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Effects of Diet and Cyclic Daily Heat Stress on Electrolyte, Nitrogen and Water Intake, Excretion and Retention by Colostomized Male Broiler Chickens

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Abstract: Male Ross broiler chicks were grown in a thermoneutral environment from 0 to 14 d. From 14 to 41 d, half the birds remained in the thermoneutral room (22.5 ± 3.5°C) whereas the other half in another room were exposed daily to cyclic stress (22.5 ± 3.5°C for 14 h and 33 ± 2.0°C for 10 h). Chickens were individually caged from 22 to 41 d, with colostomy at 28 to 30 d, to evaluate effects of heat stress (d 41) and dietary electrolyte balance (DEB = Na + K – Cl mEq/kg) on serum electrolytes and on water, electrolyte and nitrogen retention (18 birds, 9/room with 3/treatment therein). The DEB levels were 140, 240, or 340 mEq/kg. The DEB levels fed from 0 to 41 d were obtained by addition of NaCl and $NaHCO_3$, plus $KHCO_3$ or NH_4Cl as needed. Feces and urine were collected separately. Nitrogen balance was least (greatest nitrogen excretion) using 140 mEq/kg in the thermoneutral room. For DEB 240, retention of Na increased compared to DEB 140 in the thermoneutral room (5.24 vs 1.75 mEq/bird/d; $P < 0.05$) and in the heat stress room (5.29 vs 2.33 mEq/bird/d; $P < 0.05$). Overall, urine Na and K increased in the 340 compared to the 240 mEq/kg treatment and Cl increased in 140 compared to 240 or 340 mEq/kg treatments. The DEB of 240 was most favorable in either temperature environment based on water, electrolyte and nitrogen metabolism results.

Key words: Broiler, electrolyte, heat stress, urinary, water

Introduction

The increase in body temperature due to the exposure to ambient temperatures above the thermal comfort zone has a negative impact on bird performance by decreasing feed intake, BW gain and livability and increasing the feed conversion ratio. After 14 d of age, the environment for broiler chicks is considered optimal when the temperature is between 20 and 25°C and relative humidity between 50 and 70% (Macari *et al*., 1994; Tinoco, 1995).

The consequences of heat stress on bird performance are well studied, but the physiological responses and the development of consistent treatments to minimize losses resulting from heat need further explanations. Under heat stress, the water and electrolyte balances are important variables for the survival and productivity of poultry flocks. Monovalent electrolytes (Na, K and Cl) have a greater absorption rate than divalent elements (Ca, Mg) due to the greater permeability of the enterocyte membrane to monovalent ions (Cunningham, 1999), with Na being absorbed by cotransportation with organic molecules (Na-Glucose), coupled with Cl and by simple diffusion (Cunningham, 1999). The stimulation of water consumption by broiler chickens using dietary electrolytes has been pointed out as one possible alternative for reducing the consequences of heat stress (Borges, 1997). On the other hand, the (Na + K – Cl) ratio has been suggested as key for the maintenance of the acid-base homeostasis in birds (Mongin, 1981). Although consequences of thermal stress on water consumption and electrolyte excretion have been documented (Belay *et al*., 1993; Belay and Teeter, 1993, 1996), there is little information on the main route of excretion, whether feces or urine.

The use of methodology involving the surgical technique of colostomy enables quantification of excretion of fluids and minerals through the urinary and fecal routes separately. The aim of this study was to assess the effects of cyclic heat stress and dietary electrolyte balance on serum electrolytes and water, nitrogen and electrolyte balances in broiler chickens near market age when most susceptible to effects of heat distress.

Materials and Methods

Animals and Housing: A total of 24 male Ross broiler chickens, reared in litter pens from 0 to 14 d under the temperature conditions recommended in the Ross Broiler Management Guide (Ross Breeders, undated) (Table 1), were placed in either thermoneutral or heat stress chambers from 14 to 21 d. They were placed in individual cages (12 birds per room; four birds/dietary treatment) from 22 to 41 d, with colostomy being performed at 28 to 30 d. The broiler chicks were fed experimental starter diets from 0 to 21 d (22% crude protein, 2,900 kcal ME/kg) and grower diets from 22 to 41 d (20% crude protein, 3,200 kcal ME/kg) (Table 2), with water and feed provided *ad libitum*.

From 0 to 14 d, normal brooding temperatures

Table 1: Daily ambient temperature and relative humidity (RH) in climatic chambers (environmentally controlled rooms)

	Thermoneutral chamber				Heat stress chamber			
	Daily Temperature (°C)			RH (%)	Temperature (°C)			RH (%)
Item Week	Max	Min	Avg		Max	Min	Avg	
1	32	28	30	49	33	28	30.5	52
2	30	25	27.5	57	31	26	28.5	50
3	28	24	26	63	35	23	29	54
4	26	21	23.5	60	35	20	27.5	51
5	25	19	22	57	33	19	26	53
6	25	19	22	58	33	19	26	52

[a]Thermoneutral temperatures were based on recommendations in a recent Ross Broiler Management Guide (Ross Breeders, Inc., undated).

(thermoneutral) shown in Table 1 were utilized and then from 14 to 41 d, separate temperatures were maintained the inside the two environmental chambers with independent temperature controls. Average maximum and minimum temperatures inside the rooms were determined daily using a dry bulb thermometer located at the center of the room. Relative humidity was obtained with a thermohygrograph (Salcas) and wet bulb thermometer (Incoterm).

Room temperatures were maintained in the following manner: Thermoneutral chamber-24 h at thermoneutral temperature (control environment).
Heat stress chamber-14 h in thermoneutral temperature and 8 to 10 h of heating at 33 ± 2°C according to the following sequence: 0700 h, 20°C; 0800 h, 25°C; 0900 h, 28°C; 1000 h, 31°C; 1100 h, 33°C; 1200 h, 34°C; 1300 h, 34°C; 1400 h, 35°C; and 1700 h, 34°C. Heaters were turned on at 0700 h and off at 1800 h, when fans were turned on. There was no Animal Use and Care Committee in place at the University at the time of this experiment, but research was conducted by a doctoral student under the auspices of a graduate committee. This study was one of a series of 18 experiments.

Dietary Treatments: The broiler chickens were fed one of three starter or grower feeds varying in dietary electrolyte balance (DEB; 140, 240, or 340 mEq/kg) from 0 through 41 d, when data were collected from colostomized birds (Table 2). Four caged broilers per diet per room in the two unreplicated rooms, thermoneutral or heat stress, were used for serum electrolyte analyses and three caged broilers per treatment per room were used for water, electrolyte and N retention studies.
To minimize variability in experimental diets, formulas were developed to contain the same amounts of corn, soybean meal, oil, phosphate, limestone, mineral and vitamin supplement, meeting requirements for protein, metabolizable energy, Ca and P suggested by the National Research Council (1994). Three DEB ratios were achieved by addition of salt (NaCl) and sodium bicarbonate ($NaHCO_3$) to all diets, plus potassium bicarbonate ($KHCO_3$) or ammonium chloride (NH_4Cl) as needed. In the DEB 140 and 240 mEq/kg diets containing added NH_4Cl, Na and Cl were varied and K was kept constant. The DEB 340 mEq/kg diets containing added $KHCO_3$ had about +0.10% extra K compared to the other DEB treatments. The DEB was calculated based on actual results from laboratory analysis of the feeds. The $NaHCO_3$ product contained approximately 27.1% Na and 71.9% HCO_3 whereas the $KHCO_3$ product had approximately 38.7% K and 60.3% HCO_3. The NH_4Cl product had approximately 66% Cl.
The DEB 140 starter diet had calculated values of 0.25% Na, 0.745% K and 0.556% Cl and grower diet had 0.25% Na, 0.666% K and 0.495% Cl. The DEB 240 starter diet had calculated values of 0.35% Na, 0.745% K, 0.366% Cl whereas the grower diet had 0.35% Na, 0.666% K and 0.294% Cl. The DEB 340 starter diet had calculated values of 0.45% Na, 0.848% K, 0.259% Cl and the grower diet had 0.45%, 0.840% K and 0.250% Cl. The DEB level in each feed was calculated by multiplying actual % Na x 434.98, % K x 255.74 and % Cl x 282.06, then doing the summation of Na + K - Cl, mEq/kg, as suggested by Hooge (1995).

Lab Analyses: Ingredients, feeds, water, feces and urine were analyzed for Na, K and N according to procedures described by AOAC (1990). The Na and K in feeds, feces, water and urine were determined by flame spectrophotometry. The Cl in feeds was analyzed by $AgNO_3$-titration (Lacroix *et al.*, 1970) and in water and urine by the Labtest kit (Labtest Diagnostica S. A., Avenida Paulo Ferreira da Costa, 600 - Vista Alegre, Minas Gerais, CP 33400-000 Brazil).

Colostomy Procedure and Collections: After 14 d of age, cyclic heat stress was imposed on half the birds

Table 2: Composition of experimental diets for the starter and grower phases

Item	Composition of Diets (by DEB[a] Treatments, mEq/kg)					
	Starter Phase (0 to 21 d)			Grower Phase (22 to 41 d)		
	140	240	340	140	240	340
	(%)	(%)	(%)	(%)	(%)	(%)
Corn	59.31	59.31	59.31	59.31	59.31	59.31
Soybean meal	34.21	34.21	34.21	30.08	30.08	30.08
Soybean oil	0.90	0.90	0.90	5.51	5.51	5.51
Dicalcium phosphate	1.86	1.86	1.86	1.35	1.35	1.35
Limestone	1.15	1.15	1.15	1.25	1.25	1.25
DL-methionine	0.07	0.07	0.07	0.00	0.00	0.00
NaCl	0.30	0.30	0.30	0.30	0.30	0.30
$NaHCO_3$	0.45	0.82	1.19	0.45	0.82	1.19
$KHCO_3$	-----	-----	0.27	-----	-----	0.44
NH_4Cl	0.46	0.16	-----	0.37	0.07	-----
Vitamin-mineral mix[a,b,c]	0.50	0.50	0.50	0.50	0.50	0.50
Builders sand	0.79	0.72	0.24	0.88	0.81	0.07
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
ME, kcal/kg	2,900	2,900	2,900	3,200	3,200	3,200
Crude protein, %	22.0	22.0	22.0	20.0	20.0	20.0
Calcium, %	1.00	1.00	1.00	0.90	0.90	0.90
Available phosphorus, %	0.45	0.45	0.45	0.35	0.35	0.35
Methionine, %	0.50	0.50	0.50	0.39	0.39	0.39
Methionine + Cystine, %	0.85	0.85	0.85	0.71	0.71	0.71
Lysine, %	1.17	1.17	1.17	1.04	1.04	1.04
Sodium, %	0.25	0.35	0.45	0.25	0.35	0.45
Chloride, %	0.566	0.366	0.259	0.495	0.294	0.250
Bicarbonate, %	0.324	0.590	1.018	0.324	0.590	1.121
Potassium, %	0.745	0.745	0.848	0.666	0.666	0.840

[a]DEB = dietary electrolyte balance, Na + K - Cl, mEq/kg. Supplements contributed the following nutrients per kg mixed diet:

[b]Starter feed - vitamin A, 2,650 IU; vitamin D_3, 500 IU; vitamin E, 2.4 mg; vitamin K_3 (menadione), 0.4 mg; thiamine, 0.2 mg; riboflavin, 2.0 mg; vitamin B_{12}, 3.5 mcg; panthotenic acid, 2.2 mg; nicotinic acid, 8.5 mg; pyridoxine, 0.4 mg; folic acid, 0.2 mg; biotin, 0.02 mg; choline, 0.15 g; monensin sodium, 0.11 g; bacitracin-MD, 0.04 g; DL-methionine, 0.3 g, ethoxyquin, 0.02 g; and carrier, 1 g.

[c]Grower feed - vitamin A, 2,300 IU; vitamin D_3, 400 IU; vitamin E, 1.8 mg; vitamin K_3 (menadione), 0.3 mg; thiamine, 0.15 mg; riboflavin, 1.4 mg; vitamin B_{12}, 3.5 mcg; panthotenic acid, 2 mg; nicotinic acid, 7 mg; pyridoxine, 0.25 mg; folic acid, 0.15 mg; biotin, 0.02 mg; choline, 0.125 g; monensin sodium, 0.125 g; bacitracin-MD, 0.03 g; ethoxyquin, 0.02 g; DL-methionine, 0.275 g; and carrier, 1 g.

[d]Trace minerals - iron, 0.035 g; copper, 0.05 g; manganese, 0.035 g; zinc, 0.03 g; iodine, 0.6 mg; selenium, 0.09 mg; and diluent, 1 g.

(heat stress room). On d 22, broilers were transferred to individual wire cages (0.59 x 0.58 x 0.48 m) equipped with linear trough feeders and waterers. During the period from d 28 to d 30, birds were colostomized. The surgical technique was described by Belay *et al.* (1993) with some adaptations. Double plastic rings were placed on a urinary tube creating a convenient site for later attachment of a rubber band over the collection bag. To facilitate the separate collection of feces and urine, a piece of this small tube was fixed with five to six separate simple stitches, using nylon thread, to the tissue underneath the cloaca and the artificial anus. On the collection day a small plastic bag for urine was attached to this tube with the help of a regular elastic band. This elastic band, around the circumference of the collection bag, was fixed in the area corresponding to the groove between the two rings. Thus, excreted urine was automatically deposited into this collection bag and feces was voided separately into another collection bag. Collections were made entirely on d 41. Collection bags for feces and urine were changed every four hours, totaling six samples per bird over the 24-h period.

Table 3: Live performance of Ross male broiler chickens, 0 to 42 d, raised under two ambient temperature regimens, thermoneutral or cyclic daily heat stress (14 to 42 d) and receiving different dietary electrolyte balances (DEB; Na + K - Cl, mEq/kg). Results for treatment groups from which broilers were colostomized (28 to 30 d) for experiment at 41 d; adapted from Borges *et al.* (2003)

DEB, mEq/kg	Weight Gain/Bird (g)	Feed Intake/ Bird (g)	Feed/Gain (g/g)	Mortality (%)
Thermoneutral Room				
40[1]	2,415[b]	4,273[b]	1.770	3.33
140	2,451[ab]	4,334[ab]	1.768	1.67
240	2,583[a]	4,491[a]	1.740	5.00
240	2,410[b]	4,330[b]	1.798	6.67
Pooled SEM	24.9	26.8	0.01	1.03
Heat Stress Room				
40	2,378	4,179	1.758	6.67
140	2,334	4,139	1.774	11.67
240	2,422	4,295	1.775	5.00
340	2,390	4,259	1.784	1.67
Pooled SEM	27.6	37.0	0.01	1.77
Combined Rooms				
40	2,396	4,226[b]	1.764	6.67
140	2,392	4,237[ab]	1.771	5.00
240	2,503	4,393[a]	1.757	5.00
340	2,400	4,295[ab]	1.791	4.17
Pooled SEM	19.8	25.7	0.01	1.03

[a - b]Means in a column and grouping without a common letter superscript differ by Tukey's test ($P < 0.05$).
[c]The DEB 40, mEq/kg, treatment results are presented here because they were included in the statistical analysis in the previous article by Borges *et al.* (2003). The formula for that diet was given in the reference and therefore is not included in Table 2 (diets) herein.

Samples were immediately sealed and frozen after each collection. After the thawing process, fecal samples were weighed and dried in a forced ventilation oven at 55 ±5°C for 72 h. After drying they were weighed again to obtain the crude feces DM content, then were ground for analysis. Final DM content of feed and excreta was determined from a 1.0 g aliquot of ground sample, placed in an oven at 105°C for 24 h, cooled and weighed to determine the final DM content by difference. Urine analyses were made with liquid urine, using a 1 ml sample to determine N (AOAC, 1990).

The amount of Na, K and Cl excreted by the urinary route was determined by multiplying the urine concentration in mEq/l by the volume in ml and this number was divided by the metabolic body size of each bird ($kg^{0.75}$). The metabolic body size was determined from average bird weight at the beginning and at the end of collection. In the feces, the amounts of Na, K and Cl were determined by multiplying the amount of these ions in mEq/kg (as collected) by the amount (g) of feces, divided by the metabolic body size of each bird ($kg^{0.75}$). The final balance was defined by the difference between consumption in mEq and the sum (Na + K - Cl) of the amounts excreted in the urine and feces in mEq.

All variables in Table 4 relating to water, electrolyte and nitrogen N, except body weight gain (g), were calculated based on the metabolic body size of each bird ($kg^{0.75}$). Feed and water consumption were determined by the difference between the quantities supplied versus quantities remaining in each cage.

Statistical Analysis: Two unreplicated environmental chambers (rooms), one per temperature regimen, were utilized. There were four cages of one bird each per dietary treatment in each room for serum electrolyte analyses and three of these cages of one bird each per treatment were used for water, electrolyte and N balances. Separate completely randomized designs were used for DEB treatments within each room and were analyzed using one-way ANOVA with three or four replicates per treatment depending on parameter.

For DEB treatments using data from rooms combined, one-way ANOVA with six or eight replicates per treatment, depending on parameter, was used. Coefficients of variation (%) were calculated for groups of means within each parameter. Means were separated using Tukey's test at the 5% level of probability (Statistix 8, 2003). Means and coefficients of variation were calculated and presented but no statistical analysis was possible by room (temperature environment) because they were unreplicated.

Results and Discussion

The analysis of the water supplied to the birds showed

Table 4: Feed intake, weight gain, water balance and nitrogen consumed:excreted ratio of 41-day-old colostomized broilers raised at different temperatures and fed various dietary electrolyte balance (DEB; Na + K - Cl, mEq/kg) levels

Item	Feed Intake (g/kg$^{0.75}$)	BW Gain (g/bird)	N Consumed/ Excreted Ratio[d]	Water Consumed		Water: Feed Ratio	Urine Output (mL/kg$^{0.75}$)	Water: Urine Ratio	Water in Feces (mL/kg$^{0.75}$)	Total Water Excreted (mL/kg$^{0.75}$)	Water Consumed/ Excreted Ratio
				Drank (mL/kg$^{0.75}$)	Total (mL/kg$^{0.75}$)						
Thermoneutral room											
140 mEq/kg	85	65	0.29[b]	233	241	2.75	76	3.35	54	130	1.97
240 mEq/kg	101	90	1.55[a]	215	225	2.15	52	4.23	71	123	1.88
340 mEq/kg	101	97	1.19[a]	287	297	2.86	90	3.23	87	177	1.69
CV (%)[c]	20.4	34.1	25.9	18.6	18.6	12.2	32.8	18.4	25.5	26.6	16.6
Heat stress room											
140 mEq/kg	98	87	1.52	318	327	3.21	78	4.10	60	138	2.35
240 mEq/kg	83	75	1.24	325	333	4.06	129	2.90	53	182	1.92
340 mEq/kg	84	70	1.24	257	266	3.06	78	3.34	63	141	1.89
CV (%)	9.2	10.1	38.7	26.7	25.9	30.3	44.6	18.2	20.1	30.6	12.7
DEB, mEq/kg											
140	92	76	0.91	275	285	2.98	77	3.73	57	134	2.16
240	92	83	1.40	270	280	3.10	90	3.56	62	152	1.90
340	92	83	1.22	272	282	2.96	84	3.29	75	159	1.79
CV (%)	17.4	27.6	46.3	27.0	26.3	30.8	47.0	21.4	25.8	29.8	14.8
Room (not analyzed statistically)[e]											
Thermoneutral	96	84	1.01	245	254	2.59	73	3.60	71	143	1.85
Heat stress	88	77	1.33	300	309	3.44	95	3.45	59	154	2.05
CV (%)	16.3	26.7	46.2	23.9	23.3	25.9	43.9	21.3	26.3	29.6	15.7

[a - b]Means in a column and grouping without a common letter superscript differ by Tukey's test ($P < 0.05$). There three replicates (birds) per treatment/room. Six feces and urine collections were made per bird over a 24-hour period.

[c]CV % = coefficient of variation which is standard deviation / mean x 100.

[d]In the thermoneutral room, birds in the DEB 140, 240 and 340 mEq/kg treatments consumed about 2.72, 3.23 and 3.23 g N/kg$^{0.75}$ in the 1-d study (based on feed intake and calculated N). Corresponding N excretions were 9.38, 2.08 and 2.71 g/kg$^{0.75}$. The N consumed/excreted ratio is termed N balance.

[e]There was one room per temperature environment, not replicated, so means and CVs were calculated but no statistical analysis was possible.

only "traces" of the three minerals Na, K and Cl. Laboratory assays confirmed that calculated and analyzed mineral levels in feeds were similar.

In the thermoneutral room (Table 3), the only the N consumed:excreted ratio (nitrogen balance) was decreased ($P < 0.05$) by dietary electrolyte balance (DEB) 140 mEq/kg compared to the other DEB treatments (0.29 vs 1.55 and 1.19, respectively). Although details of N consumption and retention are not presented, one can estimate N consumption using grower feed crude protein/6.25 to obtain percent N in the diet. In the thermoneutral room, using feed intake results, the birds in the DEB 140, 240 and 340 mEq/kg treatments consumed about 2.72, 3.23 and 3.23 g N/kg$^{0.75}$ in the 1-d study. Dividing these values by the N consumed/excreted ratios of 0.29, 1.55 and 1.19 g/kg$^{0.75}$, respectively, one can determine that the N excretions were correspondingly 9.38, 2.08 and 2.71, indicating a negative effect of DEB 140 mEq/kg on N retention (increased N excretion). The N consumed/excreted ratio is termed N balance.

In the heat stress room (Table 3), none of the DEB treatment differences were significant for feed intake, BW gain, N balance, or water and urine related parameters. For combined rooms, there were no significant DEB treatment effects on parameters presented. Although not analyzed statistically, water consumption increased by 22.4% from 254 to 300 ml/kg$^{0.75}$ in heat stress compared to thermoneutral environment whereas water:feed ratio was 24.7% greater in heat stress versus the thermoneutral environment (3.44 vs 2.59).

Table 5: Consumption, urine content, feces content and calculated retention of electrolytes (Na, K, Cl) of 41-day-old colostomized broilers raised at different temperatures and fed various dietary electrolyte balance (DEB, mEq/kg) levels

Parameter	Consumed (mEq/bird/d)			Urine (mEq/bird/d)			Feces (mEq/bird/d)			Retention (mEq/bird/d)		
	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
Thermoneutral room												
140 mEq/kg	9.3[b]	14.6	11.91	3.62[b]	6.70[ab]	5.53[a]	3.92	3.90	2.50	1.75[b]	3.95	3.88
240 mEq/kg	15.0[a]	17.1	8.32	3.80[b]	6.24[b]	2.04[b]	6.25	3.74	1.87	5.24[a]	7.12	4.41
340 mEq/kg	19.7[a]	21.7	7.10	9.07[a]	10.55[a]	1.39[b]	7.94	3.40	2.33	2.73[ab]	7.73	3.38
CV (%)[d]	15.2	18.9	27.9	9.1	22.0	38.6	35.1	32.6	23.3	32.5	25.5	44.6
Heat stress room												
140 mEq/kg	10.7[b]	16.7[ab]	13.70[a]	3.68[b]	7.08[a]	5.62[a]	4.66	3.39	2.91	2.33[b]	6.25	5.17[a]
240 mEq/kg	12.6[b]	14.1[b]	6.84[b]	3.07[b]	4.91[b]	0.59[b]	4.22	3.34	2.41	5.29[a]	5.82	3.84[ab]
340 mEq/kg	16.4[a]	18.0[a]	5.90[b]	7.94[a]	7.60[a]	1.25[b]	5.56	3.48	2.29	2.90[ab]	6.93	2.37[b]
CV (%)	9.0	8.7	9.0	7.6	7.6	19.4	23.2	2.91	34.1	27.7	22.6	21.1
DEB, mEq/kg												
140	10.0[c]	15.6[b]	12.80[a]	3.65[b]	6.89[ab]	5.58[a]	4.29	3.65	2.70	2.04[b]	5.10	4.52
240	13.8[b]	15.6[b]	7.58[b]	3.43[b]	5.58[b]	1.31[b]	5.23	3.54	2.14	5.26[a]	6.47	4.13
340	18.1[a]	19.8[a]	6.50[b]	8.50[a]	9.07[a]	1.32[b]	6.75	3.44	2.31	2.82[b]	7.33	2.87
CV (%)	15.1	16.7	20.9	11.2	21.3	33.4	33.6	25.8	28.2	27.5	25.6	34.6
Room (not analyzed statistically)[e]												
Thermoneutral	14.7	17.8	9.11	5.50	7.83	2.99	6.03	3.68	2.23	3.24	6.27	3.89
Heat stress	13.2	16.3	8.81	4.90	6.53	2.49	4.82	3.40	2.53	3.50	6.33	3.79
CV (%)	28.6	19.8	38.3	48.6	28.1	83.7	36.2	24.8	28.5	50.6	29.2	38.7

[a - c]Means in a column and group without a common letter superscript differ by Tukey's test ($P < 0.05$). There were three replicates (birds) per treatment/room. Six feces and urine samples were collected per bird over a 24-h period.

[d]CV (%) = coefficient of variation which is standard deviation / mean x 100.

[e]There was one room per temperature environment, not replicated, so means and CVs were calculated but no statistical analysis was possible.

Urine was the main pathway for water elimination (Table 3) and this emphasizes the importance of the urinary excretion route for the maintenance of water homeostasis in heat-challenged birds. Only about 10 ml water/kg$^{0.75}$ was consumed from the feed and the remainder came from water intake, varying from 225 to 297 ml/kg$^{0.75}$ in the thermoneutral room to 266 to 333 ml/kg$^{0.75}$ in the heat stress room. Broiler chickens have the ability to offset the volume of water lost from the body by evaporation, feces and urine (insensible water loss) due to heat stress by increasing water consumption (Belay and Teeter, 1993; Ait-Boulahsen *et al.*, 1995). The amount of body water is kept within limits to facilitate physiological functions in the bird.

In studies with colostomized broilers, Belay and Teeter (1993) reported that fecal water loss was not influenced by ambient temperature. An increase in excreta moisture usually observed during heat stress is a consequence of increased urine flow rather than the direct passage of water through the digestive tract. Edens and Siegel (1975) stated that the excretion of diluted urine is stimulated by the increase in body temperature, as well as by the expansion of the extra-cellular fluid volume and hemodilution.

Water is the body's general solvent and the main medium in which all metabolic processes take place. Birds have a well-developed water consumption control. Water balance is established by the balance between the intracellular, interstitial compartments and the plasma. Water movement is started when there is a disturbance in osmolality between the two

Table 6: Serum sodium, potassium and chloride levels of 41-day-old colostomized broilers raised at different temperatures and fed various DEB (mEq/kg) levels

Parameter	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)
Thermoneutral room[a]			
140 mEq/kg	144.8	5.40	109.3
240 mEq/kg	143.8	5.70	109.8
340 mEq/kg	145.8	5.30	110.8
CV (%)[b]	1.31	10.9	1.92
Heat stress room[a]			
140 mEq/kg	144.5	5.75	111.3
240 mEq/kg	142.8	6.95	111.8
340 mEq/kg	144.5	5.90	110.0
CV (%)	1.58	15.9	3.08
DEB, mEq/kg[1]			
140	144.6	5.58	111.0
240	143.3	6.32	110.8
340	145.1	5.60	109.6
CV (%)	1.39	15.00	2.46
Room (not analyzed statistically)[c]			
Thermoneutral	144.8	5.47	109.9
Heat stress	143.9	6.20	111.0
CV (%)	1.44	14.5	2.42

[a]There were four replicates (birds) per treatment/room. There were no significant differences between treatments by ANOVA and Tukey's test ($P < 0.05$).

[b]CV (%) = coefficient of variation which is standard deviation / mean x 100.

[c]There was one room per temperature environment, not replicated, so means and CVs were calculated but no statistical analysis was possible.

compartments, with the deficit resulting in a reduction in blood volume and an increase in plasma osmolality. Regardless of the reason for dehydration, the intracellular, interstitial (extra-cellular) fluids and plasma share the water deficit (Macari, 1996).

In the specific case of heat stress, water loss may affect blood volume. A reduced blood volume will stimulate the juxtaglomerular cells of the kidney to produce renin that converts the angiotensinogen into angiotensin I and II, triggering a stimulus at the thirst center, thus increasing water consumption. Additionally, the increase in plasma osmolality activates osmoreceptors that stimulate the thirst center, increasing water consumption. The stretch receptors present in the large veins and in the atrium can also be stimulated by a drop in venous return, with these baroreceptors acting by the afferent vagal pathway, stimulating the thirst centers. Thus, water consumption in birds can be induced by cellular dehydration and by the renin-angiotensin system (Macari, 1996). In the specific case of birds exposed to heat stress and receiving electrolyte supplementation, the changes in both blood volume and osmolality can stimulate water consumption and urinary excretion.

Heat stress causes rapid shallow breathing (panting), inducing respiratory alkalosis which is associated with a reduction in plasma CO_2 and HCO_3 concentrations, increasing blood pH, urine pH (Teeter *et al.*, 1985; Branton *et al.*, 1986; Belay and Teeter, 1993) and urinary flow, while reducing urine osmolality (Deetz and Ringrose, 1976; Van Kampen, 1981; Bottje and Harrison, 1985; Deyhim *et al.*, 1990; Belay and Teeter 1993, 1996).

Electrolyte (Na, K and Cl) consumption, excretion in urine and in feces and retention are presented in Table 4. The series of DEB diets, from 140 to 340 mEg/kg, had progressively higher Na levels (0.25, 0.35 and 0.45%) whereas Cl diminished (0.566, 0.366 and 0.259% in starters and 0.495, 0.294 and 0.250% in grower feeds) and K was constant at DEB 140 and 240 but increased at DEB 340 due to $KHCO_3$ addition. Therefore, alterations in intake of Na, K and Cl were due not only to decreased feed intake in heat stress but to the dietary levels of each. As expected, Na consumption increased ($P < 0.05$) with increasing DEB level in the thermoneutral room (9.3 vs 15.0 and 19.7 mEq/bird/d) and in the heat stress room (10.7 and 12.6 vs 16.4 mEq/bird/d). For rooms combined, all DEB treatments differed in Na intake (least to highest DEB, 10.0 vs 13.8 vs 18.1 mEq/bird/d). The K consumption increased for DEB 340 compared to DEB 240 in the heat stress room (18.0 vs 14.1 mEq/bird/d), with DEB 140 intermediate (16.7 mEq/bird/d) and for rooms combined (15.6 and 15.6 vs 19.8 mEq/bird/d, DEB 140 to 340). The Cl consumption

was greater ($P < 0.05$) for the DEB 140 treatment than the DEB 240 and 340 treatments in the heat stress rooms (13.70 vs 6.84 and 5.90 mEq/bird/d) and for rooms combined (12.80 vs 7.58 and 6.50 mEq/bird/d).

Urinary Na level (Table 4) increased substantially in the DEB 340 compared to DEB 140 and 240 treatments in the thermoneutral (+151 and +139%) and heat stress (+116 and +159%) rooms and for combined rooms (+133 and +148%), indicating probable excess of dietary Na intake with the DEB 340. Urinary K was least for the DEB 240 treatment in each room and for combined rooms, indicating better conservation of K. Urinary Cl was greatest for the DEB 140 treatment in each room and overall, indicating excessive Cl intake. Fecal electrolyte levels were not significantly affected by DEB levels. Retention of electrolytes equals consumed electrolytes minus urinary plus fecal electrolytes. The Na retention increased for the DEB 240 compared to the DEB 140 treatment in the thermoneutral room (5.24 vs 1.75 mEq/bird/d), the heat stress room (5.29 vs 2.33 mEq/bird/d) and combined rooms (5.26 vs 2.04 mEq/bird/d). In the heat stress room only, there was an increase in Cl retention with decreasing DEB and the differences between DEB 140 and 340 (5.17 vs 2.37 mEq/bird/d) were significant ($P < 0.05$).

There have been reports of heat stress reducing urinary loss of Cl (Belay and Teeter, 1996) and increasing plasma Cl^- concentration (Khone and Jones, 1975). Usually the acid-base imbalance (greater pH of blood in respiratory alkalosis) is partly corrected by renal exchange of HCO_3 for Cl (Mongin, 1981) because Cl in plasma increased and urinary excretion decreased (Belay and Teeter, 1996) in heat stress. The kidneys attempt to correct the acid-base balance that has deviated from normal due to heat stress challenge by regulating body electrolyte content (Mongin, 1981; Hulan *et al.*, 1986). Respiratory alkalosis in blood can be accompanied by metabolic acidosis in tissues.

Broiler chickens receiving diets with DEB 340 were under metabolic alkalosis and the increase in K excretion may have been be a physiological response inasmuch as this ion is alkalogenic - its loss results in acidification of body fluids. On the other hand, the more simple explanation would be that diets with DEB 340 had more K and extra K was absorbed by pericellular passive diffusion due to the concentration gradient. Thus, as the K concentration increases in the intestinal lumen, a gradient favorable to the absorption of this ion is generated. In addition, K absorption is directly linked to water absorption (Cunningham, 1999). Although total K retention was not affected by temperature (Table 4), an increase in K retention was observed with increasing DEB in the feed for combined rooms as a simple body compensatory mechanism (Ait-Boulahsen *et al.*, 1995). Birds subjected to chronic heat stress from 14 to 41 d of age had less urinary Na excretion when compared to birds kept under thermoneutrality (4.90 vs 5.50 mEq Na/bird/d), as shown in Table 5 (not analyzed statistically; unreplicated rooms), even though serum Na levels remained relatively constant (Table 6; 143.9 vs 144.8 mmol/l for heat stress vs thermoneutral room). This was apparently related to the mechanism to preserve blood volume and maintain appropriate hydration of tissues.

Favorable results for the DEB 240 mEq/kg treatment (improved N balance, less K excretion) may be in some way associated with greater BW gain in broilers in performance trials carried out in the same laboratories as the present trial (Borges *et al.*, 1999).

Implications: Broiler chickens regulated a larger volume of water consumption by greater urinary excretion. Electrolyte levels (Na, K and Cl mEq/bird/d) excreted in urine depended on concentrations in feed and on ambient temperature. In heat stress, water:feed ratio increased whereas urine Na and K mEq/bird/d decreased. In each temperature environment, retention of Na increased in the 240 compared to 140 mEq/kg treatment (340 mEq/kg intermediate). Overall, urine Na and K increased in the 340 compared to 240 mEq/kg treatment and Cl increased in 140 compared to 240 or 340 mEq/kg treatments. Dietary electrolyte balance (DEB) of 240 mEq/kg was most favorable for broilers in either temperature environment based on water, electrolyte and N metabolism results. These data appear to confirm the DEB concept of Mongin (1981) and partly help to explain live performance benefits at around DEB 240 mEq/kg observed by the authors in previous broiler trials at research facilities in Brazil.

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