Maternal hyperthyroidism is associated with a decreased incidence of cold-induced ascites in broiler chickens

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ABSTRACT A hypothesis was tested that providing the breeder hens with exogenous thyroxine (T_4) would help their offspring to better survive the ascites-inducing condition during the growing period. In total, 132 broiler breeder hens were randomly assigned to one of 3 treatments: control (CON), hypothyroid [HYPO; 6-*N*-propyl-2-thiouracil (PTU)-treated], and hyperthyroid (HYPER; T_4-treated). The hens were artificially inseminated, and the hatching eggs (n = 1,320) were incubated. No eggs in the HYPO group hatched. The 1-d-old male chicks (n = 288) from other groups were reared for 42 d under standard or low ambient temperature to induce ascites. Blood samples were drawn from the hens, embryos, and broilers for determination of T_4 and triiodothyronine (T_3). The hematocrit was also determined in broilers. The PTU-treated hens had an increased BW along with lower plasma T_3 and T_4 concentrations. Plasma T_4 was higher in the HYPER hens compared with CON hens, but T_3 concentration was not different between these groups. The fertility rate was not affected by either hypo- or hyperthyroidism. The embryos in the HYPO group had lower plasma T_3 and T_4 concentrations at d 18 of embryonic development and internal pipping. Higher plasma T_4 was recorded in the HYPER birds at internal pipping, although plasma T_3 concentration was not affected at this stage. Maternal hyperthyroidism decreased the overall incidence of ascites in the cold-exposed chickens (10.0 vs. 33.4% for HYPER and CON groups, respectively). Although the effect of maternal PTU or T_4 treatment on plasma thyroid hormones and on the right ventricle-to-total ventricular weight ratio in the broilers was not significant, the cold-exposed healthy CON chicks showed higher hematocrit values, compared with the HYPER birds. It was concluded that maternal hyperthyroidism could decrease the incidence of cold-induced ascites in broiler chickens; however, probable causal mechanisms remain to be elucidated.

Key words: ascites, hyperthyroidism, cold stress, breeder hen, broiler

2012 Poultry Science 91:1165–1172
http://dx.doi.org/10.3382/ps.2011-02021

INTRODUCTION

The ascites syndrome, also known as pulmonary hypertension syndrome, is a metabolic disorder in broilers believed to be caused by the high oxygen requirement of rapidly growing tissues in the contemporary meat-type chickens (Julian, 1993). This metabolic disorder, accounting for over 25% of overall mortality (Guo et al., 2007), is a severe cause of loss to the broiler industry in many countries, because of the high rate of mortality, decreased weight gain, and increased condemnations at slaughter (Julian, 1993). Several methods have been proposed to combat ascites incidence, including genetic selection (Pavlidis et al., 2007), intermittent lighting (Buys et al., 1998), feed restriction (Shlosberg et al., 1991; Acar et al., 1995), and feeding prebiotics (Solis de los Santos et al., 2005), clenbuterol (Ocampo et al., 1998), coenzyme Q_{10} (Geng et al., 2004), or potassium bicarbonate in the drinking water (Shlosberg et al., 1998). Nevertheless, decreasing the ascites incidence still appears to be a challenge in the broiler industry.

Received November 14, 2011.
Accepted January 19, 2012.
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From an endocrinological standpoint, the role of thyroid hormones (**TH**) in the pathogenesis of broiler ascites seems to be confounded in the literature. On the one hand, it has been reported that providing the broiler chicks with supplementary TH caused a marked increase in the incidence of ascites (Buys et al., 1998), which has been attributed to increased basal metabolic rate and O_2 consumption in most tissues (McNabb et al., 1991). On the other hand, ascitic chickens were found to be unable to maintain sufficient concentrations of both 3,5,3′-triiodothyronine (**T_3**) and thyroxine (**T_4**), which were reduced significantly during the development of the syndrome (Luger et al., 2001). This observation led to claims that broilers are susceptible to ascites due to hypothyroidism. Another report on decreased mortality rate due to ascites in cold-exposed hyperthyroid chicks has shed light on the roles of the TH during the development of the syndrome (Luger et al., 2002). Etiologically, the metabolic problems (e.g., lower TH), associated with the ascites syndrome might have existed since embryonic development; although, the occurrence of ascites is more pronounced between wk 5 and 6 posthatch (Decuypere et al., 2000). In comparing 2 divergently selected lines, Dewil et al. (1996) found that embryos from the ascites-sensitive lines had lower TH levels during incubation and a longer hatching process compared with the eggs from the ascites-resistant lines. On the other hand, hypoxemia in ascitic chicks, as Decuypere et al. (2005) stated, although primarily originates from cardiovascular problems, may be related to the lungs thereafter, where TH appear to be necessary for the maturational events preparatory to lung inflation in birds (Wittmann et al., 1983).

As far as we know, there are no reports on the association between maternal thyroid status and the prevalence of ascites in broilers. Therefore, the objective of the present study was to elucidate the effect of providing the developing embryos with extra T_4, by adding the hormone to the drinking water of breeder hens, on the incidence of cold-induced ascites during a posthatch 42-d growing period. We hypothesized that maternal hyperthyroidism would affect the embryos developmentally and decrease the incidence of the syndrome. For comparison, a group of hypothyroid-induced hens was also included in the trial. The findings might also help in better understanding the effects of factors causing fluctuations in maternal TH, which may influence the events during the incubation and rearing periods.

MATERIALS AND METHODS

Breeder Stock, Experimental Treatments, and Sampling

All procedures in the current study were approved by the Animal Care and Welfare Committee of our institute. In total, 132 twenty-six-week-old wing-banded broiler breeder hens (Babolkenar Arian Line Breeding Center, Babolkenar, Iran) were randomly allotted into one of 3 treatment groups (4 replicates of 11 hens per treatment) as control (**CON**), hypothyroid (**HYPO**), and hyperthyroid (**HYPER**), housed in trap-nested floor pens covered with wood shavings. Both 6-*N*-propyl-2-thiouracil (**PTU**) and T_4 were added at a level of 100 and 1 mg/L (Luger et al., 2002) to drinking water of the HYPO and HYPER groups, respectively (starting at wk 30 up to the end of wk 33). A severe thyroid inhibition in adult hens has been reported to be associated with complete cessation of egg laying (Decuypere et al., 1991; McNabb, 2007). A pilot study, using a group of age-matched birds of the same strain confirmed the efficacy of the administration levels of PTU and T_4 in terms of an efficient alteration in the levels of TH, with no apparent health or welfare problems as well as obtaining an adequate number of hatching eggs, especially in the HYPO group. The CON group was provided with normal drinking water. The breeder males of the same strain (26-wk-old; n = 20) were adapted (2 wk) to abdominal massage for semen collection. The hens were artificially inseminated with pooled semen diluted in homogenized and pasteurized low-fat milk (at a semen-to-milk ratio of 1 to 5) on a weekly basis. Hatching eggs (n = 1,320) were collected during the last 14 d of the treatment period, fumigated (20 min), and stored (12.5°C, 75% RH). All birds were maintained under similar environmental and management specifications of the breeder company (21°C and a 15L:9D photoschedule). The hens were provided with a standard pelleted diet containing 2,700 kcal of ME/kg, 14% CP, 2.99% calcium, and 0.36% phosphorus. At weekly intervals (29–35 wk of age), the birds were weighed and the blood samples were drawn from the brachial vein into EDTA-coated tubes. The samples were centrifuged (12 min, 1,800 × *g*, 18°C) and the plasma was stored at −20°C until analyzed for total levels of T_3 and T_4, using commercially available kits (Adaltis, Rome, Italy). Briefly, to validate for parallelism and recovery rate in the chicken, samples were diluted at a rate of 1 to 5 with the dilution buffer. The levels of T_3 and T_4 were calculated from a standard curve, ranging between 0.5 to 300.0 and 0.03 to 10.0 ng/mL for $T_4$and T_3, respectively ($r > 0.99$). Using a calibrator solution (T_4 = 0.00 ng/mL), serial dilutions (at the ratios of 1:2, 1:4, 1:8, and 1:16) were made for 4 plasma samples with known T_4 concentrations to control for linearity. Each sample was assayed in duplicate. The mean recovery rates were 90, 96, 103, and 109% for the dilution rates of 1:2, 1:4, 1:8, and 1:16, respectively. This procedure was also used for T_3, with recovery rates of 92, 95, 101, and 104% for the serially diluted samples. The intraassay and interassay CV were 4.7 and 5.5% for T_3 and 4.3 and 7.4% for T_4, respectively.

Hatchery

After an 8-h preincubation period at 24°C, the eggs were set on turning trays allotted to the same side of trolleys for minimal possible positional effect and trans-

ferred to the same incubator (Petersime, Zulte, Belgium; specific dry-bulb temperature of 37.6°C and wet-bulb temperature of 29°C for 18 d) to avoid interassay variability. On transfer to the hatcher (d 18), 5 eggs per replicate (60 eggs total) were randomly selected for embryonic blood sampling, and the remaining eggs were transferred to the hatching baskets. The eggs from each hen were placed under a special wire-mesh breeding basket. Blood samples were taken at internal pipping (**IP**; n = 5 per replicate, 60 eggs total) and at hatching (n = 5 per replicate, 40 chicks total). At the end of incubation (d 21.25), the number of hatched chicks, the fertility rate (fertile eggs divided by total eggs set), and the fertile hatchability rate (chick number divided by fertile eggs) were determined. A total of 288 one-day-old vent sexed male chicks of good quality (144 chicks for each CON and HYPER group) was wing-banded and reared for 42 d.

Broilers, Experimental Treatments, and Sampling

As none of the eggs from the HYPO hens resulted in chick emergence, the broiler rearing experiment was conducted using the chicks from the CON and HYPER groups. In 2 temperature-controlled rooms (±1°C) and under a 23L:1D photoperiod, the 1-d-old chicks were raised in the standard (**S**; 6 replicates of 9 chicks from each maternal treatment group; 108 in total) or in the ascites-inducing cold temperature (**C**) treatment (10 replicates of 9 chicks from each maternal treatment group; 180 in total). The design was a 2 × 2 factorial experiment with maternal treatment group (CON or HYPER) and rearing temperature (S or C) as the factors. Each replicate was reared on a floor pen (1.0 × 0.8 m) covered with wood shavings. Birds in the S group were raised under standard conditions until 21 d of age (gradual reduction from 32 to 26°C) and were then exposed to a constant 22°C for the rest of the study (Luger et al., 2002). The temperature in the C group was 32 and 30°C during wk 1 and 2, lowered to 15°C during wk 3, and maintained between 10 and 15°C until 42 d (Iqbal et al., 2001). The birds were randomly assigned to the replicates and were fed ad libitum a conventional mash broiler diet containing 2,806 kcal/kg of ME and 21.8% CP during the starter (1–14 d), 2,935 kcal/kg and 20.1% for the grower (15–28 d), and 2,945 kcal/kg and 18.0% for the finisher (29–42 d) periods. Experimental diets meet or exceed the NRC (1994) requirements as appropriate. All the birds had free access to water.

Blood samples were taken from the brachial vein into EDTA-containing tubes, and BW were determined at weekly intervals. A portion of blood was stored at 4°C for determination of the hematocrit (**HCT**) after centrifugation (7 min at 14,000 × *g*, 18°C). Another portion was centrifuged (1,800 × *g*, 12 min, 18°C) and the plasma was stored at −20°C, pending T_3 and T_4 assays. Mortalities were recorded and necropsied daily for diagnosis of ascites (Luger et al., 2002). At the end of 42 d, all birds were killed, the heart was removed, and the right ventricle was dissected from the left ventricle and the septum, which were then weighed separately. The birds with a right ventricle-to-total ventricular weight (**RV:TV**) ratio greater than 0.27 to 0.30 and with accumulation of abdominal fluid were considered ascitic (Wideman et al., 1998; Balog, 2003). The overall ascites incidence was presented as the cumulative ascites mortality throughout wk 6, plus ascites observed at necropsy on d 42.

Statistical Analysis

The data recorded for the breeder hens and embryos were analyzed using the Proc GLM in SAS (2002). The BW was included as a covariate for ANOVA and the treatment means were compared by the Duncan's multiple-range test. Similarly, the broiler data were analyzed for each of the 6 wk for the treatment groups, including chicks from the CON and HYPER hens reared under the standard temperature and cold-exposed healthy and ascitic birds. The incidence of ascites was analyzed using the logistic regression model by the GENMOD procedure in SAS (2002), and the treatment effect was declared significant ($P \leq 0.05$) based on the Wald chi-squared values.

RESULTS

Breeder Hens

The effect of transient hypo- and hyperthyroidism on the BW, thyroid hormone concentrations, fertility, and fertile hatchability in breeder hens is presented in Table 1. The induced hypothyroidism resulted in an increased BW; however, no difference was observed between HYPER and CON hens. The PTU-treated hens had the lowest plasma T_3 and T_4 concentrations. Plasma T_4 level in HYPER hens was higher than CON birds; but T_3 concentrations were not different between these groups. Although thyroid-manipulating treatments had no effect on fertility, the hatching process was completely inhibited due to hypothyroidism. The fertile hatchability, however, was not affected by T_4 administration to hens (83 vs. 78% for CON and HYPER hens, respectively).

Embryonic Thyroid Hormones

The embryos in the HYPO group had lower T_3 and T_4 concentrations at embryo day $(\mathbf{ED})_{18}$ and at IP (Table 2). Embryonic TH concentrations were not influenced by maternal hyperthyroidism at ED_{18} and at hatching; however, at IP, a higher T_4 value was recorded in the HYPER than in CON embryos (7.81 vs. 5.3 ng/mL, respectively). Plasma T_3 level was not different between these groups at this stage.

Table 1. The effect of induced hypo- and hyperthyroidism on BW (g), plasma levels (ng/mL) of triiodothyronine (T_3) and thyroxine (T_4), fertility (%), and fertile hatchability (%) in broiler breeder hens (mean ± SE)[1]

Item	Control	Hypothyroid	Hyperthyroid
BW	3,335 ± 21.3[b]	3,422 ± 21.3[a]	3,361 ± 21.3[b]
T_3	1.60 ± 0.21[a]	0.87 ± 0.06[b]	1.62 ± 0.07[a]
T_4	11.88 ± 0.77[b]	8.00 ± 2.54[c]	36.08 ± 3.76[a]
Fertility	98.1 ± 0.7	96.5 ± 1.1	96.3 ± 1.0
Fertile hatchability[2]	83.1 ± 3.5[a]	0.00[b]	78.2 ± 3.9[a]

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

[1]Propylthiouracil (100 mg/L) for hypothyroid, thyroxine (1 mg/L) for hyperthyroid, and common water for control treatment were provided (wk 30–33; n = 44 hens/treatment).

[2]Number of hatchlings divided by fertile eggs set; no chick emerged for hypothyroid hens.

Mortality and BW in Broilers

Maternal hyperthyroidism significantly decreased the overall ascites incidence in the cold-exposed chickens (Table 3; 10.0 vs. 33.4% for HYPER and CON, respectively). More than 78% of total mortality could be attributed to ascites through wk 6 and the remainder to birds found ascitic at necropsy. However, this mortality-attenuating effect was absent at standard rearing temperatures (Table 3). Although, final BW in broilers was not influenced by treating the dams with exogenous T_4, cold exposure was associated with a decreased BW from both the CON and HYPER mothers (Table 3).

Thyroid Hormones and HCT in Broilers

Table 4 shows the weekly T_3, T_4, and HCT values in broilers of the HYPER and CON hens. Maternal hyperthyroidism, irrespective of rearing temperature, did not significantly affect the plasma T_3 concentrations in broilers. However, higher values were recorded for cold-exposed birds. No difference was noted in plasma T_4 concentrations between broilers from HYPER and CON hens, but exposure to low ambient temperature decreased the plasma T_4 concentrations, as compared with the birds raised under standard temperature. Minimum T_4 concentration was found in healthy birds at 4 wk. When compared with their counterparts, cold-exposed ascitic birds showed a decrease in T_3 and T_4 concentrations 1 wk before death; however, the decrease in T_4 for the birds that died at wk 6 was significant from wk 4 onwards (Table 4).

Although maternal treating with excess T_4 did not affect the HCT values under the standard temperature, the healthy chickens from CON hens reared under cold temperature showed higher HCT values that were not observed in the HYPER counterparts. The HCT values increased with increasing age in the ascitic birds and the difference became significant 2 wk before death.

RV:TV Ratio

Hyperthyroidism in dams did not influence the RV:TV ratio in their offspring (Figure 1). In comparison with the birds under the standard rearing temperature (CON and $HYPER_S$), the ratio was higher in the cold-stressed broilers. The highest ratios were recorded in the cold-exposed ascitic broilers of CON or HYPER hens (0.44 and 0.42, respectively).

DISCUSSION

In the current study, we used PTU and T_4 to induce transient maternal hypo- and hyperthyroidism, respectively. Although the T_4 level was higher in HYPER hens, the concentration of T_3 was not different between these birds and the CON hens. This could be due to rapid and almost total conversion of T_4 to the metabolically inactive reverse-T_3 (**rT_3**; Decuypere et al., 1987). As an antithyroid agent, PTU inhibits iodide oxidation by inhibiting peroxidase, thereby decreasing TH concentrations (Taurog, 1976).

Treatment with PTU, resulted in the complete failure of the hatching process, which can be attributed to

Table 2. The effect of induced maternal hypo- and hyperthyroidism on plasma levels (ng/mL) of triiodothyronine (T_3) and thyroxine (T_4) in embryos at 18 d (ED_{18}), internal pipping (IP), and hatching (mean ± SE)

	ED_{18}		IP		At hatching[2]	
Treatment[1]	T_3	T_4	T_3	T_4	T_3	T_4
Control	0.16 ± 0.02[a]	2.58 ± 0.11[a]	2.93 ± 0.36[a]	5.33 ± 0.54[b]	1.73 ± 0.24[a]	8.43 ± 0.79[a]
Hypothyroid	0.06 ± 0.01[b]	1.28 ± 0.02[b]	0.41 ± 0.04[b]	1.47 ± 0.24[c]	—	—
Hyperthyroid	0.17 ± 0.03[a]	2.44 ± 0.12[ab]	2.79 ± 0.29[a]	7.81 ± 0.42[a]	1.71 ± 0.27[a]	8.29 ± 0.84[a]

[a–c]Within columns, values with different superscripts differ significantly ($P \leq 0.05$); n = 20 embryos/treatment per stage.

[1]Propylthiouracil (100 mg/L) for hypothyroid, thyroxine (1 mg/L) for hyperthyroid, and common water for control treatment were provided for hens (wk 30–33).

[2]No chick emerged for the hypothyroid hens.

Table 3. The effect of induced maternal hyperthyroidism on BW (mean ± SE) and ascites incidence in broiler chickens reared under a standard (S) or a cold (C) temperature condition[1]

Item	CON_S	$HYPER_S$	CON_C	$HYPER_C$
Final BW (g), d 42	2,576 ± 42.1[a]	2,569 ± 45.3[a]	2,174 ± 56.7[b]	2,167 ± 53.4[b]
Overall ascites incidence, %	9.2[b]	7.4[b]	33.4[a]	10.0[b]
Ascites mortality,[2] %	5.5	3.7	26.7	7.8
Ascitic at necropsy,[2] %	3.7	3.7	6.7	2.2
Estimated odds ratio (95% CI)	0.222 (0.098–0.503)*	0.204 (0.074–0.566)*	Referent	0.160 (0.053–0.485)*

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

[1]Thyroxine (1 mg/L) for hyperthyroid (HYPER) and common water for control (CON) treatments were provided (wk 30–33).

[2]Cumulative ascites mortality through wk 6, ascites at necropsy on d 42, and overall ascites incidence as a percentage of birds raised (n = 54 chicks for CON_S or $HYPER_S$; n = 90 for CON_C or $HYPER_C$).

*Denotes different from the CON_C group.

low embryonic plasma T_3 concentration. It is known that T_3 is important for the hatching process (McNabb, 2007). There are reports demonstrating that maternal TH are transferred into the eggs (Hilfer and Searls, 1980; Sechman and Bobek, 1988; Prati et al., 1992; Wilson and McNabb, 1997; McNabb, 2002), which may affect embryonic development (Wilson and McNabb, 1997). Failure to hatch may also be due to the effect of PTU on the embryos, as it has been shown to be capable of entering from maternal circulation into the eggs (Moseley and Landauer, 2003). The hatchability rate was not affected in the HYPER group in the present work, although it was previously suggested that preincubational in ovo administration of low doses of T_4 resulted in improved hatchability in turkeys (Christensen, 1985).

As far as we are aware, this is the first report demonstrating that provision of exogenous T_4 in the drinking water could be associated with a decreased incidence of ascites syndrome in cold-exposed broilers (10.0 vs. 33.4% for HYPER and CON broilers, respectively). The nonsignificant difference in plasma TH between the CON and HYPER broiler groups in either standard or cold conditions prompted the search for probable causal mechanisms during the incubation period. The embryonic thyroid gland is under hypothalamic-pituitary axis control from mid ED_{11}, such that the embryonic blood T_4 concentration is low from the first one-third to mid-incubation period (McNabb, 2006). Prior to the start of TH secretion from the embryonic thyroid gland, maternal T_4 can enter the egg yolk mainly in lipoprotein or transthyretin-bound forms that then can affect embryonic development (Wilson and McNabb, 1997). Administration of thyroid-suppressing compounds to the hens or eggs before incubation, suggested a greater effect of these hormones on the embryonic organogenesis compared with the administration during the late incubation (McNabb, 2002).

Table 4. The effect of induced maternal hyperthyroidism[1] on plasma levels of triiodothyronine (T_3), thyroxine (T_4), and hematocrit in healthy (h) or ascitic broiler chickens reared under a standard (S) or a cold (C) temperature condition[2] (mean ± SE)

					Cold-exposed ascitic broilers based on age at mortality		
Item	CON_S	$HYPER_S$	$CON_{C\text{-}h}$	$HYPER_{C\text{-}h}$	4 wk	5 wk	6 wk
T_3, ng/mL							
2 wk	1.09 ± 0.28	1.11 ± 0.51	1.11 ± 0.69	1.17 ± 0.63	1.14 ± 0.58	1.27 ± 0.97	1.22 ± 0.31
3 wk	1.43 ± 0.57[b]	1.39 ± 0.49[b]	1.93 ± 0.51[a]	1.89 ± 0.59[a]	1.70 ± 0.89[b]	1.76 ± 0.81[ab]	1.65 ± 0.57[b]
4 wk	1.71 ± 0.14[b]	1.76 ± 0.27[b]	2.29 ± 0.81[a]	2.38 ± 0.81[a]	1.19 ± 0.14[c]	1.90 ± 1.09[b]	2.27 ± 0.21[a]
5 wk	0.89 ± 0.63[b]	0.92 ± 0.49[b]	1.84 ± 0.59[a]	1.69 ± 0.67[a]		0.89 ± 0.16[b]	0.64 ± 0.12[b]
6 wk	0.86 ± 0.51[b]	0.84 ± 0.31[b]	1.85 ± 0.58[a]	1.76 ± 0.64[a]			0.67 ± 0.09[c]
T_4, ng/mL							
2 wk	8.8 ± 0.64	8.3 ± 0.53	8.3 ± 0.57	8.5 ± 0.61	7.8 ± 0.63	7.6 ± 0.73	8.1 ± 0.97
3 wk	5.7 ± 0.48[a]	5.9 ± 0.51[a]	4.3 ± 0.18[bc]	4.3 ± 0.21[bc]	3.8 ± 0.27[c]	4.1 ± 0.30[bc]	4.2 ± 0.41[bc]
4 wk	5.2 ± 0.38[a]	5.1 ± 0.32[a]	3.1 ± 0.12[b]	3.2 ± 0.21[b]	2.7 ± 0.11[c]	2.9 ± 0.39[bc]	2.6 ± 0.23[c]
5 wk	7.0 ± 0.51[a]	7.1 ± 0.56[a]	4.5 ± 0.16[b]	4.6 ± 0.19[b]		1.3 ± 0.09[c]	1.8 ± 0.10[c]
6 wk	7.6 ± 0.49[a]	7.6 ± 0.40[a]	5.7 ± 0.43[b]	5.4 ± 0.57[b]			2.1 ± 0.18[c]
Hematocrit, %							
2 wk	32.3 ± 0.29	33.1 ± 0.39	32.6 ± 0.41	32.4 ± 0.75	33.1 ± 0.66	32.8 ± 0.57	32.1 ± 0.63
3 wk	33.5 ± 0.54[b]	33.3 ± 0.49[b]	34.4 ± 0.69[ab]	32.4 ± 0.57[b]	38.2 ± 1.32[a]	36.4 ± 1.08[ab]	33.6 ± 1.05[b]
4 wk	31.9 ± 0.67[d]	32.4 ± 0.61[d]	37.6 ± 0.88[c]	33.1 ± 0.23[d]	46.9 ± 1.66[a]	46.3 ± 1.81[a]	40.4 ± 2.06[b]
5 wk	34.1 ± 0.66[c]	35.3 ± 0.73[c]	39.8 ± 1.01[b]	33.4 ± 0.38[c]		50.9 ± 2.33[a]	48.7 ± 2.34[a]
6 wk	33.7 ± 0.85[c]	34.2 ± 0.67[c]	38.7 ± 1.13[b]	32.9 ± 0.87[c]			51.6 ± 2.57[a]

[a–d]Within each row, values with different superscripts differ significantly ($P \leq 0.05$).

[1]Thyroxine (1 mg/L) for hyperthyroid (HYPER) and common water for control (CON) treatment were provided for hens (wk 30–33).

[2]n = 10 for broilers emanating from control (CON_S) or hyperthyroid ($HYPER_S$) hens and raised under standard environmental temperature; the same number for broilers emanating from control ($CON_{C\text{-}h}$) or hyperthyroid ($HYPER_{C\text{-}h}$) hens and exposed to cold ambient temperature but remained healthy (h). For ascitic chickens, n varied with week of age (a total of 4, 22, and 10 birds died due to ascites during 4, 5, and 6 wk of age, respectively).

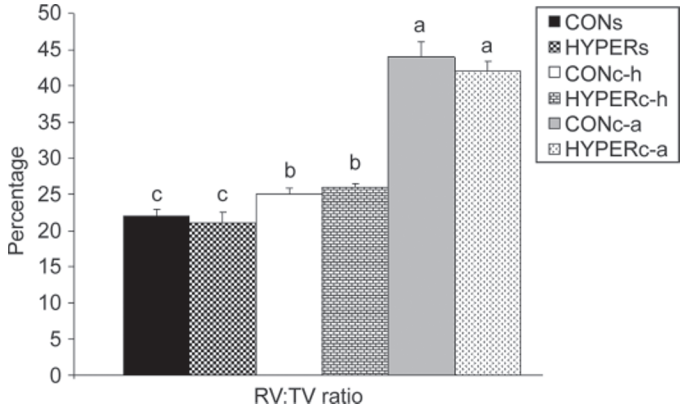

Figure 1. The effect of transient maternal hyperthyroidism on the right ventricle to total ventricular (RV:TV) ratio in healthy (h) and ascitic (a) broiler chickens reared under a standard (S) or a cold (C) temperature condition. Thyroxine (1 mg/L) for hyperthyroid (HYPER) and common water for control (CON) treatments were provided for hens (wk 30–33). n = 10 for broilers emanating from control (CON_S) or hyperthyroid ($HYPER_S$) hens and raised under standard ambient temperature; the same number for broilers emanating from control ($CON_{C\text{-}h}$) or hyperthyroid ($HYPER_{C\text{-}h}$) hens and exposed to cold ambient temperature but remained healthy (h). For ascitic chickens from control and hyperthyroid hens that were exposed under cold temperature ($CON_{C\text{-}a}$ and $HYPER_{C\text{-}a}$), 10 and 9 birds were evaluated, respectively. [a-c]Means with different letters differ significantly ($P \leq 0.05$).

The decreased incidence of ascites in the cold-exposed HYPER group might be due to the effects of TH on the pulmonary development and the enhanced efficiency of O_2 and CO_2 exchange. Thyroid hormones appear to be necessary for the maturational events preparatory to lung inflation in birds (Wittmann et al., 1983). Hypoxemia in the ascitic chicks, as Decuypere et al. (2005) stated, is primarily the result of cardiovascular derangement; however, it may also be related to the lungs thereafter. Van der Geyten et al. (2002) showed that iodothyronine deiodinases 3 expression (converting T_4 to rT_3) in the lungs was low during the last week of incubation, coinciding with an intermediate iodothyronine deiodinases 1 (converting T_4 to T_3) expression. It could, therefore, be assumed that T_3 production in the lungs as a result of T_4 deiodination, might influence the pulmonary system. It has also been stated that T_4 and T_3 are essentially of equal physiological potency in birds (McNabb, 2000). It also is likely that other forms of TH, in addition to T_3, affected the results in the present work, as T_4 and rT_3 are said to be more potent than T_3 (Hulbert, 2000). Whether alternative forms of TH, including 3,5-T_2, were involved in the present study remains to be elucidated. The vascular endothelial growth factor is increased during thyroidal hypersecretion in human (Fagin, 2005). Determining the contribution of this factor in the developing bird pulmonary capillary network (Maina, 2005) would strengthen its probable involvement in our work, which necessitates more elaborate studies.

The effects of TH on the gut function (Wasan et al., 2005) may be another mechanism for the lower incidence of ascites in the cold-exposed HYPER group. It was suggested that factors in the amnion or yolk influence developmental function of the intestine (Uni et al., 2003). The gastrointestinal tract requires a large amount of oxygen (Yen et al., 1989), and gut development has been demonstrated to be inhibited in commercial broilers suffering from hypoxemia (de los et al., 2005). Triiodothyronine, which is at least partially produced by the intraintestinal deiodination, is needed for the intestinal differentiation during the latter part of the embryonic life (Van der Geyten et al., 2002). The intestine might have been affected by the TH in such a way to decrease the oxygen demand of the gut, thereby enabling the cold-exposed HYPER birds to better tolerate the hypoxemic stress during the cold exposure. Further studies are required to substantiate this hypothesis.

The proportional rate of yolk TH transportation into the embryo is considerably important. Transthyretin mRNA expression in the early chick embryos contributes to the transport of these hormones into the embryo (Southwell et al., 1991). Thyroxine in the yolk of high T_4 eggs might be deiodinated in embryonic tissues to T_3 or rT_3 or both (Wilson and McNabb, 1997). However, if TH transport occurs primarily via yolk lipid uptake by the yolk sac membrane, then most of the movement of maternal TH into the embryo would occur toward the end of embryonic period (McNabb, 2002). Noble (1987) suggested that yolk uptake in chicken eggs increased up to approximately 1 g/d during the last 2 d of the incubation period. It might be assumed that T_4 deiodination to rT_3 would be decreased, as this time corresponds to low iodothyronine deiodinases 3 levels but highly expressed iodothyronine deiodinases 1 (De Groef et al., 2008). Assuming that most of the yolk TH transportation into the embryo occurs during late incubation, then the decrease in embryonic TH secretion might be partial, because the negative feedback inhibition could not sufficiently inhibit the hypothalamic-pituitary-thyroid axis (De Groef et al., 2008). Even though high levels of TH at IP, observed in the embryos from HYPER dams, would have been capable of negative regulation of embryonic thyroidal secretions, a reservoir of T_4 is yet present that would not be affected meanwhile, namely the yolk sac. Alternatively, maternal hyperthyroidism might permanently alter the hypophyseal-pituitary set-point for TH secretion.

Summarized, the probable causal mechanisms of lower incidence of ascites in the cold-exposed HYPER chicks might be: 1) the effects of T_3 and T_2, produced locally in the embryonic lungs or of embryonic plasma T_4, on pulmonary developmental events in favor of more efficient O_2 and CO_2 exchange; and 2) the probable effects on gut morphology and the reduced O_2 demand. It is noteworthy that the HCT values were larger in the cold-exposed healthy broilers in the CON group than in their HYPER counterparts during the latter part of the 42-d rearing period. This could imply less-intense hypoxemic stress in the broilers from HYPER hens. A higher HCT value in the ascitic birds was also reported by Luger et al. (2002). Absence of the ascites-atten-

uating effect of maternal hyperthyroidism under the standard rearing temperature might indicate that the standard condition was presumably optimal enough to either maternal treatment groups, not challenging for vital organs affecting the O_2 availability to show their functional efficiency. Contrary, challenging to the harsh low ambient temperature could reveal the efficiency of the HYPER group to combat the hypoxemic stress, thereby resulting in a lower ascites incidence compared with the control.

In conclusion, induced maternal hyperthyroidism has been associated with the decreased incidence of ascites in the cold-exposed broilers during a 42-d growing period. The data provided new insights into the efficiency of TH during the development of ascites. Of practical terms, it may appear that adding T_4 in the drinking water is more convenient for the breeder producers, as compared with using T_4 some time later during the incubation or broiler growing periods. On the other hand, providing the hens with extra T_4 for 4 wk did not adversely affect the fertility and fertile hatchability rates. However, any recommendation should be made after any undesirable effect(s) of long-term T_4 administration in the breeder hens and consequences on the broiler performance have been clarified.

ACKNOWLEDGMENTS

Grateful thanks are extended to A. N. Eghbalian (the production manager) and the farm employees at Babolkenar Arian Line Breeding Center in Iran for diligent cooperation and time.

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