IJAS Iranian Journal of Applied Animal Science

The Influence of Drinking Water Containing Lemon Juice and Thyme Supplemented Diet on Performance and some Blood Parameters of Broilers under Heat Stress

Research Article

H. Behboudi[1], O. Esmaeilipour[1*], R. Mirmahmoudi[1] and M. Mazhari[1]

[1] Department of Animal Science, Faculty of Agriculture, University of Jiroft, Jiroft, Iran

Received on: 27 Mar 2015
Revised on: 20 Apr 2015
Accepted on: 30 Apr 2015
Online Published on: Mar 2016

*Correspondence E-mail: omid.esmaeilipour@ujiroft.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

This study was conducted to find out the effects of drinking water containing lemon juice and thyme supplemented diet on growth performance, serum concentration of cholesterol and triglycerides, heterophils (H), lymphocytes (L), H/L ratio and enzyme activity of glutathione peroxidase (GPX) in broilers under heat stress condition. The experiment was carried out as a 2 × 3 factorial arrangement with 2 levels of lemon juice (0 and 2 mL/L of drinking water) and 3 levels of thyme (0, 0.5 and 1 % of diet). Each dietary treatment consisted of 4 replicate pens (9 birds/pen). The birds were kept at 34 ˚C ± 2 (9.00 to 17.00) from 25 to 42 d of age. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), H/L ratio and GPX activity and serum cholesterol concentration were not affected (P>0.05) by individual addition of lemon juice or thyme; whereas, combination effect of the two additives improved performance and reduced H/L ratio and serum triglycerides concentration (P≤0.05). Concurrent usage of 0.5 thyme and 2 mL/L of lemon juice increased FI and BWG by 229 and 165 g, respectively. Addition of 0.5 and 1% thyme to the diet and 2 mL/L lemon juice to drinking water reduced the serum triglycerides concentration by 9.8, 11.1 and 8.5 mg/dL, respectively (P≤0.05). It is concluded that the concurrent administration of 0.5% of thyme to diet and 2 mL/L lemon juice to drinking water provides a potential protective management strategy in preventing heat-stress-related reduction in the performance of broiler chickens.

KEY WORDS chicken, heat stress, heterophil, lemon juice, lymphocyte, thyme.

INTRODUCTION

In the subtropical regions, the high ambient temperature is a major concern that adversely influences the physiological status and hence reduces the performance of boiler chickens. It has been demonstrated that heat stress reduces feed intake (FI), body weight gain (BWG), feed efficiency and immunity of broiler chickens (Siegel, 1995; Borges *et al*. 2003; Lin *et al*. 2006). Furthermore, it has been found that the plasma concentration of antioxidant vitamins such as vitamin C and E reduced and oxidative damage increased in birds during heat stress condition (Sahin *et al*. 2002). Several studies have reported that administration of vitamin C alleviates the deleterious effects of heat stress on performance and metabolism of broiler chickens (Kutlu and Forbes, 1993; Sahin *et al*. 2002; Gursu *et al*. 2004). In fact, vitamin C is the first line of defense against reactive oxygen species in the body (McDowell, 1989). In birds, it is generally synthesized in kidneys, but its quantity is not enough during heat stress condition since the rate of its usage for scavenging of the free radicals is increased. The ascorbic acid content in lemon (*Citrus limon*) juice obtained by fruit squeezing is reported to be 54.74 mg/100 mL (Pisoschi *et al*. 2011). It is reported that addition of lemon juice to drinking

water improved immunity of broiler chickens under heat stress condition (Kadam *et al*. 2009). Recently, studies have been focused on the application of herbs as growth promoters instead of antibiotics in poultry nutrition. Thyme has been recognized as an important phytogenic feed additive which contains thymol and carvacrol. These components have antioxidant and antimicrobial properties (Lambert *et al*. 2001; Hippenstiel *et al*. 2011), which can improve the nutrient digestibility (Amad *et al*. 2011) as well as immunity (Radwan *et al*. 2008; Toghyani *et al*. 2010; Hippenstiel *et al*. 2011). In this regards, Nedyalka *et al*. (1999) reported that thymol and carvacrol inhibit lipid peroxidation. It is suggested that the addition of a mixture of herbs can be more efficient in improvement of the immunity and performance in broilers, which could be due to the synergistic and suppressing effects of their active components (Ertas *et al*. 2005; Hippenstiel *et al*. 2011). For example, Thanissery *et al*. (2014) reported that thyme-orange combination showed a synergetic effect against *Salmonella*. The present experiment was therefore undertaken to evaluate the effects of drinking water containing lemon juice and thyme supplemented diet on performance and some blood parameters in broiler chickens under heat stress condition.

MATERIALS AND METHODS

Birds, housing and diets

A total of 216 (1-d-old) male Ross 308 broiler chicks were obtained from a commercial hatchery. The birds were reared under a standard broiler management program until 24 d of age. The average body weight (BW) of birds at the beginning of the experiment (on d 25) was 910 ± 21 g. The birds were then randomly distributed into 24 floor pens (pen size was 1 m×1.5 m) in an environmentally controlled house.

The experiment was conducted as a 2 × 3 factorial arrangement with 2 levels of fresh lemon juice (0 and 2 mL/L of drinking water) and 3 levels of thyme powder (0, 0.5 and 1% of diet). Each of the 6 treatment groups was replicated 4 times, with 9 birds per pen (1×1.5 m). The experiment lasted 18 days from 25 to 42 d of age. Feed ingredients and nutrients composition of the basal diet are presented in the Table 1. The basal diet was formulated to meet or exceed all nutrient recommendations published in the Ross rearing guideline (Aviagen, 2007).

Air-dried *Thymus vulgaris* plant materials were ground to pass a 2 mm screen, and then used in supplemented groups. Lemon juice was extracted from fruits which were harvested at appropriate maturity in September from orchards of Jiroft region in south of Iran. The birds in treatment group were given drinking water supplemented with lemon juice at a final concentration of 2 mL/L.

Birds had free access to feed and fresh water throughout the experiment.

Table 1 Composition and calculated analyses of the basal diet

Item (% unless noted)	25 to 42 d
Corn	60.63
Soybean meal, 44% CP	30.85
Sunflower oil	4.85
Limestone	1.30
Dicalcium phosphate	1.21
Vitamin and mineral premix[1]	0.50
Sodium chloride	0.29
DL-methionine	0.25
L-lysine-HCl	0.13
Calculated analysis	
Metabolizable energy (kcal/kg)	320
Crude protein	19.0
Lys	1.09
Met + Cys	0.86
Thr	0.82
Arg	1.43
Ca	0.85
Available P	0.42

[1] Vitamin and mineral mix supplied the following per kilogram of diet: vitamin A: 9000 IU; vitamin D_3: 215 IU; vitamin E: 18 IU; vitamin K_3: 2 mg; vitamin B_1: 18 mg; vitamin B_2: 6.6 mg; vitamin B_6: 3 mg; vitamin B_{12}: 0.015 mg; Nicotinic acid: 10 mg; Folic acid: 1 mg; Pantothenic acid: 12 mg; Choline chloride 60%: 500 mg; Mn: 100 mg; Zn: 84.7 mg; Cu: 10 mg; Se: 2 mg; I: 1 mg and Fe: 50 mg.

The compact fluorescent bulbs were used continuously. The temperature was maintained at 33 ˚C from d 1 to 3 and then gradually reduced to 24 ˚C by end of the third week. During the experimental period, the mean relative humidity was 50 ± 10% and the basal temperature was maintained at 22 ± 2 ˚C. It was gradually increased to 34 ± 2 ˚C during 9 am to 10 am, and this high temperature then was maintained for 7 h (until 5 pm).

Growth trial procedures

On d 25 of age, the birds were weighed by pen, and at the end of experiment BW, feed intake and feed to gain ratio (FCR) were calculated. The birds were inspected daily and the BW of dead birds and date of death were recorded. When calculating feed efficiency, the BW of the dead birds was taken into consideration.

Blood characteristics

On d 42, after 5 hours fasting, one bird per pen was randomly selected. Blood samples were collected as quickly as possible from the wing vein using 3 mL syringe with 25 G needle and carefully moved into EDTA tubes (0.5 mL), for measuring the glutathione peroxidase enzyme (GPX) activity and heterophils to lymphocytes (H:L) ratio and non-heparinized tubes (1.5 mL) to estimate the cholesterol and triglycerides concentration. The blood samples were then immediately transferred to the laboratory for analysis of parameters. At the time of blood sampling, two smears slides were prepared.

The H:L was recorded after fixation by methanol, and stained with May-Grünwald-Gimsa stain (Gross and Siegel, 1983). Approximately one hundred cells (H and L) from each slide were counted according to the morphological criteria under an optical microscope. In order to measure the concentration of total cholesterol and glucose of serum, the samples were analyzed with bionic kit, an automatic analyzer (Hitachi 912, Boehringer Mannheim, Ingelheim am Rhein, Germany), by using the colorimetric method. Glutathione peroxidase enzyme was measured using a Randox kits (Randox, Co. Antrim, United Kingdom). The animal ethics committee of the university approved this experiment.

Statistical analysis

All data were analyzed using the GLM procedures of SAS software (SAS, 2001). Differences among the treatment means were tested using Duncan's multiple comparison tests ($P<0.05$).

RESULTS AND DISCUSSION

Growth performance

The effects of dietary supplementation with thyme and drinking water contained lemon juice on performance of broilers under heat stress are presented in Table 2. Neither the main effect of thyme nor that of lemon juice significantly affected birds' performance ($P>0.05$); whereas, combination effect of the two additives improved the FI ($P\leq0.05$), BWG ($P\leq0.01$) and FCR ($P\leq0.05$). All combination of the two additives significantly increased FI and BWG compared to control group, except for the combination of 2 mL/L lemon juice and 1% thyme. However, the value of BWG obtained by inclusion of 0.5% thyme without lemon juice group was not significantly different with that in control group (1395 *vs.* 1307 g). In comparison with the control group, concurrent usage of 0.5 thyme and 2 mL/L of lemon juice increased FI and BWG by 229 and 165 g, respectively.

The lowest and greatest FCR were belong to the combination usage of 1% thyme without lemon juice and the treatment group which was offered 1% thyme and 2 mL/L lemon (1.569 and 1.726, respectively).

Blood characteristics

The effects of dietary supplementation with thyme and drinking water contained lemon juice on the serum concentration of cholesterol and triglycerides and enzyme activity of GPX in broilers under heat stress are shown in Table 3. Both main effects of lemon juice and thyme and their interaction significantly decreased serum concentration of triglycerides ($P\leq0.01$).

Addition of 2 mL/L lemon juice, 0.5 and 1% thyme decreased serum concentration of triglycerides by 8.5, 9.75 and 11.13 mg/dL, respectively, compared to the control group.

Table 2 Effects of thyme and lemon juice supplementation on the performance of broiler chickens under heat stress condition

Treatment	FI (g/d)	BWG (g/d)	FCR
Thyme (% diet)			
0	127.56	78.39	1.63
0.5	131.44	79.61	1.65
1	129.55	79.00	1.65
SEM	2.25	1.79	0.02
Lemon juice (mL/L of water)			
0	127.39	78.11	1.63
0.2	131.67	79.78	1.65
SEM	1.76	1.46	0.02
Lemon juice × thyme			
0 × 0	119.94^{b}	72.61^{b}	1.65^{ab}
0 × 0	135.17^{a}	84.17^{a}	1.61^{b}
0 × 0.5	130.22^{a}	77.50^{ab}	1.68^{ab}
0.2 × 0.5	132.67^{a}	81.78^{a}	1.63^{ab}
0 × 1	131.94^{a}	84.17^{a}	1.57^{b}
0.2 × 1	127.17^{ab}	73.89^{b}	1.73^{a}
SEM	3.05	2.53	0.04
	P-value		
Thyme	0.459	0.891	0.786
Lemon juice	0.100	0.382	0.497
Thyme × lemon juice	0.013	0.001	0.011

The means within the same column with at least one common letter, do not have significant difference ($P>0.05$).
SEM: standard error of the means.
FI: feed intake; BWG: body weight gain and FCR: feed conversion ratio.

The lowest level of serum triglycerides concentration (83.75 mg/dL) was observed in the treatment group which offered the combination of 2 ml/lit lemon juice without thyme.

Neither the main effects of the two additives nor their interaction significantly influenced the serum concentration of cholesterol and enzyme activity of GPX ($P>0.05$).

The effects of dietary supplementation with thyme and drinking water contained lemon juice on H, L and H/L ratio of broilers under heat stress are presented in Table 4. Thyme and lemon juice alone did not affect H and L percentage and H/L ratio, whereas, their interaction significantly decreased H percentage ($P\leq0.05$) and H/L ratio ($P\leq0.01$) and increased L percentage ($P\leq0.05$).

To the best of our knowledge, this is the first study that evaluates the potential usage of thyme as a phytogenic feed additive along with lemon juice to ameliorate the adverse effects of heat stress on broiler production. The negative effects of heat stress on the productivity of broiler chickens is the most commonly occurring challenge in several production systems worldwide.

In this investigation, individual usage of thyme or lemon juice did not significantly increase FI and BWG, although

the greater numerical values were obtained in the supplemented groups.

Table 3 Effects of thyme and lemon juice supplementation on serum cholesterol and triglycerides concentrations and enzyme activity of glutathione peroxidase (GPX) in broiler chickens under heat stress condition

Treatment	Cholesterol (mg/dL)	Triglycerides (mg/dL)	GPX (U/gHb)
Thyme (% of diet)			
0	125.50	96.13[a]	15.60
0.5	124.60	86.38[b]	15.92
1	117.10	85.00[b]	15.49
SEM	3.49	1.98	0.74
Lemon juice (mL/L of water)			
0	119.50	93.42[a]	16.26
0.2	125.30	84.92[b]	15.07
SEM	2.85	1.62	0.60
Lemon juice × thyme			
0 × 0	122.80	108.50[a]	16.11
0.2 × 0	128.30	83.75[b]	15.06
0 × 0.5	117.70	85.75[b]	16.91
0.2 × 0.5	131.50	87.00[b]	14.93
0 × 1	118.00	86.00[b]	15.76
0.2 × 1	116.30	84.00[b]	15.22
SEM	4.93	2.81	1.05
		P-value	
Powdered thyme	0.302	0.002	0.911
Lemon juice	0.165	0.002	0.184
Thyme × lemon juice	0.314	0.000	0.790

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In case of application of thyme during heat stress condition, no published data were found, Nonetheless, similar results were reported by Hosseini *et al*. (2013) working on broilers using thyme powder, Sengül *et al*. (2008) on Japanese quails by adding thyme extract and Radwan *et al*. (2008) on lying hens after supplementation with thyme powder, under non-stress conditions. Lemon juice is a rich source of vitamin C (Pisoschi *et al*. 2011). It has been found that dietary inclusion of vitamin C increased body weight, FI and feed efficiency in broilers under heat stress condition (McKee *et al*. 1997; Tatli Seven *et al*. 2008; Elagib and Omer, 2012).

In this regard, the results of the current study showed that FI, BWG as well as feed efficiency of broilers improved by concurrent supplementation of thyme and lemon juice. This could be attributed to the combination effects of thyme and lemon juice that may exploit benefits by creating synergistic or positive associative effects between active compounds present in the two additives. Furthermore, the increased BWG of treated birds with the concurrent usage of the two additives could be partly due to an increase in FI. The starting point of most deleterious impacts of heat stress conditions on birds' production is presumably decreased FI, which it reduces BWG and feed efficiency (Hippenstiel *et al*. 2011).

Therefore, the most important finding of the present study was that the incorporation of thyme and lemon juice together has satisfactory enhanced FI and consequently improved the broiler performance.

Table 4 Effects of thyme and lemon juice supplementation on heterophils (H), lymphocytes (L) and H:L of broiler chickens under heat stress condition

Treatment	H (%)	L (%)	H:L
Thyme (% diet)			
0	10.25	86.13	0.12
0.5	8.75	88.25	0.10
1	8.88	87.38	0. 10
SEM	0.50	0.73	0.01
Lemon juice (mL/L of water)			
0	9.67	86.42	0.11
0.2	8.92	88.08	0.10
SEM	0.41	0.60	0.01
Lemon juice × thyme			
0 × 0	12.00[a]	83.25[b]	0.15[a]
0.2 × 0	8.50[b]	89.00[a]	0.10[b]
0 × 0.5	8.50[b]	88.50[a]	0.10[b]
0.2 × 0.5	9.00[b]	88.00[a]	0.10[b]
0 × 1	8.50[b]	87.50[a]	0.10[b]
0.2 × 1	9.25[b]	87.25	0.11[b]
SEM	0.71	1.04	0.01
		P-value	
Thyme	0.088	0.847	0.071
Lemon juice	0.209	0.615	0.147
Thyme × lemon juice	0.012	0.011	0.008

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In a present investigation, similar to our results associated with growth performance, the considerable improvement has been occurred in the blood measurements of broiler chickens which supplemented by thyme and lemon juice together. In this regard, H and L counts, H/L ratio, and blood triglyceride concentration have been changed toward the establishment of a stronger immune system. Several experiments have been conducted to find out the effects of high temperatures on the immune responses of broiler chickens.

For example, Mashaly *et al*. (2004) reported that exposure to the heat stress condition increased H/L ratio in broiler chickens, which has been suggested as a reliable indicator of stress in birds (Gross and Siegel, 1983). In this investigation, H/L ratio in broilers under heat stress condition (34±2 ˚C) promisingly reduced by 34% after supplementation with thyme and lemon juice together.

Thymol and carvacrol are the major phenolic components of thyme which their antioxidant and antimicrobial properties are well documented (Lawrence, 2005). Recently, thymol and carvacrol have received considerable attention as

alternatives to the traditional antibacterial feed additives in poultry nutrition (Hippenstiel *et al*. 2011). Hashemipour *et al*. (2013) and Ertas *et al*. (2005) reported that dietary supplementation with thymol and carvacrol enhanced the performance of broiler chickens.

In birds, vitamin C is synthesized in kidneys. However, due to an increase in the rate of usage for scavenging the free radicals, its quantity becomes insufficient under heat stress condition.

The physiological functions of vitamin C are largely dependent on its antioxidative properties. As an antioxidant, it is also the first line of defense against reactive oxygen species in the body (McDowell, 1989). It is suggested that the addition of a mixture of herbs could be more efficient for improvement of immunity and growth performance in broilers. Such beneficial effects may be attributed to the synergistic and suppressing effects of active components in the phytogenic plants (Hippenstiel *et al*. 2011; Thanissery *et al*. 2014).

CONCLUSION

In conclusion, the results of the present investigation demonstrated that concurrent supplementation of 0.5% thyme to diet and 2 mL/L lemon juice to drinking water provide a potential protective management tools in ameliorating the adverse effects of heat stress on broiler chickens. These positive impacts may be associated to the synergistic effects of active components of thyme and lemon juice. However, these possible synergistic effects need to be studied in more details and under standardized conditions.

ACKNOWLEDGEMENT

This experiment was partially funded by Department of Animal Science, Faculty of Agriculture, University of Jiroft, Jiroft, Iran.

REFERENCES

Amad A.A., Männer K., Wendler K.R, Neumann K. and Zentek J. (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poult. Sci.* **90,** 2811-2816.

Aviagen. (2007). Parent Stock Management Manual: Ross 308. Aviagen Ltd., Newbridge, UK.

Borges S., Da Silva A.F., Ariki J., Hooge D. and Cummings K. (2003). Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat stress environments. *Poult. Sci.* **82,** 428-435.

Elagib H.A. and Omer H. (2012). Effect of dietary ascorbic acid on performance and immune response of heat stressed broiler chicks. *Pakistan J. Nutr.* **11,** 216-220.

Ertas O.N., Güler T., Çiftçi M., Dalkilic B. and Simsek U.G. (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *Int. Poult. Sci.* **4,** 879-884.

Gross W.B. and Siegel H.S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian. Dis.* **27,** 972-979.

Gursu M.F., Onderci M., Gulcu F. and Sahin K. (2004). Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress *in vivo*. *Nutr. Res.* **24,** 157-164.

Hashemipour H., Kermanshahi H., Golian A. and 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. *Poult. Sci.* **92,** 2059-2069.

Hippenstiel F., Abdel-Wareth A.A.A., Kehraus S. and Südekum K.H. (2011). Effects of selected herbs and essential oils and their active components on feed intake and performance of broilers: a review. *Arch. Geflügelk.* **75,** 226-234.

Hosseini S.A., Meimandipour A., Alami F., Mahdavi A. and Mohiti-Asli M. (2013). Effects of ground thyme and probiotic supplements in diets on broiler performance, blood biochemistry and immunological response. *Italian J. Anim. Sci.* **12,** 116-120.

Kadam A.S., Lonkar V.D., Patodkar V.R., Kolangath S.M. and Bhosale T.A. (2009). Comparative efficacy of supplementation of natural (citrous limon juice), herbal and synthetic vitamin C on the immune response of broiler chicken during summer stress. *Asian J. Poult. Sci.* **3,** 57-62.

Kutlu H. and Forbes J. (1993). Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. *Livest. Prod. Sci*. **36,** 335-350.

Lambert R.J.W., Skandamis P.N., Coote P.J. and Nychas G.J.E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* **91,** 453-462.

Lawrence B.M. (2005). Antimicrobial / Biological Activity of Essential Oils. Allured Publishing Corporation, Illinois, United States.

Lin H., Jiao H., Buyse J. and Decuypere E. (2006). Strategies for preventing heat stress in poultry. *World's Poult. Sci. J*. **62,** 71-86.

Mashaly M.M., Hendricks G.L., Kalama M.A., Gehad A.E., Abbas A.O. and Patterson P.H. (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poult. Sci.* **83,** 889-894.

McDowell L. (1989). Vitamins in Animal Nutrition Comparative Aspects to Human Nutrition. Academic Press, London, UK.

McKee J., Harrison P. and Riskowski G. (1997). Effects of supplemental ascorbic acid on the energy conversion of broiler chicks during heat stress and feed withdrawal. *Poult. Sci.* **76,** 1278-1286.

Nedyalka V., Yanishlieva E.M., Marinovaa M.H., Gordon V. and Raneva G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem.* **64,** 59-66.

Pisoschi A.M., Pop A., Negulescu G.P. and Pisoschi A. (2011). Determination of ascorbic acid content of some fruit juices and wine by voltammetry performed at pt and carbon paste electrodes. *Molecules.* **16,** 1349-1365.

Radwan N.L., Hassan R.A., Qota E.M. and Fayek H.M. (2008). Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. *Int. J. Poult. Sci.* **7,** 134-150.

Sahin K., Sahin N. and Yaralıoglu S. (2002). Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites and mineral concentrations of laying hens reared at high ambient temperature. *Biol. Trace Elem. Res.* **85,** 35-45.

SAS Institute. (2001). SAS®/STAT Software, Release 8. SAS Institute, Inc., Cary, NC. USA.

Sengül T., Yurtseven S., Cetin M., Kocyigit A. and Sogut B. (2008). Effect of thyme (*Thymus vulgaris*) extracts on fattening performance, some blood parameters, oxidative stress and DNA damage in Japanese quails. *J. Anim. Feed Sci.* **17,** 608-620.

Siegel H.S. (1995). Stress, strains and resistance. *Br. Poult. Sci.* **36,** 3-22.

Tatli Seven P., Seven I., Yılmaz M. and Simsek G. (2008). The effects of Turkish propolis on growth and carcass characteristics in broilers under heat stress. *Anim. Feed Sci. Technol.* **146,** 137-148.

Thanissery R., Kathariou S. and Smith D.P. (2014). Rosemary oil, clove oil, and a mix of thyme-orange essential oils inhibit *Salmonella* and *Campylobacter in vitro*. *J. Appl. Poult. Res.* **23,** 221-227.

Toghyani M., Tohidi M., Gheisari A.A. and Tabeidian S.A. (2010). Performance, immunity, serum biochemical and hemato-logical parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. *African J. Biotechnol.* **9,** 6819-6825.