens) for each treatment. A total of 420 one-day-old male chicks of the Ross 308 strain (Aviagen, Newbridge, Scotland, UK) was allotted to 21 pens of 20 birds each, such that mean pen body weights were similar for each pen. Environmental conditions were kept similar for all treatments. The treatments were as follows:

Treatment 1: Control – only drinking water after all vaccinations

Treatment 2: Drinking water + antibiotic sulfamet (0.25 ml/L) for 4 d after each vaccination
Treatment 3: Drinking water + C-Vet-50 (0.1 g/L) for 4 d after each vaccination
Treatment 4: Drinking water + antibiotic sulfamet + C-Vet-50 for 4 d after each vaccination
Treatment 5: Drinking water + probiotic Primalac for 4 d after each vaccination (0.12 g/L for one to 21 d of age, and 0.06 g/L for 22 to 42 d of age)
Treatment 6: Drinking water + butyric acid (5cc/L) for 4 d after each vaccination
Treatment 7: Drinking water + extract of *E. purpurea* (2.5cc/L) for 4 d after each vaccination

Antibiotic sulfamet includes 400 mg/mL sulphadiazine Na and 80 mg/mL tri methoperium. Primalac was added as a lyophylized mix containing 1×10^8 CFU/g of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium*. C-Vet-50 included 500 g/kg ascorbic acid and butyric acid, and *Echinacea purpurea* extract was purchased from a local supplier.

All chickens were fed according to the producer's feeding instructions. The ingredients and calculated nutrient composition in the starter (one to 21 d of age) and finisher (22 to 42 d of age) diets are given in Table 1.

Before the beginning of the experiment, the facility, drinkers, and feeders were thoroughly cleaned, and the facility was disinfected using Aqua GPC® 10 (Rasht, Iran). All drinkers and feeders were immersed in a 20% solution of benzalkonium chloride (germ killer). The facility was left to dry for 2 days. Thereafter nonflammable parts were flamed up, including the floor and metal walls of the pens (2 × 1 m). Walls were subsequently sprayed with water and lime. After drying, all equipment to be used during the rearing period, including buckets, sandals, cardboard rolls, temperature gauges, and all drinkers and feeders were returned to the facility, and all joints, windows, and ventilation were gasified with Formalex solution, and doors and windows were left shut for 48 hours. Ventilation was turned on to optimize the climate 24 h before the broilers were brought in. The facility was equipped with 8 ventilators and 2 strong ventilators.

Table 1. Ingredients and calculated nutrient composition in the starter (1 to 21 d of age) and finisher (22 to 42 d of age) diets.

	Starter diet (1 to 21 d of age)	Finisher diet (22 to 42 d of age)
Ingredients		
Corn	56.9	58.7
Soybean meal (43% CP)	33.1	30
Fish meal	3.4	3.5
Soybean oil	2.0	3.5
Di Calcium Phosphate	1.55	1.55
Oyster shell	1.03	1.18
DL-Methionine	0.01	0.01
Vitamin premix*	0.5	0.5
Mineral premix**	0.5	0.5
NaCl	0.26	0.26
Sand	0.75	0.75
Nutritional contents		
Metabolizable Energy (kcal/kg)	2910	3030
Crude protein (N × 6.25) (%)	20.1	19.0
Crude Fat (%)	4.60	6.14
Calcium (%)	0.95	0.90
Total phosphorus (%)	1.23	1.06
Available phosphorus	0.45	0.36
Methionine	0.50	0.38
Lysine	1.01	1.0
Methionine + Cysteine	0.83	0.71

*vitamin A, 3600000 IU; D3, 800000 IU; vitamin E, 7200 IU; vitamin B1, 710 mg; vitamin B2, 2640 mg; vitamin B6, 1176 mg; vitamin B9, 400 mg; vitamin B12, 6 mg; vitamin k3, 800 mg; pantothenic acid, 3920 mg;; vitamin Biotin, 40 mg; vitamin Niacin, 12000 mg and choline chloride, 200000 mg.

**Mn, 40000 mg; Fe, 20000 mg; Zn, 33900 mg; Cu, 4000 mg; I, 400 mg and Se, 80 mg.

Two heaters were used and the temperature program was set according to the instructions for Ross 308 broilers (Aviagen, Newbridge, Scotland, UK). Air humidity was kept at 55 to 65% in the early growing phase by spraying water on the floor. One-hundred-watt lamps were installed at a height of 2.2 meters above the floor. The lights were left on for 23 h daily, and for one h the house was left dark throughout the trial until slaughter at d 42.

Sanitation principles and health measures for raising chickens were applied. Drinkers were washed and cleaned daily. The birds were vaccinated against bronchitis disease (one and 18 d of age), Newcastle disease (one and 18 d of age), influenza disease (one d of age), and Gumboro disease (14 and 24 d of age).

Growth Performance and Carcass Characteristics

Feed intake and body weight (group) were recorded weekly. Feed conversion ratio was calculated based on conventional protocol.

At the age of 42 d and after 4 h of fasting for the complete evacuation of the gut, 2 birds from each replicate were selected. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. These birds were used for measuring carcass yield and distribution of meat and gastrointestinal tract characteristics. Birds were fully pecked by the dry pecking method. Feet were separated from the carcass in the tibiotarsal joint. The neck, wingtips, gut, and liver were removed, and the empty or edible carcass was weighed and intestinal segment dimensions were recorded. Various parts of the carcasses, i.e., abdominal fat, gizzard, liver and bile, thigh and breast, were dissected and weighed separately.

Blood Chemistry

Before blood collection for plasma constituent determination, feed was removed from all the birds for 4 h to allow stabilization of the various plasma constituents, and all blood sampling was done in the morning to further reduce the variability of the plasma constituents to be measured. At 42 d of age, 5 mL of venous blood was collected from the ulnaris vein in the wing of one bird from each replicate. Care was taken to choose the most representative birds with respect to body weight compared to the group mean body weight. The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulant ethylene diamine tetra acetic acid (**EDTA**). Blood samples were centrifuged at 3,000 rpm for 20 min to separate the blood cells from the plasma. Plasma was collected and stored at -20°C until plasma constituent analyses were made.

The levels of plasma cholesterol and triglyceride were determined using enzymatic methods (TeifAzmoon Pars, Co., Tehran, Iran). On the other hand, HDL cholesterol and LDL cholesterol were measured directly with HDL-C and LDL-C diagnostic kits (TeifAzmoon Pars Co, Tehran, Iran). The colorimetric determination of cholesterol in blood plasma samples involved the use of the cholesterol oxidase, which is based on the formation of a colored red-purple quinoneimine dye, produced by oxidative condensation of a phenolic compound with 4-aminoantipyrine in the presence of hydrogen peroxide [13]. The absorbance of the quinoneimine dye measured spectrophotometrically has a direct relationship with the amount of cholesterol in the sample.

Plasma triglycerides were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. The glycerol was converted to pyruvate and then to lactate. Decreased absorbance, measured spectrophotmetrically, is proportional to the triglyceride concentration in the sample [14].

A glucose oxidase kit (TeifAzmoon Pars, Co., Tehran, Iran), based on oxidase-peroxidase procedure, was used to measure plasma glucose. In this assay, glucose is oxidized in the presence of the glucose oxidase catalyst into H_2O_2 and gluconic acid. The reactions among gluconic acid, hydrogen peroxide, a phenolic compound, and 4-aminoantipyrine form a red-violet colored quinoneimine, and the absorbance of the quinoneimine chromagen, measured by spectrophotometer, is directly associated with the amount of glucose in the sample.

A uric acid-uricase enzyme kit (TeifAzmoon Pars, Co., Tehran, Iran), based on the oxidase-peroxidase procedure [15], was used to measure plasma uric acid. In this procedure uric acid is oxidized with the uricase in the presence of the generated hydrogen peroxide, a phenolic compound, and 4-aminoantipyrine and forms a red-colored quinoneimine, and the absorbance of the quinoneimine chromagen, measured by spectrophotometer, is directly associated with the amount of uric acid in the sample [16].

Two blood samples per replicate were diluted 20 times with a diluter fluid (3 mL acetic acid

glacial + 97 mL distilled water + Leishman stain). Differential leukocyte counts were examined using 2 samples per replicate on Giemsa stained blood smears using a light microscope. One hundred cells were counted and differentiated into heterophils (**H**), lymphocytes (**L**), and monocytes. The mean H/L ratio was calculated from individual H/L ratios.

Microbiota Measurements

At the end of the trial, 2 chickens from each replicate were slaughtered and the ileum was removed. Agar plates were streaked and samples were sent to the laboratory along with intact intestinal segments for further culture. To determinate bacterial growth and colony counts, the agar plates streaked on site of slaughter were used. Collecting tubes were weighed, wrapped with aluminum foil, and autoclaved. The culture media were prepared and poured into the petri dish 24 h before the sample collection. The deMan Rogosa Sharpe (**MRS**) agar media were used to culture *Lactobacilli*, and Eosin Methylene Blue (**EMB**) agar media were used to culture *Escherichia coli*.

Samples were transferred to the laboratory in the listed tubes and again weighed. The amount of sample in each tube was calculated from the differences between these 2 values. Tubes were shaken for approximately 30 min for bacterial isolation from gastrointestinal contents and preparation of suspensions. One mL of the suspensions was added into 9 mL phosphate buffer saline (**PBS**) in another tube to prepare a series of dilutions, i.e., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. A 100 μl sample was removed from the 10^{-4}, 10^{-5}, and 10^{-6} dilutions and poured into petri dishes containing the media and spread uniformly. Incubations for bacterial growth were performed at 37°C under anaerobic conditions in anaerobic jars, and total aerobic bacteria were counted after 48 h using a colony counter.

Statistical Analysis

Data were analyzed by a completely randomized experimental design using the General Linear Model procedures of the Statistical Analysis System v8, considering each pen (20 birds) as an experimental unit within each experimental group (3 replicates [pens] for each).

Differences among means ($P \leq 0.05$) were assessed using Tukey's range test. Statements of significance were declared at $P < 0.05$.

RESULTS

Treatments had no effect ($P > 0.05$) on feed intake during the starter, finisher, or total period (Table 2). During the starter, finisher, and total periods, the treatment drinking water + sulfamet + C-Vet-50 resulted in the highest numeric body weight and the control treatment the lowest. The treatments were clustered in 3 groups, i.e., the treatments drinking water + extract of *E. purpurea*, drinking water + C-Vet-50, and drinking water +sulfamet + C-Vet-50 were in one group; the treatments drinking water + Primalac, drinking water + sulfamet, drinking water + extract of *E. purpurea*, and drinking water + C-Vet-50 in the next; and the treatments drinking water

Table 2. Effect of experimental treatments on feed intake (g) of broilers.

Treatments	Starter period (1 to 21 d of age)	Finisher period (22 to 42 d of age)	Total (1st-42nd days of age)
Drinking water (control)	1084[a]	3055[a]	4139[a]
Drinking water + antibiotic sulfamet	1122[a]	3078[a]	4200[a]
Drinking water + C-Vet-50	1167[a]	3371[a]	4328[a]
Drinking water + antibiotic sulfamet + C-Vet-50	1177[a]	3256[a]	4433[a]
Drinking water + probiotic Primalac	1123[a]	3101[a]	4224[a]
Drinking water + butyric acid	1100[a]	3044[a]	4144[a]
Drinking water + extract of *E. purpurea*	1155[a]	3091[a]	4246[a]
SEM	27.6	75.4	89.2
P-value	0.219	0.474	0.274

[a]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

Table 3. Effect of experimental treatments on body weight (g) of broilers.

Treatments	Starter period (1 to 21 d of age)	Finisher period (22 to 42 d of age)	Total period (1st-42nd days of age)
Drinking water + antibiotic sulfamet + C-Vet-50	745[a]	1341[a]	2066[a]
Drinking water + C-Vet-50	727[a,b]	1305[a,b]	2032[a,b]
Drinking water + extract of *E. purpurea*	725[b]	1267[b]	1992[b]
Drinking water + probiotic Primalac	705[b]	1271[b]	1979[b]
Drinking water + antibiotic sulfamet	706[b]	1261[b]	1966[b]
Drinking water + butyric acid	665[c]	1192[c]	1857[c]
Drinking water (control)	660[c]	1188[c]	1848[c]
SEM	11.52	18.2	21.5
P-value	0.0009	0.0003	0.0001

[a-c]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

Table 4. Effect of experimental treatments on feed conversion ratio (g/g) of broilers.

Treatments	Starter period (1 to 21 d of age)	Finisher period (22 to 42 d of age)	Total period (1st-42nd days of age)
Drinking water (control)	1.64[a]	2.57[a]	2.24[a]
Drinking water + antibiotic sulfamet	1.60[a]	2.44[a]	2.17[a]
Drinking water + C-Vet-50	1.61[a]	4.43[a]	2.13[a]
Drinking water + antibiotic sulfamet + C-Vet-50	1.58[a]	2.43[a]	2.13[a]
Drinking water + probiotic Primalac	1.59[a]	2.44[a]	2.14[a]
Drinking water + butyric acid	1.65[a]	2.55[a]	2.23[a]
Drinking water + extract of *E. purpurea*	1.59[a]	2.441[a]	2.13[a]
SEM	0.049	0.069	0.049
P-value	0.918	0.588	0.437

[a]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

(control) and drinking water + butyric acid in the last group (Table 3). There were no ($P > 0.05$) differences observed among treatments in feed conversion ratio (g/g) of broilers (Table 4).

There were no ($P > 0.05$) differences reported among treatments in carcass characteristics (%) of broilers for proportions of abdominal fat, gizzard, liver and bile, or thigh and breast (Table 5).

There were no ($P > 0.05$) differences observed among treatments in blood constitutes (mg/dl) of broilers for total protein, glucose, or uric acid (Table 6). The treatments drinking water (control), drinking water + butyric acid, and drinking water + sulfamet had higher ($P < 0.05$) triglyceride values than the treatments drinking water + Primalac, drinking water + extract of *E. purpurea*, and drinking water + C-Vet-50. The treatment drinking water + sulfamet + C-Vet-50 showed intermediate effect.

The treatments of drinking water (control) and drinking water + butyric acid had higher ($P < 0.05$) cholesterol than the treatments drinking water + C-Vet-50, drinking water + Primalac, and drinking water + extract of *E. purpurea*. The treatments drinking water + Primalac, drinking water + sulfamet + C-Vet-50, and drinking water + sulfamet showed intermediate effect.

The treaments drinking water + butyric acid, drinking water (control), and drinking water + sulfamet had higher ($P < 0.05$) LDL cholesterol and lower ($P < 0.05$) HDL cholesterol than the treatments drinking water + extract of *E. purpurea* and drinking water + C-Vet-50. The treatments drinking water + sulfamet + C-Vet-50 and drinking water + Primalac showed intermediate effect on both LDL and HDL cholesterol.

The treatments drinking water + Primalac, drinking water + C-Vet-50, drinking water + sulfamet + C-Vet-50, and drinking water + extract of *E. purpurea* had higher ($P < 0.05$) lymphocytes and lower ($P < 0.05$) heterophils than the treatments drinking water (control), drinking water + butyric acid, and drinking water + sulfamet (Table 7).

Table 5. Effect of experimental treatments on carcass characteristics (%) of broilers.

Treatments	Carcass	Breast	Thigh	Liver and bile	Gizzard	Abdominal fat
Drinking water (control)	71.26[a]	31.07[a]	26.65[a]	2.44[a]	1.50[a]	1.67[a]
Drinking water + antibiotic sulfamet	72.26[a]	34.10[a]	27.15[a]	2.51[a]	1.44[a]	1.67[a]
Drinking water + C-Vet-50	71.80[a]	32.75[a]	27.20[a]	2.45[a]	1.46[a]	1.62[a]
Drinking water + antibiotic sulfamet + C-Vet-50	72.44[a]	34.53[a]	26.80[a]	2.54[a]	1.46[a]	1.66[a]
Drinking water + probiotic Primalac	72.39[a]	33.65[a]	27.33[a]	2.46[a]	1.56[a]	1.64[a]
Drinking water + butyric acid	71.45[a]	31.07[a]	26.40[a]	2.46[a]	1.46[a]	1.63[a]
Drinking water + extract of *E. purpurea*	71.68[a]	32.27[a]	26.75[a]	2.46[a]	1.47[a]	1.55[a]
SEM	1.139	1.021	1.005	0.032	0.023	0.108
P-value	0.983	0.222	0.993	0.307	0.307	0.989

[a]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

Table 6. Effect of experimental treatments on blood constitutes (mg/dl) of broilers.

Treatments	Total protein	Glucose	Cholesterol	Triglyceride	HDL cholesterol (High density lipoproteins)	LDL cholesterol (Light density lipoproteins)	Uric acid
Drinking water (control)	4.29[a]	97.0[a]	104.5[a]	68.5[a]	41.05[b]	49.29[a]	4.45[a]
Drinking water + antibiotic sulfamet	4.54[a]	88.8[a]	98.8[a,b]	67.7[a]	39.8[b]	46.47[a]	4.71[a]
Drinking water + C-Vet-50	4.45[a]	94.0[a]	92.3[b]	49.1[b]	53.27[a]	29.26[b]	4.55[a]
Drinking water + antibiotic sulfamet + C-Vet-50	4.17[a]	92.2[a]	98.8[a,b]	59.8[a,b]	47.37[a,b]	39.51[a,b]	4.99[a]
Drinking water + probiotic Primalac	4.12[a]	85.4[a]	91.1[a,b]	53.9[a,b]	41.25[a,b]	39.08[a,b]	4.75[a]
Drinking water + butyric acid	4.25[a]	95.4[a]	106.7[a]	68.3[a]	40.53[b]	52.46[a]	4.57[a]
Drinking water + extract of *E. purpurea*	4.12[a]	92.2[a]	93.2[b]	51.2[b]	51.74[a]	31.17[b]	4.47[a]
SEM	0.127	17.24	2.66	5.00	3.101	4.503	0.542
P-value	0.302	0.14	0.005	0.045	0.024	0.017	0.991

[a,b]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

Table 7. Effect of experimental treatments on immunity of broilers.

Treatments	Heterophils (%)	Lymphocytes (%)	Hetrophil/ lymphocyte
Drinking water (control)	33.15[a]	52.27[b]	0.63[a]
Drinking water + antibiotic sulfamet	32.50[a]	51.50[b]	0.63[a]
Drinking water + C-Vet-50	27.30[b]	58.60[a]	0.46[c]
Drinking water + antibiotic sulfamet + C-Vet-50	31.60[a]	58.60[a]	0.54[b]
Drinking water + probiotic Primalac	28.41[b]	59.75[a]	0.74[c]
Drinking water + butyric acid	32.45[a]	52.15[b]	0.62[a]
Drinking water + extract of *E. purpurea*	27.83[b]	58.20[a]	0.48[c]
SEM	0.002	0.074	0.0001
P-value	0.002	0.874	0.012

[a–c]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

The treatment drinking water (control) had higher ($P < 0.05$) *E. coli* count than all other treatments. The treatments drinking water + Primalac and drinking water + extract of *E. purpurea* had the highest ($P < 0.05$) *Lactobacilli* count followed by the treatments drinking water + sulfamet, drinking water + butyric acid, drinking water + C-Vet-50, and drinking water + sulfamet + C-Vet-50. The treatment drinking water (control) had the lowest ($P < 0.05$) *Lactobacilli* count (Table 8).

DISCUSSION

The production of high-quality and profitable poultry totally depends upon the maintenance of gut health and the immune function.

Table 8. Effect of experimental treatments on ileal microbiota (log CFU/g) of broilers.

Treatments	*Escherichia coli*	*Lactobacilli*
Drinking water (control)	7.57[a]	6.77[c]
Drinking water + antibiotic sulfamet	6.46[b]	8.15[a,b]
Drinking water + C-Vet-50	6.51[b]	8.02[a,b]
Drinking water + antibiotic sulfamet + C-Vet-50	6.54[b]	7.70[b]
Drinking water + probiotic Primalac	6.45[b]	8.65[a]
Drinking water + butyric acid	6.46[b]	8.10[a,b]
Drinking water + extract of *E. purpurea*	6.44[b]	8.46[a]
SEM	0.036	0.219
P-value	0.0001	0.0008

[a-c]Means within same column with different superscript letters are significantly different ($P < 0.05$).
SEM: Standard error of mean.

The dietary composition affects the innate and cellular immune system of the broiler chicken. Modern poultry production can be enhanced by improving the immune response and resistance to pathogens through efficient supply of the nutrients. Higher production and efficient feed conversion are the necessity of the modern broiler industry and can be achieved by supplementing the specific feed additives into the diet or drinking water.

In the present investigation, treatments had no impact on feed intake during the starter, finisher, or total period. Similarly, there were no differences observed among treatments in feed conversion ratio (g/g) of broilers. On the other hand, the treatment drinking water + antibiotic sulfamet + C-Vet-50 resulted in the highest numeric body weight of the broiler chicken when compared to the control. The finding of the present study regarding body weight gains coincides with the reports of Kopecky et al. [17] who demonstrated a significant increase in the average body weight ($P < 0.05$) for a citric acid supplemented diet when compared with the control. Similar results were observed by Afsharmanesh and Pourreza [18] who stated an increment in the body weight gain of broiler rations in the presence of citric acid. Surprisingly, our results do not favor the findings of Ozpinar et al. [19] who demonstrated that body weights of the broiler chicks were unaffected by the addition of vitamin C into the diet over an experimental period. In the present context, the improvement in the body weight of the broiler chicken after the supplementation into drinking water might be due to the beneficial effect of vitamin C on the gut flora. Antibiotics have been used as growth promoters and to control intestinal health in order to improve growth efficiency in poultry. However, antibiotic resistance has led to a ban on antibiotic use in livestock industries in many countries. Moreover, the present study showed improvement in the weight gain of broiler chickens after the supplementation of sulfamet. Our results were in complete agreement with the findings of Yakhkeshi et al. [20] who observed that the supplementation of antibiotics improved the body weight when compared to the unsupplemented group.

There were no differences observed among treatments in carcass characteristics of broilers for proportions of abdominal fat, gizzard, liver and bile, or thigh and breast. In the line of our study, Islam et al. [21] also noted that carcass characteristics were unaltered after the supplementation into the diet. Dietary supplements did not show any improvement in the carcass, heart, kidney, liver, gizzard, or abdominal fat yield [22]. Our findings were consistent with Hernandez and Madrid [23] and Celik and Ozturkcan [24] who found that dietary supplementation had no effect on the improvement of carcass traits. However, few reports demonstrated the increased carcass [25], breast [25], liver, heart, spleen, and gizzard weight [26]. On the other hand, Sahin et al. [26] also observed the reduction in the abdominal fat pad after the supplementation into the diet of the broiler chickens. As discussed above, the variation among our results and previous findings related to carcass traits could be due to the different mode of mechanisms of feed additives. However, they may show similar physiological features by altering the carcass characteristics.

In the present investigation, the dietary supplementation into the drinking water did not affect the blood constituents of broiler chickens for total protein, glucose, or uric acid. Similar observations were reported by Konca et al. [22] who showed that the supplementation of ascorbic acid unaltered the serum total protein and

glucose levels. Contrary to our findings, Pourakbari et al. [27] reported a higher blood glucose level in the probiotic supplemented treatments. These improved effects in the previous study could be due to the higher absorptive capacity of the intestinal mucosa and efficient digestion of the diet because of increased intestinal enzyme activity [28].

The present context showed a significant reduction in the triglyceride level of the blood when the drinking water was supplemented separately with the probiotic Primalac, extract of *E. purpurea*, and C-Vet-50. Our results were contrary to the findings of Konca et al. [22] and Mckee et al. [29] who demonstrated the neutral effect of ascorbic acid as a supplement into the diet on the triglyceride level of blood of broiler chickens. But the present study strongly favors the finding of Clegg et al. [30] and Pourakbri et al. [27] who reported reduced triglycerides in ascorbic acid and probiotic supplemented treatments, respectively. The significant reduction in triglyceride level of blood might be because probiotics lead to a reduction in acetyl Co-A carboxylase of liver and tissue, causing a reduction in HDL and triglyceride levels [31]. In line with the findings of Sakine et al. [32] and Rahimi et al. [33], who demonstrated the positive effect of medicinal plants on reduced triglyceride level of broiler chicks, we also observed a reduction in the triglyceride level after the supplementation of *E. purpurea* extract into the drinking water.

The treatments that were fed separately with probiotic Primalac, extract of *E. purpurea*, and C-Vet-50 showed significant reduction in the cholesterol level of blood when compared to the butyric acid supplemented and unsupplemented treatments. The observations of the present study were in complete agreement with the findings of Pourakbari et al. [27] and Rahimi et al. [33] who reported a reduction in the cholesterol level of blood after the supplementation of probiotics and medicinal plants, respectively. In like manner, Panda et al [34], Ahmadi [35], and Jouybari et al. [36] reported that the use of probiotic supplement significantly reduced the serum cholesterol level of the broiler chickens. Similar to our investigation, Sahin et al. [26] also observed a reduction in the cholesterol level of serum using ascorbic acid as a dietary supplement. In general, medicinal plants target 3-hydroxy-3-methylglutaryl-coenzyme A (a key enzyme in cholesterol synthesis regulation), resulting in inhibition of re-synthesis of cholesterol [37]. On the other hand, probiotics de-conjugate the bile salts and thus may interfere with the absorption mechanism of cholesterol in the gut [38].

Additionally, the treatments consisting of drinking water + *E. purpurea* and drinking water + C-Vet-50 showed a reduction in LDL when compared to the control treatment (drinking water). On the other side, supplementation with sulfamet, butyric acid, and the unsupplemented treatment revealed reduced HDL. Contrary to this, Pourakbari et al. [27] observed a significant reduction in LDL and improvement in HDL levels of the blood in the treatment consisting of probiotic.

The variations in immune response or in intestinal physiology could be proposed as defense strategies against the microorganisms [39]. In the present investigation, a significant increase in the lymphocytes and decrease in the heterophils of broiler chickens were observed when the drinking water was supplemented separately with probiotic Primalac, C-Vet-50, *E. purpurea* extract, and a combined treatment containing antibiotic sulfamet and C-Vet-50. The findings of the present study were in complete agreement with the reports of Dehkordi et al. [40] and Cundell et al. [41] who illustrated increased total counts of lymphocytes using *E. purpurea* as a supplement. In another report, it had been observed that the ethanolic juice of *Echinacea* increased the total lymphocyte counts in hens and pigs [42]. On the other hand, Skaudickas et al. [43] reported that *Echinacea* activated the rat immune system by increasing the number of lymphocyte counts. Contrary to the present study, Dehkordi et al. [40] observed increased heterophil counts using *E. purpurea* as a supplement. In fact, a significant increase in the lymphocyte counts represents an improved immune response because these immune cells identify the antigens and modulate the epithelial response [44]. In addition to this, investigations also reported that the heterophil/lymphocyte (H/L) ratio was an indicator for pathological, environmental, and nutritional stresses in chickens [45, 46].

Probiotics have beneficial effects on the host animal and increase the metabolism by altering

the intestinal physiology. In the present study, Primalac increased the total counts of *Lactobacilli* in the intestine while it decreased the total counts of *E. coli*. Additionally, the treatments other than control showed a reduction in the total counts of *E. coli*. The findings of the present study favored the reports observed by Pourakbari et al. [27] and Mountzouris et al. [47] who demonstrated a significant increment in the *Lactobacilli* counts after the supplementation of probiotic in the diet of broilers. On the contrary, the present investigation showed partial agreement with the study of Giannenas et al. [48] who observed that there was no difference in the *Lactobacilli* count, but there were lower total counts of *E. coli* in the broilers fed a probiotic supplemented diet. In line with our study, Jamroz et al. [49] and Jang et al. [50] observed a significant reduction in the total counts of *E. coli* when the diet of the broilers was supplemented with medicinal plant extract and antibiotic, respectively, when compared to the control diet. Similar to our results, Jamroz et al. [49] also observed a significant increase in the *Lactobacillus* counts after the supplementation of plant extract into the broiler diet.

CONCLUSIONS AND APPLICATIONS

1. The supplementation of Primalac and C-Vet-50 into the drinking water improved the body weight of broiler chickens.
2. There was no difference observed in treatments for carcass characteristics or blood constitutes of broilers for total protein, glucose, and uric acid.
3. The supplementation of Primalac, C-Vet-50, *E. purpurea* extract, and sulfamet indicated reduced triglyceride and cholesterol levels in broilers' blood.
4. The treatments investigated in the present study illustrated an increased lymphocyte count as well as *Lactobacillus* numbers. On the other hand, the treatments reduced the total counts of *E. coli* when compared to the unsupplemented treatment.
5. The supplements used in this study may be incorporated into the drinking water of broiler chickens as growth promoters and for improved performance.
6. As a result of these findings, a further, wider supplementation study is required to understand the performance, immune system, variation in the intestinal microbial counts, and any other possible alteration in the intestinal biota of the broilers.

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