Environ Sci Pollut Res (2017) 24:15816–15826
DOI 10.1007/s11356-017-9251-7

RESEARCH ARTICLE

Impact of thyme powder (*Thymus vulgaris L.*) supplementation on gene expression profiles of cytokines and economic efficiency of broiler diets

Fardos A. M. Hassan[1] · **Ashraf Awad**[1]

Received: 26 May 2016 / Accepted: 10 May 2017 / Published online: 23 May 2017

Abstract This study was conducted in an attempt to evaluate the impact of thyme powder supplementation on broiler diets with respect to cytokine and mucin2 gene expressions. It was also our aim to evaluate the growth performance, blood biochemical and hematological profiles, and economic efficiency of the diet. A total of 120 1-day old chicks (Cobb 500) were divided into four groups on the basis of the diet. One group received a basal diet (control) while the others received a basal diet supplemented with 2, 5, or 8 g/kg of thyme powder. At 42 days of age, the chickens were weighed and euthanized, and then blood and tissue samples were collected for the purpose of analysis. Results obtained clearly indicated that thyme supplementation of the diet, especially at 5 g/kg, resulted in a significant increase in body weight and caused the increased body weight gain and feed intake ($P < 0.05$) as well as augmented WBC and lymphocyte count and IgG and NO levels ($P < 0.001$). The economic evaluation showed that birds fed on 8 g/kg thyme yielded the lowest net revenue and highest feed cost to produce 1 kg of live weight compared to the other treatments, while birds fed on 2 and 5 g/kg thyme did not differ significantly from values obtained for the controls. The lipid profile of the broilers was affected by thyme supplementation at 2 and 5 g/kg as represented by a significant decrease in serum cholesterol levels as well as low density lipoprotein levels, which is known to be associated with a corresponding increase in high-density lipoprotein ($P < 0.001$). Furthermore, supplementation of thyme downregulated the pro-inflammatory mediators and increased the expression of mucin2 mRNA in the jejunum of chickens. It can be concluded that thyme supplementation in the diet of broilers at 5 g/kg has the potential to favorably influence productive performance via an improvement in the immune status of the broiler.

Keywords Cytokines · Gene expression · Growth · Costs · Thyme · Broiler

Responsible editor: Philippe Garrigues

✉ Ashraf Awad
ashrafgenetic20000@yahoo.com

[1] Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

Introduction

Phytogenic feed additives (PFAs) or their products, such as cold pressed oil, essential oils, and their extracts, are indispensable in the field of animal and poultry production because of their positive impact with respect to improving live body weight, body weight gain, feed conversion ratio, immune response, antioxidant status, carcass traits, and quality along with lowered morbidity and mortality rates (Ashour et al. 2014; Farag et al. 2014; Alagawany et al. 2015a, b; Dhama et al. 2015). Supplementation of herbs in the form of PFA has the potential to benefit broiler health and performance through its antioxidant activity (Hoffman-Pennesi and Wu 2010), antimicrobial potency (Steiner 2009), and digestion enhancement through stimulation of endogenous enzymes such as proteases, amylases, and lipases (Al-Khdri 2013).

Thyme (*Thymus vulgaris L.*) is a popular herb grown predominantly in the Mediterranean regions. It is a plant that has recently been receiving a great deal of interest largely due to its medicinal properties. Thyme is used in poultry nutrition in the form of herbal feed additive as it is known that its contents, such as thymol and carvacrol, have a positive impact on broiler performance and feed utilization, which in turn results in enhanced economic profits. This improvement in performance can be attributed to activation of the digestive system structure

and function which causes an enhanced absorption and metabolism of the nutrient supplement and its ability to alter the gut microbiota (Abd El-Hack et al. 2016). Apart from thymol and carvacrol, essential oil of thyme has also been shown to possess potent antioxidant (Grigore et al. 2010), antimicrobial (Windisch et al. 2008), and anti-inflammatory activity (Fachini-Queiroz et al. 2012).

Cytokines are tiny regulatory proteins that are created by immune cells; these proteins are known to play an important role in processes such as differentiation, maturation, and activation of the immune system. Cytokines can either exert a pro-inflammatory or an anti-inflammatory effect or both, depending on the specific local microenvironment. They are also well established as critical mediators that bridge the innate and adaptive immune systems (Su et al. 2012). Experimental data obtained in various animal models suggest that the beneficial effects of the plant extracts can be mediated through their effects on the cytokine production system (Rogler and Andus 1998; Fournout et al. 2000).

The intestinal tract is lined with a mucus layer composed of mucin glycoproteins secreted by goblet cells. The mucus layer plays an essential role in protecting the gut against acidic chyme, digestive enzymes, and pathogens. Also, it acts as lubricant and facilitates nutrition, transport between the luminal contents and the epithelial cells (Montagne et al. 2004). Mucin2 is the major intestinal mucin gene; it was initially isolated from a human jejunum complementary DNA (cDNA) library (Sadasivan et al. 2011).

Recently, the inclusion of PFA in livestock species for the purpose of supplementing gut health and positively impacting performance has received a great deal of attention and concern (Ashour et al. 2014). However, reports that investigate or analyze the molecular basis through which herbal plants exert their effect on immune status and digestion efficiency in the broiler system are few and far between (Bozkurt et al. 2014). The current study was conducted in an attempt to fill this lacuna by evaluating the effects of introducing thyme powder in broiler diets as defined by changes in the cytokines and the mucin2 gene expression profile. In addition, it is also attempting to evaluate the performance, carcass traits, blood hematological and biochemical parameters, and economic efficiency of thyme supplementation.

Materials and methods

This study was performed at the Poultry Research Farm, Faculty of Veterinary Medicine, Zagazig University, Egypt. All of the experimental procedures were carried out in accordance with the regulations of the Local Experimental Animals Care Committee and approved by the institutional ethics committee.

Birds, diets, and management

A total of 120 1-day-old male Cobb 500 chicks (44.61 ± 1.26 g) obtained from the United Poultry Company, Egypt, were used in this study. A mash corn and soybean-based feed was provided to all groups. The basal corn-soya bean meal diets in both starter and grower periods (Table 1) were formulated to meet the broiler requirements as per NRC recommendations (1994). Feed and water were supplied ad libitum during the entire experimental period. The chicks were raised in floor pens containing fresh wood shavings to a depth of 5 cm and exposed to 24 h of constant light (12 h on day light plus an equivalent amount of artificial lighting through 100-w bulbs). The environmental temperature was about 32 °C during the first week, which was gradually decreased so as to reach 25 °C by day 21 and thereafter kept constant till the end of the experiment. All chicks were vaccinated against Gumboro and Newcastle, as per the vaccine manufacturer's instructions.

Experimental design

The chicks were indiscriminately divided into four experimental groups of 30 chicks each. Each group was composed of three replicates of ten chicks each. The dietary treatments included a (1) control group (fed basal diet with no supplement), (2) basal diet with 2 g/kg of thyme powder, (3) basal diet with 5 g/kg of thyme powder, and (4) basal diet with 8 g/kg of thyme powder. The experiment was conducted till the chicks were about 42 days of age. Thyme powder was obtained as a commercial product from the Free Trade Company, Egypt, and mixed with premix during ration formulation.

Analysis of thyme powder components

The essential oils were extracted from thyme powder by hydrodistillation in a Clevenger-type apparatus for 2 h as described in the European Pharmacopoeia (1975). The obtained oils were analyzed by gas chromatography/mass spectrometry (GC-MS) using an agilent 6890/5973 GC-MS (Hewlett-Packard, Palo Alto, CA, USA) according to Vasudeva and Sharma (2012), while the phenolic compounds were extracted according to Park et al. (2012) and identified by high performance liquid chromatography using an agilent 1100 HPLC (Hewlett-Packard, CA, USA) according to Ozkan and Ozcan (2014).

Performance traits

All chicks were weighed individually on day 1 (initial body weight) and on day 42 (final body weight). The feed intake (FI) was measured over this period on day 42 per replicate. The feed was withheld for 2 h before weighing the birds. The

Table 1 Ingredients and chemical composition of the basal diet (as fed basis)

Ingredients %	Starter (0 to 21 days)	Grower (21 to 42 days)
Yellow corn	54.57	61.08
Soybean meal, 44%	27.00	22.70
Corn gluten, 60%	5.00	5.00
Fish meal, 66%	5.00	3.00
Soybean oil	5.00	5.00
Calcium carbonate	1.40	1.55
Cal. dibasic phosphate	1.20	0.90
Sodium chloride	0.30	0.30
Premix[a]	0.30	0.30
Anticoccidial	0.02	0.02
Antimycotoxin	0.10	0.10
DL-methionine, 98%	0.11	0.01
L-lysine, 78%	0.00	0.04
Calculated chemical composition[b]		
Metabolizable energy, Kcal/kg	3183	3253
Crude protein, %	22.72	20.10
Ether Extract, %	7.61	7.72
Crude fiber, %	3.20	3.03
Calcium, %	1.01	0.91
Available phosphorus, %	0.46	0.35
Lysine, %	1.17	1.00
Methionine, %	0.55	0.39
Cysteine	0.36	0.33
Methionine + cystine	0.91	0.73

[a] Supplied per kg of diet: vitamin A, 12,000 IU; vitamin D3, 2200 IU; vitamin E, 26 IU; vitamin K3, 6.25 mg; vitamin B1, 3.75 mg; vitamin B2, 6.6 mg; vitamin B6, 1.5 g; pantothenic acid, 18.8 mg; vitamin B12, 0.31 mg; niacin, 30 mg; folic acid, 1.25 mg; biotin, 0.6 mg; Fe, 50 mg; Mn, 60 mg; Cu, 6 mg; I, 1 mg; Co, 1 mg; Se, 0.20 mg; Zn, 50 mg; choline chloride, 500 mg

[b] Calculated according to NRC (1994) tables

body weight gain (BWG) was calculated by subtracting the final weight from the initial weight of chicks. Feed conversion rate (FCR) was calculated for each replicate as feed intake divided by body weight gain (Wanger et al. 1983).

Sample collection and carcass traits

At the end of the experimental period, six birds from each group with an average body weight for the respective group were selected for blood sampling and slaughter, before which they were fasted for 12 h. Blood samples were harvested from the brachial vein of birds, with one part being used in a gel activator tube for separation of serum by centrifugation (3000 rpm; 15 min; 4 °C), and other parts stored in a vacutainer tube containing EDTA as an anticoagulant. Chickens were weighed and euthanized prior to being manually feathered and eviscerated. Giblets (gizzard, liver, and heart), lymphoid organs (thymus gland and spleen), eviscerated carcass, and breast muscle were weighed and their percentages were calculated relative to live body weight. The jejunum was rapidly excised and flushed with ice-cold phosphate-buffered saline (PBS). A 2-cm portion of the jejunum was snap-frozen in liquid nitrogen for RNA isolation.

Economic efficiency of diet

The economic efficiency of the experimental diet was calculated from the input-output analysis as per the prevailing market price of the experimental diets and broiler live body weight at the time of the experiment. This was done as mentioned below:

Total feed cost = total feed intake per bird × cost of 1 kg diet.

Feed cost/kilogram weight gain = feed conversion × cost of 1 kg diet (Tag-El-Din et al. 1999).

Net revenue/kilogram gain = revenue/kilogram gain (price/kilogram meat) − feeding cost/kilogram gain

Economic efficiency = net revenue/feed cost per kilogram gain.

Hematological evaluation

The whole blood sample was used to determine the total red blood cells (RBCs), hematocrit (HCT), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, lymphocytes, and heterophils as per the method previously described by Feldman et al. (2000). A Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy) was used for the purpose. Total leukocyte count was determined using an automated analyzer while differential leukocyte counts were calculated using the manual method described by Dacie and Lewis (1991).

Serum and immunological parameter analysis

Serum total protein, albumin, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and immunoglobulin G (IgG) levels were analyzed using a commercially available diagnostic kit (Spinreact Co., Santa Coloma, Spain). Globulin levels were calculated by subtracting the albumin value from total protein value and expressed in grams per deciliter. Serum concentrations of nitric oxide (NO) were measured using a

commercially procured ELISA kit as per the manufacturer's instructions.

Cytokines and mucin2 gene expression in jejunum by qReal-Time PCR

Total RNA was extracted from the jejunum using the GeneJET RNA Purification Kit (Fermantus, UK) as per the manufacturer's protocol. The concentration and purity of total RNA were estimated through a GeneQuant spectrophotometer (Pharmacia Biotech, Freiburg, Germany). Total RNA was reverse transcribed into cDNA using a Quantitect® Reverse Transcription kit (Qiagen, Germany) in accordance with the manufacturer's instructions. Real-time PCR was performed using a Rotor-Gene Q instrument (Qiagen, Germany) and QuantiTect® SYBR® Green PCR kit (Qiagen, Germany) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) acting as the internal control gene. Chicken MUC2, GAPDH, IL6, TNF-α, IFN-γ, and NF-κB P50 were amplified using gene-specific primer sequences (Table 2).

The qRT-PCR was performed in a reaction volume of 20 μl. The composition of the reaction mix was as follows: 10 μl of SYBR Green PCR master mix (Qiagen, Germany), 1 μl of forward primer (10 μM), 1 μl of reverse primer (10 μM), 2 μl of cDNA, and 6 μl of ddH2O. The cycling parameters were as follows: 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 15 s at 60 °C, and 15 s at 72 °C. Melt-curve analysis was performed (from 65 to 95 °C, using 0.5 °C temperature increments with 5 s hold in each step) in triplicate. Relative fold change in expression of target genes was calculated using the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). ΔΔCT is the difference between the mean ΔCT of the treatment group and that of the control group where ΔCT is the distinction between the mean CT of the gene of interest and the mean CT of the internal control gene for every sample.

Statistical analysis

Data resultant from the study was analyzed using the SAS statistical system (SAS Institute 2003). Data were analyzed using one-way analysis of variance (ANOVA), after verifying normality using the Kolmogorov-Smirnov test. The model used for data analysis was as follows: $Y_{ij} = \mu + A_i + e_{ij}$, where Y_{ij} =observed value for a particular character, μ = overall mean, A_i = effect of the ith treatment, and e_{ij} = random residual error term. Tukey's HSD multiple comparison test was used to test the significance of the differences between the mean values. Variability in the data was expressed as the pooled SEM and the alpha level for determination of significance was 0.05.

Results

Thyme powder components

The analysis of thyme powder showed that it is very complex natural mixtures which contain many components of essential oils at different concentrations. The major essential oils were thymol (50.48%), γ-terpinene (11.03%), P-cymene (9.77%), and carvacrol (4.30%). The main phenolic acids in thyme extract were salicylic acid (2450.03 ppm), ellagic acid (1240.42 ppm), benzoic acid (440.08 ppm), chlorogenic acid (330.50 ppm), caffeic acid (136.55 ppm), gallic acid (41.22 ppm), vanillic acid (30.12 ppm), and cinnamic acid (18.66 ppm), while the main flavonoid compounds were hesperidin (35,598.0 ppm), rosmarinic (8650.08 ppm), quercitrin (4617.73 ppm), narerigin (1850.48 ppm), rutin (233.52 ppm), quercetin (490.33 ppm), narenginin (210.03 ppm), kaempferol (950.15 ppm), epicatechin (355.22 ppm), and luteolin (89.96 ppm).

Table 2 Primer sequences used for qRT-PCR analysis

Target gene	Sequences (5 → 3)	Size	Accession no.
MUC2	F: CTGTTGTGGATGGGCGGATTG R: CCAAACTTGCTGTCCAGCTCC	157	XM_421035.2
GAPDH	F: ACATCATCCCAGCGTCCA R: CATCAGCAGCAGCCTTCAC	189	NM_204305
IL6	F:GAAATCCCTCCTCGCCAATC R:CCCTCACGGTCTTCTCCATAAAC	107	NM_204628
TNF-α	F:GAAGCAGCGTTTGGGAGTG R:GTTGTGGGACAGGGTAGGG	203	NM_204267
IFN-γ	F:GCTGACGGTGGACCTATT R:CACCTTCTTCACGCCATC	198	NM_205149
NF-κB P50	F:TGCGTCTTATGTTTACTGCCTTTC R:CCGCTGTCCTGTCCATTCTTA	145	D13719.1

F forward primer, *R* reverse primer, *MUC2* mucin2, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon, *NF-κB* nuclear factor kappa B

Effect of dietary thyme powder on performance traits

As shown in Table 3, broilers fed on a diet that had been supplemented with 5 g/kg thyme recorded significantly ($P = 0.017$) higher body weights at 42 days of age as compared to those of the control group. Birds fed on 2 and 8 g/kg thyme did not differ significantly from values obtained for the controls. At 42 days of age, the highest BWG was attained by broilers that received thyme powder at 5 g/kg, while birds receiving 2 and 8 g/kg thyme exhibited a non-significant increase in BWG in comparison with the control birds. The FI of birds fed on 5 g/kg thyme showed a significant increase ($P = 0.034$) as compared to the group that received the control diet (Table 3). It is to be noted that in the case of FCR, supplementing broiler diet with different concentrations of thyme powder had no significant effect when compared with the control group ($P > 0.05$).

Effect of dietary thyme powder on carcass traits and relative weights of lymphoid organs

Data regarding characteristics of the carcass and immune organ weights are presented in Table 4. No significant differences ($P > 0.05$) were observed between the different treatment groups with respect to the percentages of carcass, breast, and giblet organs (liver, gizzard, heart). In addition, the results showed non-significant differences amongst dietary groups in terms of the relative weight of the spleen. However, a significant difference ($P = 0.001$) was observed regarding the thymus gland relative weight, as the birds fed on 5 g/kg thyme had the highest thymus weight as compared to the other groups.

Effect of dietary thyme powder on economic efficiency of diet

Economic calculations revealed that birds fed on diets containing 8 g/kg thyme recorded the highest feed cost to produce 1 kg of live weight as compared to other treatments ($P = 0.037$, Table 5). Accordingly, birds that received thyme powder at 8 g/kg thyme showed a significant decrease in net revenue and economic efficiency as compared with other treatments, while statistically similar values were observed in birds fed on diets supplemented with 2 and 5 g/kg thyme compared with the group on the control diet.

Effect of dietary thyme powder on hematological parameters

The effect of dietary thyme on blood-based parameters of broilers is listed in Table 6. Blood constituents were not significantly ($P > 0.05$) influenced by thyme supplementation except for WBC and lymphocyte counts, which were significantly higher ($P < 0.001$) in the thyme-supplemented dietary groups, especially in case of the 5 g/kg, as compared with control.

Effect of dietary thyme powder on serum and immunological parameters

The influence of thyme on total protein, albumin and globulin was observed to be less than significant at all levels of dietary supplementation ($P > 0.05$). However, the results of the lipid profile analysis showed a significant decrease in the total cholesterol level for birds fed on 5 g/kg thyme (149.03, $P = 0.001$) when compared to other treatments. It was also observed that thyme at levels of 2 and 5 g/kg resulted in a significant increase in HDL-cholesterol concentrations (45.21, 44.67, respectively, $P = 0.002$) and a significant decrease in LDL-cholesterol concentrations (90.30, 80.17 respectively, $P < 0.001$) as compared to the control group. Besides, no significant differences were detected in triglyceride concentration. The IgG value showed a significant increase in birds fed on thyme-supplemented diets in comparison to control ($P < 0.001$). NO level exhibited an elevation, which was statistically significant in the case of birds fed diets supplemented with 5 g/kg thyme (0.185, $P = 0.036$) but less than significant in other thyme dietary groups compared to control (Table 7).

Table 3 Effect of dietary thyme powder on the performance traits of broiler chickens

Parameters	Control	Thyme, g/kg of control diet			SEM	*P* value
		2	5	8		
Body weights, g						
Initial	44.60	44.65	44.72	44.45	0.126	0.892
Final	2704.9^{b}	2787.8^{ab}	2835.0^{a}	2719.9^{ab}	17.47	0.017
Body weight gain, g	2660.3^{b}	2743.1^{ab}	2790.2^{a}	2675.4^{ab}	18.04	0.032
Feed intake, g	4217.4^{b}	4272.2^{ab}	4363.4^{a}	4305.4^{ab}	19.94	0.034
Feed conversion ratio	1.58	1.55	1.56	1.61	0.010	0.156

Different superscript letters within the same row are significantly different ($P < 0.05$)

SEM standard error of the mean

Table 4 Effect of dietary thyme powder on the carcass traits and relative weights of lymphoid organs (% of body weight) of broiler chickens

Parameters	Thyme, g/kg of control diet				SEM	*P* value
	Control	2	5	8		
Carcass, %	77.92	78.09	77.55	78.10	0.363	0.957
Breast, %	48.34	48.31	47.49	48.07	0.281	0.726
Liver, %	1.96	1.97	2.15	1.91	0.071	0.548
Heart, %	0.46	0.47	0.46	0.46	0.012	0.991
Gizzard, %	1.85	1.53	1.64	1.77	0.075	0.474
Spleen,%	0.13	0.12	0.12	0.13	0.004	0.804
Thymus, %	0.34^{b}	0.35^{b}	0.40^{a}	0.34^{b}	0.007	0.001

Different superscript letters within the same row are significantly different ($P < 0.05$)

SEM standard error of the mean

Effect of dietary thyme powder on cytokine transcripts and mucin2 genes expression

Relative changes in the messenger RNA (mRNA) transcript levels for cytokines (IL6, TNF-α, IFN-γ, NF-κB P50) as well as mucin2 gene are presented in Fig. 1. The results clearly demonstrated that the broilers whose diet was supplemented with varying amounts of thyme exhibited a decreasing tendency towards the production of pro-inflammatory cytokines when compared to the control group. There were no significant differences observed in the relative expression levels of IFN-γ and NF-κB P50 mRNAs within the different thyme groups though TNF-α showed a distinct downregulation in the case of broilers supplemented with 5 and 8 g/kg thyme. In case of IL6 expression, a non-significant downregulation was seen in broilers whose diet was supplemented with 2 and 5 g/kg thyme while a significant decrease was seen in broilers supplemented with 8 g/kg. Regarding expression of the mucin2 gene, in comparison to the control group, a significant tendency towards upregulation was seen in the broilers supplemented with 5 and 8 g/kg thyme, while a similar but non-significant trend was observed in broilers supplemented with just 2 g/kg thyme.

Discussion

There is a developing enthusiasm in the development of herbal feed additives as growth promoters in livestock production because of the prohibition on utilization of certain antibiotics and harmful residual impacts as well as cost viability. Based on the results of our study, we propose that birds fed on diet containing thyme at 5 g/kg demonstrate superior estimates for BW and BWG. Such amelioration can be ascribed to the favorable effect of the active principles of thyme on digestion of feed, the microbial balance of the gut, and stimulation of digestive enzymes (Langhout 2000; Williams and Losa 2001). In addition, thyme can also participate in enhancing growth and diminishing the incidences of disease and mortality through its antioxidant and antibacterial impacts in the intestine (Nascimento et al. 2000). In parallel with our results, a study conducted by Toghyani et al. (2010) also reported that broilers receiving 5 g/kg thyme had a significantly higher BW at day 42 of age, while a report published by Kamali Sangani et al. (2014) claimed the opposite by demonstrating that no significant effect was recorded.

Our study also showed that thyme supplementation at 5 g/kg significantly increased the feed consumption. This positive effect can be evaluated on the basis of different perspectives as thyme, in the form of a natural feed additive, is known to improve diet palatability by influencing the main components of the thymus that stimulate appetite and the digestive process (Windisch et al. 2008). These results are in the same line with the results published by Bölükbaşi et al. (2006), Al-Kassie (2009), and Abid (2013); they showed that chicks fed with thyme supplement had significantly higher ($P < 0.05$) feed intake, and body weight gain compared with the control

Table 5 Effect of dietary thyme powder on economic efficiency of broilers (at 42 days of age)

Parameters	Control	Thyme, g/kg of control diet			SEM	*P* value
		2	5	8		
Total feed cost/bird, $	2.15^{b}	2.22^{b}	2.31^{a}	2.37^{a}	0.027	0.000
Feed cost / kg gain, $	0.81^{b}	0.81^{b}	0.83^{b}	0.89^{a}	0.006	0.037
Net revenue / kg gain, $	0.47^{a}	0.47^{a}	0.45^{a}	0.39^{b}	0.004	0.007
Economic efficiency (EEF)	0.58^{a}	0.58^{a}	0.54^{a}	0.44^{b}	0.014	0.001
Relative economic efficiency*	100.0^{a}	100.0^{a}	93.10^{a}	75.86^{b}	2.962	0.000

Cost kg^{-1} diet (including herbal cost) = 0.51$ for control, 0.52$ for 2 g/kg thyme, 0.53$ for 5 g/kg thyme, and 0.55$ for 8 g/kg thyme; price kg^{-1} meat = 1.28$. Different superscript letters within the same row are significantly different ($P < 0.05$)

SEM standard error of mean

*Assuming that the relative economic efficiency of control diet is equal to 100

Table 6 Effect of dietary thyme powder on hematological parameters of broiler chickens

Parameters	Thyme, g/kg of control diet				SEM	*P* value
	Control	2	5	8		
RBCs, 10^6/μl	2.53	2.42	2.54	2.44	0.025	0.213
Hb, g/dl	8.80	8.06	8.53	8.53	0.104	0.081
HCT, %	29.96	27.83	30.06	29.63	0.357	0.082
MCV, fl	118.27	114.93	118.60	121.37	1.104	0.198
MCH, pg	34.73	33.33	33.70	34.96	0.341	0.097
MCHC, g/dl	29.36	29.00	28.40	28.80	0.162	0.208
WBCs, 10^3/μl	19.00^c	21.30^b	23.40^a	21.00^b	0.397	0.000
Lymphocyte, 10^3/μl	12.24^c	14.42^b	15.84^a	13.87^b	0.307	0.000
Heterophil, 10^3/μl	4.63	5.00	5.52	5.17	0.144	0.234
H/L ratio	0.38	0.35	0.35	0.37	0.005	0.110

Different superscript letters within the same row are significantly different ($P < 0.05$)

SEM standard error of the mean

group. In contrast to our results, some studies have claimed that the supplementing broiler diet with thyme powder has no significant effect on feed intake during the entire period of study (Toghyani et al. 2010; Mansoub and Myandoab 2011; Ali 2014). The non-significant effect of different dietary thyme levels on FCR of broilers was in line with findings reported by Demir et al. (2008) and Kamali Sangani et al. (2014). On the other hand, Foroughi et al. (2011) and Ali (2014) found that supplementation of broiler diet with thyme had a significant positive effect on FCR of broilers. The different responses of growth performance may be due to the health status of birds, hygienic status of experimental place, environmental conditions, and diet composition (Hong et al. 2012).

In the current study the obtained data clearly demonstrated that carcass traits and relative weight of lymphoid organs were not significantly different for the treatment groups except for the relative weight of the thymus gland. These results are similar to those reported by Rahimi et al. (2011), Pourmahmoud et al. (2013), and El-Faham et al. (2015). However, studies published by Abdulkarimi et al. (2011a) and Rafiee et al. (2013) have claimed the contrary and reported significant increases in carcass yield, breast yield, and relative weight of gizzard in broilers fed on diets supplemented with thyme extract. Najafi and Torki (2010) did not observe any statistically significant effect of thyme supplementation on the relative weight of the thymus gland in broiler chicks. The results above suggest that lymphoid compartments differ in their responses to thyme residue inclusion in chicken which may be due to the different roles in the immune system. The bursas of Fabricius and thymus are sites of B cell and T cell differentiation, respectively (Abdel-Fattah et al. 2008).

Economical evaluation of the experimental diets showed a significant decrease in the net revenue and economic efficiency by dietary thyme supplementation at the level of 8 g/kg. In addition, in spite of improvement in growth by supplementing

Table 7 Effect of dietary thyme powder on serum and immunological parameters of broiler chickens

Parameters	Thyme, g/kg of control diet				SEM	*P* value
	Control	2	5	8		
Total protein, g/dl	2.85	3.41	3.36	3.24	0.081	0.052
Albumin, g/dl	1.37	1.44	1.49	1.46	0.016	0.063
Globulin, g/dl	1.48	1.97	1.87	1.78	0.096	0.298
Cholesterol, mg/dl	171.40^a	158.43^{ab}	149.03^b	170.03^a	2.498	0.001
Triglyceride, mg/dl	104.96	107.94	104.31	105.23	1.381	0.821
HDL cholesterol, mg/dl	37.81^b	45.21^a	44.67^a	41.17^{ab}	0.864	0.002
LDL cholesterol, mg/dl	112.60^a	90.30^b	80.17^b	107.82^a	3.048	0.000
IgG, mg/dl	7.20^b	8.80^a	9.30^a	8.71^a	0.181	0.000
Nitric oxide	0.148^b	0.181^{ab}	0.185^a	0.171^{ab}	0.005	0.036

Different superscript letters within the same row are significantly different ($P < 0.05$)

SEM standard error of the mean

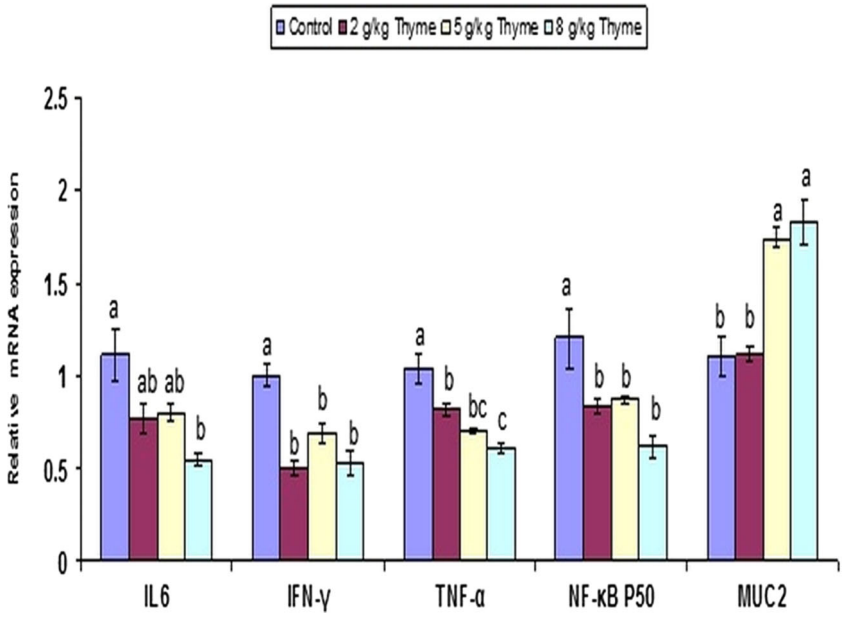

Fig. 1 Effect of dietary thyme powder on the relative expression of IL6, IFN-γ, TNF-α, NF-κB P50, and MUC2 mRNA in the jejunum of broilers. The expression abundance of gene mRNA was normalized against the internal control gene GAPDH using quantitative real-time PCR technique. *Each bar carrying different letters* (*a*, *b*, *c*) was significantly different ($P < 0.05$) (mean ± SE, $n = 3$)

thyme at 5 g/kg diet, there is no significant impact on net revenue and economic efficiency. This is due to the fact that improvement in growth occurred along with significant concurrent increase in the total feed cost. These results are in disagreement with a report by Osman et al. (2010) who claimed that the inclusion of thyme feed additives in broiler diets demonstrated the least cost/kilogram gain and the highest percentage of economic efficiency when compared with that of an un-supplemented diet.

Blood parameters are a good benchmark for judging the health of an animal and are broadly viewed as crucial indicators of the nutritional and physiological status of birds and animals (Abd El-Hack and Alagawany 2015). The results of the hematological analysis showed that dietary thyme supplementation in broiler diets at different levels did not induce any statistically significant effect on RBCs, HCT, Hb, MCV, MCH, and MCHC levels. Similar to our results, Toghyani et al. (2010) have also reported that thyme supplementation failed to elicit any statistically significant impact on the hematological parameters of broilers. In contrast, studies conducted by Saleh et al. (2014) and Jameel et al. (2014) found significant increases in RBCs, Hb, and PCV levels in birds fed on diets that were supplemented with thyme powder. With respect to the total and differential leukocyte count, our results indicated that dietary thyme supplementation at 5 g/kg improved the WBC and lymphocyte count while no such significant difference was observed for heterophil count and H/L ratio. This is in agreement with Jameel (2008), Al-Kassie and Jameel (2009), and Najafi and Torki (2010), who reported that using thyme in broiler diets resulted in significantly elevated total leukocyte and lymphocyte count as compared to the control group. In direct opposition, Toghyani et al. (2011) claimed that dietary thyme supplementation in broiler diets did not induce any significant impacts on WBCs and lymphocyte counts. Souri et al. (2014) concluded that the addition of 1% thyme extract in drinking water of broilers significantly increased heterophil percentage as well as the H/L ratio while reducing the lymphocyte percentage. In former studies, it was accounted for that medicinal herbs and their ingredient could activate such immune functions as lymphocyte proliferation, phagocytosis, and boost WBC counts (Hashemipour et al. 2013; Alagawany et al. 2015b).

A strong correlation is known to exist between hematopoietic and immune cells and the ability to guard against foreign pathogens and stressors. As a result of this, there is a growing need to induce hematological and immunological improvement by using various immunostimulants. A large number of plants are known to have immunomodulatory role and have been used to present an alternative to ordinary chemotherapy for a wide range of diseases particularly those related to immunodeficiency (Kumar et al. 2011; Farag et al. 2014). In the poultry industry, it is critical to hoist the immune system in order to decrease or prevent the infectious disease occurrence. Utilizing immune promoters is a pivotal solution to improve immune status and reduce the susceptibility to infectious disease in poultry farms. PFAs that are rich in flavonoids are known to extend the biological activity of vitamin C, function as an antioxidant, and have the potential to upgrade immune function (Acamovic and Brooker 2005). Adding thyme to broiler diets may improve the immune system by virtue of elevating the IgG and NO levels.

The use of different levels of thyme did not appear to have any significant effect on the total protein, albumin, and globulin values. This is in line with findings published by Toghyani et al. (2010, 2011) and in direct contradiction to those reported by Hosseini et al. (2013), who claimed that

thyme in broiler diets adversely influenced serum protein and albumin concentrations. Our results regarding the lipid profile showed that dietary thyme supplementation of broiler diets decreased the total serum cholesterol and LDL and increased HDL but did not affect the triglyceride (TG) concentration. The reduction in cholesterol levels can be attributed to the negative effect of thymol and carvacrol on 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), an enzyme that catalyzes the rate limiting enzyme of cholesterol synthesis (Lee et al. 2003). Thyme contains saponins, which react with cholesterol in digesta forming insoluble complexes furthermore hinder the intestinal absorption of endogenous and exogenous cholesterol (Oakenfull and Sidhu 1990). Some studies reported that dietary thyme supplementation decreases serum cholesterol (Abdulkarimi et al. 2011b; Ali 2014) and increases HDL cholesterol (Rahimi et al. 2011) in broiler chickens. In contrast, Najafi and Torki (2010) reported that plasma total cholesterol, TG, and HDL levels were not affected by dietary thyme oil inclusion in broiler diets. Bölükbaşi et al. (2006) stated that thyme oil supplementation led to an increased plasma level of cholesterol, TG, and LDL cholesterol in broilers fed 200 mg/kg thymol.

Our results revealed that supplementation of thyme downregulated the expression of pro-inflammatory mediators in the jejunum of broilers. These results suggest that thyme has an anti-inflammatory effect as well as lowers the production of TNF-a, enterotoxins A and B, and alpha-hemolysin (Qiu et al. 2011). Moreover, thyme is able to modulate transcription factors such as NF-kB P50 that are known to play critical roles in processes such as inflammation, immunity, cell proliferation, differentiation, and survival in both in vitro and in vivo conditions (Paur et al. 2010). In addition, carvacrol is known to decrease TNF-α and IL-1b levels in intoxicated rats through the suppression of cycloxygensae-2 (COX-2) mRNA and protein thereby causing a repression of the inflammatory process (Tsai et al. 2011). This finding is in agreement with other studies that have stated that thyme extracts significantly downregulate gene expression as well as production of pro-inflammatory mediators like TNF-α, IL-1B, and IL-6 and have a significant opposite effect on anti-inflammatory cytokines such as IL-10 (Paur et al. 2010; Gulec et al. 2013; Abdel-Aziem et al. 2014; Liang et al. 2014; Abu-Raghif et al. 2015; Kara et al. 2015). Ocaña and Reglero (2012) analyzed the inflammatory effects of thyme extracts from three different species on cytokine production and gene expression. They reported that thyme extracts significantly reduced production and gene expression of the pro-inflammatory mediators TNF-α, IL-1B, and IL-6 and significantly increased the production of IL-10. Changes were dose dependent and varied as per the thyme content of each species.

Supplementation of thyme to basal diets upregulated the expression of mucin2 in the jejunum of broilers. It may be attributed to the bioactive substances present in these herbs that may affect the concentration of HapA, which is an extracellular proteinase that promotes secretion and accumulation of mucus in the digestive system. The bioactive substances may also perturb the activity of transcription factors that regulate mucin2 gene expressions in chickens (Van der Sluis et al. 2008). This finding was in line with the results of Kamali Sangani et al. (2014), who reported that the expression of the mucin2 gene was reduced in chickens that were fed the condensed diet; however, the expression was observed to increase upon supplementation with turmeric, thyme, and cinnamon.

In conclusion, our data suggest that thyme supplementation in broiler diet at the level of 5 g/kg could elicit favorable influences on growth performance and feed intake through an enhancement of mucin2 expression. Results also show that this effect can be achieved without any detrimental impact on economic profit. Moreover, the results proved that thyme supplementation can elevate the immune status of broilers through modulation of cytokine gene expression. Further studies should be conducted in order to accurately assess and evaluate the efficiency of this dietary thyme level against the impact of pathogens and other immunosuppressive stressors.

Compliance with ethical standards All of the experimental procedures were carried out in accordance with the regulations of the Local Experimental Animals Care Committee and approved by the institutional ethics committee.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abd El-Hack ME, Alagawany M (2015) Performance, egg quality, blood profile, immune function, and antioxidant enzyme activities in laying hens fed diets with thyme powder. J Anim Feed Sci 24:127–133

Abd El-Hack ME, Alagawany M, Farag MR, Tiwari R, Karthik K, Dhama K, Zorriehzahra J, Adel M (2016) Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. J Essent Oil Res 28(5):365–382

Abdel-Aziem SH, Hassan AM, El-Denshary ES, Hamzawy MA, Mannaa FA, Abdel-Wahhab MA (2014) Ameliorative effects of thyme and calendula extracts alone or in combination against aflatoxins-induced oxidative stress and genotoxicity in rat liver. Cytotechnology 66(3): 457–470

Abdel-Fattah SA, El-Sanhoury MH, El-Mednay NM, Abdel-Azeem F (2008) Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int J Poult Sci 7:215–222

Abdulkarimi R, Aghazdeh AM, Daneshyar M (2011a) Growth performance and some carcass characteristics in broiler chickens supplemented with thymus extract (*Thymus vulgaris*) in drinking water. Report and Opinion 3(11):26–32

Abdulkarimi R, Daneshyar M, Aghazadeh AM (2011b) Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. Ital J Anim Sci 10:e20

Abid AR (2013) Productive performance of broilers (Ross 308) diet supplemented with thyme, garlic and combination. Journal of Kerbala University 11(4):293–301

Abu-Raghif AR, Qasim BJ, Abady AH, Sahib HB (2015) Effects of aqueous thyme extract against cisplatin induced nephrotoxicity in rabbits. Int J Pharm Sci Rev Res 30(1):190–194

Acamovic T, Brooker JD (2005) Biochemistry of plant secondary metabolites and their effects in animals. Proc Nutr Soc 64:403–412

Alagawany MM, Farag MR, Dhama K, Abd El-Hack ME, Tiwari R, Alam GM (2015a) Mechanisms and beneficial applications of resveratrol as feed additive in animal and poultry nutrition: a review. Int J Pharmacol 11:213–221

Alagawany MM, Farag MR, Dhama K (2015b) Nutritional and biological effects of turmeric (*Curcuma longa*) supplementation on performance, serum biochemical parameters and oxidative status of broiler chicks exposed to endosulfan in the diets. Asian J Anim Vet Adv 10: 86–96

Ali AHH (2014) Productive performance and immune response of broiler chicks as affected by dietary thyme leaves powder. Egypt Poult Sci 34:71–84

Al-Kassie GAM (2009) Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pak Vet J 29(4): 169–173

Al-Kassie GAM, Jameel YJ (2009) The effect of adding *Thyme vulgaris* and *Cinnamomum zeylanicum* on production performance and some blood traits in broiler chicken. The Iraqi Journal of Veterinary Medicine 33(2):84–90

Al-Khdri AMA (2013) Effect of ginger (*Zingiber officinale*) and thyme (*Thymus vulgaris*) dietary supplementation on productive and immunological performance of broiler. Master thesis, Faculty of Agriculture and Forestry School of Animal Production Kurdistan Regional Government, Iraq

Ashour EA, Alagawany M, Reda FM, Abd El-Hack ME (2014) Effect of supplementation of *Yucca schidigera* to growing rabbits diets on growth performance, carcass characteristics, serum biochemistry and liver oxidative status. Asian J Anim Vet Adv 9:732–742

Bölükbaşi ŞC, Erhan MK, Özkan A (2006) Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. South African Journal of Animal science 36(3):189–196

Bozkurt M, Hippenstiel F, Abdel-Wareth AAA, Kehraus S, Küçükyilmaz K, Südekum K-H (2014) Effects of selected herbs and essential oils on performance, egg quality and some metabolic activities in laying hens—a review. Eur Poultry Sci 78:1–15

Dacie JV, Lewis SM (1991) Practical hematology, 7th edn. Churchill Livingstone, London, pp 659–661

Demir E, Kilinc K, Yildirim Y, Dincer F, Eseceli H (2008) Comparative effects of mint, sage, thyme and flavomycin in wheat-based broiler diets. Archiva Zootechnica 11(3):54–63

Dhama K, Latheef SK, Mani S et al (2015) Multiple beneficial applications and modes of action of herbs in poultry health and production—a review. Int J Pharmacol 11:152–176

El-Faham AI, Ahmed AMH, El Sanhoury MHS (2015) Thyme leaves or its extracted oil for enhancing productive and physiological status of broiler chickens. Egyptian Poultry Science Journal 35:215–236

European Pharmacopoeia (1975) Maisonneuve SA, Sainte-Ruffine. vol 3, pp 68–71

Fachini-Queiroz FC, Kummer R, Estevao-Silva CF, Carvalho MDB, Cunha JM, Grespan R, Bersani-Amado CA, Cuman RKN (2012) Effects of thymol and carvacrol, constituents of *Thymus vulgaris* L. essential oil, on the inflammatory response. Evid Based Complement Alternat Med 2012:657026 **10 pages**

Farag MR, Alagawany MM, Dhama K (2014) Antidotal effect of turmeric (*Curcuma longa*) against endosulfan-induced cytogenotoxicity and immunotoxicity in broiler chicks. Int J Pharmacol 10:429–439

Feldman BF, Zinkl JG, Jain NC (2000) Schalm's veterinary hematology, 5th edn. Lippincott, London, pp 1219–1924

Foroughi AR, Torghabeh HM, Saleh H (2011) The effect of essential oil of thyme (*Thimus vulgaris*) on to performance and humoral immune response broilers chicken. Agric J 6(6):299–302

Fournout S, Dozois CM, Odin M, Desautels C, Pérès S, Hérault F, Daigle F, Segafredo C, Laffitte J, Oswald E, Fairbrother JM, Oswald IP (2000) Lack of a role of cytotoxic necrotizing factor 1 toxin from *Escherichia coli* in bacterial pathogenicity and host cytokine response in infected germfree piglets. Infect and Immun 68(2):839–847

Grigore A, Paraschiv INA, Colceru-Mihul S, Bubueanu C, Draghici E, Ichim M (2010) Chemical composition and antioxidant activity of *Thymus vulgaris L*. volatile oil obtained by two different methods. Romanian Biotechnological Letters 15(4):5436–5443

Gulec AK, Danabas D, Ural M, Seker E, Arslan A, Serdar O (2013) Effect of mixed use of thyme and fennel oils on biochemical properties and electrolytes in rainbow trout as a response to *Yersinia ruckeri* infection. Acta Vet Brno 82(3):297–302

Hashemipour H, Kermanshahi H, Golian A, Veldkamp T (2013) Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poultry Sci 92:2059–2069

Hoffman-Pennesi D, Wu C (2010) The effect of thymol and thyme oil feed supplementation on growth performance, serum antioxidant levels, and cecal Salmonella population in broilers. J Appl Poult Res 19:432–443

Hong JC, Steiner T, Aufy A, Lien TF (2012) Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livestock Sci 144(3):253–262

Hosseini SA, Meimandipour A, Alami F, Mahdavi A, Mohiti-Asli M, Lotfollahian H, Cross D (2013) Effects of ground thyme and probiotic supplements in diets on broiler performance, blood biochemistry and immunological response to sheep red blood cells. Ital J Anim Sci 12:116–120 **e19**

Jameel YJ (2008) The effect of adding *Thyme vulgaris* and *Cinnamomum zeylanicum* on production performance and some blood traits in broiler chicken. M.V.Sc Thesis in Science of Public Health, College of Veterinary Medicine, University of Baghdad, Iraq

Jameel YJ, Abed AR, Al-Shimmary FO (2014) Influence of adding garlic and thyme and their combination on immune response and some blood parameters in broiler. Sci Agric 2(2):102–106

Kamali Sangani A, Masoudi AA, Hosseini SA (2014) The effects of herbal plants on *mucin 2* gene expression and performance in ascetic broilers. IJVM 8(1):47–52

Kara M, Uslu S, Demirci F, Temel HE, Baydemir C (2015) Supplemental carvacrol can reduce the severity of inflammation by influencing the production of mediators of inflammation. Inflammation 38(3): 1020–1027

Kumar SV, Kumar SP, Rupesh D, Nitin K (2011) Immunomodulatory effects of some traditional medicinal plants. J Chem Pharmacol Res 3:675–684

Langhout P (2000) New additives for broiler chickens. World Poult 16(13):22–27

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. Brit Poultry Sci 44:450–457

Liang D, Li F, Fu Y, Cao Y, Song X, Wang T, Wang W, Guo M, Zhou E, Li D, Yang Z, Zhang N (2014) Thymol inhibits LPS-stimulated inflammatory response via down-regulation of NF-κB and MAPK signaling pathways in mouse mammary epithelial cells. Inflammation 37(1):214–222

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4):402–408

Mansoub NH, Myandoab MP (2011) The effects of different levels of thyme on performance, carcass traits, blood parameters of broilers. Annals of Biological Research 2(4):379–385

Montagne L, Piel C, Lalles JP (2004) Effect of diet on mucin kinetics and composition: nutritionand health implications. Nutr Rev 62:105–114

Najafi P, Torki M (2010) Performance, blood metabolites and immunocompetaence of broiler chicks fed diets included essential oils of medicinal herbs. J Anim Vet Adv 9(7):1164–1168

Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazil J Microbiol 31(4):247–256

NRC (1994) Nutrient requirements of poultry, 9th revised edn. National Academy Press, Washington

Oakenfull DG, Sidhu GS (1990) Could saponins be a useful treatment for hypercholesterolemia? Eur J Clin Nutr 44:79–88

Ocaña A, Reglero G (2012) Effects of thyme extract oils (from *Thymus vulgaris*, *Thymus zygis*, and *Thymus hyemalis*) on cytokine production and gene expression of oxLDL-stimulated THP-1-macrophages. J Obes 2012:104706 **11 pages**

Osman M, Yakout HM, Motawe HF, Ezz El-Arab WF (2010) Productive, physiological, immunological and economical effects of supplementing natural feed additives to broiler diets. Egypt Poult Sci 30:25–53

Ozkan G, Ozcan MM (2014) Some phenolic compounds of extracts obtained from Origanum species growing in Turkey. Environ Monit Assess 186:4947–4957

Park SJ, Jung JM, Lee HE, Lee YW, Kim DH, Kim JM, Hong JG, Lee CH, Jung IH, Cho YB, Jang DS, Ryu JH (2012) The memory ameliorating effects of INM-176, an ethanolic extract of angelica gigas, against scopolamine-or Aβ1–42-induced cognitive dyes function in mice. J Ethnopharmacol 143:611–620

Paur I, Balstad TR, Kolberg M, Pedersen MK, Austenaa LM, Jacobs DR, Blomhoffl R (2010) Extract of oregano, coffee, thyme, clove, and walnuts inhibits NF-κB in monocytes and in transgenic reporter mice. Cancer Prev Res 3(5):653–663

Pourmahmoud B, Aghazadeh AM, Sis NM (2013) The effect of thyme extract on growth performance, digestive organ weights and serum lipoproteins of broilers fed wheat-based diets. Ital J Anim Sci 12(3):337–341

Qiu J, Zhang X, Luo M, Li H, Dong J, Wang J, Leng B, Wang X, Feng H, Ren W, Deng X (2011) Subinhibitory concentrations of perilla oil affect the expression of secreted virulence factor genes in *Staphylococcus aureus*. PLoS One 6(1):e16160. doi:10.1371/journal.pone.0016160

Rafiee A, Rahimian Y, Zamani F, Asgarian F (2013) Effect of use ginger (*Zingiber officinale*) and thymus (*Thymus vulgaris*) extract on performance and some hematological parameters on broiler chicks. Sci Agric 4(1):20–25

Rahimi S, Teymouri Zadeh Z, Karimi Torshizi MA, 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 Agri Sci Tech 13:527–539

Rogler G, Andus T (1998) Cytokines in inflammatory bowel disease. World J Surg 22(4):382–389

Sadasivan V, Narpala S, Budil D, Sacco A, Carrier R (2011) Modeling the human intestinal mucin (MUC2) C-terminal cystine knot dimer. J Mol Model 17:2953–2963

Saleh N, Allam T, Abd El-latif A, Ghazy E (2014) The effects of dietary supplementation of different levels of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Global Veterinaria 12(6):736–744

SAS, Institute (2003) SAS/STAT users guide. SAS Institute Inc., Cary, NC, USA

Souri H, Khatibjoo A, Taherpoor K, Abadi AH, Fattahnia F, Askari M (2014) Effect of *Thymus vulgaris* and *Satureja khuzestanica* ethanolic extracts on broiler chickens performance and immune response. Iranian Journal of Applied Animal Science 5(2):437–446

Steiner T (2009) Application and benefits of phytogenics in egg production. In: Steiner T (ed) Essential oils—biochemistry, production and utilisation. Phytogenics in animal nutrition, Natural concepts to optimize gut health and performance. Nottingham University Press, Nottingham, pp 157–167

Su DL, Lu ZM, Shen MN, Li X, Sun LY (2012) Roles of pro- and anti-inflammatory cytokines in the pathogenesis of SLE. J Biomed Biotechnol 2012:347141 **15 pages**

Tag-El-Din TH, Ali MA, Ismail FSA, Ali RAM (1999) Utilization of corn stalk in feeding rabbits. Egyptian Journal of Rabbit Science 9(1):25–42

Toghyani M, Tohidi M, Gheisari AA, Tabeidian SA (2010) Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr J Biotechnol 9(40):6819–6825

Toghyani M, Toghyani M, Mohammadrezaei M, Gheisari A, Tabeidian SA, Ghalamkari G (2011) Effect of cocoa and thyme powder alone or in combination on humoral immunity and serum biochemical metabolites of broiler chicks. In: 2nd International Conference on Agricultural and Animal Science, IPCBEE vol. 22, IACSIT Press, Singapore

Tsai ML, Lin CC, Lin WC, Yang CH (2011) Antimicrobial, antioxidant, and anti-inflammatory activities of essential oils from five selected herbs. Biosci Biotechnol Biochem 75(10):1977–1983

Van Der Sluis M, Vincent A, Bouma J, Korteland-Van Male AB, Van Goudoever JB, Renes I, Van Seuningen I (2008) Forkhead box transcription factors Foxa1 and Foxa2 are important regulators of Muc2 mucin expression in intestinal epithelial cells. Biochem Biophys Res Commun 369:1108–1113

Vasudeva N, Sharma T (2012) Chemical composition and antimicrobial activity of essential oil of Citrus limettioides Tanaka. Journal of Pharmaceutical Technology Drug Research 2050(120):1–7

Wanger DD, Furrow RD, Bradley BD (1983) Subchronic toxicity of growth promoters in broiler chickens. Vet Path 20:253–359

Williams P, Losa R (2001) The use of essential oils and their compounds in poultry nutrition. World Poult 17(4):14–15

Windisch W, Schedle K, Plitzner C, Kroismayr A (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 86(14 Suppl):E140–E148