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Shell Thickness of Turkey Eggs Affects Cardiac Physiology and Embryo Survival[1]

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Abstract: Supplementing 500 ppm of a chelated calcium proteinate (CCP) to a commercial breeder diet resulted in thicker shells and improved embryo livability. The CCP diet was fed to one half of a flock of breeders on a commercial farm that was suffering shell problems, and a standard commercial diet was fed to the remaining half. Egg production, eggshell thickness, fertility and hatchability of eggs were all monitored over an 18 wk laying period. Feeding CCP increased shell thickness and reduced numbers of cull eggs after 8 wk of lay compared to the controls. When differences in eggshell thickness were seen after 10 weeks of egg production, embryo survival and cardiac physiology were examined in three trials comparing the thicker shells to thin. Thick shells (0.44 versus 0.39 mm) improved embryo survival 2% by decreasing numbers of embryos dying late in development compared to controls and affected cardiac physiology. Thus, thick shells may improve embryo viability by affecting cardiac health during the plateau stage in oxygen consumption.

Key words: Shell thickness, embryo survival, cardiac physiology

Introduction

Prior research (Grimes *et al*., 2004) indicated that feeding a chelated calcium proteinate supplement (CCP) to turkey breeder hen diets improved embryo survival in aging turkey breeder hens. The reason given was that shell quality may have been improved late in the laying period, but it is not known how shell thickness affects embryo survival. In subsequent studies (Christensen *et al*., 2003; Christensen *et al*., 2005), the functional property of the shell, embryo cardiac physiology and poult growth and quality for 3 d post-hatching were all improved following the addition of CCP to the maternal diet. Eggshell conductance increased with maternally fed CCP accompanied by larger neonate hearts and reduced heart rates (Christensen *et al*., 2005). The myocardia of embryos displayed lower concentrations of lactate indicating less myocardial fatigue during pipping of the shell and hatching. Other studies have shown that elevated cardiac glycogen as well as increased CK activity is typical of cardiomyopathy in poults (Czarnecki, 1991). The objective of the current study was to determine the effect of thicker shells on embryo cardiac physiology, survival, and poult quality in a commercial setting.

Materials and Methods

A flock of 20,000 Large White hens of the Orlopp strain was kept on a commercial breeder farm. Half of the flock was assigned randomly to be fed a diet supplemented with 3.11% calcite and 0.05% Calkey® (Chelated Minerals Corporation, Salt Lake City, UT) (CCP). The remaining breeders were fed the standard diet of the company containing 3.16% calcite. All diets were available *ad libitum* beginning at photostimulation (29 wk of age). The period of egg laying for the study began in May and terminated in September; therefore, eggs were produced in hot weather so egg production rates were depressed and shells were thinner. Because of requirements for the hatchery, eggs for research were available for only 18 weeks. Egg production and shell thicknesses were recorded on a weekly basis for 18 wk. Because no replication of dietary treatments was possible, no statistical analysis was performed on egg production data and no inferences can be made about the diets.

Eggshell thickness was measured for 1,096 eggs during initial 14 wk of the study. Approximately 50 eggs were selected randomly weekly from each treatment, broken open, the albumen was rinsed away with water and the shell with membrane intact was air dried. A piston micrometer (Ames, Amherst, MA) was used to measure the thickness at 4 points at the equator of each egg. Individual shells were the experimental unit.

Following determination that birds fed CCP produced thicker shells. Embryo physiology and survival in eggs of thick (THCK) and thin (THIN) shells was tested at weeks 10, 14 and 18 of egg production. Approximately 5,000 eggs from each shell thickness group were set at the same time in a commercial hatchery. Twenty eggs from each group were selected within each setting to verify eggshell thicknesses. In all three settings, eggs from the CCP fed house showed thicker shells (10 wk, THCK = 0.44 mm; THIN = 0.41mm; 14 wk THCK = 0.44 mm, THIN = 0.37 mm; 18 wk, THCK = 0.43 mm; THIN = 0.36

mm). Within each trial, eggs of both thicknesses were set randomly distributed in the same cabinets and incubated under the normal temperature and humidity profiles used by the hatchery. Oxygen was supplemented to the incubators at a rate of 11 liters per min because the eggs were incubated at high altitude (ca. 3000 m). All eggs were transferred at d 25 of development to the same cabinet for hatching. At the time of transfer, 136 eggs were placed randomly into each of the hatching trays, and the embryo survival on each tray was measured. Trays (39/thickness/trial) served as the experimental unit for the embryo survival analysis. The number of poults hatching on each tray was counted and the remaining eggs were broken and the contents visualized determining true fertility or embryo death data. The percentage of fertilized eggs that hatched on each tray was used in the analysis.

Within each trial, the time poults hatched was noted by observing the number of poults freed from the shell in each of the 13 replicate trays (one rack) from each flock beginning at 630 h of development and at 6 h intervals thereafter. The percentage of the total poults hatched on each tray at each time was used in the analysis. Times for embryos to attain a stage of development were also recorded at the same times on 25 randomly selected eggs from each flock using a candling light. The hours of incubation required for each embryo to attain a stage were used in the analysis. Embryos were sampled for tissues beginning at day 27 of development as described in our prior experiment (Christensen *et al.*, 2005). Individual embryos or poults were the experimental unit.

Poult quality was assessed by marking hatchlings of each of the 3 hatches from two incubator racks (breeder eggs collected at 10, 14 and 18 wk of production) with paint and placing them randomly into brooder houses within the company system. Poult mortality for approximately 1,200 toms and 1,200 hens was recorded for 7 days following placement in each of the three independent trials. Mortalities were autopsied to determine cause of death. Each hatch was the experimental unit for the statistical analysis.

Heart rates were measured using an oscilloscope (Avitronics, UK) as described previously (Christensen *et al.*, 2005). At external pipping and hatching, five embryos or five hatched poults were selected randomly from trays within each treatment and were weighed with and without the yolk and sampled for blood, heart and liver tissues. Blood plasma was analyzed for CK and LDH activities as well as for glucose and lactate concentrations. Cardiac and hepatic tissues were assayed for glycogen and lactate concentrations. All procedures were described previously (Christensen *et al.*, 2005). Individual embryos or poults were the experimental unit for this analysis.

Table 1: Eggshell thickness (mm) of turkey eggs from dams fed two sources of calcium[1]

	Diet[1]	
Weeks of lay	CCP[1]	CON
1	0.44[a]	0.44[a]
2	0.45[a]	0.41[bc]
3	0.40[bc]	0.43[a]
4	0.42[b]	0.41[bc]
5	0.42[b]	0.42[b]
6	0.44[a]	0.42[b]
7	0.41[bc]	0.41[bc]
8	0.46[a]	0.41[bc]
9	0.44[a]	0.42[bc]
10	0.44[a]	0.43[a]
11	0.41[bc]	0.39[c]
12	0.44[a]	0.39[c]
13	0.44[a]	0.41[bc]
14	0.44[a]	0.37[d]
Mean	0.43	0.42
Mean±SEM	0.42±0.01	
Probabilities	Diet	0.0001
	Week	0.0001
N = 1096	Diet x Week	0.0001

[1]CCP=fed 0.05% chelated calcium proteinate plus 3.14% calcite as a calcium source; CON= fed 3.16% calcite as a calcium source. [a,b,c]Means with a different superscript differ significantly (P< 0.05).

Data were analyzed using the general linear models procedure of SAS (1998). Eggshell thickness treatments were determined by comparing 50 eggs from each house each wk of egg production. All remaining data were grouped into a two eggshell thicknesses (THCK and THIN) by three ages (Weeks 10, 14 and 18 of egg production) factorial for analysis. Age of breeder flock was considered a fixed factor in the analysis. Means differing significantly were separated with the least square means. Probability was based on $P < 0.05$ unless otherwise noted.

Results

After the 10th wk of production, greater differences in thicknesses were apparent with eggs collected from the group fed CCP having thicker eggshells than did the controls (Table 1). The group fed CCP produced fewer cull eggs than the controls but showed not differences in egg production rates (data not shown).

THCK shells increased the percentage of embryos surviving compared to the THIN shells (Table 2). Fewer embryos died at wk 4 of development in the THCK shells than in THIN shells. An eggshell thickness by trial interaction was noted for externally pipped eggs (day 27 of development). The percentage of pipping embryos dying was greater in the THIN than THCK shells in trials at weeks 10 and 18 of egg production but no differences were seen at week 14.

THCK prolonged development time of embryos compared to THIN (Table 3). In each trial, embryos in

Table 2: Embryo survival and times of development at which mortality occurred for eggs produced by turkey breeder hens of two thicknesses[1]

Shell[1]	Trial	Embryo survival (%)	Mortality at week 1 (%)	Mortality at week 4 (%)	Mortality at pipping (%)
Thin	1	89.8	0.2	5.5	3.8[a]
	2	91.7	0.2	3.7	2.2[bc]
	3	88.7	0.6	7.0	3.2[a]
	Mean	90.1[b]	0.3	5.4[a]	
Thick	1	92.8	0.2	3.3	2.7[ab]
	2	92.1	0.5	3.7	2.9[ab]
	3	92.0	0.6	5.7	1.8[c]
	Mean	92.3[a]	0.4	4.3[b]	
Mean±SEM		91.2 ± 0.3	0.4 ± 0.3	4.8 ± 0.2	2.7 ± 0.1
Probabilities	Shell	0.0008	NS	0.01	0.04
	Trial	NS	NS	0.0001	NS
	Shell x Trial	NS	NS	NS	0.007

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm. [a,b]Means with a different superscript differ significantly (P< 0.05).

Table 3: Time of hatching (% of total hatched) for turkey eggs of two thicknesses[1]

	Hours of incubation				
Shell[1]	636	642	648	654	660
Thick	7.3[b]	15.3[b]	31.8[b]	64.7[b]	100.0
Thin	15.1[a]	28.5[a]	43.4[a]	74.2[a]	100.0
Mean±SEM	11.2 ± 1.6	21.9 ± 1.8	37.6 ± 1.5	69.5 ± 1.2	
Probability					
Shell	0.008	0.0007	0.001	0.0003	
Trial	0.0001	0.0001	0.0001	0.0001	
Shell x Trial	NS	NS	NS	NS	

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.
[a,b]Means with a different superscript differ significantly (P< 0.05).

Table 4: Time for embryos to attain a stage of development and remain at a stage when developing in turkey eggs of two thicknesses[1]

	Stage of development				
	Internal pipping		External pipping		Hatched
Shell[1]	Time	D time[3]	Time	D time[3]	Time
Thick	615[a]	12.0[b]	627	20.0	647
Thin	608[b]	17.8[a]	626	19.6	646
Mean±SEM	612 ± 1	14.9 ± 1.1	627 ± 1	19.8 ± 0.9	647 ± 1
Probability					
Shell	0.0009	0.004	NS	NS	NS
Trial	0.01	0.007	NS	NS	NS
Shell x Trial	NS	NS	NS	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.
[3]D time = the amount of time that the embryo remained at that stage before advancing to the next.
[a,b]Means with a different superscript differ significantly (P< 0.05).

THCK had longer developmental times than did embryos from the THIN. Table 4 indicates that the longer development time was due primarily to an extended time required for embryos in THCK eggs to pip internally than for THIN.

Hatchlings from THCK were approximately 2 g heavier without the yolk than their counterparts from THIN (Table 5), and poults from THCK also displayed less residual yolk that did those from THIN. Hearts in 27-d old embryos from THCK weighed more than those from THIN on both relative and absolute bases (Table 6). Embryo and neonate liver weights did not differ between the dietary treatments (data not shown). Poults from eggs with THCK did not display better poult livability than THIN in brooder houses (Table 7).

Table 8 shows that heart rates for 25 d embryos from THCK and those of the THIN did not differ, but at 26 d, embryo heart rates from the THIN were greater than those from the THCK.

Embryos from the THCK had elevated cardiac glycogen concentrations at d 27 but depressed levels at d 28 of development compared to THIN (Table 9). No significant

Table 5: Body and yolk weights (g) of embryos and poults from turkey eggs of two thicknesses[1]

		Day of development	
Shell[1]	Trial	27	28
Without yolk			
Thin	1	47.3	49.4
	2	47.9	48.4
	3	52.2	51.4
		49.7[b]	
Thick	1	48.7	50.0
	2	50.9	52.4
	3	50.8	51.8
			51.4[a]
Mean±SEM		49.6±0.4	50.5±0.4
Probabilities	Shell	NS	0.10
	Trial	0.04	NS
	Shell x Trial	NS	NS
			Yolk
Thin	1	12.9	9.9
	2	14.6	10.7
	3	15.3	10.8
			10.4[a]
Thick	1	13.9	8.4
	2	14.2	8.9
	3	13.3	10.2
		9.2[b]	
Mean±SEM		14.0±0.4	9.8±0.2
Probabilities	Shell	NS	0.02
	Trial	NS	NS
	Shell x Trial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.

[a,b]Means with a different superscript differ significantly (P< 0.05).

Table 6: Heart weights of embryos and poults from eggs of two thicknesses[1]

		Day of development	
Shell[1]	Trial	27	28
Absolute (mg)			
Thin	1	236	288
	2	240	272
	3	302	336
		259[b]	
Thick	1	280	302
	2	290	322
	3	278	326
		283[a]	
Mean±SEM		271±4	308±6
Probabilities	Shell	0.01	NS
Trial		0.02	NS
Shell x Trial		NS	NS
		Relative (%)	
Thick	1	0.50	0.58
	2	0.50	0.56
	3	0.58	0.65
		0.52[b]	
Thin	1	0.58	0.61
	2	0.57	0.62
	3	0.55	0.63
		0.57[a]	
Mean±SEM		0.55±0.08	0.61±0.01
Probabilities	Shell	0.03	NS
	Trial	0.03	NS
	Shell x Trial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.

[a,b]Means with a different superscript differ significantly (P< 0.05).

differences were seen in cardiac lactate concentrations. Hatchlings (28-d) from the THCK had depressed hepatic glycogen concentrations compared to THIN (Table 10), and pipping embryos (27-d) from THCK displayed elevated hepatic lactate concentrations compared to THIN. When the total amounts of glycogen and lactate in the liver relative to one another were examined, no differences were seen at pipping, however, the hatched poults had more hepatic lactate than glycogen indicating cardiomyopathy.

Activity of CK was elevated in poults from THIN compared to THCK at hatching indicating greater tissue oxygen debt and cardiomyopathy in those poults (Table 11). No differences were noted in the activities of LDH at any time of development.

THCK depressed plasma glucose concentrations in hatching poults but not in pipping embryos compared to those in THIN (Table 12). No differences were noted in the concentrations of plasma lactate at either stage of development examined.

Discussion

Increased shell thickness improved embryo survival under field conditions in the current study at a commercial turkey breeding company. The flock examined suffered shell quality problems because of hot weather. In support of prior data (Grimes *et al.*, 2004; Christensen *et al.*, 2005), adding CCP to the commercial diet increased shell thickness in half of the ages examined with the difference in eggshell thickness being greater as the hens aged. In the embryo survival experiments, approximately 2% more embryos from THCK survived development in the current study than did those from THIN. Most of the difference in survival was in embryos from THCK shells that survived during 25 to 28 d of development compared to THIN. Thus, THCK improved embryo livability at the end of development. In contrast to a prior study (Grimes *et al.*, 2004), the improvement in embryo survival was independent of the age of the breeder hen. A major difference between the current and prior studies (Grimes *et al.*, 2004; Christensen *et al.*, 2005) was the presence of thin shells in flocks prior to feeding CCP to breeders in the current study. Most flocks within the company at the time of the experiment were producing eggs with thin shells. Prior studies suggested that shell thickness may also affect eggshell conductance (Christensen *et al.*, 2005). We were unable to measure eggshell conductance in the current study because of precluding factors in the commercial hatchery operation, but peripheral data (i.e.

Table 7: Mortality (%) at 7days post hatching of hatchlings from eggs of two thicknesses[1]

Shell[1]	Trial	Tom mortality (%)	Hen mortality (%)
Thick	1	1.76	1.41
	2	1.67	1.54
	3	1.62	0.70
	Mean	1.68	1.21
Thin	1	1.90	1.50
	2	1.75	1.04
	3	1.46	0.94
	Mean	1.70	1.16
Mean±SEM		1.44±0.01	
Probabilities	Shell	NS	NS
	Trial	NS	NS
	Shell x Trial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.
[a,b]Means with a different superscript differ significantly (P< 0.05).

Table 8: Heart rates (bpm) of embryos and poults from turkey eggs of two thicknesses[1]

		Day of development	
Shell[1]	Trial	25	26
Thin	1	228	243
	2	222	242
	3	224	219
	Mean	225	234[a]
Thick	1	226	223
	2	229	214
	3	220	217
		225	219[b]
Mean±SEM		225±2	226±2
Probabilities	Shell	NS	0.0001
	Trial	NS	0.0001
	Shell x Trial	NS	NS
	Trial means	Trial	Heart rate
		1	233[a]
		2	228[a]
		3	218

[b1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.
[a,b]Means with a different superscript differ significantly (P< 0.05).

Table 9: Cardiac glycogen and lactate (mg/g of wet tissue mass) in embryos and poults from eggs of two thicknesses[1]

		Day of development	
Shell[1]	Trial	27	28
		Glycogen	
Thin	1	0.45	0.86
	2	0.51	0.63
	3	1.86	1.39
	Mean	0.94[b]	0.96[a]
Thick	1	1.14	0.22
	2	1.36	0.16
	3	2.54	1.18
	Mean	1.68[a]	0.52[b]
Mean±SEM		1.31±0.08	0.74±0.06
Probabilities	Shell	0.0006	0.003
	Trial	0.0001	0.0001
	Shell x Trial	NS	NS
		Lactate	
Thin	1	1.68	2.44
	2	2.06	2.24
	3	1.89	2.28
Thick	1	2.12	2.55
	2	1.90	2.24
	3	1.95	2.28
Mean±SEM		1.93±0.06	2.34±0.09
Probabilities	Shell	NS	NS
	Trial	NS	NS
	Shell x Trial	NS	NS
Ratio (Total glycogen to total lactate)			
Thin	1	0.28	0.35
	2	0.24	0.29
	3	1.01	0.60
		0.51[b]	0.41[a]
Thick	1	0.54	0.08
	2	0.75	0.07
	3	1.35	0.51
		0.88[a]	0.22[b]
Mean±SEM		0.70± 0.05	0.32±0.02
Probabilities	Shell	0.004	0.007
	Trial	0.0001	0.0001
	Shell x Trial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.
[a,b]Means with a different superscript differ significantly (P< 0.05).

delayed hatching times and increased shell thickness) suggest that the functional property of the eggshell may have been affected by shell thickness as well. It was concluded from the current study that increased shell thickness resulted in longer embryo developmental periods and improved survival rates when compared to eggs with thin shells.

In agreement with prior studies (Christensen *et al.*, 2005), THCK in the current study affected embryo cardiac physiology. In the prior study, hearts grew faster the initial 3 d of life when the poult hatched from a thicker shelled-egg. It is speculated that the alterations may have been mediated through the shell and eggshell conductance of the vital gases of oxygen and carbon dioxide and conductance constants that prolonged the embryo developmental period (Christensen *et al.*, 2003). THCK decreased heart rates on d 26 of development and increased heart weights on day 27 of development. No differences in heart weights were seen at hatching, however, the myocardium showed residual effects of elevated glycogen and plasma CK as seen in poults suffering cardiomyopathy (Czarnecki, 1991). The myocardium exhibited increased glycogen prior to hatching but the reverse was true following hatching. Thus, mortality seen in embryos at 25, 26 and 27 d of development may reflect failed compensations in cardiac physiology made by embryos to different eggshell conductance when they were incubated in an oxygen enriched environment at high altitude.

As soon as the shell was broken at pipping and the embryo or poult adjusted its respiration rate as opposed to relying upon the shell, heart weights recovered quickly

Table 10: Hepatic glycogen and lactate (mg/g of wet tissue mass) in embryos and poults from eggs of two thicknesses[1]

		Day of development	
Shell[1]	Trial	27	28
		Glycogen	
Thin	1	1.29	0.91
	2	0.63	0.68
	3	1.54	0.82
	Mean	1.09[b]	
Thick	1	1.62	0.91
	2	0.37	0.91
	3	1.70	1.73
	Mean	1.21[a]	
Mean±SEM		1.19±0.21	0.99±0.07
Probabilities	Shell	0.05	NS
	Trial	NS	NS
	Shell x Trial	NS	NS
		Lactate	
Thin	1	0.80	1.11
	2	1.02	0.98
	3	1.04	1.20
		0.96[b]	
Thick	1	1.46	1.28
	2	1.06	1.19
	3	0.97	1.13
		1.16[a]	
Mean±SEM		1.06±0.04	1.15±0.04
Probabilities	Shell	0.02	NS
	Trial	NS	NS
	Shell x Trial	NS	NS
	Ratio (Total glycogen to total lactate)		
Thin	1	1.52	0.94
	2	0.58	1.02
	3	1.58	1.45
		1.14[a]	
Thick	1	0.85	0.70
	2	0.37	0.60
	3	1.62	0.73
		0.67[b]	
Mean±SEM		1.09±0.11	0.91±0.11
Probabilities	Shell	NS	0.05
	Trial	NS	NS
	Shell x Trial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.

[a,b]Means with a different superscript differ significantly (P< 0.05).

and were not affected by shell thickness at hatching. Survival of hatchlings following placement in brooders in our current study was identical for THCK and THIN poults in all three trials. Enhanced growth of poults to 3-d post-hatching from eggs treated similarly has been reported previously (Christensen *et al.*, 2005). We were unable to weigh the poults at 3 d post-hatching as had been reported previously (Christensen *et al.*, 2005) to assess poult quality. Spontaneous cardiomyopathy (turkey roundheart disease) is readily observed in the commercial company located at high altitude. Autopsy of the poults that died in the current study showed no consistent trends in the incidence of spontaneous cardiomyopathy probably because so few poults died (< 5 in some treatments) in the current study. Larger numbers of poults need to be placed to assess correctly the incidence of spontaneous cardiomyopathy.

Earlier studies in cardiomyopathy poults (Czarnecki *et al.*, 1975) noted excessive glycogen in various tissues, specifically in left and right ventricular tissue; with the right ventricle having a greater increase in glycogen content (Czarnecki and Evanson, 1980). Avian myocardium has no ability to recycle lactate via the Cori cycle so it is dependent upon the liver for that function. In cardiomyopathic poults, glycogen granules were observed in hepatic lysosomes (Staley *et al.*, 1978) which were hypothesized to result from a block in the citric acid cycle preventing the complete breakdown of glycogen and resulting in altered hepatic metabolism, including decreased protein synthesis and increased metabolism of fat, possibly associated with liver damage. It was determined that glycogen branching levels were unaltered; therefore, the best explanation for the altered levels was a change in degradation of glycogen (Czarnecki and Evanson, 1980; Czarnecki *et al.*, 1978; Mirsalimi *et al.*, 1990). Elevated lactate and increased glycogen concentrations were seen in poults hatching from THIN in the current study and poults hatching from THIN exhibited increased amounts of lactate relative to glycogen (Ratio) in hepatic tissue indicated the slower recycling reported in cardiomyopathy previously (Staley *et al.*, 1978).

Lactate concentrations in the myocardium of pipping embryos were not affected by thicknesses, but the ratio of total glycogen to total lactate was. THIN depressed the amount of glycogen relative to lactate in heart tissue in poults, and THIN also caused elevated plasma CK activity in poults. Elevated CK activities as well as reduced glycogen to lactate ratios in liver indicate cardiomyopathy (Czarnecki, 1991; Czarnecki and Evanson, 1980) suggesting that poults hatching from THIN may have been in oxygen debt. Thus, reduced shell thickness may affect myocardial energy metabolism and myocardium fatigue may be factors in the observed reduced heart weight and embryonic mortality. Thus, observations from the current study may support the idea that thick shells improved embryo survival via adjustments in cardiac physiology, but shell thickness had no effect on the survival of the hatchling despite displaying elevated CK and altered total glycogen to lactate ratios at hatching.

The reasons that THIN may have resulted in cardiac problems are unknown. Previous research indicated no relationship between egg specific gravity, an estimate of shell thickness, and cardiomyopathy in turkeys (Gadzinski *et al.*, 1993). In our study, changes in cardiac physiology were readily observed, and may have been related to eggshell conductance and eggshell conductance constants instead of just shell thickness

Table 11: Blood plasma creatine kinase (CK) and lactate dehydrogenase (LDH) activities (U/L) of embryos and poults from eggs of two thicknesses[1]

		Day of development	
Shell[1]	Trial	27	28
		CK	
Thin	1	1,373	2,840
	2	1,347	2,206
	3	1,398	2,108
			2,387[a]
Thick	1	1,782	2,141
	2	1,449	2,108
	3	1,451	2,248
			2,166[b]
Mean±SEM		1,468±91	2,277±66
Probabilities	Shell	NS	0.05
	Trial	NS	NS
	ShellxTrial	NS	NS
		LDH	
Thin	1	382	521
	2	376	553
	3	397	588
Thick	1	513	558
	2	408	494
	3	404	599
Mean±SEM		413±18	552±10
Probabilities	Shell	NS	NS
	Trial	NS	NS
	ShellxTrial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.

[a,b]Means with a different superscript differ significantly (P< 0.05).

Table 12: Blood plasma glucose and lactate concentrations (mg/dL) in embryos and poults from eggs of two thicknesses[1]

		Day of development	Shell[1]
	Trial	27	28
		Glucose	
Thin	1	196	234
	2	202	255
	3	187	230
			240[a]
Thick	1	188	228
	2	206	225
	3	201	222
			232[b]
Mean±SEM		197±3	232±2
Probabilities	Shell	NS	0.02
	Trial	NS	NS
	ShellxTrial	NS	NS
		Lactate	
Thin	1	13	15
	2	12	19
	3	13	18
Thick	1	14	18
	2	11	19
	3	18	13
Mean±SEM		14±1	16±1
Probabilities	Shell	NS	NS
	Trial	NS	NS
	ShellxTrial	NS	NS

[1]Thick=shells were measured to be 0.44 mm. Thin=shells were measured to be 0.39 mm.

[a,b]Means with a different superscript differ significantly (P< 0.05).

(Christensen *et al.*, 2005). The conductance property of an egg differs from specific gravity because it is functional. It ensures three requirements are met in a timely manner for successful hatching (Rahn, 1981). One of the requirements is that the total amount of oxygen that will have been consumed is about 100 mL/g of egg weight. Poults from thin-shelled eggs hatched earlier than those from thick-shelled eggs suggesting that the embryos may not have had sufficient time to consume 100 mL of oxygen per g prior to emerging from the shell (Rahn, 1981; Christensen *et al.*, 1997; Christensen *et al.*, 1999b). If embryos from thin-shelled eggs did not meet this requirement, then embryonic tissues must make adjustments for anaerobic metabolism (Christensen *et al.*, 1999a). One of the adjustments made by hypoxic turkey embryos is a reduction in heart weight (Christensen *et al.*, 1999b). Thus, observations of the current study may suggest that altered hatching times due to thin shells is a response to hypoxia that affects cardiac tissue and increases embryonic mortality at 25, 26 and 27 d of development.

In conclusion, thicker shells resulted in 2% better embryo livability at the commercial hatchery and can improve the survival of turkey embryos. This observation may be particularly useful for eggs produced in hot weather. Although thicker shells affected embryo cardiac physiology and survival, it did not affect the survival of the hatchlings to 7 d of age.

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[1]The mention of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned.