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Incubator Temperature and Oxygen Concentration at the Plateau Stage Affect Cardiac Health of Turkey Embryos[1]

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Abstract: The plateau stage in oxygen consumption of turkey embryos occurs at 25 and 26 days of incubation and most embryonic deaths occur at that time (Rahn, 1981 and Christensen *et al.*, 2003). At the plateau stage, vital gas diffusion through the shell is insufficient for oxygen to drive metabolism or for expelling the metabolic by products of water vapor and carbon dioxide. The objective of the experiments reported here was to determine the effect of environmental temperature and oxygen concentration during hypoxia and hypercapnia of the plateau stage on turkey embryo cardiac health. Three experiments were conducted. In Experiment 1, turkey embryos at the plateau stage were exposed to 36, 37, 38 or 39°C. In Experiment 2, embryos at the plateau stage were exposed to 17, 19, 21 or 23% oxygen concentrations, and in Experiment 3, the highest and lowest levels of temperature and oxygen treatments were combined to determine interaction effects on cardiac physiology. Temperatures greater than 37 C depressed heart weight but not BW and decreased cardiac tissue energy metabolism. Oxygen concentrations greater than 21% increased BW and improved the cardiac glycogen to lactate ratio with no effect on heart weight. When examined together, the two factors interacted to affect cardiac energy metabolism. It was concluded that the physiologic action of oxygen during the plateau stage favored BW whereas temperature affected cardiac tissue but not BW. Temperature and oxygen interact during the plateau to affect cardiac muscle energy metabolism.

Key words: Plateau stage, oxygen, temperature, and turkey embryo

Introduction

Physiological responses by turkey embryos to environmental conditions are not well understood. When a portion of a chicken eggshell was covered with an impermeable plastic membrane, growth was retarded (Metcalfe *et al.*, 1981 and McCutcheon *et al.*, 1982). McCutcheon *et al.* (1982) also observed a significant reduction in chick embryo heart and liver weights when eggshells were covered. High altitude slows the growth of chick and poult embryos (Smith *et al.*, 1969; Bagley and Christensen, 1989), and Stock and Metcalfe (1984) suggested that metabolism is depressed by low oxygen pressure; thus embryonic growth would be limited normally by the availability of oxygen. Metabolic rate increases with increasing temperature in both chicken and duck embryos (Janke *et al.*, 2002). Increased incubation temperature is also stressful at almost all ages to developing embryos and limits growth and increases mortality (French, 2000), but some data suggest that high temperature may be more stressful at the end of incubation (Ande and Wilson, 1981).

Tissue oxygen requirements in late developing embryos from all precocial species exceed the eggshell functional properties (Rahn, 1981 and Dietz *et al.*, 1998). Vital gas diffusion late in incubation is insufficient to provide adequate oxygen to the embryo and ventilate metabolic by products. This time is called the plateau stage in oxygen consumption. The plateau in metabolic rate creates a paradox for the embryo because growth and maturation must continue (Dietz *et al.*, 1998) with little or no oxygen. Turkey embryo cardiac tissue may be assisted during the plateau by increased thyroid output (Nobukuni *et al.*, 1987), anaerobic metabolism or an increase in the size of the heart (Christensen *et al.*, 2003).

Few if any data exist reporting the range of temperature and oxygen concentrations during the plateau stage resulting in optimal cardiac health. Therefore, the hypothesis tested was temperatures and oxygen concentrations in incubators during the plateau might affect the turkey embryo heart.

Materials and Methods

Experimental incubation cabinets simulating commercial incubators were manufactured and used to control ambient temperature and 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 desired gaseous concentrations.

Experiment 1--Temperature: Fertilized turkey eggs from a commercial strain of turkey breeders were obtained on

the day of oviposition and incubated until the 25th day when they were candled to determine embryo viability. The 25th day of incubation for turkeys is the beginning of the plateau stage for that species (Rahn, 1981). Following candling and removal of nonviable embryos, randomly selected viable embryos were transferred to one of the four experimental cabinets. Each cabinet operated at one of the treatment temperatures normally found in incubators (36, 37, 38 or 39°C). Blood samples were collected into vials containing 10 mg of EDTA at external pipping (EP) (approximately 26 to 27 d of incubation) and hatching (28 d of incubation). The heart and liver were quickly excised (< 60 sec). The blood was placed immediately on ice and heart and liver tissues were placed into cold 7% perchloric. Plasma was obtained by centrifugation under refrigeration (4°C) at 700 x g for 20 min. The plasma was decanted, frozen (-22°C) and stored for later analysis. Ten embryos were sampled per treatment at each stage of development. Each cabinet was operated at one of the treatment temperatures of 36, 37, 38 or 39°C.

Plasma glucose was assayed using glucose oxidase (Donaldson and Christensen, 1991). Heart and liver tissue glycogen and lactate concentrations were analyzed as described by Christensen *et al.* (2003).

Experiment 2--Oxygen: Four oxygen concentrations (17, 19, 21 and 23%) were the treatments in the second experiment. The fractional concentrations at sea level (Raleigh, NC) corresponded to oxygen partial pressures of 129, 144, 160 and 175 mm Hg. Concentrations lower than ambient oxygen concentration (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 using an oxygen meter and the flow rates from adjacent oxygen or nitrogen storage tanks were adjusted at hourly intervals to maintain the desired oxygen level.

Eggs were incubated as in Experiment 1 until the beginning of the 25th day of development, then candled and selected. Embryo tissue samples were collected and analyzed identically as in Experimental 1.

Experiment 3-Temperature and Oxygen: The most extreme temperatures (36 and 39°C) and oxygen (17 and 23%) levels in the previous experiments were combined in a factorial arrangement for Experiment 3. The incubator temperatures and oxygen concentrations were arranged as a 2 x 2 factorial. All treatments were maintained identically as described in the previous experiments. Fertilized eggs were again incubated 25 d in an incubator when viable embryos were assigned randomly to one of the four cabinets. The conditions were 36°C with 17 or 23% oxygen or 39°C with 17 or 23% oxygen. 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 Inst. 1998). Experiments 1 and 2 were analyzed as four levels of oxygen or temperature treatment. In Experiment 3, the data were analyzed as two temperatures by two oxygen concentrations factorial design. 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

Experiment 1--Temperature: Temperature had no effect on BW, but 39°C compared to 36 and 37, but not 38°C, increased the amount of residual yolk (Table 1). At EP the 36°C temperature increased heart weight compared to all other temperatures, but at hatching all heart weights were equivalent (Table 2). Liver weights at EP were equivalent, but at hatching the exposure to 37 or 39°C increased liver weights compared to 36 and 38°C treatments. Liver weights at 36°C were heavier than those at 38°C.

The lowest temperature (36°C) increased cardiac glycogen concentration at EP but not at hatching compared to all other treatments. Temperature had no effect on cardiac lactate concentrations (Table 3). Because glycogen was increased, the ratio of glycogen to lactate in cardiac tissue also displayed a temperature by day interaction. The ratio in EP embryos exposed to 36°C was increased whereas that in embryos exposed to 39°C was depressed compared to 37 or 38°C. No differences were noted in hatched poults.

At EP, hepatic glycogen in embryos at 36°C was greater than all other treatments, but no response was noted in hatchlings (Table 4). At 36°C, EP and hatching embryos reduced hepatic lactate concentrations compared other treatments. The 36°C temperature increased the glycogen to lactate ratio in EP embryos, but had no effect on hatchling ratios.

The 39°C treatment increased plasma glucose concentrations compared to all other temperatures at both developmental stages, while 36 and 38°C depressed glucose concentrations compared to the other treatments (Table 5). The 37°C embryos were intermediate.

Experiment 2--Oxygen: Significant oxygen by day interactions were seen for BW with yolk, BW without yolk and

Table 1: Body and yolk weights (g) of turkey embryos incubated at four temperatures during the plateau stage in oxygen consumption

Temperature (C)	Day of incubation		
	27	28	√
		Body weight with yolk	
36	64.7	63.5	64.1
37	64.8	62.7	63.8
38	67.4	61.5	64.4
39	70.2	64.6	67.4
√	66.8[a]	63.1[b]	
× ± SEM	64.9 ± 0.7		
Probabilities	Temperature (T)	NS	
	Day (D)	0.006	
	T x D	NS	
		Body weight without yolk	
36	52.9	54.2	53.5
37	53.6	53.3	53.5
38	54.0	51.5	52.8
39	56.1	53.8	54.9
√	54.1	53.2	
x ± SEM	53.7 ± 0.5		
Probabilities	Temperature (T)	NS	
	Day (D)	NS	
	T x D	NS	
		Yolk	
36	11.8	9.3	10.6[b]
37	11.2	9.4	10.3[b]
38	13.4	10.0	11.7[a]
39	14.2	10.9	12.5[a]
√	10.5[a]	7.6[b]	
√ ± SEM	11.3 ± 0.2		
Probabilities	Temperature (T)	0.001	
	Day (D)	0.0001	
	T x D	NS	

Table 2: Organ weights (mg) of turkey embryos incubated in four temperatures during the plateau stage in oxygen consumption

Temperature (C)	Day of incubation		
	27	28	√
		Heart weights	
36	307[a]	281[b]	
37	270[b]	285[ab]	
38	271[b]	265[bc]	
39	260[c]	275[b]	
√			
√ ± SEM	277 ± 4		
Probabilities	Temperature (T)	NS	
	Day (D)	0.05	
	T x D	0.004	
		Liver weights	
36	971[c]	1,161[b]	
37	971[c]	1,273[a]	
38	929[c]	1,076[c]	
39	972[c]	1,248[a]	
√			
√ ± SEM	1,075 ± 11		
Probabilities	Temperature (T)	0.001	
	Day (D)	0.0001	
	T x D	0.05	

Table 3: Cardiac glycogen and lactate concentrations (mg/g of wet tissue mass) of turkey embryos incubated in four temperatures during the plateau stage in oxygen consumption

	Day of incubation		
Temperature (C)	27	28	√
		Glycogen	
36	5.0[a]	2.7[cd]	
37	3.3[bc]	2.3[d]	
38	3.4[b]	2.4[d]	
39	2.5[d]	2.1[d]	
√			
√ ± SEM	3.0 ± 0.09		
Probabilities	Temperature (T)	0.0001	
	Day (D)	0.0001	
	T x D	0.007	
		Lactate	
36	0.81	1.17	0.99
37	0.93	1.09	1.01
38	0.82	1.22	1.02
39	0.99	1.26	1.12
√	0.89[b]	1.18[a]	
√ ± SEM	1.03 ± 0.02		
Probabilities	Temperature (T)	NS	
	Day (D)	0.0001	
	T x D	NS	
		Ratio (glycogen/lactate)	
36	6.14[a]	2.38[c]	
37	3.91[b]	2.09[c]	
38	4.68[b]	1.95[c]	
39	2.77[c]	1.76[c]	
√			
√ ± SEM	3.21 ± 0.11		
Probabilities	Temperature (T)	0.0004	
	Day (D)	0.0001	
	T x D	0.01	

residual yolk (Table 6). At EP 23% oxygen increased BW with yolk compared to all other treatments, but at hatching, 21% oxygen increased BW with yolk compared to all treatments. The BW without yolk at EP displayed a similar pattern, but at hatching only the 17% BW without yolk was smaller than the 19% with no other treatment differences. Residual yolk displayed the same interaction as was seen with the body weights, but the means differed conversely. At EP 17 and 19% had more yolk than 21 but not 23%, but at hatching 21 and 23% had more residual yolk that 17 or 19%.

Oxygen treatment did not affect heart weight in the experiment (Table 7). At EP, oxygen concentrations of 23% resulted in heavier livers than 19 or 17% and 21% was intermediate. At hatching, livers in poults at 19% oxygen were heavier than all other treatments.

Oxygen at EP and hatching resulted in greater cardiac glycogen concentrations at 17% oxygen than at other oxygen levels (Table 8). The 21 and 23% oxygen concentrations increased cardiac lactate compared to 17 or 19% at both stages. The 17% oxygen elevated cardiac glycogen to lactate ratios compared to all other treatments, and 19% had greater ratios than either 21 or 23%. The 21 and 23% values did not differ from each other.

Oxygen had no effect on hepatic glycogen at EP, but at hatching 23% oxygen elevated glycogen nearly 3-fold that observed at the other levels (Table 9). At EP, 23% oxygen increased hepatic lactate compared to 17% but did not affect lactate at hatching. Oxygen had no effect on the ratio of glycogen to lactate in hepatic tissue at EP, but at hatching, 23% increased the ratio compared to all other treatments.

Increased oxygen concentrations elevated plasma glucose concentrations (Table 10). The 21 and 23% oxygen had greater glucose concentrations than 17 or 19%.

Experiment 3-Temperature and Oxygen: Neither temperature nor oxygen affected BW with yolk (Table 11), however, when the yolk was removed, 23% oxygen increased BW compared to 17%. A temperature by day

Table 4: Hepatic glycogen and lactate concentrations (mg/g of wet tissue mass) of turkey embryos incubated in four temperatures during the plateau stage in oxygen consumption

	Day of incubation		
Temperature (C)	27	28	√
		Glycogen	
36	19.0[a]	5.8[bc]	
37	4.3[c]	10.0[b]	
38	6.2[bc]	8.4[b]	
39	4.2[c]	9.2[b]	
√			
√ ± SEM	8.1 ± 1.6		
Probabilities	Temperature (T)	0.02	
	Day (D)	NS	
	T x D	0.0001	
		Lactate	
36	0.15	0.20	0.17[b]
37	0.19	0.20	0.19[a]
38	0.19	0.21	0.20[a]
39	0.18	0.22	0.20[a]
√	0.17[b]	0.21[a]	
√ ± SEM	0.19 ± 0.004		
Probabilities	Temperature (T)	0.04	
	Day (D)	0.0005	
	T x D	NS	
		Ratio (glycogen/lactate)	
36	138.17[a]	31.11[b]	
37	25.8[b]	51.3[b]	
38	35.0[b]	40.2[b]	
39	25.5[b]	43.5[b]	
√			
√ ± SEM	48.8 ± 5.3		
Probabilities	Temperature (T)	0.003	
	Day (D)	NS	
	T x D	0.0001	

Table 5: Blood plasma glucose concentrations (mg/dL) in turkey embryos incubated at four temperatures during the plateau stage in oxygen consumption

	Day of incubation		
Temperature (C)	27	28	√
		Plasma glucose	
36	203	220	211[c]
37	228	236	232[b]
38	209	224	217[c]
39	242	256	248[a]
√	220[b]	234[a]	
√ ± SEM	227 ± 2		
Probabilities	Temperature (T)	0.0001	
	Day (D)	0.002	
	T x D	NS	

interaction was noted at EP when 36°C BW was greater than that at 39°C. No difference in BW was noted at hatching. Embryos in 17% oxygen exhibited more residual yolk than did 23%.

Temperature by day and oxygen by day interactions affected heart weights (Table 12). The 36°C treatment resulted in heavier hearts at EP and hatching than did 39°C, but 36°C poults increased heart weight nearly 30 g from 27 to 28 days of incubation whereas 39°C poults increased them only 7 g. The 36°C temperature and 23% oxygen increased liver weights compared to 39°C and 17%, respectively.

Table 6: Body and yolk sac weights (g) of turkey embryos incubated in four oxygen concentrations during the plateau stage in oxygen consumption

	Day of incubation		
Oxygen (%)	27	28	√
		Body weight with yolk sac	
17	59.4[b]	55.0[c]	
19	60.7[b]	57.4[c]	
21	59.3[b]	58.0[b]	
23	65.1[a]	56.7[c]	
√			
√ ± SEM	59.0 ± 0.5		
Probabilities	Oxygen (O)	0.07	
	Day (D)	0.0001	
	O x D	0.05	
		Body weight without yolk sac	
17	49.0[bc]	47.8[c]	
19	49.9[b]	50.9[b]	
21	49.6[b]	49.6[bc]	
23	54.2[a]	48.5[bc]	
√			
√ ± SEM	49.9 ± 0.4		
Probabilities	Oxygen (O)	0.06	
	Day (D)	0.06	
	O x D	0.01	
		Yolk sac	
17	10.4[ab]	7.2[d]	
19	10.8[a]	6.5[e]	
21	9.7[b]	8.4[c]	
23	10.8[a]	8.2[c]	
√			
√ ± SEM	9.0 ± 0.2		
Probabilities	Oxygen (O)	NS	
	Day (D)	0.0001	
	OxD	0.02	

Table 7: Heart and liver weights (mg) of turkey embryos incubated in four oxygen concentrations during the plateau stage in oxygen consumption

	Day of incubation		
Oxygen (%)	27	28	√
		Heart	
17	233	279	256
19	242	294	268
21	248	262	255
23	264	263	263
√	246[b]	274[a]	
√ ± SEM	260 ± 3		
Probabilities	Oxygen (O)	NS	
	Day (D)	0.0001	
	O x D	NS	
		Liver	
17	856[d]	1,106[b]	
19	871[d]	1,238[a]	
21	896[cd]	1,117[b]	
23	966[c]	1,109[b]	
√			
√ ± SEM	1,017 ± 11		
Probabilities	Oxygen (O)	NS	
	Day (D)	0.0001	
	O x D	0.008	

Table 8: Cardiac glycogen and lactate concentrations (mg/g of wet tissue mass) of turkey embryos incubated in four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation		
Oxygen (%)	27	28	√
		Glycogen	
17	4.4	3.3	3.9[a]
19	3.5	3.2	3.4[b]
21	3.3	2.9	3.1[b]
23	3.8	2.5	3.1[b]
√	3.8[a]	3.0[b]	
√ ± SEM	3.4 ± 0.1		
Probabilities	Oxygen (O)	0.006	
	Day (D)	0.0001	
	O x D	NS	
		Lactate	
17	0.93	0.82	0.88[c]
19	0.97	0.91	0.94[bc]
21	1.17	0.98	1.07[a]
23	1.01	0.97	0.99[ab]
√	1.01[a]	0.92[b]	
√ ± SEM	0.97 ± 0.02		
Probabilities	Oxygen (O)	0.007	
	Day (D)	0.02	
	O x D	NS	
		Ratio (glycogen/lactate)	
17	4.90	4.26	4.58[a]
19	3.87	3.74	3.80[b]
21	2.88	3.07	2.98[c]
23	3.80	2.61	3.20[c]
√	3.86[a]	3.42[b]	
√ ± SEM	3.64 ± 0.11		
Probabilities	Oxygen (O)	0.0001	
	Day (D)	0.05	
	O x D	NS	

Temperature and oxygen interacted such that at 36°C, 23% oxygen increased cardiac glycogen compared to 17%, but at 39°C, 17 and 23% were equivalent (Table 13). Oxygen and day interacted such that 23% increased glycogen at EP compared to 17%, but at hatching no differences were seen. Temperature and oxygen interacted such that at 39°C, 23% oxygen increased cardiac lactate compared to 17%, but at 36°C no differences were noted. Oxygen and day interacted at hatching in that 23% oxygen at hatching increased cardiac lactate concentrations compared to 17% but no differences were seen at EP. Temperature, oxygen and day interacted to change the glycogen to lactate ratios. At 27 d 36°C and 23% oxygen increased the ratio compared to all other treatments and 39°C and 17% oxygen depressed the ratio compared to all other treatments with 36°C and 17% oxygen and 39°C and 23% oxygen being intermediate. At hatching only the 36°C and 17% oxygen ratios were elevated compared to the 39°C and 23% oxygen ratios.

Temperature and day interacted to affect hepatic glycogen concentrations (Table 14). At EP, 39°C depressed hepatic glycogen concentrations compared to 36°C but had no effect at hatching. Temperature, oxygen and day interacted at EP so that 36°C in combination with 23% oxygen depressed hepatic lactate compared to all other treatments. At hatching 17% oxygen and 39°C depressed hepatic lactate concentrations compared to 23% and 36°C, respectively. Temperature and day interacted such that hepatic ratios of glycogen to lactate at EP 36°C increased the hepatic ratio compared to 39, but no effects were seen at hatching.

Temperature and day affected the plasma glucose concentrations (Table 15). At EP temperature and day had no effect on plasma glucose concentrations, but at hatching 39°C treated embryos displayed greater glucose concentrations than did 36°C. No other factors affected blood plasma glucose concentrations.

Discussion

The hypothesis tested in the current study was that environmental conditions in the incubator during the plateau stage in oxygen consumption might affect embryo cardiac health. 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

Table 9: Hepatic glycogen and lactate concentrations (mg/g of wet tissue mass) of turkey embryos incubated in four oxygen partial pressures during the plateau stage in oxygen consumption

	Day of incubation		
Oxygen (%)	27	28	√
		Glycogen	
17	4.2[b]	6.9[b]	
19	3.0[b]	8.0[b]	
21	3.7[b]	10.0[b]	
23	2.7[b]	27.0[a]	
√			
√ ± SEM	8.1 ± 1.6		
Probabilities	Oxygen (O)	0.0001	
	Day (D)	0.0001	
	O x D	0.05	
		Lactate	
17	0.24[c]	0.29[a]	
19	0.28[ab]	0.28[ab]	
21	0.28[ab]	0.28[ab]	
23	0.26[b]	0.27[ab]	
√			
√ ± SEM	0.27 ± 0.004		
Probabilities	Oxygen (O)	0.05	
	Day (D)	0.01	
	O x D	0.01	
		Ratio (glycogen/lactate)	
17	17.8[b]	24.4[b]	
19	11.2[b]	28.6[b]	
21	13.2[b]	35.5[b]	
23	10.4[b]	94.5[a]	
√			
√ ± SEM	29.2 ± 5.2		
Probabilities	Oxygen (O)	0.09	
	Day (D)	0.002	
	O x D	0.04	

Table 10: Blood plasma glucose concentrations (mg/dL) of turkey embryos incubated in four oxygen concentrations during the plateau stage in oxygen consumption

	Day of incubation		
Oxygen (%)	27	28	√
		Plasma glucose (mg/dL)	
17	218	226	222[c]
19	222	232	227[bc]
21	224	248	236[a]
23	224	238	231[ab]
√	222[b]	236[a]	
√ ± SEM	229 ± 2		
Probabilities	Oxygen (O)	0.05	
	Day (D)	0.0005	
	O x D	NS	

not (Dietz *et al.*, 1998). Because the plateau stage in altricial species is very short, it has been speculated that extended periods of hypoxia and hyperthyroidism of the precocial species enhance organ maturation to improve neonate survival outside the shell (Dietz and Prinzinger, 1997). The time during development of the plateau and increased thyroid hormone secretion are determined by eggshell conductance (Rahn, 1981; Christensen and Biellier, 1982; Christensen *et al.*, 2002). Temperatures in the current study greater than 37°C and oxygen concentrations less than 21% affected cardiac health. This is in agreement with the previous observations that hypoxia and low

Table 11: Poult weights (g) with and without yolk when exposed to different temperature or oxygen treatments during the plateau stage in oxygen consumption

		Day of incubation		
Temperature (C)	Oxygen (%)	27	28	√
			Body weight with yolk	
36	17	67.3	63.3	
	23	67.0	62.4	
39	17	65.3	61.9	
	23	66.0	64.5	
	Day √	66.4[a]	63.0[b]	
	√ ± SEM	64.7 ± 0.5		
Probabilities	Temperature (T)	NS		
	Oxygen (O)	NS		
	Day (D)	0.02		
	T x O	NS		
	T x D	NS		
	O x D	NS		
	T x O x D	NS		
			Body weight without yolk	
36	17	51.6	52.3	
	23	54.2	51.1	
	√	52.9[a]	51.7[ab]	
39	17	48.8	54.2	
	23	51.0	54.2	
	Day √	49.9[b]	52.2[a]	
	Oxygen √	17 = 50.7[b]		
		23 = 52.6[a]		
	√ ± SEM	51.7 ± 0.5		
Probabilities	Temperature (T)	NS		
	Oxygen (O)	0.05		
	Day (D)	NS		
	T x O	NS		
	T x D	0.05		
	O x D	NS		
	T x O x D	NS		
			Yolk weight	
36	17	15.7	11.0	
	23	12.8	11.2	
39	17	16.5	11.7	
	23	15.1	10.3	
	Day √	15.2[a]	11.1[b]	
	√ ± SEM	13.0 ± 0.6		
	Oxygen √	17 = 13.7[a]	23 = 12.4[b]	
Probabilities	Temperature (T)	NS		
	Oxygen (O)	0.05		
	Day (D)	0.0001		
	T x O	NS		
	T x D	NS		
	O x D	NS		
	T x O x D	NS		

eggshell conductance lead to reduced heart weight and altered intermediary metabolism of cardiac tissue (McCutcheon *et al.*, 1982; Bagley, 1987 and Christensen *et al.*, 2003).

Temperature : High temperature reduced embryonic BW at EP uniquely, as BW with yolk was heavier but only because of residual yolk. Yolk utilization was clearly impaired by temperatures greater than 37°C without actually affecting body tissue mass. More residual yolk suggests that higher temperatures increase gluconeogenesis rather

Table 12: Heart and liver weights (mg) of poults exposed to different temperature or oxygen treatments during the plateau stage in oxygen consumption

			Day of incubation		
Temperature (C)	Oxygen (%)		27	28	√
				Heart	
36	17		275	321	
	23		295	307	
	√		285[b]	314[a]	
39	17		233	263	
	23		261	262	
	√		278[c]	285[b]	
	O x D √	17	254[c]	292[a]	
		23	278[b]	285[ab]	
	√ ± SEM		277 ± 4		
Probabilities	Temperature (T)		0.0001		
	Oxygen (O)		NS		
	Day (D)		0.001		
	T x O		NS		
	T x D		0.01		
	O x D		0.05		
	T x O x D		NS		
				Liver	
36	17		1,019	1,321	1,193[a]
	23		1,114	1,320	
39	17		881	1,156	1,050[b]
	23		921	1,242	
	Day √		1,094[b]	1,260[a]	
	Oxygen √		17 = 1,094[b]		
			23 = 1,149[a]		
	√ ± SEM		1,122 ± 13		
Probabilities	Temperature (T)		0.0001		
	Oxygen (O)		0.04		
	Day (D)		0.0001		
	T x O		NS		
	T x D		NS		
	O x D		NS		
	T x O x D		NS		

than utilize yolk lipid. Presumably, high temperature increased metabolism requiring more oxygen at the plateau stage, thus, hypoxia may be responsible for this action. Therefore, at the plateau an higher temperatures turkey embryos require more energy for function than growth (Schmalhausen, 1930 and Dietz *et al.*, 1998). Reduced BW from High temperature was not seen at hatching when the shell was broken and more oxygen was available.

Further evidence for vacillation between growth and function comes from the observations of cardiac tissue. Temperatures above 37°C consistently depressed heart weight and depressed glycogen while increasing lactate concentrations in cardiac muscle. Thus, high temperatures created a negative energy balance in cardiac muscle and reduced its growth. Therefore, to maximize the use of cardiac glycogen, incubators should be operated at temperatures lower than 37°C during the plateau stage in oxygen consumption.

Because cardiac tissue cannot recycle lactate, a metabolic by-product of cardiac muscle contraction, the liver provides lactate recycling for the heart via the Cori cycle (Pearce and Brown, 1971; Donaldson and Christensen, 1991). The blood carries lactate to the liver, where it is converted to glucose and returned to the heart for energy. Because of this inter-organ relationship, hepatic tissue was also examined in the current study. Temperatures of 37°C or greater during the hypoxia of the plateau stage did not affect liver weight but did depress the ratio of hepatic glycogen to lactate. More lactate compared to glycogen may indicate increased recycling. The elevated plasma glucose concentrations seen at higher temperatures may also be supporting evidence for increased lactate recycling. Therefore, we conclude that for optimal hepatic lactate recycling for cardiac tissue metabolism, incubator temperature at the time of the plateau stage in oxygen consumption must be less than 37°C.

Table 13: Cardiac glycogen and lactate concentration (mg/g of wet tissue mass) incubating eggs were exposed to different temperature or oxygen treatments during the plateau stage in oxygen consumption

Temperature (C)	Oxygen (%)	Day of incubation 27	28	√
			Glycogen	
36	17	2.62	1.96	2.29[b]
	23	3.54	2.07	2.80[a]
39	17	2.34	1.69	2.01[c]
	23	2.22	1.49	1.85[c]
	O x D √	17	2.48[b]	1.82[c]
	23	2.88[a]	1.78[c]	
	√ ±SEM	2.21 ± 0.05		
Probabilities	Temperature (T)	0.0001		
	Oxygen (O)	NS		
	Day (D)	0.0001		
	T x O	0.001		
	T x D	NS		
	O x D	0.05		
	T x O x D	NS		
			Lactate	
36	17	1.08	1.12	1.09[b]
	23	1.01	1.32	1.17[b]
	√			
39	17	0.99	1.18	1.09[b]
	23	1.14	1.41	1.27[a]
	√			
	O x D √	17 1.03[c]	1.15[b]	
		23 1.05[bc]	1.36[a]	
	√ ±SEM	1.22 ± 0.2		
Probabilities	Temperature (T)	0.001		
	Oxygen (O)	NS		
	Day (D)	0.0001		
	T x O	0.008		
	T x D	NS		
	O x D	0.03		
	T x O x D	NS		
			Ratio (glycogen/lactate)	
36	17	2.53[b]	1.77[cd]	
	23	3.64[a]	1.59[cde]	
	√			
39	17	2.62[b]	1.44[de]	
	23	2.01[c]	1.07[e]	
	√			
	√ ±SEM	2.10 ± 0.09		
Probabilities	Temperature (T)	0.001		
	Oxygen (O)	NS		
	Day (D)	0.0001		
	T x O	0.008		
	T x D	NS		
	O x D	NS		
	T x O x D	0.03		

Oxygen: In contrast to temperature, oxygen concentrations of at least 21% during the plateau stage consistently increased BW and increased the utilization of yolk by turkey embryos. This observation is in agreement with the observation of Stock and Metcalfe (1984) that oxygen limits the growth of the chick embryo. Thus, for maximal embryonic growth, incubators during the plateau in oxygen consumption should be ventilated to maintain at least 21% oxygen.

Table 14: Hepatic glycogen and lactate concentration (mg/g of wet tissue mass) incubating eggs were exposed to different temperature or oxygen treatments during the plateau stage in oxygen consumption

		Day of incubation	
Temperature (C)	Oxygen (%)	27	28
			Glycogen
36	17	9.6	6.8
	23	12.9	13.7
	√	9.8[b]	13.3[a]
39	17	9.9	3.7
	23	13.7	15.7
	√	5.2[c]	14.7[a]
	√ ± SEM	10.7 ± 0.5	
Probabilities	Temperature (T)	NS	
	Oxygen (O)	NS	
	Day (D)	0.0001	
	T x O	NS	
	T x D	0.004	
	O x D	NS	
	T x O x D	NS	
			Lactate
36	17	0.27[b]	0.27[b]
	23	0.23[c]	0.28[ab]
	√		
39	17	0.27[b]	0.30[a]
	23	0.28[ab]	0.28[ab]
	√		
	√ ± SEM	0.27 ± 0.005	
Probabilities	Temperature (T)	0.01	
	Oxygen (O)	NS	
	Day (D)	0.04	
	T x O	NS	
	T x D	NS	
	O x D	NS	
	T x O x D	0.003	
			Ratio (glycogen/lactate)
36	17	36.8	48.1
	23	43.3	49.4
	√	40.1[b]	48.7[ab]
39	17	25.7	47.0
	23	14.6	57.4
	√	20.2[c]	52.2[a]
	√ ± SEM	40.3 ± 1.9	
Probabilities	Temperature (T)	0.03	
	Oxygen (O)	NS	
	Day (D)	0.0001	
	T x O	NS	
	T x D	0.003	
	O x D	NS	
	T x O x D	NS	

In an unexpected observation, the concentration of oxygen did not affect heart weights, but it did affect those of liver. Oxygen concentrations also increased cardiac (> 19%) and hepatic (> 21%) glycogen and lactate concentrations. Greater oxygen concentrations also increased plasma glucose concentrations indicating increased energy being shuttled to the heart when the incubator oxygen concentration is at least 21%. Energy for embryonic cardiac muscle contraction during the plateau stage in oxygen consumption may become limiting when oxygen concentrations fall below 19%. Oxygen concentrations of less than 19% occur frequently at the plateau during normal incubation when humidity is increased prior to hatching (Christensen, unpublished data).

Table 15: Blood plasma glucose concentration (mg/dL) in poults exposed to different temperature or oxygen treatments during the plateau stage in oxygen consumption

		Day of incubation		
Temperature (C)	Oxygen (%)	27	28	√
			Glucose	
36	17	230	260	
	23	228	256	
	√	229[c]	258[b]	
39	17	215	271	
	23	227	279	
	√	221[c]	275[a]	
	√ ± SEM	246 ± 3		
Probabilities	Temperature (T)	NS		
	Oxygen (O)	NS		
	Day (D)	0.0001		
	TxO	NS		
	TxD	0.05		
	OxD	NS		
	TxOxD	NS		

Temperature and Oxygen: Both temperature and oxygen environments were tested simultaneously for their interaction effects. A 23% oxygen concentration increased poult BW by 2 g and decreased yolk weight by 1.5 g independent of temperature. Because no interactions were seen, it is clear that the primary influence on embryonic growth during the plateau is from oxygen. Incubators must be ventilated during the plateau to maintain sufficient oxygen to optimize embryonic BW. The 23% oxygen concentration also ameliorated the negative effect of 39°C on BW during the plateau stage.

Temperature and oxygen affected heart and liver weights independently but interacted to affect cardiac glycogen to lactate ratios. Thus, oxygen concentrations of 17% may be stressful to cardiac tissue at either temperature, but the 23% oxygen concentration may improve the effect of temperature on heart weights. The 39°C temperature elevated the blood glucose concentrations but increased oxygen did not. This observation suggests that heat stress plays the primary role in detrimental effects on cardiac tissue and the role of oxygen is only minor and modifies the effects of temperature.

Conclusion

The plateau stage in oxygen consumption can be detrimental to the cardiac health of turkey embryos if incubator temperatures exceed 37°C or if oxygen concentrations fall below 21%. The data from the current study indicate separate actions of temperature and oxygen concentration on cardiac physiology. Increased oxygen stimulated the growth of the entire embryo whereas higher temperatures depressed specific organ weights with no effect on overall growth. Thus, oxygen lower than 17% and temperatures higher than 37°C should be avoided during the plateau stage in oxygen consumption. High temperature effects on cardiac tissue may be ameliorated by an oxygen concentration of 23%.

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