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Incubator Temperature and Oxygen Concentration at the Plateau Stage in Oxygen Consumption Affects Intestinal Maturation of Broiler Chicks

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Abstract: Incubator temperature and oxygen concentrations were tested as factors determining the intestinal maturation of two lines of broiler chickens. One line was a Low G line selected because its eggs display low eggshell conductance. The second line was a High G line that grew at a reduced rate and its eggs show high eggshell conductance values. All eggs were incubated normally until the 18th day of development or the beginning of the plateau stage in oxygen consumption. At that time the eggs were divided randomly and placed into experimental cabinets operating at 36, 37 38 or 39ºC in experiment 1 or with 17, 19, 21 or 23% oxygen in experiment 2. In experiment 3, the best and worst conditions observed in experiments 1 and 2 were combined in a factorial arrangement. Body weight and intestinal maturation were measured by assaying for maltase and alkaline phosphatase activities in intestinal tissues. Increasing temperatures suppressed intestinal maturation whereas increasing oxygen concentrations enhanced intestinal maturation. When examined together in a factorial arrangement, it was clear that the effects of temperature and oxygen on the embryos were independent because they did not interact. The effects of temperature and oxygen were greater on Low G broiler embryos than they were on High G type embryos. It is concluded that incubator temperatures greater than 37° C, and oxygen concentrations less than 21% are detrimental to intestinal maturation in broiler chicks.

Key words: Broiler chicks, intestinal maturation, maltase, alkaline phosphatase

Introduction

Previous research indicated that incubator temperature and oxygen concentrations during the plateau stage in oxygen consumption for turkey embryos (25 and 26 days of development) impaired intestinal (Christensen *et al.*, 2004a) and cardiac development (Christensen *et al.*, 2004b). At that stage of development embryos require more oxygen and expel more carbon dioxide than the functional properties of an eggshell can provide creating a constraint on oxygen flux and a paradox for the embryo (Dietz *et al*., 1998). The embryo must sustain life and continue to grow. The constraint creates a plateau effect in oxygen consumption (Rahn, 1981). Little is known about the effects of the environmental conditions during the plateau stage on chick embryo development.

A consequence of the plateau stage for chick embryos may be an insufficiency of anaerobic energy that delays intestinal maturation. Major maturation of the intestine occurs at the plateau stage in oxygen consumption (Black, 1978) and is an energy-demanding process (Fan *et al*., 1997). Additionally, glycogen for cardiac and skeletal muscle glycolysis is required to hatch, and energy is required for other vital tissue maturation (Dietz *et al*., 1998). If stressed during the plateau, embryo growth and organ function may become antagonistic and additional energy could be required to adapt. Therefore, the hypothesis was proposed that temperature and oxygen consumption might affect the growth and maturation of chick embryo intestine. Additionally, two genetic lines were tested that represented a rapid growing modern-type high yield strain of broiler with low eggshell conductance (Low G) and a slower growing line with high eggshell conductance (High G).

Materials and Methods

Experimental incubator cabinets simulating commercial incubators were manufactured and used to control ambient temperature or oxygen concentrations. Each cabinet contained one incubator tray with capacity for 100 eggs. Digitized thermostats, connected to microprocessors with temperature sensitivity of ± 0.1 C, controlled the wet and dry bulb temperatures. Digital thermometers were used in each cabinet to verify set point temperatures, and ports were used to infuse the desired gaseous concentrations.

Temperature: Fertilized broiler chicken eggs from two commercial strains (Low conductance = Low G; High conductance = High G) were obtained on the day of oviposition and incubated until the 18th day using standard conditions when they were candled to

Table 1: Body and yolk 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[2]	High G[2]	Means
	Without yolk				
36	38.4	38.1	39.8	37.7	
37	39.5	37.8	41.4	38.7	
38	39.6	38.0	41.3	40.5	
39	36.9	38.1	38.8	38.5	
Day means					
Line means	39.5[a]	38.3[b]			
Mean ± SEM		38.8±0.3			
Probability	Temperature	NS			
	Line (L)	0.06			
	Day (D)	0.05			
	T x L	NS			
	T x D	NS			
	L x D	NS			
	T x L x D	NS			
	Yolk				
36	9.4	9.5	7.2	6.2	
37	10.3	10.5	8.3	7.0	
38	8.7	11.3	5.5	8.0	
39	10.7	11.2	6.5	6.2	
Day means					
Overall mean ± SEM		8.6±0.2			
Probability	Temperature	NS			
	Line (L)	NS			
	Day (D)	0.0001			
	T x L	NS			
	T x D	NS			
	L x D	NS			
	T x L x D	NS			

[1]Degrees centigrade inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

determine embryo viability. The 18th day of development for chick embryos is the beginning of the plateau stage in oxygen consumption (Rahn, 1981). Following candling and removal of infertile eggs and nonviable embryos, randomly selected viable embryos were transferred to one of the four experimental cabinets. Each cabinet operated at one of the treatment temperatures (36º, 37º, 38º or 39º C).

Ten embryos or chicks per line were selected randomly from each incubator at 20 days of development (external pipping) and at 21 days of development (hatching). Chick body (nearest 0.1g) and intestine (nearest 0.01 mg) weights were recorded and intestinal function was evaluated by maltase and alkaline phosphatase (ALP) activities. Maltase hydrolyzes readily available carbohydrate (maltose) to glucose during the initial days of life outside the shell, and ALP is a ubiquitous enzyme found in nearly every tissue of the body that indicates intestinal maturation (Moog, 1950). The chicks were decapitated and the intestine was exposed and dissected using the following protocol. The jejunum was dissected from the pancreas to Meckel's diverticulum. Each segment of the intestine was weighed; the unstretched length was measured and immediately frozen in physiological saline (-22°C). Each segment was assayed for both specific and total maltase and ALP activity using the procedures of Black (1978). The entire length of each jejunum was used in the assay. Intestinal activity was expressed per unit of protein and per jejunum.

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 hourly to maintain the desired oxygen level.

Eggs were incubated in one machine until the beginning

Table 2: Jejunal weight and length 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[2]	High G[2]	Means
	Weight (mg)				
36	235	188	333	295	262[b]
37	210	199	337	268	253[b]
38	227	185	446	330	297[a]
39	166	184	352	328	258[ab]
Day mean	199[b]		336[a]		
Line mean	288[a]	247[b]			
Overall mean ± SEM		266 ± 7			
Probability	Temperature	0.05			
	Line (L)	0.003			
	Day (D)	0.0001			
	T x L	NS			
	T x D	NS			
	L x D	NS			
	T x L x D	NS			
	Relative weight (%)				
36	0.61	0.49	0.84	0.78	0.67[b]
37	0.53	0.52	0.82	0.69	0.64[c]
38	0.59	0.49	1.09	0.83	0.75[a]
39	0.46	0.49	0.91	0.84	0.68[b]
Day mean	0.52[b]		0.85[a]		
Line mean	0.73[a]	0.64[b]			
Overall mean ± SEM		0.68 ± 0.01			
Probabilities	Temperature	0.05			
	Line (L)	0.01			
	Day (D)	0.0001			
	T x L	NS			
	T x D	NS			
	L x D	NS			
	T x L x D	NS			

[1]Degrees centigrade inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

of the 18th day of development, candled to determine viability and selected as described in Experiment 1. Embryo intestines were collected and analyzed as in Experiment 1.

Temperature and oxygen: The most extreme temperatures (36º and 39º 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 28 days in an incubator when viable embryos were assigned randomly to one of the four cabinets. The conditions were 36º C or 39º C with 17 or 23% oxygen in a factorial arrangement. Embryos or hatchlings were sampled identically as described in the previous experiments.

Statistical analysis: Data for all three experiments were analyzed using the general linear models procedure (SAS Inc., 1998). Experiments 1 and 2 were analyzed as four levels of temperature or oxygen treatments by two lines factorial. In Experiment 3, the data were analyzed as two temperatures by two oxygen concentrations by two lines factorial arrangement. Means determined to differ significantly were separated by the least square means procedure. All means given in tables are least square means. All possible main and interaction effects were tested for significance. 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. Temperatures greater than 37°C increased jejunum weight and Low G chicks had consistently heavier jejuna than did High chicks (Table 2). Temperatures greater than 37°C also increased jejuna length compared with cooler temperatures (data not shown). Intestinal maltase was analyzed as specific and total activities. Specific

Table 3: Jejunal maltase activity 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[2]	High G[2]	Mean
	Specific activity (µmol glucose/h/µg of protein)				
36	2.8	2.4	6.5	7.4	
37	4.4	4.8	6.9	4.8	
38	1.8	1.6	13.0	8.1	
39	2.4	1.4	7.8	8.2	
T x D Means	Temperature	Day 20	Day 21		
	36	2.6^{ef}	7.0^{bc}		
	37	4.6^{de}	5.9^{cd}		
	38	1.7^{f}	10.5^{a}		
	39	1.9^{f}	8.0^{b}		
Overal mean ± SEM		5.1 ± 0.3			
Probability	Temperature	NS			
	Line (L)	NS			
	Day (D)	0.0001			
	T x L	NS			
	T x D	0.001			
	L x D	NS			
	T x L x D	NS			
	Total activity (µmol glucose/h /jejunum)				
36	49.2^{e}	33.6^{f}	127.6^{b}	137.0^{b}	
37	70.7^{d}	71.7^{d}	106.3^{c}	76.2^{d}	
38	30.0^{f}	23.2^{g}	419.7^{a}	177.2^{b}	
39	46.5^{e}	19.6^{g}	171.0^{b}	165.6^{b}	
Mean					
Overall mean ± SEM		99.4 ± 8.0			
Probabilities	Temperature	NS			
	Line (L)	NS			
	Day (D)	0.0001			
	T x L	NS			
	T x D	0.0001			
	L x D	NS			
	T x L x D	0.04			

[1]Degrees centigrade inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

maltase activity displayed a temperature by day of development interaction (Table 3) at 20 days temperatures lower than 37°C increased specific activity compared to greater temperatures, but at 21 days, temperatures lower than 37°C decreased specific maltase activity compared to greater temperatures. A significant line by temperature by day of development interaction occurred for total maltase. The same pattern seen for intestine functional development differed between Low and High G lines. Embryos and chicks in the High G line exhibited an exaggerated pattern of increase in total maltase activity from 20 to 21 days compared to that of Low G embryos and chicks. Jejunal ALP is also reported as specific and total activities. Specific ALP activity showed no significant effect, but total ALP activity displayed temperature by line and temperature by day interactions (Table 4). The Low G line increased total ALP activity to a greater extent in response to higher temperatures than did High G line. No response to temperature was noted in total ALP at day 20, but at day 21 total ALP activity increased as temperature increased.

Oxygen: Oxygen increased BW of both lines as the fractional concentration increased above 21% (Table 5), and Low G embryos and chicks were heavier than High. Significant oxygen by line as well as significant line by day interactions were seen in yolk weights. At day 20 oxygen concentrations greater than 21% increased the utilization of yolk by the embryo, but all embryos had used the same amount of yolk by hatching. Low G embryos and hatchlings had significantly more residual yolk at both 20 and 21 days of development than High. Both absolute and relative intestine weights showed oxygen by line and line by day interactions (Table 6). Oxygen greater than 21% increased the growth of intestine in Low G line embryos and chicks and only 19% oxygen increased the growth of High G line embryos. Oxygen concentration of 17% depressed jejunum weight at both 20 and 21 days of development,

Table 4: Jejunal alkaline phosphatase activity 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[2]	High G[2]	Mean
	Specific activity (µmol phosphorus/h/µg of protein)				
36	0.40	0.39	0.60	0.70	
37	0.46	0.43	0.77	0.63	
38	0.49	0.43	0.77	0.72	
39	0.36	0.35	0.77	0.80	
Day mean	0.40[b]		0.74[a]		
Overall mean ± SEM		0.56 ± 0.02			
Probability	Temperature	NS			
	Line (L)	NS			
	Day (D)	0.0001			
	T x L	NS			
	T x D	NS			
	L x D	NS			
	T x L x D	NS			
	Total activity (µmol phosphorus/h /jejunum)				
Temperature	Low G		High G		
36	6,238[de]		12,520[b]		
37	7,007[d]		11,742[c]		
38	6,811[d]		22,487[a]		
39	5,811[e]		15,478[b]		
T x D means	Temperature	Day 20	Day 21		
	36	6,238[c]	12,520[b]		
	37	7,007[c]	11,742[b]		
	38	6,811[c]	22,487[a]		
	39	5,811[c]	15,478[b]		
Overall mean ± SEM		10,542 ± 593			
Probability	Temperature	0.02			
	Line (L)	0.03			
	Day (D)	0.0001			
	T x L	0.05			
	T x D	0.02			
	L x D	NS			
	T x L x D	NS			

[1]Degrees centigrade inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

but 19% oxygen increased the weight of the intestine between 20 and 21 days to a greater extent than all other treatments. Oxygen concentrations less than 21% hindered intestine growth between 20 and 21 days. Jejunum was longer in the 23% oxygen environment than in all other. Specific and total maltase activity indicated significant oxygen by day by line interactions (Table 7). At 20 days, no differences were noted, but at day 21 23% oxygen increased maltase activity in Low G chicks to a greater extent than it increased in High G chicks. The remaining oxygen concentrations had no effect on maltase activity. Specific and total ALP activities also displayed similar oxygen by day and line by day interactions (Table 8). Greater oxygen concentrations increased ALP activities to a greater extent on day 21 than on day 20, and Low G chicks increased ALP activity to a greater extent on day 21 than did High G chicks.

Temperature and Oxygen. When examined together, temperature and oxygen had similar but independent effects on BW (Table 9). Oxygen concentration of 23% increased BW compared to 17% and 39° C depressed BW compared to 36° C. Residual yolk weights indicated a significant temperature by oxygen interaction at day 20. An environment of 39°C and 17% oxygen increased residual yolk in embryos compared to all other treatment combinations. At 21 days both oxygen and temperature interacted with line to affect residual yolk. Low G chicks at 17% oxygen had more residual yolk than High G chicks at 17%, but they did not differ in 23% oxygen. Low G chicks at 39° also had more residual yolk that all other treatment combinations. High temperature depressed intestine growth at 20 days and interacted at 21 days such that Low G chicks increased intestine weights in 36° compared to 39° C to a greater extent than did High G chicks (Table 7). The 36° C temperature increased specific maltase activity in all chicks at days 20 and 21 of development, and 23% oxygen increased specific maltase activity at day 20 compared to 17%. Similar results were seen for total maltase activity with an additional significant line effect at day 20 when High G

Table 5: Body and yolk weights (g) of chick embryos from two lines incubated at four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation				
	20		21		
Oxygen[1]	Low G[2]	High G[2]	Low G[2]	High G[2]	Oxygen mean
	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 mean	37.9[b]		39.5[a]		
Line mean	39.7[a]	37.8[b]			
Overall mean ± SEM		38.7±0.3			
Probability	Oxygen	0.001			
	Line (L)	0.0001			
	Day (D)	0.0001			
	O x L	NS			
	O x D	NS			
	L x D	NS			
	O x L x D	NS			
	Yolk				
17	10.3[a]		8.6[bc]		
19	10.5[a]		7.5[d]		
21	9.3[b]		8.2[cd]		
23	8.7[bc]		7.8[d]		
L x D means	Line	Day 20	Day 21		
	Low G	11.0[a]	8.4[b]		
	High G	8.7[b]	7.3[c]		
Overall mean ± SEM		8.9±0.2			
Probability	Oxygen	0.03			
	Line (L)	0.0001			
	Day (D)	0.0001			
	O x L	0.05			
	O x D	NS			
	L x D	0.04			
	O x L x D	NS			

[1]Percentage oxygen inside incubator. 2High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. a,bMeans with different superscripts differ significantly ($P < 0.05$).

embryos increased total maltase to a greater extent than did Low G embryos. Specific and total ALP activities displayed the same differences (Table 8). Increasing temperature depressed ALP activity at day 20 in all embryos, and High G embryos showed greater ALP activity than did Low G until day 21 when Low G had greater activity than did High G chicks.

Discussion

The hypothesis tested in the current study was that environmental conditions during the plateau stage in oxygen consumption affect embryo well-being. 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 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), intestinal weight and function (Christensen et al, 2003a). Increased adrenal cortical hormones may prolong the developmental period (Hayward *et al.*, 2006). Data from the current study show clearly that elevated temperature and depressed oxygen in the incubator at the plateau stage deter the process of intestinal maturation and preparation for life outside of the shell. Low G type broilers responded differently than did High G type broilers supporting a possible link between the selection for rapid growth and embryo development.

Thyroid and adrenal hormones play major roles at the plateau in maturation of intestines (Black, 1978) and improved neonate survival (Davis and Siopes, 1989; Christensen *et al.*, 2003b). In a prior study we showed that conditions similar to those in the current study affected plasma concentrations of thyroid hormones

Table 6: Jejunal weight and length of chick embryos from two lines incubated at four oxygen partial pressure during the plateau stage in oxygen consumption

	Day of incubation				
	20		21		
Oxygen[1]	Low G[2]	High G[2]	Low G[2]	High G[2]	Oxygen mean
O x L means					
17	234[d]	246[d]			
19	272[abc]	286[ab]			
21	293[a]	254[c]			
23	290[ab]	264[bc]			
LxD means	Line	Day 20	Day 21		
	Low G	209[c]	335[a]		
	High G	216[c]	309[b]		
Overall mean±SEM		267 ± 5			
Probability	Oxygen	0.001			
	Line (L)	NS			
	Day (D)	0.0001			
	O x L	0.03			
	O x D	NS			
	L x D	0.03			
	O x L x D	NS			
	Relative weight (%)				
O x L means					
17	0.61[c]	0.67[bc]			
19	0.69[b]	0.77[a]			
21	0.72[a]	0.65[bc]			
23	0.72[a]	0.68[b]			
L x D means	Line	Day 20	Day 21		
	Low G	0.53[c]	0.83[a]		
	High G	0.59[b]	0.80[a]		
Overall mean±SEM		0.69±0.01			
Probability	Oxygen	0.01			
	Line (L)	NS			
	Day (D)	0.0001			
	O x L	0.03			
	O x D	NS			
	L x D	0.03			
	O x L x D	NS			

[1]Percentage oxygen inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly (P < 0.05).

Table 7: Jejunal maltase activity of chick embryos from two lines incubated at four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation			
	20		21	
Oxygen[1]	Low G[2]	High G[2]	Low G[2]	High G[2]
	Specific activity (µmol of glucose/h/µg of protein)			
17	5.3[d]	6.2[d]	9.6[c]	8.8[c]
19	4.6[d]	5.9[d]	8.4[c]	8.6[c]
21	6.5[d]	5.7[d]	9.1[c]	8.6[c]
23	4.8[d]	6.0[d]	19.4[a]	13.3[b]
Overall mean ± SEM		8.2 ± 0.3		
Probability	Oxygen	0.0001		
	Line (L)	NS		
	Day (D)	0.0001		
	O x L	0.07		
	O x D	0.0001		
	L x D	0.007		
	O x L x D	0.02		
	Total activity (µmol of glucose/h/jejunum)			
17	51.4[ef]	61.2[e]	103.8[c]	93.1[d]
19	50.1[ef]	68.2[e]	112.5[bc]	124.0[b]
21	69.9[e]	62.0[e]	117.9[bc]	92.4[d]
23	44.9[f]	57.5[ef]	216.0[a]	130.5[b]
√				
Overall mean ± SEM		90.8 ± 6.3		
Probability	Oxygen	0.0005		
	Line (L)	NS		
	Day (D)	0.0001		
	O x L	0.02		
	O x D	0.0001		
	L x D	0.003		
	O x L x D	0.03		

[1]Percentage oxygen inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly (P < 0.05).

Table 8: Jejunal alkaline phosphatase activity of chick embryos from two lines incubated at four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation			
	20		21	
Oxygen[1]	Low G[2]	High G[2]	Low G[2]	High G[2]
	Specific activity (µmol of phosphorus/h /µg of protein)			
17	0.51[e]		1.49[c]	
19	0.56[e]		1.39[c]	
21	0.67[de]		1.68[b]	
23	0.78[d]		2.17[a]	
L x D means	Line	Day 20	Day 21	
	Low G			
	High G			
Overall mean± EM		1.15 ± 0.03		
Probability	Oxygen	0.0001		
	Line (L)	0.03		
	Day (D)	0.0001		
	O x L	NS		
	O x D	0.05		
	L x D	0.003		
	O x L x D	NS		
	Total activity (µmol of phosphorus/h/jejunum)			
17	4,796[e]		15,355[c]	
19	6,203[de]		18,780[b]	
21	6,935[d]		19,714[b]	
23	7,454[d]		22.753[a]	
L x D means	Line	Day 20	Day 21	
	Low G	6,278[c]	20,864[a]	
	High G	6,416[c]	17,438[b]	
Overall mean ± SEM		12,712 ± 420		
Probability	Oxygen	0.0001		
	Line (L)	0.007		
	Day (D)	0.0001		
	O x L	NS		
	O x D	0.05		
	L x D	0.003		
	O x L x D	NS		

[1]Percentage oxygen inside incubator. [2]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

(Christensen *et al.*, 2005). The current study indicates that such alterations may also deter intestinal maturation.

Temperature: Data indicated that temperatures greater than 37°C slowed yolk-free body growth, but also spared residual yolk. Thus, although chicks may appear to be heavy at hatching at high temperatures, more residual yolk mass was responsible for the difference and not tissue mass. Temperatures above 37°C also depressed jejunum weight and function as measured by maltase and ALP activities. Elevated temperatures also suppressed thyroid hormone concentrations at the plateau stage (unpublished data). Thus, we conclude that incubator temperatures greater than 37°C at the plateau stage in oxygen consumption depress intestinal weight and yolk utilization. The actions of temperature on thyroid hormones and intestinal maturation seem to be related. Temperature-related jejunum growth effects on Low G embryos and chicks were more pronounced than they were on High G perhaps indicating greater temperature sensitivity for those embryos. Also, the maltase and ALP activities were both increasing as embryos matured, but ALP was increasing more rapidly in Low than High G embryos in response to high temperatures.

Oxygen: In contrast to increased temperatures, increased concentrations of oxygen in the incubators increased embryo weights and nutrient utilization. Thus, increased oxygen concentrations greater than 21% enhance both growth and nutrient utilization during the plateau stage. Greater oxygen concentrations increased jejunum weights faster in Low G broiler embryos than in High G embryos, but when oxygen was examined in the presence of higher temperatures; its effects on jejunum growth were minimized. Thus, the concentration of oxygen in an incubator at the plateau stage has minimal effect on intestinal tissue unless the line of broiler has been selected for high yields. From these data, it can be inferred that a minimum concentration of 21% oxygen is

Table 9: Body and yolk 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

		Day of incubation			
		20		21	
Temperature[1]	Oxygen[2]	Low G[3]	High G[3]	Low G[3]	High G[3]
		Body weight without yolk			
36	17	37.4	38.5	38.0	38.2
	23	38.4	38.4	40.0	37.9
	Mean	38.2[a]			
39	17	34.8	35.9	37.1	37.1
	23	37.2	36.6	38.3	38.8
	Mean	36.1[b]			
Oxygen means		17% = 36.7[b]	23% = 37.6[a]	17% = 37.6[b]	23% = 38.8[a]
Overall mean ± SEM		37.2 ± 0.2		38.2 ± 0.3	
Probabilities	Temperature	0.0003		NS	
	Oxygen (O)	0.05		0.05	
	Line (L)	NS		NS	
	T x O	NS		NS	
	T x L	NS		NS	
	O x D	NS		NS	
	T x O x L	NS		NS	
		Yolk			
36	17	10.8	10.9	7.8	7.7
	23	11.8	8.6	7.6	9.1
	√				
39	17	13.8	10.9	10.3	8.2
	23	11.0	9.5	8.9	8.3
Line means					
T x O means	Oxygen	36 C	39 C		
	17%	10.8[b]	13.3[a]		
	23%	10.2[b]	10.2[b]		
O x L means			Oxygen	Low G	C
			17%	9.0[a]	7.9[b]
			23%	8.2[ab]	8.7[ab]
T x L means			Temperature		
			36 C	7.6[b]	8.4[b]
			39 C	9.6[a]	8.3[b]
Overall mean ± SEM		11.1 ± 0.2		8.5 ± 0.2	
Probabilities	Temperature	0.01		0.05	
	Oxygen (O)	0.0006		NS	
	Line (L)	0.01		NS	
	T x O	0.02		NS	
	T x L	NS		0.02	
	O x D	NS		0.05	
	T x O x L	NS		NS	

[1]Degrees centigrade inside incubator. [2]Percentage oxygen inside incubator. [3]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

required to maximize embryo growth, but jejunum growth can probably be sustained with 19% oxygen in the cabinet. The effects of low oxygen on these measurements parallel circulating thyroid hormone concentrations (Christensen *et al.*, 2005). We speculate that this may be a physiological mechanism preparing the bird for life outside the shell. If the embryo is stressed at hatching, one of its greatest needs following hatching is a readily available source of carbohydrate (Christensen and Donaldson, 1991). Under such conditions, a functional intestine would be requisite to supply glucose for metabolism.

Jejunal maltase and ALP activities were enhanced by increasing concentrations of oxygen. The enhancement at 23% was greater in the Low than High G embryos suggesting a preparation for readily available carbohydrate in the line immediately following hatching. The preparation may indicate a greater preparation by Low G embryos for growth and readily available carbohydrate following the hypoxia of the plateau stage (Donaldson and Christensen, 1991). Because of the increased ALP activity, we can also conclude that the intestinal tissue in Low G chicks is much more metabolically active than that in High G chicks. Thus, we conclude that optimal oxygen concentration for maltase and ALP activities is dependent upon the genetic predisposition of the chick for growth. Evidence in the current study suggests that for optimal maltase and ALP the concentration during the plateau stage not be less than 21%. Concentrations less than that may cause metabolic stress that prohibits the embryo from developing both growth and function of intestinal tissue.

Table 10: Jejunal weights of embryos and chicks from two lines incubated at two temperatures and two oxygen partial pressures during the plateau stage in oxygen consumption

		Day of incubation			
		20		21	
Temperature[1]	Oxygen[2]	Low G[3]	High G[3]	Low G[3]	High G[3]
		Absolute weight (mg)			
36	17	225	226	381	322
	23	220	231	356	316
	Mean	226[a]		344[a]	
39	17	196	163	233	273
	23	175	188	257	273
	Mean	180[b]		259[b]	
T x L means			Temperature	High Yield	High G
			36 C	368[a]	319[b]
			39 C	245[c]	272[c]
Overall mean ± SEM		203 ± 9		301 ± 6	
Probability	Temperature	0.01		0.0001	
	Oxygen (O)	NS		NS	
	Line (L)	NS		NS	
	T x O	NS		NS	
	T x L	NS		0.003	
	O x L	NS		NS	
	T x O x L	NS		NS	
		Relative weight (%)			
36	17	0.60	0.58	1.00	0.84
	23	0.57	0.60	0.89	0.84
	√				
39	17	0.56	0.45	0.63	0.73
	23	0.47	0.52	0.67	0.70
T x L means			Temperature	High Yield	High G
			36 C	0.95[a]	0.84[b]
			39 C	0.65[c]	0.71[c]
Overall mean ± SEM		0.54 ± 0.02		0.79 ± 0.01	
Probability	Temperature	0.06		0.0001	
	Oxygen (O)	NS		NS	
	Line (L)	NS		NS	
	T x O	NS		NS	
	T x L	NS		0.01	
	O x D	NS		NS	
	T x O x L	NS		NS	

[1]Degrees centigrade inside incubator. [2]Percentage oxygen inside incubator. [3]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly ($P < 0.05$).

Table 11: Jejunal maltase activity of chick embryos from two lines incubated at two temperatures and two oxygen partial pressures during the plateau stage in oxygen consumption

		Day of incubation			
		20		21	
Temperature[1]	Oxygen[2]	Low G[3]	High G[3]	Low G[3]	High G[3]
		Specific activity (µmol of glucose/h/µg of protein)			
36	17	3.3	4.3	7.4	6.5
	23	3.9	4.9	8.0	6.9
Mean		4.1[a]		7.2[a]	
39	17	2.0	2.1	2.8	3.3
	23	2.7	3.0	2.2	1.8
Mean		2.4[b]		2.5[b]	
Oxygen means		17% = 2.9[b]	23% = 3.6[a]		
Overall mean ± SEM		3.3 ± 0.1		4.9 ± 0.2	
Probability	Temperature	0.0001		0.0001	
	Oxygen (O)	0.02		NS	
	Line (L)	NS		NS	
	T x O	NS		NS	
	T x L	NS		NS	
	O x L	NS		NS	
	T x O x L	NS		NS	

(Table 11 cont.)

		Day of incubation			
		20		21	
Temperature[1]	Oxygen[2]	Low G[3]	High G[3]	Low G[3]	High G[3]
		Total activity (µmol of glucose/h/jejunum)			
36	17	42.9	20.3	143.7	104.7
	23	53.5	71.4	132.1	117.6
Mean		53.5[a]		124.5[a]	
39	17	15.3	20.3	44.3	59.2
	23	28.7	37.0	35.4	28.7
Mean		25.3[b]		41.9[b]	
Oxygen means		17% = 34.7[b]	23% = 47.7[a]		
Line means		HY = 35.1[b]	C = 47.3[a]		
Overall mean ± SEM		41.5 ± 2.5		83.2 ± 5.0	
Probability	Temperature	0.0001		0.0001	
	Oxygen (O)	0.02		NS	
	Line (L)	0.02		NS	
	T x O	NS		NS	
	T x L	NS		NS	
	O x L	NS		NS	
	T x O x L	NS		NS	

[1]Degrees centigrade inside incubator. [2]Percentage oxygen inside incubator. [3]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly (P < 0.05).

Table 12: Jejunal alkaline phosphatase activity of chick embryos from two lines incubated at two temperatures and two oxygen partial pressures during the plateau stage in oxygen consumption

		Day of incubation			
		20		21	
Temperature[1]	Oxygen[2]	Low G[3]	High G[3]	Low G[3]	High G[3]
		Specific activity (µmol of phosphorus/h/µg of protein)			
36	17	0.48	0.60	0.80	0.63
	23	0.61	0.56	1.08	0.61
Mean		0.56[a]			
39	17	0.26	0.46	0.83	0.61
	23	0.35	0.53	0.67	0.65
Mean		0.40[b]			
Line means	Low G	0.43[b]		0.84[a]	
	High G	0.54[a]		0.63[b]	
Overall mean ± SEM		0.49 ± 0.02		0.74 ± 0.04	
Probability	Temperature	0.0006		NS	
	Oxygen (O)	NS		NS	
	Line (L)	0.01		0.01	
	T x O	NS		NS	
	T x L	NS		NS	
	O x L	NS		NS	
	T x O x L	NS		NS	
		Total activity (µmol of phosphorus/h/jejunum)			
36	17	6,319	9,104	15,325	10,987
	23	8,115	8,017	18,065	10,289
Mean		7,889[a]			
39	17	2,070	4,732	12,900	10,741
	23	3,738	6,605	10.394	10,231
Mean		4,286[b]			
Line means	Low G	5,060[b]		14,171[a]	
	High G	7,115[a]		10,562[b]	
Overall mean ± SEM		6,138 ± 399		12,367 ± 776	
Probability	Temperature	0.0001		NS	
	Oxygen (O)	NS		NS	
	Line (L)	0.01		0.02	
	T x O	NS		NS	
	T x L	NS		NS	
	O x L	NS		NS	
	T x O x L	NS		NS	

[1]Degrees centigrade inside incubator. [2]Percentage oxygen inside incubator. [3]High G = line of broiler with high eggshell conductance (G); Low G = line of broilers with low eggshell conductance. [a,b]Means with different superscripts differ significantly (P < 0.05).

Temperature and oxygen: Temperature and oxygen interacted only when embryos at the plateau stage were placed in 17% oxygen at 39°C. Under those conditions, embryos used less yolk at the plateau stage. Otherwise, no interactions affected intestinal physiology in the current study. Lower incubation temperature increased jejunum weight as had been seen in the prior experiment and greater concentrations of oxygen increased by jejunum weight and length. Therefore, it is clear that the incubator temperature exerts the primary influence on embryonic jejunum growth in chick embryos at the plateau. High temperature at the plateau stage depressed intestinal growth throughout the hatching process and had a greater effect on Low G chicks than on High chicks.

In a prior study egg weight, eggshell conductance and the length of the incubation period interacted to affect intestinal growth and function (Christensen *et al.,* 2003a). The timing of the plateau stage in oxygen consumption is a function of the three egg measurements (Ar and Rahn, 1978), but the conditions of the plateau can become a life-threatening situation if environmental conditions surrounding the egg are not controlled (Dietz *et al.,* 1998). Evidence from the current study suggests the conditions may be even more critical with the Low G lines of broilers than with the High G lines. The data indicate separate actions of incubator temperature and oxygen concentration on intestinal maturation at the plateau stage. The plateau stage can delay intestinal development especially of Low G chicks if temperatures exceed 38° C or if oxygen concentrations decline below 21%. Thus, at days 18 to 21 of incubation, temperatures greater than 38°C and oxygen concentrations of less than 21% should be avoided.

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