

Assessment of Extracellular Fluid Deficit and Electrolyte Levels in Stressed Marwari Sheep

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Abstract: In the present investigation, the Extracellular Fluid (ECF) volume and serum electrolytes were determined in healthy adult female Marwari sheep during control, thirst, drinking and recovery periods. The control mean value of ECF volume was 9.187 ± 0.332 . Advancement in thirst period resulted in gradual deficit in ECF volume and on days 5 of thirst period 35.278% change was observed in ECF volume. Immediately after rehydration, replenishment was only 13.824%. Even on day 10 of recovery period, replenishment was not complete in ECF volume. The mean values of various serum electrolytes viz., calcium, phosphorus, magnesium, chloride and sodium increased significantly ($p \leq 0.05$) as the days progressed except potassium which decreased significantly ($p \leq 0.05$). On day 5 of thirst period, the values of calcium, phosphorus, magnesium, chloride and sodium were 37.892, 45.654, 61.687, 34.454, 21.805 and 24.428% higher, respectively than the corresponding mean value during control period. Drinking resulted in lowering of all the electrolytes than their respective day 5 thirst period values except potassium which showed an increase at hour 1/2 of drinking period. At 72 h of drinking period all the serum electrolytes showed non-significant ($p > 0.05$) differences from respective control mean values. In present study no clinical signs of oedema, staggering or other illness was observed after thirst period and drinking periods. It was concluded that a thirst period of 5 days caused a state of dehydration resulting in water loss from ECF compartment. Owing to slow replenishment in the ECF volume, close monitoring of the dehydrated animals is essential to replenish fluid volume along with fluid therapy for a longer period.

Key words: ECF, electrolytes, Marwari, sheep, serum

INTRODUCTION

Scientific know-how of fluid and electrolyte status is prerequisite feature to execute fluid therapy in veterinary clinical practice. In animals isotonic dehydration is common which can be observed in the conditions of acute diarrhoea and inappropriate diuretic administration (Kaneko *et al.*, 1999). Further control of turnover of water and electrolyte is significant in the survival of animals in arid areas. For the physiological events, proper maintenance of volume and composition of body fluid is primary requirement of the body. From nerve conduction and muscular contraction to metabolic reactions, transmembrane movement of electrolytes is essential. Fluid imbalance affects virtually all systems of the body. The total amount of water in the body remains relatively constant from day to day however, considerable variation

is observed in total body water content of an animal due to conditions related with water lack (Kataria *et al.*, 2003). During water scarcity periods, homeostasis largely depends upon the ability of the animal to conserve water. One of the common problems experienced by the sheep in arid tracts is dehydration (Kataria and Kataria, 2006).

Water loss from an animal during dehydration may be reduced by various physiological and behavioural strategies like drawing of water from various body water compartments and the degree by which these compartments are depleted during dehydration differs from species to species and can be used as an indicator of an animal's ability to withstand dehydration (Denny and Dawson, 1975). For crisis management of animals with water losses, an understanding of body fluid profile along with the levels of different electrolytes is mandatory for veterinary clinical practice. Dehydration can also affect

properties of constituents of plasma. Decrease in protein hydration provide a plausible physical mechanism through which microwaves enhance aberrant protein folding and aggregation (Bren and Janezic, 2012).

Sodium is the osmotic skeleton for the extra cellular fluid (Kaneko *et al.*, 1999). Potassium is the chief ion of intracellular fluid. Changes in electrolyte composition may bring about volume changes. Decreased sodium concentration results in deficit in extra cellular fluid volume, whereas sodium excess is related with an increase in body water.

Marwari sheep along with camel and goat is considered as desert species. Certain specific situations like drought and lower water table result into long thirst periods for the animals in field conditions. They have to walk longer distances during grazing without water. Therefore, the animal's water conservation mechanism should be strong enough to tide over the period of water scarcity or deprivation. This would come at a physiological cost to the animal for the maintenance of homeostasis (Sihag *et al.*, 1999). Tolerance of animals to water deficit can be best adjudged by determining certain serum electrolytes (Kataria *et al.*, 2002). For proper health management of animals, evaluation of fluid and electrolyte status is a key factor to unlock many physiological reactions related with adaptability of the animals particularly in arid and semi arid tracts where water scarcity compounded with higher ambient temperature imposes great stress on the animal body. Looking towards the paucity of research on this aspect in Marwari sheep, an investigation was carried out to assess extracellular fluid deficit and electrolyte levels during thirst periods.

MATERIALS AND METHODS

Animals and sampling: Six apparently healthy adult female Marwari sheep ageing 1-2 years were used for assessment of extracellular fluid deficit and serum electrolyte determination by the permission of Institutional Animal Ethical Committee (IAEC), College of Veterinary and Animal Science, Bikaner, Rajasthan, India. Standard management practices were used to keep animals stress free. Animals were fed with sole roughage diet of dry *Ziziphus nummularia* leaves and watered *ad libitum*. The experiment was divided into four periods of control (30 days for adjustment of the animals), thirst (5 days), drinking (10 days) and recovery (10 days) periods using the same animals. The mean maximum and minimum ambient temperatures were $39.38 \pm 0.43^\circ\text{C}$ and $24.06 \pm 0.60^\circ\text{C}$, respectively. In the control period, animals were fed and watered *ad libitum*. During thirst period, the animals were kept under same feeding conditions but with complete water restriction to induce thirst mechanism.

Drinking period started after the end of thirst period during which water was provided *ad libitum*. The recovery period started after the end of drinking period during which feeding and watering were same as that in control period. This period was used to assess the per cent recovery in each parameter.

Analysis: Extra Cellular Fluid (ECF) volume was estimated by using sodium thiocyanate (Wrenn *et al.*, 1962; Kataria *et al.*, 2003). For determination of ECF, 330 mg sodium thiocyanate was mixed with 10 mL of distilled water by boiling for 1 min. Final volume was made to 20 mL by adding sterile normal saline solution. Before each infusion whole blood sample was collected and then the solution was injected into the jugular vein within 90 sec. The completion time was noted accurately and subsequently four blood samples were collected at 30, 60, 90 and 120 min post infusion. Zero time reading was obtained by extrapolation. Extra cellular fluid volume was determined during control period, days 2 and 5 of thirst period, hours 1/2 and 24 of drinking period and on day 10 of recovery period.

Serum electrolytes included sodium, potassium, chloride, calcium, phosphorus and magnesium. Sodium and potassium were determined by using flame photometry, calcium by OCPC Methods of kit (Wipro), phosphorus by Phosphorus Molybdate Method of kit (Wipro), magnesium by Titan Yellow Method and chloride by method of Schales and Schales. Body weight was recorded daily in the morning before feeding throughout the course of experiment to calculate ECF volume per kg body weight.

Statistics: For each parameter mean \pm SEM values were determined in each period and their subsets. The mean values for every parameter in periods of control, thirst, drinking and recovery were compared with respective mean values in control period to find out significance in differences statistically (Steel and Torrie, 1980).

RESULTS

The mean \pm SEM values of extra cellular fluid volume and serum electrolytes during different periods of experiment are presented in Table 1 and 2, respectively. The mean values of ECF volume during different periods are presented in litres (L), mL per kg body weight (mL kg^{-1} b.wt.), body weight % and change %. Advancement in thirst period resulted in gradual deficit in ECF volume. A 35.278% change was observed in ECF volume on day 5 of thirst period. Immediately after rehydration, replenishment was only 13.824%. Even on day 10 of recovery period, replenishment was not

Table 1: Extracellular Fluid (ECF) changes in Marwari sheep during control, thirst, drinking and recovery periods (Mean±SEM, N = 6)

ECF volume	Control	Thirst periods (days)		Drinking periods (h)		Recovery period (Day 10)
		2	5	1/2	24	
ECF (L)	9.187±0.332	7.572±0.335 ^b	5.946±0.358 ^b	6.768±0.402 ^b	7.381±0.368 ^b	7.883±0.360 ^b
ECF (mL kg ⁻¹ b.wt.)	297.658±3.859	266.998±4.001 ^b	237.138±4.934 ^b	233.465±5.177 ^b	261.291±7.709 ^b	263.136±6.881 ^b
ECF (% b.wt)	29.766 ±0.385	26.70±0.400 ^b	23.714±0.493 ^b	23.347±0.517 ^b	26.129± 0.77 ^b	26.314±0.688 ^b
Change in ECF (%)	-	-17.579	-35.278	+13.824	+24.134	+32.398

Sub-class means within a given row superscribed by letter b differ significantly (p≤0.05) from respective control mean value. N = Number of sheep; b.wt. = Body weight

Table 2: Serum electrolytes (mmol L⁻¹) of Marwari sheep during control, thirst, drinking and recovery periods (Mean±SEM, N = 6)

Periods	Days	Calcium	Phosphorus	Magnesium	Chloride	Sodium	Potassium
Control		1.601±0.035	1.1780±0.029	0.8530±0.022	120.983±1.875	133.000±3.559	5.117±0.2010
Thirst	1	1.667±0.022 ^b	1.267±0.026 ^b	0.949±0.043 ^b	125.05±0.084 ^b	143.667±3.63 ^b	5.0000±0.129
	2	1.786±0.026 ^b	1.345±0.029 ^b	1.191±0.030 ^b	130.133±2.572 ^b	144.833±5.394 ^b	4.717±0.164 ^b
	3	1.974±0.065 ^b	1.391±0.041 ^b	1.256±0.036 ^b	138.267±4.361 ^b	156.667±2.789 ^b	4.250±0.112 ^b
	4	2.057±0.07 ^b	1.592±0.057 ^b	1.308±0.042 ^b	148.433±6.620 ^b	158.000±4.107 ^b	4.0000±0.129 ^b
	5	2.208±0.075 ^b	1.716±0.054 ^b	1.379±0.025 ^b	162.667±5.606 ^b	162.000±1.693 ^b	3.867±0.102 ^b
Drinking (h)	1/2	1.801±0.067 ^b	1.346±0.029 ^b	1.156±0.060 ^b	145.383±6.573 ^b	141.500±2.473 ^b	4.083±0.083 ^b
	24	1.678±0.058	1.267±0.053 ^b	0.944±0.0500	127.083±2.911 ^b	138.333±2.108	4.333±0.105 ^b
	72	1.654±0.061	1.242±0.0410	0.916±0.0510	124.94±2.2410	136.620±2.146	4.528±0.1830
Recovery	10	1.639±0.035	1.229±0.0390	0.900±0.0360	123.8±2.21700	135.500±2.089	4.667±0.2470

N = Number of animals; sub-class means within a column superscribed by letter b differ significantly (p≤0.05) from control mean value

complete in ECF volume. The deficit in ECF volume was evident by the significant (p≤0.05) difference between the values of ECF (L) in recovery and control periods mean values. The former was significantly (p≤0.05) lower than latter.

The mean values of various serum electrolytes viz., calcium, phosphorus, magnesium, chloride and sodium increased significantly (p≤0.05) as the days progressed except potassium which decreased significantly (p≤0.05). On day 5 of thirst period the values of calcium, phosphorus, magnesium, chloride and sodium were 37.892, 45.654, 61.687, 34.454, 21.805 and 24.428% higher, respectively than the corresponding mean value during control period. Drinking resulted in lowering of all the electrolytes than their respective thirst period values (day 5) except potassium which showed an increase at hour 1/2 of drinking period. At hour 1/2 of drinking period the values of calcium, phosphorus, magnesium, chloride and sodium were 18.422, 21.536, 16.155, 10.625, 14.488 and 5.586% lower, respectively than the respective values on day 5 of thirst period. At 72 h of drinking period all the serum electrolytes showed non-significant (p>0.05) differences from respective control mean values. Recovery period also showed non-significant (p>0.05) differences in the mean values of electrolytes from respective control mean values. In present study no clinical signs of oedema, staggering or other illness was observed after thirst period and drinking periods.

DISCUSSION

The control mean value of ECF was similar to the values reported by Degen and Kam (1992) and Kataria and

Kataria (2007) in sheep. It was observed that on mL kg⁻¹ body weight basis, the Extra Cellular Fluid (ECF) volume in the present investigation was towards higher limit of ECF volume in adult animals ranging from 0.15-0.3 L kg⁻¹ body weight as reported earlier (Khan *et al.*, 1978; Spurlock *et al.*, 1985). The ECF consist of all the fluids located outside the cells. Pattern of variation in ECF volume due to thirst and drinking periods was similar to that observed earlier (Kataria and Kataria, 2007). Significantly lower value of ECF volume during recovery period in comparison to control period indicated slow replenishment of ECF volume.

Pattern of variation in serum electrolytes due to thirst period was more or less similar to that recorded in camel (Kataria *et al.*, 2002). Healy and Falk (1974) observed an increase in calcium, phosphorus and magnesium due to water deprivation of 6 days in sheep. Dehydration stress can also affect the level of electrolytes (Tollersrud *et al.*, 1971). Hyprnatraemia in the present study during thirst period probably helped the animals to retain water (Kataria *et al.*, 2002). Sodium homeostasis is essential for renal functions in sheep (Singh *et al.*, 2010). It is reported that aldosterone secretion increases during dehydration thus helping the animal to hold sodium and water (Finberg *et al.*, 1978). Further decrease in serum potassium levels substantiated the fact that increased aldosterone concentration caused the increased secretion of potassium, thereby causing lowering of potassium in the serum. However, Kataria *et al.* (2002) reported that potassium levels were not much affected by the dehydration in camels.

Changes in serum chlorides followed the serum sodium concentration to maintain the electrolyte balance. The excretion, absorption and distribution of chloride are passive and accompany sodium levels (Kataria *et al.*, 2002). Chloride has a key role in the regulation of body fluids, electrolyte balance, the preservation of electrical neutrality and acid-base status. Chloride is an important component of diagnostic tests because abnormal chloride levels alone usually signify a more serious underlying metabolic disorder such as metabolic acidosis or alkalosis (Berend *et al.*, 2012).

The increase in serum electrolytes could be the result of haemoconcentration due to water loss from ECF volume as evident by the results. However, other underlying mechanisms are also responsible for causing changes in serum electrolyte levels. Physiological concentrations of electrolytes are essential for proper maintenance of the cellular functions of the animal. The normal concentrations of these electrolytes in different tissues mainly depend on the dietary concentration, absorption and also on concentration of other tissue elements, homeostatic control mechanism of the body and the species of animal involved (Joshi *et al.*, 2012). Lowering of all the electrolytes due to drinking indicated haemodilution effect (Kataria *et al.*, 2002). Slight increase in potassium level can be attributed to lowering of aldosterone levels upon drinking. In the present study normal levels of calcium, magnesium and sodium were attained on 24 h of drinking period while for the rest of the electrolytes on 72 h of drinking period.

CONCLUSION

It was concluded that a thirst period of 5 days caused a state of dehydration indicated by water deficit in ECF volume. This produced haemoconcentration. Probably this could be one of the reasons that concentration of serum electrolytes increased. However, other underlying physiological mechanisms also modulated the serum electrolyte concentrations during thirst and drinking periods. A slow replenishment in the ECF volume was observed. During recovery period serum electrolyte levels differed non-significantly ($p>0.05$) from respective control mean values whereas ECF volume was still lower significantly ($p\leq 0.05$) from respective control mean volume. The result suggests close monitoring of the dehydrated animals to replenish fluid volume along with fluid therapy for a longer period.

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