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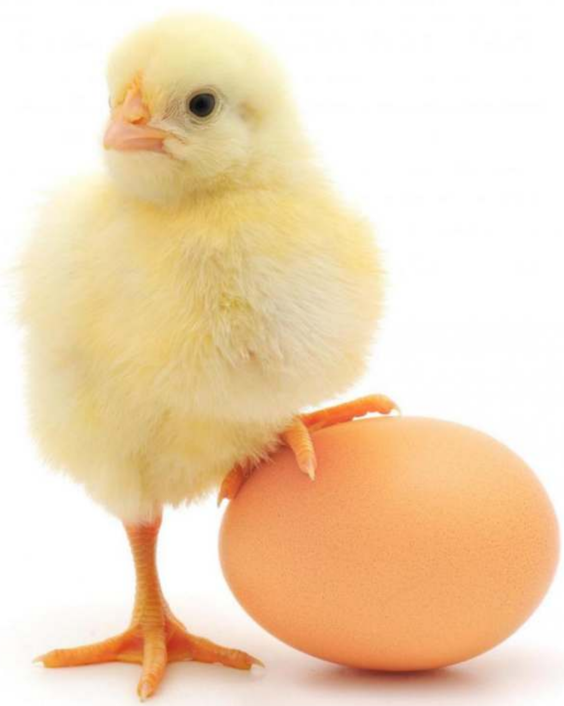

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Relationship of the Eggshell Conductance Constant to Neonatal Cardiac Physiology[1]

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Abstract: The hypothesis was proposed that changes in functional qualities of eggs, or the eggshell conductance constant (k), may affect cardiac weight and physiology and predispose poults to a weakened condition. Improved knowledge of this relationship may allow selection of k to optimize hatchling cardiac health. Egg weights (EW), eggshell conductance (G) and lengths of the incubation period (IP) (the three components of k) were manipulated to determine their effect on the heart. Eggs were selected based on EW and G in Experiment 1. In Experiment 2, eggs from the same strain were obtained from flocks of different ages so they differed in EW. Half of the eggs were exposed to increased temperature treatments resulting in shorter incubation periods (IP). Interactions of EW and G affected heart weight and metabolism in Experiment 1. In Experiment 2, imposing short IP on different EW reduced cardiac weights as well as elevated glycogen to lactate ratios in the heart. Post-hatching growth was also depressed by k due to interactions of EW and IP. Thus, k affects cardiac weight and function and may contribute to weak poults.

Key words: Eggshell conductance, weak poults, cardiac physiology, intestinal physiology

Introduction

Avian embryos exposed to lower oxygen tensions reduced heart weights (Bagley and Christensen, 1989). Reduced heart weights are the opposite response of that seen in adults. Weak poults display reduced heart weights and glycogen content (Christensen *et al.*, 2003). A possible etiology of reduced heart weight may be the functional property of eggshell (G) that determines the length of the incubation period (or timing of the plateau stage in oxygen consumption) (Rahn, 1981; Rahn and Paganelli, 1991). Ar and Rahn (1978) proposed that three egg measurements, G, egg weight (EW) and the incubation period (IP), are interdependent and consequently change proportionally to one another. Across species, the ratio of the product of G and IP divided by EW was termed the conductance constant (k). The value of k was computed to be 5.13. To the best of our knowledge, little is known of this relationship within a species.

The hypothesis tested in the current experiments was that k could influence cardiac weight and physiology of neonatal turkeys by affecting the timing and duration of the plateau stage in oxygen consumption. If this hypothesis is true, egg characteristics can be identified that may prevent weak poults.

Materials and Methods

Experiment 1: Fertilized turkey eggs from a commercial strain[2] were weighed (nearest 0.01g) prior to setting in an incubator. Eggs were incubated using commercially accepted techniques for operating the machines (Christensen *et al.*, 2000a). The eggs were weighed again at the completion of 25 d of incubation and eggshell conductance was determined using the technique of Tullett (1981). The mean EW and G values were measured and values that were greater than ± 2 SD from the means were selected for sampling. Selected eggs were placed into four categories based on EW and G. Two EW categories (Large and Small) and two G categories (High and Low) were examined in a factorial arrangement. All data were arranged in a completely random 2 (EW) x 2 (G) factorial arrangement of treatments (n = 50 for each treatment combination).

At hatching, 10 poults from each of the four EW and G categories were sampled for tissues. The poults were decapitated quickly and trunk blood was collected into a tube containing 10 mg of EDTA and placed immediately into an ice bath. The plasma was decanted following centrifugation (700xg) for 20 min at 4 ^{o}C and frozen at -22 ^{o}C. The heart, liver and thigh muscles were dissected, weighed (nearest 0.01 mg) and placed immediately into an appropriate volume of cold 7% perchloric acid in preparation for glycogen and organic acid analysis.

Blood plasma glucose concentration was measured using the technique of Donaldson and Christensen (1991). Plasma lactate dehydrogenase activity (LDH) was analyzed using the technique of Kachmar and Moss

[1]The mention of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned. [2]British United Turkeys, P. O. Box 727, Lewisburg, WV 24901

Abbreviation Key: LDH = lactate dehydrogenase, CK = creatine kinase, G = eggshell conductance, EW = egg weight, IP = incubation period

Table 1: Body weight (g) of poults hatching from two eggs sizes and two eggshell conductance values

EW[1]	G[2]	Body weight with yolk	Body weight without yolk	Yolk weight
Large	High	69.0	61.0	8.0[b]
	Low	74.4	60.4	14.0[a]
	√	71.7[a]	60.7[a]	
Small	High	59.8	51.8	8.0[b]
	Low	61.1	54.1	7.1[b]
	√	60.4[b]	52.9[b]	
	High √	64.4[b]	56.4	
	Low √	67.8[a]	57.3	
Overall √ ± SEM		66.1± 0.7	56.8± 0.5	9.3± 0.5
	Factor	Probabilities		
	EW	0.0001	0.0001	0.01
	G	0.04	NS	0.05
	EW x G	NS	NS	0.01

[a,b]Columnar means with different superscripts differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = egg weight greater than 2 SD above the mean. Small = egg weight less than 2 SD below the mean. [2]G = Eggshell conductance $(mg\ H_2O/d/torr)^2$; High = eggshell conductance greater than 2 SD above the mean. Low = eggshell conductance 2 SD below the mean.

Table 2: Organ weight (mg) of poults hatching from two egg sizes and two eggshell conductance values

EW[1]	G[2]	Heart (mg)	Relative heart (%)	Liver (mg)	Relative liver (%) (mg)	Skeletal muscle muscle (%)	Relative skeletal
Large	High	398	0.65[b]	1,726	2.83	1,424	2.33
	Low	430	0.71[ab]	1,450	2.41	1,458	2.40
	√	414[a]		1,588	2.62[b]	1,441[a]	2.37
Small	High	388	0.75[a]	1,624	3.14	1,214	2.34
	Low	386	0.71[ab]	1,496	2.77	1,302	2.40
	√	387[b]		1,560	2.96[a]	1,258[b]	2.36
	High √	393		1,675[a]	2.98[a]	1,319	2.33
	Low √	415		1,473[b]	2.59[b]	1,380	2.40
Overall √ ± SEM		401 ± 7	0.71±0.01	1,574 ± 34	2.79 ± 0.06	1,350 ± 37	2.37±0.05
	Factor	Probabilities					
	EW	0.05	0.08	NS	0.03	0.04	NS
	G	NS	NS	0.01	0.01	NS	NS
	EW x G	NS	0.05	NS	NS	NS	NS

[a,b]Columnar means with different superscripts differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = egg weight greater than 2 SD above the mean. Small = egg weight less than 2 SD below the mean. [2]G = Eggshell conductance $(mg\ H_2O/d/torr)^2$; High = eggshell conductance greater than 2 SD above the mean. Low = eggshell conductance 2 SD below the mean.

(1976); Zimmerman and Henry (1979) and creatine phosphatase activity (CK) was measured using the technique of Oliver (1963). Heart, liver and skeletal muscle tissues were analyzed for glycogen using the technique of Christensen *et al.* (2000a).

Experiment 2: Because k is computed using the time of hatching, the effects of k on embryos prior to hatching cannot be determined using the design of Experiment 1. Therefore, fertilized eggs of two different EW were obtained by selecting from flocks at the initial stages of lay (1 wk = Small) or after peak lay (16 wk = Large). The flocks were of the same strain as used in Experiment 1. Approximately 200 fertilized eggs from each EW and T group were weighed individually (nearest 0.01 g) prior to setting in incubators and again at 25 d of incubation and the G of each group was calculated (Tullett, 1981). To create different IP, half of the eggs of both EW were exposed initially to higher incubation temperatures (T) similar to single stage incubation as described previously (Christensen *et al.*, 2002) compared to a 37.5 C control group.

Table 3: Organ glycogen concentration (mg/g of wet tissue mass) of poults hatching from two eggs sizes and two eggshell conductance values

EW[1]	G[2]	Cardiac	Hepatic	Muscle
Large	High	4.60[a]	10.7	0.80
	Low	3.91[ab]	8.5	0.76
	√		9.6	0.78
Small	High	3.15[b]	11.9	0.75
	Low	4.23[a]	8.1	0.95
	√		10.0.	0.85
	High √		11.3[a]	0.88
	Low √		8.2[b]	0.86
Overall √ ± SEM		3.97± 0.25	9.8 ± 0.7	0.81±0.05
	Factor	Probabilities		
	EW	0.10	NS	NS
	G	NS	0.05	NS
	EW x G	0.01	NS	NS

[a, b]Columnar means with different superscripts differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = egg weight greater than 2 SD above the mean. Small = egg weight less than 2 SD below the mean. [2]G = Eggshell conductance (mg H_2O/d/torr)^2; High = eggshell conductance greater than 2 SD above the mean. Low = eggshell conductance 2 SD below the mean.

Table 4: Eggshell conductance (G), egg weights (EW) and incubation periods and the resulting conductance constant of turkey eggs in Experiment 2.

EW[1]	Temperature[2]	Weight (g)	G (mg of H_2O/d/mmHg)	Incubation period (h)	k[3]
Large	Control	90.2	19.9	652[a]	5.98
	High	87.4	20.9	641[c]	6.29
	√	88.8[a]	20.4[a]		6.13[a]
Small	Control	80.1	17.6	649[b]	5.90
	High	78.5	17.3	642[c]	5.94
	√	79.3[b]	17.4[b]		5.92[b]
	Control √	85.2	18.8		5.94[b]
	High √	83.0	19.1		6.11[a]
Overall √ ± SEM		84.0 ± 0.1	18.9 ± 0.1	644 ± 0.1	6.02±0.01
	Factor	Probabilities			
	EW	0.0001	0.0001	NS	0.04
	Temperature (T)	NS	NS	0.0001	0.05
	EW x T	NS	NS	0.01	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = egg weight greater than 2 SD above the mean. Small = egg weight less than 2 SD below the mean. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control). [3]k = Conductance constant computed by dividing the product of G and the incubation period divided by EW.

At external pipping (e27), hatching (0 d) and 3 d post hatching, embryos or hatchlings were selected randomly from each treatment group and sampled for tissues as described in Experiment 1. Stage of development was determined at 4 h intervals beginning at 25 d of age using a candling light. Each group was sampled when greater than half attained the desired stage of development. Tissue lactate concentration was also determined on the heart and liver tissue (Fleisher, 1970).

Statistical Analysis: Data from Experiment 1 were analyzed in a completely random design as a 2 x 2 factorial arrangement of treatments. The treatments were EW (Large and Small) and G (High and Low). All main effect and interaction means were tested for significance (SAS Inst., 1998). Means determined to differ significantly were separated by the least square means procedure of SAS.

Experiment 2 was analyzed by stage of development as a completely random design and a 2 x 2 factorial

Table 5: Body weights (g) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	62.0	61.6	93.8
	High	60.5	63.5	90.0
	√	61.3[a]	62.6[a]	91.9[a]
Small	Control	54.7	56.1	84.4
	High	58.2	52.6	78.0
	√	56.6[b]	54.5[b]	81.1[b]
	Control √	58.4	58.9	89.1
	High √	59.3	58.1	84.0
Overall √ ± SEM		58.9 ± 1.1	58.5 ± 0.9	86.6 ± 1.2
	Factor	Probabilities		
	EW	0.05	0.001	0.03
	Temperature (T)	NS	NS	NS
	EW x T	NS	NS	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

Table 6: Relative heart weights (mg/g of BW) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	0.42	0.63	0.65
	High	0.40	0.46	0.67
	√	0.41	0.55	0.66
Small	Control	0.46	0.61	0.63
	High	0.43	0.49	0.61
	√	0.45	0.55	0.62
	Control √	0.44	0.62[a]	0.64
	High √	0.41	0.48[b]	0.64
Overall √ ± SEM		0.43 ± 0.01	0.55 ± 0.02	0.64 ± 0.01
	Factor	Probabilities		
	EW	NS	NS	NS
	Temperature (T)	NS	0.005	NS
	EW x T	NS	NS	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

arrangement. The main factors for Experiment 2 were EW (Large and Small) and T (High and Control). All possible main effects and interactions were tested for significance and means determined to differ significantly were separated by the least square means procedure of SAS (SAS Inst., 1998). Probability of a Type I error was based on $P \leq 0.05$ in both experiments.

Results

Experiment 1: Large eggs produced heavier poults than Small and Low G eggs produced heavier poults than High (Table 1). When the yolk was removed, the differences in BW were observed only between EW. Yolk mass displayed an EW by G interaction. Large eggs with Low G hatched with more residual yolk than any other treatment combination.

Heart and skeletal muscles were heavier in Large than Small eggs, but liver weights were not (Table 2). High G increased liver weights compared to Low, but G had no effect on heart and skeletal muscle. Cardiac glycogen concentrations were depressed in Small eggs with High G compared to all other interaction means (Table 3).

Table 7: Cardiac glycogen concentration (mg/g of wet heart tissue) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	6.25	2.32[bc]	1.12
	High	7.06	2.76[ab]	1.14
	√	6.65		1.13
Small	Control	7.08	1.82[c]	1.45
	High	5.96	3.33[a]	1.34
	√	6.52		1.39
	Control √	6.67		1.28
	High √	6.51		1.24
Overall √ ± SEM		6.59 ± 0.28	2.55 ± 0.12	1.26 ± 0.01
	Factor	Probabilities		
	EW	NS	NS	NS
	Temperature (T)	NS	0.001	NS
	EW x T	NS	0.05	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

Table 8: Cardiac lactate concentration (mg/g of wet heart tissue) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	0.68[ab]	0.97	0.59
	High	0.51[b]	1.16	0.60
	√		1.06	0.60
Small	Control	0.52[b]	0.89	0.84
	High	0.86[a]	0.94	0.93
	√		0.92	0.86
	Control √		0.93[b]	0.72
	High √		1.05[a]	0.76
Overall √ ± SEM		0.64 ± 0.05	0.99 ± 0.04	0.74 ± 0.04
	Factor	Probabilities		
	EW	NS	NS	NS
	Temperature (T)	NS	0.001	NS
	EW x T	0.03	NS	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

High G increased hepatic glycogen concentrations compared to Low, but no other differences were noted in liver tissue. No treatment effects on skeletal muscle glycogen concentrations were noted due to either EW or G. No main effects or their interactions affected blood plasma glucose concentrations, LDH or CK activities (data not shown).

Experiment 2: Conductance constants were computed for individual eggs in Experiment 2 (Table 4). Resulting conductance constants displayed both EW and T effects but no interaction. Large eggs displayed greater k than Small eggs and High eggs had greater k than did Controls. The resulting IP revealed a significant EW by T interaction as eggs shortened IP to a greater degree among Large than Small eggs in response to the High temperature. Age of the flock elevated both EW and G used for computation of k.

Large EW resulted in heavier BW at external pipping, hatching and 3 d posthatching (Table 5). Relative heart

Table 9: Ratio of cardiac glycogen to lactate concentrations (mg/g of wet heart tissue) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	9.71^{b}	2.59^{b}	2.59
	High	14.07^{a}	2.42^{b}	4.24
	√			3.41
Small	Control	13.75^{a}	2.03^{b}	1.80
	High	8.01^{b}	3.58^{a}	1.44
	√			1.62
	Control √			2.19
	High √			2.84
Overall √ ± SEM		11.36 ± 0.82	2.66 ± 0.09	2.51 ± 0.50
	Factor	Probabilities		
	EW	NS	NS	NS
	Temperature (T)	NS	0.05	NS
	EW x T	0.006	0.03	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

Table 10: Blood plasma glucose (mg/dL) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	227^{b}	297	362
	High	225^{b}	242	320
	√		270	341
Small	Control	206^{b}	295	353
	High	253^{a}	258	313
	√		277	333
	Control √		296^{a}	357
	High √		250^{b}	316
Overall √ ± SEM		228 ± 5	273 ± 5	337 ± 12
	Factor	Probabilities		
	EW	NS	NS	NS
	Temperature (T)	0.05	0.001	NS
	EW x T	0.03	NS	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1]EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

weights differed only at hatching (Table 6). High T resulted in shorter IP and reduced heart weights in both EW groups. No effects were noted at external pipping or 3 d posthatching.

Cardiac glycogen data indicated a significant EW by T interaction also occurring at hatching (Table 7). Among Small eggs, High T elevated cardiac glycogen concentrations compared to Controls, but among Large eggs no significant difference was detected. The treatments did not affect cardiac glycogen concentrations at any other time examined.

Cardiac lactate concentrations displayed a significant interaction at external pipping but only a T effect at hatching (Table 8). Small EW eggs exposed to High T elevated lactate compared to all other treatment combinations except Large EW eggs given the Control IP. High T elevated cardiac lactate concentrations compared to Controls in both EW categories at hatching. The ratios of cardiac glycogen and lactate at each of the sampling times indicate a clearer picture of the

Table 11: Blood plasma lactate dehydrogenase (LDH) activity (U/L) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	397	725	431
	High	381	627	415
	√	389[b]	676	423[b]
Small	Control	458	850	552
	High	496	563	613
	√	477[a]	707	582[a]
	Control √	428	788[a]	492
	High √	439	595[b]	514
Overall √ ± SEM		433 ± 16	691 ± 38	503 ± 24
	Factor	Probabilities		
	EW	0.02	NS	0.05
	Temperature (T)	NS	0.02	NS
	EW x T	NS	NS	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1] EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

adjustments to the plateau stage in oxygen consumption being made by the embryo. Significant EW by T interactions were evident at both external pipping and hatching (Table 9). At external pipping in Large EW eggs greater amounts of glycogen than lactate were found when embryos were exposed to High T compared to Controls, but among Small EW eggs, the opposite was noted. At hatching, only embryos in Small eggs exposed to High T elevated glycogen compared to lactate. Blood plasma glucose concentrations also indicate embryo attempts to provide energy to the heart as a significant EW by T interaction was noted at external pipping when embryos from Small eggs at High elevated plasma glucose compared to all other treatments (Table 10).

Plasma LDH activities were elevated in Small EW eggs compared to Large EW eggs at 27 d and 3 d post hatching (Table 11). At 28 d poults in eggs exposed to a High had reduced LDH activity compared to Controls. Data from plasma CK activity displayed significant differences only at hatching (Table 12). Large EW eggs caused elevated CK compared to Small EW eggs and High depressed CK compared to Controls.

Discussion

The hypothesis proposed in the current study was that k and its effect on timing of the plateau stage in oxygen consumption might alter cardiac weight and physiology. Interdependence of EW, G and IP (Ar and Rahn, 1978), the three components of k, was clearly evident in the current study as evidenced by various two way interactions. Depressed k reduced heart weight much the same as had been noted at high altitudes (Bagley and Christensen, 1989). Additionally, reduced k increased cardiac glycogen and lactate and elevated plasma glucose concentrations all of which affect cardiac intermediary metabolism (Guarner *et al.*, 2002). To our knowledge, little was known previously of the k relationship in any poultry species, but data from the current study indicate a relationship between heart weight, cardiac metabolism and k.

Fetal cardiac tissue metabolic requirements are met primarily by carbohydrate in contrast to fatty acids used by adult hearts (Guarner *et al.*, 2002). When an embryo is experiencing hypoxia and hypercapnia at the plateau stage (Ar and Rahn, 1978) with its consequent anaerobic metabolism, reduced heart growth may occur because of insufficient carbohydrate metabolism in cardiac tissue. In the current study EW and G depressed cardiac glycogen with minor effects on hepatic and skeletal muscle glycogen concentrations. Therefore, k and its action on the intermediary metabolism of cardiac tissue may selectively depress cardiac tissue growth as seen in the current study and previously (Christensen *et al.*, 2000ab). In birds selected for growth, energy may also be diverted to other tissues for their growth in preference to the heart (Lilja, 1983; Lilja and Olsson, 1987; Ricklefs, 1987). Therefore, the data may infer that poults hatched from High T with short IP hatch with smaller hearts because of altered intermediary metabolism.

Both LDH and CK can be elevated by cardiac insufficiency (Guyton, 1976). The enzyme LDH catalyzes the conversion of lactate into pyruvate during anaerobic metabolism whereas CK frees high-energy phosphate groups from muscle creatine for energy metabolism during times of oxygen deficit. The EW and IP affected

Table 12: Blood plasma creatine kinase (CK) activity (U/L) of poults hatched from eggs of two EW when exposed to different incubation periods (IP)

EW[1]	Temperature[2]	External pip	0 d	3 d
Large	Control	444	1,117	943
	High	568	810	1,084
	√	506	963[a]	1,013
Small	Control	542	854	1,138
	High	547	654	1,686
	√	544	759[b]	1,412
	Control √	493	985[a]	1,041
	High √	558	737[b]	1,385
Overall √ ± SEM		525 ± 63	861 ± 58	1,213 ± 103
	Factor	Probabilities		
	EW	NS	0.05	NS
	Temperature (T)	NS	0.02	NS
	EW x T	NS	NS	NS

[a,b]Columnar means followed by a different superscript differ significantly ($P < 0.05$). [1] EW = Egg weight (g); Large = eggs were from a flock 16 wk into egg production. Small = eggs were from a flock 2 wk into egg production. [2]Temperature = Eggs were incubated using a higher temperature (High) for the initial two weeks of incubation to shorten the incubation period versus a constant temperature control (Control).

LDH and CK independently in Experiment 2, but attempts to demonstrate cardiac insufficiency by measuring elevated LDH and CK were unsuccessful in Experiment 1. Therefore, embryos in Experiment 2 from Small eggs or with shorter IP displayed symptoms of cardiac insufficiency, but those in Experiment 1 did not. Lactate, glycogen, CK and LDH are involved with many physiological processes. Tissue glycogen and lactate concentrations are functions of the fed-state of the whole bird or an individual tissue (Garcia *et al.*, 1987). Starved animals have depressed cardiac glycogen and lactate concentrations and birds fed a high protein diet have unchanged glycogen with moderately depressed cardiac lactate concentrations compared to controls. Based on these criteria, the depressed cardiac elevated cardiac glycogen to lactate ratios in the current study infers that cardiac tissue existed on protein substrates. This speculation may have merit because very little glucose remains in eggs at the stages of development examined. Cardiac glycogen was depressed in poults from Small eggs with High G in Experiment 1 and elevated in poults from Small EW exposed to short IP in Experiment 2.

In precocial avian embryos, the plateau in oxygen consumption immediately prior to hatching (Ar and Rahn, 1978) is accompanied by increased plasma thyroid hormone concentrations (McNabb *et al.*, 1981; McNabb *et al.*, 1984). Presumably to foster organ maturation and improve neonate survival outside the shell in the absence of extensive maternal care. Timing of the plateau and the surge in thyroid hormones are determined by k (Christensen *et al.*, 2002). Physiologically, the plateau is also related to lung inflation (Bagley, 1987) and hypoxia stimulates establishment blood acid-base balance (Eramus *et al.*, 1970/71) both of which are related to cardiac weight and function. Therefore, maturation of other physiological processes may also be integrated by k at the plateau stage and be integrated in cardiac health. Thus, the effects seen in the current study may reflect physiological integration rather than a single process on cardiac tissue.

In summary, k or the product of G and IP divided by EW determines cardiac growth and metabolism at the end of incubation. Large, Low G eggs (low k) reduced heart weight and altered the intermediary metabolism of embryonic heart compared to greater k. When the IP was shortened, the condition was worse. Thus, if the G of an egg is perfectly matched to its EW and IP then cardiac growth and function are normal (Christensen *et al.*, 2002). It is recommended that extremes in egg size be avoided and that incubation temperatures be moderated to extend incubation periods to optimize cardiac health.

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