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PAPER

Ajwain (*Trachyspermum copticum*) extract in broiler diets: effect on growth performance, carcass components, plasma constituents, immunity and cecum microflora

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ABSTRACT

Hypothesis is that dietary ajwain (*Trachyspermum copticum* L.), a phytogenic feed additive, could improve the performance of broiler chickens. On a total of 200 Ross 308 male broilers, during the 42-d growing period (starter: day 1–14; grower: day 15–28 and finisher: day 29–42), varying levels of ajwain alcoholic extract (0, 150, 350 and 450 ppm) were added to the drinking water. A control treatment containing the Virginiamycin antibiotic was also studied. Within each treatment and period, the performances were evaluated and the Economic index calculated. On day 42, two birds per pen were killed and the carcase and organ weights determined. On day 42, on two birds per pen, blood samples were collected on the farm to study the serum parameters. On birds, vaccinated against sheep red blood cells at 14 and 35 days, blood samples were collected at 21 and 42 days of age to determine total Ig, IgG and IgM antibodies and, at day 42, the spleen and bursa of Fabricius were weighed. The microflora on the caecum content was counted. Ajwain extract significantly ($p < .05$) influenced productive performances, blood parameters, immune parameters and gut microbiota, indicating its possible role as a substitute for antibiotics in a sustainable poultry industry.

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Poultry diet; ajwain extract; performance; blood and immunity parameters; caecum microflora

Introduction

There is great interest in animal nutrition in the study of additives aimed at improving the animal health and performance. Among the additives defined as nutraceuticals, plant extracts and their essential oils can represent a valid alternative to synthetic additives (Shim et al. 2010; Apajalahti and Vienola 2016; Xue et al. 2020; Nooreh et al. 2021). One of these nutraceuticals is ajwain (*Trachyspermum copticum* L.) (Mahboubi and Kazempour 2011; Mabood et al. 2017).

Ajwain, despite its unique English name, is often confused with other plants such as celery, lovage, nigella or with non-culinary plants (goutweed, toothpick weed).

The parts of the plant used are the small caraway-like fruits. These are sometimes mislabelled as lovage seed, although they are not at all.

Ajwain is characterised, among its main components, by the essential oil (from 2.5 to 5% in dried fruit) rich in thymol (2-isopropyl-5-methylphenol, from 35 to 60%) and in smaller quantities, α-pinene, p-cymene, limonene and γ-terpinene. Isothymol (50%) is mainly represented in the essential oil obtained from the flowers and leaves of the ajwain grown in Algeria, followed by p-cymene, thymol, limonene and γ-terpinene. However, it is doubtful that the name isothymol refers to the compund 2-isopropyl-4-methylphenol or 3-isopropyl-6-methylphenol (carvacrol) (Chialva et al. 1993).

As there is no report on its effects on broilers, this study tested the hypothesis that feeding ajwain could affect broiler performance during a 42-day production cycle. In particular, the aim of this study was to evaluate *in vivo* and *post-mortem* performance, blood biochemistry, immunity and microflora of the caecum, in relation to the different concentrations of Ajwain extracts in the broiler diet.

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Materials and methods

This study was carried out on a commercial poultry farm in Guilan, Iran. The experimental protocol was approved by the Animal Ethic Committee of the Islamic Azad University (approval number 971218), and the experiment was performed according to the International Guidelines (Directive 2010/63/EU 2010).

A total of 200 Ross 308 male broiler chicks were purchased and reared at thermoneutral environment temperature in accordance with the standard hatching practice for bird breeding stages (Aviagen 2002). The breeding area was illuminated for 24 hours for the 1st day; while, in the following days, for 23 hours every day. Broiler diets were formulated for 3 growth stages (starter: day 1 to day 14; grower: day 15 to day 28 and finisher: day 29 to day 42) (Table 1). The birds were vaccinated against influenza on day 1, infectious bronchitis on days 1 and 8, Gumboro on days 16 and 32, and Newcastle on days 1 and 8.

The ajwain alcoholic extract was purchased from the Institute for Medicinal Plants Research of Academic Jihad (Karaj, Iran) (http://imp.acecr.ac.ir) and added to the water.

Two hundred birds were assigned to 20-floor pens (1.0 m × 1.0 m) containing 10 birds each. The 20-floor pens were divided into 5 groups in relation to each treatment and each treatment was represented by four replicates. The birds in the four groups received four different supplementations of ajwain extract (0, 150, 300 and 450 ppm) in their drinking water for each group; birds of the fifth group (40 animals) received treatment with the antibiotic Virginiamycin of 0.2 g/kg. The distribution of birds (200 animals) per group (5 treatments) and per replicate (4 replicates) was based on a completely randomised design. Feed and water supply were *ad libitum* during the 42-day growing period.

During the starter period, grower period, finisher period, and whole study period (day 1–42), the body weight and feed intake (calculated by the difference of offered feed and refused feed) were measured (data not shown), and the average daily feed intake, average daily weight gain and gain to feed ratio were calculated.

Economic index was calculated using the following formula:

$$EI = \frac{[\text{bodyweightat}42^{nd}\text{dayofage(gr)} \times 10]}{[\text{feedconversionratiofrom}1^{st} - 42^{nd}\text{daysofage} \times 42]}$$

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

Treatment	Starter (from 1 to 14 d)	Grower (from 15 to 28 d)	Finisher (from 29 to 42 d)
Ingredients (%, on fed basis)			
Corn	54.5	58.5	62.7
Soybeanmeal	37.5	33.2	29.5
Soybeanoil	4.0	4.0	4.0
Calcium carbonate	1.2	1.2	1.1
Dicalciumphosphate	1.6	1.5	1.5
NaCl	0.23	0.26	0.25
Vitamin and mineral premix[a]	0.6	0.6	0.6
Sodiumbicarbonate	0.12	0.14	0.1
DL-Methionine	0.18	0.21	0.15
L-Lysine	0.07	0.09	0.1
Chemical composition			
Metabolisable energy[b] (kcal/kg)	3010	3050	3100
Crude protein (%)	21.04	19.60	18.18
Lysine (%)	1.27	1.10	0.97
Methionine + Cystine (%)	0.94	0.84	0.76
Methionine (%)	0.47	0.42	0.36
Arginine (%)	1.31	1.14	1.02
Tryptofan (%)	0.20	0.18	0.16
Calcium (%)	1.05	0.90	0.85
Availablephosphorus (%)	0.50	0.45	0.42
Maganesium (%)	0.05	0.06	0.05
Sodium (%)	0.21	0.19	18.18
Chloride (%)	0.17	0.17	0.16
Potassium (%)	0.50	0.40	0.40
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)	120.0	120.0	120.0
Selenium (mg/kg)	0.30	0.30	0.30
Zinc (mg/kg)	100.0	100.0	100.0
Vitamin A (IU/kg)	11000	9000	9000
Vitamin E (IU/kg)	75	50	50
VitaminK (mg/kg)	3	3	2
VitaminB12 (mg/kg)	0.016	0.016	0.010
Riboflavin (mg)	8	6	5

[a]Amount per kg: vitamin A, 5,000 IU; vitamin D_3, 500 IU; vitamin E, 3 mg; vitamin K_3, 1.5 mg; vitamin B_2, 1 mg; calciumpantothenate, 4 mg; niacin, 15 mg; vitamin B_6, 13 mg; Cu,3 mg; Zn, 15 mg; Mn, 20 mg; Fe, 10 mg; K, 0.3 mg.
[b]Metabolic availability (from UFFDA output; Pesti& Miller, 1993). Metabolizable energy was estimated using the Carpenter and Clegg equation.

At the end of the growth period (day 42), two bird per pen (totally 40 birds, 8 birds per each treatment) randomly selected, were weighed and killed by cervical dislocation. The weights of the whole defeathered carcase and of the carcase without head and drumsticks were recorded; then, after the removal of the viscera and abdominal fat, the carcase yield and the relative weights of the abdominal fat and of the anatomical parts (breast, drumsticks, wings, and neck) were calculated and expressed as a percentage of the eviscerated carcase (Golrokh et al. 2016). Furthermore,

at the end of the growth period (day 42), on 40 birds (two bird per replicate for a total of 8 birds per each treatment), randomly selected, the immune response was evaluated and, on the same animals, killed by cervical dislocation, the relative weights of organs related to the immune system function (spleen and bursa of Fabricius) were calculated (Shabani et al. 2015).

On day 42, on the farm, after 2 hours of fasting, before transfer to the slaughterhouse, on two bird per replicate (in total 40 birds, 8 birds per each treatment), blood samples (1.5 mL) were taken from the wing vein and collected into EDTA tubes.

The blood was centrifuged at 1500 g for 10 min using a Rotofix 32 A centrifuge (Hettich, Germany) and the sera were stored at −18 °C until the analysis. Levels of uric acid, total cholesterol, triglycerides, high density lipoprotein (HDL), glucose, total protein and albumin were determined using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran).

Forty birds (2 bird per replicate per treatment) of similar weight, at 14 and 35 days of age, were vaccinated against sheep red blood cells (SRBC) by subcutaneous administration of SRBC suspension in 5% PBS. At 21 and 42 days of age, total and the IgG antibodies against SRBC were evaluated on blood samples, taken from the wing vein and pooled by replication, in order to assess the systemic antibody response (Dibaji et al. 2015). All antibody titres were recorded as $\log_{10}$ of the maximum serum dilution which agglutinated an equal volume of a 0.5% SRBC suspension in PBS. The IgM titre was calculated from the difference between the total immunoglobulin (IgT) titre and the IgG titre.

The caecal microbiota was counted according to the method proposed by Dibaji et al. (2014). As the culture medium, de Man, Rogosa, Sharpe agar for the lactobacilli and MacConkey agar for coliforms were used. Bacteria were counted using a colony counter and the results expressed as the logarithm of the number of bacteria per gram of the sample.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) using the GLM procedure of IBM SPSS Statistics 21 software for Windows® (SPSS 1997) in a completely randomised design with a model containing treatment as the main effect. For growth parameters, the experimental unit was represented by the pen while, for carcase characteristics, blood constituents, immune response and caecal microflora, the experimental unit was the individual bird. The probability values of $p \leq .05$ were declared significant. When the treatment effect was significant, differences between treatment means were identified using Tukey's test.

Results

The results are summarised in Tables 2–5.

Table 2 shows the broiler production performance and the economic index. As regards the body weight, from the beginning to the end of the trial (1st–42nd days of age) the ajwain 300 ppm group showed the greatest ($p < .05$) weight gain ($p < .05$).

During the whole period of the trial, the ajwain 150 ppm group had the highest ($p < .05$) feed intake (Table 2) while, the Virginiamycin and the ajwain 450 ppm groups had the lowest ($p < .05$) values; the other groups showed intermediate values.

The Feed Conversion Rate (Table 2) during the whole period (1st–42th days of age), showed the best ($p < .05$) value in the ajwain 450 ppm group and the worst ($p < .05$) in the ajwain 150 ppm group.

As regards the economic index (Table 2), the effect of *Trachyspermum copticum extract* integration into the broiler diet during the total trial period (1st to the 42nd days of age) showed the highest ($p < .05$) values in the ajwain 450 ppm group and the lowest in Virginiamycin group.

As shown in Table 3, chickens fed the diet with 300 ppm of *Trachyspermum copticum* extracts showed significantly ($p < .05$) higher carcase weight, breast, drumsticks, abdominal fat, heart and liver weight values than those of the other groups. The ajwain 150 ppm group showed the highest ($p < .05$) weight of gizzard. Chickens fed the diet supplemented with the highest level of *Trachyspermum copticum* extracts (ajwain 450 ppm) showed lower ($p < .05$) values for

Table 2. Performance of Ross 308 broilers fed the different levels of *Trachyspermum copticum extract* during the whole period.

	Initial Body Weight (g)	Final Body Weight (g)	Feed intake (g/chick/period)	Feed conversion ratio	Economic index
Virginiamycin (0.2 g/kg)	53	2645^{a}	3658^{b}	1.54^{ab}	370^{b}
Ajwain (0 ppm)	54	2415^{ab}	3756^{bc}	1.52^{ab}	389^{ab}
Ajwain (150 ppm)	53	2460^{ab}	4037^{a}	1.61^{a}	373^{ab}
Ajwain (300 ppm)	53	2763^{a}	3959^{ab}	1.54^{ab}	399^{ab}
Ajwain (450 ppm)	54	2258^{b}	3682^{b}	1.47^{b}	407^{a}
P-value		0.049	0.011	0.045	0.043
SEM	0.58	60.37	46.61	0.02	5.56

[a,b,c]Mean values with different superscript letters in a column indicate significant differences at $p < .05$.

Table 3. Mean (±SEM) of carcase and organs weights (g) of Ross broilers at 42nd days fed different levels *Trachyspermum copticum extract* from 1st to 6th weeks of age.

	Carcase	Breast	Drumsticks	Wings	Abdominal fat	Heart	Liver	Gizzard
Virginiamycin (0.2 g/kg)	2150^{a}	710^{ab}	548^{a}	56.50	40.25^{bc}	12.25^{ab}	63.75^{a}	44.00^{ab}
Ajwain (0 ppm)	1988^{ab}	656^{bc}	508^{ab}	53.25	41.50^{bc}	10.25^{b}	56.25^{ab}	41.25^{b}
Ajwain (150 ppm)	2050^{ab}	663^{bc}	565^{a}	56.00	46.50^{ab}	10.50^{b}	44.00^{c}	54.75^{a}
Ajwain (300 ppm)	2240^{a}	780^{a}	606^{a}	56.00	66.50^{a}	13.75^{a}	61.00^{a}	42.25^{b}
Ajwain (450 ppm)	1858^{b}	586^{c}	450^{b}	54.75	34.25^{c}	12.50^{ab}	48.75^{bc}	50.25^{ab}
p-value	.047	.003	.027	.908	.0001	.042	.009	.049
SEM	46.33	18.42	17.21	1.09	2.88	0.47	2.24	1.83

^{a,b,c}Means within each column without common superscript have significant difference at $p < .05$.

Table 4. Mean (±SEM) of blood parameters (mg/dL) at 42nd days of age in Ross 308 broilers fed different levels of *Trachyspermum copticum extract* from 1st to 6th weeks of age.

	Glucose	Total cholesterol	Triglycerides	HDL	LDL	VLDL	Albumin	Total protein	Uric acid	LDL/HDL
Virginiamycin (0.2 g/kg)	211.50	150.00	81.00^{b}	61.25^{a}	75.00^{a}	16.25	1.69	4.86^{b}	5.20^{b}	0.83^{b}
Ajwain (0 ppm)	223.00	143.00	92.00^{ab}	61.00^{a}	62.10^{b}	18.02	1.55	3.48^{c}	5.69^{a}	0.99^{ab}
Ajwain (150 ppm)	222.25	145.50	113.00^{a}	60.75^{ab}	61.80^{b}	22.20	1.74	5.42^{a}	4.53^{c}	0.99^{ab}
Ajwain (300 ppm)	271.25	135.75	103.25^{ab}	57.75^{ab}	53.35^{b}	19.65	1.69	3.53^{c}	5.82^{a}	1.08^{a}
Ajwain (450 ppm)	223.00	136.25	100.25^{ab}	56.25^{b}	58.55^{b}	20.45	1.58	4.77^{b}	5.41^{ab}	0.98^{ab}
p-value	.396	.289	.023	.041	.006	.399	.400	.0001	.0001	.046
SEM	10.14	2.42	4.56	0.74	2.13	0.99	0.03	0.18	0.12	0.03

^{a,b,c}Means within each column without common superscript have significant difference at $p < .05$. HDL: High-Density Lipoproteins. LDL: Low-Density Lipoproteins. VLDL: Very Low-Density Lipoproteins.

Table 5. Mean (±SEM) of immunity parameters (log_{10}) against sheep red blood cells at different days of age, organ weights (g) related to immunity and caecum microflora parameters in Ross broilers at 42nd days fed different levels of *Trachyspermum copticum extract* from 1st to 6th weeks of age.

	28th day of age			42nd day of age			42nd day of age			
	IgG	IgM	IgT	IgG	IgM	IgT	Bursa of Fabricius	Spleen	Coliform bacteria (CFUg-1)	Lactobacillus spp (CFUg−1)
Virginiamycin (0.2 g/kg)	4.50^{a}	2.50^{b}	7.00^{ab}	4.00	5.50	9.50^{a}	4.00^{a}	4.50	2.42^{a}	1.03^{b}
Ajwain (0 ppm)	4.75^{a}	1.75^{c}	6.50^{bc}	4.50	4.75	9.25^{a}	3.25^{b}	3.00	2.00^{a}	2.27^{a}
Ajwain (150 ppm)	2.50^{c}	3.00^{b}	5.50^{c}	5.25	4.25	9.50^{a}	4.00^{a}	3.50	1.88^{a}	1.75^{ab}
Ajwain (300 ppm)	3.50^{b}	2.50^{b}	6.00^{bc}	3.75	4.50	8.25^{b}	4.00^{a}	3.25	0.87^{b}	1.31^{b}
Ajwain (450 ppm)	4.00^{ab}	4.00^{a}	8.00^{a}	4.00	5.00	9.00^{ab}	3.25^{b}	3.25	2.50^{a}	2.24^{a}
p-value	.0001	<.0001	.006	.503	.757	.056	.003	.244	.001	.007
SEM	0.21	0.19	0.26	0.28	0.30	0.16	0.10	0.22	0.72	0.15

^{a,b,c}Means within each column without common superscript have significant difference at $p < .05$.

live body weight, empty abdomen, carcase weight, breast, drumsticks and abdominal fat weights. The ajwain 150 ppm group exhibited the lowest ($p < .05$) hearth and weights. The other groups showed intermediate values.

Regarding blood parameters (Table 4), Triglycerides showed the highest ($p < .05$) content in ajwain 150 ppm group and the lowest ($p < .05$) value in Virginiamycin group; the HDL showed the highest ($p < .05$) level in ajwain 0 ppm and Virginiamycin groups while the lowest ($p < .05$) value was observed in the ajwain 450 ppm group; LDL showed the highest ($p < .05$) value in Virginiamycin group; the LDL/HDL ratio showed the highest value in the ajwain 450 ppm group and the lowest in the Virginiamycin group.

As regard the protein metabolism (Table 4), the total protein showed the highest ($p < .05$) value in the ajwain 150 ppm group and the lowest ($p < .05$) values in the ajwain 0 ppm and 300 ppm groups; on the contrary, the uric acid content was significantly ($p < .05$) higher in the ajwain 0 ppm and 300 ppm groups and significantly ($p < .05$) lower in the ajwain 150 ppm group than those in the other groups.

Glucose, Total Cholesterol, Very Low-Density Lipoprotein and Albumin were not affected by the integration of *Trachyspermum copticum* extracts into the chicken diet (Table 4).

Regarding the immunity parameters against sheep red blood cells (Table 5), during the first four weeks of age (from 1st to 4th week of age), IgG showed higher ($p < .05$) values in the Virginiamycin and ajwain 0 ppm groups and the lowest ($p < .05$) value in the ajwain 150 ppm group; IgM showed the highest ($p < .05$) value in the ajwain 450 ppm group and the lowest ($p < .05$) value in the ajwain 0 ppm group; finally, the IgT exhibited the highest ($p < .05$) value in the ajwain 450 ppm group and the lowest ($p < .05$) value in the ajwain 150 ppm group.

From the 4th to the 6th week of age, IgG and IgM were not affected ($p > .05$) by the integration of

Trachyspermum copticum extracts in the chicken diet while IgT showed higher ($p < .05$) values in the Virginiamycin, ajwain 0 ppm and 150 ppm groups and the lowest ($p < .05$) values in the ajwain 300 ppm group (Table 5).

Table 5 shows the organ (spleen and Bursa of Fabricius) weights relative to the immunity of broilers at the 42nd day of age. Spleen weight did not show significant ($p > .05$) differences among the treatments while, Bursa of Fabricius weight showed a significantly higher ($p < .05$) values in the Virginiamycin, ajwain 150 ppm and 300 ppm groups than those observed in the ajwain 0 and 450 ppm groups.

Finally, Table 5 shows some parameters of the caecal microflora (coliform bacteria and lactobacilli) in the broilers at the 42nd day of age. *Trachyspermum copticum* extracts affected both coliform bacteria, showing the lowest ($p < .05$) value in the ajwain 300 ppm group, and the *Lactobacillus* spp., showing significantly ($p < .05$) higher values in the ajwain 0 ppm and 450 ppm groups than those observed in the Virginiamycin and ajwain 300 ppm groups.

Discussion

The integration of *Trachyspermum copticum* extracts in broiler drinking water positively affected the weight gain and feed intake in accordance with the results reported in literature on the integration of plant extracts into the diet of broiler chickens (Alali et al. 2013; Hafeez et al. 2020). Khattak et al. (2014) reported an increase of BW and an improvement in FCR in the broiler chickens fed a diet containing a mixture of essential oils extracted from basil, ajwain, bay leaf, lemon, sage, and thyme.

Although it has been proposed that immunostimulation may have some negative effects on growth performance, due to the repartitioning of nutrients to synthesise antibodies and to develop immune organs, resulting in decreased nutrient availability for growth (Hevener et al. 1999; Takahashi et al. 2000), our study shows the positive effect of supplementing ajwain in the broiler diet, highlighting an immunostimulating effect, without any negative effect on growth performance. Performances are closely linked to gut health and microflora; the underdeveloped gastrointestinal tract and the microflora of chicks, in the first weeks after hatching, are often unable to successfully fight pathogenic organisms, as bacteria, such as *Escherichia coli, Salmonella* spp., *or Clostridium* spp., which proliferate much faster than natural strains of the intestinal microbiota (Adaszyńska-Skwirzyńska and Szczerbińska 2019). However, some natural extracts, by acidifying the intestinal environment, can reduce the populations of some pathogenic microbial species (Gopi et al. 2014) by influencing intestinal morphology. In fact, the reduction of pathogenic bacteria in the intestine can improve the capacity of epithelial cells in terms of regeneration of the intestinal villi. The result of these actions is an increase in the nutrients absorption (Choi et al. 2015; Zeng et al. 2015). According to these observations, in our study broiler chickens receiving a supplementation of *Trachyspermum copticum* extracts in their drinking water showed higher weight gain and feed intake during the entire period of the trial. These findings may be due to the effect of carvacrol and thymol contained in ajwain, which are 'generally recognized as safe' by the Food and Drug Administration (Burdock 2010; Pliego et al. 2020). Carvacrol, a monoterpenoid phenol, has a marked bioactivity on poultry metabolism (Reiner et al. 2009) and, thanks to its antioxidant activity, reduces lipid peroxidation. Similarity, thymol, another phenolic compound, is used as a growth promoter (Yanishlieva et al. 1999). According to our results, Lee et al. (2003) observed a greater efficiency in the feed utilisation with the integration of thymol and carvacrol; Hernandez et al. (2004) observed an improvement in the performance of broilers fed thymol and carvacrol supplementation and Luna et al. (2010) suggested carvacrol as an ideal natural additive for improving animal performances in poultry industries.

Our data show that supplementing ajwain at ae dose of 300 ppm significantly increased final live body weight, empty abdomen carcase, abdominal fat and breast. The latter result is very important because breast weight is one of the most economically important traits in the poultry industry (Shakeri et al. 2018). Data on dressing percentage (calculated from the empty abdomen carcase / live body ratio, data not shown) showed an improvement in relation to the supplementation of *Trachyspermum copticum* extracts as observed by Attia et al. (2019) with an integration of essential oils containing cinnamaldehyde and thymol.

As regards the blood parameters, our study revealed that the addition of *Trachyspermum copticum* alcoholic extracts had no significant effects on total cholesterol and glucose, in accordance with other Authors (Najafi and Torki 2010; Khattak et al. 2014; Ghazanfari et al. 2015; Kim et al. 2016; Adaszyńska-Skwirzyńska and Szczerbińska 2019), which did not show any effect on the biochemical parameters of the blood following the dietary supplementation of

essential oils or plant extract in the chicken breeding .On the contrary, Valiollahi et al. (2014) reported that the inclusion of 0.2 g/kg of ajwain powder in the basal diet of broiler birds reduced the cholesterol level; also Chowdhury et al. (2018) observed a decrease in cholesterol level with the integration of 0.4 g/kg of ajwain oil in broiler diet. A possible explanation could be the cholestatic effect of ajwain in the liver through an increased removal or catabolism of lipoproteins or the inhibition of hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in the liver (Lee, Jeon, et al. 2003; Lee, Everts, et al. 2003) resulting in a decrease in LDL cholesterol (Javed et al. 2009), as observed in our study. It is widely accepted that the level of total proteins in the blood is related to the body condition of broilers (Shabani et al. 2015). In our study, the higher plasma total protein content observed in the broilers receiving the ajwain supplementation reflected the greater weight gain in broiler chickens fed a *Trachyspermum copticum* supplement in drinking water.

Regarding the parameters of immunity against SRBC and immunity-related organ weight, the results showed a significantly greater effect of ajwain supplementation on the immunoglobulin levels at 28th day of age than that observed at 42nd day of age, and a significantly higher effect on the weight of bursa of Fabricius than that of spleen. After the production of highly specific IgG antibodies during microbial infections or autoimmune diseases by activated B cells, these antibodies recruit innate immune effector cells, which abundantly express cell surface receptors specific to the different Fc-fragments of the antibody. These Fc receptors link the specificity of the humoral immune system to the powerful effect or functions provided by the cells of the innate immune system (Nimmerjahn and Ravetch 2010). Some positive effects have been reported for plant extracts on the immune system such as an increase in lymphocyte proliferation and phagocytic rate, immunoglobulins (Reis et al. 2018) and on the size of lymphatic organs, spleen and bursa of Fabricius (Qu et al. 2017). One explanation could be that some phenolic monoterpenes in plant extracts could act as additional ligands of Fc protein receptors of immunoglobulin molecules, which bind to IgG and stimulate the immune response (Ahmed et al. 2013).

Furthermore, some plant bioactive compounds regulate the expressions of various genes involved in immune response (Liu et al. 2014). However, the mechanisms mediating the suppressive effects of plant extracts on inflammation and stimulate immune responses in chickens and other animals are still unknown (Chowdhury et al. 2018).

In the poultry industry, gastrointestinal diseases are one of the most important problems, and there are many concerns about antibiotic resistance (Dibaji et al. 2014). Overall, our results showed an increase of the lactobacilli population and a decrease in coliform bacteria in broilers receiving ajwain dietary supplementation, although, the control group (ajwain 0 ppm) exhibited large amounts of lactobacilli and the group with the highest dosage of ajwain (450 ppm) showed a large number of coliforms.

Carvacrol has a stimulating effect on the proliferation of *Lactobacillus* spp.(Rahimi et al. 2011); the increase in lactobacilli, modifying intestinal acidity, causes a reduction in the activity of harmful bacteria such as *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Clostridium perfringens, Campylobacter* sp. and *Salmonella* sp. (Vegas 2004; Choi et al. 2015; Zeng et al. 2015). The qualitative and quantitative composition of the intestinal microflora in poultry is influenced by many factors, and, among these, by the dietary supplementation of some active substances contained in essential oils (Roberts et al. 2015). Roberts et al. (2015) found that adding *Trachyspermum copticum* to broiler drinking water had a bacteriostatic effect, reducing coliform bacteria and increasing probiotic bacteria. Zomorodian et al. (2011) showed that the use of ajwain could inhibit *E. coli* in the intestinal tract. Valiollah et al. (2014) observed a decrease of *E. coli* and an increase of lactobacilli in broiler chicks fed a diet supplemented with 0.02% of ajwain. The main constituents, thymol and carvacrol, of *Trachyspermum copticum* are well known for their antibacterial, anthelminthic, hypocholesterolaemic and antioxidant effects (Rajput et al. 2012).

Conclusions

The addition of ajwain in broiler feed has shown some beneficial effects on the productive performances, metabolic status, immunity parameters and gut microbiota through pathogen control, resulting in improved animal production and food safety for the consumer. Our results indicate that ajwain may represent a good dietary additive to poultry feed as a potential substitute for antibiotics, thereby contributing to an efficient production in a sustainable way, promoting healthy and robust animals.

However, further research is needed to clarify the phytochemical composition, mode of action and dosage level appropriate to use ajwain extract as a

supplement in broiler diets and to establish the efficay and safety of this additive in the poultry industry.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Adaszyńska-Skwirzyńska M, Szczerbińska D. 2019. The effect of lavender (*Lavandula angustifolia*) essential oil as a drinking water supplement on the production performance, blood biochemical parameters, and ileal microflora in broiler chickens. Poult Sci. 98(1):358–365.

Ahmed ST, Hossain ME, Kim GM, Hwang JA, Ji H, Yang CJ. 2013. Effects of resveratrol and essential oils on growth performance immunity, digestibility and fecal microbial shedding in challenged piglets. Asian Australas J Anim Sci. 26(5):683–690.

Alali WQ, Hofacre CL, Mathis GF, Faltys G. 2013. Effect of essential oil compound on shedding and colonization of Salmonella enterica serovar Heidelberg in broilers. Poult. Sci. 92(3):836–841.

Apajalahti J, Vienola K. 2016. Interaction between chicken intestinal microbiota and protein digestion. Anim Feed Sci Technol. 221:323–330.

Attia Y, Al-Harthi M, El-Kelawy M. 2019. Utilisation of essential oils as a natural growth promoter for broiler chickens. Ital. J. Anim. Sci. 18(1):1005–1012.

Aviagen. 2002. ROSS broiler management manual. Newbridge, Scotland: Aviagen Ltd.

Burdock GA. 2010. Fenaroli's handbook of flavor ingredients. New York, NY: Taylor & Francis Group.

Chialva F, Monguzzi F, Manitto P, Akgül A. 1993. Essential oil constituents of *Trachyspermum copticum* (L.) Link Fruits. J. Essent. Oil Res. 5(1):105–106.

Choi KY, Lee TK, Sul WJ. 2015. Metagenomic analysis of chicken gut microbiota for improving metabolism and health of chickens – a review. Asian-Australas J Anim Sci. 28:1217–1225.

Chowdhury S, Mandal GP, Patra AK, Kumar P, Samanta I, Pradhan S, Samanta AK. 2018. Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. Anim Feed Sci Technol. 236: 39–47.

Dibaji SM, Seidavi A, Asadpour L, Moreira da Silva F. 2014. Effect of a synbiotic on the intestinal microflora of chickens. J Appl Poult Res. 23(1):1–6.

Dibaji SM, Seidavi A, Asadpour L, Sánchez Chopa F, Laudadio V, Casalino E, Tufarelli V. 2015. Effect of Biomin®IMBO on the humoral immune response of broiler chickens. Eur Poult Sci. 79:1–8.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance, 2010.

Ghazanfari SI, Mohammadi ZI, Adib Moradi M. 2015. Effects of coriander essential oil on the performance, blood characteristics, intestinal microbiota and histological of broilers. Braz J Poult Sci. 5:419–426.

Golrokh AJ, Bouyeh M, Seidavi A, van den Hoven R, Laudadio V, Tufarelli V. 2016. Effect of different dietary levels of atorvastatin and L-carnitine on performance, carcass characteristics and plasma constitutes of broiler chicken. Jpn Poult Sci. 53(3):201–207.

Gopi M, Karthik K, Manjunathachar HV, Tamilmahan P, Kesavan M, Dashprakash M, Balaraju L, Purushothaman MR. 2014. Essential oils as a feed additive in poultry nutrition. Adv Anim Vet Sci. 2(1):1–7.

Hafeez A, Sohail M, Ahmad A, Shah M, Din S, Khan I, Shuiab M, Nasrullah SW, Iqbal M, Khan RU. 2020. Selected herbal plants showing enhanced growth performance, ileal digestibility, bone strength and blood metabolites in broilers. J Appl Anim Res. 48(1):448–453.

Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD. 2004. Influence of two plant extracts on broiler performance, digestibility, and digestive organ size. Poult Sci. 83(2): 169–174.

Hevener WP, Routh A, Almond GW. 1999. Effects of immune challenge on concentrations of serum insulin-like growth factor-I and growth performance in pigs. Can Vet J. 40: 782–786.

Javed I, Zia-Ur-Rahman N, Khan MZ, Muhammad F, Aslam B, Iqbal Z, Sultan JI, Ahmad I. 2009. Anti hyperlipidaemic efficacy of *Trachyspermum ammi* in albino rabbits. Acta Vet Brno. 78(2):229–236.

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. Poult Sci. 93(1):132–137.

Kim SJ, Lee KW, Kang CW, An BK. 2016. Growth performance, relative meat and organ weights, cecal microflora, and blood characteristics in broiler chickens fed fiets containing different nutrient density with or without essential oils. Asian Australas J Anim Sci. 29(4):549–554.

Lee JS, Jeon SM, Park EM, Huh TL, Kwon OS, Lee MK, Choi MS. 2003. Cinnamate supplementation enhances hepatic lipid metabolism and antioxidant defense systems in high cholesterol-fed rats. J Med Food. 6:183–191.

Lee KW, Everts H, Kappert HJ, Frehner M, Losa R, Beynen AC. 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br Poult Sci. 44(3): 450–457.

Liu Y, Song M, Che TM, Lee JJ, Bravo D, Maddox CW, Pettigrew JE. 2014. Dietary plant extracts modulate gene expression profile in ileal mucosa of weaned pigs after *Escherichia coli* infection. J Anim Sci. 92(5):2050–2062.

Luna A, Labaque MC, Zygadlo JA, Marin RH. 2010. Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. Poult Sci. 89(2):366–370.

Mabood F, Gilani SA, Hussain J, Alshidani S, Alghawi S, Albroumi M, Alameri S, Jabeen F, Hussain Z, Al-Harrasi A, et al. 2017. New design of experiment combined with UV–Vis spectroscopy for extraction and estimation of polyphenols from Basil seeds, Red seeds, Sesame seeds and Ajwan seeds. Spectrochim Acta A Mol Biomol Spectrosc. 178:14–18.

Mahboubi M, Kazempour N. 2011. Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. Iran J Microbiol. 3(4):194.

Najafi P, Torki M. 2010. Performance, blood metabolites and immunocompetence of broiler chicks fed diets included essential oils of medicinal herbs. J Anim Vet Adv. 7: 1164–1168.

Nimmerjahn F, Ravetch JV. 2010. Antibody-mediated modulation of immune responses. Immunolog. Rev. 236: 265–275.

Nooreh Z, Taherpour K, Gharaei MA, Shirzadi H, Ghasemi HA. 2021. Effects of a dietary direct-fed microbial and Ferulago angulata extract on growth performance, intestinal microflora, and immune function of broiler chickens infected with *Campylobacter jejuni*. Poult Sci. 100(3): 100942.

Pliego AB, Tavakoli M, Khusro A, Seidavi A, Elghandour MM, Salem AZ, Márquez-Molina O, Rene R-CR. 2020. Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: a review. Anim Biotechnol. 2020:1–23.

Qu D, Huang X, Han J, Man N. 2017. Efficacy of mixed adsorbent in ameliorating ochratoxicosis in broilers fed ochratoxin A contaminated diets. Ital J Anim Sci. 16(4): 573–579.

Rahimi S, Teymouri ZZ, Karimi TM, Omidbaigi R, Rokni H. 2011. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. J Agric Sci Technol. 13(4):527–539.

Rajput MA, Rafeeq AKH, Qazi N, Feroz Z. 2012. Effect of methanol extract of ajwain (*Trachyspermum Ammi* L) on blood coagulation in Rats. J Liaquat Univ Med Health Sci. 11:105–108.

Reiner GN, Labuckas DO, Garcia DA. 2009. Lipophilicity of some GABAergic phenols and related compounds determined by HPLC and partition coefficients in different systems. J Pharm Biomed Anal. 49(3):686–691.

Reis JH, Gebert RR, Barreta M, Baldissera MD, Dos SI, Wagner R, Campigotto G, Jaguezeski AM, Gris A, de Lima JLF, et al. 2018. Effects of phytogenic feed additive based on thymol, carvacrol and cinnamic aldehyde on body weight, blood parameters and environmental bacteria in broilers chickens. Microb Pathog. 125:168–176.

Roberts T, Wilson J, Guthrie A, Cookson J, Vancraynest D, Schaeffer K, Moody R, Clark S. 2015. New issues and science in broiler chicken intestinal health: emerging technology and alternative interventions. J Appl Poult Res. 1: 1–10.

Shabani S, Seidavi A, Asadpour L, Corazzin M. 2015. Effects of physical form of diet and intensity and duration of feed restriction on the growth performance, blood variables, microbial flora, immunity, and carcass and organ characteristics of broiler chickens. Livest Sci. 180:150–157.

Shakeri M, Cottrell JJ, Wilkinson S, Ringuet M, Furness JB, Dunshea FR. 2018. Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. Animals. 8(10):162.

Shim YH, Shinde PL, Choi JY, Kim JS, Seo DK, Pak JI, Chae BJ, Kwon IK. 2010. Evaluation of multi-microbial probiotics produced by submerged liquid and solid substrate fermentation methods in broilers. Asian Australas J Anim Sci. 23(4):521–529.

SPSS. 1997. SPSS Base 7.5 for Windows. Chicago (IL): SPSS.

Takahashi KT, Mashiko Y, Akiba Y. 2000. Effect of dietary concentration of xylitol on growth in male broiler chicks during immunological stress. Poult Sci. 79:743–747.

Valiollahi MR, Gholami M, Namjoo AR, Rahimian Y, Rafiee A. 2014. Effect of using Sumac (Rhuscoriaria L.) and Ajwain (*Trachyspermum copticum*) powders on performance and intestinal microbial population in broiler chicks. Res Opin Anim Vet Sci. 4:545–549.

Vegas JL. 2004. Prebiotics, probiotic, acid fires and antibiotic growth promoters. In Poultry diseases: a guide for farmers and poultry professionals. International Book Distributing Co., Charbagh, Lucknow, India, 339–346.

Xue F, Shi L, Li Y, Ni A, Ma H, Sun Y, Chen J. 2020. Effects of replacing dietary Aureomycin with a combination of plant essential oils on production performance and gastrointestinal health of broilers. Poult Sci. 99(9):4521–4529.

Yanishlieva NV, Marinova EM, Gordon MH, Raneva VG. 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem. 64(1): 59–66.

Zeng Z, Zhang S, Wang H, Paio X. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J Anim Sci Biotechnol. 6:7–15.

Zomorodian K, Moein M, Rahimi MJ, Pakshir K, Ghasemi Y, Abedi S, Sharbatfar S. 2011. Possible application and chemical compositions of Carumcopticum essential oils against food borne and nosocomial pathogens. Middle East J Sci Res. 9:239–245.