Arterial Blood Gas, pH, and Bicarbonate Values in Laying Hens Selected for Thick or Thin Eggshell Production[1]

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ABSTRACT Bicarbonate, pH, carbon dioxide partial pressure (pCO_2), and oxygen partial pressure (pO_2) were measured in blood samples collected anaerobically from the brachial arteries of domestic fowl from lines selected for thick (TK) or thin (TN) eggshell production. The blood values of TK and TN hens were compared 6 hr prior to oviposition and continued at 2-hr intervals until 10 hrs postoviposition. Percent shell values were measured for eggs laid 2 days prior to and during blood sampling. Hens with TK shells had significantly ($P<.001$) higher percent shell values than hens with TN shells. The measured blood parameters (bicarbonate, pH, pCO_2, and pO_2) did not differ significantly ($P>.05$) when TK and TN hens were compared at the time of oviposition. However, between 2 and 6 hr postoviposition, TN hens had significantly lower blood pH, pO_2, and bicarbonate than did TK hens. Arterial pCO_2 tended to be higher in TN hens than in TK hens, but this difference was significant only at 6 hr preoviposition. These results show that TN hens develop metabolic acidosis relative to TK hens during the first 6 hr postoviposition.
(Key words: arterial blood gas, acid-base, percent shell)

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INTRODUCTION

Calcium and carbonate are the major constituents of eggshells (Mongin, 1968). During shell calcification, the carbonate used for shell formation is derived from carbon dioxide produced by the metabolic activity of the shell gland (Hodges and Lorcher, 1967; Hodges, 1969; Simkiss, 1975; Cipera, 1980; Junqueira *et al.*, 1983). Shell gland carbonic anhydrase has been implicated in the generation of eggshell carbonate (Bernstein *et al.*, 1968; Simkiss, 1975; Junqueira *et al.*, 1983). Carbonate formation is associated with hydrogen ion production, and these hydrogen ions move from the shell gland into the blood, causing blood pH and bicarbonate concentrations to decrease (Mongin and Lacassagne, 1966; Mongin, 1968; Hodges, 1969; Simkiss, 1975). The development of metabolic acidosis during eggshell calcification therefore serves as an indirect index of shell gland carbonate formation (Gutowska and Mitchell, 1945; Mongin, 1968; Simkiss, 1975).

The role of blood calcium in shell formation has been investigated using domestic fowl from lines selected for thick (TK) or thin (TN) eggshell production (Buss, 1982; Wideman and Buss, 1984). When blood samples were collected between 2 and 4 hr postoviposition, mean plasma calcium concentrations for three TK lines exceeded mean plasma calcium concentrations for three TN lines whenever the TK lines had significantly higher percent shell values than the TN lines (Wideman and Buss, 1984). The possible role of shell gland carbonate formation had not been evaluated in these lines.

Hamilton (1981) measured calcium, pH, oxygen partial pressure (pO_2), carbon dioxide partial pressure (pCO_2), and bicarbonate in venous blood from hens selected for low (LSG) or high (HSG) specific gravity egg production. There were no differences ($P>.05$) between LSG and HSG hens for any of the measured parameters when blood samples were withdrawn without regard to the time of oviposition.

The objective of the present study was to determine if TK and TN lines of hens from two strains of domestic fowl differ significantly in their arterial blood pH, pO_2, pCO_2, and bicarbonate values when blood samples are collected at known intervals before and after oviposition.

MATERIALS AND METHODS

Experimental Animals. Family selection was used to select thick- and thin-shell lines from

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two strains of domestic fowl (Buss *et al.*, 1977; Buss, 1982). White thick (WTK) and thin (WTN) lines were bred from Single Comb White Leghorn parent stock. Brown thick (BTK) and thin (BTN) lines were derived from crosses between black and Rhode Island Red parent stocks. The black stock had been bred as a test stock for feather color by crossing broiler-type males with Rhode Island Red hens. The development of these TK and TN lines was described previously (Wideman and Buss, 1984). All of the hens used in the present study were 55 to 60 weeks old. They were housed individually in cages in the same layer house at The Pennsylvania State University poultry facility. Cages measured 33 cm deep, 27 cm wide, and 42.5 cm high in the front. Lights were on for 14 hr per day, starting at 0300 hr. Water and commercial layer ration were available *ad libitum*. The layer ration contained 17.5% protein, 3.5% calcium, and .47% available phosphorus. Hens were excluded from the study if they laid fewer than three eggs per week for the 2-weeks prior to sampling.

Samples were collected on two separate days from 44 hens, including 15 WTK, 8 BTK, 11 WTN, and 10 BTN hens. Due to the small population size, data for the TK hens were pooled, as were data for TN hens. Pooling the data allowed an overall comparison of TK *vs.* TN birds.

Experimental Protocol. For each hen, a brachial artery was cannulated with 20 to 30 cm of PE-50 polyethylene tubing filled with heparinized saline (200 units ammonium heparin/ml saline). Lidocaine (2%) was injected intracutaneously as a local anesthetic prior to cannulation. The cannula was tied in place with suture thread wrapped around the brachial artery. The incision was closed with wound clips; then the cannula was fastened to the skin of the wing with suture thread. Furazolidone aerosol powder (Veterinary Products Industries, Phoenix, AZ) was applied to the incision. The cannula was flushed with fresh heparinized saline, and a knot was tied at the free end of the cannula. The cannula was coiled and secured to the wing with tape. Cannulas were flushed daily with heparinized saline to prevent clotting.

Hens were selected for blood sampling when palpation indicated they would lay an egg during the morning (0400 to 1200 hr). Blood sampling began at 0400 hr, and subsequent samples were collected every 2 hr until oviposition. The time of oviposition was noted by visually inspecting the cages at 15-min intervals. Therefore, a blood sample was taken within 15 min after oviposition. Postoviposition samples were taken every 2 hr for the next 10 hr. For hens that laid an egg on the day following the blood sampling period, all samples collected up to 10 hr postoviposition were included in the data set. For hens that failed to lay an egg on the day following blood sample collections, only the samples collected during the first 4 hr postoviposition (noncalcifying phase) were included in the data set. Blood samples collected prior to oviposition were assigned to the temporally closest preoviposition intervals of −2, −4, or −6 hr.

Blood samples were collected by uncoiling the cannula, cutting off the knot, and allowing arterial pressure to push the saline from the dead space in the cannula. Then a 23-gauge needle attached to a 3-ml syringe was inserted into the cannula, and 1 to 2 ml of blood was withdrawn anaerobically. Dead spaces in the needle and syringe were filled with heparinized saline. Within 3 min, the sample was injected into an ABL-2 Acid-Base Laboratory (Radiometer Copenhagen), and the readings were internally corrected for a body temperature of 41 C. The ABL-2 calculates bicarbonate concentrations from the Henderson-Hasselbalch equation:

$$pH = pK' + \log \frac{(HCO_3^-)}{\alpha pCO_2}$$

where $pK' = 6.099$ at pH = 7.40 and $pK' = 6.079$ at pH = 7.80. The solubility constant for carbon dioxide (CO_2) in plasma (α) used by the ABL-2 is .0306. Blood values for pH, pCO_2, pO_2, and bicarbonate were accepted if the ABL-2 was operating within recommended limits as indicated by internal automatic standardization and external "Qualicheck" standardization. Calibrations were conducted every 30 min. Because pH is a log function, all pH values were converted to hydrogen ion concentrations before calculating means and standard errors. Then, the mean and standard error values were reconverted to pH for graphic representation.

Statistical Analysis. Student's t test was used to compare TK *vs.* TN blood values at each 2-hr interval in the egg formation cycle.

TABLE 1. *Percent shell values for thick- and thin-shell lines of white and brown strains of chickens (mean ± SE, n)*[1]

Line	Shell	n
	(%)	
White thick	9.5 ± .1	44
White thin	6.3 ± .1	18**
Brown thick	9.3 ± .1	27
Brown thin	8.2 ± .1	27**
Combined thick	9.4 ± .1	71
Combined thin	7.6 ± .1	45**

[1] Significant differences (P<.001) between thick and thin values are denoted by **.

RESULTS

Table 1 shows percent shell values (weight of dried shell plus membranes/total egg weight × 100) for eggs collected from TK and TN hens 2 days prior to and during 2 separate days of blood sample collections. The WTK hens had significantly greater percent shell values than WTN hens (P<.001), and BTK hens had significantly greater percent shell values than BTN hens (P<.001). The percent shell value for combined TK hens was significantly greater than the combined percent shell value for TN hens (Table 1).

Blood values for pH, bicarbonate, pO_2, and pCO_2 are shown in Figures 1 to 4, respectively. None of these parameters differed significantly (P>.05) at oviposition (time 0). However, by 4 hr postoviposition, pH, bicarbonate, and pO_2 all were significantly lower (P<.05) in TN hens than in TK hens. The values for pCO_2 tended to be higher in TN hens when compared with TK hens, but this difference was significant only for blood samples collected 6 hr prior to oviposition (Fig. 4). Overall, TN hens developed metabolic acidosis relative to TK hens during the 6 hr postoviposition, as indicated by the combination of lower blood pH (Fig. 1) and lower bicarbonate (Fig. 2) in TN hens. The TN hens did not exhibit respiratory compensation for the metabolic acidosis, since blood pCO_2 was higher in TN hens than in TK hens (Fig. 4).

DISCUSSION

The arterial blood values for pH, bicarbonate, pCO_2, and pO_2 obtained in the present study (Figs. 1 to 4) fall within the ranges reported for arterial blood values in unanesthetized domestic fowl in other studies (Burton *et al.*, 1968; Cohen and Hurwitz, 1974; Arad, 1983). Blood gas and acid-base parameters can be affected by the composition of the diet (Hamilton and Thompson, 1980), by the pK′ or ∝ values used to calculate blood bicarbonate concentrations (Helbacka *et al.*, 1964), by ambient temperatures (Vo *et al.*, 1978; Arad, 1983), by age (Vo *et al.*, 1978), and by altitude or hypoxia (Burton *et al.*, 1968). The TK and TN hens in the present study were the same age; they were located in the same layer house exposed to the same environmental temperatures (17 to 20 C), and they were fed the same commercial layer rations. It therefore is possible to make direct qualitative and quantitative comparisons of TK *vs.* TN blood gas and acid-base values.

Mongin and Mueller (1973) considered the acid-base balance of laying hens to be "normal" 15 min postoviposition. Because blood gas and acid-base values for samples collected 15 min

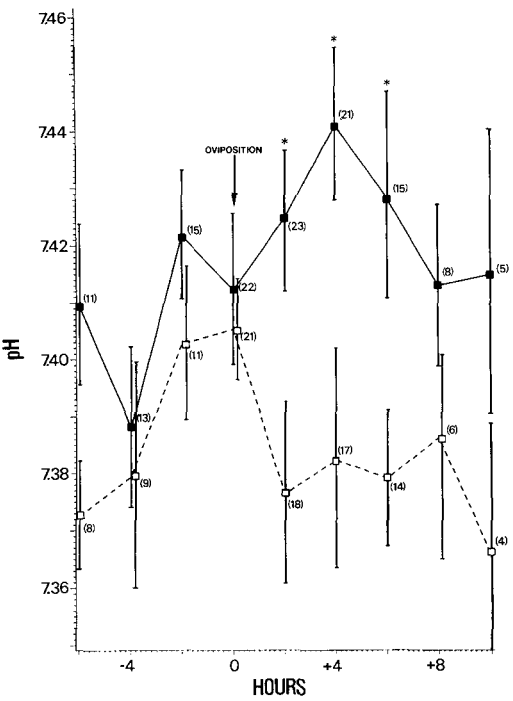

FIG. 1. Arterial blood pH for thick-shell (—■—) and thin-shell (- - -□- - -) lines of hens from 6 hr before oviposition (−6) to 10 hr postoviposition (+ 10) with oviposition shown as time zero (mean ± SE, n). *P<.05.

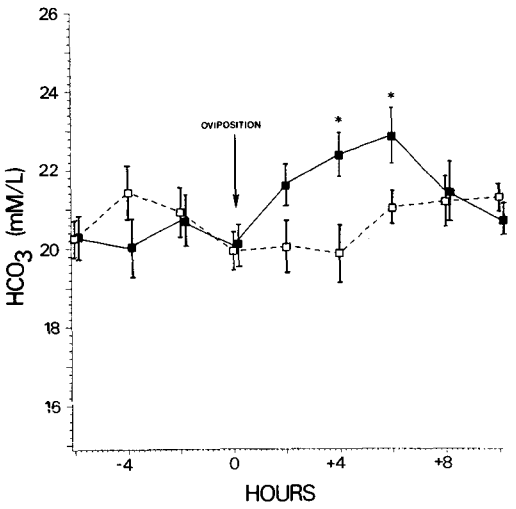

FIG. 2. Arterial blood bicarbonate (HCO_3) concentrations for thick-shell (—■—) and thin-shell (- - -□- - -) lines of hens (mean ± SE). *P<.05.

postoviposition in the present study fall within the ranges reported by other investigators *(vide supra)*, and because values for samples collected 15 min postoviposition were not significantly different for TK *vs.* TN comparisons, it can be concluded that "normal" acid-base and blood gas values near the time of oviposition had not been altered by selection for TK and TN shell lines. Blood gas values were not compared for males of the TK and TN lines.

The results of this study clearly demonstrate the need to relate blood sampling times to the stage of eggshell formation. Given the interindividual scatter of values obtained for all measured parameters, as shown by the error bars in Figures 1 to 4, it is clear that significant differences between TK and TN hens would not have been detected if the samples had been collected without regard to the stage of egg formation.

Other investigators have shown that the peak of metabolic acidosis occurs approximately 22 hr postoviposition during the later stages of shell calcification (Mongin and Lacassagne, 1966; Hodges, 1969). The 6 hr preoviposition and 10-hr postoviposition pH and bicarbonate values in the present study (Figs. 1 and 2) suggest that TK hens did not develop significant metabolic acidosis during shell calcification. Too few samples were collected during in-

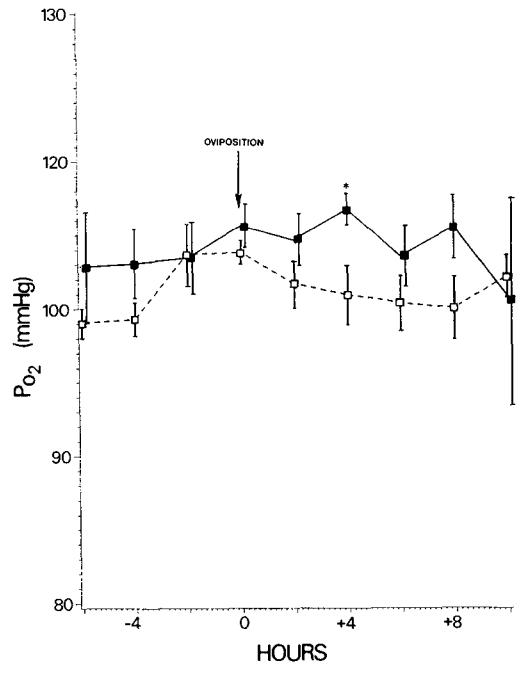

FIG. 3. Arterial blood partial pressure of oxygen (pO_2) for thick-shell (—■—) and thin-shell (- - -□- - -) lines of hens (mean ± SE). *P<.05.

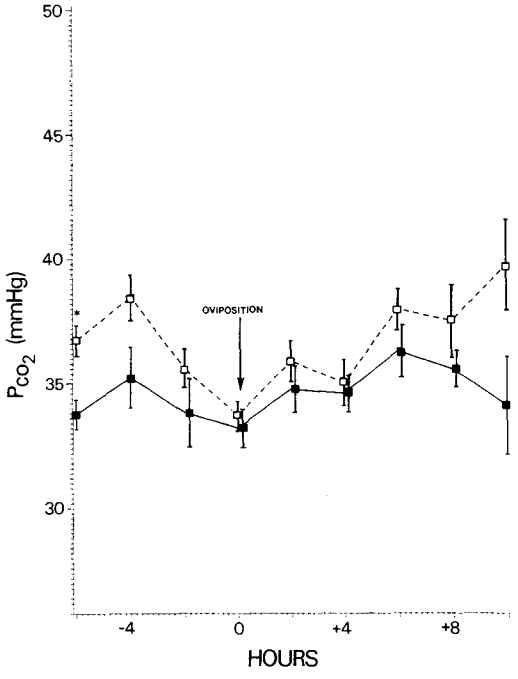

FIG. 4. Arterial blood partial pressure of carbon dioxide (pCO_2) for thick-shell (—■—) and thin-shell (- - -□- - -) lines of hens (mean ± SE). *P<.05.

termediate stages of calcification to allow reliable interpretation of acid-base balance between 12 and 18 hr postoviposition. It is clear, however, that TN hens did develop acidosis during the early and late stages of shell formation (Fig. 1). These results suggest that profound differences exist between TK and TN hens.

Differences in the development of metabolic acidosis during or prior to shell calcification (2 to 6 hr postoviposition) may be related either to differences in carbonate generation by the shell gland (see Introduction) or to differences in acid excretion in the urine. The former possibility would suggest that the shell glands of TN hens generate relatively more carbonate than the shell glands of TK hens, which would indicate that carbonate is not a limiting factor in determining shell quality. The latter possibility would suggest that increasing the renal capacity to excrete an acid load may contribute to improved shell quality. It can be estimated that 98% of the fixed acid generated by the shell gland is excreted by the kidney with only 2% of that acid being buffered by the blood (Mongin and Mueller, 1973). It has been shown that urinary bicarbonate excretion and pH decrease during shell calcification (Anderson, 1967). Renal mechanisms of acid-base balance remain to be tested in TK and TN lines of chickens.

Previous work had shown that genetic selection for TK and TN eggshell lines resulted in coselection for plasma calcium concentration in three strains of chickens (Wideman and Buss, 1984). It now appears that selection for TK and TN lines also resulted in coselection for changes in acid-base balance.

REFERENCES

Anderson, R. S., 1967. Acid-base changes in excreta of the laying hen. Vet. Rec. 80:314–315.

Arad, Z., 1983. Thermoregulation and acid-base status in the panting dehydrated fowl. J. Appl. Physiol. 54:234–243.

Bernstein, R. S., T. Nevalainen, R. Schraer, and H. Schraer, 1968. Intracellular distribution and role of carbonic anhydrase in the avian *(Gallus domesticus)* shell gland mucosa. Biochim. Biophys. Acta 159:367–376.

Burton, R. R., E. L. Besch, and A. H. Smith, 1968. Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. Am. J. Physiol. 214:1438–1442.

Buss, E. G., 1982. Genetic differences in avian egg shell formation. Poultry Sci. 61:2048–2055.

Buss, E. G., R. M. Leach, and J. T. Stout, 1977. Egg shell quality for chickens in selected lines, F_1's and F_2's. Poultry Sci. 56:1699–1700.

Cipera, J. D., 1980. Source of carbon for the biosynthesis of eggshell carbonate in the hen. Comparison of six ^{14}C labeled compounds as sources of carbon in eggshells, albumen, and yolk. Poultry Sci. 59:1529–1537.

Cohen, I., and S. Hurwitz, 1974. The response of blood ionic constituents and acid-base balance to dietary sodium, potassium and chloride in laying fowl. Poultry Sci. 53:378–383.

Gutowska, M. S., and C. A. Mitchell, 1945. Carbonic anhydrase in the calcification of the egg shell. Poultry Sci. 24:159–167.

Hamilton, R.M.G., 1981. The effects of strain, age, time after oviposition, and egg specific gravity on acid-base balance in White Leghorn hens. Poultry Sci. 60:1944–1950.

Hamilton, R.M.G., and B. K. Thompson, 1980. Effects of sodium plus potassium to chloride ratio in practical-type diets on blood gas levels in three strains of White Leghorn hens and the relationship between acid-base balance and egg shell strength. Poultry Sci. 59:1294–1303.

Helbacka, N.V.L., J. L. Casterline, Jr., C. J. Smith, and C. S. Schaffner, 1964. Investigation of plasma carbonic acid pK′ of the chicken. Poultry Sci. 43:138–144.

Hodges, R. D., 1969. pH and mineral ion levels in the blood of the laying hen *(Gallus domesticus)* in relation to egg shell formation. Comp. Biochem. Physiol. 28:1243–1257.

Hodges, R. D., and K. Lorcher, 1967. Possible sources of the carbonate fraction of egg shell calcium carbonate. Nature 216:609–610.

Junqueira, O. M., R. D. Miles, and R. H. Harms, 1983. The inability of sodium bicarbonate to induce an improvement in eggshell quality in the presence of sulfanilamide. Poultry Sci. 62:2062–2064.

Mongin, P., 1968. Role of acid-base balance in the physiology of egg shell formation. World's Poult. Sci. J. 24:200–230.

Mongin, P., and L. Lacassagne, 1966. Equilibre acido-basique du sang et formation de la coquille de l'oeuf. Ann. Biol. Anim. Biochim. Biophys. 8:93–100.

Mongin, P., and W. J. Mueller, 1973. Normal buffer lines for oxygenated and deoxygenated blood of the laying hen. Comp. Biochem. Physiol. 44A: 221–227.

Simkiss, K., 1975. Calcium and avian reproduction. Symp. Zool. Soc. Lond. 35:307–337.

Vo, K. V., M. A. Boone, and W. E. Johnston, 1978. Effect of three lifetime ambient temperatures on growth, feed and water consumption, and various blood components in male and female Leghorn chickens. Poultry Sci. 57:798–803.

Wideman, R. F. Jr., and E. G. Buss, 1984. Percent shell and plasma mineral concentrations in three strains of domestic fowl selected for thick or thin egg shell production. Poultry Sci. 64:388–395.