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The effect of drinking thyme essence on proteins, enzymatic activity, serum biochemical parameters and hematological characteristics in broiler chicks

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Abstract

This experiment was performed to investigate serum biochemical profile and hematological characteristics of broiler chicks supplemented with different levels of drinking thyme essence (DTE). Five hundred one-day old male chicks (Ross-308) were assigned based on a completely randomized design (CRD) and divided into 4 treatments with 5 replicates. Treatments were arranged in: Control group (plain water), 0.1 ml/lit (0.1%), 0.15 ml/lit (0.15%), and 0.2 ml/lit (0.2%) of thyme essence added to drinking water. At 21 and 42 days of age blood samples were collected from wing vein. According to the results, comparing to control group all treatments significantly decreased serum total cholesterol (TC) and triglyceride (TG) but glucose was increased significantly ($p<0.05$). Also response of serum total protein, albumin and globulin to different levels of DTE were significantly higher than control group ($p<0.05$). Serum calcium, phosphorus, uric acid, hepatic enzymes activity including ALT, AST and ALP were not significantly differing than control. Hemoglobin (HG), hematocrit percentage (HCT %), red blood cell (RBC), white blood cell (WBC), and heterophile (HT) counts were significantly ($p<0.05$) increased by treatments at both 21 and 42 days. In contrast lymphocyte (LY) count was decreased significantly ($p<0.05$) at both period in experimental groups than control. As a final result, use of 0.1 ml/lit of DTE initially, and afterward the levels of 0.15 or 0.2 ml/lit of DTE in broiler nutrition can improve the blood biochemical and hematological parameters as well as serum proteins without any detrimental effect on liver and health of the birds.

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Introduction

The challenge of antibiotic-resistant bacteria persuades researches to follow other alternatives and substitutes (Grashorn, 2010- Hashemi and Davoodi, 2010- Windisch *et al.*, 2008). Some consideration like reduction the number of certain pathogenic bacteria and its challenges, improving gut health, as well as better pictures of blood metabolites, are beneficial effects of adding organic matters such as phytogenic feed or water additives in poultry nutrition (Griggs and Jacob, 2005- Langhout, 2000). They can improve performance (Acamovic and Cross, 2007- Alçiçek *et al.*, 2004- Ghazalah and Ali, 2008- Herawati, 2010- Lee *et al.*, 2004), and other parameters such as physiological and biochemical (Lee *et al.*, 2003- Mátéová *et al.*, 2008- Najafi and Torki, 2010- Nworgu *et al.*, 2007- Tolba and Hassan, 2003), and hematological parameters (Al-Kassie, 2010- Ghazalah and Ali, 2008- Paul *et al.*, 2010), liver and kidney function (Annongu *et al.*, 2010- Mátéová *et al.*, 2008- Tollba *et al.*, 2010), improving the health and immunity (Al-Jaff, 2011- Al-Kassie, 2010- Gulfraz *et al.*, 2008- Stef *et al.*, 2009- Tollba *et al.*, 2010), organoleptic properties (Dahal and Farran, 2011- Simsek *et al.*, 2007), antioxidant effect (Hoffman-Pennesi and Wu, 2010- Radwan Nadia *et al.*, 2008), and some important blood constituents (Al-Jaff, 2011- Annongu *et al.*, 2010).

Thyme (*Thymus Vulgaris L.*) is a popular medicinal plant belonged to *Lamiaceae sp*. and mostly grown in Mediterranean regions (Toghyani *et al.*, 2013). This herb has been paid more attention due to its antioxidant (Bolukbasi *et al.*, 2006), antibacterial (Dorman and Deans, 2000)[Mitsh *et al.*, 2004] anticoccidial (Giannenas *et al.*, 2003- Jamroz *et al.*, 2003), antifungal and antioxidant properties (Dorman and Deans, 2000- Hertrampf, 2001). Research results on thyme have been demonstrated to have change effects on performance and serum biochemical parameters (El-Ghousein and Al-Beitawi, 2009), as well as number and ratio of blood cells and hematological characteristics (Ghasemi *et al.*, 2010), or enzymatic activities (Tollba *et al.*, 2010). The major derived components of thyme plant are thymol and carvacrol, the phenolic compounds which have shown antioxidant and antibacterial activities (Demir *et al.*, 2008- Najafi and Torki, 2010). These compounds exhibit beneficial effects in poultry health and production (Jamroz *et al.*, 2003- Lee *et al.*, 2004- Mitsch *et al.*, 2004).

The current experiment was undertaken to investigate the potential of supplemented thyme in drinking water on some blood biochemical profile, proteins, enzymatic activity and hematological characteristics in broiler chicken.

Materials and method

Animals and Treatments

This study was conducted at the research poultry farm of Qom's agricultural research center in IRAN. Totally 500, in each treatment 125 day-old male broiler chicks from Ross-308, were randomly assigned to one of four treatments with five replicates of 25 birds. This experiment was arranged based on a completely randomized design (CRD). Treatments including: Control group (plain water), 0.1 ml/lit (0.1%), 0.15 ml/lit (0.15%), and 0.2 ml/lit (0.2%) of thyme (*Thymus Vulgaris L.*) essence added to drinking water. A basal diet, iso-nitrogenous and iso-caloric with the similar nutrients was used among treatments during different periods according to table 1. All birds were fed according to recommendation of the National Research Council (Nutrition, 1984).

At 21 and 42 day of age blood samples were collected from wing vein at two sets, through non-heparinized and heparinised tubes for biochemical parameters and hematological characteristics respectively.

Serum Biochemical Parameters

Blood samples were taken after 12 hours fasting using non-heparinised tubes at day 21 and 42 of trial from 10 birds in each treatment through wing vein. Blood samples were centrifuged at 3000 rpm for 10 minutes for serum extraction. Serum samples were kept in freezer (-20^{o}C) for future determination of total cholesterol (TC), triglyceride (TG), glucose (GLC), uric acid (UA), calcium (Ca) and phosphorus (P).

Analyses of samples were performed by spectrophotometer using commercial kits (BioSystem® Company, Barcelona, Spain).

Serum Proteins and Enzymatic Activity

In order to measure total protein concentration, albumin, globulin and determine hepatic enzymes Alanine Amino Trasferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP), spectrophotometric method was employed using the kit package (BioSystem® Company, Barcelona, Spain).

Hematological Characteristics

At day 21 and 42 of age, blood samples were collected from 10 birds in each treatment by tubes containing EDTA. Slides of blood samples were prepared and stainted using Gimsa color. 100 cells per sheet were counted to determine the number of heterophile and lymphocyte, also heterophile to lymphocyte ratio was calculated and recorded (Gross and Siegel, 1983). Haemoglobin (HB) concentration was measured spectrophotometrically by cyanomethaemoglobin method. The red blood cell (RBC) and white blood cell (WBC) count were done by hemocytometer and hematocrit (HCT) values measured by microhematocrit tubes (Kececi *et al*., 1998).

Statistical Analysis

A Complete Randomized Design (CRD) model was employed to analyze data of experimental traits. The data were analyzed using the ANOVA procedures of the statistical software. Comparisons of means were done using Duncan's multiple range tests, assuming error level of 0.05.

Results

Biochemical Parameters

The effect of different levels of DTE on serum biochemical parameters at the age of 21 and 42 are presented in table 2. According to the results of this table, TC, TG and GLC were significantly ($p< 0.05$) influenced by the inclusion of DTE to water both at the 21 and 42 of age, against UA, Ca and P were not influenced. The amount of TC and TG in serum was decreased, but GLC was increased as compared with the control group.

Table 1. Ingredients and composition of the basal diet.

Ingredients (g/kg)	Starter (0-7 d)	Grower (8-21 d)	Finisher (22-42 d)
Corn (7.88 % CP)	525	533.5	602.5
Soy bean meal (44 % CP)	409.4	381.5	316
Soy bean oil	10	30	30
Calcium carbonate	16	16	20
Dicalcium phosphate[1]	17.2	17	11
DL-Methionine	4.4	4	3.5
L-Lysine	10	10	9
Vitamin and Mineral premix[2]	5	5	5
Salt	3	3	3
Total	1000	1000	1000
Calculated values			
Metabolazable energy (kcal/kg)	3025	3140	3177
Crude protein (g/kg)	23	21.51	19.07
Calcium (g/kg)	0.99	0.92	0.84
Available phosphorus (g/kg)	0.45	0.43	0.33
Methionine + Cystine (g/kg)	13.51	15.46	17.94
Lysine (g/kg)	14.3	14.5	11.7

1.Dicalcium phosphate contained: 16.5% phosphorous and 23% calcium.

2.Vitamin and mineral premix per kg of complete diet contained: vitamin A (retinol), 8400 IU; vitamin D3 (cholecalciferol), 1800 IU; vitamin E (tocopheryl acetate), 150 mg; vitamin K, 24 mg; B1, 8 mg; B2, 16.6 mg; B6, 13 mg; B12, 5 mg; panthothenic acid, 12 mg; niacin, 36 mg; biotin, 10 mg; folic acid, 2.2 mg; choline chloride, 128.8 mg; antioxidant, 100 mg. Fe ($FeSO_4$, 20.1% Fe), 95 mg; Mn ($MnSO_4$, 32.5% Mn), 120 mg; Zn (ZnO, 80.5% Zn), 120 mg; Cu ($CuSO_4$, 30.3% Cu), 35 mg; I (KI, 58% I), 5 mg; and Se ($NaSeO_3$, 45.5% Se), 2.2 mg.

Serum Proteins

Effect of different levels of DTE on serum proteins are presented in table 3. In this study, use of DTE had significant ($p< 0.05$) effect on total protein, ALB, GLO and ALB/GLO ratio at the age of 21 and also 42. The amounts of total protein, ALB, GLO and ALB/GLO ratio were recorded as higher in DTE treatments than control group.

Activity of Enzymes

The effect of inclusion of different levels of DTE on hepatical ALT, AST and ALP of broiler chicks at 21 and 42 days of age are presented in table 4. According to the results, the hepatic enzymes were not significantly affected by different levels of DTE neither at 21 nor at 42 of age.

Table 2. Effect of different levels of drinking thyme essence on serum biochemical parameters.

Items	Control	0.1 ml/lit	0.15 ml/lit	0.2 ml/lit	SEM	ANOVA P value
At the age of 21 days						
TC (mg/dl)	115.50 [a]	102.83 [b]	103.09 [b]	98.50 [c]	0.381	0.0001
TG (mg/dl)	130.17 [a]	118.67 [b]	114.83 [c]	103.83 [d]	0.392	0.0001
GLC (mmol/dl)	12.11 [c]	14.39 [b]	14.33 [b]	15.54 [a]	0.105	0.0001
UA (mg/dl)	3.89	3.75	3.81	3.87	0.065	0.0660
Ca (mg/dl)	9.78	9.74	10.20	9.94	0.133	0.9200
P (mg/dl)	7.23	7.17	7.27	7.19	0.144	0.1420
At the age of 42 days						
TC (mg/dl)	129.18 [a]	117.66 [b]	116.44 [b]	112.35 [c]	0.375	0.0001
TG (mg/dl)	134.23 [a]	125.12 [b]	124.54 [b]	117.12 [c]	0.363	0.0001
GLC (mmol/dl)	15.36 [c]	16.45 [b]	17.28 [a]	17.25 [a]	0.082	0.0001
UA (mg/dl)	4.16	4.05	3.79	3.86	0.125	0.3921
Ca (mg/dl)	9.33	9.56	9.73	9.78	0.155	0.5160
P (mg/dl)	6.14	6.13	6.21	5.93	0.125	0.7650

Values in the same row not sharing a common superscript are significantly different ($p<0.05$); SEM: Standard error of means.

TC: total cholesterol, TG: triglyceride, GLC: glucose, UA: uric acid, Ca: calcium, P: phosphorus.

Hematological Characteristics

Data in table 5 shows the effect of different levels of DTE on some hematological characteristics at the age of 21 and 42. All of items were significantly ($p< 0.05$) influenced by different levels of DTE. Except of LY, whole of items were progressed and increased in amount or number as compared with the control group.

Discussion

Results presented in table 2 showed that, the chicken receiving different levels of DTE had a significant ($p<0.05$) lower TC and TG, but higher glucose during two period compared with the control. There were no differences in Ca, P and UA among treatments neither at 21 nor 42 days of age. Decreasing effect on TC and TG or increasing on GLC, and lack of significant response in Ca, P and UA, using herbs and plant derives are common pictures among the research reports. Some factors including: age, sex and type of bird, environmental, nutritional and physiological situations, as well as purity and variation of compounds in herbs and herb products directly influences on the results (Al-Jaff, 2011- Annongu *et al.*, 2010- Tollba *et al.*, 2010).

Mateova *et al.* (Mátéová *et al.*, 2008) declared that activity of alkaline phosphatase can improve

metabolism of osteogenous mineral substances and it could be reflected in the insignificant increase in blood calcium and phosphorus in comparison with control.

Nworgu *et al.* (Nworgu *et al.*, 2007) claimed that increase in serum uric acid due to different herbal compounds may have resulted in their anti nutrients and toxic effects, or increasing the rate of nitrogen metabolism in body and consequently suffering the kidneys and health condition of chicks. The results of current study about TC and TG are consistence with the results, which was reported in poultry by Tollba *et al* (Tollba *et al.*, 2010). The decrease of TC and TG may attributed to the lowering effect of thymol and carvacrol on hepatic 3-hydroxy-3-methyglutaryl coenzyme A (HMG-COA), the rate-limiting enzyme of cholesterol synthesis (Case *et al.*, 1995- Lee *et al.*, 2003).

Table 3. Effect of different levels of DTE on serum proteins.

Items	Control	0.1 ml/lit	0.15 ml/lit	0.2 ml/lit	SEM	ANOVA P value
At the age of 21 days						
Total protein (g/l)	33.16 ^{c}	41.52 ^{a}	38.65 ^{b}	38.35 ^{b}	0.223	0.0001
ALB (g/dl)	1.88 ^{b}	2.12 ^{a}	2.28 ^{a}	2.24 ^{a}	0.065	0.0090
GLO (g/l)	2.64 ^{b}	2.74 ^{a}	2.76 ^{a}	2.72 ^{a}	0.011	0.0003
ALB/GLO ratio	0.71 ^{b}	0.72 ^{b}	0.83 ^{a}	0.79 ^{a}	0.021	0.0420
At the age of 42 days						
Total protein (g/l)	40.22 ^{c}	43.67 ^{a}	36.78 ^{d}	41.79 ^{b}	0.233	0.0001
ALB (g/dl)	2.15 ^{b}	2.18 ^{ab}	2.17 ^{ab}	2.32 ^{a}	0.055	0.0002
GLO (g/l)	2.08 ^{b}	2.11 ^{ab}	2.17 ^{a}	2.22 ^{a}	0.021	0.0090
ALB/GLO ratio	1.03 ^{b}	0.79 ^{c}	1.05 ^{b}	1.12 ^{a}	0.015	0.0024

Values in the same row not sharing a common superscript are significantly different ($p<0.05$); SEM: Standard error of means.

ALB: albumin, GLO: globulin, ALB/GLO: albumin to globulin ratio.

Reports have shown that thymol and carvacrol may inhibit HMG-COA reductase activity, which is a key regulatory enzyme and consequently having the hypocholeterolemic effect (Bolukbasi *et al.*, 2006- Lee *et al.*, 2004). Some reports have shown that using of different aromatic herb extracts can reduces the level of lipids in blood and egg of birds (Bolukbasi *et al.*, 2006- El-Ghousein and Al-Beitawi, 2009- Mátéová *et al.*, 2008- Tollba *et al.*, 2010). The effect of thyme extract on serum glucose level may have resulted in stimulatory effect of thyme compounds on gut and pancreas enzymes and consequently increase the level of glucose in serum (Acamovic and Cross, 2007- Lee *et al.*, 2003- Windisch *et al.*, 2008).

The results of table 3 have shown that the levels of DTE especially at the level of 0.1%, in both 21 and 42 days of age, significantly ($p<0.05$) increased total protein, albumin and globulin. These finding indicate that DTE have an enhancement effect on serum proteins which is in agreement with report of Nworgu *et al.* (Nworgu *et al.*, 2007); Ghazalah and Ali (Ghazalah and Ali, 2008); and Al-Jaff (Al-Jaff, 2011). Tollba *et al.* (Tollba *et al.*, 2010) have stated that adding aromatic herbal extracts alone or blended with organic acids to diets significantly ($p<0.05$) increased total protein, as well as albumin and globulin comparing to un-supplemented control group. The increase in serum proteins may be due to effects of plant-derived compounds to stimulate protein synthesis in relative organs. In addition, as illustrated by Houghton *et al.* (Houghton *et al.*, 1995), the increase in the globulin fraction indicates the effective role of using experimental substance in increasing immunity due to its role in developing and protecting cells and inhibiting non-enzymatic oxidation.

Amounts of ALT, AST and ALP in supplemented birds with different levels of DTE were not significantly different comparing to control group (table 4). Alanine Amino Trasferase (ALT) and AST considered as liver enzymes indicating liver damage, thus no increase in serum concentration of ALT and AST may provide evidence to protect of liver against hepatocellular degeneration [Al-Jaff., 2011].

Table 4. Effect of different levels of drinking thyme essence on enzymes activity.

Items	Control	0.1 ml/lit	0.15 ml/lit	0.2 ml/lit	SEM	ANOVA P value
At the age of 21 days						
ALT (u/l)	18.66	18.99	18.47	18.29	0.215	0.330
AST (u/l)	110.62	110.93	111.70	111.57	0.185	0.411
GLP (u/l)	298.15	297.35	298.63	298.13	0.415	0.112
At the age of 42 days						
ALT (u/l)	19.18	20.70	19.69	21.06	0.612	0.362
AST (u/l)	105.34	106.27	107.85	107.54	0.911	0.358
GLP (u/l)	298.31	298.36	301.30	298.80	0.975	0.162

ALT: Alanine Amino Trasferase , AST: Aspartate Amino Transferase , ALP: Alkaline Phosphatase;
SEM: Standard error of means.

Elevated level of ALP might suggest bone growth or damage, since chicks used for this experimental were day old, thus they were in growth phase of bone. In addition, very low activity of the enzymes will predispose to danger the cells, tissues or organs where enzymes are found (Annongu *et al.*, 2010). Tollba *et al.* (Tollba *et al.*, 2010), reported that adding the aromatic herbal extract to broiler diet did not alter the ALT and AST when compared to control. They suggested that using of thyme extract exhibit healthy, non-pathological and non-toxic on liver or kidney. Similar findings were cited by Abdel-Malak *et al.* (Abdel-Malak *et al.*, 1995), when they added biotic as herbal feed additive.

Table 5. Effect of different levels of drinking thyme essence on hematological characteristic.

Items	Control	0.1 ml/lit	0.15 ml/lit	0.2 ml/lit	SEM	ANOVA P value
At the age of 21 days						
HG (g/dl)	7.04^{b}	7.11^{b}	8.02^{a}	8.07^{a}	0.066	0.0001
HCT (%)	30.78^{c}	31.68^{b}	31.01^{bc}	32.85^{a}	0.213	0.0001
RBC (10^{6}/ml)	2.24^{b}	2.35^{c}	2.23^{b}	2.53^{a}	0.032	0.0001
WBC (10^{3}/ml)	2.08^{b}	2.14^{b}	2.84^{a}	2.82^{a}	0.028	0.0001
HT (10^{3}/µl)	27.50^{c}	30.67^{a}	30.17^{b}	31.07^{a}	0.240	0.0001
LY (mg/µl)	59.33^{a}	58.33^{b}	57.03^{c}	57.61^{bc}	0.233	0.0001
HT/LY ratio	0.46^{b}	0.53^{a}	0.53^{a}	0.55^{a}	0.010	0.0001
At the age of 42 days						
HG (g/dl)	7.31^{b}	8.01^{a}	7.22^{b}	8.15^{a}	0.053	0.0001
HCT (%)	30.48^{c}	32.36^{a}	31.19^{b}	32.53^{a}	0.110	0.0001
RBC (10^{6}/ml)	2.24^{c}	2.53^{b}	2.54^{b}	2.86^{a}	0.023	0.0001
WBC (10^{3}/ml)	1.98^{b}	2.01^{b}	2.24^{a}	2.32^{a}	0.030	0.0001
HT (10^{3}/µl)	28.70^{d}	29.30^{c}	30.37^{b}	31.23^{a}	0.120	0.0001
LY (mg/µl)	58.35^{a}	57.31^{b}	57.16^{b}	56.20^{c}	0.220	0.0001
HT/LY ratio	0.49^{b}	0.51^{a}	0.53^{a}	0.56^{a}	0.020	0.0020

Values in the same row not sharing a common superscript are significantly different ($p<0.05$); SEM: Standard error of means.
HG: hemoglobin, HCT: hematocrit, RBC: red blood cell, WBC: white blood cell,
HT: heterophile, LY: lymphocyte, HT/LY: heterophile to lymphocyte ratio.

According to the results presented in table 5, different levels of DTE significantly ($p<0.05$) increased the amount of hemoglobin and the percent of hematocrit when compared with control. The present results are in agreement with Tollba *et al.* (Tollba *et al.*, 2010) and Hertrampf (Hertrampf, 2001), who reported that this improvement may be due to antioxidant activity of essential oil components in thyme products.

Other blood parameters such as the count of RBC, WBC, HT and LY significantly ($p<0.05$) altered by treatments. The number of RBC, WBC and HT were higher, but LY was lower than control. The heterophile to lymphocyte ratio (HT/LY) for different levels of DTE were significantly ($p<0.05$) higher than control. Thus water containing thyme essence exhibits a positive effect on changing of hematological characteristics. Tollba *et al.* (Tollba *et al.*, 2010), illustrated that different components of herbal extracts due to nutritive and anti-oxidative effects could stimulate the blood cells producer organs. Similar results have been gained by essential oil of cinnamon (Friedman *et al.*, 2004). Also Ibrahim *et al.* (Ibrahim *et al.*, 2000) have reported that RBC count, hemoglobin and Packed cell volume (PCV) with 0.5% thyme were significant ($p<0.05$) increased in rabbit. Al-Kassi [2009], showed that using dietary essential oil of thyme and cinnamon caused significant ($p<0.05$) increase in the amounts of hemoglobin, RBC, WBC and HCT.

Conclusion

In conclusion, the results of this study indicated that inclusion of thyme essence as drinking water at levels of 0.1, 0.15 and 0.2 ml/lit in both period could significantly decrease the serum total cholesterol and total triglyceride, whilst increase glucose content. But can not influence on amount of Ca, P and uric acid. Also drinking thyme essence cause to elevate serum total protein, albumin and globulin fraction and increase the ALB/GLO ratio. Moreover, the activity of hepatic enzymes like ALT, AST and alkaline phosphatase were not significantly differing when compared with control. Hematological characteristics including hemoglobin, hematocrit, red and white blood cells and heterophile elevated using different levels of drinking thyme essence. Also the HT/LY ratio was higher in experimental groups than control. Therefore, using drinking thyme essence improves the biochemical and hematological parameters as well as serum proteins, so have positive effect on health status with no detrimental effect on the birds.

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