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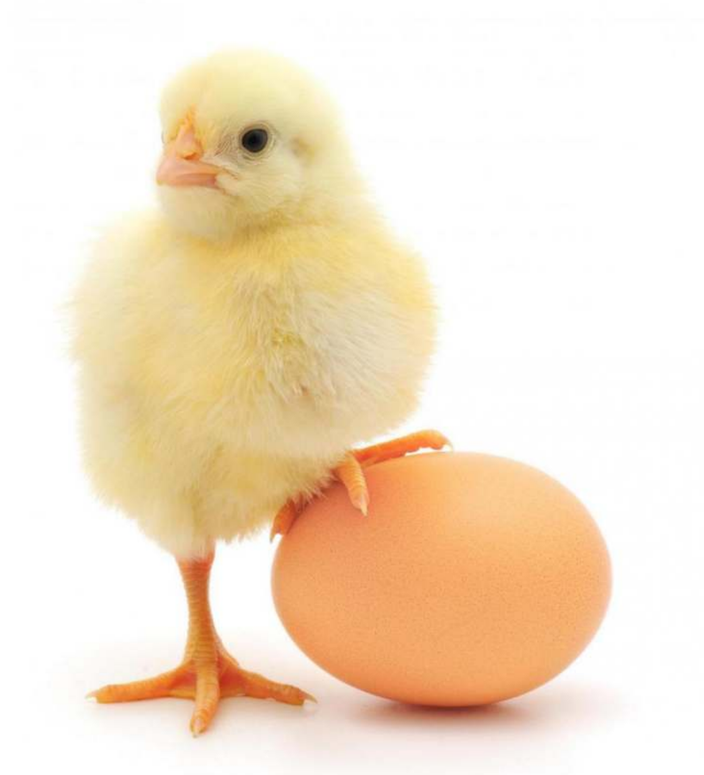

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Eggshell Conductance and Incubator Ventilation as Factors in Embryo Survival and Poult Quality[1]

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Abstract: Eggshell conductance (G) and incubator ventilation (VENT) were hypothesized to affect embryo viability and growth of poults following hatching. Nearly 6,000 eggs were weighed on the day of oviposition to determine eggs of like weight but of different G. From the 6,000 eggs, 4,000 were selected that were within 2 standard deviations of the mean. The eggs were randomly divided equally between two incubator cabinets. One cabinet operated with a closed VENT and a second operated with it open. 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 that time exhibiting high (Hi), average (Avg) or low (Low) G. The eggs within each group were placed into hatching trays of 100 eggs each and placed into he same incubation cabinet for hatching. Weights were recorded for cardiac, hepatic and intestinal tissues, and blood was collected from each treatment. The tissues were subsequently assayed for energy substrates. Embryo viability was noted and growth was observed up to 6 wk of age. More embryos in eggs of Hi or Avg G survived than did those in Low G eggs, but neonates at 6 wk from Hi G eggs weighed less than those from Avg or Low G eggs. Low G embryos had reduced heart, liver and intestinal weight and function. Embryo thyroid hormone concentrations were elevated in Hi G eggs but suppressed by Low G and Closed VENT. Thus, in the developmental process of the embryonic turkey, G may determine energy balance and maturity of each hatchling and may affect its survival and growth rates following hatching.

Key words: Poultry quality, embryo survival, incubator ventilation, eggshell conductance

Introduction

The functional property of an eggs called eggshell conductance (G) ensures three requirements are met for successful hatching (Rahn, 1981). The first requirement is that an egg loses approximately 15% of the initial egg mass as water vapor. The remaining two requirements pertain to the uptake of oxygen to drive metabolism during embryo growth and development or the output of carbon dioxide as a byproduct of that growth and development. Rahn (1981) described the remaining requirements as follows: 2. the total amount of oxygen that will have been consumed is about 100 mL/g of egg weight, and 3. the oxygen concentration in the air cell shortly before pipping will have fallen to 14% while that of carbon dioxide should increase to about 6%. Little is known about reduced ventilation in the incubator cabinet and its interaction with G and the effect on embryo survival.

Ar and Rahn (1978) indicated that G must be matched to the initial egg mass and the length of the incubation period to determine the conductance constant (k). Across avian species, the product of G and the length of the incubation period were directly proportional to k while egg mass was inversely related. They proposed that k = 5.13 would create a hatchling of the characteristic maturity of the species. Thus, k greater or smaller than 5.13 may create hatchlings of lesser quality. The current studies tested the hypothesis that turkey eggs of the same weight but of different k may interact with the ventilation of vital gases in the incubator cabinet to create offspring of varying maturities.

Materials and Methods

Approximately 6,000 fertilized turkey eggs were weighed (nearest 0.01 g) individually on the day of oviposition to obtain a population of eggs of 4,000 eggs of the same weight. Eggs were selected to represent eggs of the same weight (± 2 standard deviations), and the weights were used additionally to measure G of each egg (Tullett, 1981). A commercial flock of turkey breeders (Hybrid, Inc., Kitchner, ON) in its 21st week of lay produced the eggs. Selected eggs were transported to the hatchery where they were set randomly into two incubator cabinets (Natureform I 14, Jacksonville, FL). Both cabinets operated at a dry bulb temperature set point of 37.5^{o}C and a relative humidity set point of 50%. The vent on one cabinet was left open (OPN), but it was closed (CLS) on the second machine to alter the gas

[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]To whom correspondence should be addressed.

concentrations inside the cabinet. The eggs were distributed randomly between the two cabinets until the 25th day of development. At the beginning of the 25th day, all eggs were weighed a second time to determine the G of each individual egg (Tullett, 1981). G values were evaluated using the computer assisted program to identify High (Hi), Average (Avg) and Low (Low) G groups. Each of the three groups was transferred randomly to the same machine for hatching. The machine operated at a dry bulb set point of 36.9°C and a RH of 75%.

The length of the incubation period for all three G groups was measured by noting times of hatching at 3 h intervals during days 27 and 28 of development. Embryo survival was measured by counting the number of poults hatching from replicate trays of 100 eggs each. All eggs that did not hatch were broken open and examined following 28 days of development to estimate by inspection the time at which the embryo died. Tray data were used to calculate embryo survival rates and times of embryo death.

Tissue sampling: Tissues were sampled at days 27 and 28 of development from 10 randomly selected embryos (or poults) from each of the G by VENT treatments. Blood was collected following decapitation into a vial containing 10 mg EDTA. The blood was immediately centrifuged at 700xg for 15 minutes under refrigeration (4°C). The body and residual yolk were weighed (nearest 0.01 g) then the heart, liver and jejunum were quickly dissected and weighed (nearest 0.0001 g). Following weighing, the heart, liver and jejunum were quickly placed into a vial containing cold 7% perchloric acid and stored at 4°C preparatory to analysis for glycogen and lactate concentrations. The jejunum was dissected and weighed similarly then placed into a vial containing physiological saline and frozen (-22°C) immediately preparatory to analysis for maltase and alkaline phosphatase. The blood plasma was recovered following centrifugation, was placed in vials and frozen (-22°C) preparatory to analysis for glucose and thyroid hormone concentrations. Tissue glycogen and lactate concentrations and plasma glucose and lactate concentrations were determined as described previously (Christensen *et al.*, 2003a). Analyses for intestinal enzymes have also been described previously (Christensen *et al.*, 2003b). Thyroid hormone concentrations were measured using the procedures described for T_3 and T_4 by Christensen *et al.* (2002).

Carcasses from each of the treatments were dried (37.5°C for 2 days) to determine the percentage of water in each carcass. 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 the drying process so differences in percentage water were measured between the two compartments.

Growth of the hatchlings: At hatching, poults were separated by sex and wing bands were applied to identify birds by treatment. The hen poults were placed in an adjacent brooder house. The hens were grown to 6 weeks of age, and body weight and feed conversion ratios were measured at weekly intervals to determine long-term incubator effects on the growth and well-being of the poults. The house was 19 x 48 m and contained 36 pens housing 12 hens each. Feed and water were provided *ad libitum*. Pen was the experimental unit for the measurement of feed consumption and feed conversion ratios, and the individual birds were the units for body weights.

Statistical analysis: Data were analyzed as a 2 levels of ventilation (CLS or OPN) by 3 levels of G (Hi, Avg, and Low) factorial arrangement of a completely random experimental design using the general linear models procedure (SAS Institute, 1998). Means determined to differ significantly ($P < 0.05$) were separated using the least square means procedure.

Results

Neither VENT nor G affected embryo weights at 27 days of development, but at hatching Avg G eggs produced heavier poults than did either Hi or Low (Tables 1). Residual yolk at 27 days of development weighed more in Low eggs than Avg or Hi eggs, and Avg G embryos had significantly heavier yolks than Hi G eggs. At hatching CLS resulted in less yolk in poults than did the OPN, and Low G poults had significantly more residual yolk than either Hi or Avg G.

Neither VENT nor G caused differences in body weights at 3 weeks of age; however, by 6 weeks, the poults from the OPN VENT incubator were heavier than those from CLS, and High G poults weighed significantly less than those of Avg or Low G (Table 2). There were no differences in posthatching BW due to the VENT.

Differences in heart weights paralleled those of BW except in one case (Table 3). Heart weight relative to BW at 27 days of development was significantly heavier in Avg G eggs than either Hi or Low. Otherwise, absolute heart weight was similar to BW.

Liver weight was affected by both VENT and G (Table 4). CLS increased liver weights at 27 days of development compared to OPN, and Hi G increased liver weights compared to Low G but not Avg G. At hatching VENT and G interacted such that eggs of different G values acted differently depending upon the VENT. Eggs with Avg G had the smallest livers in CLS but had the larger livers in OPN compared to Hi or Low G.

Hi G increased jejunum weight at both 27 and 28 d of development compared to Avg or Low G (Table 5), but VENT had no effect on the weight of the intestine in the current study. Relative to the BW, jejunum weights increased stepwise as G increased from Low to Avg to Hi G.

Table 1: Body and yolk weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
		Body weight without yolk	
Closed	Hi	45.9	46.7
	Avg	47.4	48.4
	Low	47.9	46.7
	Mean		
Open	Hi	49.0	45.8
	Avg	49.1	48.9
	Low	47.8	46.8
	Mean		
G means	Hi		46.3[b]
	Avg		48.6[a]
	Low		46.7[b]
Mean±SEM		48.0±0.3	47.2±0.3
Probabilities	Vent opening	NS	NS
	G	NS	0.03
	Vent x G	NS	0.04
		Yolk	
Closed	Hi	6.1	5.7
	Avg	11.7	6.5
	Low	11.7	10.0
	Mean		7.4[b]
Open	Hi	7.6	6.4
	Avg	9.3	6.9
	Low	12.9	11.1
	Mean		8.1[a]
G means	Hi	6.9[c]	6.0[b]
	Avg	10.5[b]	6.7[b]
	Low	12.4[a]	10.5[a]
Mean±SEM		9.7±0.3	7.2±0.2
Probabilities	Vent opening	NS	0.05
	G	0.0001	0.0001
	Vent x G	NS	NS

Table 6 shows elevated cardiac glycogen in Avg G eggs compared to Hi or Low G at 27 days of development. Cardiac lactate concentration was elevated at the same stage in Hi G eggs compared to Avg and Low G. CLS vents on the cabinet reduced cardiac glycogen and elevated lactate concentrations compared to OPN. At hatching a VENT by G interaction affected both cardiac glycogen and lactate. Hatchlings from Low G eggs displayed elevated cardiac glycogen and reduced lactate concentrations in the CLS cabinet but not in the OPN compared to all other treatments. At hatching the CLS cabinet and Low G eggs poults displayed greater cardiac lactate concentrations than did Hi or Avg G. No differences were noted in the OPN cabinet.

Hepatic glycogen concentrations were also affected by both VENT and G (Table 7). At 27 days CLS reduced hepatic glycogen and elevated lactate concentrations compared to OPN, and Avg G elevated poult hepatic glycogen compared to Hi or Low G. Hepatic lactate at day 27 was elevated in Hi G poults compared to Low or

Table 2: Weights (g) of poults from eggs of high (Hi), average (Avg) and low (Low) eggshell conductance when incubated with Closed or Open vents

		Weeks of age	
Vent opening	Conductance	3	6
Closed	Hi	637	2,351
	Avg	635	2,234
	Low	579	2,067
	Mean	617[b]	2,218[b]
Open	Hi	648	2,344
	Avg	665	2,383
	Low	631	2,245
	Mean	648[a]	2,348[a]
	Conductance means		
	Hi		2,156[b]
	Avg		2,309[a]
	Low		2,348[a]
Mean ± SEM		633±9	2,274±23
Probabilities	Vent opening	0.10	0.07
	Conductance (G)	NS	0.05
	Vent x G	NS	NS

Avg G. At hatching, a VENT by G interaction was noted as CLS Low G poults elevated hepatic lactate compared to all other treatments. OPN poults from Hi G increased lactate concentrations compared to Avg G with Low G eggs being intermediate.

Both maltase and ALP activity are indicative of intestinal functional maturity and metabolic activity (Moog, 1950). Both VENT and G affected total jejunum maltase and ALP (Table 8 and 9), but only Low G reduced jejunum weights relative to BW compared to Avg and Hi (Table 4) at each stage of development. Low G embryos reduced total maltase activity but not specific activity compared to Avg or Hi G eggs whereas total ALP and specific ALP activity both were elevated in Hi G embryos compared to Low G, and total ALP activity in Hi G eggs was greater than Avg G eggs, but specific ALP activity was not. At day 28, VENT and G interacted to depress total maltase in poults hatching in CLS from Low G compared to Avg and Hi G poults, but specific maltase was elevated in Hi G poults compared to Low G poults with Avg poults being intermediate. In the OPN cabinet, Avg G poults elevated total and specific maltase activity compared to either Hi or Low G. Jejunum ALP activity in hatched poults from the CLS vent machine and Low G eggs displayed elevated total ALP activity compared to Hi and Avg G eggs. In the OPN machine, Low G eggs displayed the lowest total ALP activity compared to Hi and Avg G eggs. Specific ALP activity displayed the exact opposite pattern. Neither incubator VENT nor G changed blood plasma glucose or lactate concentrations in the current study (Data not shown). However, both VENT and G affected thyroid hormone concentrations (Table 10). Hi G elevated thyroxine (T_4) at 27 days of development compared to Low G but not Avg G, but no differences were noted at day 28. The CLS treatment reduced triiodothyronine (T_3) concentrations compared to the

Table 3: Heart weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
		Absolute weight (mg)	
Closed	Hi	231	288
	Avg	258	288
	Low	228	285
	Mean	239[b]	
Open	Hi	242	274
	Avg	275	304
	Low	234	259
	Mean	250[a]	
G means	Hi	237[b]	281[ab]
	Avg	266[a]	297[a]
	Low	231[b]	272[b]
Mean ± SEM		243 ± 3	287±3
Probabilities	Vent opening	0.01	NS
	G	0.0003	0.0001
	Vent x G	NS	NS
		Relative weight (%)	
Closed	Hi	0.51	0.60
	Avg	055	0.63
	Low	0.47	0.55
	Mean		
Open	Hi	0.49	0.62
	Avg	0.56	0.60
	Low	0.49	0.61
	Mean		
G means	Hi	0.50[b]	
	Avg	0.55[a]	
	Low	0.48[b]	
Mean ± SEM		0.51±0.01	0.60±0.01
Probabilities	Vent opening	NS	NS
	G	0.002	NS
	Vent x G	NS	NS

Table 4: Liver weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
		Absolute weight (mg)	
Closed	Hi	972	1,180[ab]
	Avg	1,034	1,146[bc]
	Low	853	1,128[cd]
	Mean		
Open	Hi	972	1,072[d]
	Avg	865	1.215[a]
	Low	878	1,015[e]
	Mean		
G means	Hi	973[a]	
	Avg	949[a]	
	Low	865[b]	
Mean ± SEM		914±12	1.126±12
Probabilities	Vent opening	NS	0.01
	G	0.02	0.0003
	Vent x G	NS	0.0005
		Relative weight (%)	
Closed	Hi	2.13	2.54[a]
	Avg	2.18	2.37[b]
	Low	1.78	2.42[a]
	Mean	2.03[a]	
Open	Hi	1.99	2.34[b]
	Avg	1.76	2.49[a]
	Low	1.84	2.17[c]
	Mean	1.86[b]	
G means	Hi	2.05[a]	
	Avg	1.97[ab]	
	Low	1.81[b]	
Mean ± SEM		2.13±0.03	2.39±0.02
Probabilities	Vent opening	0.03	0.02
	G	0.01	0.02
	Vent x G	0.07	0.003

OPN on day 27, but the opposite was observed on day 28 as CLS elevated T_3 compared to OPN. At hatching G also affected T_3 concentrations Low G depressed T_3 in poults compared to Hi and Avg G eggs.

CLS treated embryos hatched earlier, had lower survival rates, and grew slower than OPN, yet Table 2 shows heavier embryo weights (without residual yolk) in the CLS machine than in the OPN. Low G eggs with lower survival rates and depressed growth posthatching had heavier embryo weights than Avg or Hi G eggs regardless of the VENT.

In contrast to the observations with embryos, poults at hatching showed an incubator VENT by G interaction for BW as Low and Avg G eggs increased hatchling BW compared to Hi. In the OPN only weights of hatchlings from Avg G eggs were increased compared to Hi or Low G eggs. CLS decreased the residual 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 decreased residual yolk weight compared to the other G treatments. The weight of the poult relative to the initial egg mass did not differ between incubator VENT, but poults from Low G eggs hatched at 71.0% of the initial egg mass, Avg G eggs hatched at 69.1% and Hi G eggs hatched at 65.7% of the initial egg mass. All percentages differed significantly from each other (Overall mean ± SEM = 68.6% ± 0.1). CLS increased the percentage of body moisture by about 1% compared to OPN, 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).

Discussion

The growth and feed conversion measured in the current study indicate that poults hatching from Hi G eggs have impaired ability to grow and convert feed to muscle tissue, but poults hatching from Low G eggs do not when compared to Avg G eggs. Embryo survival also depended upon G as fewer embryos in Low G egg survived to hatching than did those in Hi and Avg G eggs. The data show clearly that eggshell conductance (or its consequent length of the incubation period) may be

Table 5: Jejunum weights of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
		Absolute weight (mg)	
Closed	Hi	329	373
	Avg	260	359
	Low	263	333
	Mean		
Open	Hi	312	347
	Avg	290	362
	Low	249	301
	Mean		
G means	Hi	321[a]	360[a]
	Avg	275[b]	360[a]
	Low	256[b]	317[b]
Mean ± SEM		286 ± 6	345±6
Probabilities	Vent opening	NS	NS
	G	0.003	0.01
	Vent x G	NS	NS
		Relative weight (%)	
Closed	Hi	0.71	0.80
	Avg	0.55	0.74
	Low	0.55	0.71
	Mean		
Open	Hi	0.64	0.75
	Avg	0.59	0.74
	Low	0.52	0.64
	Mean		
G means	Hi	0.67[a]	0.78[a]
	Avg	0.57[b]	0.74[a]
	Low	0.53[b]	0.67[b]
Mean ± SEM		0.60±0.01	0.73±0.01
Probabilities	Vent opening	NS	NS
	G	0.001	0.01
	Vent x G	NS	NS

Table 6: Cardiac glycogen and lactate concentrations of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
Glycogen (mg/g of wet tissue mass)			
Closed	Hi	2.20	2.17[ab]
	Avg	2.71	2.14[ab]
	Low	2.13	2.16[ab]
	Mean	2.35[b]	
Open	Hi	2.38	1.96[b]
	Avg	3.39	1.94[b]
	Low	2.82	2.58[a]
	Mean	2.85[a]	
G means	Hi	2.29[b]	
	Avg	3.05[a]	
	Low	2.47[b]	
Mean±SEM		2.60±0.09	2.16±0.08
Probabilities	Vent opening	0.03	NS
	G	0.05	NS
	Vent x G	NS	0.07
Lactate (mg/g of wet tissue mass)			
Closed	Hi	2.07	1.61[cd]
	Avg	1.44	1.58[d]
	Low	1.69	2.16[a]
	Mean	1.73[a]	
Open	Hi	1.53	2.03[ab]
	Avg	1.17	1.84[b]
	Low	1.04	1.73[c]
	Mean	1.25[b]	
G means	Hi	1.80[a]	
	Avg	1.30[b]	
	Low	1.36[b]	
Mean ± SEM		1.48±0.04	1.83±0.03
Probabilities	Vent opening	0.0001	NS
	G	0.0001	0.05
	Vent x G	NS	0.0001

major components of fitness of turkey poults and their subsequent growth. The current data also support previous observations (Christensen *et al.*, 2003ab) suggesting that G affects mechanisms optimizing the maturation of the cardiovascular and digestive systems. When similar observations were made in the prior studies (Christensen *et al.*, 2003ab), it was concluded that a combination of egg size and conductance affected each system. Large eggs in combination with low eggshell conductance or prolonged length of the incubation period depressed intestinal maturation, impaired cardiac function and BW at hatching (Christensen *et al.*, 2003a), but in the current study, the changes were independent of egg weight.

The functional property of eggshells (G) affected nearly every physiological variable measured in the current study. When the vents on the incubator cabinets were closed, some interaction with G occurred, but G and VENT affected the embryo primarily independently. The concept of eggshell conductance as the functional quality of an egg indicates that three conditions must be satisfied at the time the embryo attains the plateau stage in oxygen consumption (Rahn, 1981). The conditions are: 1) the egg must have lost approximately 15% of its initial mass as water vapor. 2) embryos must have consumed 100mL oxygen/g, and 3) air cell concentrations of oxygen must have fallen to 14% and the carbon dioxide concentration must have increased to 6%. This study examined the second and third requirements specified by Rahn (1981), i.e. that of oxygen uptake and carbon dioxide output while the first requirement was examined in the later study (Christensen *et al.*, unpublished data),

The well-being of an animal is complex and involves integration of various physiological systems by neural and endocrine factors. Previous studies have shown that k may affect the cardiovascular and digestive systems (Christensen *et al.*, 2003a; 2003b). Data from the current study indicate that reduced G affected the well-being of an embryo by suppressing both T_4 and T_3 in the circulation as well. Closed VENT in an incubator cabinet also suppressed thyroid hormone concentrations indicating additive effects augmenting the deleterious

Table 7: Hepatic glycogen and lactate of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
Glycogen (mg/g of wet tissue mass)			
Closed	Hi	2.14	2.11
	Avg	2.72	2.17
	Low	2.32	2.19
	Mean	2.39[b]	
Open	Hi	2.43	1.99
	Avg	4.46	1.95
	Low	3.01	2.63
	Mean	3.29[a]	
G means	Hi	2.28[b]	
	Avg	3.58[a]	
	Low	2.67[b]	
Mean ± SEM		2.88±0.12	2.17±0.08
Probabilities	Vent opening	0.004	NS
	G	0.007	NS
	Vent x G	NS	NS
Lactate (mg/g of wet tissue mass)			
Closed	Hi	0.19	0.16[c]
	Avg	0.17	0.16[c]
	Low	0.19	0.21[a]
	Mean	0.18[a]	
Open	Hi	0.18	0.20[ab]
	Avg	0.15	0.15[c]
	Low	0.13	0.17[bc]
	Mean	0.15[b]	
G means	Hi	0.19[a]	
	Avg	0.16[b]	
	Low	0.16[b]	
Mean ± SEM		0.17±0.01	0.17±0.01
Probabilities	Vent opening	0.03	NS
	G	0.05	0.006
	Vent x G	NS	0.01

Table 8: Jejunum maltase activity of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
Total maltase activity (imol/min/jejunum)			
Closed	Hi	133	297[a]
	Avg	134	262[a]
	Low	104	194[b]
	Mean		
Open	Hi	125	180[b]
	Avg	109	281[a]
	Low	79	110[c]
	Mean		
G means	Hi	129[a]	
	Avg	121[ab]	
	Low	91[b]	
Mean ± SEM		111±6	221±12
Probabilities	Vent opening	NS	0.02
	G	0.05	0.001
	Vent x G	NS	0.008
Specific malatase activity (imol/min/ig of protein)			
Closed	Hi	5.5	10.5[a]
	Avg	6.1	9.5[ab]
	Low	5.2	8.1[bc]
	Mean		
Open	Hi	5.8	6.6[c]
	Avg	7.5	10.0[a]
	Low	5.8	5.7[c]
	Mean		
Mean ± SEM		5.8±0.3	8.4±0.3
Probabilities	Vent opening	NS	0.01
	G	NS	0.01
	Vent x G	NS	0.05

effects of low G and perhaps aiding Hi G eggs.

The k is calculated by dividing the product of G and the incubation period in days by the egg weight in g (Ar and Rahn, 1978), so the well-being of a poult may be influenced by G, the length of the incubation or by egg weight. In nature, the measurements are critical because they determine offspring survival by the characteristic maturity at hatching and subsequent survival of a species (Ricklefs and Starck, 1998). In the current study, egg weight was kept constant by selecting similar eggs so only G and the consequent length of the incubation period were variables. Incubation conditions can also be adjusted to fit k and each type of shell (Christensen *et al.*, 2001) to enhance embryo survival.

Cardiac physiology: Low G created a longer period of time for 100 mL of oxygen/ g of egg weight to diffuse across the shell and drive metabolism whereas Hi G created a shorter incubation period to provide adequate oxygen. Thus, it is highly likely that some embryos utilize gluconeogenesis to a greater extent, especially at the plateau stage in oxygen consumption (Rahn, 1981). Immediately following the plateau stage, both Hi and Low G depressed cardiac and hepatic glycogen, but at hatching only a VENT by G interaction affected cardiac glycogen. When VENT was CLS, all G groups were equivalent. When the VENT was OPN, hatchlings from Low G eggs elevated glycogen. Elevated glycogen may be a symptom of cardiomyopathy (Czarnecki and Evanson, 1980; Czarnecki, 1991). Cardiac lactate levels showed the opposite response to glycogen. When glycogen was elevated, lactate was depressed. Thus, turkey embryos developing in large eggs with low G showed symptoms of muscle fatigue and perhaps of cardiomyopathy (Christensen *et al.*, 2003a). In contrast to our results, Gadzinski *et al.* (1993) saw no correlation between eggshell oxygen permeability and the development of cardiomyopathy in turkeys. In the current study, G affected not only cardiac glycogen adversely, but in the current study, embryos pipping the shell in eggs with Low or Hi G suffered heart weight reduction as well. By hatching, embryos in Hi G eggs recovered and weighed as much as Avg G eggs, but not those in Low G eggs. CLS VENT reduced heart weight at pipping as well, but compensation in heart weights also occurred

Table 9: Jejunum alkaline phosphatase (ALP) activity of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
Total ALP activity (imol/min/jejunum)			
Closed	Hi	3,507	7,629^{b}
	Avg	2,373	7,606^{b}
	Low	1,814	12,493^{a}
	Mean		
Open	Hi	3,075	14,173^{a}
	Avg	2,001	13,781^{a}
	Low	1,584	3,860^{c}
	Mean		
G means	Hi	3,291^{a}	
	Avg	2,187^{b}	
	Low	1,699^{b}	
Mean ± SEM		2,373±176	9,923±409
Probabilities	Vent opening	NS	NS
	G	0.003	0.01
	Vent x G	NS	0.0001
Specific ALP activity (imol/min/ig of protein)			
Closed	Hi	0.15	0.56^{a}
	Avg	0.14	0.57^{a}
	Low	0.10	0.18^{b}
	Mean		
Open	Hi	0.14	0.27^{b}
	Avg	0.11	0.27^{b}
	Low	0.10	0.52^{a}
	Mean		
G means	Hi	0.15^{a}	
	Avg	0.12^{ab}	
	Low	0.10^{b}	
Mean ± SEM		0.12±0.01	21.1±0.5
Probabilities	Vent opening	NS	0.07
	G	0.07	NS
	Vent x G	NS	0.0001

Table 10: Plasma thyroid hormone concentrations of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Days of development	
Vent opening	G	27	28
		Triiodothyronine (ng/mL)	
Closed	Hi	11.6	8.3
	Avg	4.9	6.6
	Low	5.6	5.5
Open	Hi	8.6	7.6
	Avg	9.5	8.3
	Low	6.9	7.5
G means	Hi	10.1^{a}	
	Avg	7.2^{ab}	
	Low	6.2^{b}	
Mean ± SEM		8.1±0.6	7.3±0.3
Probabilities	Vent opening	NS	NS
	G	0.05	NS
	Vent x G	NS	NS
		Thyroxine (ng/mL)	
Closed	Hi	4..5	8.3
	Avg	5.1	6.4
	Low	7.0	4.9
	Mean	5.5^{b}	6.5^{a}
Open	Hi	10.5	6.5
	Avg	14.3	5.4
	Low	10.1	4.2
	Mean	11.6^{a}	4.9^{b}
G means	Hi		6.7^{a}
	Avg		5.9^{a}
	Low		4.5^{b}
Mean ± SEM		9.2±1.0	5.7±0.2
Probabilities	Vent opening	0.02	0.001
	G	NS	0.02
	Vent x G	NS	0.08

prior to hatching. Low heart weight may reflect the effects of a delayed or prolonged plateau stage in oxygen consumption for eggs of differing G as they struggle to stay alive.

Hepatic physiology. 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 VENT and G on liver weight and physiology were complex. Hepatic tissue was obviously adjusting to a unique nutrient storage and metabolism within each egg that would assure the survival and growth of embryos emerging from eggs of different functional qualities. Embryos in Hi G eggs with CLS increased liver mass at pipping whereas those with increasingly lower G in CLS increased liver mass later in development. Assuming that these weight differences represent nutrient storage suggest the differences may be interpreted several ways. The length of the developmental period may alter the amount of residual yolk in eggs of different G. Low G eggs (longer incubation period) showed more residual yolk than Hi. Thus, slower retraction and absorption of residual yolk in the Low G eggs may decrease the weight of lipids found in the liver. Alternatively, longer incubation periods under the conditions of Low G may increase depletion of stored hepatic glycogen in embryos compared to Hi thus reducing glycogen and consequent liver weight.

Digestive physiology. 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 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 process significant amounts of carbohydrates, gluconeogenesis must be the primary source of energy until the intestine is mature (Donaldson and Christensen, 1991). Gluconeogenesis can require catabolism of existing tissues or additional catabolism of critical nutrients in residual yolk. If greater maturation could be attained prior to hatching, the poult may be better able to perform the prehension and digestive functions characterizing precocity thus precluding the requirement for gluconeogenesis. Eggs

Table 11: Plasma thyroid hormone T_4 to T_3 ratios of poult embryos in eggs of high (Hi), average (Avg) and low (Low) eggshell conductance (G) when incubated with Closed or Open vents

		Day of development	
Vent opening	G	27	28
Closed	Hi	3.44	1.90[a]
	Avg	1.27	1.33[b]
	Low	0.97	1.30[b]
	Mean		1.89[a]
Open	Hi	1.58	1.07[c]
	Avg	0.94	1.43[b]
	Low	0.73	1.71[a]
	Mean		1.08[b]
G means	Hi		2.51[a]
	Avg		1.11[b]
	Low		0.85[b]
Mean ± SEM		1.48±0.14	1.46±0.09
Probabilities	Vent opening	0.05	NS
	G	0.001	NS
	Vent x G	NS	0.03

Table 12: Time of hatching (hours of incubation) of poult embryos in eggs of high (Hi), average (Avg) and low (Lo) eggshell conductance when incubated with Closed or Open vents

Vent opening	Conductance (G)	Time
Closed	Hi	612
	Avg	614
	Lo	618
	Mean	615[b]
Open	Hi	613
	Avg	615
	Lo	622
	Mean	617[a]
	Conductance means	
	Hi	613[c]
	Avg	615[b]
	Lo	620[a]
Mean ± SEM	615 ± 1	
Probabilities		
Vent	0.0001	
Conductance	0.003	
H x G	NS	

Table 13: Poult yield (hatchling weight/initial egg mass) of from eggs of high (Hi), average (Avg) and low (Lo) eggshell conductance when incubated with Closed or Open vents

Vent opening	Conductance (G)	Yield (%)
Closed	Low	70.1
	Avg	69.0
	High	65.6
	Mean	
Open	Low	72.0
	Avg	69.2
	High	65.7
	Mean	
Conductance means		
	High	65.7[c]
	Avg	69.1[b]
	Low	71.0[a]
Mean ± SEM	68.6 ± 0.1	
Probabilities		
Vent	NS	
Conductance	0.0001	
H x G	NS	

Table 14: Hatchability (% of fertile eggs) of eggs of high (Hi), average (Avg) and low (Lo) eggshell conductance when incubated with Closed or Open vents

Vent opening	Conductance (G)	Hatchability (%)
Closed	Low	70.3
	Avg	91.3
	High	89.4
	Mean	83.5[b]
Open	Low	81.4
	Avg	95.9
	High	94.1
	Mean	90.4[a]
	Conductance means	
	High	91.7[a]
	Avg	93.6[a]
	Low	75.8[b]
Mean ± SEM	87.0 ± 0.9	
Probabilities		
Vent	0.03	
Conductance	0.0009	
H x G	NS	

with Hi G had greater jejunum weight and function compared to the other groups, but greater intestinal mass did not result in better growth or feed conversion following hatching. The contradictory data from growth and feed conversion indicate that poults hatching from Hi G eggs may have permanently impaired some physiological system affecting the ability to grow and convert feed to muscle tissue. Poults hatching from Low G eggs weighed more than the remaining G treatments but had less jejunum tissue relative to BW as had been noted previously (Suvarna *et al.*, 2004).

Thyroid function: Buys *et al.* (1998) closed the vents on an incubator containing two lines of broiler embryos and increased incidence of ascities as well as suppressed embryo thyroid output. Hi G consistently elevated T_3 and T_4, and Low G depressed both hormones. CLS elevated T_4 consistently across all G values in agreement with Buys *et al.* (1998). Thus, reduced embryo viability and neonate growth may be associated with thyroid function. Weak poults display depressed T_4 concentrations (Czarnecki, 1991; Christensen *et al.*, 2003c). Thyroid hormones enhance the development of homeothermic thermoregulation chick embryos (Nichelmann *et al.*, 2001) and high incubator temperature, and low oxygen concentrations can suppress both T_3 and T_4 concentrations (Christensen *et al.*, 2002). Both hormones are also involved in numerous maturational processes like cardiac and intestinal function prior to hatching. Therefore, it is concluded that reduced embryo survival and postnatal growth in the current study may have been associated with thyroid effects on an unknown tissue.

We conclude that current data are the first evidence known to the authors showing the critical importance of G in the production of poultry. Both embryo survival and subsequent growth were related to G, and VENT on the incubator was additive to the G effects. The long-term effects on growth were unexpected and may be mediated systemically by the thyroid (Buys *et al.*, 1998; Christensen *et al.*, 2003c).

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