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Research Article

Effect of Thyme Species Extracts on Performance, Intestinal Morphometry, Nutrient Digestibility and Immune Response of Broilers

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Abstract

The study was performed to investigate the effect of hydroalcoholic extract of different thyme species on performance, intestinal morphometry, nutrient digestibility and immune response of male broiler chickens. A total of 160 day-old Ross 308 broiler chicks were randomly allotted to five treatments including a control basal diet or diets supplemented with 0.1% of four thyme extracts *(Thymus daenensis, T. kotschyanus, T. lancifolius,* and *T. transcaspicus)* with four replicates of eight birds each. The experiment was lasted for 28 days. There were no significant effects of treatments on broilers performance during the first three weeks of age. At fourth weeks of age and throughout the experimental period, the chickens on *T. daenensis* extract showed the best weight gain among the treatments ($P<0.05$), while feed intake and feed conversion ratio were not affected by the treatments throughout the experimental period. The birds receiving *T. daenensis* extract had higher jejunal villus height and villus height to crypt depth ($P<0.05$) than those on the control. All thyme extracts increased villus surface area ($P<0.05$). Supplementation of diet with *T. daenensis* extract improved digestibility of organic matter, dry matter, crude carbohydrate, crude ash and ether extract ($P<0.05$). The immune response to Newcastle disease (ND) vaccine and sheep red blood cell (SRBC) were not affected by the treatments. The results indicated that the response of broiler chickens to thyme extract depends on birds' age and genotype of the plant.

Keywords: *Chicken, Digestibility, Mucosal morphology, SRBC, Thyme spp.*

Etlik Piliçlerde Kekik Ekstraktının Performans, Barsak Morfometrisi, Besin Sindirilebilirliği ve Bağışıklık Yanıtı Üzerine Etkileri

Öz

Bu çalışma erkek broiler piliçlerde çeşitli kekik cinslerinin hidroalkolik ekstraktının performans, barsak morfometrisi, besin sindirilebilirliği ve bağışıklık yanıtı üzerine etkilerini araştırmak amacıyla yapılmıştır. Toplam 160 adet bir günlük Ross 308 broiler civciv her bir grupta 8 civciv ve dört tekrar olmak üzere rastgele olarak 5 gruba ayrılarak kontrol grubuna bazal diyet, diğerlerine ise %0.1 oranında farklı dört kekik *(Thymus daenensis, T. kotschyanus, T. lancifolius,*ve *T. transcaspicus)* ekstraktı verildi. Deney süresi 28 gün sürdü. İlk üç ay süresince broiler performansı üzerine denemelerin anlamlı bir etkisi olmadı. Dördüncü haftada ve deneysel süre boyunca *T. daenensis* verilen piliçler diğer denemeler arasında en iyi ağırlık kazanımını gösterirken ($P<0.05$) bu grupta yem tüketimi ve yem konversiyon oranında deneysel aşama süresince bir etki gözlemlenmedi. Kontrol grubu ile karşılaştırıldığında *T. daenensis* verilen piliçler daha yüksek jejunal villus yüksekliğine ve villus yüksekliği kript derinliği oranına sahiptiler ($P<0.05$). Tüm kekik ekstraktları villus yüzey alanında artmaya neden oldu ($P<0.05$). *T. daenensis* ekstraktı ilave edilen diyet organik materyalin sindirilebirliğini artıtdı ve kuru madde, ham karbohidrat, ham kül ve eter ekstraktı değerlerinde iyileşmeye neden oldu ($P<0.05$). Newcastle hastalığı aşısı ve koyun kırmızı kan hücrelerine bağışıklık cevabı deneysel uygulamalardan etkilenmedi. Elde edilen sonuçlar broiler piliçlerin kekik ekstraktından etkilenmesinin civcivin yaşına ve bitkinin genotipine bağlı olduğunu göstermiştir.

Anahtar sözcükler: *Tavuk, Mukozal morfoloji, Koyun kırmızı kan hücresi, Thyme spp.*

INTRODUCTION

Phytochemicals have been recently considered by researchers and producers as feed additives in poultry diets. It has been shown that adding medicinal plant extracts and essential oils into the diets can improve growth performance [1], gut function [2] and immune responses [3] of poultry.

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Thyme *(Thymus spp.,* L) is one of the aromatic plants that belong to the Lamiaceae family. It contains different essential oils such as thymol, carvacrol, linalool, α-terpineol, 1,8 cineole, α- pinen, p-simenn, camphene, limonene, and several other compounds [4]. Numerous beneficial properties such as antimicrobial [5-7], antifungal [8], anti-oxidant [4] and improved nutrients digestion [6] have been reported for thyme and its derivatives.

It is demonstrated that the quantity of bioactive components of medicinal plants and their activity varies by plant species [7,9], climatic and environmental conditions [10] harvesting stage, drying process and extracting methods [11].

The amount of carvacrol and thymol, as two major essential oils of thyme, is variable between thyme species (2 to 42 and 1 to 50 percent of total essential oils, respectively) [10] and even between populations of one species [7]. In one study, Amiri [9] has revealed the variation in terpene compounds by reporting the range of 16.4 to 42.6 percent for thymol, 7.6 to 52.3 percent for carvacrol and 3 to 11.4 percent for γ-terpinene of three wild-growing Thymus species in the west of Iran.

There are evidences that essential oils stimulate the secretion of digestive enzymes in broilers [1,5] and increase fat [12] and amino acid digestibilities [11]. The increase of *Lactobacillus* to *E. coli* ratio, bifidobacteria and propionibacteria populations have been also illustrated as the effect of essential oils in gastrointestinal tract of monogastric animals [13].

The modulatory effect of some medicinal plants [1], especially flavonoids rich plants such as thyme [14], has been shown on immune and defence system of poultry. The effect has been pronounced by increasing thymus, bursa of Fabricius and spleen weights [15], enhancing antibody titre against infectious bronchitis [16] and Newcastle disease (ND) vaccine [17] in broilers.

Although there are plenty of reports on thyme and its derivatives, to our knowledge, there is no study to compare the physiological effects of different species of thyme on broilers. Hence, the objective of this study was to investigate the influence of dietary supplementation with a hydroalcoholic extract of different thyme species on growth performance, gut morphometry, nutrient digestibility and immune response of broiler chickens.

MATERIAL and METHODS

Preparation of Plant Materials and Extraction

To minimize the environmental effects, the studied thyme species including *T. daenensis, T. kotschyanus, T. lancifolius* and *T. transcaspicus* were prepared from the same harvesting place, Agricultural and Natural Resources Research Centre of Ardabil, Iran. The plant materials were dried at room temperature in shade, and grounded using a laboratory mill. The hydroalcoholic extract of plants aerial parts was obtained by maceration technique using 50% ethanol as solvent at 10:1 solvent to plant ratio [18]. The extract was then concentrated in a rotary evaporator (Bibby RE 200 B, England) at 50°C and air-dried, then standardized based on 8% dry matter following the yield assessment [19]. The phenolic acid compounds of thyme extracts were evaluated by HPLC analysis [20,21] and expressed as µg/g dry weight. Total phenolic content of the extract was determined by folin-ciocalteau colorimetric method [22] and expressed as mg gallic acid equivalents (GAE)/L of extract *(Table 1)*.

Birds and Experimental Diets

A total of 160 one-day old Ross 308 male broiler chicks were used in an *in vivo* study. The birds were distributed into 20 wire floor cages with equal group weight, and were randomly assigned to one of five experimental diets. Therefore, a completely randomized design with five treatments including a control diet with no extract and four diets with 0.1% of four thyme species extract was employed with four replicates of 8 birds per each replicate. The diets were formulated according to the Ross 308 strain catalogue recommendations [23], and were fed to the chickens in three feeding phases of 1-10, 11-24 and 25-28 days of age *(Table 2)*.

The trial was lasted 28 days and conducted in an

Table 1. *Concentration of phenolic acid compounds (µg/g DW) and total phenolics (mg/L) of different thyme species extracts*

Phenlics	***T. daenensis***	***T. kotschyanus***	***T. lancifolius***	***T. transcaspicus***
Chlorogenic acid	187	129	175	110
Vanilic acid	175	48	107	66
Caffeic acid	190	162	242	117
Syringic acid	977	730	626	565
P_Cumaric acid	119	0	185	60
Rosmarinic acid	665	1047	870	1420
Sinapic acid	78	63	116	0
Total phenolics	707	615	570	666

Table 2. *Composition of experimental diets in different periods of the experiment*

Ingredient (%)	Starter (1-10 day)	Grower (11-24 day)	Finisher (25-28 day)
Maize	51.16	53.96	59.03
Soybean meal (44% CP)	40.00	36.17	31.40
Vegetable oil	4.07	5.76	5.65
Dicalcium phosphate	1.88	1.65	1.58
Oyester shell	1.34	1.11	1.09
Common salt	0.37	0.37	0.37
Vitamin premix[a]	0.25	0.25	0.25
Mineral permix[b]	0.25	0.25	0.25
DL-methionine	0.38	0.30	0.25
L-Lysine hydrochloride	0.30	0.17	0.13
Total	100	100	100
Calculated compositions			
ME, MJ/kg	12.66	13.18	13.39
Crude protein, %	22.41	21.00	19.28
Methionine, %	0.72	0.66	0.56
Methionine + cystine, %	1.07	1.00	0.87
Lysine, %	1.43	1.24	1.11
Arginine, %	1.45	1.33	1.21
Thereonine, %	0.83	0.83	0.75
Calcium, %	1.05	0.90	0.86
Available Phosphorus, %	0.50	0.43	0.43
Sodium, %	0.16	0.16	0.16

[a] Provided per kg of diet: vit. A, 9.000 IU; vit. D_3, 2.000 IU, vit. E, 18 mg; vit. K_3, 2 mg; vit B_1, 1.8 mg; vit B_2, 6.6 mg; Pantothenate calcium, 30 mg; Niacin, 10 mg; vit B_6, 3 mg; vit B_9, 1 mg; vit B_{12}, 0.015 mg; Biotin, 0.1 mg; Choline chloride, 500 mg
[b] Provided per kg of diet: Mn, 100 mg; Fe, 85 mg; Zn, 100 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg

environmentally controlled room. Feed and water were provided *ad-libitum*, except at weighing times when feed was withdrawn for 4 h to ensure the emptiness of digestive system contents [24]. Feed intake and weight gain were measured weekly and feed conversion ratio was calculated. Light was provided for 23 h/day, while temperature was gradually reduced from the initial 31°C to approximately 21°C by day 21, and was kept constant until the end of the experimental period. All procedures used in the experiment approved by research council of the University of Mohaghegh Ardabili.

Nutrients Digestibility

To determine the total tract nutrient digestibility coefficients, 0.3 percent chromic oxide, as an inert marker, was added to the experimental diets, and fed to chickens from 25 to 28 days. The excreta samples of each replicate were collected twice daily and mixed together, then kept in the freezer (-20°C) for subsequent analyses. The samples were oven dried (60°C, 72 h) and ground prior to analysis. The chemical compositions of feed and excreta samples, including dry matter, organic matter, ether extract and crude protein were measured according to the AOAC [25] method. The crude carbohydrate content of samples was calculated by following formula [26]:

Crude Carbohydrate (%) = 100 - (% Nitrogen × 6.25) - % Crude Ash - % Ether Extract

The chromium oxide content of excreta and feed samples was measured according to the method described by Fenton and Fenton [27], then the apparent nutrients digestibility and nitrogen retention was determined using the following equation [28]:

$$\text{Digestibility (\%)} = [\,1 - (\frac{\%\ \text{diet}\ Cr_2O_3}{\%\ \text{excreta}\ Cr_2O_3} \times \frac{\%\ \text{excreta nutrient}}{\%\ \text{diet nutrient}})] \times 100$$

Morphometry

At the end of the experiment, two birds per cage (replicate) were randomly selected, weighed and euthanized by CO_2 asphyxiation. After eviscerating, a tissue sample (3 cm) from proximal jejunum was obtained and its digesta being washed with saline buffer was fixed in 10% buffered formalin. The fixed tissue samples in formalin solution were processed and embedded in paraffin. Sections were prepared at a thickness of 5 µm and stained with haematoxylin and eosin. The morphometric indices were determined using computer-aided light microscope image analyses (SPOT 3.1; Diagnostic Instruments, Sterling Heights, MI, USA). The measurements of villus height (from the tip of villus to the villus-crypt junction), villus width, crypt depth, villus height: crypt depth ratio and thickness of muscle layer were made. Apparent villus surface area was calculated from the villus height and width [29]. The values of means from 9 adjacent and vertically orientated villi and crypts from each bird were used for further analysis.

Immune Response to ND Vaccine and SRBC

All chicks were vaccinated against Newcastle disease (ND) at 7 days of age, by eye-drop and subcutaneous injection, simultaneously. Vaccination was carried out according to the regional vaccination program routine. At 14 days of age blood samples were collected from birds' wing in tubes to evaluate the immune response to ND virus by haemagglutination inhibition (HI) test according to Hossain et al.[30].

Sheep red blood cells (SRBC) were used to assay the antibody response. Two birds per cage were injected intramuscularly with SRBC (3% suspension in PBS, 1 mL/chick) at 17 days of age. Blood sampling of birds was done on the 7th days after injection. After inactivation of the serum at 56°C temperature for 30 min, 50 µL of serum was put in an equal amount of PBS in the first column of a 96-well V-shaped bottom plate, and the solution was incubated at 37°C for 30 min. Then a serial dilution was made (1:2), and 50 µL of

2% SRBC suspension was added to each well. After 30 min of incubation at 37°C, total antibody titre was read. The well immediately prior a well with a distinct SRBC button was considered as the endpoint titre for agglutination. To assess IgG titre, 50 µL of 0.01 M 2-mercaptoethanol in PBS was used, followed by the procedure mentioned above for total antibody titre. Antibody level of IgM was calculated from the difference between the total and the IgG [31].

Statistical Analysis

All collected data were subjected to statistical analysis using the General Linear Model procedure of SAS software 9.1 [32]. Duncan's Multiple Range test was used to detect the differences ($P< 0.05$) between different means.

RESULTS

Growth Performance

During the first 3 weeks of the experiment, none of the thyme extracts had a significant effect on chickens weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) *(Table 3)*. In the fourth weeks of the experiment, supplementation of diet with *T. daenensis* extract increased WG of the chickens ($P<0.05$) and a significant decrease in FI and FCR of birds on *T. lancifolius* extract was also observed ($P<0.05$) although there was no significant difference between FCR of broilers on *T. daenensis* and *T. lancifolius* extracts. Throughout the entire period of the experiment (1 to 28 d), WG of chickens was only improved by *T. daenensis* extract ($P<0.05$). FI and FCR were not significantly affected by the treatments.

Morphometry

At 28 days of age, the birds on *T. daenensis* extract had significant high villus height and villus height to crypt depth ratio compared with control *(Table 4*; $P<0.05$). Jejunal crypt depth was increased by *T. kotschyanus* extract in comparison with control ($P<0.05$). Villus surface area

Table 3. *Effect of different thyme species extracts on growth performance of broilers during different weeks of age and throughout the experimental periods*

Period	Parameters	Treatments					Statistics	
		Control	*T. daenensis*	*T. kotschyanus*	*T. lancifolius*	*T. transcaspicus*	SEM	*P* Value
Day 1 to 7	WG (g)	104.70	104.40	108.12	110.83	106.57	3.013	0.559
	FI (g)	146.00	142.75	146.00	144.16	141.50	2.385	0.607
	FCR	1.40	1.37	1.35	1.30	1.33	0.033	0.339
Day 8 to 14	WG (g)	231.17	236.75	229.75	236.80	241.27	5.073	0.514
	FI (g)	346.50	353.25	340.00	345.50	355.50	5.243	0.275
	FCR	1.50	1.49	1.48	1.46	1.47	0.034	0.464
Day 15 to 21	WG (g)	367.30	386.85	372.73	363.33	371.70	7.566	0.285
	FI (g)	495.75	487.00	494.00	500.00	497.25	7.563	0.794
	FCR	1.35	1.27	1.33	1.38	1.34	0.036	0.284
Day 22 to 28	WG (g)	561.00 ^{b}	605.13 ^{a}	555.38 ^{b}	567.10 ^{b}	567.10 ^{b}	10.18	0.027
	FI (g)	826.50^{a}	849.50 ^{a}	825.50^{a}	779.25 ^{b}	830.75 ^{a}	12.37	0.015
	FCR	1.47 ^{a}	1.40 ^{ab}	1.49 ^{a}	1.37 ^{b}	1.47 ^{a}	0.028	0.043
Day 1 to 28	WG (g)	1263.43^{b}	1333.03^{a}	1265.93 ^{b}	1278.05^{b}	1284.13 ^{b}	15.37	0.037
	FI (g)	1815.00	1832.25	1805.25	1768.50	1825.25	16.89	0.120
	FCR	1.43	1.37	1.43	1.38	1.42	0.019	0.139

^{a,b} Means with different superscripts in the same row differ significantly (P≤.05); SEM: Standard error of means

Table 4. *Effects of different thyme species extract on broilers jejunal mucosal morphometry at 28 days of age*

Parameters	Treatments					Statistics	
	Control	*T. daenensis*	*T.kotschyanus*	*T. lancifolius*	*T. transcaspicus*	SEM	*P* Value
VH (µm)	865.75^{b}	1101.00^{a}	640.25^{b}	1036.75^{ab}	1098.00^{ab}	26.63	0.0001
CD (µm)	151.75 ^{b}	148.75 ^{b}	168.00 ^{a}	161.50 ^{ab}	159.75 ^{ab}	4.21	0.036
VS (mm^{2})	0.08^{c}	0.09^{b}	0.11 ^{a}	0.12 ^{a}	0.11 ^{a}	0.004	0.0001
VH to CD	5.72^{c}	7.43^{a}	5.61^{c}	6.43^{bc}	6.90^{ab}	0.286	0.0017
TML (µm)	152.75	161.250	175.75	165.50	171.25	5.91	0.109

^{a,b} Means with different superscripts in the same row differ significantly (P≤0.05); SEM: Standard error of means; VH: Villus height; CD: Crypt depth; VS: Villus surface area; VH to CD: Villus height to Crypt depth; TML: Thickness of muscle layer

Table 5. *The effect of different thyme species extract on apparent digestibility of nutrients (%) at 28 days of age*

Parameters*	Treatments					Statistics	
	Control	*T. daenensis*	*T. kotschyanus*	*T. lancifolius*	*T. transcaspicus*	SEM	*P* Value
DM	69.40 ^{bc}	73.57 ^{a}	68.39 ^{cd}	70.38 ^{b}	67.61 ^{d}	0.429	0.0001
OM	72.35 ^{bc}	76.09 ^{a}	71.51 ^{cd}	73.47 ^{b}	70.93 ^{d}	0.403	0.0001
EE	68.34 ^{c}	73.37 ^{a}	69.70 ^{bc}	71.87 ^{ab}	67.49 ^{c}	2.466	0.0002
NR	67.08 ^{abc}	70.10 ^{a}	63.53 ^{c}	68.19 ^{ab}	64.61 ^{bc}	1.431	0.026
CC	74.07 ^{bc}	78.91 ^{a}	73.69 ^{bc}	75.12 ^{b}	73.28 ^{c}	0.532	0.0001
ASh	32.39 ^{b}	39.86 ^{a}	27.04 ^{cd}	29.46 ^{bc}	23.23 ^{d}	1.295	0.0001

^{a,b} Means with different superscripts in the same row differ significantly (P≤.05); DM: Dry Matter; OM: Organic Matter; EE: Ether Extract; NR: Nitrogen Retention; CC: Crude Carbohydrate; SEM: Standard error of means

Table 6. *The effect of different thyme species extract on immune response of chickens to Newcastle disease (ND) vaccine and sheep red blood cell (SRBC) injection*

Parameters	Treatments					Statistics	
	Control	*T. daenensis*	*T. kotschyanus*	*T. lancifolius*	*T. transcaspicus*	SEM	*P* Value
ND titre (14 days)	2.25	2.00	3.00	2.00	2.00	0.270	0.319
Total Ig^{a} (28 days)	4.87	5.37	5.37	5.37	4.75	0.378	0.612
Ig G^{a} (28 days)	2.13	2.13	2.50	2.75	1.87	0.389	0.534
Ig M (28 days)	2.75	3.25	2.87	2.63	2.87	0.442	0.889

^{a} Inresponse to sheep red blood cells injection; SEM: Standard error of means

was increased by all thyme extracts (P<0.05). There were no significant differences between thyme extracts on the thickness of muscle layer.

Nutrients Digestibility

Interestingly, the highest digestibility of dry matter, organic matter, ether extract, crude carbohydrate and crude ash (P<0.05) was observed as the effect of *T. daenensis* extract *(Table 5)*.

Digestibility of ether extract was similar between *T. daenensis*and *T. lancifolius* extracts. Nitrogen retention was not changed significantly by different thyme extract compared with control.

Immune Response

Antibody titres against ND vaccine, total antibody titre, Ig G and Ig M were not significantly affected by the treatments *(Table 6)*.

DISCUSSION

Growth performance of broiler chickens was not influenced by thyme extract during the first three weeks of age. Such results have been also observed by other researchers who used thyme extract in drinking water [33] and diet [34] of chickens, and found no significant changes in performance parameters. However, at fourth weeks of age and within the experimental period, among four thyme extracts, *T. daenensis* extract improved birds growth response and *T. lancifolius* decreased FI and FCR compared with control. To compare the obtained results, there were no available reports on different thyme species supplementation in broilers diet. However, similar results have been reported by supplementation of a mixed essential oil, including thyme oil, to drinking water of quails [35]. There are contradictory effects of thyme on broiler performance in literatures. Beside the reports indicating affectless of thyme product, as mentioned above, in agreement with beneficial effect of one thyme extract in this study, there are some observations representing the positive effect of thyme on broilers growth response. Al-Kassie [1] used 100 and 200 ppm thyme essential oils and observed improved WG. Such finding was also reported by Hernandez et al.[36] using 5000 ppm *Labiatae* extract including thyme.

Most of herbal bioactive components leave their effects on broiler performance through their antimicrobial activity [5]. At an early stage of life, the population of microorganisms in digestive tract of broilers is limited and then develops with birds age [37,38]. For instance, the increases of *Streptococcus*, *Enterobacteriaceae* and *Clostridium perfringens* [37], Coli-forms, *Clostridium* species and total anaerobic bacteria numbers [38] have been reported. In digestive tract, the microorganisms compete with the host to get the nutrients such as carbohydrates and amino acids [39]. Thus, it seems that any factor could

eliminate the competition between the bacteria and the host, for example, by decreasing intestinal microbial population, can increase feed efficiency for the host. The antimicrobial property of thyme main active components including carvacrol and thymol has been illustrated [11,40]. Moreover, the differences in quantity of the active components between different thyme species have also been proven [7,9]. Therefore, the different growth response of broilers to the studied thyme species would be acceptable and a part of the beneficial effect of *T. daenensis* extract compared with other extracts on performance of aged birds might be explained due to relatively high total phelolics *(Table 1)*. The same explanation might be applied to different intestinal morphometry and nutrients digestibility as the effect of supplemental extracts because of their probable different phytochemicals *(Table 4* and *Table 5)*.

In contrast to some studies which used thyme phytochemicals [1,11] and observed their growth promoting effect, in this study, thyme extracts failed to show much improvement in growth response of broilers, except one case. The probable reason behind this observation might be related to housing system. Because in the mentioned studies, the birds were raised in deep litter pens, while in the present trial the wire floor cages were used for raising. It is well known that in deep litter systems, birds directly contacts with litter microorganisms which can affect their intestinal microbial population [41]. However, in cages the birds cannot access to the litter as an infection source.

Villus height to crypt depth ratio is considered as a health indicator for the gut. Increased crypt depth and decreased the ratio means high nutrients demand for intestine maintenance [42]. Therefore, the higher ratio indicates that more nutrients have been directed to body growth. Moreover, increased digestion and absorption of nutrients can also be expected by higher villus height and villus surface area. The higher digestibility of nutrients by *T. daenensis* extract compared with the control *(Table 5)* confirms this concept. Hence, the improved weight gain of birds fed with this extract might be described by this explanation too. The increased nutrients digestibility as the effect of thyme extracts have also been reported by other researchers [37,43].

High villus height induced by thyme extract, specifically *T. daenensis*, might be related to the presence of high antioxidant agents in this extract *(Table 1)*. Furthermore, thyme extract contains essential oils such as thymol and carvacrol which their antioxidant activity, similar to alpha tocopherol and butylated hydroxytoluene, has been elicited [4]. It is likely that the compounds with antioxidant property can trigger villus development by preventing the damage of free radicals produced from digestion processes. Moreover, it could be mediated by anti microbial activity of thyme phytochemicals [5-7]. Because shorter intestinal villi height and lower cell mitosis have been observed due to bacterial toxins such as ammonia [44].

In keeping with our results, application of thyme extract [16], thyme powder [45] and thyme essential oil [46] in broiler diet did not revealed any significant effects on antibody titre against ND vaccine. The finding on SRBC is similar to that obtained by Toghyani et al.[45], who also did not observe any improvement in broiler humoral immune response using thyme powder in the diet. It is noted that the plants rich in flavonoids such as thyme can stimulate immune system through increasing vitamin C activity and antioxidant properties [14]. The lack of positive result of using thyme extract in this experiment on immune response is probably related to the dose of supplemental extract [3,45] and absence of secondary challenge with SRBC [47]. Furthermore, preparing relatively high hygiene condition in wired cages for broilers might be another possible reason for our findings on bird's immunity. It is shown that the influence of essential oils is more pronounced in microbial challenged broiler chickens [48].

In summary, it can be concluded that the response of broilers to dietary inclusion of thyme extracts depends on the plant genotype. The role of bird age was also illustrated in this regard, as the response was more pronounced after 3 weeks of age. The improvement in broiler performance as the effect of thyme extract was well related to improved intestinal mucosal architecture and increased nutrients digestibility.

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