Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol.25 No.3 Ser. No.88 2024

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Comparative effects of herbal additive, symbiotic and antibiotic on growth performance, blood constituents, gut microbiota, and immune response in broiler chickens

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10.22099/ijvr.2024.49567.7298

(Received 24 Feb 2024; revised version 20 Aug 2024; accepted 5 Oct 2024)

Abstract

Background: Using medicinal plants in broiler diets has been gaining attention as an alternative to synthetic additives due to their potential health benefits and lower risk of residue accumulation. **Aims:** The present study primarily aimed to evaluate and compare the effects of herbal additives, specifically barberry (*Berberis vulgaris*), sumac (*Rhus coriaria*, L), symbiotic, and antibiotic on broiler chickens. **Methods:** A total of 384 one-day-old broiler chicks (Ross 308) were assigned to eight different dietary treatments, with six replicates per treatment. Experimental diets included control diet (CON), and other experimental groups were supplemented with 0.2 g/kg virginiamycin (VM), symbiotic (SS), 1 g/kg (B_1) and 2 g/kg (B_2) barberry seed powder, 1 g/kg (S_1) and 2 g/kg (S_2) sumac seed powder and 1 g/kg sumac seed powder + 1 g/kg barberry seed powder ($B_1 + S_1$). **Results:** The study results indicated that body weight increased in birds fed with VM and SS-supplemented diets ($P<0.05$) during 1 to 42 days of age. All dietary treatments except CON increased the count of *Lactobacillus* spp. and decreased the number of coliforms versus at the end of the experiment ($P<0.05$). Antibody titers against Gambaro disease were higher in birds fed diets B2 and B1 + S1 ($P<0.05$). The results also indicated that the heterophil to lymphocyte ratio was significantly lower in the SS and B1 + S1 groups as compared with the control group. **Conclusion:** A mix of sumac and barberry (1%) powdered seeds has the potential to improve performance, and disease responsiveness and intestinal microbiota in broiler.

Key words: Barberry, Broiler chickens, Immune system, Microbiota, Sumac

Introduction

Over the past few decades, the importance of gastrointestinal health and its role in the health of birds has been an important subject of poultry research. Due to high-stocking densities and genetic selection for faster-growing birds, poultry farms always need a proper implementation of biosecurity programs to enhance the immune system, balance of microorganisms in the gut and resistance to pathogens. Gut microbiota impose several adverse and positive impacts on bird's health and productivity where they compete with the host animal in obtaining nutrients from the gastrointestinal tract (GIT) and by destroying and altering bile acids, important entities in fat digestion and absorption (Gaskins *et al*., 2002).

Furthermore, according to Kogut *et al*. (2020), the relationship between the gut microbiota and the innate immune system of broiler chickens may result in an adaptive immune response and mucosal cells. Based on the findings of this study, it appears that manipulating the gut microbiota in broiler chickens through the use of feed additive supplements, either individually or in combination, could be a promising strategy. This could potentially improve growth performance, enhance immune response, and positively impact the development of mucosal cells in broiler chickens.

Following the European Union ban on antibiotics as growth promoters (AGP) in animal feed in 2006 (Phillips, 2007), several investigations have been conducted to find alternatives to antibiotics for use in poultry feed. The results of these studies have led to the introduction of a few classes of feed additives known as "non-antibiotic feed additives", including probiotics, prebiotics, symbiotics, and organic acids. Symbiotics, specifically, refer to a synergistic combination of prebiotics and probiotics designed to improve the survival and replacement of dietary microbial supplements in the gastrointestinal tract (GIT) (Dibaji *et al*., 2014). Symbiotics are not only used for improving the survival of beneficial microorganisms added to food or feed, but also for stimulating the proliferation of specific native bacterial strains present in GIT.

Another class of non-antibiotic feed additive that has received high attention is phytogenic. Phytogenics are known for their ability to improve growth performance, stimulate the secretion of endogenous digestive enzymes, activate the immune response, and exhibit antibacterial, antiviral, and antioxidant actions (Alsamri *et al*., 2021), as well as their effectiveness against the most common poultry pathogens such as *Clostridium perfringens*, *Campylobacter jejuni*, and *Escherichia coli*. According to Greene *et al*. (2020), plant-based products can be used as feed additives to improve the performance of broilers under heat stress conditions. In the poultry industry, the perspective of health management through nutrition is one of the most significant and popular topics. Many of the beneficial properties of photogenic compounds are due to their bioactive molecules. *Berberis vulgaris* (family Berberidaceae) is a candidate herbal remedy that contains an isoquinoline alkaloid, exhibiting many biological activities such as boosting the immune system, antibacterial, antioxidant, anti-inflammatory, hypotensive, anti-cholesterolemic, and hepatoprotective effects (Abushouk *et al*., 2017).

Berberis is extensively cultivated in Iran (typically in Birjand), central and southern Europe and the northeastern United States (Tabeshpour *et al*., 2017). Sumac (*Rhus coriaria* L) is another herbal medicine belonging to the Anacardiaceous family. It is a wild-growing plant mainly found in Mediterranean bordering countries, South Europe, North Africa, Iran, and Afghanistan (Abu-Reidah *et al*., 2014). Sumac contains flavanols, tannins, phenolic acids, organic acids, and anthocyanin and many studies have confirmed antioxidant effects (Abu-Reidah *et al*., 2015), hypocholesterolemic and antibacterial effects against both gram-negative and gram-positive bacteria for this plant (Nasar-Abbas and Halkman, 2004). Azizi *et al*. (2020) by examining different levels (0.05, 0.1, 0.15, and 0.2%) of sumac observed significant effects in reducing cholesterol and LDL cholesterol concentration as well as the amount of *E. coli* in the ileum digesta in chickens fed with 0.1% of sumac powder decreased as compared with other treatments. In a study conducted by Mansoub (2011), different levels of sumac (0.75%, 1%, 1.5%, and 2%) were added to the diet of broiler chickens. The results showed that the groups fed with 1.5% and 2% sumac had higher percentages of breast and thigh, and the lowest percentage of abdominal fat was observed in the 1.5% sumac group.

In recent years, the plant compounds have been investigated due to availability, high distribution, low cost, and fewer side effects as new food additives with natural properties and prevention of liver damage and lipid peroxidation. Given the promising results of previous studies on the use of sumac and barberry fruit in broiler feed, including improved meat quality and potential health benefits for humans, this study aimed to investigate the effects of feeding powdered sumac and barberry fruit in broiler diets. Specifically, the study aimed to evaluate the impact of these dietary supplements on growth performance, organ characteristics, serum lipid profile, immunity, hematology, and Gut microbiota in broiler chickens. The results of these interventions will be compared with those of symbiotic and a well-documented antibiotic growth promoter, virginiamycin.

Materials and Methods

Ethics statement

By adhering to the guidelines approved by the Animal Health and Care Committee of Ilam Agricultural University, this experiment ensured the ethical treatment and welfare of the animals involved. Throughout the trial, the birds were handled in compliance with local laws and regulations.

Birds and management

A total of 384 Ross 308 broiler chickens (one-day male) with an average initial weight of 43 ± 0.05 and randomly allocated to one of the eight dietary treatments. There were six repetitions of each treatment, each involving eight chicks. All the chickens were raised in wire cages where the lightening regimen was 23 h light/1 h darkness. On the 28th, the temperature ranged from 33°C to 22°C and then remained constant. Experimental treatments consisted of:

Table 1: Constituents and chemical compositions of basal diets

Ingredient (kg)	Starter (days 1-21)	Grower (days 22-42)
Maize	645.90	681.90
Soybean meal (44%)	291.0	270.0
Fish meal	28.70	14.10
Dicalcium phosphate	14.70	14.60
Oyster shells	10.60	10.60
Mineral premix[a]	2.50	2.50
vitamin premix[b]	2.50	2.50
Common salt	0.25	0.25
DL-Methionine	1.40	1.30
L-Lysine (HCL)	0.20	0.40
Energy (ME) (MJ. Kg^{-1})	12.14	12.35
Crude protein	204.30	188.5
Crude fiber (%)	3.57	3.94
Ether extract (%)	2.82	2.88
Ca (%)	9.20	9.60
Available phosphorous	4.60	4.20
Na	1.50	1.40
Methionine	5.20	4.70
Methionine + cysteine (%)	8.30	7.60
Lysine	1.16	1.02

[a, b] Vitamin-mineral premix provided the following nutrients per kg of diet: vit A, 9000 IU; vit D3, 2000 IU; vit E, 18 IU; K3, 2.1 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; niacin, 30 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; biotin, 0.1 mg; choline chloride, 100 g; folic acid, 1 mg; cyanocobalamin, 0.015 mg; phosphorous, 1.6 mg; calcium, 2.3 mg; Zn, 100 mg; Mn, 100 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 0.2

1) A maize and soybean seed basal diet in mash form (Table 1) as control diet (CON)
2) CON + 0.2 g/kg virginiamycin (VM)
3) CON + 0.5 g/kg symbiotic (SS)
4) CON + 1 g/kg barberry seed powder (B_1)
5) CON + 2 g/kg barberry seed powder (B_2)
6) CON + 1 g/kg sumac seed powder (S_1)
7) CON + 2 g/kg sumac seed powder (S_2)
8) CON + 1 g/kg sumac +1 g/kg barberry (B_1+S_1)

Virginiamycin was received form Tolide Darouhai Dami Iran Co., Tehran, Iran. The symbiotic (Biomin®IMBO) was prepared from a commercial producer (Protexin aquatic, Nikotak, Tehran). It is made up of probiotic strain *Enterococcus faecium* prebiotic fructo-oligosaccharides, cell wall fragments (made of β-glucans, lipopolysaccharides, peptidoglycans and nucleotides), and phycophytic substances derived from sea algae. The sumac and barberry seeds were obtained from the nearby herbal market in Esfahan, Iran. They were dried in a dark place and ground into a powder using a mixer grinder. Chemical analysis of *Rhuscoriaria* L including 95.5% DM, 4.55% CP, 22% crude fiber, 18.5% ether extract and 6% ash and *Berberis vulgaris* 94.5% DM, 2.30% CP, 24% crude fiber, 8% ether extract and 5% ash were determined using the method of the Association of Official Analytical Chemists (AOAC International, 2000). Diet was prepared in accordance with Ross 308's guidelines to satisfy the nutritional needs of broilers. The basal control diet was changed according to the bird's age (grower diet 1-21 d; finisher diet 22-42 d) and did not include anticoccidials or antibiotics. Broilers had *ad libitum* access to experimental feed and tap water. The birds were regularly vaccinated against infectious bronchitis, Newcastle disease and Gambaro disease, but were not drugged for the entire experimental period.

Performance parameters

Feed intake (FI) and body weight (BW) were measured for all birds in a cage on days 10, 28, and 42 of age. The ratio of feed intake (FI) to body weight gain (BWG) was used to compute the feed conversion ratio (FCR) for the phases of 1 to 10 d, 11 to 21 d, 22 to 42 d, and 1 to 42 d. Mortality was recorded upon occurrence. We did not investigate the mortality rate because there were just two deaths over the entire growth period. At day 42 of age, ten birds from each treatment were randomly chosen and killed by cervical dislocation to evaluate relative weight (expressed as a percentage of the eviscerated carcass) of abdominal fat and immune organs (spleen, bursa of Fabricius, and thymus).

Blood sampling

At day 42 of age, blood sample were collected from the wing vein of 10 birds per group (2 birds/replicate) into plasma-separating tubes containing lithium heparin (100 IU/5 ml). Blood samples were centrifuged at 3000 × g for 10 min; plasma was then collected and stored at -20°C until analysis. Plasma glucose (Glu), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and total protein (TP) were measured using standard protocols of commercial laboratory kits (Pars Azmoon, Co., Tehran, Iran). For heterophils (H) and lymphocytes (L), a blood smear was prepared using Giemsa staining, and the number of H and L were counted to a total of 100 cells following a schematic diagram and using a Leica DM500 microscope with a magnification of ×100 immersion oil (Fidan *et al*., 2017). The H/L ratio was calculated by dividing the number of heterophil cells by lymphocyte cells.

Bacteriological analysis

Ten birds were selected for one treatment and killed by dislocation of the cervical vertebrae on day 42. The carcass was then opened and the entire gastrointestinal tract removed under aseptic conditions. The jejunum (from the most distal insertion of the duodenal mesentery to the junction with the Meckel's diverticulum), the ileum (from the junction with the Meckels diverticulum to the ileocaecal junction), and the cecum (from the mouth to the end of each cecum) were then excised. Samples (1 g) of the contents of the jejenum, ileum and caecum were immediately collected in glass containers and were mixed with 9 ml of sterile dilution blank solution, and homogenized for three minutes. Then, serial dilutions (10^{-1} to 10^{-7}) were prepared for further analysis by Jin *et al*. (1998). All microbial analyzes were performed in duplicate, and mean values were used for statistical analysis. *Lactobacillus* species were grown on in MRS (de Man, Rogosa, and Sharpe) agar (Merck, Darmstadt, Germany) after incubation in anaerobic chamber at 37°C for 48 h, and coliform bacteria were grown on McConkey agar (Merck, Darmstadt, Germany). The selective agar used to enumerate coliforms was incubated under aerobic condition, at 37°C for 24 h. The number of bacteria was expressed as l g CFU/g digestion of jejunum, ileum, or caecum.

Humoral immune response

Newcastle (NDV) and Gambaro (IBD) antibody levels were measured in sera samples through inhibition (HI) test according to Cunningham (1971) and enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the instructions provided by the manufacturer (Pars Azmoon, Co., Tehran, Iran). To do so, blood samples were collected from two randomly taken birds per replication on days 10 and 20 after the related immunization. All antibody titers were recorded as $\log_2$ of the highest dilution of serum that agglutinated an equal volume of a 0.5% suspension in PBS.

Statistical analysis

Data were subjected to one-way ANOVA in a randomized complete block design using the GLM procedure of the statistical analysis system (SAS Institute, 2001). Cages were used as the experimental unit for data analysis. Differences between means were tested using Tukey's multiple range method, and $P<0.05$ was statistically significant.

Results

Growth performance and carcass composition

No significant effect of dietary experimental was observed on FI, BW and FCR in 1-10 d (P>0.05). Mean BW was greater in the birds fed on the feed additive supplementation-diets compared with those fed on the CON diet during days 11 to 21 of age (P<0.05; Table 2). In the same period, FI was lower in the birds fed with diets B_1 + S_1 and SS than that in those fed with the VM, B_1 and B_2 diets (P<0.05). Mean FCR was better in all herbal supplemented groups than that of those offered by CON and B_2 diets from days 11 to 21 (P<0.05). Broilers fed with VM showed a greater BW than other groups during 22 to 42 days of age (P<0.05). In this period, mean FCR decreased in the SS and VM-fed birds compared with those fed on the CON, B_1 and S_1 diet (P<0.05). At the end of experiment, the birds fed with diets VM and SS exhibited greater body weight gains (P<0.05; Table 2). Mean FI was lower in the birds fed with the SS, B_2, S_2 and B_1 + S_1 diets than that of those received the CON, VM, and S_1 diets during 22 to 42 and 1 to 42 days of age. Feed conversion ratio was better in the birds fed with all experimental diets compared with that in the CON group during 1 to 42 days of age (P<0.05).

Blood biochemical parameters

Table 3 shows that the chicks fed with S2 diet had a greater plasma total protein level than those fed with CON or S1 diet (P<0.05). The broilers fed with the CON diet had lower plasma glucose levels (P<0.05) than the broilers fed with the SS-supplemented diet. All diets, except VM and B_1, decreased plasma TG levels as compared with the control birds. The mean blood concentration of cholesterol was larger in the birds fed on the CON diet than in the birds receiving B_1 + S_2 diet (P<0.05).

Organ indices and immunological parameters

Table 4 shows that the percentage of abdominal fat in the birds fed with the SS, B2, S2, and B_1 + S_1 diets was lower than that of the birds fed with the CON diet (P<0.05). Experimental treatments exert no influence on the weight of thymus and bursa of Fabricius at 42 days of age (Table 4; P>0.05), whereas a marked increase was observed in the weight of the spleen in broilers fed with VM, SS and S_1 diets compared with those fed with S_2 and B_1 + S_1 diets (P<0.05). Heterophil (H) and lymphocyte (L) counts and H/L ratio was affected by dietary treatments (P<0.05), where the lower heterophil counts and H/L ratio were observed in the birds fed on the SS and B_1 + S_1 diets compared with the control birds. Lymphocyte count was also greater in the birds fed on the SS diet compared with the other groups (P<0.05; Table 4).

Table 2: Effects of different dietary treatments on growth performance of broilers

Diets	1 to 10 d			11 to 21 d			22 to 42 d			1 to 42 d		
	BW (g)	FI (g)	FCR	BW (g)	FI (g)	FCR	BW (g)	FI (g)	FCR	BW (g)	FI (g)	FCR
CON	99	146.85	1.49	514.60^{b}	766.37^{abc}	1.48^{a}	1795.30^{c}	3656.67^{a}	2.03^{a}	2408.67^{c}	4642.30^{a}	1.92^{a}
VM	102.14	147.85	1.45	622.60^{a}	801.43^{a}	1.28^{bc}	1962.40^{a}	3649.47^{a}	1.86^{b}	2687.14^{a}	4598.76^{a}	1.71^{cd}
SS	102.14	144.97	1.42	594.33^{a}	720.90^{bc}	1.21^{dc}	1943.00^{ab}	3481.17^{bc}	1.76^{b}	2639.48^{a}	4347.04^{c}	1.64^{d}
B_1	100.42	146.57	1.46	609.23^{a}	818.83^{a}	1.34^{bc}	1733.37^{c}	3554.18^{abc}	2.05^{a}	2443.03^{bc}	4519.58^{ab}	1.85^{ab}
B_2	105.08	148.29	1.41	585.77^{a}	815.70^{a}	1.39^{ab}	1776.17^{c}	3440.00^{c}	1.93^{ab}	2467.02^{bc}	4403.99^{bc}	1.78^{bc}
S_1	97.85	138.20	1.41	585.87^{a}	787.97^{ab}	1.34^{bc}	1779.30^{c}	3666.83^{a}	2.06^{a}	2465.02^{bc}	4593.00^{a}	1.86^{ab}
S_2	102.57	149.42	1.46	579.87^{a}	764.37^{abc}	1.32^{bc}	1802.14^{c}	3497.33^{bc}	1.95^{ab}	2484.58^{bc}	4411.13^{cb}	1.78^{bc}
B_1+S_1	102.85	147.85	1.44	623.33^{a}	704.17^{c}	1.12^{d}	1841.28^{bc}	3579.00^{ab}	1.94^{ab}	2567.47^{ab}	4431.02^{cb}	1.72^{cd}
SEM	3.441	3.502	0.043	14.924	21.367	0.044	38.946	41.902	0.052	42.989	42.493	0.037
P-value	0.86	0.45	0.89	0.0006	0.0043	0.002	0.0020	0.0023	0.0124	0.0005	0.0001	0.0003

$^{a,\ b}$ Means within same column with different superscripts differ significantly (P<0.05), the number of observations n=48 in each group. CON: Control diet, VM: 0.2 g/kg virginiamycin, SS: Synbioti, B_1: 1 and B_2: 2 g/kg barberry powdered seeds, S_1: 1 and S_2: 2 g/kg sumac powdered seeds, and B_1+S_1: Mixture of barberry 1 g/kg + sumac 1 g/kg. BW: Body weight, FI: Feed intake, and FCR: Feed conversation ratio

Table 3: Effect of different dietary treatment on biochemical parameters of 42-day-old broilers

Diets	Protein (g/dl)	Glucose (mmol/l)	Triglyceride (mmol/l)	Cholesterol (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)
CON	3.75^{c}	12.03^{c}	5.17^{a}	8.79^{a}	3.60	4.16
VM	4.05^{abc}	12.56^{abc}	4.37^{ab}	7.79^{ab}	3.82	3.82
SS	4.04^{ab}	12.97^{a}	3.46^{bc}	6.70^{bc}	3.69	2.31
B_1	3.94^{abc}	12.27^{bc}	4.40^{ab}	7.98^{abc}	3.96	3.13
B_2	4.19^{ab}	12.75^{ab}	3.87^{bc}	7.71^{abc}	3.69	3.24
S_1	3.85^{bc}	12.29^{bc}	4.18^{bc}	8.42^{ab}	4.14	3.46
S_2	4.30^{a}	12.76^{ab}	3.35^{c}	7.47^{abc}	4.05	2.91
B_1+S_1	4.18^{ab}	12.77^{ab}	3.49^{bc}	6.59^{c}	3.58	2.30
SEM	0.13	0.09	0.11	0.19	0.12	0.22
P-value	0.05	0.03	0.002	0.002	0.9	0.2

$^{a,\ b}$ Means within same column with different superscripts differ significantly (P<0.05), the number of observations n=48 in each group. CON: Control die, VM: 0.2 g/kg virginiamycin, SS: Synbiotic, B_1: 1 and B_2: 2 g/kg barberry powdered seeds, S_1: 1 and S_2: 2 g/kg sumac powdered seeds, and B_1+S_1: Mixture of barberry 1 g/kg + sumac 1 g/kg. BW: Body weight, FI: Feed intake, and FCR: Feed conversation ratio

Table 4: Effect of different dietary treatment on organ indices and immune parameters of 42-day-old broilers

Diets	Abdominal fat (g)	Spleen (g)	Thymus (g)	Bursa of fabricius (g)	Heterophil (%)	Lymphocyte (%)	H/L (%)
CON	39.10^{a}	3.54^{ab}	3.48	3.34	22.1^{a}	59.3^{c}	0.38^{a}
VM	30.28^{ab}	4.06^{a}	4.34	3.21	20.2^{ab}	67.3^{b}	0.30^{cd}
SS	19.82^{b}	3.87^{a}	4.19	3.56	15.7^{c}	71.7^{a}	0.22^{e}
B_1	30.92^{ab}	3.37^{ab}	3.65	3.32	20.6^{ab}	59.5^{c}	0.35^{ab}
B_2	26.82^{b}	3.08^{ab}	4.10	3.47	18.9^{b}	66.8^{b}	0.28^{cd}
S_1	29.13^{ab}	3.97^{a}	3.86	3.09	18.5^{b}	58.8^{c}	0.32^{bc}
S_2	25.28^{b}	2.41^{b}	3.83	2.21	19.7^{ab}	64.5^{b}	0.30^{cd}
B_1+S_1	29.62^{b}	2.80^{ab}	3.85	2.04	18.1^{bc}	67.1^{b}	0.27^{d}
SEM	1.35	0.15	0.16	0.12	0.84	1.09	0.013
P-value	0.032	0.049	0.937	0.5006	0.011	0.0001	0.0001

$^{a, b}$ the number of observations n=48 in each group. CON: Control diet: VM: 0.2 g/kg virginiamycin, SS: Synbiotic, B_1: 1 and B_2: 2 g/kg barberry powdered seeds, S_1: 1 and S_2: 2 g/kg sumac powdered seeds, and B_1+S_1: Mixture of barberry 1 g/kg + sumac 1 g/kg

Table 5: Effects of different dietary treatment on populations of lactobacillus and coliforms jejunum, ileum, and caecum of 42-day-old broiler (log10 CFU* g^{-1} fresh digesta)

Diets	*Lactobacillus*			Coliforms		
	Jejunum	Ileum	Ceca	Jejunum	Ileum	Ceca
CON	7.85^{b}	7.88^{c}	8.00^{c}	3.40^{a}	3.96^{c}	7.53^{a}
VM	7.98^{b}	8.29^{b}	8.55^{ab}	1.90^{bc}	2.65^{b}	5.35^{b}
SS	8.64^{a}	8.44^{ab}	8.85^{a}	2.00^{bc}	2.78^{b}	5.48^{b}
B_1	8.55^{a}	8.65^{a}	8.60^{ab}	2.42^{b}	2.88^{b}	6.13^{b}
B_2	8.70^{a}	8.72^{a}	8.81^{a}	1.69^{bc}	2.62^{b}	6.08^{b}
S_1	8.36^{a}	8.68^{a}	8.53^{ab}	2.03^{c}	2.82^{b}	5.85^{b}
S_2	8.40^{a}	8.38^{ab}	8.72^{a}	1.89^{bc}	2.65^{b}	5.40^{b}
B_1+S_1	8.48^{a}	8.70^{ab}	8.78^{a}	1.95^{bc}	2.26^{b}	5.74^{b}
SEM	0.15	0.055	0.090	0.172	0.33	0.35
P-value	0.031	0.001	0.004	0.007	0.019	0.043

$^{a, b}$ Means within same column with different superscripts differ significantly (P<0.05), the number of observations n=48 in each group. CON: Control diet, VM: 0.2 g/kg virginiamycin, SS: Symbiotic, B_1: 1 and B_2: 2 g/kg barberry powdered seeds, S_1: 1 and S_2: 2 g/kg sumac powdered seeds, and B_1+S_1: Mixture of barberry 1 g/kg + sumac 1 g/kg. * CFU: Colony forming unit

Intestinal *Lactobacillus* and *Coliform* bacteria communities

The numbers of *Lactobacillus* in the jejunum of birds fed VM and CON diets were significantly (Table 5; P<0.05) lower than in the birds fed with other experimental diets. However, the total count of *Lactobacillus* in the ileum decreased when the broilers were fed on VM and CON diets (P<0.05). The cecal population of *Lactobacillus* significantly was greater in the birds receiving the SS, B_2, S_2 and B_1 + S_1 diets compared with birds fed on the CON diet at 42 days of age (P<0.05). The numbers of coliforms in the jejunum, ileum and caecum of birds fed CON diet were significantly higher compared with the birds fed with other experimental diets (Table 5; P<0.05).

Humoral immune response against Newcastle (ND) and infectious bursal disease (IBD) disease

Primary antibody titer against ND and IBD was not affected by various experimental diets (Table 6; P>0.05). Broilers fed with the SS, B2, S2 and B_1 + S_1 diets showed greater titers for total antibody titers against ND virus (P<0.05) for the secondary response (at 32 days of age) compared with those fed on the control diet. Moreover, antibody titers against Gambaro virus were greater in the birds fed with the B_2 and B_1 + S_1 diets at 42 days of age (P<0.05).

Table 6: Effect of different dietary treatment on the levels of humoral immune antibody titer against Newcastle and infectious bursal vaccine in broiler

Diets	ND titer		IBD titer	
	Primary titer	Secondary titer	Primary titer	Secondary titer
CON	5.25	4.50^{b}	9.92	8.59^{c}
VM	5.50	5.50^{ab}	10.00	9.09^{abc}
SS	5.25	5.75^{a}	10.61	9.09^{abc}
B_1	5.25	5.25^{ab}	9.92	9.10^{abc}
B_2	5.00	6.00^{a}	10.36	9.82^{a}
S_1	5.50	5.50^{ab}	9.84	8.59^{c}
S_2	5.25	5.75^{a}	10.34	9.64^{ab}
B_1+S_1	4.75	6.00^{a}	10.28	9.83^{a}
SEM	0.490	0.373	0.300	0.311
P-value	0.3102	0.0021	0.2111	0.001

$^{a, b}$ Means within same column with different superscripts differ significantly (P<0.05), the number of observations n=48 in each group. CON: Control diet, VM: 0.2 g/kg virginiamycin, SS: Symbiotic, B_1: 1 and B_2: 2 g/kg barberry powdered seeds, S_1: 1 and S_2: 2 g/kg sumac powdered seeds, and B_1+S_1: Mixture of barberry 1 g/kg + sumac 1 g/kg. ND: Newcastle disease, and IBD: Infectious bursal disease

Discussion

As our results showed, the addition of antibiotics to the diet improved weight gain and decreased FCR, while

FI was like that recorded in control chickens. This confirms the findings of previous studies that showed the addition of antibiotic growth stimulants has a positive effect on the growth of broilers due to its effect on intestinal microbiota (Khodambashi Emami *et al*., 2015). This was highlighted by Bedford (2000), that germ-free animals were not affected by growth-promoting antibiotics. The use of growth-promoting antibiotics can increase the uptake of nutrients by the host and productivity in poultry by manipulating the community of beneficial bacteria (Baskara *et al*., 2020).

In the present study, supplementation of symbiotic in the diet improved body weight and FCR of birds possibly due to providing a better gut health status and microbiota function. In agreement with our results, Chayatid *et al*. (2019) reported that symbiotics (MOS mixed with *Bacillus subtilis* and *Bacillus licheniformis*) assist to optimize digestive system health and microbial balance in the intestine, thereby improving growth performance and reduction in the mortality of poultry. It seems that the synergistic increase the availability of indigestible nutrients and beneficial changes in food metabolism by improving intestinal microbial balance, increasing the activity of digestive enzymes, and activating digestive enzymes, thereby improving feed efficiency. Studies have been conducted recently to actively characterize the impact of phytogenic in enhancing animal health and performance.

In this study, the B_1+S_1 added diet, despite no difference with VM and SS included diets, was able to increase BWG compared with CON diet. This improvement is likely realized through synergistic effects between active plant compounds in protecting the intestinal absorption surface leading to increased use of nutrients. Medicinal plants can improve body weight by reducing harmful bacteria in GIT and increasing digestion and absorption of nutrients. Reduction of pathogenic bacteria due to reduced competition with nutrients and prevention of several intestinal diseases has a positive effect on increasing access to nutrients for animal use (Baskara *et al*., 2020). Consistent with the present results, previous studies have suggested an improvement in the performance of the chickens fed with a diet supplemented with sumac (Toghyani and Faghan, 2017) and barberry (Zhu *et al*., 2021). According to Ghasemi *et al*. (2014), the improved broiler's performance with sumac-supplemented diets is due to the presence of specific biochemical components (cinnamaldehyde and eugenol) found in sumac, causing greater efficiency in the use of feed, resulting in an enhanced weight gain. In line with the findings of previous studies (Cho *et al*., 2014), the results of this study showed that supplementation of broiler diets with phytogenic feed additive reduced the proportion of the intestinal coliforms and increased lactobacilli populations, leading to improvement in nutrients digestibility of feed and beneficially alters material metabolism, the results which collectively improved the efficiency of feeding and increased weight gain of the birds.

In the present study, abdominal fat percentage decreased by 0.55, 0.57, 0.64 and 0.70% in the broilers fed with B_1 + S_1, S_2, B_2 and SS diets compared with those fed with CON (1.82%) diet, respectively. Ahmadian *et al*. (2020) reported that abdominal fat weight was primarily affected by the feed supplements, regardless of dose, being reduced by 41% for chickens fed the thyme supplements and 62% for chickens fed the sumac supplements. The presence of harmful microbes in GIT, increases the breakdown of proteins and amino acids in digestive substances, the deamination activity of proteins and amino acids consumed, and increases their rate of breakdown due to the secretion of substances such as urease by microbes. Accordingly, since medicinal plants have reduced the harmful microbial population of GIT, the rate of breakdown of proteins and amino acids in the digestive tract is reduced and more of them are absorbed and stored in the body, leading to improved carcass percentage. As a result, it reduces the conversion of protein to fat and less fat can accumulate in the body. Blood lipid-lowering effects of barberry and sumac in broiler's chicken may be attributed to the presence of alkaloid and tannins contents, as well as an increase in dietary crude fiber. Chand *et al*. (2007) observed that inclusion of 20 g/kg *Berberis lycium* decreased serum total cholesterol, triglyceride and LDL and increased HDL. The active components of barberry, such as alkaloids are associated with the up-regulation of the hepatic expression of LDL-receptors in cells.

Plant active compounds help the growth and proliferation of lactobacilli by lowering the pH of GIT, especially the intestines, and by producing enzymes that change the chemical structure of bile acids and decongest them, which in turn reduces the amount of cholesterol. In a normal broiler, *Lactobacillus* spp. are the most predominant bacteria in the crop. They are released from the lining of the crop, travel through the rest of the digestive tract, and therefore play a significant role in the populations of bacteria present in the small intestine. *Lactobacillus* strains can decrease harmful pathogens such as *E. coli* in the crop and small intestine, thereby help to maintain the microbial balance in GIT. In the present study, the populations of Lactobacilli, was lower in the birds given any of the herbal treatments compared with those fed with the control diet. Similar results were reported by Dibaji *et al*. (2014). According to Li *et al*. (2019), Bacillus species controlled the makeup of the intestinal bacterial flora, preserved the equilibrium of the GIT microbiota, and enhanced the intestinal mucosa's immune response. Dietary probiotic and prebiotic can decrease the intestinal luminal pH due to the production of lactic acid, which could affect the composition of the microbiota, especially pathogenic bacteria and reduce the development of pathogens. Bukhari *et al*. (2011) evaluated potential positive activities of barberry against *E. coli* and proteus (100-80%), pseudomonas and staphylococcus (60-70%) microorganisms compared with penicillin G. Shariatmadari and Shariatmadari (2020) also suggested that the intestinal *Lactobacillus* counts were increased in broiler fed with a sumac supplemented-diet than those receiving the control diet. Antimicrobial activity is one of the primary properties of plants against pathogenic intestinal microbiota.

In this regard, it has been confirmed that the phenolic compounds in phytogenics are among the main substituents responsible for disrupting bacterial cell wall by changing the Na+ and K permeability and reacting to cytoplasmic membrane causing leakage of intracellular compounds (Lopez Romero *et al*., 2015) and disintegration cell membrane and inhibition of ATP synthesis and eventually cell death. The H/L ratio is a stress indicator and a reduction in this ratio indicates an increase in immunoregulatory capacity of the host.

In this study, immune-stimulatory effects of symbiotic, barberry, and sumac were confirmed by lower H/L ratio in the broilers fed diets SS, B_2, S_1, S_2 and B_1 + S_1 compared with CON diet. Results of Toghyani and Faghan (2017) showed no treatment effect on antibody titers against NDV by adding 3 and 7 g/kg sumac powder in the broiler diet in comparison with an antibiotic growth promoter (AGP), but H/L ratio was decreased in response to 7 g/kg sumac and AGP. Since there was no disease challenge in this study, the ratio of heterophile to lymphocyte has not been expected to increase. Therefore, a decreased H/L ratio might show that sumac powder imposed no stress in birds. In general, the results of the present study showed that in comparison with the control group, the addition of VM in the chicken's diet showed a positive effect on growth performance and health of broilers, but due to the potential of bacterial resistance and antibiotic residues in animal products, attempts are being made to replace them with other feed additives such as symbiotics and phytogenics.

According to our results, symbiotics stimulated selective growth and activity of beneficial microorganisms in the gut and thereby benefit health. Phylogenetics can increase the number of lactic acid-producing bacteria in the ileum and cecum of broilers and decrease the number of coliforms by lowering of the pH value. Since *Lactobacillus* species play an important role in creating this balance and increasing the level of local and temporal immunity of the host. By manipulating the microorganisms of GIT and creating a proper microbial balance in this organ, the immune response and performance of the bird can be optimally improved. The results of the present study demonstrated that the sumac- and barberry-supplementation diets significantly improved body weight gain, feed conversion ratio, immune system, and intestinal microbiota, with significant benefits in reducing fat deposition in broiler chickens. Plant's effects may be due to greater efficiency in the use of feed which results in enhanced growth.

According to the study results, dietary inclusion of the sumac and barberry mixture can be applied as an alternative to antibiotic growth promoters without any adverse effects on immune responses in broiler diets. The effects of the plant may be due to greater efficiency in feed use, which leads to increased growth and modulation of gut microbiota. With better use of nutrients, it has a positive effect on nutrition, health, and growth of the host as well.

Acknowledgements

The authors would like to thank Prof. H. Khosravinia and Dr. H. A. Ghasemi for their review of the manuscript and Department of Animal Sciences, Agriculture Faculty, Ilam University, Iran for their technical support during the experiments.

Conflict of interest

The authors declare there is no conflict of interest regarding the current study and its publication.

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