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Maternal Dietary Iodide Influences Turkey Embryo Thyroid Function[1]

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Abstract: An experiment was designed to discover a mechanism for improved embryonic survival resulting from supplemental maternal dietary iodine. Commercial turkey breeder hens were fed diets containing 4 ppm supplemental iodide during a 20 wk egg production period (32 to 48 wk of age). Dietary iodide treatment depressed maternal blood thyroxine (T_4) concentrations as the hens aged. Dietary iodide depressed maternal blood 3, 5, 3'- triiodothyronine (T_3) levels at all times examined. The dietary iodide treatment increased embryonic blood concentrations of T_4 at 25 to 28 d of incubation but depressed blood concentrations of T_3 only at 27 d of incubation. In a second trial, the iodide treatment decreased embryonic T_4 concentrations as well but in a time-dependent manner. When the hens were similarly fed additional iodide in Trial 2, no effects were noted in T_3 concentrations. Iodide also accelerated the increase in embryo T_4 concentrations and initiated earlier pipping in embryos compared with non-supplemented controls. The data indicate that embryo thyroid function during hatching is affected by the maternal dietary iodide in turkey dams even though the embryo develops outside the maternal body.

Key words: Thyroid, maternal, embryo, embryo survival

Introduction

Dietary iodide and adequate maternal thyroid function are essential for good reproduction in poultry breeder hens (Rogler *et al*., 1961ab; Christensen and Donaldson, 1994). Previous data indicated that feeding increased iodide or triiodothyronine (T_3) affected embryo physiology in the latter part of the development (Christensen and Donaldson, 1994). Specifically, blood glucose concentrations increased faster via gluconeogenesis during pipping and hatching; and embryonic survival was improved. It is unknown how the maternal diet can affect the embryo when it is developing outside the maternal body. One plausible explanation may be that maternal deposits in the egg differ depending upon their diet. Therefore, the hypothesis was proposed that maternal dietary iodide may affect the embryo plasma thyroid hormone concentrations by depositing more iodine or iodohormones into the egg prior to oviposition. Increased embryonic plasma thyroid hormone concentrations may then affect carbohydrate metabolism and basal metabolic rate of the developing embryo (Czarnecki, 1991; Nobukuni *et al*., 1989). The objectives of the studies reported here were to: 1) measure plasma thyroid hormones in turkey breeder hens fed additional iodide and photostimulated at 30 wk of age to initiate a 20 wk egg laying cycle, and 2) measure plasma thyroid hormones in embryos from the treated hens to discover direct effects on the thyroid hormone concentrations of the developing embryo.

Materials and Methods

Two similar but independent trials of the experiment were conducted on subsequent years. The treatments were identical to a prior study (Christensen and Donaldson, 1994) in which supplemental iodide (4 ppm) was included in the breeder feed and compared to a non-supplemented control diet (2 ppm). Blood samples were collected from the hens at 32, 36, 41, and 48 wk, and eggs produced during these time periods were used to examine embryonic survival and thyroid function. Trial 2 was conducted in the same facility one year later than the first at the same season of the year. The same treatments were observed using more replications. The hens were sampled identical with the addition of 30 wk to establish baseline hormone values prior to feeding the diets.

In both trials, fertilized eggs for hatching the breeder[2] birds were obtained from the primary breeder in May of each year and hatched in June at a University Research Unit. One hundred and fifty hens were grown to sexual maturity (30 wk) then 144 hens and 20 toms were moved to facilities described previously (Christensen and Donaldson, 1994). The housing was equipped for egg laying and semen production. Six hens were kept in each pen in the laying house and four toms per pen were placed in an adjoining male breeder house. The hens were photostimulated to initiate reproduction by exposure to 15.5 h of light per day (0500 to 2030) and the toms by exposure to 12 h light per day (0600 to 1800).

Beginning at photo stimulation, half of the hens in Trials 1 and 2 was fed a basal diet (16% CP and 2970 ME, kcal/kg) containing 0.4 ppm iodide (Control) and the remaining half was fed the basal diet with 4 ppm iodide

Table 1: Hatchability and embryos dying during times of incubation (%) in eggs produced by turkey breeder hens fed supplemental iodide

	Measurement			
Diet[1]	Hatchability	Week 1 mortality	Week 4 mortality	Pipping mortality
Trial 1				
Control	78.2[b]	6.2	5.4	13.5[a]
Iodide	80.6[a]	5.2	5.9	10.2[b]
Mean ± SEM	79.7 ± 0.7	5.7 ± 0.3	5.6 ± 0.3	12.1 ± 0.6
Probability	0.04	NS	NS	0.004
Trial 2				
Control	72.4[b]	6.7	5.4	13.5[a]
Iodide	81.2[a]	5.3	4.8	8.2[b]
Mean ± SEM	76.2 ± 1.5	6.1 ± 0.8	5.1 ± 0.8	10.9 ± 1.0
Probability	0.01	NS	NS	0.02

[1]Control = hens were fed a basal diet containing 0.4 ppm iodine; Iodide = hens were fed a basal diet supplemented with 4 ppm iodine.
[a, b]Columnar means with a different superscript differ significantly ($P \leq 0.05$).

added as potassium iodide[3]. Feed was available throughout the trials for *ad libitum* consumption.

All hens were inseminated artificially with pooled semen from the toms at weekly intervals for the duration of both trials. Fertile eggs were collected and sanitized immediately following collection from nests and stored for 2 to 15 d in an egg cooler (75% RH and 12.8°C) before setting. Eggs were placed in incubators at biweekly intervals for a total of 10 settings. Eggs were incubated using standard procedures described previously (Christensen and Donaldson, 1994).

Embryonic thyroid function was assessed simultaneously to that of the hens by collecting blood samples from embryos in four settings of eggs. Within each setting time eight embryos were selected randomly from each treatment group at 25 d of incubation (immediately prior to pipping), at 26 d (internal pipping), at 27 d (external pipping) and at hatching (28 d). Blood samples were obtained from a total of 32 embryos per treatment for each trial. Embryos were quickly decapitated and trunk blood was collected into glass tubes containing 10 mg EDTA, placed on ice and centrifuged (700 x G) under refrigeration (4°C) for 15 min. The plasma was recovered, decanted and frozen (-22°C). The remaining sets of eggs were used to observe embryonic survival as reported as the percentage of eggs set which hatched into viable poults.

Maternal thyroid function was assessed by obtaining blood samples from the brachial vein of 48 laying turkey hens per treatment at monthly intervals for the duration of egg laying (30, 32, 36, 41, and 48 wk of age). In each trial 480 hen blood samples were analyzed. All blood was collected into tubes containing 10 mg EDTA and placed immediately into an ice bath. The samples were then centrifuged (700 x g) under refrigeration (4°C) for 15 min. Following centrifugation, plasma was decanted and frozen (-22°C) until analysis. Embryonic plasma was assayed for thyroid hormones using the technique described by Christensen *et al.* (1993), and the hen plasma was assayed using the technique of Davis *et al.* (2000). The assay of laying hen plasma required different techniques because of lipemia. All samples were analyzed using the same assay to avoid interassay variation. To test recovery, stock solutions of T_3 and T_4 were serially diluted from 32 to 0.5 ng/ml. Each serially diluted concentration of T_3 and T_4 was added (spiked) to filtered turkey plasma, each with one ml volume. The percent recovery of both T_3 and T_4 ranged from a low of 95% for the 0.5 ng/ml sample to a high of 100% for the 32 ng/ml sample. To test parallelism, stock solutions of T_3 and T_4 were serially diluted to each standard curve concentrations (range of 0.4 to 10.0 and 0.5 to 32 ng/ml, respectively). These were added (spiked) to one ml of pooled turkey plasma sample; and the logit-log plot of percentage bound *vs* concentration was compared to the standard curve. The slopes of the standard curve and spiked, pooled turkey curve were similar for both T_3 and T_4 RIA's.

Embryonic thyroid function alterations and consequent changes in energy metabolism were verified by analyzing, relative liver, heart and muscle glycogen in Trial 2. The tissues were sampled and assayed as described in a previous study (Christensen and Donaldson, 1994).

The data were analyzed as a completely randomized design (Snedecor and Cochran, 1974) with two treatments in Trials 1 and 2 (Control and Iodide). Maternal hormone data were analyzed as a treatment by time of lay interaction factorial arrangement, and the embryo data were analyzed as a treatment by day of incubation factorial arrangement using the PC SAS package (SAS Institute, 1998). All significant statistical differences were based on $P \leq 0.05$, and means determined to differ significantly were separated using the Least Square Means Procedure in SAS.

Table 2: Maternal plasma thyroid hormone concentrations in hens fed supplemental iodide or triiodothyronine

	Weeks of age				
Diet[1]	32	36	41	48	Mean
Thyroxine (ng/ml)					
Control	6.5	5.2	7.0	7.4	6.6[a]
Iodide	5.6	5.5	6.6	6.8	6.1[b]
Mean	5.9[bc]	5.3[c]	6.8[ab]	7.1[a]	
Mean ± SEM	6.4 ± 0.1				
Probabilities					
Diet	0.0001				
Age	0.0001				
Diet x Age	NS				
Triiodothyronine (ng/ml)					
Control	1.26	2.52	2.95	2.75	2.38[a]
Iodide	1.32	1.75	2.05	2.59	1.93[b]
Mean	0.28[b]	2.13[a]	2.50[a]	2.67[a]	
Mean ± SEM	2.45 ± 0.10				
Probabilities					
Diet	0.05				
Age	0.0001				
Diet x Age	NS				
Triiodothyronine:thyroxine ratio					
Control	0.26	0.78	0.40	0.39	0.46[a]
Iodide	0.29	0.40	0.32	0.41	0.36[b]
Mean	0.27[c]	0.59[a]	0.36[bc]	0.40[b]	
Mean ± SEM	0.41 ± 0.05				
Probabilities					
Diet	0.0001				
Age	0.0001				

[1]Control = hens were fed the basal diet containing 0.4 ppm iodine; Iodide = hens were fed the basal diet supplemented with 4 ppm iodine. [a, b, c] Columnar or row means followed by a different superscript differ significantly ($P \leq 0.05$).

Results

Embryonic survival: Feeding iodide increased the percentage of surviving embryos compared to control in Trial 1 (2.4%) and Trial 2 (8.8%) (Table 1). Embryo survival rates improved during the latter times of embryo development when embryonic thyroid hormone concentrations increased (i.e., pipping mortality in the iodide treatment improved 3.3% in Trial 1 and 5.3% in Trial 2).

Trial 1: Maternal dietary iodide depressed concentrations of T_4 in laying hens for the entire laying cycle (range 0.4 to 1.1 ng/ml) (Table 2). Maternal plasma T_3 concentrations and the T_3:T_4 ratios were depressed compared to controls by feeding iodide. Maternal plasma T_4, T_3 and T_3:T_4 ratios increased greatly as the hens aged to 36 wk but declined gradually at 41 and 48 wk. Although the values declined during lay they remained elevated compared to pre-lay levels.

Iodide and Day of development interacted to affect embryo plasma T_4 (Table 3). Iodide elevated plasma T_4 at 27 d of incubation compared controls but no other differences were noted. Additionally, T_3 levels were affected similarly. The T_3:T_4 ratio was elevated in the Iodide treatment compared to controls, and the ratio increased incrementally as the embryos aged from 26 to 27 and 27 to 28 d of incubation. There was no significant Diet by Day of development interaction.

Trial 2: In Trial 2, plasma was sampled before photo stimulation at 30 wk to determine baseline levels of maternal thyroid hormones prior to laying. The concentration found prior to photo stimulation was greater than that at the onset of lay but the treatment groups did not differ. Supplemental iodide depressed mean maternal plasma T_4 and the T_3:T_4 ratio concentrations during lay (Table 4). Maternal plasma T_3 did not differ between dietary treatments. Plasma T_4, T_3 and the T_3:T_4 ratio increased initially as hens began egg production but declined gradually as the hens aged but not to pre-laying levels.

A dietary treatment by Day of development interaction affected embryonic plasma T_4 (Table 5). Feeding iodide elevated plasma T_4 at 26 and 27 d of incubation and depressed it at hatching (28 d) compared to control levels. Dietary iodide did not affect embryonic plasma T_3

Table 3: Thyroid hormone concentrations in embryonic poults and hatchlings in eggs produced by hens fed supplemental iodide or triiodothyronine

	Day of incubation				
Diet[1]	25	26	27	28	Mean
Thyroxine (ng/ml)					
Control	7.0[c]	5.6[c]	14.2[b]	4.1[c]	6.3
Iodide	4.8[c]	5.6[c]	21.3[a]	3.4[c]	7.7
Mean	5.9	5.6	16.8	3.9	
Mean ± SEM	7.0 ± 0.5				
Probabilities					
Diet	NS				
Day	0.0001				
Diet x Day	0.05				
Triiodothyronine (ng/ml)					
Control	0.41d	0.61[c]	2.15[b]	1.86[b]	1.26
Iodide	0.23[d]	0.52[cd]	3.48[a]	2.28[b]	1.63
Mean	0.32	0.57	2.82		2.07
Mean ± SEM	1.50 ± 0.09				
Probabilities					
Diet	0.05				
Day	0.0001				
Diet x Day	0.05				
Triiodothyronine:thyroxine ratio					
Control	0.08	0.12	0.19	0.49	0.22[b]
Iodide	0.07	0.10	0.71	0.82	0.43[a]
Mean	0.07[c]	0.11[c]	0.45[b]	0.66[a]	
Mean ± SEM	0.35 ± 0.07				
Probabilities					
Diet	0.03				
Day	0.0001				
Diet x Day	NS				

[1]Control = hens were fed a basal diet containing 0.4 ppm iodine; Iodide = hens were fed the basal diet supplemented with 4 ppm iodine.
[a, b, c]Columnar or row means with a different superscript differ significantly ($P \leq 0.05$).

concentrations but T_3 increased between days 25 and 26 of development and decreased between days 27 and 28 of incubation. The T_3:T_4 ratio showed a Diet by Day of incubation interaction as iodide increased the ratio compared to controls at day 25 and at hatching (28 d) but not at 26 or 27 d.

Dietary iodide interacted with Day of development to depress hepatic glycogen concentration (Table 6) at d 25 compared to controls but not at any other day of development. Maternal dietary iodide decreased cardiac glycogen at all days of incubation but skeletal muscle glycogen remained unchanged compared to controls. Liver weight was reduced by feeding iodide but skeletal muscle weights were increased (data not shown).

Discussion

The current study suggests a relationship between domestic turkey maternal thyroid function and embryonic thyroid function immediately prior to hatching. Supplemental dietary iodide depressed maternal thyroid hormone concentrations, increased embryonic T_4 and improved survival rates of embryos during the latter stages of incubation. When NRC recommended levels of iodide were fed, the diet supported adequate egg production and the well-being of the hen, but insufficient iodine or hormone was available to sustain embryonic growth and maturation.

Many physiological factors are integrated at the time of hatching in precocial avian species (Mallon and Betz, 1982; Decuypere *et al.*, 1992). One critical factor in the integration of physiological functions is the onset of thyroid function in the embryo and the initial appearance of the biologically active form of the thyroid hormone, T_3 (McNabb *et al.*, 1993). Thyroid hormones of maternal origin are thought to play a role in early embryonic development (Prati *et al.*, 1992), but no effects on embryonic mortality during the first wk of incubation were noted in the current study. Late maturation of the embryo determines the characteristic stage of development at hatching (Ricklefs and Starck, 1998), and thyroid hormones play major roles in tissue differentiation and in the final maturation of many tissues just prior to hatching (Black, 1978; Mallon and Betz, 1982; McNabb, 1993; Decuypere *et al.*, 1992). Data from the current

Table 4: Maternal plasma thyroid hormone concentrations when fed supplemental iodide

	Weeks of age					
Diet[1]	30	32	36	41	48	Mean
Thyroxine (ng/ml)						
Control	7.8	5.6	5.4	7.6	8.0	6.9[a]
Iodide	7.4	4.6	4.7	6.6	6.5	5.9[b]
Mean	7.6[a]	5.1[b]	5.0[b]	7.1[a]	7.2[a]	
Mean ± SEM = 6.6 ± 0.2						
Probabilities						
Diet	0.03					
Age	0.0001					
Diet x Age	NS					
Triiodothyronine (ng/ml)						
Control	3.88	1.84	1.77	1.98	2.17	2.33
Iodide	3.92	1.91	1.93	2.19	1.86	2.36
Mean	3.89[a]	1.88[b]	1.85[b]	2.08[b]	2.01[b]	
Mean ± SEM = 2.45 ± 0.12						
Probabilities						
Diet	NS					
Age	0.0001					
Diet x Age	NS					
Triodothyronine:thyroxine ratio						
Control	0.23	0.28	0.33	0.45	0.33	0.33[a]
Iodide	0.21	0.25	0.26	0.28	0.36	0.27[b]
Mean	0.22[b]	0.26[ab]	0.30[a]	0.37[a]	0.35[a]	
Mean ± SEM = 0.28 ± 0.01						
Probabilities						
Diet	0.04					
Age	0.0001					
Diet x Age	NS					

[1]Control = hens were fed a basal diet containing 0.4 ppm iodine; Iodide = hens were fed the basal diet supplemented with 4 ppm iodine.
[a,b]Columnar or row means with different superscripts differ significantly ($P \leq 0.05$).

study describe a possible mechanism for maternal dietary iodine in excess of the maternal requirement to affect the physiology and survival of turkey embryos. These data reconfirm the essential role for dietary iodide in turkey reproduction (Christensen and Donaldson, 1994) and suggest recommended levels may be adequate for the health of the hen but insufficient to support embryonic thyroid function and subsequent survival of modern type turkeys.

The generally depressing effect of dietary iodide on maternal T_3 and T_4 concentrations was unexpected. The normal response to oral or intramuscular iodine given to goitrous animals is an increase in maternal plasma T_4 followed by improved neonatal health (Phillips and Osmond, 1989). However, the effect of maternal dietary iodine is biphasic (Tramontano *et al.*, 1989), and additional iodine fed to a non-goitrous animal can reduce thyroid size and proliferation of thyroid cells. Although depressing effects on maternal T_4 were seen in the current study, the turkey embryo was consistently elevated T_4 in response to increased dietary iodide.

It is speculated that iodide fed in excess of nutritional requirements or excess maternal hormone was deposited into the egg (Rogler *et al.*, 1959, 1961a; Sechman and Bobek, 1988; 1996; Sechman, *et al.*, 2000). The iodide or thyroid hormone was assumed to be readily available to the embryo through the yolk. Thus, hormone levels during hatching may be the total combination of T_3 and T_4 from embryonic synthesis and extra maternal hormone or iodide in excess of the metabolic requirements for maternal maintenance and egg production (Sechman *et al.*, 2000). By depositing excess iodide or thyroid hormones in the egg during formation, the hen can ensure not only the maturity of hatchlings but their survival as well.

Feeding goitrogens to block maternal T_4 synthesis has been associated with reduced embryonic survival rates and longer incubation periods (Christensen and Donaldson, 1994), and feeding additional iodide had no effect on incubation periods but resulted in improved survival rates. Observations suggest that incubation periods may need to match thyroid function in a time-

Table 5: Embryonic plasma thyroid hormone concentrations during pipping and hatching when turkey breeder hens were fed supplemental iodide

	Day of incubation			
Diet[1]	25	26	27	28
Thyroxine (ng/ml)				
Control	6.1[c]	11.7[c]	20.7[b]	29.3[ab]
Iodide	6.1[c]	34.1[a]	38.5[a]	5.8[c]
Mean	6.1	22.9	29.5	17.6
Mean ± SEM = 18.3 ± 1.9				
Probabilities				
Diet	NS			
Day	0.008			
Diet x Day	0.002			
Triiodothyronine (ng/ml)				
Control	0.17	1.48	2.10	1.45
Iodide	0.37	2.19	3.08	1.29
Mean	0.27[c]	1.85[ab]	2.59[a]	1.37[b]
Mean ± SEM = 1.57 ± 0.16				
Probabilities				
Diet	NS			
Day	0.001			
Diet x Day	0.10			
Triiodothyronine:thyroxine ratio				
Control	0.03[c]	0.15[b]	0.14[b]	0.08[bc]
Iodide	0.09[b]	0.08[bc]	0.10[b]	0.23[a]
Mean	0.06	0.12	0.12	0.15
Mean ± SEM = 0.12 ± 0.01				
Probabilities				
Diet	NS			
Day	0.10			
Diet x Day	0.04			

[1]Control = hens were fed a basal diet containing 0.4 ppm added iodine; Iodine = hens were fed basal diet containing 4 ppm supplemental iodine. [a,b,c] Columnar means followed by a different superscript differ significantly ($P \leq 0.05$).

related manner. If thyroid hormones are elevated at a time when embryos are not developmentally prepared, it can be fatal.

In a prior study (Christensen *et al.*, 1996), organ growth and glycogen use were affected by the age of the breeder hen. For example, it was noted that as laying hens aged, embryonic liver weight and pipping muscle weight increased at hatching, but that of heart did not. Present data show that thyroid hormone mediated changes in organ growth and glycogen metabolism mediated not only by seasonal changes in egg size and eggshell conductance but by increased embryonic thyroid function as well.

Increased maternal T_4 and T_3 as commercial turkey hens aged and produced eggs agrees with earlier data (Lien and Siopes, 1989). Increases seen in the previous study were speculated to be involved with the setting of biological clocks involved in seasonal egg production. This suggests that the variation seen in hatchability of turkey eggs as hens age (Christensen *et al.* 1996) may be correlated to a seasonal rhythm in thyroid function that prevails in birds with seasonal egg production cycles (Lien and Siopes, 1989). Biologically, this may mean that poults hatching at times when the maternal thyroid output is optimized have more *in ovo* maternal deposition of available thyroid hormones. Therefore, declining hatchability as hens age may be related to depressed maternal thyroid function.

Summary and Conclusion: In conclusion, the current data show that in the turkey, maternal and embryonic thyroidal function may be interdependent. Data indicate further that maternal thyroid function alters embryonic thyroid function to ensure survival of the embryo at hatching. Maternal control occurs despite the cleidoic nature of incubation in turkeys. The data also suggest

Table 6: Embryonic organ glycogen concentration (mg/g of tissue) when turkey breeder hens are fed supplemental iodide

	Day of incubation				
Diet[1]	25	26	27	28	Mean
Cardiac glycogen (mg/g of tissue)					
Control	8.1	8.2	6.3	2.9	6.4[a]
Iodide	5.9	6.0	6.2	1.5	4.9[b]
Mean	7 .0[a]	7.1[a]	6.2[a]	2.2[b]	
Mean ± SEM = 5.6 ± 0.2					
Probabilities					
Diet	0.009				
Day	0.0001				
Diet x Day	NS				
Hepatic glycogen (mg/g of tissue)					
Control	25.6[a]	7.9[c]	4.4[cd]	2.7[cd]	10.2
Iodide	14.9[b]	7.3[c]	5.1[c]	1.5[d]	7.2
Mean	20.2	7.6	4.8	2.1	
Mean ± SEM = 8.7 ± 0.8					
Probabilities					
Diet	0.06				
Day	0.001				
Diet x Day	0.05				
Skeletal muscle glycogen (mg/g of tissue)					
Control	5.3	6.2	6.6	3.2	5.4
Iodide	3.3	8.1	8.3	4.3	6.0
Mean	4.3[b]	7.1[a]	7.5[a]	3.8[b]	
Mean ± SEM					
Probabilities					
Diet	NS				
Day	0.006				
Diet x Day	NS				

[1]Control = hens were fed a basal diet containing 0.4 ppm added iodine. Iodide = hens were fed a basal diet containing 4 ppm added iodine. [a, b] Columnar means followed by a different superscript differ significantly ($P \leq 0.05$).

that the NRC iodine requirement for modern type commercial turkey breeders diets may be too low.

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[1]The use of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned.

[2]Hybrid Turkeys, 650 Riverbend Drive, Suite C, Kitchener, ON N2K 382, Canada.

[3]Supplemented as potassium iodide, Fisher Scientific Co., Raleigh, NC 27629.

Abbreviations key: T_4 = thyroxine, T_3 = 3, 5, 3-triiodothyronine, RIA = Radioimmunoassay