

ALLEVIATION OF HEAT STRESS IN FARAFRA SHEEP BY DIETARY MINERALS SUPPLEMENTATION

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ABSTRACT

Thirty five Farafra mature ewes aged 2.5 – 3.5 years and averaged 38.8 ± 0.69 kg body weight and fifteen Farafra mature rams aged 2.0-3.0 years and averaged 59.3 ± 1.30 kg body weight were used in this investigation. The experimental animals (rams and ewes) were divided into five equal groups (7 ewes + 3 rams each) as control, 1% sodium bicarbonate (G1), 1% potassium carbonate (G2), 0.5% sodium bicarbonate + 0.5% potassium carbonate (G3) and 1% sodium bicarbonate + 1% potassium carbonate (G4). Blood samples were collected from each animal every 10 days after feeding throughout the experimental period (June to August). Results indicate that dietary electrolyte insignificantly increased blood hemoglobin (Hb) while significantly ($P < 0.05$) decreased blood hematocrit (Ht) in both rams and ewes. The values of glucose, total protein, sodium and potassium significantly ($P < 0.05$) tended to increase and dietary electrolyte balance DEB values to significantly ($P < 0.01$) increase in both rams and ewes supplemented with salts. Serum urea values were insignificantly increase while serum chloride tended to significantly ($P < 0.05$) decrease in both rams and ewes with salts-fed animals.. Salts supplement led to insignificant increase in serum triiodothyronine and thyroxin of rams and ewes.

In conclusion, supplementation of some salts (sodium or potassium or both) to the diet of animals during summer (the hot season) led to alleviate heat stress in term of controlling the blood metabolites.

Key words: Blood parameters; dietary salts; heat stress; sheep;

INTRODUCTION

Sheep production plays an important role in the socio-economics of farmers and sheep owners. Minerals are considered as essential item that play a key role in animal physiology. The metabolism of the primary nutrient (fat, carbohydrates and protein) rely on minerals for their respective fates as co-factors of enzymes. The mineral ions of the diet may be positive charged cations, such as sodium (Na) and potassium (K) or negatively charged anions, such as chloride (Cl) and sulfur (S). The differences between these cations and anions (Na + K-Cl) of the diet is referred to dietary cation anion differences (DCAD) and also called dietary electrolyte balance (DEB) or dietary cation anion balance (DCAB).

Minerals are integral part of all biological functions. A specific amount and proportion of certain mineral may improve the dry matter intake by making some adjustments in blood chemistry during hot summer. The balance of K between intracellular and extracellular fluids is controlled by Na – K pump. Generally three Na ions pumped out the cell and two K ions diffused into the cell. Exchanges of Na – K ions are responsible of transmission of nerve impulses, secondary transport of nutrients such as glucose and maintenance of osmotic pressure, water balance and acid-base balance (Mohammed, 2005).

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Many researches reported that the requirement from minerals elements such as potassium and sodium increases during heat stress (West et al., 1991 and 1992; Block, 1994; Sanchez, 2003 and Beatty, et al., 2007). A lot of works have been done on cows to study the effect of dietary cation-anion difference. But regarding sheep; very limited scientific information is available in this respect. The purpose of this study is to investigate the effect of some salts supplementation (sodium or potassium or both) on some blood metabolites of sheep under heat stress conditions.

MATERIALS & METHODS

The field work of this study was carried out at Minia Governorate, Mallawi Experimental Station, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture. The chemical analysis was executed in the physiological lab of the Animal Production Department, Faculty of Agriculture, Minia University during the period from June to August (2009).

Experimental animals

A total number of fifty animals were used in this investigation. Thirty five Farafra mature ewes (aged 2.5 – 3.5 years and averaged 38.8 ± 0.69 kg body weights) and fifteen Farafra mature rams (aged 2.0-3.0 years and averaged 59.3 ± 1.30 kg body weights) were used in this experiment. All sheep were shorn in April (2006). Animals were treated for internal and external parasites at the beginning of the experiment. All animals were also subjected to the routine vaccination programs for infectious diseases (foot and mouth disease, rift valley fever, etc.).

Experimental design.

The experimental animals (rams and ewes) were divided into five equal groups (7 ewes + 3 rams each) according to their age and initial body weight as control, 1% sodium

bicarbonate (G_1), 1% potassium carbonate (G_2), 0.5% sodium bicarbonate + 0.5% potassium carbonate (G_3) and finally 1% sodium bicarbonate + 1% potassium carbonate (G_4). The first group fed the basal diet without any supplementation and several as control groups. Whereas, the other four groups fed the basal diet plus salts supplementation. Animals were fed according to NRC (1985) standards (The concentrate mixture was machinery mixed with the salt additives). The concentrate mixture contain 12% crude protein and 50% starch value. At 8.00 am animals were offered wheat straw in addition to the concentrate mixture (at 70: 30 concentrate: roughage ratio). Animals were allowed to drink *ad-libitum* fresh tap water and were confined in semi-open pens throughout the experiment. Feed samples (concentrate mixture and wheat straw) at the beginning of the experiment were analyzed for major electrolytes (Na, K and Cl). Thus, dietary cation anion balance calculated as mille equivalents (DCAB) of (Na, K and Cl) per kilogram of feed DM according to Mongin (1980).

Ambient temperature and relative humidity were recorded during the experimental period at afternoon (12-2 p.m). A mercury centigrade thermometer was used to measure ambient temperature, while a hygrometer hanging from the roof of the shed at height of two meters from the ground was used to measure relative humidity.

Blood samples

Blood samples (10 ml) were collected three hours after feeding every 10 days from the jugular vein of 7 ewes and 3 rams per group. Each blood sample was collected into two tubes (heparinized and non heparinized). Heparinized blood samples were used for measuring hemoglobin (Hb) and hematocrit (Ht). Hemoglobin concentration (Hb, g/dl) was measured by colorimetric method using kits (Diamond reagent). Hematocrit value (Ht) was measured in hematocrit capillary tubes using a hematocrit centrifuge at 3000 r.p.m. for 15

minutes. Unheparinized blood samples were centrifuged at 3000 r.p.m for 15 minutes and serum was obtained and stored at -20°C for blood metabolites and hormonal assays. Serum total protein (TP) was determined using the kits of Stanbio Company. Urea concentration was measured by enzymatic calorimetric method using the kits of Biodiagnostic Company. Glucose concentration (mg/dl) was measured by a colorimetric method using the kits of Dialab Company. Serum Na and K (mEq/l) concentrations were determined using the kits of sodium enzymatic color measuring (Biodiagnostic Company). Chloride concentration (mEq/l) was determined using thiocyanate method (QCA Company). Direct radioimmunoassay (RIA) technique was performed for determination of serum thyroid hormones. **Triiodothyronine:** The Coat-A-Count T_3 kits produced by Diagnostic Products Corporation (U.S.A) were used for the determination of serum triiodothyronine according to **Bates (1974)**. **Thyroxine:** The Coat-A-Count kits produced by Diagnostic Products Corporation (U.S.A) were used for the determination of serum thyroxine according to **Albertini (1982)**.

Statistical analysis

Statistical analyses were carried out using SPSS 11.0 for Windows (**SPSS Inc., Chicago, IL**). ANOVA was used to test the effect of treatment. **L.S.D** (least significant difference) was used to test the significance among treatments. The following model was used in ANOVA:

$$Y_{ij} = \mu + T_i + E_{ij} \quad \text{Where,}$$

Y_{ij} = Experiment observations.

μ = Overall mean.

T_i = The effect of dietary treatments.

E_{ij} = The experimental error.

RESULTS AND DISCUSSION

Climatic condition

The meteorological data during the experimental period (June to August 2006) are presented in Table (1). The mean value of the

ambient temperature during experimental was $38.83 \pm 0.49^{\circ}\text{C}$ (ranged between $36-40^{\circ}\text{C}$), whereas the corresponding value of relative humidity was $29.0 \pm 1.157\%$ (ranged between $24-35.0\%$). Temperature humidity index (THI) during experimental was calculated according to (Thom, 1959). It was 92.23 (ranged between 90-92) these values indicated that animals were under severe heat stress during the experimental period according to Davis et al. (2003) and Khalifa et al. (2005).

2-Dietary cation anion balance (DCAB)

The electrolyte content in the different experimental diets for the five experimental groups is presented in Table (2). Results indicated that the values of DCAB were increased as a result of salts supplementation to the concentrate mixture by 113.9, 140.0, 123.5 and 253.9% for G1, G2, G3 and G4, respectively in comparison with the control group.

3- Blood hematological and chemical parameters

3.1-Hemoglobin concentration

Results indicated that dietary salts was insignificantly increased blood hemoglobin (Hb) in both rams and ewes (Table 3 and 4). Generally, the present results did not match with those of **Kilmer et al., (1981)** whom found that dietary sodium bicarbonate decreased insignificantly blood Hb. Also, **Escobosa et al., (1984)** found that dietary sodium bicarbonate decreased blood Hb of dairy cows during summer.

The slight increase in blood Hb may be an indicator of normal blood oxygen load, because the prolonged exposure to excessive heat stress may decreased blood Hb. **Khalifa (1982); Ashmawy (1987) and El-Shafie (1997)** found that Hb concentration decreased by the increase of ambient temperature either for short duration (diurnal variation) or in long term (seasonal variation).

3.2-Hematocrit value

The effect of dietary salts on blood hematocrit value in rams and ewes are presented in Tables (3 and 4). Dietary salts caused significant ($P < 0.05$) decrease in blood Ht in both rams and ewes. In rams, blood Ht values were decreased by 5.45, 5.04, 5.33 and 5.41% for G₁, G₂, G₃ and G₄, respectively in comparison with the control. While, in ewes, blood Ht values were decreased by 4.83, 4.49, 4.64 and 4.35% for G₁, G₂, G₃ and G₄, respectively compared to the control. At the same time no significant differences were found among the treated groups. These results may be due either to hemo dilution or over hydration under hot climate, which resulted in increasing plasma and interstitial fluids or may be resulted from the increase of water intake under hot conditions which resulted from salts addition during heat stress to overcome the increase in sweating rate. These results agree with **Escobosa et al., (1984)**, who found that increasing level of DCAB decreased blood Ht.

The higher value of blood Ht in the control group may be a reflection of being subjected to heat stress. **Kume et al., (1998)** reported that blood Ht of heifers was increased by heat stress. However, the addition of electrolytes alleviated the effect of heat stress.

3.3-Glucose concentration

The values of serum glucose concentrations in rams and ewes are illustrated in Tables (3 and 4). The results indicated that dietary salts supplementation increased ($P < 0.05$) serum glucose concentrations in both rams and ewes. In rams, serum glucose values were increased by 4.01, 3.16, 4.72 and 3.95% for G₁, G₂, G₃ and G₄, respectively as compared to the control group. While, in ewes, serum glucose values were increased by 3.87, 3.42, 3.85 and 3.53% for G₁, G₂, G₃ and G₄, respectively in comparison with the control group. However, there were no significant differences among supplemented groups. The present results agree with **Escobosa et al., (1984)**, who examined the effect of dietary

sodium bicarbonate in the diet of cows during summer on blood glucose. They reported that the increase of blood glucose may be due to significant increase of feed intake in high sodium bicarbonate group. On the other hand, **Boisclair et al., (1987)**; **Vicini et al., (1987)**; **Belibasakis (1990)** and **Rizzi et al., (2004)** found that changing DCAD level in the diet did not significantly affected blood glucose concentration.

3.4-Total protein

The values of serum total protein concentrations for rams and ewes are illustrated in Tables (3 and 4). The results revealed that dietary salts caused significant ($P < 0.05$) increase in serum total protein concentrations in both rams and ewes. In rams, the values were increased by 3.83, 4.33, 3.84 and 3.66% for G₁, G₂, G₃ and G₄, respectively as compared to control. While, in ewes, blood total protein values were increased by 3.26, 3.43, 3.09 and 2.77% for G₁, G₂, G₃ and G₄, respectively as compared to the control group. There were no significant differences among the treated groups between rams and ewes.

Blood total protein has a particular importance in maintaining plasma volume. **Cunningham (2002)** reported that proteins plays a role in intracellular buffers within the body tissues to provide a reserve buffering capacity. This indicates that increasing blood total protein concentrations by dietary electrolytes had a beneficial effect on acid base balance. The increase in the values of serum total protein, which come from dietary electrolytes in the present study may be beneficial to maintain the osmotic pressure. **Khalifa (1982)** suggested that the increase in total protein of sheep during heat stress causes an increase in plasma colloid osmotic pressure, which may help the animal to conserve water. In addition, **Kamal (1982)** reported that during heat stress an increase in plasma total protein occurred to match the change in colloid osmotic pressure.

On the other hand, **Boisclair et al., (1987)**; **Arambel et al., (1988)**; **Cheirecato et**

al., (2003); Rizzi et al., (2004) and Belibasakis (1990) reported that the addition of electrolytes in diets had no significant effect on blood concentrations of total protein.

3.5-Urea concentration

Means of the effect of dietary electrolytes on serum urea of rams and ewes are presented in Tables (3 and 4). Urea concentrations insignificantly increase by dietary salts in both rams and ewes.

The present results agree with West et al., (1991), while Belibasakis (1990) and West et al., (1992) found that dietary electrolytes did not significantly affect blood urea concentrations. On the other hand, Wildman et al., (2007) found that blood urea nitrogen was lower for the highest DCAB diet.

3.6- Sodium ion concentration

Means of serum sodium concentrations for rams and ewes are illustrated in Tables (3 and 4). The results indicate that dietary salts caused significant ($P<0.05$) increase in serum Na. In rams, serum Na values were increased by 4.27, 3.71, 4.57 and 6.19% for G₁, G₂, G₃ and G₄, respectively as compared to the control group. Also, in ewes, blood Na values were increased by 3.98, 3.20, 4.33 and 4.98% for G₁, G₂, G₃ and G₄, respectively in comparison with the control. No significant differences were observed among the supplemented groups in both rams and ewes. These results are expected due to sodium salts addition in the diet which will lead to increase circulating blood Na that may have a beneficial effect to cover the losses in Na through renal excretion under heat stress conditions. These results are in agreement with those reported by Fredeen et al., (1988); Patience et al., (1986); Haydon et al., (1990) and West et al., (1992).

On the other hand, on bucks, Rizzi et al., (2004) examined the effect of diets differ in electrolyte balances on serum Na and found that dietary electrolytes did not

significantly affect plasma Na. Similar results were obtained by Belibasakis (1990); Delaquis and Block (1995) and Chan et al., (2005). Also, Mohammed (2005) noticed that alteration in DCAB in the diet of cattle didn't significantly affect the concentrations of serum sodium

3.7- Potassium ion concentration

The effect of different treatments on serum potassium concentrations in rams and ewes are illustrated in Tables (3 and 4). The results indicate that dietary salts caused significant ($P<0.05$) increase in serum K in rams and ewes. The higher concentrations of serum K were observed in treatment groups compared to the control group in both rams and ewes. However, no significant differences were observed among treated groups for both rams and ewes.

The present results indicate that addition of potassium salts to diet led to elevate circulating K. The supplementation of potassium salts was beneficial to prevent the severe losses of this ion through sweating. The present results are in line with those of West et al., (1992) who examined the effect of changing DCAB on serum K of dairy cows and reported that serum K was significantly increased with increasing DCAB. Our results are in contrary with those of Ross et al., (1994), who studied the effect of varying diets of DEB on blood plasma K with beef steers. They noticed that plasma concentrations of K were decreased quadratically with increasing DEB. While, Escobosa et al., (1984) examined the effect of three DCAB diets with supplementing sodium bicarbonate in the high DCAB diet during summer on blood K and reported that there was no significant effect for changing DCAB on blood K. Also, similar results were obtained by Belibasakis (1990); Delaquis and Block (1995); Rizzi et al., (2004); Chan et al., (2005) and Mohammed (2005).

3.8- Chloride concentration

The values of blood chloride concentrations in rams and ewes are illustrated in Tables (3 and 4). The results indicate that dietary salts caused significant ($P < 0.05$) decrease in serum Cl. In rams, blood Cl values were decreased by 4.67, 5.01, 4.91 and 4.03% for G₁, G₂, G₃ and G₄, respectively in comparison with the control. While, in ewes, blood Cl values were decreased by 4.19, 3.85, 3.73 and 4.06% for G₁, G₂, G₃ and G₄, respectively compared with the control. Insignificant differences were observed among salt treatments in both rams and ewes.

Chloride ion is the major anion in extracellular fluid. Loss of HCO₃ caused a relative increase in blood Cl as expected and this means that control animals were suffering from metabolic acidosis. Our results are in a harmony with those of **Tucker et al., (1988)**, who examined the effect of dietary salts on diets of dairy cows and reported a linear decrease in plasma Cl concentrations with increasing DEB. Similar results were obtained by **West et al., (1991 and 1992)**; **Ross et al., (1993 and 1994)**; **Manna et al., (1999)**; **Roche et al., (2003) and Mohammed (2005)**. On the other hand, **Delaquis and Block (1995)** found that reducing DCAB had no effect on plasma Cl concentrations of dairy cows at any sampling time. Additionally, similar results were obtained by **Rizzi et al., (2004)** and **Chan et al., (2005)**.

3.9- Dietary electrolyte balance

Means of blood dietary electrolytes balance (Na + K - Cl) mEq/l of rams and ewes are illustrated in Tables (3 and 4). It can be observed that dietary salts caused significant ($P < 0.01$) increase in serum DEB. In rams, values of blood DEB were increased by 21.00, 20.17, 22.32 and 25.18% for G₁, G₂, G₃ and G₄, respectively in comparison with the control. The highest DEB value was observed in G₄, which received high DCAD. In ewes,

blood DEB values were increased by 29.16, 24.94, 29.11 and 32.76% for G₁, G₂, G₃ and G₄, respectively as compared to the control. Insignificant differences were observed among different treatments in both rams and ewes.

These results were expected because the addition of sodium and potassium salts in the diets led to increase DEB, resulting in improving blood buffering capacity in cases of heat stress and losing of Na and K by sweating could be more severe. These results are in agreement with those noticed by **Haydon et al., (1990)**. They observed that blood DEB increased linearly as dietary DEB increased in study on swine under heat stress. A linear increase in serum DEB was also supported by other researchers (**Tucker et al., 1988**; **West et al., 1991 and 1992**; **Hu and Murphy, 2004** and **Mohammed, 2005**).

3.10- Thyroid hormones

Data of serum thyroid hormones concentrations in rams and ewes are illustrated in Tables (3 and 4). The results indicate that dietary electrolytes insignificantly increase serum thyroid hormones in both rams and ewes. The slight increase of thyroid hormones in both rams and ewes may be an indicator of increasing carbohydrate, protein and fat metabolism, which resulted from alleviating heat stress or may be from increasing feed. Reducing thyroid hormones caused decreasing of gut motility and rate of passage (**Beede and Collier, 1986**). These results are in a harmony with **Vicini et al., (1987)**, who examined the effect of 2% sodium bicarbonate in the diet of dairy cows on plasma thyroid hormones. They found insignificant increase in thyroid hormones concentrations in sodium bicarbonate group. Meanwhile, **Cheirecato et al., (2003)** reported that plasma levels of thyroid hormones were not significantly influenced by higher dietary DEB level. Also, **Rizzi et al., (2004)** found that dietary electrolyte balance did not substantially modify the thyroid hormone profile of the bucks.

CONCLUSION

From the present results it can be suggested that heat stress during summer seasons (June to August) could be alleviated by supplementation of sodium or potassium salts or both to the ration of animals. This led to beneficial effect on the physiological responses of the animals throughout controlling blood metabolites parameters.

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Table (1):- Meteorological data during the experimental period.

Months	Ambient temperature °C	Relative humidity %	THI
June	36.3 ± 0.18	34 ± 0.76	89.98
July	37.4 ± 0.23	29 ± 0.89	91.59
August	39.8 ± 0.17	24 ± 1.02	95.11
Mean	38.83 ± 0.49	29 ± 1.57	92.23

Table (2) Electrolyte minerals content of the experimental diets.

Item	<i>Electrolytes content</i>				
	<i>control</i>	<i>G₁</i>	<i>G₂</i>	<i>G₃</i>	<i>G₄</i>
Na (%)	0.297	0.567	0.297	0.432	0.567
K (%)	0.523	0.523	1.088	0.806	1.088
Cl (%)	0.563	0.563	0.563	0.563	0.563
DCAB (mEq/kg feed)	115	246	276	257	407

G₁ (1% Sodium bicarbonate) G₂ (1% Potassium carbonate).

G₃ (0.5 % Sodium bicarbonate + 0.5 % Potassium carbonate).

G₄ (1% Sodium bicarbonate + 1 % Potassium carbonate).

DCAB = Dietary cation anion balance calculated as milliequivalents of (Na + K – Cl) per kilogram of feed DM, according to **Mongin (1980)**.

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Table(3) Effect of some dietary salts on some blood hematological and chemical parameters of rams.

Parameters	Treatments					Sig
	Control	G ₁	G ₂	G ₃	G ₄	
Hemoglobin (g/dl)	10.35 ± 0.43	10.44 ± 0.45	10.47 ± 0.37	10.51 ± 0.25	10.43 ± 0.27	NS
Hematocrit (%)	33.95 ^a ± 0.54	32.10 ^b ± 0.77	32.24 ^b ± 0.67	32.14 ^b ± 0.56	32.11 ^b ± 0.57	*
Glucose (mg/dl)	72.06 ^b ± 0.85	74.95 ^a ± 0.86	74.34 ^a ± 0.61	75.46 ^a ± 0.98	74.91 ^a ± 0.61	*
Total protein (g/dl)	6.01 ^b ± 0.096	6.24 ^a ± 0.055	6.27 ^a ± 0.083	6.24 ^a ± 0.063	6.23 ^a ± 0.032	*
Urea nitrogen (mg/dl)	34.43 ± 1.62	34.56 ± 1.15	34.49 ± 1.46	34.54 ± 1.27	34.58 ± 0.91	NS
Sodium (mEq/l)	145.90 ^b ± 1.95	152.13 ^a ± 1.75	151.32 ^a ± 2.69	152.58 ^a ± 1.64	154.93 ^a ± 2.20	*
Potassium (mEq/l)	5.92 ^b ± 0.12	6.15 ^a ± 0.08	6.19 ^a ± 0.07	6.17 ^a ± 0.13	6.21 ^a ± 0.07	*
Chloride (mEq/l)	98.91 ^a ± 1.08	94.29 ^b ± 1.59	93.95 ^b ± 1.26	94.05 ^b ± 1.15	94.92 ^b ± 1.11	*
DEB (mEq/l)	52.90 ^b ± 1.36	64.01 ^a ± 1.42	63.57 ^a ± 2.24	64.71 ^a ± 2.31	66.22 ^a ± 2.23	**
Thyroxine (μg/dl)	2.19 ± 0.12	2.24 ± 0.11	2.23 ± 0.14	2.24 ± 0.15	2.25 ± 0.14	NS
Triiodothyronine (ng/dl)	128.66 ± 1.31	130.52 ± 1.52	129.81 ± 1.05	130.57 ± 1.83	130.86 ± 1.84	NS

a,b Means followed by the same superscript were not significantly different (p<0.05).

G₁ (1% Sodium bicarbonate)

G₂ (1% Potassium carbonate).

G₃ (0.5% Sodium bicarbonate + 0.5% Potassium carbonate).

G₄ (1% Sodium bicarbonate + 1% Potassium carbonate).

DEB Dietary Electrolyte Balance.

*

Significant (p<0.05).

**

Significant (p<0.01).

NS

Non – Significant .

Table(4) Effect of some dietary salts on some blood hematological and chemical parameters of ewes.

Parameters	Treatments					Sig
	Control	G ₁	G ₂	G ₃	G ₄	
Hemoglobin (g/dl)	10.38 ± 0.23	10.47 ± 0.26	10.45 ± 0.22	10.47 ± 0.19	10.44 ± 0.22	NS
Hematocrit (%)	33.57 ^a ± 0.53	31.95 ^b ± 0.45	32.06 ^b ± 0.46	32.01 ^b ± 0.49	32.11 ^b ± 0.65	*
Glucose (mg/dl)	72.82 ^b ± 1.02	75.64 ^a ± 1.05	75.31 ^a ± 0.89	75.63 ^a ± 0.93	75.39 ^a ± 0.92	*
Total protein (g/dl)	6.13 ^b ± 0.08	6.33 ^a ± 0.08	6.34 ^a ± 0.09	6.32 ^a ± 0.07	6.30 ^a ± 0.09	*
Urea nitrogen (mg/dl)	34.38 ± 1.32	34.45 ± 1.15	34.44 ± 1.08	34.49 ± 1.09	34.50 ± 1.27	NS
Sodium (mEq/l)	134.76 ^b ± 2.08	140.12 ^a ± 2.16	139.08 ^a ± 2.04	140.59 ^a ± 1.95	141.47 ^a ± 2.15	*
Potassium (mEq/l)	6.03 ^b ± 0.09	6.23 ^a ± 0.06	6.25 ^a ± 0.06	6.24 ^a ± 0.07	6.28 ^a ± 0.08	*
Chloride (mEq/l)	104.75 ^a ± 1.01	100.36 ^b ± 1.02	100.71 ^b ± 1.21	100.84 ^b ± 1.09	100.49 ^b ± 1.15	*
DEB (mEq/l)	35.56 ^b ± 1.78	45.93 ^a ± 2.01	44.43 ^a ± 2.08	45.91 ^a ± 2.11	47.21 ^a ± 2.01	**
Thyroxine (µg/dl)	2.22 ± 0.17	2.27 ± 0.16	2.25 ± 0.17	2.26 ± 0.11	2.25 ± 0.12	NS
Triiodothyronine (ng/dl)	112.83 ± 1.16	114.34 ± 1.01	114.15 ± 1.21	114.82 ± 1.61	114.51 ± 1.25	NS

a,b Means followed by the same superscript were not significantly different (p<0.05)

G₁ (1% Sodium bicarbonate) G₂ (1% Potassium carbonate).

G₃ (0.5% Sodium bicarbonate + 0.5% Potassium carbonate).

G₄ (1% Sodium bicarbonate + 1% Potassium carbonate).

DEB Dietary Electrolyte Balance. * Significant (p<0.05).

** Significant (p<0.01). NS Non – Significant .