Effects of Hot Environments and Carbonated Drinking Water on Bone Characteristics of Eight-Week-Old Broiler Chicks

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ABSTRACT Epiphyses of the femura from 8-week-old broiler chicks were examined for morphology using scanning electron microscopy and for elemental composition using energy dispersive x-ray microanalysis. Birds between the ages of 4 and 8 weeks were subjected to either 25 or 35 C environments and given tap or carbonated drinking water. The morphological appearance of the epiphyses was affected by the kind of drinking water but not the thermal environment. Elemental constituents, however, were affected by both environmental temperature and drinking water.
(Key words: bone morphology and composition, hot environments, carbonated drinking water)

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INTRODUCTION

There is substantial evidence that environmental temperatures ranging above 32 to 42 C, as those often encountered in summer months, cause a number of altered physiological and economic conditions in poultry. Some of the consistently observed changes are body temperature, heart and respiratory rates, and blood pressure and flow patterns. High environmental temperatures cause polypnea in birds, which often results in an altered acid-base balance of the blood (Linsley and Burger, 1964; Harrison and Biellier, 1969; Edens and Siegel, 1974; Barnas *et al.*, 1981). Blood pH and carbon dioxide partial pressure (PCO_2) were inversely changed under conditions of high sustained temperature, but PO_2 was not affected by the hot environment (Edens and Siegel, 1974). In addition, hyperthermia in egg laying hens has been noted to cause not only a significant reduction in egg production (Mueller, 1966; Odom *et al.*, 1985; Vo *et al.*, 1980) but also a reduction in egg weight (Mueller, 1966; Vo *et al.*, 1980) and egg specific gravity (Harrison and Beilier, 1969; Odom *et al.*, 1985). Addition of sodium bicarbonate ($NaHCO_3$) to the feed does not appear to counteract the effect of hyperthermia on egg shell quality (Baker and Harrison, 1978). The effect of carbonated drinking water on chicks in hot environments, however, was notable. The results were improved egg shell quality and the relief of possible electrolyte imbalance induced by polypnea (Bottje and Harrison, 1985; Odom *et al.*, 1985).

The egg shell consists of approximately 98% calcium carbonate and 2% glycoprotein. Calcium of the egg shell is obtained from feed and that which is dissolved from medullary bones and released into the blood (Bloom *et al.*, 1941) whereas the carbonate is metabolically generated. During thermal polypnea body, carbonate is reduced by increased respiratory ventilation, resulting in hypocapnia and renal excretion (Bottje and Harrison, 1985; Staten and Harrison, 1985). As a consequence of these reductions in carbonate, blood acid-base balance is regulated within physiological ranges during hyperthermia. The involvement of bone in electrolyte balance during thermally induced hypocapnia is unknown, but such bone involvement has been documented in hypercapnia (Schaefer *et al.*, 1980; Pasquale *et al.*, 1980). These investigators have demonstrated that the carbonate and elemental fraction of bone changes in response to hypercapnic environments and may even contribute to an alternate acid-base balance. The purpose of this study has been to determine, with the aid of

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the scanning electron microscope (SEM) and energy-dispersive x-ray analytical techniques, if carbonated drinking water affects the composition and structural morphology of bone in heat-stressed chicks *vs.* those maintained at thermoneutral (room) temperature.

MATERIALS AND METHODS

Commercial Hubbard chicks at 4 weeks of age were divided into four treatment groups: 1) thermoneutral environment (25 C) with tap drinking water, (CTW); 2) thermoneutral environment with carbonated drinking water, (CCW); 3) hot environment (35 C) with tap drinking water (HTW); and 4) hot environment with carbonated water (HCW). Birds were maintained in their respective treatments up to the age of 8 weeks. During the 4-week experimental period, the birds received *ad libitum* feed (20% protein and 2800 kcal/kg diet) and continuous incandescent lighting. Chicks were housed on wood shavings in controlled environmental chambers. Carbonated drinking water was produced by mixing CO_2 gas with the tap water (Harrison, 1985). The drinking water was maintained throughout the experiment at a mean pH of 7.2 and 5.3 for the tap and carbonated sources, respectively. At 8 weeks of age, femura of six birds from each of the treatment groups were dissected and prepared for SEM and quantitative x-ray analysis.

For SEM preparations, the dorsal epiphysis regions of the femura were either oven-dried at 38 C for 1 week or fixed in a .1 *M* cacodylate-buffered fixative mixture consisting of 2.5% glutaraldehyde and 2.0% paraformaldehyde. Following drying or fixation, the samples were immersed into 5.25% sodium hypochlorite solution for 5 hr for removal of organic substances from the bones. The specimens were then gently washed in several changes of glass double distilled water for 6 hr and dehydrated

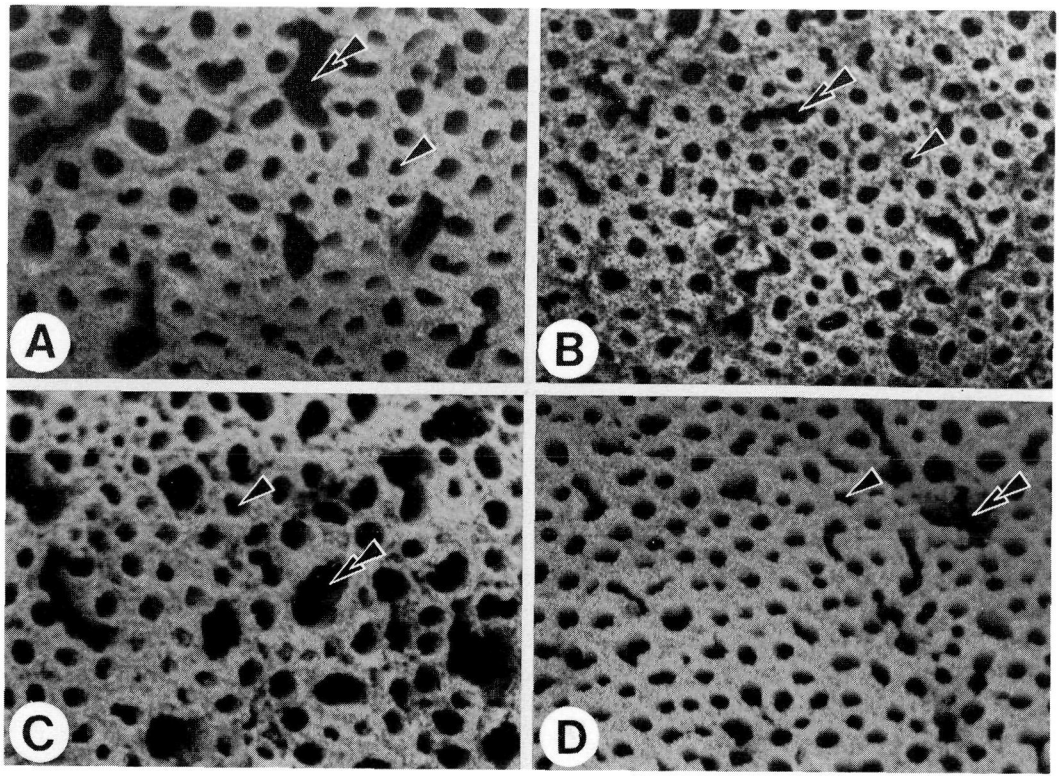

FIG. 1. Scanning electron micrographs of anorganic epiphysis surfaces. A, thermoneutral (25 C) environment with tap water; B, thermoneutral environment with carbonated water; C, hot environment (35 C) with tap water; and D, hot environment with carbonated water. Hole characteristics: Type 1, single arrow; Type 2, double arrow. ×25.

in an ascending series of ethanol solutions followed by air drying. The specimens were attached to aluminum mounting stubs with Duco cement or Dag and ion sputtered (Ca. 15 nm) with a gold-palladium alloy. Micrographs were taken with an ISI DS-130 scanning electron microscope (bottom stage operation).

For X-ray analysis, fresh proximal epiphyses of femurs were oven-dried at 38 C for 1 week. Specimens were then cut into 1 × 1 × 3-mm pieces with a razor blade cleaned with acetone and ethanol. The small sample pieces were vacuum infiltrated with soft LR White resin (Ernest F. Fullam, Inc.), using an evacuated dessicator, and polymerized at 60 C for 24 hr. Sections of the embedded bone samples were cut at a thickness of 2 μm with glass knives. In each case, sections were recovered on 300 mesh uncoated grids. A second grid was placed on top of the sections to prevent them from curling while drying. Prior to x-ray microanalysis, the top grid was removed and sample specimens were carbon coated (EMScope Model TB 500) to minimize charging effects. These specimens were observed in an Hitachi H-600 analytical electron microscope operating at 25 kV in a scanning transmission mode. The x-ray generated were analyzed using a Tracor-Northern detector (146 eV resolution) and a TN-5500 fully quantitative analyzer. This enabled full spectral elemental identification and semiquantitation of the inorganic composition of the specimen. Acquisition time for each spectrum was 150 sec (live time).

RESULTS AND DISCUSSION

Morphological results of epiphyseal surfaces of the femura obtained from each water treatment group reveal distinctly different morphologies (Fig. 1). The SEM show two types of pores; Type 1, individual small holes, and Type 2, larger irregular holes that appear to be made up of several smaller holes. Micrographs of CTW samples (Fig. 1A) showed more Type 2 holes than did CCW specimens (Fig. 1B). Similarly,

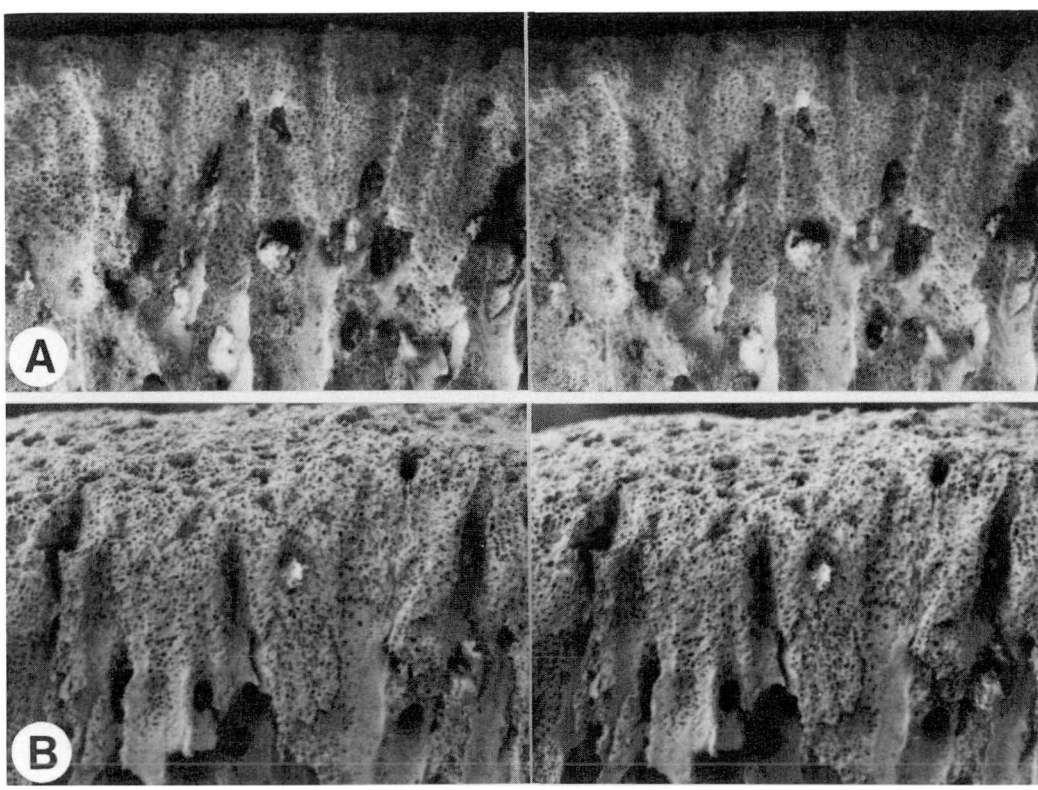

FIG. 2. Scanning electron stereomicrographs of anorganic epiphyses cut vertical to the epiphyseal surface. A, Hot environment (35 C) with carbonated water, and B, hot environment (35 C) with tap water. ×40.

TABLE 1. *Mean weight percent of bone element analyzed with energy dispersive x-ray microanalysis as influenced by environmental temperature and drinking water*

Element	Treatment[1]				Pooled SE
	CTW	CCW	HTW	HCW	
Ca	66.77	66.80	67.33	66.98	.39
P[2]	31.89[a]	31.56[ab]	31.12[abc]	30.64[bc]	.31
S	.57	.72	.48	1.40	.38
K	.37	.44	.48	.42	.12
Mg[2]	.28[ab]	.43[a]	.20[b]	.35[ab]	.06
Na	.04[a]	.04[a]	.05[a]	.19[b]	.04
Cl[2,3]	.03[a]	.02[a]	.10[b]	.02[a]	.02
Si	.06	.00	.24	.00	.12
Ca:P[2]	2.12[a]	2.12[a]	2.17[ab]	2.19[b]	.02

[a,b,c]Means in a row with same superscripts are not different (P<.05). t-Statistic was used for tests of significance.

[1] CTW = 25 C environment with tap water, CCW = 25 C environment with carbonated water, HTW = 35 C environment with tap water, HCW = 35 C environment with carbonated water.

[2] Environmental temperature is different (P<.05) when averaged over water treatment.

[3] Drinking water is different (P<.05) when averaged over temperature treatment.

the Type 2 holes observed in the micrographs of HTW samples (Fig. 1C) were greater in size and number than those seen in the HCW preparation (Fig. 1D). Wider interstitial spaces were present between the holes in the CTW samples than in the CCW samples. The morphology of HTW samples was similar to that of the CTW. The micrographs of HTW samples again clearly show that the Type 2 holes were made up of several Type 1 holes. Overall, SEM obtained from HTW and HCW demonstrated the same morphological similarity as seen between the CTW and CCW groups, respectively.

Stereomicrographs of samples of HTW and HCW cut vertical to the epipyseal surface showed a different morphology in the two groups (Fig. 2). Comparison of stereopairs taken from the HCW samples (Fig. 2A) and HTW (Fig. 2B) further suggest the HCW bone to be less porous. Observations made from the SEM also suggest a difference in bone compactness in the carbonated water treatment compared with tap water treatment. Carbonated water treatment reduced the size and number of the Type 2 holes.

Elemental x-ray analysis of all the bone samples from each group are shown in Table 1. Calcium and P components compose the major fraction of bone elements. Higher P (P<.05) was found in the control treatment (C) groups. As a consequence, the Ca:P ratio was lower (P<.05) in these groups. There were, however, no significant differences in Ca in any of the groups.

Sodium concentrations were similar in three of the groups (CTW, CCW, and HTW). Only HCW showed significantly more Na (P<.05). It is of interest to note that the increase in bone Na in the HCW group in this study was mimicked by increased labile bicarbonate (HCO_3^-) observed in a parallel study conducted in our laboratory (unpublished data). These results suggest a relationship between the Na and HCO_3^- in the chick bones that are influenced by heat stress and the consumption of carbonated drinking water.

A significantly less Cl was found in the control groups than in the heat-stressed groups (P<.05). Notably more Cl (P<.01) was observed in HTW than in CTW, CCW, and HCW. Based on the changes occurring in total blood carbonate (CO_2 and HCO_3^-) during thermal panting and respiratory alkalosis (Bottje and Harrison, 1985), the higher Cl concentrations found in the bone of HTW-treated chicks may represent a chloride ion shift in order to maintain anionic homeostasis.

Scanning electron micrographs showed that bone from the HCW chicks appeared more compact than bone from the HTW birds. Of the different experimental groups studied, x-ray analytical results demonstrated that there were no apparent differences in amount of bone Ca.

HTW = hot environment tap water

HCW = hot environment Carbonated water

The greatest elemental differences were with the anion Cl and the cation Na, which were probably associated with changes in body CO_2 and HCO_3^- during thermally induced hypocapnea and replenishment of CO_2 with carbonated water (Bottje and Harrison, 1985).

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