PHYSIOLOGY AND REPRODUCTION

The long-term oral administration of thyroxine: effects on blood hematological and biochemical features in broiler breeder hens

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ABSTRACT Published data on the beneficial effect of short-term administration of thyroxine (T_4) in broiler breeder hens to reduce the ascites incidence in their progeny chicks raises the question as to what extent might the long-term maternal administration of T_4 affect the blood hematological and biochemical attributes in breeder hens. A total of 70 broiler breeder hens (47-wk-old) were randomly allotted to control or thyroxine treated (T_4) groups. Pure T_4 (0.3 mg/bird per day) was orally administered to T_4 birds for 14 successive weeks, whereas the control group received the drinking water only. Blood samples were obtained from the brachial vein prior to the initiation of the trial as well as weeks 50, 53, 55, 57, 59, and 61 of age. Body weight was decreased but egg production was not affected by T_4 treatment. Plasma concentration of T_4, but not triiodothyronine (T_3), was increased in T_4-treated hens ($P < 0.05$). The total number of leukocytes and erythrocytes were also higher in T_4 birds. A significant effect of time was observed for erythrocyte number and plasma cholesterol concentration ($P < 0.05$). The long-term administration of T_4 did not affect the concentrations of serum calcium and plasma total protein, albumin, globulin, cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein, alanine amino transferase, and aspartate amino transferase ($P > 0.05$). However, serum concentrations of phosphorus, glucose, and alkaline phosphatase were higher in T_4 hens as compared to their control counterparts. In spite of differences in circulatory concentrations of a number of traits between the experimental groups, the recorded values were within their reference ranges. Therefore, the administration of T_4 for an extended period of time had no apparent adverse effect on the clinical profile in subjected hens, which may practically support the implementation of this preventative treatment as an approach to decrease the ascites incidence; however, a lower incidence rate in the progeny chicks produced from hens receiving T_4 for long-term periods of time remains to be elucidated.

Key words: biochemistry, blood enzyme, hematology, hyperthyroidism, thyroid hormone

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INTRODUCTION

The ascites syndrome, as a consequence of high oxygen demands of the fast growing tissues in meat type chicken (Julian, 1993) is accompanied by a marked increase in broiler mortality rate (Genget al., 2007). Thyroid hormones concentration has been reported to be reduced in ascitic chickens prior to death and thyroxine (T_4) administration was suggested to diminish the mortality rate from the ascites syndrome in broiler chickens (Luger et al., 2001, 2002). Accordingly, Akhlaghi et al. (2012) found that maternal administration of T_4 for 4 wk was associated with a decreased ascites incidence in their cold-exposed progeny chicks. The same treatment was also reported to be associated with enhanced early immune responses (Akhlaghi et al., 2013a) with no adverse effect on growth performance and intestinal morphology (Akhlaghi et al., 2013b) in their offspring.

Despite the importance of the preventive effect of maternal hyperthyroidism on ascites incidence rate and its enhancing effect on the immune function in the

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offspring, the effects of long-term administration of T_4 have not been adequately addressed. Most recently, the long-term effects of T_4-induced hyperthyroidism on the histology of oviduct (Saemi et al., 2018a) and reproductive performance (Saemi et al., 2018b) in broiler breeder hens have been reported. To our knowledge, the influence of long-term induced hyperthyroidism on the clinical profile of broiler breeder hens has not been dealt with previously. Practically, plausible adverse effects of long-term maternal administration of T_4 on hematology and biochemistry of blood would limit the use of this treatment in preventing the ascites incidence in the progeny chicks. Therefore, the aim of the present study was to ascertain whether the long-term administration of T_4 for 14 consecutive week might be associated with any changes (possibly the adverse ones) in blood hematological and biochemical attributes in Cobb 500 broiler breeder hens to provide valuable recommendations for T_4 administration in reducing the ascites incidence.

MATERIALS AND METHODS

Birds and Experimental Treatments

All procedures in the current study were approved by the Animal Care and Welfare Committee of the Department of Animal Science, College of Agriculture, Shiraz University (Shiraz, Iran). A total of seventy 47-wk-old Cobb 500 breeder hens (weighing ∼3890 g) were randomly allotted to control or extra-T_4 treated (**T_4**) groups with 5 replicates of 7 birds for each treatment group. The hens in the T_4 treatment group individually received an oral administration of pure T_4 (0.3 mg/bird per day; Iran Hormone Pharmaceutical Company, Tehran, Iran) dissolved in 1 mL of water for 14 successive weeks. A sham operation was conducted for the control counterpart. The birds were reared under the same conditions (a 15.5L:8.5Dphotoschedule photoperiod and 21°C ambient temperature) and fed a corn-soybean based diet (155 g/d per bird; 2700 kcal metabolizable energy/kg, and 14.0, 2.99, and 0.36% crude protein, calcium, and phosphorus, respectively; Table 1) with a free access to fresh drinking water.

Table 1. Ingredients and chemical composition of the experimental diet fed to breeder hens (DM basis).

Ingredient	%
Corn grain	36.60
Wheat grain	25.00
Barley grain	13.40
Soybean meal (44%)	15.76
Oyster shell	7.06
Dicalcium phosphate	1.48
Sodium chloride	0.18
Sodium bicarbonate	0.16
Vitamin premix[1]	0.10
Mineral premix[2]	0.10
DL-Methionine	0.095
L-Lys	0.040
L-Thr	0.025
Composition	
ME (kcal/kg)	2700
CP (%)	14.00
Ca (%)	2.99
P (%)	0.36

[1]Supplied per kg diet: vitamin A, 14,000 IU; vitamin D3, 3000 IU; niacin, 50 mg; vitamin E, 35 mg; calcium pantothenate, 20 mg; vitamin K_3, 4 mg; riboflavin, 7.0 mg; pyridoxine, 5.7 mg; vitamin B_{12}, 25 μg, and biotin, 50 μg.

[2]Supplied per kg diet: Fe ($FeSO_4$·H2O), 85 mg; Mn ($MnSO_4$·H_2O), 90 mg; Zn (ZnO), 67.3 mg; Cu ($CuSO_4$·$5H_2O$), 11.1 mg, and Se (Na_2SeO_3), 0.19 mg.

Blood Sampling and Hematology

Body weight and egg production were recorded on a weekly basis. Blood samples (5 mL) were obtained from the brachial vein prior to the experiment (week 47) as well as weeks 50, 53, 55, 57, 59, and 61 of age following weighing the birds. A fraction of each sample was collected in anticoagulant-free tubes to obtain serum samples and the remaining blood was collected in EDTA-coated tube to separate the plasmas. Prior to centrifugation, the whole blood samples were used to quantify the total erythrocyte and leukocyte number by hemocytometer, using the Natt–Herrick's solution (Buitenhuis et al., 2006). To determine the percentages of lymphocytes, monocytes, heterophils, eosinophils, basophils, and heterophil to lymphocyte (**H:L**) ratio, duplicate blood smears were air-dried, stained with Wright's-Giemsa (Saikin Kagaku Institute Co. Ltd., Sendai, Japan), and counted to a total of 100 cells/slide, using a Zeiss (Jena, Germany) compound light microscope (×1000 magnification).

Blood Biochemical Indices

Following the sampling for blood cells enumeration, the remaining blood samples were centrifuged (1800 × g for 12 min, 12°C), using a rotating centrifuge (International Equipment Co., Needham Heights, MA). Plasma/serum samples were then decanted and divided into 3 aliquots and stored at –20°C until analyzed for T_3 and T_4, using commercially available kits (Padtan Elm, Tehran, Iran) and an ELISA reader (Anthos2020, Biochrom Co, England). The procedures were validated for parallelism and recovery rate in chicken samples as described previously (Akhlaghi et al., 2012). Briefly, the samples were diluted at a rate of 1 to 5 with the dilution buffer. The concentrations of T_3 and T_4 were calculated from a standard curve, which ranged between 0.5 to 300.0 and 0.03 to 10.0 ng/mL for T_4 and T_3, respectively ($r > 0.99$). In order to control for linearity, serial dilutions at the ratios of 1:2, 1:4, 1:8, and 1:16 were provided for 4 plasma samples with given T_4 levels, using a calibrator solution (T_4 = 0.00 ng/mL). The samples were evaluated in duplicate. The intra- and inter-assay coefficients of variation were 12.6 and 13.2% for T_3, and 7.6 and 2.2% for T_4, respectively.

The concentrations of total protein, albumin, globulin, total cholesterol, high-(**HDL**), low-(**LDL**), very low-(**VLDL**) density lipoprotein, triglycerides,

Table 2. Effects of long-term induced hyperthyroidism on body weight, egg production, and hematological attributes in Cobb 500 broiler breeder hens (least squares means).[1]

Trait	Treatment		SE	*P*-value		
	Control	Hyperthyroid		Treatment	Time	Treatment × time
Body weight (g)	4369*	4257	38.6	0.040	NS	NS
Egg production (%)	46.01	45.84	1.09	NS	0.0003	<0.001
Leukocytes number (10^3/mL)	10.7	11.5*	0.14	<0.001	NS	NS
Erythrocytes number (10^3/mL)	2400	2600*	0.01	<0.001	<0.001	0.002
Lymphocytes (%)	49.4	50.0	0.29	NS	NS	NS
Monocytes (%)	6.3	6.1	0.18	NS	NS	NS
Heterophils (%)	42.5	42.1	0.27	NS	NS	NS
Eosinophils (%)	1.8	1.6	0.15	NS	NS	NS
Heterophils/Lymphocytes	0.9	0.8	0.01	NS	NS	NS

[1]The hens in the hyperthyroid group individually received an oral administration of thyroxine (0.3 mg/bird per day) dissolved in 1 mL of water for 14 successive weeks. A sham operation was conducted for the control group. Blood sampling was done from 47 to 61 wk of age (n = 35 hens/treatment). Blood sampling was done from 47 to 61 wk of age (n = 35 hens/treatment).

*Significant difference ($P \leq 0.05$).

NS: Not significant ($P > 0.05$).

aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), and alkaline phosphatase (**ALP**) were quantified in the plasma samples (Pars Azmoon Co., Tehran, Iran). The concentration of glucose, calcium, phosphorus, and ALP were determined in serum samples. The biochemical assessments were carried out by spectrophometric methods (Butler and Laqua, 1995). The quantification protocol for each trait was also validated for chicken plasma/serum according to the procedure described for T_3 and T_4.

Statistical Analysis

The experiment was conducted as a completely randomized design. Levene's and Kolmogorov–Smirnov tests were respectively used to test the data for equality of variances and normality. The percentage data were arc-sin transformed and the data subjected to the Proc MIXED (SAS, 2002). Body weight was included in the model as a covariate for analysis of variance. The means were compared by the least squares means and the level of significance was set at $P \leq 0.05$.

RESULTS

The concentration of T_4 was elevated in the T_4 hens (27.08 ng/mL) as compared with that in the control birds (10.24 ng/mL; $P = 0.003$); whereas plasma T_3 concentration was not influenced by the long-term administration of T_4 ($P > 0.05$). No bird died during the study. The effects of long-term induced hyperthyroidism on body weight, egg production, and hematological attributes in broiler breeder hens are presented in Table 2. A decrease in body weight was recorded in T_4 birds (4,369 and 4,257 g for control and T_4 birds, respectively; $P = 0.040$). No differences in egg production were found between the experimental groups ($P > 0.05$), although time ($P = 0.003$) and treatment by time interaction effect ($P < 0.001$; Figure 1) influenced the percentage of egg production.

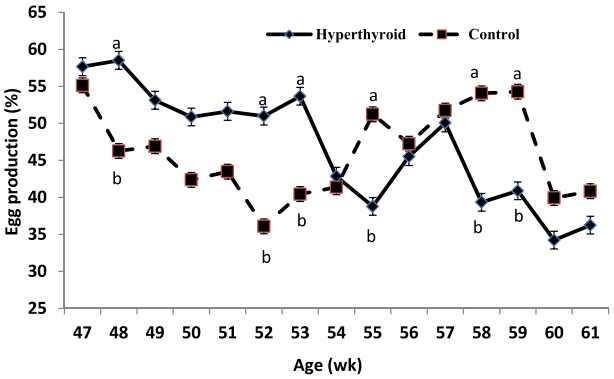

Figure 1. Thyroxine × time interaction effect on weekly egg production percentage in Cobb 500 broiler breeder hens. The hens in the hyperthyroid group individually received an oral administration of pure thyroxine (0.3 mg/bird per day) dissolved in 1 mL of water for 14 successive weeks. A sham operation was conducted for the control group. [a,b]Within each week, means with different letters differ significantly ($P \leq 0.05$). Differences were found in weeks 48, 52, 53, 55, 58, and 59.

Thyroxine administration increased the total number of leukocytes (11.5 × 10^3/mL) compared to the control (10.7 × 10^3/mL) birds ($P < 0.001$). Likewise, a higher total erythrocyte number was found in hyperthyroid hens (2600 vs. 2400 × 10^3/mL for control and T_4 birds, respectively; $P < 0.001$). Treatment × time (wk) interacted to affect the number of erythrocytes, where higher numbers in the T_4 birds were recorded as compared to the control treatment group from 50 to 61 wk of age (Figure 2). Leukocyte differential counts and H:L ratio, however, were not affected by T_4 administration, time, and their interaction effects (Table 2).

The effects of long-term induced hyperthyroidism on blood biochemical attributes in the experimental birds are presented in Table 3. Blood phosphorus level showed an increase in the T_4 hens (5.77 mg/dL) in comparison to that in the control birds (5.17 mg/dL; $P = 0.0121$), although no significant effects were found for time and treatment by time interaction effects ($P > 0.05$). Birds

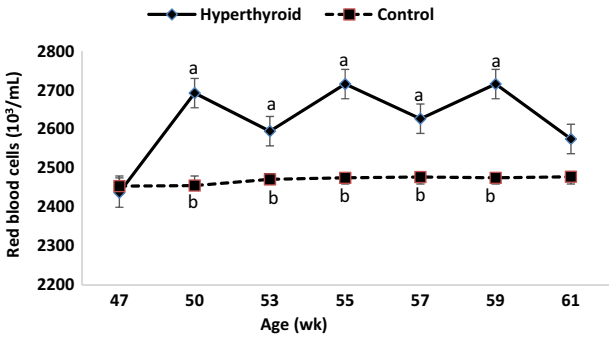

Figure 2. Thyroxine × time interaction effect on red blood cells count in Cobb 500 broiler breeder hens.The hens in the hyperthyroid group individually received an oral administration of pure thyroxine (0.3 mg/bird per day) dissolved in 1 mL of water for 14 successive weeks. A sham operation was conducted for the control group. Blood sampling was done from 47 to 61 wk of age (n = 35 hens/treatment). [a,b]Within each week, means with different letters differ significantly ($P \leq 0.05$). No differences were found in weeks 47 and 61.

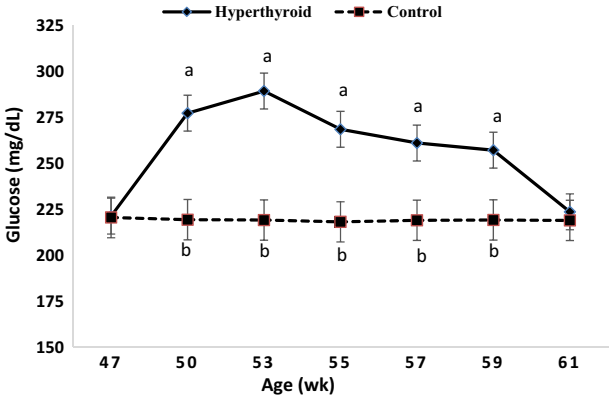

Figure 3. Thyroxine × time interaction effect on blood glucose concentration in Cobb 500 broiler breeder hens.The hens in the hyperthyroid group individually received an oral administration of pure thyroxine (0.3 mg/bird per day) dissolved in 1 mL of water for 14 successive weeks. A sham operation was conducted for the control group. Blood sampling was done from 47 to 61 wk of age (n = 35 hens/treatment). [a,b]Within each week, means with different letters differ significantly ($P \leq 0.05$). No differences were found in weeks 47 and 61.

subjected to extra T_4 recorded a higher serum glucose level (256.9 mg/dL) when compared to that in their control counterparts (219.3 mg/dL; $P < 0.0001$). The glucose level was also affected by time ($P < 0.0017$) and treatment × time interactive ($P < 0.0011$) effects, where the hyperthyroid treatment group recorded the higher levels during the trial, except for values recorded for 47 and 61 wk of age (Figure 3). Long-term administration of T_4 was associated with an increased serum level of ALP, where the T_4 birds recorded the value of 20.03 U/L as compared with that in the control birds (18.53 U/L;$P = 0.0051$). Extended period of exposure to T_4 did not influence the serum calcium and plasma concentrations of total protein, albumin, globulin, total cholesterol, triglycerides, HDL, LDL, VLDL, AST, and ALT ($P > 0.05$; Table 3).

DSCUSSION

Considering the role of thyroid hormones in regulating the ascites incidence in broilers (Suvarnaet al., 1993) and the lower cold-induced ascites incidence in progeny chicks produced by hens subjected to extra T_4 for a limited period (Akhlaghi et al., 2012), we hypothesized that the long-term administration of T_4 would affect the blood attributes in broiler breeder hens. Although the percentage of egg production was not affected by T_4 in the present work, comparable treatment in our most recent report was associated with a decrease in albumen height, Haugh unit, and egg weight as well as an increase in albumen pH (Rostami et al., 2019). Although the T_4 concentration was increased in the T_4

Table 3. Effects of long-term induced hyperthyroidism on blood biochemical attributes in Cobb 500 broiler breeder hens (least squares means).[1]

	Treatment			*P*-value		
Trait	Control	Hyperthyroid	SE	Treatment	Time	Treatment × time
Calcium (mg/dL)	11.51	11.88	0.18	NS	NS	NS
Phosphorus (mg/dL)	5.17	5.77*	0.16	0.0121	NS	NS
Glucose (mg/dL)	219.3	256.9*	3.54	<0.0001	<0.0017	<0.0011
Total protein (mg/dL)	3.52	3.46	0.05	NS	NS	NS
Albumin (mg/dL)	1.81	1.86	0.02	NS	NS	NS
Globulin (mg/dL)	1.70	1.59	0.06	NS	NS	NS
Total cholesterol (mg/dL)	138.51	137.78	1.48	NS	0.0051	NS
Triglyceride (mg/dL)	81.58	81.64	1.69	NS	NS	NS
HDL(mg/dL)	70.51	70.41	1.13	NS	NS	NS
LDL (mg/dL)	51.68	51.04	1.67	NS	NS	NS
VLDL (mg/dL)	16.32	16.33	0.34	NS	NS	NS
ALP (U/L)	18.53	20.03*	0.37	0.0051	NS	NS
ALT (U/L)	14.62	14.69	0.29	NS	NS	NS
AST (U/L)	151.55	150.68	2.31	NS	NS	NS

[1]The hens in the hyperthyroid group individually received an oral administration of thyroxine (0.3 mg/bird per day) dissolved in 1 mL of water for 14 successive weeks. A sham operation was conducted for the control group. Blood sampling was done from 47 to 61 wk of age (n = 35 hens/treatment).

*Significant difference ($P \leq 0.05$).

NS: Not significant ($P > 0.05$).

HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

hen in the current study, there was no effect of T_4 administration on plasma T_3 concentration which can be ascribed to the conversion of T_4 to inactive reversed T_3 (Decuypere et al., 1987).

Red blood cells (Maxon et al., 1975), white blood cells (Fabris, 1973; Bachman and Mashaly, 1987), and blood concentrations of calcium (Jowsey and Detenbeck, 1969), total proteins (Goodman, 2009), albumin (Harrison and Harrison, 1986), cholesterol (Campbell and Coles, 1986), triglycerides (Davey et al., 2001), lipoproteins (Duntas, 2002), and several serum enzymes (Huang and Liaw, 1995) were reported to be influenced by thyroid hormones. Although T_4 treatment increased the total number of leukocytes, we found no effect of long-term administration of T_4 on the relative number of lymphocytes, monocytes, heterophils, and eosinophils. Klecha et al. (2006) showed that thyroid hormones have an important role in regulating lymphocyte reactivity. Fabris (1973) suggested that thyroidectomy caused a decrease in total leukocytes number as a result of decreased lymphocyte number. Consistent to the present findings, Chatterjee and Chandel (1983) showed that hyperthyroidism was associated with increased white blood cells and lymphocytes numbers, and there was a positive association between thyroid hormone levels and lymphocyte numbers in birds. Hyperthyroidism was also shown to be associated with an increased lymphocyte number in human (Ultmann et al., 1963). Increased erythrocyte number in T_4 hens may be ascribed to the erythropoietic effect of thyroid hormones (Golde et al., 1977), although erythrocyte concentration was within reference ranges in the current study.

Bone mobilization in hyperthyroid individuals, as a result of increased osteoclastic activity, is associated with a trend to hypercalcemia and decreased parathyroid hormone secretion (Mosekilde et al., 1990); however, T_4 treatment over a relatively long period did not affect the serum calcium concentration in the present study. Serum phosphorous in the T_4 group was higher than that in the control treatment group. It has been shown that hyperthyroid individuals show hyperphosphatemia as a result of increased bone catabolism and decreased phosphorous clearance (Mosekilde and Christensen, 1977). Therefore, the higher levels of phosphorus in T_4 hens may be due to the direct effect of thyroid hormones on tissue phosphate metabolism and renal phosphate clearance (Dhanwal, 2011).

Alkaline phosphatase, a biochemical marker of bone formation and resorption, was increased in hyperthyroid patients (Pantazi and Papapetrou, 2000). Increased alkaline phosphatase concentration in the T_4 hens in the present work may be attributed to the stimulation of membrane-bound fragment of alkaline phosphatase in osteoblasts by T_4 (Banovac and Koren, 2000).

Birds subjected to extra T_4 recorded a higher serum glucose level in the present study. Thyroid hormones have an important role in regulation of glucose homeostasis (Brenta, 2010). Hyperthyroidism increases blood glucose levels, and thyrotoxic patients show diabetic glucose tolerance after a reversal to the euthyroid condition (Maxon et al., 1975). Thyroid hormones influence metabolism in the small intestine with T_4 in vitro for 72 h increasing glucose active transport in the duodenum of chick embryos (Black, 1988). Thyroid hormones elevate the expression of the enzymes involved in gluconeogenesis, phosphoenolpyruvatecarboxykinase (Park et al., 1999), and glucose-6-phosphatase (Suh et al., 2013), which are involved in hepatic glucose production. Hyperthyroidism causes activation of hepatic gluconeogenesis by enhancing alanine transport and its transformation to glucose (Park et al., 1999). In hyperthyroid individuals, production of endogenous glucose is elevated and does not respond to the repressive effect of insulin (Holness and Sugden, 1987).

Overall, the long-term administration of T_4 in broiler breeder hens had no adverse effects on blood hematological and biochemical characteristics, and apart from increases in the concentration of some attributes, the recorded values were within their reference ranges. According to unaffected H:L ratio, the birds orally administered by extra T_4 did not experience stress during the trial. The present study, however, did not evaluate the progeny chicks in term of ascites incidence. If the preventative effect of long-term maternal exposure to extra T_4 on the ascites incidence in progeny chicks (not evaluated here) is comparable to that of the short-term exposure (Akhlaghi et al., 2012), the long-term maternal administration of T_4 may then be a helpful alternative to decrease the incidence of ascites in the progeny of broiler breeder hens; however, application at the farm level requires further substantiation of the present findings, with an eye to immune- and growth-associated effects in progeny broilers.

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