ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 5 (8): 714-722, 2006
ISSN 1682-8356

Incubator Environment Interacts with Genetic Line of Broiler at the Plateau Stage to Affect Embryo Plasma Thyroxine and Triiodothyronine Concentrations

M.J. Wineland[1], V.L. Christensen[1], I. Yildrum[2], B.D. Fairchild[1], D.T. Ort[1] and K.M. Mann[1]
[1]Department of Poultry Science, College of Agriculture and Life Sciences,
North Carolina State University, Raleigh, North Carolina 27695-7608, USA
[2]Department of Animal Science, Agriculture Faculty,
The University of Selcuk, 42031 Campus, Konya, Turkey

Abstract: We hypothesize that temperature and oxygen in conjunction with genetic lines of broilers regulate embryo thyroid function. Thyroid response of broiler embryos of the two lines was measured at different incubator temperature and oxygen concentrations at the plateau stage in oxygen consumption (days 17 to 20 of embryo development). Each of the lines showed different eggshell conductance (G) values. Eggs of the same weight from the lines of broilers were incubated identically until the 17th day of development. On the 17th day (plateau stage in oxygen consumption), eggs were randomly distributed by line into four incubators operating at 36, 37, 38 or 39ºC in trial 1 or at 17, 19, 21 or 23% oxygen in trial 2. At external pipping (the end of the plateau stage in oxygen consumption) as well as at hatching ten embryos or chicks per treatment were sampled for blood plasma. Plasma was analyzed for thyroxine and triiodothyronine concentrations. In trial 3, temperature line of broiler and oxygen treatments were arranged factorially. Increasing temperatures suppressed hormone concentrations in embryos, and the suppression was greater with low G. Increasing oxygen increased hormone concentrations in low G embryos to a greater degree than high G. It can be concluded that incubation temperature suppresses plasma thyroid hormone concentrations in low G lines whereas oxygen increases it.

Key words: Thyroid function, plateau stage, plasma

Introduction

Organ system immaturity associated with thyroid hormones is a principal cause of morbidity associated with extreme prematurity in humans. Therapies for premature infants include the infusion of triiodothyronine (T_3) and thyroxine (T_4) (Yeung and Smyth, 2002). Organ system immaturity (Lilja and Olsson, 1987; Wineland *et al.*, 2006) and the onset of thermoregulation (Nichelmann *et al.*, 2001) both of which are mediated by thyroid hormones and are important components of broiler chick quality as well. Therefore, the hypothesis was proposed that incubator temperature and oxygen concentrations or their interaction with genetic line at the plateau stage in oxygen consumption stimulate the chick embryo thyroid to release T_4 and or T_3 into the blood.

Chick embryo plasma thyroid hormone concentrations increase developmentally as the hypothalamus matures (Christensen, 1978; McNabb *et al.*, 1984a). Differences in concentrations have been noted among embryos from lines selected for heavy or light body weights (McNabb *et al.*, 1989; 1993). The peak in thyroid hormone concentrations coincides with a time called the plateau stage in oxygen consumption identified by the hypoxic and hypercapnic conditions created for embryos (Rahn, 1981). Embryonic thyroids from different genetic backgrounds respond differently to environmental conditions (Christensen *et al.*, 2002). Temperature, atmospheric oxygen (Christensen *et al.*, 2005), carbon dioxide (Buys *et al.*, 1998), and maternal dietary iodide (Christensen *et al.*, 2002) have been identified as environmental stimuli for avian embryo thyroid responses. Incubator temperature and oxygen concentrations eliciting broiler embryo thyroid responses are unknown.

Materials and Methods

Eggs of identical weights from two lines of broiler breeders were obtained. The eggs were incubated for 17 days using commercially accepted standards. Experimental cabinets accommodating approximately 100 eggs were used for the actual hatching process. Each cabinet contained one incubator tray and was regulated by thermistors connected to microprocessors with temperature sensitivity of ± 0.05^{o}C. Humidity was controlled by a similar system using relative humidity sensors. Digital thermometers were used with each cabinet to verify set point temperatures. Atmospheric gases were measured by using an infrared detector for carbon dioxide and electrochemical cells for oxygen, each with a sensitivity of ± 0.1%. Manually operated valves infused gases into each of the cabinets, and the flow rate was adjusted based on the sensors to create the desired gas concentration. Three experiments were conducted using similar conditions. Experiment 1

Table 1: Body weights of chick embryos from two lines incubated at four temperatures during the plateau stage in oxygen consumption

	Day of incubation			
	20		21	
Temperature[1]	Low G[2]	High G[2]	Low G	High G
Without yolk				
36	38.4	38.1	39.8	37.7
37	39.5	37.8	41.4	38.7
38	39.6	38	41.3	40.5
39	36.9	38.1	38.8	38.5
Day means	38.3b		39.5^a	
	Mean ± SEM	38.8 ± 0.3		
Probabilities	Temperature (T)	NS		
	Line (L)	0.06	Low G = 39.5^a	
	Day (D)	0.05	High G = 38.3^b	
	T x L	NS		
	T x D	NS		
	L x D	NS		
	T x L x D	NS		

[1]Incubating eggs were exposed to 36, 37, 38 or 39ºC from days 18 to 21 of embryonic development. [2]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance. [a,b]Means within a factor or factors displaying different superscripts differ significantly ($P \leq 0.05$)

examined the effect of temperature on embryo thyroid function. Experiment 2 examined the effect of oxygen concentration on the embryo thyroid, and experiment 3 examined the most effective and least effective temperature and oxygen treatments in a factorial arrangement.

Temperature: Fertilized broiler chicken eggs of the same weight from two commercial lines (Low conductance = Low G; High conductance = High G) were obtained on the day of oviposition, weighed and placed into incubators for 17 d using standard conditions. On the 17th d they were candled to determine embryo viability. The 17th day of development for chick embryos is the beginning of the plateau stage in oxygen consumption (Rahn, 1981). All infertile eggs and nonviable embryos were removed prior to transfer to one of the four experimental cabinets. Each cabinet operated at one of the treatment temperatures (36, 37, 38 or 39°C).

Ten embryos or chicks per line were selected randomly from each incubator at external pipping during the 20th day and at hatching on 21 days of development. Chick body (nearest 0.1g) weights were recorded and thyroid function was evaluated by measuring blood plasma T_3 and T_4 concentrations. The chicks were decapitated and trunk blood was collected into a tube containing 10 mg EDTA. The blood was centrifuged (700 x g) for 15 min under refrigeration (4°C). The plasma was decanted and frozen (-22°C) until analysis by radioimmunoassay (Davis *et al.*, 2000). Samples from all three trials were assayed in a single assay and the intraassay variation was 1.3% for T_4 and 2.0% for T_3.

Oxygen: Four oxygen concentrations were the treatments in the second experiment. The concentrations were 17, 19, 21 or 23% of the atmosphere within the cabinets. Each fractional concentration at sea level (Raleigh, NC) corresponded to oxygen partial pressures of 129, 144, 160 and 175 mm Hg, respectively. Concentrations lower than ambient oxygen concentrations (20.9%) were maintained by infusing nitrogen gas into the cabinet at a rate that resulted in the desired concentration of 17 or 19% oxygen. Concentrations were measured with an oxygen meter and flow rates from oxygen or nitrogen storage tanks were adjusted to maintain the desired oxygen level.

Embryo samples were collected and analyzed as in Experiment 1. Eggs were incubated in the same machine until the 17th day of development, candled to determine viability and selected randomly as described in Experiment 1 and placed into each of the four cabinets.

Temperature and oxygen: The most and least effective temperatures (36° and 39°C) and oxygen (17 and 23%) levels in the prior experiments were combined in a factorial arrangement for the third experiment. The incubator temperatures and oxygen concentrations were arranged in a 2 x 2 factorial. All treatments were maintained identically as described in the previous experiments. Fertilized eggs were again incubated 18 days in an incubator when viable embryos were assigned randomly to one of the four cabinets. The conditions were 36°C or 39°C with 17 or 23% oxygen in a factorial arrangement. Embryos or hatchlings were sampled identically as described in the previous experiments.

Table 2: Plasma thyroid hormone concentrations of chick embryos from two lines incubated at four temperatures during the plateau stage in oxygen consumption

	Day of incubation				Temperature means
	20		21		
Temperature[1]	Low G[2]	High G[2]	Low G	High G	
	---------- Thyroxine (ng/mL) ---------				
36	25.9	15.5	10.4	11.6	15.8^{a}
37	20.9	14.1	13.7	11.7	15.1^{a}
38	18.6	17.8	10.2	10.8	14.4^{ab}
39	12.3	16.7	8.7	5.1	10.7^{b}
Day means	17.8^{a}		12.9^{b}		
	Mean ± SEM	13.7± 0.6			
Probabilities	Temperature (T)	0.04			
	Line (S)	NS			
	Day (D)	0.0001			
	T x S	NS			
	T x D	NS			
	T x S x D	NS			
	--------- Triiodothyronine (ng/mL) ---------				
36	18.1^{ab}	8.2^{c}	11.1^{b}	26.8^{a}	
37	9.4^{c}	14.7^{b}	13.8^{b}	15.0^{b}	
38	9.1^{c}	4.6^{d}	7.4^{c}	11.5^{b}	
39	10.1^{b}	11.7^{b}	6.5^{cd}	6.1^{cd}	
	Mean ± SEM	10.8 ± 0.7			
Probabilities	Temperature (T)	0.01			
	Line (L)	NS			
	Day (D)	NS			
	T x L	NS			
	T x D	NS			
	L x D	0.05			
	T x L x D	0.05			
	---------- Ratio ----------				
36	0.82^{c}	0.47^{d}	1.03^{bc}	5.37^{a}	
37	0.56^{cd}	0.43^{d}	1.18^{b}	1.28^{b}	
38	0.31^{d}	0.29^{d}	0.72^{c}	1.01^{bc}	
39	0.91^{bc}	1.81^{b}	0.89^{bc}	1.21^{b}	
	Mean ± SEM	8.6 ± 0.2			
Probabilities	Temperature (T)	0.0001			
	Line (L)	0.0006			
	Day (D)	0.0001			
	T x L	0.002			
	T x D	0.0001			
	L x D	0.003			
	T x L x D	0.0001			

[1]Incubating eggs were exposed to 36, 37, 38 or 39ºC from days 18 to 21 of embryonic development.

[2]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance. [a,b,c,d]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05)

Statistical analysis: Data for all three experiments were analyzed using the general linear models procedure (SAS Inc., 1998). Experiments 1 and 2 were arranged as a four levels of temperature or oxygen treatments by two lines factorial. In Experiment 3, the data were arranged as two temperatures by two oxygen concentrations by two lines factorial. Means differing significantly were separated by the least square means procedure. Means in tables are least square means. All possible main and interaction effects were tested. All probabilities were based on $P < 0.05$ unless otherwise noted.

Results

Temperature: Temperatures had no effect on BW (Table 1), but Low G chicks were heavier at hatching than were High. No differences were noted in residual yolk weights of any of the treatments. Elevated temperatures depressed embryo plasma T_4 concentrations in a nearly step-wise fashion (Table 2), and plasma T_4 declined as the embryos developed from day 20 to day 21. A significant three way interaction was noted in plasma T_3 concentrations. High G embryos were delayed in their response to increasing temperatures when compared to Low G embryos. High G embryo concentrations were

Table 3: Body weights (g) of chick embryos from two lines incubated at four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation				Oxygen means
	20		21		
Oxygen[1]	Low G[2]	High G[2]	Low G	High G	
		---------- Without yolk ----------			
17	38.6	36.3	38.5	37.1	37.6[b]
19	38.7	35.7	39.8	38.6	38.2[b]
21	39.6	37.7	41.6	39.9	39.7[a]
23	39.2	37.5	41.3	39.3	39.3[a]
Day means	37.9[b]		39.5[a]		
	Mean ± SEM	38.7 ± 0.3			
Probabilities	Oxygen (O)	0.001			
	Line (L)	0.0001	Low G = 39.7[a]		
	Day (D)	0.0001	High G = 37.8[b]		
	O x L	NS			
	O x D	NS			
	L x D	NS			
	O x L x D	NS			

[1]Incubating eggs were exposed to 17, 19, 21 or 23 % oxygen from days 18 to 21 of embryonic development. [2]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance.
[a,b]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05)

suppressed at day 21 with increasing temperature, but Low G embryos showed the suppressed response at day 20 of development. Deiodination was assessed as T_3:T_4 ratios (Table 2). A temperature by line by age of development interaction was noted for the ratio. High G line embryos responded clearly to temperature, but Low G did not. Increasing temperature increased ratios at day 20 of development, but the reverse was noted at day 21 of development when 39°C elevated ratios compared to all other temperatures.

Oxygen: In contrast to increased temperatures, increased concentrations of oxygen in the incubators increased embryo weights (Table 3). Thus, increased oxygen concentrations greater than 21% enhanced growth during the plateau stage. Increasing oxygen percentages also increased embryo T_4 concentrations and Low G embryos had greater T_4 concentrations than did High G embryos (Table 4). Two-way interactions characterized the responses seen in embryo plasma T_3 concentrations. Oxygen treatment interacted with breed of broiler to increase concentrations in embryos with High G and decrease concentrations in embryos with Low G (Table 4). Oxygen treatments also had different effects on T_3 at days 20 and 21 (Table 5). Increasing concentrations of oxygen decreased T_3 at day 20 but increased it at day 21. Decreased concentrations of oxygen in the incubators resulted in increased ratios of T_3 to T_4 at both times of development examined.

Temperature and oxygen: When temperature and oxygen were examined as factorials, both affected embryo body weight independently (Table 6). Increased temperature suppressed body weight, and increased oxygen enhanced it. Increased oxygen concentrations increased plasma T_4 concentrations at both 20 and 21 days of development, and temperature had no effect on T_4 concentrations (Table 7). Plasma T_3 concentrations were depressed by increasing temperature at day 20 of development but not at day 21. Increased temperature interacted with line to increase T_3 in embryos from eggs of High G with no consequent effect on eggs of Low G (Table 8). The changes in T_3 to T_4 ratios were identical to those reported for T_3 concentrations.

Discussion

The hypothesis was proposed that temperature, line of broiler and oxygen modulate embryonic thyroid function during the plateau stage in oxygen consumption. In mammals modulation is performed maternally primarily by the placenta (Yeung and Smyth, 2002). In the absence of maternal modulators, the control mechanisms for avian embryo thyroid are probably intrinsic but are unknown. The current study shows clearly that the embryonic broiler thyroid at the plateau stage in oxygen consumption responds to both temperature and oxygen stimuli, but the response was dependent upon the line of broiler. Data from the current study indicated as well that both factors affect monodeiodination that may be due to either synthesis or clearance due to mondiodinase I or III (Darras *et al.*, 2000). Overall, temperature was a more effective stimulus, but oxygen effects were also very prominent.

An apparent paradox in energy budgets of avian eggs and metabolism occurs at the plateau stage in incubation as heat output increases, but oxygen utilization does not (Dietz *et al.*, 1998). When confronted with life-threatening situations, embryonic organ growth

Table 4: Plasma thyroid hormone concentrations of chick embryos from two lines incubated at four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation				Oxygen means
	20		21		
Oxygen[1]	Low G[2]	High G[2]	Low G	High G	
		---------- Thyroxine (ng/mL) ----------			
17	15.1	12	14.4	7.3	
19	16.8	14.1	12.6	10.3	
21	20.9	17.3	11	8.1	
23	18.9	11	11.3	11.4	
Day means	15.8[a]	10.8[b]			
	Mean ± SEM	12.8 ± 0.6			
Probabilities	Oxygen (O)	NS			
	Line (L)	0.001	Low G = 15.1[a]		
	Day (D)	0.0001	High G = 11.4[b]		
	O x L	NS			
	O x D	NS			
	L x D	NS			
	O x L x D	NS			
O x L Means		---------- Triiodothyronine (ng/mL) ----------			
17	7.7[a]	6.1[b]			
19	6.4[ab]	7.1[a]			
21	6.9[a]	7.1[a]			
23	5.7[b]	8.3[a]			
	Mean ± SEM	7.0 ± 0.2			
Probabilities	Oxygen (O)	NS			
	Line (L)	NS			
	Day (D)	0.0001			
	O x L	0.003			
	O x D	0.01			
	L x D	NS			
	O x L x D	NS			
		---------- Ratio ----------			
17	1.08	0.5	0.44	0.88	0.73[a]
19	0.49	0.53	0.45	0.61	0.52[b]
21	0.38	0.51	0.55	0.61	0.51[b]
39	0.32	0.55	0.53	0.77	0.54[b]
	Mean ± SEM	0.59 ± 0.06			
Probabilities	Oxygen (O)	0.05			
	Line (L)	NS			
	Day (D)	NS			
	O x L	NS			
	O x D	NS			
	L x D	NS			
	O x L x D	NS			

[1]Incubating eggs were exposed to 17, 19, 21 or 23 % oxygen from days 18 to 21 of embryonic development. [2]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance.
[a,b]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05)

and function may be antagonistic (Schmalhausen, 1930). Increased plasma thyroid and adrenal hormone concentrations facilitate survival and are predetermined by genetics and developmentally by eggshell conductance (Rahn, 1981; Christensen and Biellier, 1982; Wentworth and Hussein, 1982; Christensen *et al.*, 2002). Low eggshell conductance prolongs development and reduces thyroid hormone concentrations (Christensen *et al.*, 2002; Christensen *et al.*, 2005), as well as intestinal weight and function in broiler and turkey embryos (Christensen *et al*, 2003a,b; Wineland *et al.*, 2006). Increased adrenal cortical hormones may prolong the developmental period (Hayward *et al.*, 2006) but must act in conjunction with thyroid hormones to effect intestinal maturation (Black, 1978). Elevated temperature and depressed oxygen in the incubator at the plateau stage interacted with G to affect the process of hypothalamic maturation and thyroid function preparatory for life outside of the shell differently. Low G type broilers responded differently than did High G type broilers supporting a possible relationship between the selection for rapid growth, eggshell conductance, and thyroid maturation or function.

Table 5: Means of the interaction of oxygen fractional concentration and day of incubation effects on plasma triiodothyronine hormone concentrations (ng/mL) in chick embryos from two lines incubated at four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation		
Oxygen1	20		21
17	7.8[ab]		6.0[cd]
19	8.3[a]		5.2[d]
21	8.8[a]		5.2[d]
23	7.0[bc]		7.1[b]
	Mean ± SEM	7.0 ± 0.2	
Probabilities	Oxygen (O)	NS	
	Line (L)	NS	
	Day (D)	0.0001	
	O x L	0.003	
	O x D	0.01	
	L x D	NS	
	O x L x D	NS	

[1]Incubating eggs were exposed to 17, 19, 21 or 23 % oxygen from days 18 to 21 of embryonic development.
[a,b,c]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05)

Table 6: Body weights (g) of embryos and chicks from two lines incubated at two temperatures and two oxygen partial pressures during the plateau stage in oxygen consumption

Temperature[1]	Oxygen[2]	Day of incubation			
		20		21	
		Low G[3]	High G[3]	Low G	High G
		---------- Body weight without yolk ----------			
36	17	37.4	38.5	38	38.2
	23	38.4	38.4	40	37.9
	T mean	38.2[a]			
39	17	34.8	35.9	37.1	37.1
	23	37.2	36.6	38.3	38.8
	T mean	36.1[b]			
	Mean ± SEM	37.2 ± 0.2		38.2 ± 0.3	
Probabilities	Temperature (T)	0.0003		NS	
	Oxygen (O)	0.05	17% = 36.7[b]	0.05	17% = 37.6[b]
	Line (L)	NS	23% = 37.6[a]	NS	23% = 38.8[a]
	T x O	NS		NS	
	T x L	NS		NS	
	O x D	NS		NS	
	T x O x L	NS		NS	

[1]Incubating eggs were exposed to 36, 37, 38 or 39ºC from days 18 to 21 of embryonic development.
[2]Incubating eggs were exposed to 17, 19, 21 or 23 % oxygen from days 18 to 21 of embryonic development.
[3]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance. [a,b]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05)

Thyroid and adrenal hormones play major roles at the plateau stage for intestinal maturation (Black, 1978) and improved neonate survival (Davis and Siopes, 1989; Christensen *et al*., 2003b). Both types of hormones prepare essential organs (Black, 1978) for life outside of the shell. Both T_3 and T_4 increase exponentially during the plateau stage in oxygen consumption attaining their greatest concentrations at any time in the life of the bird (Decuypere *et al*., 1991). The increased embryonic T_3 and T_4 concentrations in precocial species distinguish these species from altricial (McNabb *et al*., 1984b; McNabb, 1988; and Vyboh *et al*., 2001). Thus, thyroid hormones may be connected directly to the level of maturation at hatching. If T_3 and T_4 are depressed, then hatchling maturity may be delayed (Christensen *et al*., 2003a,b). If maturity and function are delayed in a vital tissue, then hatchling health and neonate health may be jeopardized as in humans (Christensen and Biellier, 1982; Christensen *et al*., 2003a,b).

Thyroid hormone responses in avian embryos have been suggested to be of two types, i.e., developmental and functional (Christensen *et al*., 2005). Incubator environment stimulates embryo functional thyroid responses in unique ways (Christensen *et al*., 2002). Embryos from different lines may have the same developmental thyroid characteristics but may differ in functional response to temperature or ambient oxygen concentrations (Christensen *et al*., 2005). Turkey

Table 7: Plasma thyroid hormone concentrations of embryos and chicks from two lines incubated at two temperatures and two oxygen partial pressures during the plateau stage in oxygen consumption

Temperature[1]	Oxygen[2]	Day of incubation			
		20		21	
		Low G[3]	High G[3]	Low G	High G
		------------------- Thyroxine (ng/mL) ------------------			
36	17	20.6	23.8	11.6	15.8
	23	27.9	25.7	16	17.7
39	17	17.2	22.5	10	11.7
	23	24.4	20.4	15.3	14.8
	Mean ± SEM	21.9 ± 1.0		13.9 ± 0.9	
Probabilities	Temperature (T)	NS		NS	
	Oxygen (O)	0.05	17% = 21.0[b]	0.03	17% = 12.2[b]
	Line (L)	NS	23% = 24.6[a]	NS	23% = 16.0[a]
	T x O	NS		NS	
	T x L	NS		NS	
	O x L	0.08		NS	
	T x O x L	NS		NS	
		------------------- Triiodothyronine (mg/mL) ------------------			
36	17	18.6	23.7		
	23	10.7	11.8		
	T mean	16.2[a]			
39	17	12.4	9.9		
	23	8.1	8.4		
	T mean	9.7[b]			
	Mean ± SEM	13.3 ± 1.3			
Probabilities	Temperature (T)	0.0007			
	Oxygen (O)	0.001	17% = 16.2[a]		
	Line (L)	NS	23% = 9.8[b]		
	T x O	NS			
	T x L	NS			
	O x L	NS			
	T x O x L	NS			
		------------------- Ratio -------------------			
36	17	0.91	1.23		
	23	0.56	0.48		
	T mean	0.80[a]			
39	17	0.83	0.48		
	23	0.33	0.51		
	T mean	0.53[b]			
	Mean ± SEM	0.65 ± 0.08			
Probabilities	Temperature (T)	0.05			
	Oxygen (O)	0.04	17% = 0.86[a]		
	Line (L)	NS	23% = 0.47[b]		
	T x O	NS			
	T x L	NS			
	O x L	NS			
	T x O x L	NS			

[1]Incubating eggs were exposed to 36, 37, 38 or 39ºC from days 18 to 21 of embryonic development.
[2]Incubating eggs were exposed to 17, 19, 21 or 23 % oxygen from days 18 to 21 of embryonic development.
[3]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance. [a,b]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05).

embryos of different genetic backgrounds survive differently when exposed to elevated temperatures as well (Christensen *et al.*, 2002). Embryos with greater T_4 concentrations and perhaps greater hypothalamic maturity survive better than those with depressed levels (Christensen and Biellier, 1982). Comparing embryos of selected lines to their randombred controls revealed that both developmental and functional responses are essential to embryo survival (Christensen *et al.*, 2002). Plasma thyroid hormone concentrations were found to be depressed in weak neonatal turkeys as identified by characteristic flip-over behavior (Christensen *et al.*, 2003b). Poults exposed to excessive carbon dioxide concentrates also display hypothyroidism (Donaldson *et al.*, 1995). Thus, better thyroid responses may also indicate not only improved embryo viability but better neonate survival as well.

The broiler chick embryo at the end of development has

Table 8: Interaction means for incubator temperature and eggshell conductance effects on triiodothyronine concentrations and ratio of triiodothyronine to thyroxine concentrations in chicks from two lines incubated at two temperatures during the plateau stage in oxygen consumption.

Temperature[1]		Low G[2]	High G[2]
		-------------- Triiodothyronine (ng/mL) --------------	
36		10.3[ab]	15.0[a]
39		13.3[ab]	9.9[b]
	Mean ± SEM	12.1 ± 1.2	
Probabilities	Temperature (T)	NS	
	Oxygen (O)	NS	
	Line (L)	NS	
	T x O	NS	
	T x L	0.04	
	O x L	NS	
	T x O x L	NS	
		-------------- Ratio --------------	
36		1.06[ab]	1.41[a]
39		1.43[a]	0.68[b]
	Mean ± SEM	1.18 ± 0.14	
Probabilities	Temperature (T)	NS	
	Oxygen (O)	NS	
	Line (L)	NS	
	T x O	NS	
	T x L	0.05	
	O x L	NS	
	T x O x L	NS	

[1]Incubating eggs were exposed to 36, 37, 38 or 39ºC from days 18 to 21 of embryonic development

[2]Low G = line of broilers whose eggs display low eggshell conductance; High G = line of broilers whose eggs display high eggshell conductance. [a,b]Means within a factor or factors displaying different superscripts differ significantly (P = 0.05)

a functional thyroid as does the turkey (Christensen *et al.*, 2002) as evidenced by the effects of ambient temperature and oxygen concentration on thyroid hormones. However, the most important observation from the study was the different thyroid hormone response seen in embryos from each of the two lines of broiler. The line with Low G elevated thyroid hormones less readily than did the line with High G. Thus, it is possible that the hen confers a metabolic rate upon her embryonic progeny by the G of egg she produces. The ability of the embryo to control metabolic rates was modulated by both oxygen and temperature in the incubation cabinet. Increasing temperature generally suppressed thyroid hormone concentrations in the plasma whereas increasing oxygen concentrations increased circulating thyroid hormone concentrations.

Deiodination of T_3 to T_4 was more susceptible to temperature, oxygen and G than was the level of T_4. It has been reported that the initial detection of plasma T_3 may be at the stages of development that we examined (McNabb, 2000). Thus, it may be inferred that increased heat and hypoxia interfere with the maturation of the monodeiodination system in broiler embryos (Darras *et al.*, 2000). The effect of oxygen on deiodination was less pronounced than that for temperature.

The data from our study infer that functional maturation of the hypothalamo-pituitary-thyroid axis is at least partially complete at the plateau stage in incubation. The thyroid of broiler embryos immediately following the plateau responded clearly to both temperature and oxygen stimuli and the changes in both were dependent on line or G. Responses may be a functionally important mechanism to allow the embryo/chick to cope metabolically with its environment as well as preparation for life following the hatching process.

References

Black, B.L., 1978. Morphologic development of the epithelium of the embryonic chick intestine in culture: influence of thyroxine and low hydrocortisone. Am. J. Anat., 153: 573-600.

Buys, N., E. Dewil, E. Gonzales and E. Decuypere, 1998. Different CO_2 levels during incubation interact with hatching time and ascites susceptibility in two broiler lines selected for different growth rate. Avian Path., 27: 605-612.

Christensen, V.L., 1978. Physiological factors and hatchability in the fowl. PhD. Thesis, University of Missouri, Columbia, MO.

Christensen, V.L. and H.V. Bieller, 1982. Physiology of turkey embryos during pipping and hatching. IV. Thyroid function in embryos from selected hens. Poult. Sci., 61: 2482-2488.

Christensen, V.L., G.S. Davis and K.E. Nestor, 2002. Environmental incubation factors influence embryonic thyroid hormones. Poult. Sci., 81: 442-450.

Christensen, V.L., J.L. Grimes, M.J. Wineland and G.S. Davis, 2003a. Accelerating growth during incubation following prolonged egg storage. 2. Embryonic growth and metabolism. Poult. Sci., 82: 1869-1878.

Christensen, V.L., D.T. Ort and J.L. Grimes, 2003b. Physiological factors associated with weak neonatal poults *(Meleagris gallopavo*). Int. J. Poult. Sci., 2: 7-14.

Christensen, V.L., M.J. Wineland, I. Yildrum, B.D. Fairchild, D.T. Ort and K.M. Mann, 2005. Incubator temperature and oxygen concentrations during the plateau stage in oxygen uptake affect turkey embryo plasma T_4 and T_3 concentrations. Int. J. Poult. Sci., 4: 268-273.

Darras, V.M., S.V.D. Geyten and E.R. Kuhn, 2000. Thyroid hormone metabolism in poultry. Biotechnol. Agron. Soc. Environ., 4: 13-20.

Davis, G.S. and T.D. Siopes, 1989. Ontogeny of daily rhythmicity in plasma corticosterone and variation in sensitivity of the corticosterone response in turkey poults. Poult. Sci., 68: 423-427.

Davis, G.S., K.G. Anderson and A.S. Carrol, 2000. The effects of long-term caging and molt of Single Comb White Leghorn hens of heterophil to lymphocyte ratios, corticosterone and thyroid hormones. Poult. Sci., 79: 514-518.

Decuypere, E., E. Dewil and E.R. Kuehn, 1991. The hatching process and the role of hormones. Pages 239-256 in: Avian Incubation. S. G. Tullett, ed. Butterworth-Heineman, London, UK.

Dietz, V.M., M.J. van Kampen, M.J.M. van Griensven and S. van Mourik, 1998. Daily energy budgets of avian embryos: The paradox of the plateau phase in egg metabolic rate. Physiol. Zool., 71: 147-156.

Donaldson, W.E. and V.L. Christensen, J.D. Garlich, J.P. McMurtry and N.C. Olson, 1995. Exposure to excessive carbon dioxide: A risk factor for early poult mortality. J. Appl. Poult. Res., 4: 249-253.

Hayward, L.S., J.B. Richardson, M.N. Grogan and J.C. Wingfield, 2006. Sex differences in the organizational effects of corticosterone in the egg yolk of quail. Gen. and Comp. Endocrin., 146: 144-148.

Lilja, C. and U. Olsson, 1987. Changes in embryonic development associated with long-term selection for high growth rate in Japanese quail. Growth, 51: 301-308.

McNabb, F.M.A., F.W. Stanton and S.G. Dicken, 1984a. Post-hatching thyroid development and body growth in precocial vs. altricial birds. Comp. Physiol. Biochem., 78A: 629-635.

McNabb, F.M.A., L.J. Lyons and T.E. Hughes, 1984b. Free thyroid hormones in altricial (ring doves) versus precocial (Japanese quail) development. Endocrinol., 115: 2133-2136.

McNabb, F.M.A., 1988. Peripheral thyroid hormone dynamics in precoccial and altricial avian development. Am. Zoologist, 28: 427-465.

McNabb, F.M.A., E.A. Dunnington, T. Freeman and P.B. Siegel, 1989. Thyroid hormones and growth patterns of embryonic and posthatch chickens from lines selected for high and low juvenile body weight. Growth Dev. Aging 53: 87-92.

McNabb, F.M.A., E.A. Dunnington, P.S. Siegel and S. Suvarna, 1993. Perinatal thyroid hormones and hepatic 5'deiodinase in relation to hatching time in weight-selected chickens. Poult. Sci., 72: 1764-1771.

McNabb, F.M.A., 2000. Thyroids. Pages 461-471 in: Avian Physiology, G. Causey Whittow, ed. Academic Press, San Diego, CA.

Nichelmann, M., O. Janke and B. Tzschentke, 2001. Efficiency of thermoregulation in precoccial avian species during the prenatal period. J. Thermal Biol., 26: 273-280.

Rahn, H., 1981. Gas exchange of avian eggs with special reference to turkey eggs. Poult. Sci., 60: 1971-1980.

SAS Institute, 1998. SAS/STAT Guide for Personal Computers, Version 6 Edition, SAS Institute Inc., Cary, NC.

Schmalhausen, I., 1930. Das wachstumsgesetz als gesetz der progressiven differenzierun. Wilhelm Roux Arch. Enwicklungsmech. Org., 123: 143-178.

Vyboh, P., M. Zeman, J. Buyse, M. Jurani and E. Decuypere, 2001. Developmental changes in thyrotropic and somatotropic effect of TRH in precocial Japanese qiail and European starlings. Gen. and Comp. Endocrinol., 124: 293-299.

Wentworth, B.C. and M.O. Hussein, 1982. Plasma corticosterone in the turkey embryo and the effects of this hormone on hatching. Poult. Sci., 61:1567 (Abstract).

Wineland, M. W., V. L. Christensen, I. Yildrum, B. D. Fairchild, K., M. Mann and D.T. Ort, 2006. Incubator temperature and oxygen concentration at the plateau stage in oxygen consumption affects intestinal maturation of broiler chicks. Int. J. Poult. Sci., 5: 229-240.

Yeung, M.Y. and J.P. Smyth, 2002. Hormonal factors in the morbidities associated with extreme prematurity and the potential benefits of hormonal supplementation. Bio. Neonate, 8:1-15.

Section: The mention of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned.