Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tjas20

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To cite this article: Samantha Sigolo, Chrysostomos Milis, Mahmoud Dousti, Ebrahim Jahandideh, Ali Jalali, Noorouddin Mirzaei, Behrouz Rasouli, Alireza Seidavi, Antonio Gallo, Giulia Ferronato & Aldo Prandini (2021) Effects of different plant extracts at various dietary levels on growth performance, carcass traits, blood serum parameters, immune response and ileal microflora of Ross broiler chickens, Italian Journal of Animal Science, 20:1, 359-371, DOI: 10.1080/1828051X.2021.1883485

To link to this article: https://doi.org/10.1080/1828051X.2021.1883485

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Published online: 22 Feb 2021.

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ITALIAN JOURNAL OF ANIMAL SCIENCE
2021, VOL. 20, NO. 1, 359–371
https://doi.org/10.1080/1828051X.2021.1883485

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Effects of different plant extracts at various dietary levels on growth performance, carcass traits, blood serum parameters, immune response and ileal microflora of Ross broiler chickens

Samantha Sigolo[a], Chrysostomos Milis[b], Mahmoud Dousti[c], Ebrahim Jahandideh[c], Ali Jalali[c], Noorouddin Mirzaei[c], Behrouz Rasouli[c], Alireza Seidavi[c], Antonio Gallo[a], Giulia Ferronato[a] and Aldo Prandini[a]

[a]Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti (DIANA), Università Cattolica Sacro Cuore, Piacenza, Italy; [b]Ministry of Rural Development and Foods, Thessaloniki, Greece; [c]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

ABSTRACT

A study was conducted to evaluate the effects of different plant extracts (common nettle, coriander, dill and thyme) at various dietary levels (0, 150, 300 and 450 mg/L) on growth performance, carcass traits, blood serum parameters, immune response and ileal microflora of 650 male Ross chickens (13 treatment groups; five replicates/treatment group; 10 birds/replicate) in a 42-day trial. Plant extracts were added to drinking water. Supplementation, except for coriander, increased feed intake ($p < .05$). Thyme extracts at 300 mg/L improved both weight gain and feed efficiency ($p < .05$). Thyme or dill (300 mg/L) improved carcass and drumsticks yields ($p < .05$). Pre-slaughtered body weight was increased by supplementation with plant extracts at 300 mg/L ($p < .05$). Plant extracts positively affected blood serum parameters decreasing concentrations ($p < .05$) of total protein, albumin, urea and total cholesterol. Dill extract increased IgG against sheep red blood cells at d 28 ($p < .05$) and d 42 (IgG; tendency $p = .0698$), whereas coriander extract (450 mg/L) raised immune response against avian influence within 42 d from vaccination ($p < .05$). Plant extracts reduced coliforms (except dill at 300 mg/L level; $p < .05$) and aerobic bacteria (except dill; $p < .05$). Negative gram bacteria were increased by thyme and dill treatments at 150 mg/L ($p < .05$) whereas lactobacilli bacteria were raised by thyme at 150 mg/L level ($p < .05$). In conclusion, supplementation with thyme at 300 mg/L level had the greatest benefits on the evaluated parameters.

HIGHLIGHTS

- Thyme supplementation at 300 mg/L level could improve broiler chicken growth performance, carcass traits and blood serum parameters.
- Thyme supplementation at 150 mg/L could positively affect ileal microbiota increasing lactobacilli bacteria and reducing coliforms.
- However, no relevant effects of the thyme treatment were observed on broiler chicken immune responses.

ARTICLE HISTORY
Received 20 July 2020
Revised 25 January 2021
Accepted 27 January 2021

KEYWORDS
Common nettle; coriander; dill; growth promoter; thyme

Introduction

The possible consequences on the public health due to the use of antibiotics in livestock farms, are among the most frequent consumers' concerns related to production and consumption of meat. The capability of antibiotics at sub-therapeutic doses to increase growth and maintain the health of animals and birds was discovered in the late forties (Gustafson and Bowen 1997). Over time, the use of antibiotic growth promoters has become a common practice (Chattopadhyay 2014), making a great contribution to the profitability of the livestock industry (Sharifi et al. 2013). Although the mechanism of action remains unclear, it was hypothesised that antibiotic growth promoters modulate the intestinal microflora providing an optimal microbiota for nutrient utilisation and therefore for animal growth (Dibner and Richards 2005; Attia and Al-Harthi 2015). However, the massive

CONTACT Dr Alireza Seidavi alirezaseidavi@iaurasht.ac.ir Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht 41335-3516, Iran

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use of antibiotics for prophylaxis, growth promotion and metaphylaxis has promoted the emergence of antibiotic resistant bacteria and genes that can be transmitted from animal to human (Attia et al. 2018; Chokshi et al. 2019). This has brought to the ban for the use of antibiotics as growth promoters in animal agriculture in Europe Union on 2006 year and in US on 2017 year (van der Aar et al. 2017).

Today, there is a growing need to find efficient alternatives for antibiotics to improve performance and maintain health of livestock species, especially poultry. Poultry production is considered a high risk for antibiotic resistance emergence because poultry generally receives higher quantities of antibiotics than other livestock species (van den Bogaard 2001; Graham et al. 2017), and resistance is more likely to develop in conditions of overcrowding and poor sanitation (Rousham et al. 2018). Research efforts are increasingly directed towards using natural agents with similar beneficial effects of growth promoters (Mehdi et al. 2018). Botanical products (herbs, spices, essential oils or oleoresins) are referred to as phytogenic feed additives consisting of many bioactive molecules with antioxidant and antimicrobial properties, and stimulating effects on the digestive system (Windisch et al. 2008; Diaz-Sanchez et al. 2015; Attia, Bakhashwain, et al. 2017; Attia, Al-Harthi, et al. 2017).

Beneficial effects of phytogenic compounds have been observed on performance (Giannenas et al. 2005; Hong et al. 2012; Attia and Al-Harthi 2015; Hashemi et al. 2018), nutrient digestibility (Hernández et al. 2004; Amad et al. 2011; Mountzouris et al. 2011), activity of digestive enzymes (Lee et al. 2003; Jang et al. 2007) and antimicrobial activity (Cross et al. 2007; Reisinger et al. 2011) in broiler chickens. For against, other studies have reported no effects or negative effects on performance, and gut morphology and microbiota (Abildgaard et al. 2010; Hafeez et al. 2016; Ahsan et al. 2018).

Some of these botanicals are considered as traditional medicinal plants due to their potential benefits resulting from the presence of phenolic compounds (Wasli et al. 2018). *Urtica dioica* is reported to have effect as antidiabetic, hypolipidaemic and liver and renal damage recovering effects in rats (Abedi Gaballu et al. 2015) and leaves are used with anti-inflammatory (Hajhashemi and Klooshani 2013) and antibacterial (Körpe et al. 2013) potentials. Moreover alcoholic extract of leaves was found to be effective against coccidiosis in chicks (Rahman et al. 2015). *Anethum graveolens* show positive effects on birds' serum lipid profile and gut health, enhancing overall performance (Vispute et al. 2019). These could be possible due to the active component having ability to reduce oxidative stress (Singh et al. 2005), antibacterial (Singh et al. 2002) and hypolipidaemic (Hajhashemi and Abbasi 2008) properties. *Coriandrum sativum* is also known for its antimicrobial food borne pathogen (Kubo et al. 2004) and stimulating effect on digestive system (Lee et al. 2004) enhancing also the liver functions (Hernández et al. 2004). Also, *Thymus vulgari* is recognised to have antimicrobial and antifungicidal and antioxidant effects (Toghyani et al. 2010).

Further research is needed to investigate the effective applicability of phytogenic compounds as non-antibiotic growth promoters in poultry nutrition as well as to optimise administration level. Thus, the present study aimed to evaluate the effects of common nettle (*Urtica dioica*), dill (*Anethum graveolens*), coriander (*Coriandrum sativum*) and thyme (*Thymus vulgari*) extracts at various dietary levels on growth performance, carcass traits, blood serum parameters, immune responses and ileal microflora of broiler chickens.

Materials and methods

The study was carried out in a commercial poultry farm at Gulian, Iran during 2018. The experimental protocol was authorised by the Institutional Animal Care and Ethics Committee of Islamic Azad University (Rasht Branch, Rasht, Iran) and the experiment was performed with respect to the Directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament and Council 2010).

Animals, housing and experimental diets

The experimental trial involved 650 male Ross 308 broiler chickens (Aviagen, Newbridge, Scotland, UK). The birds (initial body weight (BW) of 41.7 ± 1.2 g) were randomly divided into 13 treatment groups with five replicates per treatment group. Each replicate of 10 male birds was allocated in a floor pen (1.0 m × 1.0 m). Lighting provision was made for 24 h on day 1 and subsequently, for 23 h per day. The temperature, humidity and general conditions were according to the standard brooding practice for the rearing stages of the birds as reported in *Ross Broiler Management Manual* (Ross 2002).

A basal diet was formulated to meet the nutritional requirements of broiler chickens as indicated by Ross (2002) for three growth periods, as follows: from d 1 to d 14 (i.e. starter period), d 15 to d 28 (i.e. grower period) and d 29 to d 42 (i.e. finisher period). In

Table 1. Ingredients, chemical composition and metabolisable energy of the starter, grower and finisher diets.

Item	Starter (from 1 to 14 d)	Grower (from 15 to 28 d)	Finisher (from 29 to 42 d)
Ingredients, g/kg			
Corn	545	585	627
Soybean meal	375	332	295
Soybean oil	40.0	40.0	40.0
Calcium carbonate	12.0	12.0	11.0
Dicalcium phosphate	16.0	15.0	15.0
Salt	2.30	2.60	2.50
Vitamin and mineral premix[a]	6.00	6.00	6.00
Sodium bicarbonate	1.20	1.40	1.00
DL-Methionine	1.80	2.10	1.50
L-Lysine	0.700	0.900	1.00
Calculated analysis			
Metabolisable energy[b], MJ/kg	12.5	12.7	12.9
Crude protein, g/kg	210	196	181
Lysine, g/kg	12.7	11.0	9.70
Methionine + cystine, g/kg	9.40	8.40	7.60
Methionine, g/kg	4.70	4.20	3.60
Arginine, g/kg	13.1	11.4	10.2
Tryptophan, g/kg	2.00	1.80	1.60
Calcium, g/kg	10.5	9.00	8.50
Available phosphorus, g/kg	5.00	4.50	4.20
Magnesium, g/kg	0.500	0.600	0.500
Sodium, g/kg	2.10	1.90	1.80
Chloride, g/kg	1.70	1.70	1.60
Potassium, g/kg	5.00	4.00	4.00
Copper, mg/kg	16.0	16.0	18.0
Iodine, mg/kg	1.25	1.25	1.25
Fe, mg/kg	40.0	40.0	40.0
Manganese, mg/kg	120	120	120
Selenium, mg/kg	0.300	0.300	0.300
Zinc, mg/kg	100	100	100

[a]Amount per kg: retinol, 5000 U; cholecalciferol, 500 U; tocopherol, 3 mg; menadione, 1.5 mg; riboflavin, 1 mg; calcium pantothenate, 4 mg; niacin, 15 mg; pyridoxine, 13 mg; Cu,3 mg; Zn, 15 mg; Mn, 20 mg; Fe, 10 mg; K, 0.3 mg.
[b]Metabolisable energy was estimated using the Carpenter and Clegg equation (Leeson and Summers 2001).

control group, birds received drinking water without plant extracts. In common nettle, dill, coriander and thyme groups, from 1 to 42 d of age, drinking water was supplemented with plant extracts respectively from common nettle (*Urtica dioica*), dill (*Anethum graveolens*), coriander (*Coriandrum sativum*) and thyme (*Thymus vulgari*) at four different levels (i.e. 150, 300 or 450 mg/L). All the plant extracts were supplied from a local provider (Avasina Co., Hamedan, Iran). Plant parts were harvested and sun-dried for 1 week. The plant extracts were obtained by infusing dried plants in boiling water at 100 °C (200 g of herb: 1 L of water, w/v) for 10 min, then cooled at room temperature and squeezed to obtain plant extracts. Water and feed (mash form) were offered *ad libitum* through automatic drinker and pan feeder. Ingredient, chemical composition and metabolisable energy of the starter, grower and finisher diets are shown in Table 1.

Growth performance and carcass characteristics

Body weights and feed intake (calculated as difference between offered feed and refused feed) were weekly recorded to determine average daily feed intake (ADFI), average daily weight gain (ADG) and gain to feed ratio (G:F) for each replicate within each treatment for the starter, grower, finisher periods and throughout the study (i.e. d 1 to d 42).

At slaughter (42 d of age), two male birds per replicate (10 birds per treatment) were carefully chosen on the basis of average BW of the group, weighed, and killed by cervical dislocation. The weights of the empty abdomen carcasses were recorded to estimate the eviscerated carcass yields. The relative weights of breast, drumsticks, wings, abdominal fat, heart, liver, spleen and bursa of Fabricius were calculated as a percentage of eviscerated carcass according to Shabani et al. (2015).

Blood serum analysis

On the last day of the experimental trial (d 42), blood samples (1.5 mL) were taken from a wing vein of three randomly chosen male birds for replicate, collected into EDTA tubes, and centrifuged (Rotofix 32A centrifuge, Hettich, Westphalia, Germany; 1500 × *g* for

Table 2. Effects of different plant extracts at various dietary levels (0, 150, 300 and 450 mg/L of water) on broiler chicken growth performance.

Item	0, control[a]	150[b]	300[b]	450[b]	Common nettle[b]	Coriander[b]	Dill[b]	Thyme[b]	√MSE	*p* Value: Treatment	*p* Value: Level	*p* Value: Treatment × level	Contrasts: Linear	Contrasts: Quadratic
ADFI, g														
d 0 to d 14	515	41.5	21.5	33.8	28.7	30.1	36.0	34.4	24.0	.818	<.05	.0751	0.316	<0.05
d 15 to d 28	1647	87.1	56.7	71.4	119	79.9	19.1	68.8	84.7	<.05	.529	.946	0.560	0.336
d 29 to d 42	3642	−124	−164	−164	26.1	−647	10.5	8.43	59.3	<.05	.0550	.691	<0.05	0.209
d 0 to d 42	5804	4.89	−86.2	−58.5	174	−538	65.5	112	114	<.05	<.05	.600	0.0860	0.0642
ADG, g														
d 0 to d 14	454	71.7	2.70	57.3	66.3	42.1	41.3	25.9	25.7	<.05	<.05	<.05	0.0800	<0.05
d 15 to d 28	854	−22.6	38.3	−59.4	−46.8	−27.6	−30.8	46.9	85.6	<.05	<.05	.810	0.181	<0.05
d 29 to d 42	1151	−146	−117	−107	−203	−189	−115	12.1	75.6	<.05	.246	.981	0.108	0.653
d 0 to d 42	2459	−97.3	−76.4	−109	−183	−175	−104	84.9	79.5	<.05	.425	.847	0.637	0.224
G:F														
d 0 to d 14	0.880	0.0667	−0.0279	0.0545	0.0794	0.0296	0.0216	−0.00617	0.0595	<.05	<.05	<.05	0.519	<0.05
d 15 to d 28	0.519	−0.0382	0.00581	−0.0555	−0.0603	−0.0387	−0.0239	0.00561	0.0493	<.05	<.05	.866	0.274	<0.05
d 29 to d 42	0.316	−0.0296	−0.0171	−0.0144	−0.0575	0.00546	−0.0322	0.00272	0.0251	<.05	.137	.978	0.0623	0.483
d 0 to d 42	0.424	−0.0166	−0.00576	−0.0140	−0.0429	0.0105	−0.0224	0.00639	0.0165	<.05	.108	.836	0.629	<0.05

ADFI: average daily feed intake; ADG: average daily gain; G:F: gain to feed ratio; MSE: mean square error.
Significance was considered at $p \leq .05$ and tendency was declared at $.05 < p \leq .10$.
[a]Average values obtained for control group.
[b]Data expressed as differences between unique control group and specific treatments.

10 min) to separate blood serum. Commercial laboratory kits (Pars Azmoon Co., Teheran, Iran; Gorlokh et al. 2016) were used to determine blood serum levels of uric acid, glucose, total protein, albumin, triglycerides, total cholesterol and high-density lipoprotein (HDL).

Immune responses

All the birds were vaccinated against avian influenza (AI; at d 1), infectious bronchitis (strain H120; at d 1 and 16), Newcastle disease (ND, strain *Viscerotropic velogenic*; at d 8 and 20) and Gumboro disease (at d 14 and 23) through administration of the vaccines via drinking water after a 3 h period of water removal.

Blood samples were taken from the brachial vein (three randomly chosen birds per replicate) and pooled per replicate. After incubation for 1 h at room temperature, blood serum was separated by centrifugation (as above) and chilled at −20 °C until analysis. Hemagglutination-inhibition test (Cunningham 1971) was used to measure antibody titres (IgG2) against ND within 1, 35 and 42 days and against AI within 28 and 42 days.

At d 14 and 35, two birds per replicate, with weights similar to the replicate average, were vaccinated against sheep red blood cells (SRBCs), through subcutaneous administration of SRBC suspension in 5% PBS, in order to evaluate the systemic antibody response (Dibaji et al. 2015). At d 28 and 42, blood samples were collected and pooled per replicate to determine total Ig against SRBC using a hemagglutination assay in serum 7 as previously described by Sigolo, Deldar, et al. (2019), Sigolo, Khazaei, et al. (2019) and Sigolo et al. (2017).

Ileal microflora

The determination of the ileal microflora was conducted on three birds/replicate according to Dibaji et al. (2014). Briefly, agar plates were streaked on the site with ileal content to determine the bacterial growth and colony counts. The culture media were prepared and poured into the Petri dish 24 hours before sample collection. The collection tubes, previously wrapped in an aluminium sheet and autoclaved for 10 min, were empty with the samples and shaken for about 30 min to isolate bacteria from gastrointestinal contents and suspension preparation. An aliquot of each resulting suspension (1 mL) was collected and mixed with 9 mL PBS in the other tube. Lactobacilli bacteria were incubated at 37 °C under anaerobic

Table 3. Effects of different plant extracts at various dietary levels (0, 150, 300 and 450 mg/L of water) on broiler chicken carcass traits, abdominal fat and anatomical parts, and organs related to immune system.

Item	0, control[a]	150[b]	300[b]	450[b]	Common nettle[b]	Coriander[b]	Dill[b]	Thyme[b]	√MSE	*p* Value: Treatment	Level	Treatment × level	Contrasts: Linear	Quadratic
Carcass traits														
Pre-slaughtered body weight, g	2424	162	220	90.5	204	220	90.5	204	332	.715	<.05	.384	<0.05	<0.05
Eviscerated carcass weight, g	1884	72.5	50.4	221	16.0	19.5	223	200	238	<.05	.0574	.208	0.0545	0.146
Carcass yield, %	78.2	−0.935	1.91	−3.16	−4.53	−6.09	6.10	1.59	6.46	<.05	.0500	.530	0.280	<0.05
Abdominal fat and anatomical parts[c], %														
Abdominal fat	0.769	0.00107	0.135	−0.0241	0.0705	−0.00450	0.0229	0.0600	0.268	.862	.143	.647	0.768	0.0521
Breast	28.0	−2.56	−2.75	20.2	−3.08	−3.01	−3.97	29.9	36.6	<.05	.0854	<.05	0.0554	0.255
Drumsticks	24.1	−0.899	−0.400	−3.23	−1.82	−2.11	−0.70	−1.41	2.01	.261	<.05	<.05	<0.05	<0.05
Wings	6.18	−0.534	−0.639	−0.713	−0.763	−0.799	−0.48	−0.468	0.754	.486	.755	.376	0.458	0.940
Organs related to immune system[c], %														
Heart	0.542	−0.0294	0.00197	−0.00519	−0.0169	−0.0354	0.0119	−0.00310	0.0662	.259	.302	.296	0.254	0.294
Liver	2.40	1.55	−0.446	−0.527	−0.236	−0.394	−0.698	2.10	3.07	.0610	.0626	<.05	<0.05	0.260
Spleen	0.134	−0.0116	0.0128	0.000826	−0.0000127	−0.00614	0.0254	−0.0165	0.0271	<.05	<.05	<.05	0.152	<0.05
Bursa of Fabricius	0.272	−0.0719	−0.0264	−0.0498	−0.0376	−0.0396	−0.0459	−0.0742	0.0512	.192	<.05	.0524	0.178	<0.05

MSE: mean square error.
Significance was considered at $p \leq .05$ and tendency was declared at $.05 < p \leq .10$.
[a]Average values obtained for control group.
[b]Data expressed as differences between unique control group and specific treatments.
[c]Relative weights calculated as a percentage of eviscerated carcass.

conditions for 72 h whereas total aerobic bacteria were incubated at 37 °C under aerobic conditions for 48 h. The bacteria count was done using a colony counter. The bacterial counts were expressed as the logarithm number of bacteria per gram of sample.

Statistical analysis

Differences between unique control group and specific treatments were analysed according a completely randomised design with a four plant extracts (i.e. common nettle, coriander, dill or thyme)×three doses (i.e. 150, 300 and 450 mg/L of water) factorial arrangement by using GLM procedure of SAS (version 9.2; Institute Inc., Cary, NC). The main tested effects in the model were plant extracts, doses and their first order interaction. When plant extracts or first order interaction resulted significant for a $p \leq .05$, lsmeans were post hoc compared by using Tukey's test by using LSMEANS option. The experimental unit was the group. When the dose effect resulted significant at a $p \leq .05$ and the first order interaction was not significant, orthogonal contrast were tested to verify linear or quadratic effects of dose levels in specific parameter difference. The coefficients adopted in orthogonal contrasts were generated by using IML procedure of SAS (version 9.2; Institute Inc., Cary, NC). In the tables, the value of each tested specific parameters was reported to obtain indication about differences.

Results

Growth performance

The effects of different plant extracts at various dietary levels on broiler chicken growth performance are shown in Table 2. In the starter period (i.e. d 0 to d 14), a treatment × level interaction effect was observed on ADFI (tendency, $p = .0751$), ADG and G:F ($p < .05$). Compared with control, the addition of plant extracts to the broiler chicken diet tended to increase ADFI. In particular, ADFI tended to be the highest for thyme and dill treatments at 450 mg/L level (compared with control, differences of 48.7 and 45.7 g, respectively) and coriander treatment at 150 mg/L level (47.3 g difference), and the lowest for dill, thyme or coriander treatments at 300 mg/L level (26.5, 12.8 and 10.3 g differences) and for common nettle treatment at 450 mg/L level (8.26 g difference). The highest ADG and G:F were obtained with common nettle at 450 mg/L level (compared with control, differences of 91.0 g and 0.162, respectively, for ADG and G:F) followed by dill (82.0 g and 0.0949 differences,

Table 4. Effects of different plant extracts at various dietary levels (0, 150, 300 and 450 mg/L of water) on blood serum parameters of broiler chickens.

										p Value			Contrasts	
Item	0, control[a]	150[b]	300[b]	450[b]	Common nettle[b]	Coriander[b]	Dill[b]	Thyme[b]	√MSE	Treatment	Level	Treatment × level	Linear	Quadratic
Glucose, mg/dL	192	8.80	6.75	5.40	23.5	6.60	−9.47	7.27	17.7	<.05	.830	.548	0.546	0.943
Total cholesterol, mg/dL	143	−16.6	−7.15	−10.7	−8.67	−21.1	−14.1	−1.87	17.1	<.05	.222	.112	0.279	0.174
Triglycerides, mg/dL	102	−11.8	−8.25	−1.15	−12.6	−12.1	−6.20	2.60	20.1	.149	.242	.115	0.100	0.748
HDL, mg/dL	72.6	7.30	12.0	10.8	14.7	6.80	5.47	13.1	10.6	<.05	.353	.856	0.306	0.308
Total protein, g/dL	4.16	−0.690	−0.680	−0.500	−0.873	−0.920	−0.260	−0.440	0.354	<.05	.173	.100	0.097	0.386
Urea, mg/dL	4.52	−0.850	−1.04	−1.07	−0.547	−1.33	−0.813	−1.24	0.737	<.05	.610	.444	0.361	0.703
Albumin, mg/dL	2.06	−0.460	−0.460	−0.370	−0.500	−0.533	−0.300	−0.387	0.241	<.05	.402	<.05	0.24	0.499

HDL: high-density lipoprotein; MSE: mean square error.
Significance was considered at $p \leq .05$ and tendency was declared at $.05 < p \leq .10$.
[a]Average values obtained for control group.
[b]Data expressed as differences between unique control group and specific treatments.

respectively, for ADG and G:F), coriander (80.0 g and 0.0718 differences, respectively, for ADG and G:F) or thyme (55.0 g and 0.0380 differences, respectively, for ADG and G:F) at 150 mg/L, whereas the lowest ADG and G:F were obtained with plant extracts at 300 mg/L level.

In the grower period (i.e. d 15 to d 28), a treatment effect ($p < .05$) was found on ADFI. Compared with control, the addition of plant extracts to the broiler chicken diet increased ADFI. In particular, common nettle treatment resulted in the highest ADFI followed by coriander and thyme treatments. Treatment ($p < .05$) and level (quadratic, $p < .05$) effects were observed on ADG and G:F. Compared with control, thyme treatment resulted in higher ADG and G:F whereas the other plant extract treatments resulted in lower ADG and G:F. However, the highest ADG and G:F were observed when plant extracts were administered at 300 mg/L level.

In the finisher period (i.e. d 29 to d 42), a treatment ($p < .05$) and level (tendency, $p = .0550$) effect was found on ADFI. Compared with control, coriander treatment reduced ADFI whereas common nettle treatment increased ADFI followed by dill and thyme treatments. However, ADFI tended to decrease (linear, $p < .05$) by increasing the supplementation level of plant extract. A treatment effect ($p < .05$) was obtained on ADG and G:F. Compared with control, common nettle, coriander and dill resulted in lower ADG. Control group showed higher G:F than common nettle and dill groups.

Throughout the study (i.e. d 0 to d 42), treatment and level effects ($p < .05$) were found on ADFI. Compared with control, common nettle treatment increased ADFI followed by thyme and dill treatments whereas coriander treatments reduced ADFI. The ADFI decreased (quadratic, $p = .0642$) through 300 and 450 mg/L level of plant extracts. A treatment effect ($p < .05$) was observed on ADG and G:F. The highest ADG and G:F were obtained adding thyme to the broiler chicken diet.

Carcass characteristics

The effects of different plant extracts at various dietary levels on broiler chicken carcass traits and relative weights of abdominal fat, anatomical parts and organs related to immune system are shown in Table 3.

A level effect (quadratic, $p < .05$) was observed on pre-slaughtered BW. Pre-slaughtered BW enhanced by increasing the level of plant extracts in the broiler chicken diet from 0 up to 300 mg/L. Treatment and level effects were found on eviscerated carcass weight (treatment, $p < .05$; level, tendency $p = .0574$; linear, $p = .0545$) and carcass yield (treatment, $p < .05$; level, $p = .0500$; quadratic, $p < .05$). The addition of dill to the broiler chicken diet resulted in the highest eviscerated carcass weight and carcass yield followed by thyme. However, eviscerated carcass weight tended to enhance by increasing the level of plant extracts in the broiler chicken diet whereas the highest carcass yield was obtained with 300 mg/L level of plant extracts.

A treatment × level interaction effect was found on relative weight of breast ($p < .05$), drumsticks ($p < .05$), liver ($p < .05$), spleen ($p < .05$) and bursa of Fabricius ($p = .0524$). The addition of thyme to the broiler chicken diet at 450 mg/L level resulted in the highest relative weight of breast (compared with control, difference of 90.1 points %), whereas the addition of the other three plant extracts resulted in lower relative weight of breast compared with the control. The plant extracts in broiler chicken diet reduced the relative weight of drumsticks except for dill and thyme both at 300 mg/L level that resulted in higher relative weight of drumsticks than control (differences of 1.75

Table 5. Effects of different plant extracts at various dietary levels (0, 150, 300 and 450 mg/L of water) on broiler chicken immune responses.

										p Value			Contrasts	
Item	0, control[a]	150[b]	300[b]	450[b]	Common nettle[b]	Coriander[b]	Dill[b]	Thyme[b]	√MSE	Treatment	Level	Treatment × level	Linear	Quadratic
ND within 1 d (IgG2)	6.40	−0.750	−0.150	−0.650	−0.267	−0.533	−0.600	−0.667	1.12	.778	.204	.934	0.779	0.0797
ND within 35 d (IgG2)	3.80	0.750	1.10	0.900	1.07	0.533	1.20	0.867	1.18	.448	.646	.643	0.690	0.400
ND within 42 d (IgG2)	7.20	−1.65	−1.00	−1.45	−1.67	−0.733	−1.67	−1.40	1.60	.342	.426	.750	0.694	0.215
SRBC at d 28 (Total Ig)	4.00	−0.950	0.400	−0.500	−1.20	−0.733	1.47	−0.933	2.20	<.05	.152	.469	0.520	0.0676
SRBC at d 28 (IgG)[c]	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SRBC d 42 (total Ig)	5.60	1.10	1.05	0.600	1.07	−0.0667	1.53	1.13	2.28	.264	.748	.255	0.491	0.750
SRBC d 42 (IgG)	–	0.500	0.500	0.600	0.400	0.133	1.20	0.400	1.13	.0698	.949	.587	0.781	0.872
AI within 28 d (IgG2)	9.00	−0.200	0.250	0.100	0.267	0.133	0.200	−0.400	1.22	.434	.502	.628	0.442	0.376
AI within 42 d (IgG2)	7.60	−0.300	−0.250	−0.350	−0.400	−0.200	−0.333	−0.267	0.438	.631	.772	<.05	0.720	0.535

AI: avian influenza; MSE: mean square error; ND: Newcastle disease; SRBCs: sheep red blood cells.
Significance was considered at $p \leq .05$ and tendency was declared at $.05 < p \leq$%.10.
[a]Average values obtained for control group.
[b]Data expressed as differences between unique control group and specific treatments.
[c]IgG absent.

and 0.353 points %, respectively). Compared with control, only the addition of 150 mg/L of thyme to the broiler chicken diet increased the relative weight of liver (difference of 7.27 points %). The relative weight of spleen was increased administering dill at 300 or 450 mg/L (compared with control, differences of 0.0589 and 0.0346 points %, respectively) and in minor measure, adding coriander at 300 mg/L (difference of 0.00574 points %) and common nettle at 150 or 300 mg/L (differences of 0.00193 and 0.00342 points %, respectively). The use of plant extracts in broiler chicken diet reduced the relative weight of bursa of Fabricius and specially thyme at 450 mg/L level (compared with control, difference of −0.120 points %) followed by thyme and dill at 150 mg/L level (differences of −0.0859 and −0.0826 points %, respectively).

Blood serum parameters

The effects of different plant extracts at various dietary levels on blood serum parameters are shown in Table 4.

A treatment effect was observed on blood serum contents of glucose ($p < .05$), total cholesterol ($p < .05$), HDL cholesterol ($p < .05$), total protein ($p < .05$) and urea ($p < .05$). Compared with control, common nettle treatment followed by thyme and coriander treatments increased levels of glucose and HDL cholesterol. The supplementation with plant extracts of the broiler chicken diet decreased blood serum contents of total cholesterol, total protein and urea. A treatment × level interaction effect ($p < .05$) was found on albumin level. The addition of plant extracts in the broiler chicken diet decreased albumin content specially coriander at 450 mg/L level (compared with control, −0.640 mg/dL difference) followed by coriander and common nettle both at 150 mg/L level (–0.620 mg/dL difference), and dill at 300 mg/L level (–0.560 mg/dL difference).

Immune responses

The effects of different plant extracts at various dietary levels on broiler chicken immune responses are shown in Table 5.

A treatment effect was observed on total Ig against SRBC at d 28 ($p < .05$). In particular, the broiler chicken antibody response was higher for dill treatment compared with all the other treatments. The addition of plant extracts in the broiler chicken diet, especially dill followed in equal measure from common nettle and thyme, tended to increase the IgG against SRBC at d 42 (treatment effect, $p = .0698$). A treatment × level interaction effect ($p < .05$) was found on antibody response against AI within 42 d from the vaccination. Compared with control, all the plant extract treatments decreased IgG2 titres except for coriander at 450 mg/L which increased them (0.200 difference).

Ileal microflora

The effects of different plant extracts at various dietary levels on broiler chicken ileal microflora are shown in Table 6.

A treatment × level interaction effect ($p < .05$) was found on lactobacilli, coliform and negative gram bacteria. Compared with control, thyme treatment at 150 mg/L level increased lactobacilli bacteria (compared with control, difference of 1.17 log10 CFU/g) followed by dill and common nettle treatments at 300 mg/L level (differences of 0.912 and 0.730 log10 CFU/g, respectively). Plant extract treatments resulted in lower coliform counts than control treatment

Table 6. Effects of different plant extracts at various dietary levels (0, 150, 300 and 450 mg/L of water) on broiler chicken ileal microflora (log10 CFU/g).

Item	0, control[a]	150[b]	300[b]	450[b]	Common nettle[b]	Coriander[b]	Dill[b]	Thyme[b]	√MSE	*p* Value: Treatment	*p* Value: Level	*p* Value: Treatment × level	Contrasts: Linear	Contrasts: Quadratic
Lactobacilli bacteria	5.63	0.207	−0.00650	−0.114	−0.342	−0.0673	0.653	−0.129	0.520	<.05	.151	<.05	0.0572	0.713
Aerobic bacteria	7.62	−0.778	−0.947	−0.590	−0.697	−1.46	0.226	−1.16	0.909	<.05	.468	.0775	0.516	0.296
Coliform bacteria	7.40	−0.917	−0.945	−0.744	−1.79	−1.35	−0.273	−0.0633	0.493	<.05	.383	<.05	0.271	0.401
Negative gram bacteria	6.33	−0.0950	−0.899	−1.37	−0.772	−1.17	−0.200	−1.02	0.341	<.05	<.05	<.05	<0.05	0.0847

MSE: mean square error.
Significance was considered at $p \leq .05$ and tendency was declared at $.05 < p \leq .10$.
[a]Average values obtained for control group.
[b]Data expressed as differences between unique control group and specific treatments.

except for dill at 300 mg/L level (difference of 0.696 log10 CFU/g). Negative gram bacteria were decreased through the addition of plant extracts in the broiler chicken diet except for dill and thyme treatments both at 150 mg/L level (differences of 1.08 and 0.368 log10 CFU/g, respectively). A treatment effect ($p < .05$) was observed on aerobic bacteria. Compared with control, only dill treatment slightly increased aerobic bacteria in broiler chicken ileal microflora.

Discussion

The results obtained on broiler chicken growth performance showed that the addition of common nettle, dill or thyme to the broiler chicken diet positively affected dietary intake in all three growth periods (i.e. starter, grower and finisher) and throughout the study (i.e. d 0 to d 42 period). Conversely, coriander decreased feed intake in the finisher period and this decrement was reflected throughout the study. The spices and their extracts are a source of phytogenic components which affect organoleptic properties of feed, such as flavour and palatability, stimulating dietary intake in poultry (van der Aar et al. 2017). For the same herbs tested in the current study, the literature reports conflicting results about their effects on broiler chicken feed intake. No effect on ADFI of heat stressed broiler chickens (Behboudi et al. 2016) or a depressing effect on ADFI of broiler chickens under normal environmental conditions (Hashemipour et al. 2013) have been reported for thyme. Authors found increased ADFI in broiler chickens fed diets added with coriander (Naeemasa et al. 2015; Taha et al. 2019), whereas other ones found no effect on ADFI in broiler chickens fed diets containing common nettle (Keshavarz et al. 2014) or dill (Vispute et al. 2019). However, these discrepancies could depend on the different experimental conditions such as administered herb form (e.g. seed powder, essential oil or alcoholic extract), supplementation dosage, concentration of bioactive molecules in herbs, breeding environmental conditions (heat stress condition or not) (Attia and Al-Harthi 2015; Attia, Al-Harthi, et al. 2017; Attia, Bakhashwain, et al. 2017; Attia et al. 2018).

Concerning weight gain and dietary efficiency results, although in the starter period broiler chickens receiving the diet added with common nettle extract at 450 mg/L level achieved the highest weight gains and feed efficiencies, throughout the study broilers chickens receiving the diets containing thyme extract, grew more and had higher feed efficiency compared with broiler chickens of the other experimental groups. In particular, the positive effects of thyme extract both on ADG and G:F were observed in the grower period at administering level of 300 mg/L, and on ADG in the finisher period regardless of the supplementation level. The digestive tract of the broiler chickens plays an important role on growth performance as it is involved in the nutrient adsorption process (Giannenas et al. 2018). The positive effects of thyme extract on broiler chicken growth performance could be ascribed to the antioxidants and phenolic substance present in thyme such as thymol and carvacrol (Attia, Al-Harthi, et al. 2017; Attia, Bakhashwain, et al. 2017). The latter, thanks their antibacterial activity, can reduce the harmful bacterial populations in the gastrointestinal tract and improve nutrient adsorption (Hernández et al. 2004; Ragaa et al. 2016). Additionally, carvacrol and thymol seem to stimulate digestive and pancreatic secretions improving nutrient digestion and utilisation (Lee et al. 2003; Hernández et al. 2004). Our findings obtained on weight gain and feed efficiency of broiler chickens fed the diets added with thyme extract were in agreement with those obtained by some authors (Cross et al. 2007; Hashemipour et al. 2013; Ragaa et al. 2016; Attia, Bakhashwain, et al. 2017) but in disagreement with those observed by other ones (Cross et al. 2003; Ragab et al. 2013; Behboudi et al. 2016).

The results of carcass traits showed that the supplementation of the broiler chicken diets with plant extracts at 300 mg/L level can increase pre-slaughtered BW. However, only dill or thyme extracts at 300 mg/L level improved the carcass and drumstick yields. Furthermore, thyme extract at 450 mg/L level increased the breast yield. The beneficial effects of thyme extract observed on broiler chicken growth performance, probably due to the activity of the substances which it contains (i.e. thymol and carvacrol), as mentioned above, were also confirmed by the data of broiler chicken carcass traits in accordance with Mansoub and Myandoab (2011) and Ragaa et al. (2016). Moreover, in the current study, the thyme treatment at 150 mg/L level increased relative weight of liver. Anyway, increased liver antioxidant enzyme activities in broiler chickens fed diets added with thymol and carvacrol have been reported by Hashemipour et al. (2013).

Concerning dill, this plant contains mainly carron and limonene which make more than 90% of the extract (Sintim et al. 2015) and is widely used in traditional medicine to treat gastrointestinal disorders (Hosseinzadeh et al. 2002; Abd El-Hack et al. 2020). Furthermore, it is known for its strong antioxidant potential (Satyanarayana et al. 2004). According to Rahimian et al. (2017) dill, like thyme, could improve digestive process and digestibility of feed. However, studies conducted on broiler chickens fed diet supplemented with dill reported no relevant effect of dill on carcass traits (Bahadori et al. 2013; Rafiei-Tari et al. 2016; Vispute et al. 2019).

In the current study, the supplementation of the broiler chicken diet with plant extracts, regardless of the treatment, positively affected blood serum parameters decreasing the concentrations of total protein, albumin, urea and total cholesterol. Serum protein levels are important for preserving the immune system and can increase under disease and stress conditions, such as toxicity (Tekce and Gül 2017). In birds, serum total protein consists mainly of albumin and globulin (Scholtz et al. 2009). Thus, high total protein levels are accompanied by high serum concentrations of albumin and globulin and vice versa (Sigolo, Deldar, et al. 2019). Urea and uric acid are protein metabolites and are useful indicators of nitrogen utilisation. In birds, a decreased serum concentration of urea and/or uric acid is related to an increased amino acid incorporation into tissue muscle proteins (Donsbough et al. 2010). In accordance with our results, the beneficial role of herb supplementation on serum cholesterol levels and distribution of cholesterol lipoproteins, from low density lipoproteins to HDL, has been reported (Abd El-Hack et al. 2020). However, our findings showed that only dill treatment positively affected serum glycaemia value. In birds, serum glucose concentration is a stress biochemical indicator. The glucocorticoids, produced under stress conditions, promote gluconeogenesis from muscle tissue proteins raising serum glucose level (Tawfeek et al. 2014).

Immune-stimulating properties, as well as protective capacity of the gastrointestinal tract, have been attributed to phytogenics (Alp et al. 2012; Kim, Lillehoj, Lee, Jang, Lillehoj, et al. 2013; Kim, Lillehoj, Lee, Jang, Park, et al. 2013; Attia and Al-Harthi 2015; Attia, Al-Harthi, et al. 2017; Attia, Bakhashwain, et al. 2017; Attia et al. 2018). Nevertheless, the action mechanisms of phytochemicals, the active constituents in phytogenics, are still unclear. In our study, dill treatment increased broiler chicken immune response against SRBC, whereas coriander treatment at 450 mg/L level raised immune response against AI within 42 d from the vaccination in disagreement with Hosseinzadeh et al. (2014). However, there is still a lack of information about the effects of herb feed additives on broiler chicken immune response. In the ileal tract, plant extracts reduced coliforms (except dill at 300 mg/L level) and aerobic bacteria (except dill regardless of the level). The negative gram bacteria were increased by thyme and dill treatments both at 150 mg/L level, whereas lactobacilli bacteria were specially raised by thyme at 150 mg/L level. A microbiota changes to higher lactic acid bacteria populations can determine acidification of the gastrointestinal tract promoting the growth and multiplication of beneficial bacteria, such as the lactic acid bacteria. The latter, by producing short-chain fatty acids, increase gastrointestinal acidity preventing the growth of unfavourable microorganisms, such as *Salmonella spp*. and coliforms (Giannenas et al. 2018). In agreement with our findings, Tiihonen et al. (2010) found that a dietary blend of essential oils including thymol when fed to broiler chickens increased the proportions of *Lactobacillus* and *Escherichia coli* (negative gram bacterium) in caecal microbiota. Higher *Lactobacillus* and *Bifidobacterium* counts but lower *Escherichia coli* levels in the ileum of broiler chickens fed diet supplemented with thyme essential oil were reported by Khaksarzareha et al. (2012).

Conclusions

In conclusion, taking into account all the results of the trial, it emerged that among the tested plant extracts at different levels, dietary supplementation with thyme at 300 mg/L level could concurrently improve broiler chicken growth performance in terms of feed intake, gain weight and feed efficiency as well as carcass traits in terms of carcass and drumsticks yields, and blood serum parameters such as total protein, albumin, urea, total cholesterol and HDL. Furthermore, dietary addition with thyme extract at 150 mg/L could positively affect ileal microbiota increasing lactobacilli bacteria and reducing coliforms. No relevant effects of the thyme treatment were observed on broiler chicken immune responses. Conversely, the dill treatment improved broiler chicken immune response against SRBC. Due to possible synergistic effects among different herbal extracts, future research is needed to study if the combined use of plant extracts could be more advantageous than the individual use of herb extracts.

Ethical approval

The experimental protocol was authorised by the Institutional Animal Care and Ethics Committee of Islamic Azad University (Rasht Branch, Rasht, Iran).

Disclosure statement

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the Rasht Branch, Islamic Azad University [Grant number 17.16.4.18418].

ORCID

Samantha Sigolo http://orcid.org/0000-0001-9081-0565
Chrysostomos Milis http://orcid.org/0000-0002-5274-8155
Alireza Seidavi http://orcid.org/0000-0002-1903-2753
Antonio Gallo http://orcid.org/0000-0002-4700-4450
Giulia Ferronato http://orcid.org/0000-0001-9153-8540
Aldo Prandini http://orcid.org/0000-0002-8650-8766

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