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Eggshell Conductance and Incubator Humidity as Factors in Embryo Survival and Poult Growth[1]

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Abstract: Eggshell conductance (G) and incubator humidity (RH) were hypothesized to affect poult embryo survival and hatchling growth. Nearly 4,000 fertilized eggs of the same weight were selected (within 2 standard deviations of the mean). Selected eggs were divided randomly between two incubators. One cabinet operated at 65% RH whereas the second operated at 50% RH, and both cabinets had the same temperature (37.2ºC). At the completion of the 24th day of development, all eggs were weighed a second time to determine eggshell G. Three groups were formed at this time by calculating eggshell conductance and sorting into groups of eggs exhibiting high (Hi), average (Avg) or low (Low) G. The eggs were then placed in the same incubation cabinet for hatching. Measurements were made of embryo cardiac and intestinal physiology. Samples were collected at external pipping and hatching from each of the groups. Tissues were assayed for plasma glucose and lactate, cardiac and hepatic glycogen and lactate. The RH and G effects on survival were noted, and poult weights were recorded for the first 6 weeks of age. More embryos from eggs of Hi or Avg G survived than Low G eggs, but poults from Hi G eggs did not grow as well as those from Avg or Low G eggs. Low G poults showed depressed cardiac glycogen and elevated lactate and had less mature intestines. Thus, in the developmental process of turkey embryos, G and RH may determine organ maturity at hatching thereby influencing survival and growth.

Key words: Eggshell conductance, incubator humidity, embryo survival

Introduction

Many measurements have been made of physical characteristics of eggshells, some of which relate to embryo livability (Burley and Vadehra, 1989; Meijerhof, 1992). Simkiss (1980) stated that the role of eggshell porosity had been overemphasized in avian embryonic development, and that the embryo tolerates a wide range of shell characteristics and incubation conditions by metabolic mechanisms that are not fully understood. However, few measurements have been made of the functional quality of eggshells called eggshell conductance (G) (Rahn, 1981) in relation to offspring viability (Christensen and McCorkle, 1982). G is a measurement of the diffusion of vital gases across the shell to support metabolic processes creating the new hatchling. G determines the length of the incubation period (Rahn, 1981; Rahn and Paganelli, 1991) by matching the length of incubation and the initial egg mass to the correct water loss and oxygen uptake and carbon dioxide expiration criteria for growth and development, to create the conductance constant (k = 5.13) (Ar and Rahn, 1978). Thus, k varies directly with G and the length of the incubation period but varies inversely with initial egg mass. Ar and Rahn (1978) proposed across avian species that proper k would create a hatchling with the characteristic maturity of the species. Greater or smaller k due to any factor in its calculation may create hatchlings of lesser maturity or quality.

One of three proposed functions of G involves humidity (RH) to ensure that an egg loses 15% of its initial weight as water vapor during embryo development prior to the plateau stage in oxygen consumption (Rahn, 1981). Proper water vapor gradients across the shell are prerequisite to embryo survival (Hulet *et al*., 1987). The current study tested viability of turkey embryos in eggs of the same weight but different G incubated in high RH.

Materials and Methods

Over 6,000 eggs were weighed (nearest 0.01 g) individually on the day of oviposition, and approximately 4,000 eggs of the same weight (± 2 standard deviations) were selected for the experiment. All eggs were from a commercial flock of turkey breeders (Hybrid, Inc., Kitchner, Ontario) in its 14th week of lay. The selected eggs in the experimental population were transported to a hatchery at Salisbury, NC and were set randomly into two incubator cabinets (Natureform I40, Natureform Co., Jacksonville, FL). Both cabinets operated at a temperature of 37.2ºC, but the RH of one cabinet was 65% (High) whereas that of the second cabinet was 50% (Control). The eggs were distributed randomly between the two machines and were incubated for 24 days. At the conclusion of the 24th day, all eggs were weighed a second time to calculate their eggshell G (Tullett, 1981) using computerized scales. G values

were calculated and eggs were identified by high (Hi), average (Avg) G and low (Low) values. Each of the three groups was transferred randomly to individual trays by RH and G groups and combined in the same machine for hatching. The hatching machine operated at a dry bulb set point of 36.9°C and a RH of 75%.

The length of the incubation period for the G group was noted at 3 h intervals on days 27 and 28 of development. Embryo survival was recorded by counting the number of poults hatching from 15 trays of 100 eggs. All eggs that did not hatch were broken open and examined following 28 days of incubation to determine the stage at which embryo death occurred. These data were used also to calculate survival rates.

Tissue sampling: At days 27 and 28 of development, 10 embryos (or poults) were selected randomly from each of the G and RH treatment groups. Blood was collected following decapitation with a tube containing 10 mg of EDTA then centrifuged for 15 min at 700 x g under refrigeration (4°C). The body and residual yolk were weighed to the nearest 0.01 g then the heart, liver and jejunum were quickly dissected. The heart and liver were weighed immediately (nearest 0.0001 g) then placed into a vial containing cold 7% perchloric acid and stored at 4°C preparatory to analysis for glycogen and lactate. The jejunum was dissected and weighed then placed into a vial containing physiological saline and frozen quickly (-22°C) preparatory to analysis for maltase and alkaline phosphatase activities. The blood plasma was frozen (-22°C) preparatory to analysis for glucose, lactate and thyroid hormone concentrations. Tissue glycogen and lactate concentrations and blood plasma glucose concentrations and intestinal enzyme activities were assayed by techniques described previously (Christensen *et al*., 2003ab). Thyroid hormones (T_3 and T_4) were assayed using RIA procedures described by Christensen *et al*. (2005).

Ten carcasses from each of the treatments were dried (37.5°C for 2 days) to determine the percentage water. Carcasses were weighed (nearest 0.01 g) prior to placement into the oven and immediately after removal from the oven. The carcass and yolk were separated prior to drying to separate weights of the two compartments.

Growth of poults: Hatchlings were divided post-hatching by treatment, identified by a wing band, sexed then the hen poults were placed in a brooder house and grown to 6 weeks of age. Body weight and feed conversion ratios were measured weekly to examine effects of RH and G on the growth and well-being of the poults. The house had 36 pens of 9 square meters with 12 hens each. Feed and water were provided *ad libitum*. Pen was the experimental unit in the determination of feed consumption and feed conversion ratios, and each bird was weighed to determine the body weights.

Statistical analysis: Data were analyzed as a 2 levels of RH (High and Control) by 3 levels of G (Hi, Avg, Low) factorial arrangement of treatments in a completely random experimental design (SAS Inst., 1998). Means determined to differ significantly ($P<0.05$) were separated using least square means.

Table 1: Weights (g) of poults from eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Weeks of age	
Humidity[1]	G[2]	3	6
High	Hi	637	2,351
	Avg	635	2,234
	Low	579	2,067
	Humidity mean	617[b]	2,218[b]
Control	Hi	648	2,344
	Avg	665	2,383
	Low	631	2,245
	Humidity mean	648[a]	2,348[a]
	G means		
	Hi		2,156[b]
	Avg		2,309[a]
	Low		2,348[a]
Mean ± SEM[3]		633±9	2,274±23
Probabilities	Humidity	0.1	0.07
	G	NS	0.05
	Humidity x G	NS	NS

[1]High = 65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean. a,b Means followed by a different superscript differ significantly.

Results

Embryos survived better in the Control than in the High RH machine (Control = 90.4%; High = 83.5%), and more Hi and Avg G eggs hatched than Low G eggs (Hi = 91.7%, Avg = 93.6% and Low = 75.8%; Overall mean ± SEM = 87.0g ± 0.9). The High RH poults hatched earlier at 645 hours of incubation than those in the Control (647 hours). G also affected time of hatching as Hi G eggs hatched (643 hours) in significantly less time than Avg (645 hours). Low G eggs hatched slowest (650 hours) (Overall mean ± SEM = 645 h ± 1).

Neither RH nor G affected body weights at 3 weeks of age; however, by 6 weeks, the poults from the Control RH incubator were heavier than those from High RH, and Hi G poults weighed significantly less than those of Avg or Low G (Table 1). There were no differences in posthatching body weights due to RH.

High RH embryos hatched sooner, had lower survival rates, and grew slower than Controls, yet Table 2 shows heavier embryo weights (without residual yolk) in the High RH machine than in the Control. Low G eggs with lower survival rates and depressed growth post-hatching had heavier embryo weights than Avg or Hi G eggs regardless of the RH. Conversely, RH and G

Table 2: Body and yolk weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Body weight without yolk	
High	Hi	48.5	47.4[bc]
	Avg	51.4	49.0[ab]
	Low	49.8	49.4[a]
	Humidity mean	49.9[a]	
Control	Hi	45.2	44.5[d]
	Avg	50.9	49.2[ab]
	Low	47.6	45.7[cd]
	Humidity mean	47.9[b]	
	G means		
	Hi	46.8[c]	
	Avg	51.1[a]	
	Low	48.7[b]	
Mean±SEM[3]		48.9±0.4	47.5±0.3
Probabilities	Humidity	0.02	0.001
	G	0.0008	0.0001
	Humidity x G	NS	0.04
		Yolk	
High	Hi	9.3	4.3
	Avg	9.3	5.9
	Low	11.2	9.3
	Humidity mean		6.5[b]
Control	Hi	8.2	6.5
	Avg	9.9	6.9
	Low	13.1	10.3
	Humidity mean		7.9[a]
	G means		
	Hi	8.8[b]	5.4[c]
	Avg	9.6[b]	6.4[b]
	Low	12.1[a]	9.8[a]
	Mean ± SEM3	10.2±0.3	7.2±0.2
Probabilities	Humidity	NS	0.005
	G	0.0003	0.0001
	Humidity x G	NS	0.04

[1]High = 65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean. a,bMeans followed by a different superscript differ significantly.

Table 3: Heart weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Absolute weight (mg)	
High	Hi	254	277
	Avg	269	289
	Low	261	314
	Humidity mean	261[a]	293[a]
Control	Hi	225	260
	Avg	260	292
	Low	255	287
	Humidity mean	247[b]	280[b]
	G means		
	Hi	240[b]	268[c]
	Avg	264[a]	291[b]
	Low	258[a]	301[a]
Mean±SEM[3]		254±3	287±3
Probabilities	Humidity	0.05	0.07
	G	0.0001	0.0001
	Humidity x G	NS	NS
		Relative weight (%)	
High	Hi	0.53	0.59
	Avg	0.52	0.59
	Low	0.52	0.64
	Humidity mean		
Control	Hi	0.5	0.59
	Avg	0.51	0.59
	Low	0.54	0.63
	Humidity mean		
	G means		
	Hi		0.59[b]
	Avg		0.59[b]
	Low		0.63[a]
Mean±SEM[3]		0.51±0.01	0.60±0.01
Probabilities	Humidity	NS	0.NS
	G	NS	0.04
	Humidity x G	NS	NS

[1]High = 65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean. a,bMeans followed by a different superscript differ significantly.

interacted to increase poult weights in Low and Avg G eggs compared to Hi. In Control RH, poults from Avg G eggs weighed more than Hi or Low G eggs. High RH reduced yolk weights at hatching, and Low G increased yolk weights at both 27 and 28 days of embryo development compared to Avg or Hi G. At day 28 Hi G reduced residual yolk compared to the other G.

RH did not affect the weight of the poult relative to the initial egg weight, but poults from Low G eggs hatched at 71.0% of the initial egg mass, Avg G eggs at 69.1% and Hi G eggs at 65.7%. All percentages differed significantly from each other (Overall mean ± SEM = 68.6% ± 0.1). High RH increased the percentage of body moisture by about 1% compared to Controls, and Low G increased body moisture (81.5%) compared to Avg (78.9%), and both Low and Avg showed increased body water compared to Hi G (78.2%)(Overall mean ± SEM = 79.5% ± 0.1).

High RH hearts at both days of development weighed more than the Control on an absolute basis but not relative to body weight (Table 3). Low G eggs resulted in heavier hearts than those in Avg or Hi G eggs.

At 27 days of development, relative liver weight of poults from eggs of Hi G was greater than those in Avg or Low G (Table 4). However, at hatching a RH by G interaction affected the weights. High RH with Low G had the heaviest liver absolute weight and Control RH with Low G as well as High RH with Hi G livers weighed the least.

Table 4: Liver weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Absolute weight (mg)	
High	Hi	1,075	1,315[c]
	Avg	1,082	1,397[ab]
	Low	1,005	1,423[a]
Control	Hi	1,075	1,390[b]
	Avg	983	1,394[b]
	Low	981	1,279[c]
Mean ± SEM[3]		1,034±16	1,366±13
Probabilities	Humidity	NS	NS
	G	NS	NS
	Humidity x G	NS	0.006
		Relative weight (%)	
High	Hi	2.22	2.78[b]
	Avg	2.11	2.85[b]
	Low	2.02	2.89[b]
Control	Hi	2.39	3.14[a]
	Avg	1.94	2.83[b]
	Low	2.07	2.80[b]
	G means		
	Hi	2.31[a]	
	Avg	2.02[b]	
	Low	2.05[b]	
Mean±SEM[3]		2.13±0.03	2.88±0.03
Probabilities	Humidity	NS	NS
	G	0.003	NS
	Humidity x G	NS	0.01

[1]High=65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean.
a,bMeans followed by a different superscript differ significantly.

Table 5: Cardiac glycogen and lactate of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Glycogen (mg/g of wet tissue mass)	
High	Hi	2.66	1.67[b]
	Avg	2.84	2.12[a]
	Low	3.08	1.06[c]
Control	Hi	2.65	1.00[c]
	Avg	3.42	1.56[b]
	Low	3.09	1.38[bc]
Mean±SEM[3]		2.96±0.12	1.46±0.06
Probabilities	Humidity	NS	0.02
	G	NS	0.0008
	Humidity x G	NS	0.007
		Lactate (mg/g of wet tissue mass)	
High	Hi	1.04	1.03
	Avg	1.03	1.03
	Low	0.99	0.95
Control	Hi	1.01	0.88
	Avg	1	0.98
	Low	0.98	0.96
Mean±SEM[3]		1.01±0.03	0.97±0.02
Probabilities	Humidity	NS	NS
	G	NS	NS
	Humidity x G	NS	NS

[1]High=65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean.
a,bMeans followed by a different superscript differ significantly.

All other treatment weights were intermediate. Control RH interacted with Hi G to increase relative liver weight compared to Avg and Low G.

At 27 days of development, neither RH nor G affected cardiac glycogen, but a RH by G interaction affected the glycogen at hatching (Table 5). At Control RH, Avg G had more glycogen than Hi but not Low. At High RH poults from eggs with Avg G had more glycogen than either Low or Hi G, and poults from Hi G had more glycogen than poults from Low G eggs. The treatments had no effect on cardiac lactate.

High RH increased hepatic glycogen at 27 days compared to Control, and Low and Hi G elevated glycogen compared to Avg G (Table 6). At hatching, poults at High RH reduced glycogen compared to Control. Poults from Avg G displayed elevated glycogen compared to Hi G or Low G, and Low G showed the lowest glycogen. Hepatic lactate differed only at hatching and the values mirrored those of glycogen. All treatments with elevated hepatic glycogen showed depressed lactate.

A High RH by Avg G interaction at hatching elevated plasma glucose compared to Hi or Low (Avg = 309; Hi = 283 and Low = 289 mg/dL; Overall Mean ± SEM = 286 ± 6), but a Control RH by Avg G interaction depressed glucose compared to Hi or Low (Avg = 287; Hi = 308 and Low = 303 mg/dL; Overall Mean ± SEM = 296 ± 3).

At every developmental stage, High RH and Hi G increased jejunum weight compared to the other treatments (Table 7). No differences in maltase activity were noted at High RH, but in Control RH, Hi G poults had elevated maltase compared to Avg or Low G poults (Table 8). High RH increased embryo jejunal ALP activity at 27 days of development compared to Controls, and Avg G elevated ALP compared to Hi or Low G (Table 9). The High RH machine caused a stepwise decline in ALP as the G values decreased from Hi to Low, but in the Control RH machine Hi and Avg G eggs had greater ALP than Low G eggs but did not differ from each other.

High RH depressed embryo plasma T_3 and T_4 concentrations at day 27 compared to Control, and Low G eggs showed depressed T_3 concentrations compared to Avg G eggs, but neither differed from Hi G (Table 10). No differences due to G were noted in plasma T_4. At hatching, High RH elevated plasma T_3 compared to Control, and Avg G elevated T_4 and T_3 compared to Low G but not Hi G.

Table 6: Hepatic glycogen and lactate of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Glycogen (mg/g of wet tissue mass)	
High	Hi	3.63	1.78
	Avg	2.67	2.18
	Low	4.59	1.08
	Humidity mean	3.63[a]	1.68[b]
Control	Hi	2.95	2.12
	Avg	2.04	3.45
	Low	2.83	1.63
	Humidity mean	2.61[b]	2.40[a]
	G means		
	Hi	3.29[a]	1.95[b]
	Avg	2.36[b]	2.81[a]
	Low	3.71[a]	1.36[c]
Mean±SEM[3]		3.12±0.23	1.46±0.06
Probabilities	Humidity	0.02	0.0009
	G	0.05	0.0001
	Humidity x G	NS	NS
		Lactate (mg/g of wet tissue mass)	
High	Hi	0.18	0.12
	Avg	0.18	0.11
	Low	0.11	0.17
Control	Hi	0.19	0.15
	Avg	0.12	0.12
	Low	0.16	0.18
	G means		
	Hi		0.14[b]
	Avg		0.12[c]
	Low		0.17[a]
Mean±SEM[3]		0.16±0.01	0.14±0.01
Probabilities	Humidity	NS	NS
	G	NS	0.0004
	Humidity x G	NS	NS

[1]High=65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean.
a,bMeans followed by a different superscript differ significantly.

Table 7: Jejunum weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Absolute weight (mg)	
High	Hi	387	667
	Avg	356	598
	Low	337	552
	Humidity mean		606[a]
Control	Hi	365	572
	Avg	317	567
	Low	339	517
	Humidity mean		552[b]
	G means		
	Hi		620[a]
	Avg		582[ab]
	Low		534[b]
Mean±SEM[3]		350±11	579±13
Probabilities	Humidity	NS	0.04
	G	NS	0.03
	Humidity x G	NS	NS
		Relative weight (%)	
High	Hi	0.79	1.41
	Avg	0.7	1.22
	Low	0.68	1.12
Control	Hi	0.8	1.29
	Avg	0.62	1.15
	Low	0.72	1.13
	G means		
	Hi	0.80[a]	1.35[a]
	Avg	0.66[b]	1.19[b]
	Low	0.70[b]	1.12[b]
Mean±SEM[3]		0.72±0.02	1.22±0.03
Probabilities	Humidity	NS	NS
	G	0.05	0.006
	Humidity x G	NS	NS

[1]High=65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean.
a,bMeans followed by a different superscript differ significantly.

Discussion

The concept of eggshell conductance constant (Rahn, 1981) may have an impact on poultry production. Previously, Simkiss (1980) stated that the role of eggshell porosity had been overemphasized in avian embryonic development, and that the embryo tolerates a wide range of shell characteristics and incubation conditions by metabolic mechanisms that are not fully understood. Data from the current study show clearly that when G was factored with RH, embryo survival decreased in Low G eggs, and Hi G depressed the long-term growth of poults compared to Avg or Low. These data indicate that the turkey embryo may not have to ability to compensate to G and RH as suggested by Simkiss (1980). High RH also played a role in survival and growth by interacting with G to affect maturation of the cardiovascular and digestive systems.

The maturity of a neonate is complex and involves integration of physiological systems by neural and endocrine factors. Egg weight, G and the consequent length of the developmental period are interdependent (Ar and Rahn, 1978) and determine maturity of a hatchling (Christensen *et al.*, 2003ab; Christensen *et al.*, 2005). The three variables form an equation to compute the conductance constant (k) (Ar and Rahn, 1978). Previous studies have shown that k may affect cardiovascular and digestive health (Christensen *et al.*, 2003a; 2003b), and data from the current study indicate that reduced G may affect the well-being of an embryo by suppressing both T_4 and T_3 in the circulation. Elevated RH also suppressed thyroid hormone concentrations indicating additive effects of High RH and Low G. The current data show clearly that G and the length of the

Table 8: Jejunum maltase activity of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity.

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Total maltase activity (µmol/min/jejunum)	
High	Hi	162	710[ab]
	Avg	103	682[ab]
	Low	106	652[b]
Control	Hi	150	777[a]
	Avg	101	499[c]
	Low	97	432[c]
Mean±SEM[3]		120±11	1,366±13
Probabilities	Humidity	NS	NS
	G	0.07	NS
Humidity x G	NS	0.006	
		Specific malatase activity (µmol/min/µg of protein)	
High	Hi	8.5	22.4[b]
	Avg	5.6	22.0[b]
	Low	5.1	20.2[bc]
Control	Hi	9	26.9[a]
	Avg	6	18.4[cd]
	Low	6	16.7[d]
Mean±SEM[3]		6.7±0.6	21.1±0.5
Probabilities	Humidity	NS	NS
	G	NS	0.0001
	Humidity x G	NS	0.005

[1]High = 65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean. a,bMeans followed by a different superscript differ significantly.

Table 9: Jejunum alkaline phosphatase (ALP) activity of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at High or Control humidity

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Total ALP activity (µmol/min/jejunum)	
High	Hi	6,859	31,780[a]
	Avg	4,726	21,947[b]
	Low	3,203	13,768[c]
	Humidity mean	4,929[a]	
Control	Hi	4,204	15,828[bc]
	Avg	3,524	18,917[b]
	Low	2,902	6,410[d]
	Humidity mean	3,544[b]	
	G means		
	Hi	4,125[ab]	
	Avg	5,532[a]	
	Low	3,053[b]	
Mean±SEM[3]		4,236±365	18,108±830
Probabilities	Humidity	0.08	0.0001
	G	0.04	0.0001
	Humidity x G	NS	0.01
		Specific ALP activity (µmol/min/µg of protein)	
High	Hi	0.36	1.00[a]
	Avg	0.25	0.71[b]
	Low	0.15	0.42[d]
Control	Hi	0.25	0.55[cd]
	Avg	0.2	0.69[bc]
	Low	0.19	0.25[e]
	G means		
	Hi	0.31[a]	
	Avg	0.23[ab]	
	Low	0.17[b]	
Mean±SEM[3]		0.23±0.02	21.1±0.5
Probabilities	Humidity	NS	0.0003
	G	0.07	0.0001
	Humidity x G	NS	0.008

[1]High=65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean. a,bMeans followed by a different superscript differ significantly.

incubation period may be major components of the mechanism optimizing the maturation of the cardiovascular and digestive systems, the embryonic survival and subsequent growth of turkeys. These observations support the idea that k is a valid concept. Because k is calculated from three interdependent variables, the well-being of a poult may be influenced by either egg weight, eggshell conductance or the length of the incubation period. In nature, k is critical because it determines survival of the hatchling maturity and subsequent viability of offspring (Ricklefs and Starck, 1998). In the current study, egg weight was not a factor because eggs of the same weight were selected. Thus, G and the consequent length of the incubation period were variables. High G (or a shorter incubation period) affected cardiac physiology adversely, and depressed intestinal maturation and growth at hatching.

Large eggs with Low G may be at risk for cardiomyopathy (Christensen *et al.*, 2003a). In the current study, poults hatching from eggs of the same weight with Low G exhibited heavier hearts but did not display the elevated cardiac glycogen that is characteristic of cardiomyopathy (Czarnecki and Evanson, 1980; Czarnecki, 1991).

Normal intestinal maturation exhibits straight-line growth with the body in avian neonates (Konarzewski *et al.*, 1990), and some estimates indicate that 60% of the total energy of a hatchling may be devoted to increased maturation and growth of intestinal tissue in the first few days following hatching (Fan *et al.*, 1997). Because the neonatal intestine is immature and not able to digest significant amounts of carbohydrates, gluconeogenesis may be the primary source of energy until the intestine matures (Donaldson and Christensen, 1991). Gluconeogenesis requires recycling of lactate by the Cori cycle primarily in the avian liver, catabolism of existing tissues or catabolism of available nutrients in residual yolk by the liver and kidney. If greater maturation

Table 10: Plasma thyroid hormone concentrations of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated at different humidity.

		Days of development	
Humidity[1]	G[2]	27 day embryo	28 day poult
		Triiodothyronine (ng/mL)	
High	Hi	14.4	6.8
	Avg	11.9	8.8
	Low	10.1	4.8
	Humidity mean	12.1[b]	
Control	Hi	23.4	6.7
	Avg	25.5	8.5
	Low	11.7	6.1
	Humidity mean	20.1[a]	
	G means		
	Hi		6.7[b]
	Avg		8.6[a]
	Low		5.4[c]
Mean±SEM[3]		16.2±1.9	7.0±0.3
Probabilities	Humidity	0.04	NS
	G	NS	0.0007
	Humidity x G	NS	NS
		Thyroxine (ng/mL)	
High	Hi	8.5	8
	Avg	9.6	10.9
	Low	6.4	3.9
	Humidity mean	8.2[b]	7.6[a]
Control	Hi	11.5	4.9
	Avg	18.8	6.3
	Low	10.3	3.2
	Humidity mean	13.5[a]	4.8[b]
	G means		
	Hi	10.0[ab]	6.5[ab]
	Avg	14.2[a]	8.6[a]
	Low	8.3[b]	3.6[b]
Mean±SEM[3]		10.8±0.9	6.1±0.6
Probabilities	Humidity	0.01	0.04
	G	0.08	0.01
	Humidity x G	NS	NS

[1]High = 65% RH; Control = 50% RH. [2]G = eggshell conductance; Hi = eggs with high G; Avg = eggs with average G; Low = eggs with low G. [3]Overall mean ± standard error of the mean. a,bMeans followed by a different superscript differ significantly.

could be attained prior to hatching, the poult may perform better the prehension and digestion characteristic of precocity thus precluding the requirement for gluconeogenesis. As noted previously (Christensen *et al.*, 2003b), eggs with Hi G had heavier jejunum weight and better function compared to the other groups, but the precocity did not result in better growth or feed conversion following hatching. The growth and feed conversion data from the current study indicate that Hi G poults had impaired ability to grow and convert feed to tissue, and poults from Low G eggs grew better than all others.

The liver is the metabolic center of the body (Lehninger, 1975), and as such coordinates many physiological events including nutrient storage and utilization. The effects of RH and G on liver were complex. Hepatic tissue was adjusting to metabolism within each egg type that would assure the survival and growth of embryos emerging from the eggs. Embryos in Hi G eggs with High RH increased liver mass at pipping whereas those with Low G in High RH increased liver mass later in development. The differences suggest several possibilities. Two possible causes may be the longer developmental period or the amount of residual yolk remaining in eggs of different G. More residual yolk was in Low than in Hi G eggs when development was prolonged. Thus, slower retraction and absorption of more residual yolk in the Low G eggs may affect the weight of lipids found in the liver. Alternatively, longer incubation periods under the conditions of Low G may increase glycogen depletion and decrease the weight of stored hepatic glycogen in Low compared to Hi G.

Turkey embryo plasma thyroid concentrations increase developmentally as the hypothalamus matures (Christensen and Biellier, 1982). The peak coincides with the plateau stage in oxygen consumption identified by the hypoxic and hypercapnic condition created for embryos (Rahn, 1981). Embryo thyroids from different genetic lines respond differently to iodide and the length of the incubation period (Christensen *et al.*, 2002). Carbon dioxide has also been proposed as a stimulus for chick embryo thyroid hormone release (Buys *et al.*, 1998). High temperature and low oxygen concentrations elicit embryonic thyroid responses as well (Christensen *et al.*, 2005). Our data show clearly that G or the incubation period may be factors for elevating thyroid hormones.

In conclusion, the data are the first evidence known to the authors showing the critical importance of eggshell G in the production of turkeys. Both survival and growth were shown to be related to G. The effects of G were seen as long as 6 weeks following hatching. The results from thyroid hormones suggest they may mediate the effects seen.

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