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Effect of Chelated Calcium Proteinate Fed in the Maternal Diet of Turkey Breeders on Embryo Cardiac Physiology and Poult Quality[1]

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Abstract: Embryo and hatchling survival diminish as turkey breeder hens age. Recent data indicated that a chelated calcium proteinate (CCP) additive given to turkey breeder hens improved embryo survival as hens aged but did not affect shell thickness. We hypothesized that the mechanism by which this occurred may be by improved functional shell quality and its consequent effect on cardiac physiology. To test the hypothesis, CCP was supplemented to the diet of Large White turkey breeder hens for a 25 week egg production period and compared with controls without supplementation. Eggshell conductance, conductance constants, poult growth and cardiac physiology were measured at weeks 10, 18 and 25 of production. Because elevated temperatures increase heart rates and reduce heart weight and survival, half of the eggs was incubated at 37.9°C whereas the remaining eggs were incubated at 37.5°C. Embryos and poults from the CCP group exhibited increased heart weights and improved cardiac health. The hatching poults from CCP-fed hens also grew faster for the first 3 d of life. We conclude that CCP improves eggshell conductance, and the subsequent eggshell conductance constant (k) of eggs from turkey breeder hens. The change in k improved embryo cardiac health and poult BW after hatching.

Key words: Chelated calcium, heart, embryo

Introduction

As turkey breeder hens age, embryo survival diminishes (Fairchild *et al.*, 2002) and the quality of the hatchling declines (Grimes *et al.*, 2004). Previous experiments indicated that supplementation of a chelated calcium proteinate product (CalKey® (CCP) Chelated Minerals Corporation, Salt Lake City, UT) to turkey breeder hen diets, improved the livability of embryos by decreasing the number of embryos dying at pipping (Grimes *et al.*, 2004). The improvement was especially evident when the hens were older than 54 weeks of age. The improvement in embryo viability occurred independent of changes in eggshell thickness. We speculate that the actions of the added calcium may have been on functional rather than physical properties of the eggshell or on the action of calcium ions directly on the heart. Functional characteristics may differ because conductance involves not only shell thickness, but includes pore number and cross-sectional area (Christensen *et al.*, 2005). Alternatively, ionic calcium may have a direct effect on the embryo heart (Rahn *et al.*, 1980) because of its effect on myocardium action potentials. Thus, the results of prior studies may also have been due to other factors not tested in prior studies. Increased incubator temperatures have been known for many years to increase heart rates of embryos (Mountcastle *et al.*, 1974) as well as diminish poult quality. Therefore, the hypothesis was proposed that the additional stress of increased incubator temperature on cardiac health may be ameliorated by feeding a maternal source of chelated calcium proteinate (CCP). The CCP was compared to controls fed limestone flour as a calcium source.

Materials and Methods

Nicholas turkey breeder hens were distributed randomly to 12 pens with 6 birds each and fed identical turkey diets. Birds were divided into two treatment groups with six pens being fed the control diet containing 2.75% calcium from limestone flour, and the remaining six pens being fed the treatment diet containing 2.70% calcium from limestone flour supplemented with 0.05% calcium from CCP (Grimes *et al.*, 2004). Pens served as the experimental unit for the egg production data. At 32 wk of age the hens were photo stimulated to begin an egg production cycle and given the treatment diets.

The diets were available to the breeder hens on an *ad libitum* basis beginning at photo stimulation. A total of 25 weeks of egg production was observed for the experiment. Hens were inseminated weekly with semen from sires of the same age and strain. Eggs were collected daily and stored for 3 to 15 days prior to setting. The eggs were sorted by pens then were divided randomly into two incubation temperature groups. One group was incubated at 37.5°C (C) and the other at 37.9°C (HI). Heated and control eggs were set in five different incubators, and the treatments were assigned to different incubators for each set so incubator was not a factor in the experiment. Within weeks 9, 17 and 24 of production, approximately 600 eggs representing all treatments (*ca*. 150/treatment combination) were weighed at setting and at transfer to determine eggshell conductance values using the calibrated egg technique (Romanoff and Sochen, 1938).

The functional characteristic of each eggshell (mg of water vapor/day/mm Hg) was further evaluated by determining the length of the incubation period for each egg by noting the time each poult hatched at 6 h intervals beginning at 600 h of development. Individual eggs from weeks 9, 17 and 24 were the experimental unit for the eggshell measurements. These data were used to compute of the conductance constant (Rahn, 1981). The value for k indicates the proper time for hatching that optimizes hatchling maturity (Christensen *et al*., 2005) depending upon egg weight and eggshell conductance. At weeks 10, 18 and 25, embryo heart rates were observed for randomly selected eggs using an oscilloscope designed for incubating eggs (Avitronics, UK). Heart rates were recorded for 20 eggs per diet and temperature treatment combination. Rates were recorded at 4-d intervals between 12 and 24 d of development. Randomly selected individual eggs representing all pens in a treatment were the experimental unit for the heart rate analysis (N = 80 per d). Within each of the three trials, heart tissue was collected daily at incubation days 27 and 28 from 12 randomly selected embryos as well as from poults at 3 d posthatching. Randomly selected individual embryos/poults representing all pens were the experimental units for the tissue analyses (N = 36 per d). Blood was collected following decapitation, the body with and without yolk was weighed (nearest 0.01g), hearts were removed and weighed, and cardiac glycogen and lactate concentrations were subsequently measured (Tullett *et al*., 1981).

On each of the sampling weeks following hatching, 36 poults from each of the treatments (144 total poults) were placed into a battery brooder (Petersime, Gettysburg, OH 45328). The pens were randomly assigned to treatments with 3 pens of 12 poults each per treatment combination (n = 108 per treatment). The poults were weighed individually at placement, 3 and 7 d, and the feed consumed per pen (n = 9 per treatment) was determined over the same time periods. Individual poults were the experimental unit for the BW data, and pens were the experimental unit for the feed consumption data.

Data were analyzed as a 2 diets by 2 temperatures factorial arrangement (Christensen *et al*., 2005). Data were sorted by day of development prior to analysis. Trial (hatches or weeks of egg production) was used as a fixed factor in the analysis. Individual bird data were used in the analysis for all variables. Poult growth data also utilized pen as a fixed factor in the analysis, but no significant pen effects were seen so they will not be presented. Means that differed significantly were separated by the least square mean procedure. The level of significance was set at $P < 0.05$ unless otherwise noted.

Results

Heart rates: HI increased heart rates in 12 d embryos and interacted with maternal dietary calcium to affect heart rates in 20 d embryos (Table 1). At 20 d HI increased heart rates if the dams were fed the C diet, but HI lowered heart rates if the dams were fed CCP. At 24 d of development both CCP and HI increased heart rates compared to controls.

Heart weights: HI lowered heart weights in 27 d embryos regardless of diet (Table 2). At 28 d CCP reduced heart weights compared to the Control diet. In 3 d old poults, diet and temperature interacted such that maternal CCP increased heart weights in poults exposed to HI to be equivalent to those from C, but when dams were fed the control diet, HI temperature depressed heart weights compared to C.

Cardiac physiology: Embryo cardiac glycogen concentrations were not affected by diet or incubator temperature (Table 3), but HI elevated myocardial lactate in embryo hearts compared to C, and dietary CCP prevented lactate accumulation in myocardium at 27 d of embryo development compared to those in embryos from hens fed the Control diet. No diet effects were seen at 28 d.

Eggshell conductance: Dietary CCP increased eggshell conductance compared to the Control diet (Table 4), and diet and temperature interacted to affect hatching times. The CCP diet shortened developmental times of control embryos by nearly 4 h compared to Control. When embryos were exposed to HI, the overall development times were shorter but did not differ between diets. Conductance constants (k) were computed as a function of egg weight, eggshell conductance and hatching times (Romanoff and Sochen, 1938) CCP increased k compared to Control. Diet had no effect on egg production rates (data not shown) or egg weights in the current experiment.

Poult BW: Poult quality was tested by weighing hatchlings at hatching and 3 d following hatching (Table 5). A diet by temperature interaction affected the poult weights. Among poults from hens fed the Control diet, HI reduced hatchling BW compared to C, but CCP interacted with temperature and increased BW to be equivalent with the controls. No differences were noted in feed consumption (data not shown). HI increased residual yolk at hatching compared to C.

Table 1: Heart rate (beats per min, n = 80) of turkey embryos in eggs from dams fed chelated calcium proteinate (CCP) then exposed to high incubation temperatures (37.9ºC) compared to controls (37.5ºC)

		Poult age[3]			
Diet	Temperature	12	16	20	24
CCP	High	235	222	219[b]	242
	Control	213	224	229[a]	234
					238[a]
Control	High	232	225	229[a]	229
	Control	224	224	221[b]	222
					226[b]
Temperature means					
High		234[a]			235[a]
Control		218[b]			228[b]
Mean ± SEM		227 ± 3	223 ± 2	224 ± 2	232 ± 2
Probabilities					
Diet		NS	NS	NS	0.02
Temperature		0.05	NS	NS	0.05
Diet x Temperature		NS	NS	0.006	NS

[1]Diet = CCP-treated breeder hens were fed 2.70% calcium as limestone flour with 0.05% chelated calcium proteinate; CON-breeder hens were fed 2.75% calcium. [2]Temperature = High were incubated were incubated at 37.9°C; Remaining eggs were incubated at 37.5°C. [3]Poult age is the number of days of embryo development.

Table 2: Heart weights (mg, n = 36) of turkey embryos in eggs from dams fed chelated calcium proteinate (CCP) then exposed to high incubation temperatures (37.9°C) compared to controls (37.5°C).

		Poult age[3]		
Diet[1]	Temperature[2]	27 d embryo	28 d embryo	3 days
CCP	High	348	424	550[ab]
	Control	369	403	596[a]
			413[b]	
Control	High	346	427	582[a]
	Control	358	439	509[b]
			433[a]	
Temperature means				
High		346[b]		
Control		364[a]		
Mean ± SEM		356 ± 8	423 ± 9	559 ± 12
Probabilities				
Diet		NS	0.03	NS
Temperature		0.05	NS	NS
Diet x Temperature		NS	NS	0.01

[1]Diet = CCP-treated breeder hens were fed 2.70% calcium as limestone flour with 0.05% chelated calcium proteinate; CON-breeder hens were fed 2.75% calcium. [2]Temperature = High were incubated were incubated at 37.9°C; Remaining eggs were incubated at 37.5°C. [3]Poult age = 27 days of embryo development = e27; hatching = e28; three days post hatching = 3d.

Discussion

Maternal dietary calcium affected eggshell G and subsequent poult health via cardiac physiology, thus, we accept our hypothesis. Shell thickness declines (Grimes *et al*., 2004) and G increases (SAS Institute, 1998) as hens age, and feeding CCP to turkey breeder hens may be a mechanism to increase G and improve the body weight of poults from aging hens.

The data relating cardiac physiology and eggshell G are novel but probably not surprising. Many environmental factors have been implicated in cardiomyopathy in perinatal turkeys including diet, rapid growth and viral infection (Christensen *et al*., 1996, Magwood and Bray, 1962, Hunsaker, 1971; Dewar and Sillar, 1971; Jankus *et al*., 1972; Onderka and Bhatnagar, 1982; Frame *et al*., 2003), but to the authors' knowledge, no one has implicated G. The idea that the functional property of eggshells may limit oxygen diffusion (Roberson, 2005) to the developing embryo and consequently alter cardiac health (Tullett , 1981) may be unique to this study.

Table 3: Cardiac glycogen and lactate concentrations (mg/g of wet tissue mass, n = 36) of turkey embryos in eggs from dams fed chelated calcium proteinate (CCP) then exposed to high incubation temperatures (37.9°C) compared to controls (37.5°C).

		Poult age[3]		
Diet[1]	Temperature[2]	27 d embryo	28 d embryo	3 days
			Glycogen	
CCP	High	4.88	3.39	1.13
	Control	4.76	3.25	1.04
Control	High	5.09	3.12	1.23
	Control	5.28	3.39	1.20
Mean ± SEM		5.00 ± 0.20	3.29 ± 12	1.15 ± 0.04
Probabilities				
Diet		NS	NS	NS
Temperature		NS	NS	NS
Diet x Temperature		NS	NS	NS
			Lactate	
CCP	High	0.56[ab]	0.91	0.88
	Control	0.56[ab]	0.95	0.96
Control	High	0.70[a]	0.86	0.99
	Control	0.46[b]	1.04	0.88
Temperature means				
High			1.01[a]	
Control			0.89[b]	
Mean ± SEM		0.58 ± 0.03	0.94 ± 0.02	0.99 ± 0.01
Probabilities				
Diet		NS	NS	NS
Temperature		0.05	0.03	NS
Diet x Temperature		0.05	NS	NS

[1]Diet = CCP-treated breeder hens were fed 2.70% calcium as limestone flour with 0.05% chelated calcium proteinate; CON-treated breeder hens were fed 2.75% calcium. [2]Temperature = High were incubated were incubated at 37.9°C; Remaining eggs were incubated at 37.5°C. [3]Poult age = 27 days of embryo development = e27; hatching = e28; three days post hatching = 3d.

Table 4: Functional characteristics of eggs produced by turkey hens fed two sources of calcium

		Measurement			
Diet[1]	Temperature[2]	Initial egg weight (g)	Conductance (mg/d/mmHg)	Hatching time (h)	Conductance constant
CCP	Control	102.4	23.7	653[b]	6.24
	High	102.2	24.3	647[c]	6.43
	Mean	24.0[a]		6.29[a]	
CON	Control	102.7	23.3	657[a]	5.99
	High	101.5	23.0	646[c]	5.95
	Mean		23.1[b]	5.97[b]	
	Mean ± SEM	102.3 ± 0.1	23.5 ± 0.1	652 ± 0.5	6.03 ± 0.05
Probabilities	Diet	NS	0.03	0.05	0.002
	Temperature	NS	NS	0.0001	NS
	Diet x Temperature	NS	NS	0.02	NS

[1]Diet = CCP-treated breeder hens were fed 2.70% calcium as limestone flour with 0.05% chelated calcium proteinate; CON-breeder hens were fed 2.75% calcium. [2]Temperature = High were incubated were incubated at 37.9 C;° Remaining eggs were incubated at 37.5 C. °

Body and heart weight: The poult growth data from the current study showed that poults hatching from eggs with increased eggshell permeability had an advantage in growth for the initial 3 d outside the shell. Increased poult was associated with heavier embryo hearts in eggs with greater G from CCP-fed dams. High incubator temperatures also increased heart weights, but when CCP was fed no heart enlargement was noted. Thus, it is concluded that eggs with greater G show improved posthatching growth and better cardiac health that

Table 5: Body and yolk weights (g) of poults from hens fed two sources of calcium when exposed to a higher incubation temperature

		Poult age[3]		
Diet[1]	Temperature[2]	e27	e28	3d
			BW without yolk	
CCP	High	60.1	58.3[a]	84.4[a]
	Control	59.3	59.6[a]	88.5[a]
CON	High	57.9	55.9[b]	84.7[a]
	Control	61.7	60.6[a]	78.2[b]
Temperature means				
High		58.6[b]		
Control		60.9[a]		
	Mean ± SEM	59.8 ± 0.5	57.4 ± 0.9	84.0 ± 0.2
Probability	Diet (D)	NS	NS	0.04
	Temp	0.05	NS	NS
	Diet x Temperature	NS	0.01	0.03
			Yolk	
CCP	High	16.5	11.6	2.0
	Control	16.3	10.2	1.8
CON	High	15.7	13.2	1.6
	Control	15.1	9.6	2.6
Temperature means				
High			12.4[a]	
Control			9.9[b]	
	Mean ± SEM	15.9 ± 0.2	11.1 ± 0.2	2.0 ± 0.3
	Diet (D)	NS	NS	NS
	Temp (T)	NS	0.03	NS
	Diet x Temperature	NS	NS	NS

[1]Diet = CCP-treated breeder hens were fed 2.70% calcium as limestone flour with 0.05% chelated calcium proteinate; CON-treated breeder hens were fed 2.75% calcium. [2]Temperature = High were incubated were incubated at 37.9°C; Remaining eggs were incubated at 37.5°C. [3]Poult age = 27 days of embryo development = e27; hatching = e28; three days post hatching = 3d.

enables adjustment to high incubator temperature at the end of embryo development.

Cardiac physiology: Greater G of eggs from hens fed CCP was associated with greater heart rates regardless of incubator temperature, but elevated temperatures also increased rates during embryo development in the current study. Maternal dietary CCP slowed temperature-induced increases in heart rates at 20 d, but CCP elevated heart rates at 24 d of development regardless of temperature. Thus, it may be that G changes heart rates depending upon respiratory needs of the embryo during the plateau stage in oxygen consumption.

Cardiac glycogen and lactate: Early studies in cardiomyopathy poults (Roberson, 2005) noted excessive glycogen in various tissues, specifically in left and right ventricular tissue; with the right ventricle having a greater increase in glycogen content (Rahn, 1981). Glycogen granules were observed in lysosomes 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 (Czarnecki *et al.*, 1975). 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 *et al.*, 1980). Other reports exist for cardiomyopathy effects on tissue glycogen indicating either no change in glycogen levels (Czarnecki *et al.*, 1975) or decreased levels of glycogen in the poults (Staley *et al.*, 1978). Embryo cardiac glycogen concentrations increased in the current study only as the breeder hen aged (Trial effects). However, lactate concentrations increased in myocardium of pipping embryos from CON-fed hens but not in those fed CCP. Thus, temperature and hen age both increased the amount of lactate found in myocardium suggesting myocardial energy metabolism and possible muscle fatigue may be factors in the reduced heart weight. Thus, myocardial glycogen and lactate concentrations observed in the current study support the idea that CCP improved survival via cardiac health.

Eggshell conductance: Previous research indicated no relationship between shell thickness and cardiomyopathy in turkeys (Czarnecki *et al.*, 1978). Differences in the current study were due to functional eggshell qualities of eggshell conductance (G) and eggshell conductance constant (k) rather than shell thickness. The conductance property of an egg is functional because it ensures three requirements are met in a timely manner for successful hatching (Mirsalimi *et al.*, 1990). The first requirement is that an egg loses approximately 15% of the initial egg mass as water vapor. He (Mirsalimi *et al.*, 1990) 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%. The three conditions ensure that the poult hatches with the characteristic maturity or quality of the species. Others (Gazdinski *et al.*, 1993) indicated that conductance must be additionally matched to the initial egg mass and the length of the incubation period to determine a conductance constant (k). The k then ensures that the hatchling has the characteristic maturity of the species. Across avian species, the product of conductance 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. It may be suggested from the results of the current study that feeding CCP to turkey breeder hens increased conductance, shortened hatching times and had no effect on egg weight such that k was increased. Feeding CCP did not alter egg weights in the current study. Greater k increased heart weights and improved cardiac health such that poults gained more weight the initial 3 days of life. It may be concluded that the poult quality was improved by the maternal diet by increasing eggshell conductance in large eggs.

Conclusions and applications:

1. Feeding CCP to turkey dams improved embryo cardiac health compared to Controls.
2. The CCP dietary treatment also increased the growth rate of hatchling poults to 3 days of age compared to Controls.
3. Cardiac health of poults may be mediated either by increased functional shell properties or increased free calcium ions.

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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.

Abbreviation Key: CCP = Diet containing chelated calcium proteinate feed supplement; Control = control diet with no supplement; LDH = lactate dehydrogenase; HI = Eggs were incubated at 39.0°C; C = Eggs were incubated at 37.5°C; G = eggshell conductance; k = eggshell conductance constant.