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Performance, immunity, and serum biochemical parameters in broiler chickens fed diet supplemented with *Zataria multiflora* essential oil

Khosrow Ghazvinian[1], Atefeh Araghi[2], Mohaddeseh Abouhosseini Tabari[2*]

[1]*Clinical Sciences Dept., University of Semnan, Semnan, I.R. Iran;* [2]*Veterinary Medicine Dept., Amol University of Special Modern Technologies, Amol, I.R. Iran.*

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ABSTRACT

Background and aims: *Zataria multiflora* Boiss (ZM) is a thyme-like plant reported to have immunomodulatory properties. This study was aimed to examine effects of ZM essential oil on performance, biochemical and immunological parameters in broilers.

Methods: Two hundred and forty, broiler chickens were divided into 4 groups. CON considered as control group received basal diet, T100, T200 and T400 treated by basal diet supplemented with ZM 100, 200 and 400 mg/kg feed, respectively. Body weight and feed intake were recorded at 14, 28, and 42 days of age. At the end of experiment, eight broilers per group were slaughtered and carcass portion yield was calculated. Humoral immune response efficacy by injection of 5% sheep red blood cell into the pectoral muscle of birds was evaluated.

Results: This study showed that supplementation of ZM promoted the weight gain in T200 as compared to other groups ($P<0.05$). Also, the best food conversion ratio was achieved in the same group. On the other hand, ZM supplementation in all of the doses used, did not cause significant increase in carcass portion yield ($P<0.05$), except for liver weight, which was significantly increased in T400. Serum biochemical analysis showed that levels of total protein, albumin and globulin were highest in T200 while the lowest were in T400; addition of ZM especially at the dose of 200 mg/kg improved immune responses in broilers ($P<0.05$).

Conclusion: According to the results of present study, supplementing broilers diet with ZM could have favorable impact on performance and immunity.

Keywords: *Zataria multiflora*, Growth performance, Serum biochemical parameters, Immunity, broilers.

Original article

INTRODUCTION

In recent years, the restriction and avoidance of the use of antibiotic growth promoters in poultry industry made scientists to find appropriate alternatives to improve the health of consumers and performance of broilers.[1] From that time onward, some authors reported the beneficial effect of herbal extracts and essential oils (EOs) as performance enhancers, antimicrobials, immune stimulants, and anti-oxidants.[2-4]

[*]**Corresponding author:** Mohaddeseh Abouhosseini Tabari, Veterinary Medicine Dept., Amol University of Special Modern Technologies, Amol, I.R. Iran, Tel: 00981144265008, E-mail: m.abouhosseini@ausmt.ac.ir

Zataria multiflora Boiss (ZM), also known as Zataria bracteata Boiss; Zataria multiflora var. elatior Boiss, is a thyme-like plant belonging to the Lamiaceae family that geographically grows wild in restricted regions including central and southern in Iran, Pakistan and Afghanistan.[5] It poses some chemical and pharmacological similarities to Thymus vulgaris, the well-known and widely investigated medicinal plant. It is also called Avishan-e-Shirazi (Avishan meaning thyme in the Persian language and Shiraz being the name of a city in southern Iran).[6] It was previously stated that ZM aerial parts is not only a condiment, but is also used in traditional medicine for its antiseptic, analgesic, carminative, anthelmintic and antidiarrheal properties. Based on studies, ZM essential oil analysis showed carvacrol and thymol as main compounds which have biological properties such as antinociceptive, antimicrobial, spasmolytic and anti-inflammatory effects.[7,8]

Recently the immunomodulatory effect of ZM essential oil was demonstrated either in in vivo and in vitro models.[9-12] Several studies have evaluated effects of thyme while few studies have been examined the ZM effect on growth performance of chickens. The current study was conducted to detect the beneficial effect of dietary supplementation of ZM essential oil on performance, hematological, biochemical and immunological parameters of Ross broiler chickens.

METHODS

Two hundred and forty one day-old male commercial Ross 308 broiler chicks, weighing 41.5 ± 0.5 g, purchased from a local hatchery and for an experimental study were randomly divided into 4 groups each of 4 replicates with 15 birds per replicate. The first group (CON) considered as control received basal diet without any treatment, group 2 (T100), 3 (T200) and 4 (T400) treated by a basal diet supplemented with ZM essential oil at the level of 100, 200 and 400 mg/kg feed, respectively.[13] The treatment was applied daily and finished at day 42. Based on the time of experiment, three different ingredients and calculated nutrition composition of the basal diet, including starter from 0 to 14, grower from 15 to 28 and finisher from 29 to 42 days of age, were use *ad libitum*. The chicks kept at the broiled (Table 1). Fresh tap water was supplied r floor pen unit in faculty of Veterinary Medicine, university of Semnan, Shahmirzad, Iran. Animal care followed the official guidelines and protocol of the study was approved by Ethics Committee of Semnan University, Faculty of veterinary Medicine, Iran.

The aerial parts of the plant were collected and air dried. Then, they were submitted to water distillation for 4 h by using a Clevenger apparatus. The obtained essential oil was dried over anhydrous sodium sulphate, and stored at 4°C until posterior use.[13]

Body weight and feed intake were recorded at 14, 28, and 42 days of age. Mortality was recorded daily. Any bird that died was weighed and feed conversion ratios were calculated by dividing total feed intake by weight gain of live plus dead birds. At 42 days of age and after overnight fasting, two birds per replicate randomly chosen were slaughtered and abdominal fat, liver, gizzard, heart, thigh and breast were collected, weighed, and calculated as a percentage of live body weight.

Table 1: Ingredients and basal diet composition

Ingredient %	Starter (0-14 day)	Grower (15-28 day)	Finisher (29-42 day)
Corn	46.09	50.91	48.88
Fish meal	3	3	-
Protein meal	3	3	-
Oil	4.56	5.45	7.38
Soybean meal	40	35	39.97
Methionine D - L	0.29	0.23	0.17
Lysine	0.04	-	-
Threonine	0.03	-	-
Ca 22% P 18%	0.99	0.75	1.46
Lime stone	0.98	0.76	1
HCO3	0.05	-	-
Salt	0.37	0.37	0.45
KCL	-	0.03	
Vitamin - Mineral mix	0.60	0.50	0.50
Metabolize energy	3025	3150	3200
Crude protein	24.9	23	22
Calcium (%)	1.05	0.9	0.85
Phosphorus (%)	0.5	0.45	0.42
Lysine (%)	1.41	1.13	1.22
Sodium (%)	0.23	0.23	0.2
Methionine + cysteine (%)	1.05	-	0.77

To determine the impact of ZM essential oil on biochemical and immunological parameters, blood samples from wing vein were collected in non-heparinized tubes at 42 days of age from five birds in each replicate and centrifuged for 10 min at 1200 rpm. Serum samples were stored at -20 °C until posterior analysis. The level of total serum protein (TP), albumin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, triglyceride, glucose, Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were measured using commercial kits (Pars Azmoon, Tehran, Iran). In addition, globulin concentration in serum was computed by subtracting albumin concentration from total serum proteins, consequently albumin to globulin ratio was calculated.

Sheep red blood cell (SRBC) 5% suspension in sterile phosphate buffer solution (PBS) was injected intramuscularly into the pectoral muscle of 12 birds per treatment group at 14 and 28 days of age, to evaluate the humoral immune response efficiency. Then, by agglutination test the total antibody (Ab) titers against SRBC were determined in sera from all tested birds. Fourteen days after each

sensitization (day 28 and 42), antibody titers against SRBC were measured and expressed as the log 2 of the reciprocal of the highest serum dilution giving complete agglutination.[14,15]

Statistical analysis was conducted using the general linear models procedure (Repeated measure ANOVA) of SAS software (SAS Institute, 2005). When significant effect was revealed, the differences between dietary treatment means were followed by a multiple comparison Duncan test. Differences at level of $P<0.05$ were considered as significant.

RESULTS

The present study examined the effect of ZM essential oil on different parameters in Ross broiler in 42 days period. It was notified that ZM essential oil had positive effect to increase the average weight of chicken carcasses (Table 2).

Table 2: The impact of dietary supplementation of different levels of *Zataria multiflora* essential oils on the growth performance of Ross broilers

	Variables	CON	T100	T200	T400	SEM
Starter (0-14)	Weight gain (g/bird)	339.27b	326.08b	361.97a	318.23c	1.54
	Feed intake (g)	515.32a	519.14a	517.66a	500.14b	3.80
	Feed conversion ratio	1.52b	1.59b	1.43a	1.57b	0.02
	Final weight (g bird-1)	379.66b	367.13b	401.55a	360.23c	2.69
Grower (14-	Weight gain (g/bird)	895.56b	924.21a	930.22a	840.20c	8.11
	Feed intake (g)	1610.45a	1621.13a	1598.77a	1500.22b	27.12
	Feed conversion ratio	1.80a	1.75a	1.72a	1.79a	0.02
	Final weight (g bird-1)	1275.22b	1291.34b	1331.77a	1200.43c	9.52
Finisher (28-	Weight gain (g/bird)	927.55b	922.92b	961.38a	933.45b	10.33
	Feed intake (g)	2360.44a	2370.72a	2400.55a	2250.51b	31.18
	Feed conversion ratio	2.54a	2.57a	2.50a	2.41a	0.03
	Final weight (g bird-1)	2202.77b	2214.26b	2293.15a	2133.88c	22.44

CON as control, T100 received Zataria multiflora essential oil at dose of 100 mg/kg of feed, T200 received Zataria multiflora essential oil at dose of 200 mg/kg of feed and T400 received Zataria multiflora essential oil at dose of 400 mg/kg of feed. a, b, c Values in the same row with a different superscript differ significantly at P<0.05.

Noticeably, our results showed that supplementation of 200 mg ZM essential oil per kg of feed promoted the weight gain on the whole trial period as compared to other groups. But, there was no direct relationship between the dose of ZM essential oil and weight gain. Surprisingly, chickens which received 100 mg ZM essential oil per kg of feed (T100) during starter and grower period showed more weight gain as compared to the group received essential oil 400 mg/kg feed (T400) ($P<0.05$). Feed intake and feed conversion ratio were influenced by herbal extracts supplementation 42 days of age. It also seemed that the use of high dose of ZM essential oil as much as 400 mg/kg of feed, failed to contribute marked effect on daily feed intake at early age($P>0.05$). The chickens fed on the diet containing 200 mg essential oil (T200) gained the best food conversion ratio (FCR) index in three different period times($P<0.05$) (Table 2). It is worthy to note that FCR could improve

with time which it presumably may relate to the adaptation of intestinal flora to new condition.

We also determined the effects of ZM essential oil on relative weight (% bodyweight) of some internal organs of broilers shown in Table 3. The carcass yield, abdominal fat, gizzard; breast and thigh were not significantly affected by dietary supplementation (P>0.05). A marked increase in the percentage of liver weight was observed in broilers in T400 group compared to other treatment and control groups (P<0.05).

Table 3: The effect of *Zataria multiflora* essential oil supplementation on broilers carcass yield and organ size

Groups	Carcass	Breast weight (%)	Thigh weight (%)	Gizzard (%)	Liver (%)	Heart (%)	Abdominal fat (%)
CON	60.73 a	36.91 a	30.45 a	1.95a	2.00a	0.59a	2.13 a
T100	61.25 a	37.82 a	30.67 a	1.79a	1.98a	0.61a	2.55 a
T200	62.28 a	36.67 a	30.21 a	1.83a	1.95a	0.61a	1.92 a
T400	63.00 a	37.11 a	30.72 a	1.94a	2.42b	0.60a	1.86 a
SEM	0.04	0.01	0.01	0.06	0.07	0.012	0.01

CON as control, T100 received Zataria multiflora essential oil at dose of 100mg/Kg of feed, T200 received Zataria multiflora essential oil at dose of 200mg/Kg of feed and T400 received Zataria multiflora essential oil at dose of 400mg/Kg of feed. a, b Values in the same column with a different superscript differ significantly at P<0.05.

Serum samples were examined for total serum protein (TP), total cholesterol, HDL, LDL, cholesterol, triglyceride, glucose, ALT and ALP to detect the effects of dietary supplementation of ZM essential oil (Table 4).

Table 4: The influence of dietary supplementation of different levels of ZM essential oils on serum biochemical parameters in broilers

Groups	Globulin (mg/dL)	Albumin (mg/dL)	TP (mg/dL)	Glucose (mg/dL)	ALT (U/I)	ALP (U/I)
CON	2.16a	1.2a	3.36ab	262a	289.66a	280.18a
T100	2.12a	1.28a	3.40ab	268a	275.60a	271.62a
T200	2.20a	1.39b	3.59a	263a	280.22a	262.23a
T400	1.86b	1.21a	3.07b	264a	281.16a	259.69a
SEM	0.05	0.06	0.132	4	2.23	3.76

CON as control, T100 received Zataria multiflora essential oil at dose of 100 mg/kg of feed, T200 received Zataria multiflora essential oil at dose of 200 mg/kg of feed and T400 received Zataria multiflora essential oil at dose of 400 mg/kg of feed. a, b, c Values in the same row with a different superscript differ significantly at P<0.05.

Biochemical analysis revealed that ALP, ALT, and glucose concentrations did not change significantly between different groups (P>0.05). Serum levels of TP, albumin and globulin showed highest increase in T200 group whereas the lowest level of TP and globulin recorded in broilers received ZM 400 mg/kg of feed (T400). The current experiment did show significant differences in ALP and ALT activity between groups.

The lowest activity of serum ALP and ALT were recorded for T200 and T100, respectively.

The addition of ZM essential oil after 28 days improved the immune system efficacy against foreign body in treated birds as compared to control but did not reach to significant level (Table 5). The highest antibody titer was determined in T200 birds, treated with essential oil in dose of 200 mg/kg of feed after 42 days (P<0.05).

Table 5: The effect of experimental diets on SRBS at day 28 and 42

Antibody response to SRBC (log 2)	CON	T100	T200	T400
Day 28	4.39±0.56	5.09±0.41	4.73±0.17	4.52±0.23
Day 42	5.91±0.74a	6.51±0.15b	5.98±0.59a	5.56±0.23a

Data expressed as Means ± SE. CON as control, T100 received Zataria multiflora essential oil at dose of 100 mg/kg of feed, T200 received Zataria multiflora essential oil at dose of 200 mg/kg of feed and T400 received Zataria multiflora essential oil at dose of 400 mg/kg of feed. a, b Values in the same row with a different superscript differ significantly at P<0.05.

DISCUSSION

Many authors stated that providing herbal extracts in the diet was effective in protecting and growth performance of broilers.[14-16] The current study illustrated the effects of different doses of *Zataria multiflora* essential oil at three different stages of broiler production. Similar to previous experiments which confirmed the protective effect of other essential oils on broilers, we, here, revealed that *Zataria multiflora* essential oil had positive effect on broiler performance, growth rate, health and immune responses. Several experiments demonstrated the positive effect of essences and their dose dependent activity on broilers performance and carcass yield.

Najafi et al. reported that the group fed on thyme-included diet had significantly better body weight and feed conversion ratio in comparison to the control group. These discrepancies have presumably come from the different strains of birds, dosages of herbs, type and amount of basal diets and different environmental condition.[16] Foroughi and co-workers stated that chicks fed with diet contain 225 mg kg-1 and 270 mg kg-1 thymol essential oils had significantly decrease feed intake (P<0.05) as it was showed in group T400.[17] While, in the same study the birds fed diet contained 180 mg kg-1 thymol supplementation showed increase in the feed intake and had the lowest FCR, similar to current study in which FCR was improved in T200 group, received 200 mg/kg ZM essential oil. In line with the results obtained in present study, Toghyani et al. also showed that carcass yield and abdominal fat was not influenced by the dietary treatments.[4] These results are in consistent with work of Demir et al. and Lee et al. in both of them thyme has no influence on performance of broilers.[1,2] Some researchers have reported that Feeding thyme oil at the rate of 100 and 200 ppm decreased abdominal fat.[18,19]

Toghyani et al. reported that the low dosage (5 g/kg) of thyme had significant effect on broilers body weight and their feed conversion ratio, while the high dosage (10 g/kg) did not show this effect.[4] In a research done by Rahimi et al. dose of 0.1% thyme in poultry diet did not cause any significant difference in relative weight of carcass, fat pad, liver, pancreas and gizzard. But, they confirmed the positive effect of thyme on FCR.[15]

The results of serum biochemistry clarified a significant increase in serum concentrations of total protein and albumin especially in T200 group. Saleh et al. revealed significant increase in serum concentrations of total protein and globulin in broilers consuming thyme 200 mg/kg of feed. Serum ALT and AST activity did not show any significant changes, these results are in accordance with the findings of present study.[20] Tawfeek and Mustafa reported the addition of Thymus vulgaris as much as 1000 mg/kg of body weight did not affect ALT level while two-fold concentration could alter ALT activity.[21]

In research of Toghyani et al., thyme treatments failed to have any statistical effect on SRBC antibody titer at 28 day of age ($P>0.05$).[4] Hashemipour et al. also demonstrated the combination of thymol and carvacrol linearly increased primary and secondary response against SRBC antigen in broiler chickens 15. Rahimi et al. reported that 0.1% dose of thyme in poultry diet improved antibody response to at 42 days of age.[16] In line with these findings, the present study revealed beneficial effects of ZM especially at the dose of 200 mg/kg on immune responses to SRBC.

CONCLUSION

According to the results of present study, it concludes that supplementing broilers diet with 200 mg/Kg ZM could indicate favorable influences on performance of broilers without any detrimental impact on hematological and biochemical parameters and also could improve immune responses. Further studies are recommended to determine the major functional constituents of ZM essential oil in broilers performance and immunity.

CONFLICT OF INTEREST

Authors have declared that no conflicts of interest exist.

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