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Effects of Long-term Induced Hyperthyroidism on Egg Quality Traits in Cobb 500 Broiler Breeder Hens

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Abstract

Published data on 4-week-long administration of exogenous thyroxine in broiler breeder hens to decline the cold-induced ascites in their progeny suggest that the long-term maternal hyperthyroidism would affect egg quality characteristics traits in Cobb 500 broiler breeder hens. Seventy 47-w-old broiler breeder Cobb 500 hens (5 replicates and 7 hens each) were assigned in separate cages and allotted to two treatments, control and hyperthyroid. Thyroxine was orally administered to the hyperthyroid group (0.3 mg hen/day) for a period of 100 days consecutively. Simultaneously, distilled water was orally administered to the control group. The blood sampling was done every two weeks to analyze T_3, T_4, and estrogen assays, using commercially kits and the egg quality attributes were evaluated for weeks 0, 3, 6, 9, and 12. Thyroxine treatment resulted in an increase in plasma concentration of T_4; however, the T_3 level and estrogen were not affected. The results of this study showed that the long-term administration of thyroxine had adverse effect on the most of egg quality traits in broiler breeder hens; although the results may be distinct for treatments that using other birds, doses and duration of treatments, among the different egg quality traits of broiler breeder hens evaluated in this research. Therefore, further studies should be done to make a final conclusion to use of long-term maternal hyperthyroidism treatment to reduce the ascites incidence.

Introduction

Thyroid hormones are required for normal reproductive function (Sechman, 2013). The considerable influence of thyroxine on egg characteristics of White Leghorn hens was reported (Peebles *et al*., 1994). Moreover, the effect of thyroid function on avian egg shell feature was reported in White turkey and Leghorn Chicken (Christensen and Ort, 1990; Peebles *et al*., 1992). Pulmonary hypertension syndrome (fluid-filled peritoneal cavity), better known as ascites syndrome, is a metabolic disorder mostly appears in fast-growing broiler chickens (Luger *et al*., 2002). This non-infectious disorder accounts for twenty-five percent of overall mortality (Zheng *et al*., 2007).

Several practices have been suggested to cope with this syndrome which include: supplementing probiotics, ubiquinone, clenbuterol and potassium bicarbonate to feed and drinking water (Ocampo *et al*., 1998; Shlosberg *et al*., 1991; Geng *et al*., 2004; Solis de los Santos *et al*., 2005). Furthermore, genetic selection and modifying lighting schedule was reported to be effective (Buys *et al*., 1998; Pavlidis *et al*., 2007). In a study Akhlaghi *et al*. (2012) reported that 4-week administering of exogenous thyroxine to broiler breeder hens decreased ascites in progeny broiler chicks. In the other study, induced maternal hyperthyroidism not only did not weaken immune function but also improved early immune response in their offspring (Akhlaghi *et al*., 2013).

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Despite the importance of preventive effect of maternal hyperthyroidism on ascites and immune function stimulator in progeny chicks, there are some concerns remain on the effect of hyperthyroidism on hen itself. By the way, one of the main concerns is the reproductive functionality of hen. As a sequel to our recent report (Saemi *et al*., 2018a) evaluating the egg quality traits gives a valuable key that can bring us one step closer to prescribe thyroxine administration. Therefore, the aim of the current study was to assess whether the ascites-diminishing effect of long-term hyperthyroidism is correlated with various egg quality characteristics changes in Cobb 500 broiler breeder hens.

Materials and Methods

Birds and experimental design

The procedures of this study were approved by the Animal Care and Welfare Committee of the Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Seventy 47-w-old broiler breeder Cobb 500 hens (5 replicated and 7 hens each) were assigned in separate cages and allotted to two treatments included control and hyperthyroid. Cages assignment to treatments and hens to cages were on a random basis. Thyroxine (Iran Hormone, Tehran, Iran) was orally administered (0.3 mg hen/day) for a period of 100 days consecutively. Simultaneously, distilled water at the same volume was orally administered to the control group. Birds were maintained under controlled condition (at average 21°C, and a relative humidity of 70%, 16L: 8D photo schedule at 40 lux light intensity). Hens were provided restrictedly with standard commercial diet (2,700 kcal of ME/kg, 14.5% CP, 2.99% Ca, and 0.36% Pa; Table 1).

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

Ingredient (%)	Value
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
Vitamin premix[1]	0.10
Mineral premix[2]	0.10
Sodium chloride	0.18
Sodium bicarbonate	0.16
DL-Methionine	0.095
L-Thr	0.025
L-Lys	0.04
Composition	
ME (kcal/kg)	2700
CP (%)	14.05
Ca (%)	2.99
Pa (%)	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 ($FeSO_4 \cdot H2O$), 85 mg; Mn ($MnSO_4 \cdot H_2O$), 90 mg; Zn (ZnO), 67.3 mg; Cu ($CuSO_4 \cdot 5H_2O$), 11.1 mg, and Se (Na_2SeO_3), 0.19 mg.

Egg quality traits assessment

Briefly, six eggs from each experimental replicate were collected every three weeks. Totally, 60 eggs were randomly collected, in a 12 weeks of experimental period. Then egg quality attributes such as the egg weight (mass), the shell thickness, the yolk an albumen height, the pH of yolk and albumen were evaluated in weeks 0, 3, 6, 9, and 12. Furthermore, the Haugh unit and the egg shape index were calculated.

Hormonal analysis

The blood sampling was done every two weeks to analyze T_3, T_4, and estrogen assays. The blood samples were obtained from brachial vein and collected into EDTA-coated tubes and centrifuged for 15 min (1800 × *g* in 20°C). The plasma was stored at –20°C to analyze for aforementioned hormones using commercial ELISA kit (Padtan Elm Co. Ltd.) validated for chicken plasma (Akhlaghi *et al*., 2012). 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.

Statistical analysis

This study was carried out in a completely randomized design. The data were tested for normality (SAS, 2003). Data were subjected to the GLM procedure, but repeated measures data were analyzed by the PROC MIXED. Differences between means were compared by the least squares procedure and the level of significance was set at $P \leq 0.05$.

Results

The result of the current study illustrated plasma concentration of T_4 was increased; however, the T_3 level was not affected (Table 2). The effect of long-term hyperthyroidism on egg quality traits of broiler breeder hens is presented in Table 3. All egg quality variables except for shell thickness and yolk index were adversely affected by the treatments. Generally, the most of traits were declined in hyperthyroid group as compared with the control group. The albumen height (P=0.0003) and Haugh unit (P=0.0012) decreased in hyperthyroid group, whereas, the pH increased (P=0.0001). Interaction of time and treatment was statistically significant for all variables except for shell thickness.

Table 2. The effect of long-term hyperthyroidism on plasma levels of triiodothyronine (T_3), thyroxine (T_4), T_3:T_4 ratio, and estrogen (Lsmean ± *SE*) in Cobb 500 broiler breeder hens[1].

Trait	Control	Hyperthyroid	*P*-value
T_3 (ng/mL)	1.59 ± 0.26	1.64 ± 0.24	NS
T_4 (ng/mL)	10.24 ± 0.93 [b]	27.08 ± 0.89 [a]	< 0.001
T_3:T_4 ratio	0.152 ± 0.007 [a]	0.059 ± 0.007 [b]	0.002
Estrogen (pg/mL)	175.26 ± 2.18	180.03 ± 2.18	NS

[a,b] Within rows, values with different superscripts differ significantly ($P \leq 0.05$).

[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment group).

NS: Non-significant.

Table 3. The effect of long term hyperthyroidism on egg quality traits in broiler breeder hens[1]

Trait	Control	Hyperthyroid	*P*-value		
			Treat	Week	Treat × Week
Albumen height (mm)	6.26±0.12	5.57 ± 0.12	0.0003	0.0119	0.0002
Albumen pH	7.67±0.07	8.36 ± 0.07	<0.0001	<0.0001	0.0003
Egg length (mm)	50.19 ± 0.41	49.57 ± 0.41	0.2954	0.0383	0.0002
Egg weight (g)	69.68 ± 0.79	66.46 ± 0.79	0.0067	0.2370	0.0085
Egg width (mm)	34.44 ± 0.19	33.92 ± 0.19	0.0644	0.0013	0.0188
Haugh unit	74.95 ± 0.92	70.38 ± 0.92	0.0012	0.0012	0.0005
Shape Index	68.66±0.46	68.59 ± 0.46	0.9164	0.0476	0.0046
Shell thickness	0.34 ± 0.25	0.72 ± 0.25	0.3031	0.3948	0.4183
Yolk diameter (mm)	33.81 ± 0.20	33.04 ± 0.20	0.01	0.0001	0.0199
Yolk height (mm)	19.46 ± 0.14	19.49 ± 0.14	0.90	<0.0001	<0.0001
Yolk index	57.59 ± 0.50	58.99 ± 0.50	0.0584	0.2856	0.0138
Yolk pH	5.71 ± 0.07	6.42 ± 0.07	<0.0001	0.0785	0.0430

[a,b] Within rows, values with different superscripts differ significantly ($P \leq 0.05$).

[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment group).

NS: Non-significant.

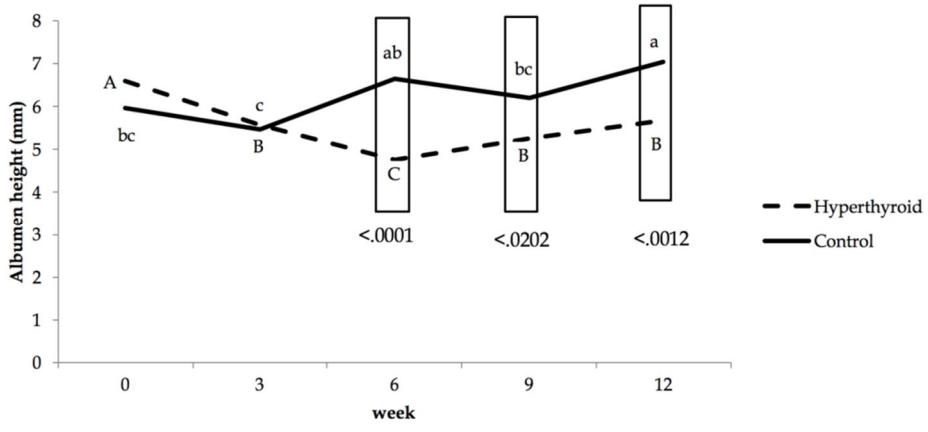

Figure 1. Effect of thyroxine × time (wk) interaction on albumen height (mm) in Cobb 500 broiler breeder hens[1].

[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled SEM = 0.27). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the *P*-value under it, means the statistical significance of differences between two groups in each week.

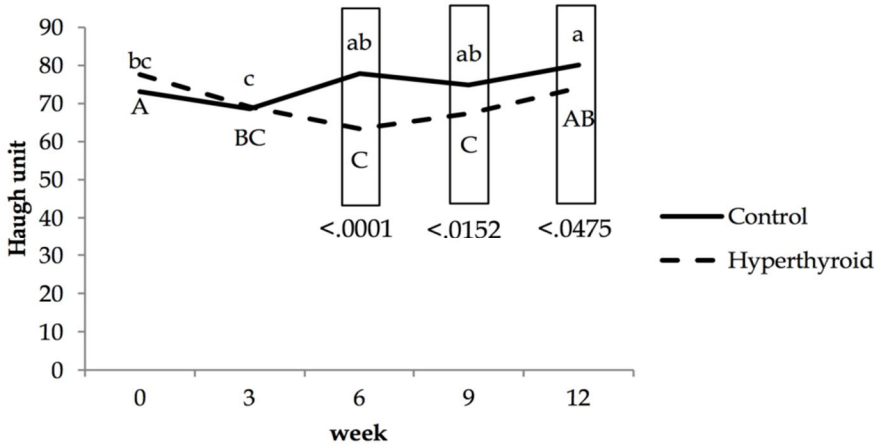

Figure 2. Effect of thyroxine × time (wk) interaction on Haugh unit in Cobb 500 broiler breeder hens[1].
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled *SEM* = 2.07). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten *P*-value means the statistical significance of differences between two groups in each week.

The albumen height and Haugh unit from the third week onward decreased significantly in hyperthyroid group compared with control group (Figures 1 and 2, respectively). After 6 weeks of thyroxine administration when the albumen height and Haugh unit reached the lowest point, an increasing trend in hyperthyroid group was observed. On the other hand, in control group a fairly increasing trend was observed.

As shown in Figure 3, the albumen pH was higher in hyperthyroid group throughout the period. The highest pH was observed at week 0 and 6 in the control and hyperthyroid groups, respectively. There was a clear trend in decreasing of albumen pH in the control group; however, the albumen pH fluctuated in hyperthyroid group during the experimental period.

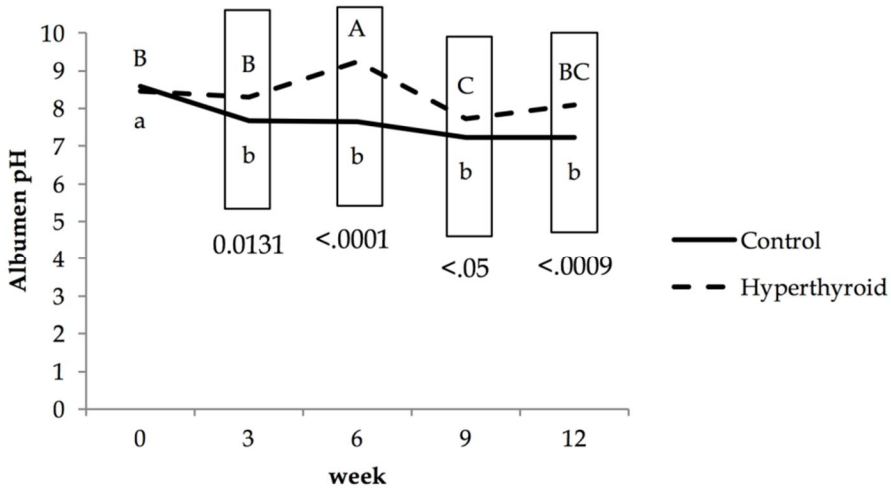

Figure 3. Effect of thyroxine × time (wk) interaction on albumen pH in Cobb 500 broiler breeder hens[1].
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled *SEM* = 0.17). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten *P*-value means the statistical significance of differences between two groups in each week.

As illustrated in Figure 4, the egg length (mm) in both groups compared to each other was unchanged during all experiment except for week in which the egg length sharply reduced in hyperthyroid group in comparison to control group 12. In contrast to hyperthyroid group, egg length in control group had a gradual increase from week 6, with the highest level in week 12.

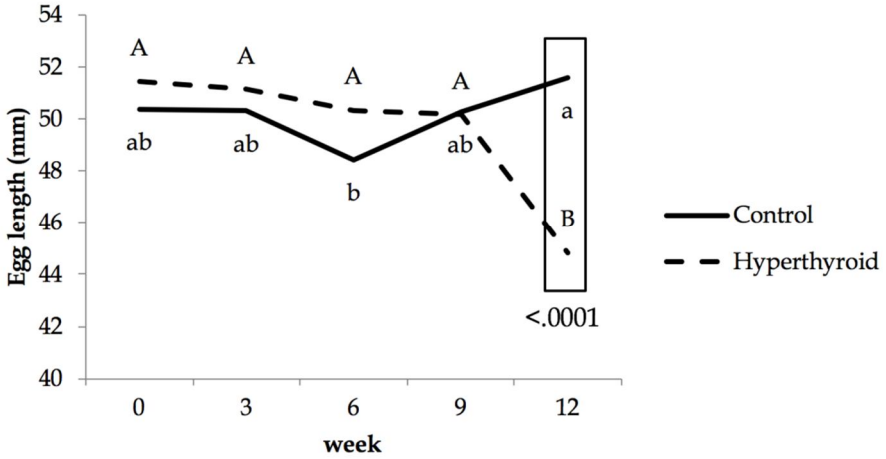

Figure 4. Effect of thyroxine × time (wk) interaction on Egg length (mm) in Cobb 500 broiler breeder hens[1].
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled SEM = 0.92). $^{a–c}$ Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). $^{A–C}$ Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten P-value means the statistical significance of differences between two groups in each week.

As it is apparent in Figures 5 and 6 the egg weight (g) and egg width (mm), respectively, almost followed an almost identical pattern as egg length. There was no difference between control and hyperthyroid group to week ninth. The egg weight (g) and egg width (mm) did not change during the study in control group; however, in hyperthyroid group a remarkable reduction in egg weight and egg width occurred in week 12. These reductions in egg weight and width were so much that caused the interactions between treatment and time to be significant (Fig. 5 and 6; P<0.0001 and P=0.0002 for egg weight and egg width, respectively).

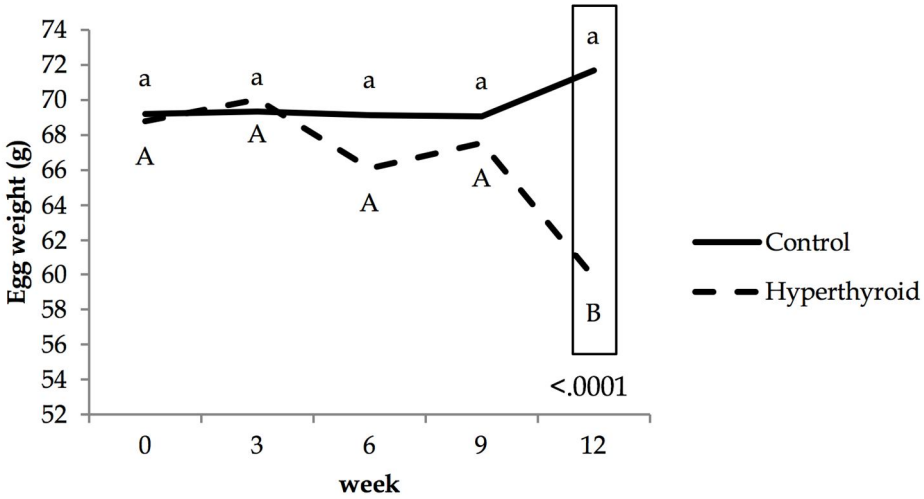

Figure 5. Effect of thyroxine × time (wk) interaction on Egg weight (g) in Cobb 500 broiler breeder hens.[1]
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled SEM = 1.78). $^{a–c}$ Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). $^{A–C}$ Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten P-value means the statistical significance of differences between two groups in each week.

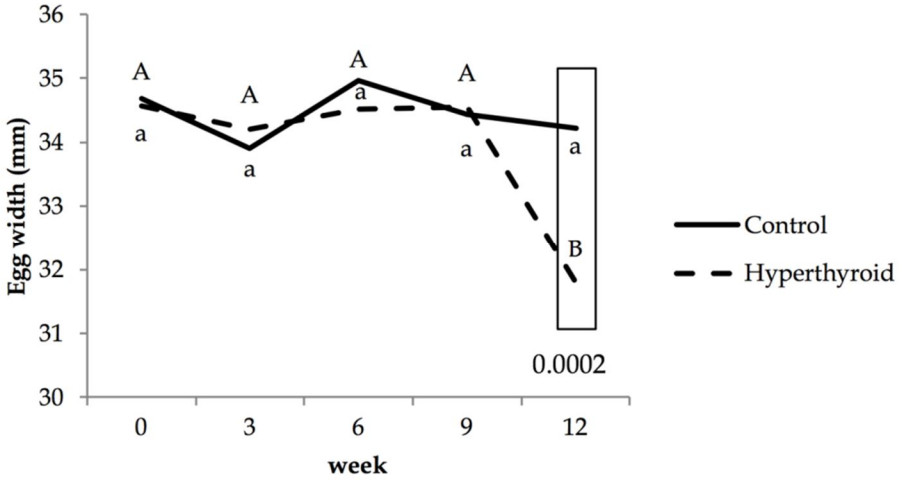

Figure 6. Effect of thyroxine × time (wk) interaction on Egg width (mm) in Cobb 500 broiler breeder hens[1].
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; *n* = 35 hens/treatment; pooled *SEM* = 0.42). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten *P*-value means the statistical significance of differences between two groups in each week.

The shape index was shown in Figure 7. The shape index in hyperthyroid group increased weekly with the highest level in week 12. In contrast, shape index in the control group had a steady state all over the experiment; however, only a sharp increase took place in week 6.

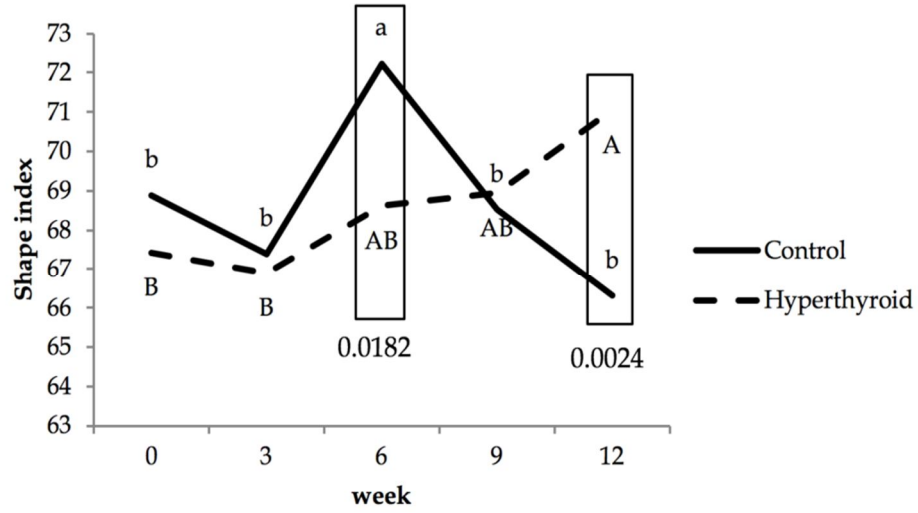

Figure 7. Effect of thyroxine × time (wk) interaction on shape index in Cobb 500 broiler breeder hens[1].
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; *n* = 35 hens/treatment; pooled *SEM* = 1.03). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten *P*-value means the statistical significance of differences between two groups in each week.

The yolk diameter (mm) in the control group as well as, the hyperthyroid group decreased in the third week of beginning the experiment (Figure 8). The yolk diameter in the control group remained constant until the end of the study; however, in the hyperthyroid group the yolk diameter reduced significantly in week 12 (*P*=0.0006).

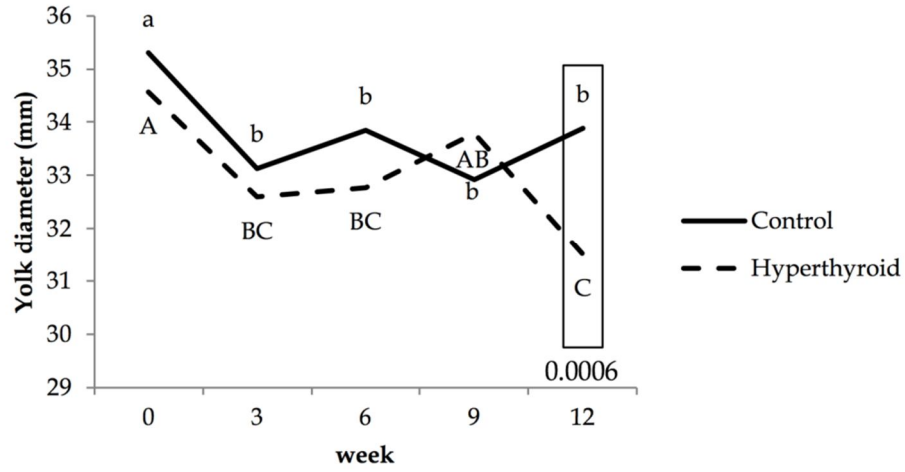

Figure 8. Effect of thyroxine × time (wk) interaction on yolk diameter (mm) in Cobb 500 broiler breeder hens[1]. [1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled SEM = 0.45). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten P-value means the statistical significance of differences between two groups in each week.

The yolk height (mm) change trend was nearly similar to that of the yolk diameter (Fig. 9). In which at the third week of experiment the yolk height in control group reduced compared with the hyperthyroid group (P=0.0036). In other weeks, the yolk height changes were similar to yolk diameter.

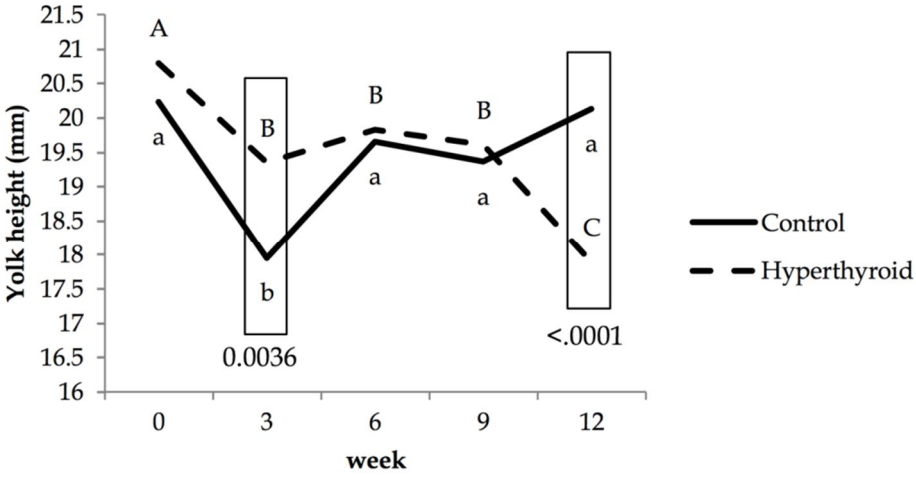

Figure 9. Effect of thyroxine × time (wk) interaction on yolk height (mm) in Cobb 500 broiler breeder hens[1]. [1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled SEM = 0.31). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten P-value means the statistical significance of differences between two groups in each week.

The yolk index in the control group and the hyperthyroid group followed a different trend (Fig. 10) in which the yolk index in the hyperthyroid group decreased in week 12. In the control group almost remained in a steady state. A significant difference was observed in week 3 between two groups (P=0.0031). The yolk pH in weeks 0, 6 and 12 was higher in the hyperthyroid group (Fig. 11). In both groups the yolk pH was not changed throughout the experiment.

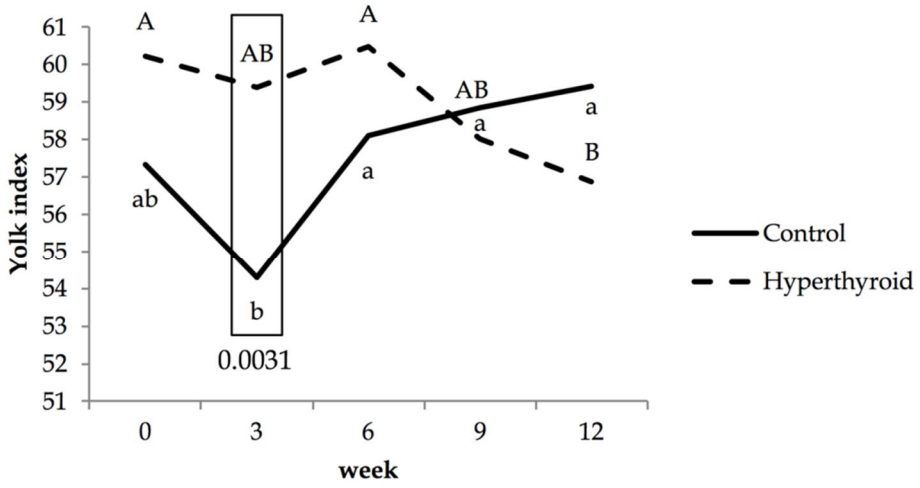

Figure 10. Effect of thyroxine × time (wk) interaction on yolk index in Cobb 500 broiler breeder hens[1].
[1]Thyroxine (T_4) was orally administered to the hyperthyroid group (0.3 mg/bird/day) and the control group received the drinking water (wk 47–64; n = 35 hens/treatment; pooled SEM = 1.13). [a–c] Within each treatment among weeks in control group, least squares means with different letters differ significantly ($P \leq 0.05$). [A–C] Within each treatment among weeks in hyperthyroid group, least squares means with different letters differ significantly ($P \leq 0.05$). The rectangle and the underwritten P-value means the statistical significance of differences between two groups in each week.

Discussion

Studies on long-term hyperthyroidism in broiler breeder hens revealed that ascites could be diminished via maternal hyperthyroidism in broiler chickens (Akhlaghi *et al*., 2012). Comparable study demonstrated that maternal hyperthyroidism regulated immune system in chickens without negative effect on host immunity (Akhlaghi *et al*., 2013). The present study aimed to induce hyperthyroidism, as it was improved ascites condition in broiler stock, on broiler breeder hens to understand its effect on egg quality traits. In spite of our expectations, a profound decrease in egg quality traits was found in hen receiving thyroxine.

CO_2 egress from egg caused albumen pH raised from 7.6 to 9.7, during storage. Concurrently, the liquefaction (egg white thinning) took place, in which the formation of thick egg white changes into liquid form. It is well established pH value increased during storage (Lapão *et al*., 1999), regarding the higher albumen pH in hyperthyroid group, it can be concluded that hyperthyroidism decreased egg quality at least by increasing the albumen pH. The higher pH value in albumen was recorded for hyperthyroid group in all experimental period in comparison to control group. Normal pH of egg albumen, as one the influential factor in the development of chick embryos, was reported between 7.9 and 8.4 (Reijrink, 2010), from this point of view the higher pH was recorded for weeks 0 in both control and hyperthyroid group and week 6 in hyperthyroid group.

As regards, the buffering capacity of albumen is weakest between 7.5 and 8.5 (albumen pH at oviposition =7.6), the increase of albumen pH in hyperthyroid group might be associated with CO_2 conductance. This event can be in relation with two factors: the albumen liquefaction and the shell thickness. As the shell thickness was not affected by hyperthyroidism status in this experiment, liquefaction of albumen in hyperthyroid group may be the primary factor in increasing the albumen pH. Furthermore, decreasing trend in albumen pH in both control and hyperthyroid group was observed in this experiment; however, the reason for this was not clear. In the current work the hatchability rate was not evaluated during experimental period; therefore, the probable contribution of these factors with hatchability could not be determined.

It is now well established that egg storage results in albumen liquefaction (Brake *et al*., 1997), which associated with a decrease in albumen height and Haugh unit. Some factors were reported to be associated with albumen liquefaction which includes modifying α- and β- ovomucin structure, the relation between ovomucin and lysozyme, actions of enzymes and ovomucin disulfide bonds in this phenomenon (Benton *et al*., 2001).

The present data suggested that albumen height and Haugh unit followed the same pattern. Decreased albumen height after 3 weeks of administering thyroxin can be interpreted in two ways; the first one is that the hyperthyroidism might be involved in protein degradation in magnum, as it was reported that egg quality traits can be affected by albumen proteins, because variation in albumen quality indices

are related to albumen proteins (Akhlaghi *et al*., 2013). The next one, which gives a plausible explanation, is associated with oviduct histology. As reported by Saemi *et al*. (2018b) the hyperthyroid group had lower tubular glands in the magnum compared with control group. In connection with this point, the tubular glands of the magnum are extended and expanded their openings to the surface epithelium.

From the histological point of view, any changes in epithelial cells of magnum tubular gland were associated with albumen quality. Furthermore, it was shown that tubular glands in the magnum produce albumen proteins (Davidson, 1986; Roberts, 2004). As a result, reduction of albumen quality may be as a consequence of long-term thyroxin supplementation.

From the data, it is apparent that the same dramatic reduction occurred in the yolk height, yolk diameter, egg width, egg weight and egg length in the 12^{th} week of thyroxin administration. All of them are indicative of egg shrinkage in size. An important concept that emerged from the data is that hyperthyroid effect on the egg dimensions was bring about after the action on egg internal characteristics such as yolk pH, albumen pH, and albumen height. Furthermore, it is possible to hypothesize that these condition make molting happening possible. In other point of view, significant reduction in both height and diameter of yolk took place. These results are in accord with study indicated that thyroid hormones have important role on ovarian follicles function in laying hens (Sechman, 2013). Some studies have revealed the effects of gonadal steroids on development of reproductive system (Kohler *et al*., 1969) and the influence of thyroid hormones on clearance of gonadal steroids (Klandorf *et al*., 1992), the decrease in some reproductive traits might be attributed to the plausible lower level of gonadal steroids in hyperthyroidism state, although the plasma estrogen concentration was not affected by long-term induced hyperthyroidism.

Conclusion

Since long-term administration of extra thyroxine in broiler breeder hens had undesirable effects on egg quality traits, in the case of evaluating the possible effects of this treatment on the basal metabolic rate, hatchability and the chicks' performance and quality, in addition to use of various doses and duration of treatments, it might be recommended the use of thyroid hormones commercially to reduce the incidence of ascites in breeder hens.

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