Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v47i1.71940

Evaluation of drinking water supplementation of two different herbal blends on productive performance and immune responses of broiler chickens

Amir Hossein Alizadeh-Ghamsari, Seyed Adel Moftakharzadeh* and Seyed Abdullah Hosseini

[1]Animal Science Research Institute of Iran, Agricultural Research, Education and Extension Organization, zip code: 3146618361, Karaj, Iran. *Author for correspondence. E-mail: adelmoftakharzadeh@gmail.com

ABSTRACT. This study was carried out to evaluate the effects of herbal mixture on the growth performance, intestinal microbial population, and immune responses of broiler chickens. In all, 600 day-old as-hatched Ross 308 broiler chickens were assigned in a completely randomized design with four treatments, five replicates, and 30 birds in each replicate. The four treatments were: the control group (not treated) and, the second group received 1 mL L^{-1} herbal solutions (Bioherbal®), and two other groups were received 16.6, and 33.3 mL L^{-1} commercial herbal solutions (Orex®), respectively. Results showed that while water supplementation of the herbal mixture had no significant effect on feed intake (FI), body weight (BW), and feed conversion ratio (FCR) of broiler chickens ($p > 0.05$), the productive efficiency index (PEI) improved by dietary inclusion of herbal blend at 33.3 mL L^{-1} ($p < 0.05$). Furthermore, the *lactobacilli* population increased and *E. coli* population was reduced by both herbal mixture addition ($p < 0.05$). The application of both Herbal growth promoters (Orex® and Bioherbal®) boosted cellular and humoral immunity and decreased the heterophil-to-lymphocyte ratio ($p < 0.05$). The present study indicated the positive effect of Orex® addition to water at both concentrations (16.6 or 33.3 mL L^{-1}) on livability, PEI, ileal microbial populations, and immune system function.

Keywords: chicken; herbal plants; immune response; microbial population.

Received on April 15, 2024.
Accepted on November 29 2024.

Introduction

In recent years, the application of antibiotics has been confined in the poultry industry due to several limiting factors such as bacterial resistance and accumulation in animal products. On the other hand, there is increasing general attention to food safety and environmental health (Attia et al., 2010; Oliveira et al., 2020). Considering these issues, it is the poultry scientist's new responsibility to shoulder and introduce possible antibiotic alternatives to improve feed efficiency along with economic profit per unit (Baurhoo et al., 2009).

Medicinal and herbal plants found in nature have the potential to be substituted by antibiotics because of their inherent antioxidant, antimicrobial, and biochemical modulation. The beneficial effects of dietary of a wide range of medicinal herbs on feed efficiency, microbial population, coccidiosis, and immunity in farm animals have been reported due to bioactive compound (Tajodini et al., 2015; Al-Mashhadani et al., 2013; Ancsin et al., 2009; Ogwuegbu et al., 2021).

Rosemary *(Rosmarinus officinalis*) (known to be an aromatic, dense, and evergreen perennial small shrub) contains compounds such as carnasol, rosmanol, α-pinene, myrcene, 1, 8-cineole. The positive impacts of dietary inclusion of rosemary on intestinal function and morphology (Ogwuegbu et al., 2021), reduction of free radicals and the amount of malondialdehyde in the liver (Ancsin et al., 2009), and the performance of broiler chickens (Ghazalah & Ali, 2008) is well documented.

Mugwort (*Artemisia vulgaris*) is characterized by isocoumarin, coumarin, diterpenelactone, and flavonoids which appear to have anti-inflammatory properties (Kang et al., 1995). In addition, Monoterpene compounds found in cherry blossom (*Prunus yedoensis*) have enabled this plant to show antipathogenic properties (Kim ChunSuk et al., 2013; Zhang et al., 2014). *Sargassum sp.* is a naturally brown alga that has been widely used in the animal industry as a growth promotor and it is claimed improved the growth of broilers alongside with increase stability of broiler's meat and reduce cholesterol in poultry products (Armin et al., 2015; Hussein, 2018).

Although some researchers investigated the effects of rosemary and algae supplementation to broiler chicken's diets, there are limited or no studies related to the drinking water supplementation of a blend of mugwort (*Artemisia vulgaris*), colt's foot (*Tussilago farfara*) and cherry blossom (*Prunus yedoensis*) alone or in combination on broilers performance. Therefore, the current trial aimed to examine the effects of drinking water supplementation of the herbal mixture containing alcoholic extract of rosemary (*Rosmarinus officinalis*), mugwort (*Artemisia vulgaris*) colt's foot (*Tussilago farfara*), cherry blossom (*Prunus yedoensis*) and brown algae (*Sargassum sp.*) on the growth performance, immune responses and intestinal microbial population of broiler chickens. In addition, the results were compared to another common herbal drinking water mix.

Material and method

Composition of the herbal blend and microbial safety

The commercial herbal extract compound called as Orex® (Shamim Vision Asia, Theran, Iran) used in the current study consisted of rosemary (*Rosmarinus officinalis*), mugwort (*Artemisia vulgaris*), colt's foot (*Tussilago farfara*), cherry blossom (*Prunus yedoensis*), and brown algae (*Sargassum sp* . To examine microbial safety, the herbal mixture extract was analyzed using Eaton et al. (2005) method. The active compounds of Orex® was measured using SPME-GC-MS method as described by (Siqueira et al., 2013).

Experimental site

The experiment was carried out at the poultry farm of the Animal Science Research Institute of Iran (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.

Ethics approval

Experimental procedures regarding to the chicken rearing and care in this study were reviewed and approved by the Animal Ethics Board of Animal Science Research Institute of Iran (Experimental Authorization No. ASRI-2019- 961079).

Birds and diets

A total of 600 Ross 308 chicks (1 d old), males and females obtained from a local commercial hatchery were allocated randomly to 4 dietary treatments using a randomized complete block design. Each treatment replicated 5 times containing 30 chicks per each floor pen. A corn-soybean meal diet formulated to meet or exceed Ross 308 broiler nutrition specifications for macro- and micronutrients at starter, grower, and finisher, and its calculated composition is presented in Table 1. Four treatments were applied in our experiment in such a way that each group had commercial feed *ad libitum* and drinking water with or without supplement. Group 1 was not received any supplement in drinking water and considered as control treatment, the second group received 1 mL L^{-1} herbal solutions (Bioherbal®), and two other groups were received 16.6, and 33.3 mL L^{-1} commercial herbal solutions (Orex®) (rosemary, mugwort, colt's foot, cherry blossom, and brown algae), respectively. Environment temperature was kept at 34°C during the first 3 days of the trial and then reduced gradually according to age until reaching 22 at 21°C.

Table 1. Ingredient and calculated nutrient contents (%) of the experimental diets given to broiler chickens at starter, grower and finisher period.

Feed Ingredients	Starter (1 to 14 days)	Grower (15 to 28 days)	Finisher (29 to 42 days)
Corn	58.27	59.95	65.11
Soybean meal	36.12	33.62	28.39
Soya oil	1.44	2.96	2.81
Dicalcium phosphate	1.74	1.5	1.56
Caco3	1.34	1.11	1.15
Common salt	0.2	0.2	0.2
Vitamin premix1	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25
L-lysine-HCL	0.15	-	0.1
DL- methionine	0.24	0.16	0.18
Calculated nutrient contents			
Metabolizable energy (kcal kg^{-1})	2094	3024	3070

Protein (%)	21.11	20.16	17.92
Calcium (%)	1	0.87	0.86
Available phosphorous (%)	0.48	0.43	0.4
Sodium (%)	0.1	0.1	0.1
Chloride (%)	0.17	0.17	0.17
Digestable lysine (%)	1.21	1.06	0.94
Digestable methionine (%)	0.56	0.47	0.44
Digestable methionine+cystine (%)	0.9	0.8	0.73

[1]Provided the following (per kg of diet): vitamin A (transretinyl acetate), 9,000 IU; vitamin D_3 (cholecalciferol), 2,000 IU; vitamin E (alfa- tocopherol acetate), 18 IU; vitamin K (bisulfate menadione complex), 2 mg; riboflavin, 6.6 mg; pantothenic acid (D-calcium pantothenate), 10 mg; pyridoxine (pyridoxine HCl), 3 mg; folic acid, 1 mg; thiamin (thiamin mononitrate), 1.8 mg; vitamin B_{12} (cyanocobalamin), 15 µg; D-biotin, 0.1 mg; niacin, 30 mg; choline (choline chloride), 500 mg and ethoxyquin, 0.1 mg. [2]Provided the following (per kg of diet): Se (Na2SeO3), 0.2; I (KI), 1; Cu (CuSO4 .5H2O), 10; Fe (FeSO4 .7H2O), 50; Zn (ZnO), 85 and Mn (MnSO4.H2O), 100 mg.

Growth performance

The growth performance was evaluated by recording feed intake (FI), body weight (BW) at days 14, 28, and 42. The feed conversion ratio (FCR) was calculated accordingly for each period. Productive efficiency index (PEI) and the livability was calculated using the following equation for whole period.

The components of the PEI formula are body weight, livability, bird age, and feed conversion ratio, and then calculated:

$$\text{PEI (\%)} = \frac{\text{Body weight (kg)} \times \text{ livability (\%)} \times 100}{\text{Age(days)} \times \text{feed conversion ratio}}$$

Carcass characteristics

At 42 days of age, two birds per pen were selected (with near-average body weight) and slaughtered to measure carcass characteristics. Breast, thigh, liver, spleen, bursa of Fabricius, and abdominal fat were weighed separately and the results were expressed as a percentage of live weight.

Ileal microbial populations

On d 42, the 2 chicks were slaughtered by neck cut for extraction of ileal contents. The ileal contents of each bird were pooled for serial dilution. Microbial populations were determined by serial dilution (10-4 to 10-6) of ileal samples in anaerobic diluents before inoculation onto Petri dishes of sterile agar as described by (Bryant & Burkey, 1953). *Lactobacilli* were grown on Rogosa SL agar, *E. coli* was grown on eosin methylene blue agar, and *coliforms* were grown on McConkey agar (Darmstadt, Germany). Plates for *Lactobacillus* were incubated anaerobically (73% N: 20% CO2:7% H2) at 37°C. *Escherichia coli* and coliforms were incubated aerobically at 37°C. colonies were counted 24 hours after inoculation. Colony-forming units were defined as distinct colonies measuring at least 1 mm in diameter.

Immune response

Cell-mediated responses

To assess cell-mediated responses, phytohemagglutinin-induced cutaneous basophil hypersensitivity was used (Corrier & DeLoach, 1990). At d 14, the thickness of toe web between the two and third digit of the right foot was measured in millimeters. Then, 100 µg of phytohemagglutinin (suspended in 0.10 mL of sterile saline) was injected into the toe web (two birds per pen). The toe web swelling was measured 24 (1st measurement) and 48 (2nd measurement) hours after injection. The response was determined by subtracting the skin thickness of the first measurement from the skin thickness of the second measurement (Corrier & DeLoach, 1990). On the same day, two other birds from each replicate were selected and the skin of the birds was anointed with 0.1 mL of DNCB solution. An area of approximately 10 cm2 without feathers on the left lateral abdomen was chosen for the challenge with DNCB. The same area on the right side was treated with the solvent alone. The skin thickness (on both sides) before and 24h after the challenge was measured to assess reactions. Differences before and after the challenge were calculated to determine the mean increase in skin thickness in each bird. For each bird, the average of 3 repeat measurements was used for analysis (Verma et al., 2004).

Heterophils, lymphocytes, and SRBC antibodies

At 42 d of age, two birds per pen were selected and their blood samples were collected using heparin-containing syringes to avoid blood clot formation for hematological analysis. Blood smears were prepared on

slides and painted by Giemsa method (Lucas, 1961). One hundred leukocytes per sample were counted by heterophil and lymphocyte separation under an optical microscope, and then the heterophil/lymphocyte ratio (H/L) was measured. For antibody Response, 3 birds per pen were injected intravenously with sheep red blood cells (SRBC) at d 21 and 28 d, 1.0-mL suspension (5% vol/vol) of SRBC was injected intraperitoneally. At 7 d after the second injection, the serum from each sample was collected, heat-inactivated at 56°C for 30 min., and then analyzed for total and IgM (mercaptoethanol-sensitive) and IgG (mercaptoethanol-resistant) anti-SRBC antibodies as described by (Peterson et al., 1999). The antibody data were expressed as the log of the reciprocal of the highest dilution displaying visible agglutination.

Statistical analysis

All data were analyzed as a randomized complete design using SAS (SAS, 2002) and the general linear model procedure, and Duncan's multiple range test was used to compare treatments mean ($p < 0.05$).

Results and discussion

Chemical composition

The major components found in the herbal mixture were 1,8-cineol, thymol, α-Pinene, camphor, β – Pinene, p-cumaric acid, linalool, carnosol, caffeic acid, borneol, carvacrol, and rosmarinic acid. According to the company recommendation, the main compounds observed in the bioherbal® solution were aliicin, thymol, carvacrol, rosmarinic acid, α-Pinene, p-Cumaric acid, Linalool, and quercetin.

Productive performance

The effect of treatments on the performance of broiler chicks in all periods is presented in Table 2. Throughout the entire period, feed intake (FI), body weight (BW), average daily gain (ADG), and feed conversion ratio (FCR) of broilers were not significantly affected by the treatments ($p > 0.05$). Only at the starter phase, the broilers fed Bioherbal® showed a significantly lower BW than the control group ($p < 0.05$). The results of growth performance during the whole period are consistent with Botsoglou et al. (2004) and Hernandez et al. (2004). Ahsan et al. (2018) reported that the addition of phytogenic compounds to diet did not influence the feed intake and FCR of broiler chickens. On the contrary, Perić et al. (2010), and Scheuermann et al. (2009) reported that herbal components enhance the growth performance parameters may be due to increasing the efficiency of nutrient utilization in the gastrointestinal tract.

Table 2. Effect of dietary treatments on the growth performance of broilers in each experimental periods.

Periods	Treatments				*P*-value
	Control	Bioherbal® (1 mL L^{-1})	Herbal mixture (16.6 mL L^{-1})	Herbal mixture (33 mL L^{-1})	
Daily Feed Intake (g day^{-1})					
1-14	35.7	32.8	35.6	34.3	0.159
0-28	65	61.8	63.9	63.7	0.318
0-42	97.3	94.6	99.8	99.3	0.41
Live body weight (g)					
14	459^{a}	428.8^{b}	454.3^{ab}	439.5^{ab}	0.115
28	1325.9	1297.9	1303.9	1315.5	0.834
42	2316.9	2292.2	2402.7	2331.4	0.459
Average daily gain (g day^{-1})					
0-14	28.4	26	28	26.9	0.224
0-28	43.7	42.1	43.2	43.6	0.685
0-42	52.4^{ab}	50.6^{b}	54.8^{a}	53.7^{ab}	0.107
Feed conversion ratio					
0-14	1.25	1.26	1.27	1.27	0.697
0-28	1.49	1.47	1.48	1.46	0.576
0-42	1.86	1.87	1.82	1.85	0.664
Livability (0-42d) (%)	94^{b}	87.3^{c}	98.6^{a}	98.6^{a}	0.001
Productive efficiency Index (0-42d)	293.5^{bc}	268.2^{c}	326.2^{a}	310.9^{ab}	0.007

^{a,b,c}Means with different superscript for the same parameters in each row are significantly different ($p < 0.05$).

The effects of medicinal plants and their derivatives on the performance of broiler chickens were diverse from having positive effects (Khattak et al., 2014; Tajodini et al., 2015), showing no effect (Ahsan et al., 2018) or depressing the growth (Ghazalah & Ali, 2008; Lee et al., 2004). The effects of herbal supplementation on broilers depend on several factors such as the derivatives, the part of the plant, the method, and the number of additives (plant powder in feed, essential oil in feed, extract in feed or drinking water, or infused in drinking water) (Al-Mashhadani et al., 2013); Even though the herbal mixture addition had no significant effect on growth parameters in our trial, FCR and ADG were numerically better for both herbal-treated groups in comparison with the control group. The livability significantly increased by dietary inclusion of herbal blends at both levels of supplementation. The PEI at 33.3 mL L^{-1} of the herbal blend was improved which is consistent with the results of Devi et al. (2018). According to the formula, the group that drank 33.3 mL L showed significantly higher PEI, possibly as a result of higher livability, and numerically better FCR and body weight of broiler chickens.

Carcass characteristics

According to Table 3, There was no significant difference among treatments in terms of carcass yield and the relative weight of thigh, breast, heart, liver, pancreas, gizzard, bursa of Fabricius, spleen, and abdominal fat ($p > 0.05$). Our findings on carcass characteristics are in agreement with the observations of Amouzmehr et al. (2012), but in contrast with the reports of Alcicek et al. (2004).

Ileal microbial populations

As can be seen from the Table 3, adding the herbal mixture or Bioherbal® to drinking water significantly enhanced the population of *lactobacillus* and decreased *E.coli* population of ileal contents ($p < 0.05$). The improvement observed in the population of microbial composition in the ileum contents by the herbal extract mixture supplementation in the present study was similar to the results of Mathlouthi et al. (2012), who observed that a blend of essential oils containing *rosemary* had antimicrobial activity against pathogenic bacteria such as *Escherichia coli*, *Salmonella indiana*, and *Listeria innocua*. The addition of colt's foot t to the diet resulted in decrease in the population of *E.coli,* and *Saccharomyces cerevisiae* in the *in vitro* studies (PetrovÃ et al. (2013). It has been reported that water-soluble polysaccharides and phenolic compounds such as dicaffeoylquinic acids, and quercetin pentoside are responsible for antimicrobial properties associated with colt's foot application (Dobravalskyte et al., 2013). Mugwort has been used as an analgesic and antimicrobial agent in rats (Kwon et al., 1993). Moreover, Kim ChunSuk et al. (2013) reported that the inclusion of mugwort increased the total phenol content and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity in broiler meat. According to our literature, there is a lack of information regarding the effect of cherry blossom on poultry species, but using methanol extracts and the ethyl acetate fraction demonstrated anti-inflammatory properties in the in vitro studies (Stankovic et al., 2014).

Table 3. Carcass yield and relative weight of organs of broiler chickens as affected by treatments at 42 day.

Parameters (% of LW)	Treatments				
	Control	Bioherbal® (1 mL L^{-1})	Herbal mixture (33 mL L^{-1})	Herbal mixture (16.6 mL L^{-1})	*P*-value
Carcass yield	64.95	66.17	65.12	64.23	0.643
breast	25.11	25.77	25.09	25.07	0.916
thigh	18.19	17.10	17.65	17.14	0.19
Heart	0.53	0.47	0.42	0.49	0.22
liver	2.37	2.62	2.48	2.38	0.63
Gizzard	2.86	3.3	2.97	3.1	0.819
Pancreas	0.19	0.14	0.24	0.17	0.334
bursa of Fabricius	0.07	0.05	0.06	0.06	0.25
Spleen	0.11	0.11	0.11	0.12	0.992
Abdominal fat	1.06	1.28	1.18	1.03	0.811
log10 cfu g^{-1} of DM					
Lactobacillus	6.12^{d}	6.47^{c}	7.8^{a}	7.24^{b}	0.001
E.coli	7.73^{ab}	6.82^{b}	6.21^{d}	6.54^{c}	0.001

^{a,b,c,d}Means with different superscript for the same parameters in each row are significantly different ($p < 0.05$).

Another possible reason for the reduced *E.coli* population is brown algae. Al-Khalaifah et al. (2022) stated that dietary supplementation of brown algae (*Sargassum sp.*) promoted the health of the gastrointestinal tract of broiler chickens via reducing *E.coli* and *Salmonella sp.* population. Similarly, Kang et al. (1995) observed an improvement in the production of *Lactobacillus* bacteria in the intestinal microflora when seaweed was added

to the diet. It seems some antimicrobial, antiviral, and antioxidant compounds such as polyphenols and carotenoids extracted from brown algae may be responsible for these microbial regulations (Cho et al., 2007; Mehdinezhad et al., 2016). However, the highest population of *lactobacillus* and the lowest population of *E. coli* belonged to the control group fed 33.3 mL L^{-1} level of herbal mixture ($p < 0.05$).

Immune response

The Table 4 demonstrates the effect of experimental treatments on Humoral immune responses**,** skin hypersensitivity reaction 24 hours after the injection of dinitrochlorobenzene (DNCB) and phytohemagglutinin phosphate (PHA-P) at the age of 30 days, as well as the differential count of white blood cells at 42 d. The birds received either Bioherbal® or herbal mixture extract showed significantly higher total antibody titer and IgY in the serum as compared to unsupplemented group ($p < 0.05$), the IgM titer showed no difference among treatments ($p >0.05$) (Table 4). The highest IgY antibody titers belonged to the herbal plant mixture treated group at 16.6 mL L^{-1} significantly different with control and herbal mixture groups ($p < 0.05$) and not differ with Bioherbal®-supplemented group ($p > 0.05$).

Table 4. Immune response of broiler chickens as affected by treatments.

Parameters	Treatments				*P*-value
	Control	Bioherbal® (1 mL L^{-1})	Herbal mixture (33 mL L^{-1})	Herbal mixture (16.6 mL L^{-1})	
PHA-P* thickness (mm) (30 day)	1.328[b]	1.452[a]	1.452[a]	1.438[a]	0.006
DNCB** thickness (mm) (30 day)	1.236[b]	1.396[a]	1.37[a]	1.37[a]	0.002
Total antibody	4.20[a]	6[b]	5.6[b]	6.4[b]	0.001
IGM (32 day)	1.2	1.2	1.6	1.2	0.642
IGY (32 day)	3[c]	4.8[a]	4[b]	5.2[a]	0.001
Hetrophil (%) (42 day)	11.6[a]	8.2[b]	9[b]	8.6[b]	0.001
Lymphocyte (%) (42 day)	88.4[b]	91.8[a]	91[a]	91.4[a]	0.001
Hetrophil/ Lymphocyte (42 day)	0.131[a]	0.089[b]	0.099[b]	0.094[b]	0.001

[a,b,c]Means with different superscript for the same parameters in each row are significantly different ($p < 0.05$). *Phytohemagglutinin-P. **Dinitrochlorobenzene

The addition of Bioherbal® or plant extract mixture strengthened cellular immunity (increased skin sensitivity response to PHA-P and DNCB injection) ($p < 0.05$). Besides, there was a significant decrease in the percentage of heterophils and the ratio of heterophil-to-lymphocyte (stress index) ($p < 0.05$) when plant extract mixture or Bioherbal® was added to the diet. On the other hand, the percentage of lymphocytes significantly increased when both herbal additives were used ($p < 0.05$).

Similar to our findings related to the improvement in immunity occurred by herbs supplementation, Khazaei et al. (2017) showed that Rosemary extract or leaves increased the antibodies titer against infectious viral disease. Moreover, Elnaggar (2016) indicated that dietary inclusion of rosemary stimulated innate and adaptive immunity. In their study, the levels of cytokine, as an indicator of cell-mediated Immunity, increased when rosemary was supplemented. Zhang et al. (2017) reported that mugwort improved the immune stress induced by lipopolysaccharide in broilers. Similarly, cherry blossom acted as an effective inflammatory inhibitor against lipopolysaccharide-induced Nitric Oxide production in the in vitro study (Zhang et al., 2014).

The improvement in the immune response of animals when herbal blends were added to water could be due to the presence of some bioactive compounds (especially thymol, linalool, carvacrol, and pinene) in both herb mixtures that have been confirmed by previous researchers (Kusuhara et al., 2019; Nouri, 2019). In accordance with our trial, Hashemipour et al. (2013) reported that thymol and carvacrol supplementation to diet modified the immune response of broiler chickens. However, the mechanism is not clear, but it seems that herbal bioactive compounds improve the activity of lymphocytes and macrophages, enhance phagocytosis, or stimulate interferon synthesis (FrAnKIČ, et al., 2009). Furthermore, phenolic and flavonoid compounds in the herbal blend can play a key role in reducing the activity of free radicals (Kim ChunSuk et al., 2013). So, the reduction of heterophil-to-lymphocyte ratio, as an indicator of stress, may be attributed to these compounds.

Conclusion

It is concluded that drinking water supplementation of the herbal blend (Orex®) at both concentrations (16.6 or 33.3 mL L^{-1}) can significantly improves livability, the immunity and microbial population of broiler chickens, while only addition of 33.3 mL L^{-1} of herbal plant increased PEI of birds.

References

Ahsan, U., Kuter, E., Raza, I., Köksal, B. H., Cengiz, Ö., Yıldız, M., Kızanlık, P. K., Kaya, M., Tatlı, O., & Sevim, Ö. (2018). Dietary supplementation of different levels of phytogenic feed additive in broiler diets: the dynamics of growth performance, caecal microbiota, and intestinal morphometry. *Brazilian Journal of Poultry Science*, *20*(4), 737-746. https://doi.org/10.1590/1806-9061-2017-0698

Al-Khalaifah, H. S., Al-Nasser, A., & Surrayai, T. (2022). Effects from dietary addition of sargassum sp., spirulina sp., or gracilaria sp. powder on immune status in broiler chickens. *Frontiers in Veterinary Science*, *9*, 928235. https://doi.org/10.3389/fvets.2022.928235

Al-Mashhadani, E. H., Al-Mashhadani, H., & Al-Shamire, J. S. (2013). Effect of supplementing different levels of chamomile oil on broiler performance and some physiological traits. *International journal of poultry science*, *12*(7), 426-429. https://doi.org/10.3923/ijps.2013.426.429

Alcicek, A. H. M. E. T., Bozkurt, M., & Cabuk, M. E. T. İ. N. (2004). The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. *South African Journal of Animal Science*, *34*(4), 217-222.

Amouzmehr, A., Dastar, B., Nejad, J. G., Sung, K. I., Lohakare, J., & Forghani, F. (2012). Effects of garlic and thyme extracts on growth performance and carcass characteristics of broiler chicks. *Journal of Animal Science and Technology*, *54*(3), 185-190. https://doi.org/10.5187/JAST.2012.54.3.185

Ancsin, Z., Erdélyi, M., & Mézes, M. (2009). Effect of rosemary and garlic oil supplementation on glutathione redox system of broiler chickens. *Acta Biologica Szegediensis*, *53*(suppl.), 19-21.

Armin, F., Rahimi, S., Mahdi Abkenar, A., Ghofrani Ivari, Y., & Ebrahimi, H. (2015). Effect of Sargassum sp. and vitamin E on stability of fish oil enriched meat in broiler chickens. *Iranian Journal of Applied Animal Science*, *5*(2), 385-392.

Attia, Y. A., Abdalah, A. A., Zeweil, H. S., Bovera, F., El-Din, A. T., & Araft, M. A. (2010). Effect of inorganic or organic selenium supplementation on productive performance, egg quality and some physiological traits of dual-purpose breeding hens. *Czech Journal of Animal Science, 55*(11), 505-519. https://doi.org/10.17221/1715-CJAS

Baurhoo, B., Ferket, P. R., & Zhao, X. (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poultry science*, *88*(11), 2262-2272. https://doi.org/10.3382/ps.2008-00562

Botsoglou, N. A., Christaki, E., Florou-Paneri, P., Giannenas, I., Papageorgiou, G., & Spais, A. B. (2004). The effect of a mixture of herbal essential oils or α-tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. *South African Journal of Animal Science*, *34*(1), 52-61. https://doi.org/10.4314/sajas.v34i1.4039

Bryant, M. P., & Burkey, L. A. (1953). Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. Journal of Dairy Science, *36*(8), 205-217. https://doi.org/10.3168/jds.S0022-0302(53)91482-9

Cho, S. H., Kang, S. E., Cho, J. Y., Kim, A. R., Park, S. M., Hong, Y. K., & Ahn, D. H. (2007). The antioxidant properties of brown seaweed (*Sargassum siliquastrum*) extracts. *Journal of medicinal food*, *10*(3), 479-485. https://doi.org/10.1089/jmf.2006.099

Corrier, D. E., & DeLoach, J. R. (1990). Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. *Poultry science*, *69*(3), 403-408. https://doi.org/10.3382/ps.0690403

Dobravalskyte, D., Venskutonis, P. R., Talou, T., Zebib, B., Merah, O., & Ragazinskiene, O. (2013). Antioxidant properties and composition of deodorized extracts of Tussilago farfara L. *Records of Natural Products*, *7*(3), 201.

Devi, P. C., Samanta, A. K., Das, B., Kalita, G., Behera, P. S., & Barman, S. (2018). Effect of plant extracts and essential oil blend as alternatives to antibiotic growth promoters on growth performance, nutrient utilization and carcass characteristics of broiler chicken. *Indian Journal of Animal Nutrition*, *35*(4), 421-427. https://doi.org/10.5958/2231-6744.2018.00064.6

Eaton, A. D., Clesceri, L. S., Franson, M. A. H. Association, APH, Rice, EW, Greenberg, AE, Association, AWW, & Federation, WE, (2005). *Standard Methods for the Examination of Water & Wastewater, Standard Methods for the Examination of Water and Wastewater*. American Public Health Association.

Elnaggar, A. S. (2016). Productive, physiological and immunological effect rosemary leaves meal (Rosemarinus officinalis) supplementing to broiler diet. *Egyptian Poultry Science Journal*, *36*(4), 859-573. https://doi.org/10.21608/epsj.2016.168814

FrAnKIČ, T., Voljč, M., Salobir, J., & Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. *Acta agriculturae slovenica*, *94*(2), 95-102. https://doi.org/10.14720/aas.2009.94.2.14834

Ghazalah, A. A., & Ali, A. M. (2008). Rosemary leaves as a dietary supplement for growth in broiler chickens. *International Journal of Poultry Science*, *7*(3), 234-239. https://doi.org/10.3923/ijps.2008.234.239

Hashemipour, H., Kermanshahi, H., Golian, A., & Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry science*, *92*(8), 2059-2069. https://doi.org/10.3382/ps.2012-02685

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., & Megias, M. D. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poultry science*, *83*(2), 169-174. https://doi.org/10.1093/ps/83.2.169

Hussein, E. A. (2018). Effect of dietary brown algae supplementation on the performance of broiler chicks. Menoufia *Journal of Animal Poultry and Fish Production*, *2*(1), 11-22. https://doi.org/10.21608/MJAPFP.2018.175400

Kang, Y. H., Park, Y. K., Oh, S. R., & Moon, K. D. (1995). Studies on the physiological functionality of pine needle and mugwort extracts. *Korean Journal of Food Science and Technology*, *27*(6), 978-984.

Khattak, F., Ronchi, A., Castelli, P., & Sparks, N. (2014). Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. *Poultry science*, *93*(1), 132-137. https://doi.org/10.3382/ps.2013-03387

Khazaei, R., Esmailzadeh, L., Seidavi, A., & Simões, J. (2017). Comparison between rosemary and commercial antioxidant blend on performance, caecal coliform flora and immunity in broiler chickens fed with diets containing different levels of poultry fat. *Journal of Applied Animal Research*, *45*(1), 263-267. https://doi.org/10.1080/09712119.2016.1174125

Kim ChunSuk, K. C., Lee SunJoo, L. S., Hyun ChangGu, H. C., & Lee NamHo, L. N. (2013). Chemical constituents of supercritical extracts from Prunus yedoensis, Saururus chinensis, Zanthoxylum piperitum and their anti-inflammatory activities. *International Journal Of Pharmacology*, *9*(4), 258-264. https://doi.org/10.3923/ijp.2013.258.264

Kusuhara, M., Maruyama, K., Ishii, H., Masuda, Y., Sakurai, K., Tamai, E., & Urakami, K. (2019). A fragrant environment containing α-pinene suppresses tumor growth in mice by modulating the hypothalamus/sympathetic nerve/leptin axis and immune system. *Integrative Cancer Therapies*, *18*, 1534735419845139. https://doi.org/10.1177/1534735419845139

Kwon, M. N., Choi, J. S., & Byun, D. S. (1993). Effect of flavonoid (+)-catechin as stabilizer in rats fed fresh and peroxidized fish oil. *Journal of the Korean Society of Food and Nutrition (Korea Republic)*, *22*(4), 381-391.

Lee, K. W., Everts, H., Kappert, H. J., Wouterse, H., Frehner, M., & Beynen, A. C. (2004). Cinnamaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. *International Journal of Poultry Science*, *3*(9), 608-612. https://doi.org/10.3923/ijps.2004.608.612

Lucas, A. M. (1961). *Atlas of avian hematology* (Vol. 25). US Department of Agriculture. https://doi.org/10.5962/bhl.title.6392

Mathlouthi, N., Bouzaienne, T., Oueslati, I., Recoquillay, F., Hamdi, M., Urdaci, M., & Bergaoui, R. (2012). Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: in vitro antimicrobial activities and effects on growth performance. *Journal of animal science*, *90*(3), 813-823. https://doi.org/10.2527/jas.2010-3646

Mehdinezhad, N., Ghannadi, A., & Yegdaneh, A. (2016). Phytochemical and biological evaluation of some Sargassum species from Persian Gulf. *Research in pharmaceutical sciences*, *11*(3), 243-249.

Nouri, A. (2019). Chitosan nano-encapsulation improves the effects of mint, thyme, and cinnamon essential oils in broiler chickens. *British poultry science*, *60*(5), 530-538. https://doi.org/10.1080/00071668.2019.1622078

Ogwuegbu, M. C., Oyeagu, C. E., Edeh, H. O., Dim, C. E., Ani, A. O., & Lewu, F. B. (2021). Effects of sodium butyrate and rosemary leaf meal on general performance, carcass traits, organ sizes and nutrient digestibility of broiler chickens. *Iranian Journal of Applied Animal Science*, *11*(2), 365-379.

Oliveira, N. A., Gonçalves, B. L., Lee, S. H., Oliveira, C. A. F., & Corassin, C. H. (2020). Use of antibiotics in animal production and its impact on human health. *Journal of Food Chemistry and Nanotechnology*, *6*, 40-47. https://doi.org/10.17756/jfcn.2020-082

Perić, L., Milošević, N., Žikić, D., Bjedov, S., Cvetković, D., Markov, S., Mohnl, M & Steiner, T. (2010). Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. *Archives Animal Breeding*, *53*(3), 350-359. https://doi.org/10.5194/aab-53-350-2010

Peterson, A. L., Qureshi, M. A., Ferket, P. R., & Fuller, J. C. (1999). Enhancement of cellular and humoral immunity in young broilers by the dietary supplementation of β-hydroxy-β-methylbutyrate. *Immunopharmacology and immunotoxicology*, *21*(2), 307-330. https://doi.org/10.3109/08923979909052765

PetrovÃ, J., PavelkovÃ, A., RovnÃ, K., BobkovÃ, A., & ÄŒuboÅˆ, J. (2013). Antimicrobial activity of Tussilago Farfara L. *Journal of microbiology, biotechnology and food sciences*, *2*(special issue 2), 1343-1350.

Scheuermann, G. N., Cunha Junior, A., Cypriano, L., & Gabbi, A. M. (2009). Phytogenic additive as an alternative to growth promoters in broiler chickens. *Ciência Rural*, *39*(2), 522-527. https://doi.org/10.1590/S0103-84782009000200032

Siqueira, E. M. D. A., Rosa, F. R., Fustinoni, A. M., de Sant'Ana, L. P., & Arruda, S. F. (2013). Brazilian savanna fruits contain higher bioactive compounds content and higher antioxidant activity relative to the conventional red delicious apple. *PloS one*, *8*(8), e72826. https://doi.org/10.1371/journal.pone.0072826

Stankovic, M. S., Zia-Ul-Haq, M., Bojovic, B. M., & Topuzovic, M. D. (2014). Total phenolics, flavonoid content and antioxidant power of leaf, flower and fruits from cornelian cherry (Cornus mas L.). *Bulgarian Journal of Agricultural Science*, *20*(2), 358-363.

Tajodini, M., Saeedi, H. R., & Moghbeli, P. (2015). Use of black pepper, cinnamon and turmeric as feed additives in the poultry industry. *World's poultry science journal*, *71*(1), 175-183. https://doi.org/10.1017/S0043933915000148

Verma, J., Johri, T. S., Swain, B. K., & Ameena, S. (2004). Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. *British poultry science*, *45*(4), 512-518. https://doi.org/10.1080/00071660412331286226

Zhang, P., Shi, B., Su, J., Yue, Y., Cao, Z., Chu, W., Li, K & Yan, S. M. (2017). Relieving effect of Artemisia argyi aqueous extract on immune stress in broilers. *Journal of Animal Physiology and Animal Nutrition*, *101*(2), 251-258. https://doi.org/10.1111/jpn.12553

Zhang, Y. Q., Guan, L., Zhong, Z. Y., Chang, M., Zhang, D. K., Li, H., & Lai, W. (2014). The anti-inflammatory effect of cherry blossom extract (Prunus yedoensis) used in soothing skincare product. *International journal of cosmetic science*, *36(*6), 527-530. https://doi.org/10.1111/ics.12149