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Effects of Water Deprivation during the Prelaying Period on Eggshell Quality, Wet Droppings and Blood Physiology in Laying Hens

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ABSTRACT

The influence of water deprivation at the onset of lay on subsequent eggshell quality, fecal moisture content and blood physiology was investigated in ISA Brown pullets. Kidney and bone appearances were also determined at the end of the study. The pullets were randomly divided into 3 groups; control, dehydrated, and dehydrated+NH_4Cl groups. The birds in the control group were provided a layer diet with water at all times. At 20 weeks of age, drinking water supply in the dehydrated and dehydrated+NH_4Cl groups was removed completely for 48 hours whereas feed was available at all times. Thereafter, the water was provided until the end of the trial. The birds in the dehydrated+NH_4Cl group were fed on the control diet added with ammonium chloride (NH_4Cl) at a level of 1% throughout the rehydration phase. Water deprivation for 2 days at the onset of lay did not affect eggshell quality throughout the experimental period. An incidence of poor eggshell was observed in the dehydrated+NH_4Cl group as evidenced by reduced shell weight and shell thickness at 24 weeks of age ($P<0.05$). During the 2 days of dehydration, fecal moisture contents were significantly reduced for the birds in the two groups of dehydration. However, the excreta moisture of the birds in these groups increased rapidly as the drinking water was returned and significantly greater than that of the control birds by 6 hours of water replenishment. On Week 8 of rehydration, the increase of the manure moisture was observed again for the dehydrated+NH_4Cl hens when compared with the control birds ($P<0.05$). Interruption of available drinking water increased hematocrit values for the two groups of dehydration during 48 hours of water deprival. Hematocrit values of the hens in these groups, however, were rapidly decreased as the water was reintroduced and significantly reduced on Week 8 of rehydration for the dehydrated hens fed with the acidified diet. Water deprivation was associated with increases in the concentration of sodium in plasma whereas plasma pH was inconsistent and varied among the experimental groups. Decreased humerus weight was observed in the dehydrated+NH_4Cl hens necropsied at the end of the study.

Key words: water deprivation, ammonium chloride, onset of lay, laying hens, eggshell, wet droppings, hematocrit, plasma sodium, kidneys, humerus bone

INTRODUCTION

Lacking of drinking water commonly occurs in poorly managed poultry flocks, especially over the week-end period. Water deprivation resulting from failures of mechanical equipment can be acute in such flocks. Inadequate water occurred because of improper slope or level on flowthrough or float systems, leakage, clogged lines, cups not functioning properly or improperly placed in the

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cages so birds in those cages or the adjoining cages had difficulty reaching them. The removal of drinking water in laying hens even for a few days appears to have a long-term effect on subsequent eggshell quality. Bierer *et al*. (1965) indicated that withdrawal of water for 48 hours had deleterious effects on eggshell quality as evidenced by decreased eggshell thickness. It is well documented that reduced availability of drinking water may cause avian kidney diseases (Siller, 1981; Julian, 1982). Siller (1981) demonstrated that prolonged water deprivation severely affected the kidneys in birds resulting in renal failure and sudden death. Julian (1982) indicated that water deprivation in laying hens was the major cause of an outbreak of urolithiasis and acute kidney diseases. This worker found symptoms of urolithiasis, such as renal tubule degeneration, kidney atrophy and swollen kidneys, in laying hens deprived of water for 48 hours. Onderka *et al*. (1987) showed an evidence of pale and swollen kidneys in 2-day-old broiler chickens after 120 hours of water deprivation but the investigators were unable to detect any histological lesions in kidneys of the birds deprived of water for 24 or 48 hours. Adding ammonium chloride in diets has been reported to be effective on reducing the incidence of renal disease (Glahn *et al.,* 1988).

Little is known about the influence of lacking of drinking water in pullets during the pullet-layer transition period on subsequent eggshell quality and kidney appearances. The objective of this study was to determine the effects of water deprivation for 48 hours in commercial pullets, at 20 weeks of age, on subsequent eggshell quality, fecal moisture content and blood physiology. In addition, the use of a diet acidified with 1%NH_4Cl in preventing kidney damages was also examined. Observations of long bones and asymmetrical kidneys were also undertaken at the end of the experiment.

MATERIALS AND METHODS

Three hundred ISA Brown pullets, 16 weeks of age, were used in this study. The birds were randomly divided and assigned into three treatment groups: 1) control (feed with water), 2) dehydrated (feed without water), and 3) dehydrated+NH_4Cl (feed without water+NH_4Cl). The pullets were allotted to standard laying cages (100 cages, 3 birds per cage) located in an open-sided layer house. An acclimatization period of 4 weeks was allowed. In the control group, the pullets were provided food and water *ad libitum* throughout the experimental period. In the dehydrated and dehydrated+NH_4Cl groups, the drinking water was removed completely for 48 hours of dehydration but feed available at all times. The water was then returned (rehydration) to the dehydrated and dehydrated+NH_4Cl groups following the water withdrawal period. The birds in all treatment groups were fed on a layer diet, except for the birds in the dehydrated+NH_4Cl group were received the layer diet supplemented with 1%NH_4Cl throughout the rehydration period. The layer ration used in this study was a mash commercial corn-soybean ration, formulated to have a calculated analysis of 3.25% Ca, 0.5% available phosphorous (aP), and an ME value of 2,851 cal/kg. The acidified layer diet was made by adding 0.45 kg of NH_4Cl to 44.9 kg of the layer diet. Biweekly, egg shell quality was measured by measuring shell weight and shell thickness for the first 12 weeks of production. Fecal moisture contents were recorded 1 week prior to water deprivation and during 48 hours of water deprivation. The manure moisture contents were determined again after the water was returned for 6 hours, 1 week, 2, 4, 8 and 12 weeks. Fresh manure samples were collected, over a seven-hour period, in aluminum foil containers underneath the cages, weighing the fresh droppings, drying them to constant weight at 95°C for 24 hours using a dry oven (Model Binder E) and re-weighing.

Percentage of moisture content was calculated. Meanwhile, blood samples were collected from 5 birds from each treatment groups. A 5-ml blood sample was drawn in a 10-ml syringe via vein puncture of the cutaneous ulnar vein. The time of sampling was usually between 9:00 and 11:00 a.m. Duplicate hematocrit tubes were filled with blood, sealed and centrifuged in a Hawksley Microhematocrit Centrifuge at 1,500 RPM for 10 minutes for the measurement of hematocrit. The remainder of the blood was centrifuged in a Hettich EBA 8S Zentrifugen at 3,500 RPM for 15 minutes and the plasma was removed for the measurement of pH and sodium concentrations. The concentration of sodium and pH were analyzed using an ion selective PC 5000/5500 pH/ISE Benchtop Meter at temperature 25°C.

Gross kidney and bone measurements: At the end of the study, five birds from each group were sacrificed for gross kidney and bone examination. The birds were necropsied and the weights of the body were recorded. Immediately, after the necropsy, both kidneys were taken carefully and weighed individually. Kidney asymmetry was measured by dividing the weight of the heaviest kidney within each bird by the weight of the lighter kidney (Wideman *et al*., 1983). This calculation was called the heavy: light kidney ratio. Humerus and femur bones were also collected for the determination of the length and weight.

The experiment was commenced in July, 2003 and terminated in December, 2003. The average of minimum and maximum ambient temperatures were 26.04 ± 1.98°C and 33.29 ± 0.66°C, respectively. The statistical evaluation of the data was performed by analysis of variance. Repeated measures ANOVA was used where appropriate. Mean values were compared using Duncan's multiple range test to determine significance (Snedecor and Cochran, 1980). Significance was assumed if $P<0.05$.

RESULTS

Generally, no deaths occurred during the experimental period. The birds remained seemingly in good health. Birds deprived of water drank the water rapidly and avidly after the water was returned. Normally the urine of birds is always clouded by solid deposits of uric acid. In this experiment, uric acid was obviously noticed in the urine excreted by the dehydrated birds during the dehydration.

Influences of drinking water withdrawal before the onset of egg laying on subsequent eggshell quality of the different treatment groups are shown in Table 1. At 24 weeks of age, eggshell weight of the birds in the dehydrated+NH_4Cl group (8.8%) was significantly lower than that of the hens in the dehydrated group (9.6%) ($P<0.05$). However, the average of eggshell weight of the control hens was not significantly different from that of the birds in the other two groups of dehydration. A similar pattern was observed in shell thickness at the same age. Table 2 represents fecal moisture content and hematocrit values of the experimental birds before and during water deprivation, and after rehydration occasionally. During the 2 days of dehydration, fecal moisture of the dehydrated hens decreased approximately 5.7 % from an average control value ($P<0.05$). Reversely, the hens in the two groups of deprival produced the excreta with a higher percentage than did the normally hydrated birds as the water was reintroduced within 6 hours ($P<0.05$). A significant difference of fecal moisture content was observed again on Week 8 of rehydration, in which the dehydrated+NH_4Cl hens produced wetter manure as compared with the control hens. However, fecal moisture of the birds in the dehydrated group was not significantly different from that of the hens in the other two groups ($P>0.05$). The data indicated that deprivation of water for 2 days significantly affected hematocrit values. Hematocrit values in both groups of water

deprivation were elevated approximately 15% on dehydration, and then returned to normal levels when the water was replenished, except at 8 weeks at post-rehydration. On Week 8 of rehydration, the level of hematocrit values of the birds in the dehydrated+NH_4Cl group was lower than ($P<0.05$) that of the hens in the dehydrated group.

Changes in plasma pH and sodium concentrations in the different treatment groups were summarized in Table 3. The concentration of sodium in plasma increased during the 2 days of dehydration for the dehydrated hens fed with the acidified diet as compared with the control hens. Although the difference was not statistically significant, the hens in the dehydrated group had a greater level of plasma sodium than the birds in the control group. The effect of water deprivation at the onset of lay on plasma pH was inconsistent and

Table 1 Effects of water deprivation at 20 weeks of age on subsequent eggshell quality.

Group	Age (weeks)					
	22	24	26	28	30	32
			Shell weight (%)			
Control	9.6±0.4	9.3±0.7^{ab}	9.3±0.3	9.2±0.7	9.2±0.5	10.0±0.4
Dehydrated	9.2±0.6	9.6±0.2^{a}	9.3±0.9	9.3±0.5	9.2±0.3	9.7±0.1
Dehydrated +NH_4Cl	9.4±0.3	8.8±0.6^{b}	9.0±0.3	9.3±0.3	9.3±0.2	9.6±0.6
			Shell thickness (μm)			
Control	352.2±44.0	349.5±25.6^{ab}	364.8±11.1	356.3±24.6	363.1±22.4	392.1±24.8
Dehydrated	346.4±35.0	368.8±13.8^{a}	363.7±35.9	358.7±28.4	370.3±10.9	381.2±9.2
Dehydrated +NH_4Cl	340.0±10.0	334.5±22.4^{b}	352.5±17.6	353.2±7.8	369.6±8.7	382.2±16.3

$^{a-b}$ Means within columns in each parameter with no common superscript differ significantly between groups ($P<0.05$) by the use of DMRT.

Table 2 Effects of water deprivation at 20 weeks of age on subsequent fecal moisture and hematocrit values in the different treatment groups.

Group	Pretest	48 hours-of deprived	Rehydration (weeks)					
			6 hours	1 week	2	4	8	12
					Fecal moisture (%)			
Control	73.4±6.7	74.2±0.9^{a}	80.4±2.9^{b}	76.8±4.5	75.6±6.1	84.1±6.2	80.3±4.2^{b}	87.0±4.1
-water	78.9±6.0	70.4±1.1^{b}	86.8±5.1^{a}	76.5±5.4	75.7±3.1	84.6±5.1	83.0±5.5^{ab}	87.3±6.6
-water +NH_4Cl	75.7±4.0	70.2±0.9^{b}	90.6±1.4^{a}	81.3±7.8	79.5±6.1	86.9±4.1	88.3±2.6^{a}	85.0±7.9
					Hematocrit (%)			
Control	23.4±6.7	27.4±1.1^{b}	-	26.2±2.6	24.1±0.5	25.5±1.3	26.3±2.2^{ab}	24.6±1.7
-water	28.9±6.0	31.7±2.6^{a}	-	28.2±2.1	24.9±4.4	25.2±2.7	27.5±3.2^{a}	25.9±0.8
-water +NH_4Cl	25.7±4.0	32.1±1.2^{a}	-	25.1±4.9	25.4±2.4	25.0±3.4	23.7±1.8^{b}	23.9±1.1

$^{a-b}$ Means within columns in each parameter with no common superscript differ significantly between groups ($P<0.05$) by the use of DMRT.

-water = dehydration.

varied among the experimental bird groups. Total kidney weights (left plus right) and kidney weight as a proportion of body weight, for the birds necropsied at 32 weeks of age, are given in Table 4. Total kidney weight tended to be higher in the control group than in the other two dehydrated groups. The similar tendency also occurred when the kidney weight was calculated on a body weight basis. Based on the calculated heavy/light kidney weight ratios, a tendency towards asymmetrical kidneys was noted in the dehydrated and dehydrated+NH_4Cl birds. The greatest degree of

Table 3 Effects of water deprivation at 20 weeks of age on plasma pH and Na concentrations during the first 12 weeks of production period.

Group	Pretest	48 hours-of deprived	Rehydration (weeks)				
			1 week	2	4	8	12
				Plasma Na concentration (10^4 ppm)			
Control	1.75±0.02	1.95±0.12[b]	2.09±0.20	2.21±0.09	2.20±0.37	2.27±0.22	2.27±0.08
-water	1.73±0.09	2.26±0.46[ab]	1.95±0.06	1.97±0.11	2.04±0.10	2.11±0.08	2.11±0.12
-water +NH_4Cl	1.88±0.04	2.47±0.30[a]	2.02±0.11	2.21±0.21	1.94±0.07	2.17±0.10	2.17±0.14
					Plasma pH		
Control	8.69±0.24	8.57±0.08	8.60±0.08	8.59±0.17	8.54±0.11	8.43±0.10	8.33±0.06
-water	8.60±0.10	8.50±0.13	8.45±0.10	8.49±0.01	8.40±0.11	8.51±0.07	8.50±0.03
-water +NH_4Cl	8.54±0.01	8.49±0.12	8.53±0.09	8.53±0.11	8.50±0.09	8.43±0.12	8.52±0.05

[a-b] Means within columns in each parameter with no common superscript differ significantly between groups ($P<0.05$) by the use of DMRT.

-water = dehydration.

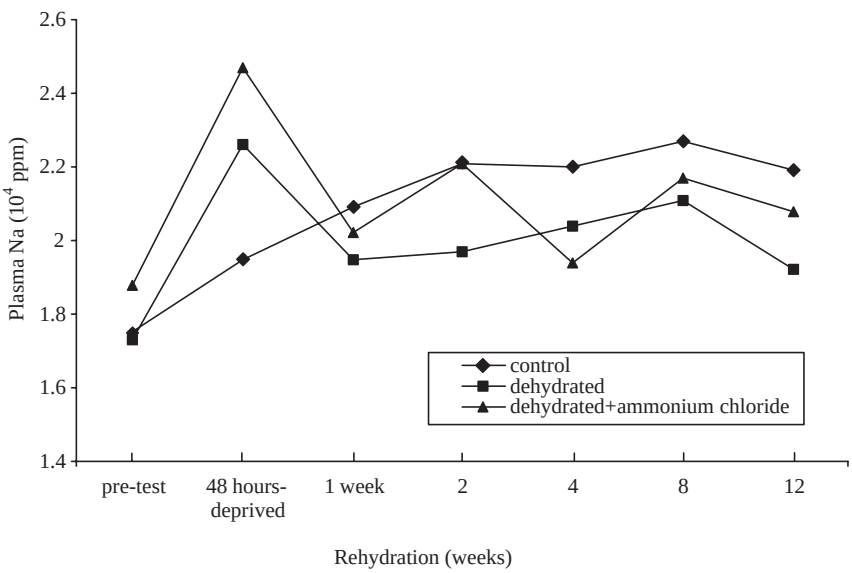

Figure 1 Changes in plasma Na concentrations in the different treatment hens before and after water deprivation.

asymmetry of the kidneys was noticed in the dehydrated+NH_4Cl group (Hen 14, 1.15). As a test of intra-individual kidney weight symmetry, left kidney weights were plotted against right kidney weights for each bird (Figure 2). Table 5 summarizes the length and weight of humerus and femur bones in the different treatment groups. There were no significant differences among the experimental bird groups for all parameters, except for humerus weight. It was apparent that the birds in the dehydrated+NH_4Cl group exhibited the lowest value of the right bone when compared with that of the hens in the other two groups ($P<0.05$).

DISCUSSION

The findings of this study, that removal of drinking water for 48 hours produced an increase in hematocrit values, were consistent with those reported by previous investigators (Koike *et al*., 1983). Consequently, plasma sodium concentrations increased during the 2 days of dehydration, although the difference of the plasma electrolyte level between the dehydrated birds and the control hens was not statistically significant.

Table 4 Gross kidney measurements of the different treatment groups recorded at 12 weeks following rehydration (32 weeks of age).

Group	BW (g)	Kidney weights (g)		Ratio of heavy/light kidney wt.	Total kidney wt. (g)	Kidney wt/BW (g/kg)
		Left	Right			
Control						
H1	1.40	5.6	5.3	1.05	11.9	8.50
H2	1.56	5.1	4.8	1.06	9.9	6.34
H3	1.40	5.3	5.3	1.00	10.6	7.57
H4	1.51	4.1	4.1	1.00	8.2	5.43
H5	1.70	5.8	5.6	1.03	11.4	6.70
Mean	1.51±0.12			1.02	10.30±1.37	6.90±1.19
Dehydrated						
H6†	1.51	5.3	4.9	1.08	10.2	6.75
H7	1.54	4.3	4.2	1.02	8.5	5.51
H8	1.65	5.4	5.2	1.03	10.6	6.42
H9	1.66	4.4	4.3	1.02	8.7	5.24
H10	1.45	4.4	4.3	1.02	8.7	6.00
Mean	1.56±0.09			1.03	9.34±0.98	5.98±0.62
Dehydrated+NH_4Cl						
H11	1.53	4.6	4.6	1.00	9.2	6.01
H12†	1.55	5.0	4.6	1.08	9.6	6.19
H13	1.50	4.4	4.5	1.02	8.9	5.93
H14†	1.39	5.1	4.4	1.15	9.5	6.83
H15	1.57	4.2	4.2	1.00	8.4	5.35
Mean	1.50±0.07			1.05	9.12±0.48	6.06±0.53

† Asymmetrical kidneys

There were no significant differences in each parameter between groups ($P>0.05$) by the use of DMRT.

BW = body weight.

Presumably, the increase in hematocrit values and the concentration of plasma sodium in the study reflected body water loss during water deprivation. The reduction in body fluid due to water deprivation has been reported previously in domestic birds (Arnason *et al*., 1986). The avian kidney contains mixture of nephrons resembling reptilian and mammalian nephrons. There are two types of nephron in the bird kidney: 1) reptilian type nephrons, which lack a loop structure between proximal and distal tubules, and 2) the mammalian type nephrons, which possess Henle's loop. Whereas the reptilian type nephrons are located superficially in the cortical lobules and empty at a right angle into the collecting ducts, the mammalian type nephrons are situated deeper, forming a medullary cone in which the loop of Henle, vasa recta, and collecting ducts are wrapped in a sheath of connective tissue (Sturkie, 1986). The mammalian type and reptilian type nephrons clearly function differently during periods of salt loading or dehydration. Reptilian type glomeruli cease filtering (glomerular intermittency) to avoid the loss of dilute or isosmotic urine, but the mammalian type nephrons continue to produce a concentrated urine (Dantzler and Braun, 1980). A reduction in

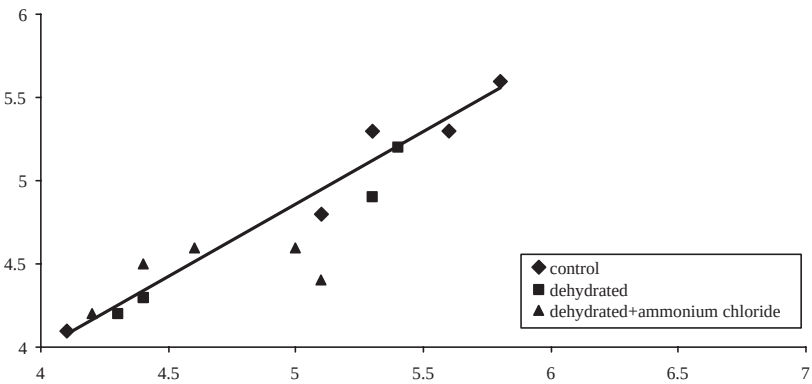

Figure 2 Scattergram of intercept points for left vs. right kidney weight ratios of individual hens on the different treatment groups.
Line = line of unity

Table 5 Averages of length and weight of humerus and femur bones in the different treatment groups measured at 12 weeks following rehydration (32 weeks of age).

Group	Bone parameters			
	Length (cm)		Weight (g)	
	Left	Right	Left	Right
		Humerus		
Control	7.78±0.22	7.72±0.21	4.58±1.05	4.00±0.48[ab]
Dehydrated	7.68±0.27	7.80±0.23	4.46±1.42	4.36±0.97[a]
Dehydrated+NH_4Cl	7.92±0.17	7.90±0.14	3.70±0.54	3.36±0.26[b]
		Femur		
Control	8.44±0.19	8.36±0.37	8.36±0.93	8.48±0.71
Dehydrated	8.44±0.13	8.44±0.13	9.02±0.58	9.20±0.45
Dehydrated+NH_4Cl	8.54±0.11	8.48±0.04	8.72±0.45	8.86±0.45

[a-b] Means with no common superscript differ significantly between groups ($P<0.05$) by the use of DMRT.

glomerular filtration rate (GFR) in response to water deprivation is well documented in various avian species (Krag and Skadhauge, 1972; Roberts, 1991). Nishimura *et al*. (1984) showed that GFR decreased while tubular reabsorption of filtered water increased during water deprivation. It has been suggested that arginine vasotocin (AVT) regulates renal water excretion by its dual actions on tubule water permeability and glomerular filtration rate (GFR) (Stallone and Braun, 1985). Skadhauge (1981) explained that the reduction in GFR seemed to be due to the release of AVT in response to high plasma osmolality. It is reasonable, therefore, to suggest that water deprivation for 48 hours in both groups of dehydration in this work would stimulate the release of AVT from neurohypophysis resulting in reduced GFR and increased renal tubule reabsorption. The reduction in GFR combined with the increase in tubular reabsorption of filtered water in the dehydrated hens would produce more concentrated urine as evidenced by reduced fecal moisture contents in the birds in both groups of dehydration (Table 2). All these mechanisms function as homeostatic mechanisms to conserve needed body water during periods of dehydration stress.

In the current work, the interruption of drinking water for 48 hours resulted in a significant increase in plasma sodium concentration observed in the dehydrated+NH_4Cl birds (Table 3). These results were in agreement with those reported by Koike *et al*. (1983), who demonstrated that water deprivation for 2 days caused an increase in plasma sodium levels in laying hens. However, such an effect was not observed in the birds in the dehydrated group, this was possibly due to variation of the individual pullets in response to dehydration. It was of interest to notice that the average of plasma sodium concentration of the dehydrated birds varied considerably. The dehydrated group tended to have a higher level of plasma sodium as compared with the control group, although the difference was not statistically significant.

The increase in the moisture of feces in the dehydrated hens as the drinking water was replenished for 6 hours would be resulted from an increase in water intake as was reported previously by Koike *et al*. (1983). The investigators demonstrated that water intake was elevated when water was returned to dehydrated birds by 156% of the normal value during the 2 days of rehydration. Takei *et al*. (1988) reported that when dehydrated birds regained access to water, they may drink substantially more water than required to restore their cellular and extracellular water deficits. A significant increase in manure moisture was noticed again on Week 8 of rehydration for the dehydrated hens fed acidified diets. The reason for the occurrence of wet droppings at this period of time was possibly due to dietary chloride consumption, leading to increased water consumption. However, such an effect was noticed only at week 8 suggested that the level of chloride in the diet was not too excessive, thus the severity of wet droppings was transient. Glahn *et al*. (1988) demonstrated that dietary acidification with ammonium chloride increased water consumption and produced wet droppings. It was of interest to note that the birds in the dehydrated+NH_4Cl group exhibited a lower level of hematocrit values as compared with the others during the similar period of time. This may reflect an increase in plasma volume due to polydipsia, and thus hypervolemia could be induced. Stallone and Braun (1986) demonstrated that an infusion of plasma into dehydrated birds caused a reduction in hematocrit values.

The results obtained from the current study indicated that deprivation of water during the onset of lay did not affect subsequent eggshell quality when the birds came to lay. An incidence of poor eggshell quality observed in the dehydrated+NH_4Cl group suggested that this was due to dietary chloride intake. A number of investigators have reported that a high level of dietary chloride reduces eggshell quality (Austic, 1984; Keshavarz and Austic, 1990). Keshavarz

and Austic (1990) revealed that renal calcium excretion was increased by consumption of the high chloride diet. Another possible explanation may lie in an understanding of the relationship between blood bicarbonate ions and intake of acidified diets. Cohen and Hurwitz (1974) demonstrated that chloride feeding caused a reduction of blood bicarbonate ions (HCO_3^-). This was confirmed by Glahn *et al*. (1988) who found that dietary acidification with NH_4Cl at a level of 1 % reduced blood HCO_3^-. Gutowska and Mitchell (1945) proposed an equilibrium reaction in which the accumulation of carbonic acid would associate with the formation of carbonate ions which were needed for the calcium carbonate of the eggshell:

$$2HCO_3^- \leftrightarrow H_2CO_3 + CO_3^-$$
$$\uparrow\downarrow \text{ (activated by carbonic anhydrase)}$$
$$H_2O + CO_2$$

where the bicarbonate ions ($2HCO_3$) in the shell gland were obtained from the blood and could react to form carbonic acid (H_2CO_3) and carbonate ions (CO_3^-).

Neither total kidney weight nor kidney weight per body weight showed consistent effects from water deprivation. However, as a test of intraindividual kidney weight symmetry, left kidney weights were plotted against right kidney weights for each bird (Figure 2). The scattergram indicated that water deprivation for 48 hours at the prelaying period had little effect on the kidney changes. An evidence of asymmetrical kidneys noticed in the dehydrated+NH_4Cl group, suggested some effects of prolonged NH_4Cl intake. Unfortunately, the damage of kidneys in the study was not sufficiently examined. This investigation suggested that a histological study in kidney lesions would be conducted in further studies.

No adverse effects were observed on bone measurement except for the weight of right humerus. The lighter bone was noticed in the dehydrated+NH_4Cl group as compared with that of the other two groups. Very little work has been done on the effect of water deprivation on bone studies. It is possible that the declination of the humerus weight results from the prolonged administration of ammonium chloride. Keshavarz and Austic (1990) stated that the concentration of calcium in ureteral urine was increased by a high level of dietary chloride. The results in this study suggested that prolonged intake of ammonium chloride would have an impact on calcium balance, and thus calcification of bone formation could be impaired.

CONCLUSION

The results from the study indicated that withdrawal of drinking water at the onset of lay, even for 48 hours produces wet droppings and high packed cell volume. However, eggshell quality during the initial period of production was not influenced. Prolonged dietary chloride intakes may cause eggshell damages, liquid excreta and kidney asymmetry. In addition, the severity of dietary acidification tends toward the structure of long bones was possibly due to calcium balance impairment, and thus calcification of the bone could be adversely affected.

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