Research Notes

Environmental Heat Stress Does Not Reduce Blood Ionized Calcium Concentration in Hens Acclimated to Elevated Temperatures

M. H. SAMARA, K. R. ROBBINS,! and M. O. SMITH

Department of Animal Science, The University of Tennessee, Knoxville, Tennessee 37901-1071

ABSTRACT Changes in blood concentrations of ionized calcium and total calcium were measured in broiler breeder hens (42 wk old) relative to egg cycle and environmental temperatures. Two environmental temperature treatments were used: 1) temperature cycled daily from a low of 10 C at 0300 h to a high of 25 C at 1600 h; and 2) temperature cycled from a low of 21 C at 0300 h to a high of 39 C at 1600 h. Serial blood samples were collected from five laying hens per temperature treatment via cutaneous ulnar vein cannula beginning at the time of oviposition and every 4 h thereafter until the next oviposition. Neither blood concentration of ionized calcium nor total plasma calcium was affected by temperature. Results suggest that the supply of calcium available in blood for shell deposition is not diminished in hens acclimated to high environmental temperatures.

(*Key words:* broiler breeder hen, heat distress, manganese biological availability, calcium, shell)

1996 Poultry Science 75:197-200

INTRODUCTION

High environmental temperatures cause a reduction in eggshell quality (Mueller, 1966; de Andrade *et ah,* 1976, 1977; Wolfenson *et al,,* 1979; Emery *et al,* 1984). Factors suggested to be responsible for this reduction in shell quality include reduced feed intake (Payne, 1966, 1967), reduced blood flow to the reproductive tract (de Andrade *et al, 1977),* disturbed blood acid-base balance (respiratory alkalosis) (Smith, 1974), and reduced blood ionized calcium concentration (Odom *et al,* 1986). However, there are several reports (Arad *et al,* 1981; Arad and Marder, 1982, 1983; Arad, 1983) suggesting that acclimation of laying hens to high temperatures prevents perturbation in the maintenance of several physiological processes, including thermoregulation and acid-base balance. Arad *et al* (1981) reported that temperature-induced decreases in total plasma calcium concentration were less in heat-acclimated fowl. Odom *et al.* (1986) observed a reduction in blood ionized calcium concentrations in hens subjected to 35 to 38 C for 3 h, In addition to the fact that the temperature was increased abruptly for a short period of time, the authors did not consider blood sampling time relative to the egg cycle, which by itself alters blood calcium levels ^ Therefore, the objective of this study was to measure changes in concentrations of blood calcium during **the_egg_oaJ£-aL** broiler breeder hens exposed to high cyclic temperatures for an extended period of time.

Received for publication March 20, 1995.
Accepted for publication July 17, 1995.
ifo whom correspondence should be addressed.

MATERIALS AND METHODS

Thirty-eight-week-old Indian River broiler breeder hens were moved to individual laying cages (60 x 45 x 45 cm) in two environmentally controlled chambers. The hens were fed a standard broiler breeder diet that provided a daily allowance of 490 kcal ME, 30 g crude protein, 5.6 g calcium, and 0.85 g available phosphorus. Water was continuously available. Hens were subjected to a 17-h light (0700 to 2400 h) and 7-h dark photoschedule. Relative humidity was not controlled but did not *exceed* 60%. Two temperature treatments were imposed: Treatments 1 (LO) and 2 (HI) were cycled daily from a low of either 10 or 21 C at 0300 h to a peak of either 25 or 39 C, respectively, at 1600 h.

The experiment was initiated following a 4-wk adaptation period. Egg laying patterns of each hen were observed and recorded during the 4-wk adaptation period. Hens that regularly laid at least three eggs in a sequence and had a 1-d pause between sequences were selected for data collection. The 1st d following an TDserve^pansein egg laying was denoted Day 1 of the experimental protocol. On Day 1, all hens were observed (five hens per treatment) that laid the first egg in a sequence between 0900 and 1000 h were fitted with cutaneous ulnar vein cannula. On Day 2, these hens were observed at 10- to 15-min intervals to verify that oviposition had occurred.

The first blood sample was collected within 10 min after oviposition of the second egg in the sequence (1100 to 1200 h) and every 4 h thereafter until the next oviposition. Heparinized syringes and test tubes were used for blood collection and storage. Two-milliliter blood samples were immediately transferred to a capped tube and immersed in ice. All samples were analyzed for pH and blood ionized calcium at 37 C using an ion selective electrode[2] within 4 h after collection. Plasma was prepared from another 1.5 mL of each blood sample by centrifugation at 1,000 x *g* for 10 min and frozen until analyzed for total calcium using atomic absorption spectrometry (Association of Official Analytical Chemists, 1980). Repeated measures ANOVA (Sokal and Rohlf, 1973) of SAS® (SAS Institute, 1985)

Blood ionized calcium (Ca^{2+}) and total calcium (TCa) concentrations during the egg cycle for hens in both temperature treatments are presented in Table 1. T he pattern of changes in Ca^2+ were affected by temperature; indeed, there was a temperature by time interaction ($P < 0.05$). Only hens maintained at LO exhibited a decrease in Ca^{2+} coincident with relative time of egg calcification. Hens at HI did not exhibit changes in blood Ca^{2+} concentration over time. Total calcium of hens housed at LO remained relatively constant. However, hens housed at HI exhibited a slight decrease ($P < 0.05$) in TCa after 12 h, coincident with onset of shell calcification. Blood pH was significantly affected by temperature (Table 1) at all but two sampling times. Blood pH was higher in hens at HI. There was a temperature by time interaction ($P < 0.05$).

DISCUSSION

Results for blood Ca^{2+} concentrations in the current study are consistent with previous research conducted under thermoneutral conditions (Taylor and Hertelendy, 1961; Luck and Scanes, 1978; Parsons and Combs, 1981; Van de Velede *et al,* 1986; Frost *et ah,* 1991), wherein blood Ca^{2+} concentrations were observed to decline as the egg entered the shell gland and remained low until 3 to 6 h before oviposition. However, at HI, blood Ca^{2+} concentrations did not follow the same pattern during temperatures.

With regard to TCa concentration, data of Luck and Scanes (1979) and Parsons and Combs (1981) showed no decline during shell calcification. However, Hertelendy and Taylor (1961) observed a progressive decline in TCa

TABLE 1. Changes in pH, blood ionized calcium, and total calcium concentrations during the egg cycle of broiler breeder hens[1] maintained under low (LO) and high (HI) environmental temperatures

Time after oviposition[2]	Ionized calcium		Total calcium		Blood pH	
	LO	HI	LO	HI	LO	HI
(h)	(mg/dL)					
0	6.19 ± 0.14^{a,x}	5.63 ± 0.14^{b,x}	21.06 ± 1.09^{a,x}	23.00 ± 0.94^{a,xy}	7.37 ± 0.013^{b,xy}	7.46 ± 0.013^{a,y}
4	6.08 ± 0.14^{x}	5.98 ± 0.14^{x}	21.08 ± 1.30^{b,x}	26.31 ± 0.94^{a,x}	7.40 ± 0.013^{b,x}	7.46 ± 0.013^{a,y}
8	5.99 ± 0.14^{xy}	5.76 ± 0.14^{x}	22.57 ± 1.28^{a,x}	25.59 ± 0.94^{a,xy}	7.37 ± 0.013^{b,xy}	7.48 ± 0.013^{a,x}
12	5.61 ± 0.14^{y}	5.92 ± 0.14^{x}	20.70 ± 1.28^{b,x}	26.45 ± 0.94^{a,x}	7.39 ± 0.013^{a,xy}	7.41 ± 0.013^{a,z}
16	5.81 ± 0.14^{xy}	5.85 ± 0.14^{x}	21.32 ± 1.28^{a,x}	23.21 ± 0.94^{a,y}	7.38 ± 0.013^{b,xy}	7.44 ± 0.013^{a,yz}
20	5.52 ± 0.14^{y}	5.87 ± 0.14^{x}	19.36 ± 1.08^{b,x}	23.79 ± 0.94^{a,xy}	7.40 ± 0.013^{a,x}	7.41 ± 0.013^{a,yz}
24	6.01 ± 0.14^{x}	5.80 ± 0.14^{x}	19.74 ± 1.64^{a,x}	21.48 ± 1.1^{a,y}	7.36 ± 0.013^{b,y}	7.43 ± 0.013^{a,yz}
Oviposition[3]	6.05 ± 0.14^{x}	5.86 ± 0.14^{x}	20.64 ± 1.10^{a,x}	23.35 ± 1.1^{a,y}	7.39 ± 0.013^{b,xy}	7.44 ± 0.013^{a,yz}

[a,b]Means within a row and the same variable with no common superscript differ significantly ($P < 0.05$).

[x-z]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Five hens per temperature treatment.

[2]Hours after oviposition indicates the interval from oviposition (1100 to 1200 h) of the second egg in the sequence.

[3]Oviposition of the third egg in the sequence. Oviposition-oviposition interval is 26 to 26.5 h. Shell formation begins at 6 h postoviposition.

[2]Beckman, LABLYTE™ 820 Electrolyte Analyzer, Beckman Instruments, Inc., Brea, CA 92621.

concentration between the middle and the end of shell formation. Van de Valede *et al.* (1986) reported that TCa concentration gradually decreased during eggshell formation from 32 to 23.3 mg/100 mL plasma. Frost *et al.* (1991), on the other hand, reported that TCa increased from 15 mg/dL at 2 h after oviposition to about 21 to 22 mg/dL at 12 to 16 h, and gradually decreased to 17 to 18 mg/dL at 22 to 24 h after oviposition. In our study, TCa concentration decreased during shell formation only in hens housed under high temperatures. This is consistent with the data of Hertelendy and Taylor (1961) and Van de Velede *et al.* (1986), but is inconsistent with those of Frost *et al.* (1991). The discrepancy among published results was considered by Frost *et al.* (1991) to be due to dilution resulting from repeated bleeding of the same bird. Because in the present study larger birds were used and smaller blood samples collected, it is unlikely that dilution affected observed calcium concentrations.

The current results differ from those of de Andrade *et al.* (1977), Wolfenson *et al.* (1979), and Odom *et al.* (1986) in that neither TCa nor Ca^{2+} concentrations were severely affected by high temperatures. The abrupt and short exposure to high temperatures (Odom *et al,* 1986) and the use of a limited number of blood samples that were not correlated to the relative stages of the egg cycle (de Andrade *et al,* 1977; Wolfenson *et al,* 1979) may explain the discrepancy in the reported data.

It has been reported that high environmental temperatures cause an immediate decline in eggshell quality (Harrison and Biellier, 1969; Miller and Sunde, 1975). We also observed a high-temperature-induced decline in eggshell quality. Eggs from hens at HI vs LO had lower ($P < 0.05$) average weight (64.9 ± 0.87 g vs 68.1 ± 0.96 g) and lower ($P < 0.05$) specific gravity (1.079 ± 0.0008 vs 1.084 ± 0.0009). De Andrade *et al.* (1977) suggested that blood flow redistribution may be one factor responsible for the low eggshell quality under heat stress conditions; however, this assumption has recently been contradicted by Arad *et al.* (1993), who reported that blood flow to the reproductive tract of the laying hen was not reduced during exposure to temperature of 35 to 45 C for 1.5 h. If this is the case, calcium supply to the shell gland may not change during shell deposition; therefore, it seems more probable that other physiological changes such as reduced calcium secretion (Bragg *et at,* 1971), reduced calcium transport in the shell gland (Odom and Harrison, 1985), or a drop in the amount of bicarbonate ^^^WM^^^^^Krrr^^mfaTosiSiU^Tn^najo^acTOrs contributing to the reduction in shell quality under high temperature conditions. Other studies suggest **that the** production of H^+ ions in the shell gland during shell formation facilitates the dissociation of the calcium-protein complex (Winget and Smith, 1962; Hodges, 1969), which in turn makes calcium available for shell on in the form of calcium carbonate. On the other hand, the source of a significant proportion of

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metabolically in the oviduct (Lorcher and Hodges, 1969). Because heat stress-induced respiratory alkalosis causes a decrease in HCO^{3-} concentration and CO2 partial pressure and a rise in blood pH (Mueller, 1966), it is likely that a drop in the concentration of bicarbonate ions occurs, which may slow the rate of formation of calcium carbonate.

The present report indicates that patterns of changes for blood ionized calcium and plasma total calcium in relation to the egg cycle is different in hens at different environmental temperatures. In addition, the supply of calcium available for shell calcification was not diminished in hens acclimated to high cyclic temperatures.

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