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Compartmental water management of Marwari sheep during dehydration and rehydration

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ABSTRACT

In order to assess the compartmental water management of Marwari sheep various body water compartments were determined during the periods of control, dehydration and rehydration. The control mean values of total body water, extracellular, plasma volume, blood volume, red cell volume, intracellular fluid volume and interstitial fluid volume were 20.0 ± 0.8, 1.80 ± 0.1, 2.8 ± 0.20, 0.56 ± 0.02, 9.3 ± 0.4, 11.0 ± 0.4 and 7.5 ± 0.3 litre, respectively, thereby forming 64.26%, 5.78, 8.99, 1.79, 29.88, 35.34 and 24.10% of the body mass, respectively. Significant (P≤0.05) decline was found with progression of dehydration in all the water compartments with a different pattern. Maximum loss was observed from the plasma volume (50%), whereas minimum loss was in the intracellular fluid compartment. Again upon rehydration, the pattern of water replenishment was different in various compartments. Immediately after rehydration the maximum gain was observed in blood volume and the slowest gain in intracellular and red cell fluid compartments. Decreased plasma volume was related with a significantly (P≤0.05) higher serum aldosterone during dehydration period. Simultaneously, dehydration functioning as a stress increased the levels of serum cortisol accompanied by increase in serum creatinine, creatine kinase, γ-glutamyl transferase and alkaline phosphatase activities significantly (P≤0.05).

Key words: aldosterone, cortisol, dehydration, rehydration, water compartments

Introduction

Adaptability to water deficiency in animals can be assessed by the rate of fluid turnover with a pattern of compartmental water movement during dehydration, which is a major problem in arid tracts arising due to intermittent watering, particularly in drought periods**.** To assess the real problem faced by the animals in the field conditions,

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and to understand crisis management, the best experimental model is dehydration and rehydration (KATARIA, 2000) with compartmental water and blood parameter analysis. Alterations in blood biochemical profile is a characteristic feature in diagnosing various conditions (PADMAJA et al., 2006). Regarding the economic importance of sheep in the arid tract and paucity of chance to explore their adaptive features during dehydration, the present investigation was taken up with the objectives to find out the water management potential during dehydration with the physiological modulations in body reactions.

Materials and methods

The experiment was conducted during moderate environmental conditions when the mean maximum environmental temperature was 25.12 ± 0.5 °C. The investigation was carried out on ten healthy adult female sheep of Marwari breed of the arid tracts. The experimental model constituted of control (5 days), dehydration (6 days) and rehydration (3 days) periods. All the animals were fed (dry *Ziziphus nummularia* leaves) and watered *ad libitum* during all the periods, but complete water restriction was imposed during the dehydration period.

To assess the compartmental water management, various body water compartments *viz*. total body water (TBW), extracellular fluid volume (ECF), plasma volume (PV), blood volume (BV), red cell volume (RCV), intracellular fluid volume (ICF) and interstitial fluid volume (ISF) were determined in all the periods as per the standard techniques described by KATARIA et al. (2003), with modifications for sheep as described below.

Measured quantities of sodium thiocyanate (330 mg), Evans' blue (8.8 mg) and urea (5 g) were mixed with 10 mL distilled water by boiling for one minute. The final volume was made to 30 mL by adding normal saline solution so as to prepare the sterile solution for infusion. Before each infusion whole blood samples were collected and then the solution was injected into the jugular vein within 90 seconds. The completion time was accurately noted and subsequently four whole blood samples were collected at 30, 60, 90 and 120 minutes post infusion to determine TBW, PV and ECF. Body fluids were determined during the control on days 2, 4, and day 6 of dehydration, hours ½, 24 and 72 of rehydration periods.

Blood samples were also collected in the morning hours before feeding and watering for blood indices and sera harvesting. Cortisol and aldosterone hormones were determined in serum samples by the ^{125}I radio immuno assay using the commercial kits (RIA kits) in radioisotope laboratory, college of Veterinary and Animal Science, Bikaner, India.

Serum γ-glutamyl transferase, creatine kinase, alkaline phosphatase and creatinine were determined with the use of commercial kits using a spectrophotometer. Blood indices including packed cell volume (PCV), and viscosity, were determined by using

standard techniques as described by SCHALM et al. (1975) and OSER (1976). Body mass of the animals were recorded with a weighing machine in the morning before feeding. Statistical analyses were carried out as per SNEDECOR and COCHRAN (1967).

Results

Body water compartment changes in sheep during dehydration and rehydration are presented in Table 1. The mean values of serum hormones, enzymes, creatinine and blood indices are given in Table 2.

During the dehydration period gradual loss in body mass was observed and after 6 days of water restriction loss was 23% of the control mean value. After rehydration body mass increased from dehydration mean value, which showed that the animal is able to restore only 89% of its body mass immediately after rehydration, and that the recovery of its body mass can reach 93% of the control mean value after 72 hours.

Compartmental mean values reflected significant ($P \leq 0.05$) decrease with advancement of dehydration. Among all the compartments the maximum loss was observed from plasma volume followed by losses in BV, ECF, ISF, RCV and ICF in a declining order. The similar trend was observed during all the days of dehydration period.

Immediately after rehydration (½ hour) the maximum gain was observed from respective day 6 values in blood volume which was followed by ISF, ECF and PV with very little difference between them. The slowest gains were observed in ICF and RCV.

During dehydration period mean values of the serum cortisol, aldosterone, γ-glutamyl transferase, creatine kinase, alkaline phosphatase and creatinine increased significantly ($P \leq 0.05$) from the respective control mean values. On day 6 cortisol marked fivefold and aldosterone marked threefold increase. Blood indices also increased significantly ($P \leq 0.05$) during dehydration period.

Immediately upon rehydration all blood indices and serum parameters did not differ significantly ($P > 0.05$) from the control mean values at hour 72, with the exception of the serum cortisol, creatinine and creatine kinase which differed significantly which differed significantly ($P \leq 0.05$), even at hour 72, from the respective control mean values.

Discussion

Water compartments and blood indices. The control mean values corroborated the earlier findings on the total body water (SARASWAT et al., 1972); plasma, blood and extra cellular fluid volumes (DEGAN and KAM,1992); intracellular fluid volume (PUROHIT et al., 1972) and interstitial fluid volume (HIX et al., 1959) in sheep.

Table 1. Changes in body water compartments of sheep during dehydration and rehydration (mean ± SE, n = 10)

Parameter	Control period	Dehydration period			Rehydration period		
		Day 2	Day 4	Day 6	Hour 1/2	Hour 24	Hour 72
Body mass (kg) % change	31.12 ± 1.2 -	28.01^{b} ± 1.2 -10.0%	26.0^{b} ± 1.12 -16.0%	24.0^{b} ± 1.10 -23%	27.81^{b} ± 1.0 +16%	28.1^{b} ± 1.0 +17%	30.1 ± 1.3 +25%
TBW(l) % BM % Change	20.0 ± 0.8 64.26%	17.92^{b} ± 0.9 63.97% -10.4%	16.38^{b} ± 1.0 63.0% -18.1%	14.40^{b} ± 1.1 60.0% -28.0%	16.12^{b} ± 1.2 57.96 +11.94%	17.98^{b} ± 1.3 63.98 +24.86	19.20 ± 1.0 63.78 +33.3 (96%)
PV(l) %BM % Change	1.80 ± 0.1 5.78	1.30^{b} ± 0.03 4.64 -27.7	1.01^{b} ± 0.02 3.88 -43.88	0.90^{b} ± 0.01 3.75 -50	1.02^{b} ± 0.01 3.66 +13.33	1.35^{b} ± 0.03 4.80 +50	1.61 ± 0.1 5.34 +78.88 (89%)
BV(l) %BM % Change	2.8 ± 0.20 8.99	2.2^{b} ± 0.11 7.85 -21.42	1.9^{b} ± 0.20 7.30 -32.14	1.61^{b} ± 0.19 6.70 -42.5	1.94^{b} ± 0.2 6.97 +20.4	2.3^{b} ± 0.18 8.18 +42.85	2.5 ± 0.20 8.30 + 55.27 (89%)
RCV(l) %BM % Change	0.56 ± 0.02 1.79	0.46^{b} ± 0.01 1.64 -17.85	0.40^{b} ± 0.01 1.53 -28.57	0.36^{b} ± 0.02 1.5 -35.71	0.4^{b} ± 0.01 1.43 +11.11	0.44^{b} ± 0.02 1.56 +22.22	0.50 ± 0.02 1.66 +38.88 (89%)
ECF(l) %BM % Change	9.3 ± 0.4 29.88	7.7^{b} ± 0.4 27.49 -17.20	6.43^{b} ± 0.3 24.73 -30.86	5.4^{b} ± 0.2 22.5 -41.93	6.2 ± 0.4 22.30 +14.81	7.38^{b} ± 0.4 28.1 +36.66	8.5 ± 0.3 28.23 +57.40 (91%)
ICF(l) BM % Change	11.0 ± 0.4 35.34	10.1^{b} ± 0.4 36.05 -8.18	9.87^{b} ± 0.38 37.96 -10.27	9.0^{b} ± 0.26 37.5 -18.18	10.0^{b} ± 0.21 35.97 +11.11	10.6 ± 0.41 37.72 +17.77	10.7 ± 0.38 35.54 +18.8 (97%)
ISF(l) BM % Change	7.5 ± 0.3 24.10	6.4 ± 0.3 22.84 -14.66	5.4 ± 0.3 20.76 -28.0	4.5 ± 0.21 18.75 -40	5.18 ± 0.3 18.63 +15.11	6.03 ± 0.1 21.45 +34.0	6.89 ± 0.31 22.89 +53.11 (91%)

Subclass means within a given parameter superscribed by letter "b" differ significantly ($P \leq 0.05$) from respective control mean value. Figures in the parentheses of hour 72 show recovery as compared to control value. Negatively marked figures show loss from respective control value and positively marked figures show gain from respective dehydrated value. N = number of sheep; BV = blood volume ICF = intracellular fluid; TBW = total body water; RCV = red cell volume; ISF = interstitial fluid; PV = plasma volume; ECF = extra cellular fluid; BM = body mass.

Table 2. Changes in serum hormones, enzymes, creatinine and blood indices in sheep during dehydration and rehydration (mean ± SE, n = 10)

Parameter	Control Period	Dehydration period			Rehydration period		
		Day 2	Day 4	Day 6	Hour 1/2	Hour 24	Hour 72
Cortisol, ng/mL	6.5 ± 0.13	9.2 ± 0.13[b]	14.5 ± 0.14[b]	35.1 ± 0.9[b]	19.4 ± 0.16[b]	15.5 ± 0.13[b]	9.8 ± 0.10[b]
Aldosterone ng/dL	1.81 ± 0.02	2.4 ± 0.03[b]	3.5 ± 0.02[b]	5.5 ± 0.03[b]	2.1 ± 0.05[b]	2.5 ± 0.04[b]	1.88 ± 0.01
γ-Glutamyl transferase, U/L	25.2 ± 2.2	35.3 ± 1.1[b]	42.2 ± 3.3[b]	53.5 ± 3.1[b]	40.3 ± 4. 2[b]	30.2 ± 1.12[b]	28.1 ± 1.1
Creatine Kinase U/L	8.5 ± 0.12	15.8 ± 1.3[b]	20.7 ± 1. 2[b]	30.3 ± 2.1[b]	19.0 ± 1.0[b]	15.4 ± 0.12[b]	12.6 ± 0.12[b]
Alkaline phosphatase U/L	70.6 ± 7.12	85.9 ± 9.2	125.4 ± 13. 2[b]	321.6 ± 12.1[b]	125.4 ± 5.0[b]	100.5 ± 9. 2[b]	81.4 ± 8.0
Creatinine mg/dL	1.2 ± 0.02	1.6 ± 0.02[b]	2.5 ± 0.02[b]	4.9 ± 0.09[b]	2.9 ± 0.04[b]	2.0 ± 0.03[b]	1.7 ± 0.05[b]
Packed cell volume,%	35.5 ± 1.12	38.3 ± 1.5	41.5 ± 1.3[b]	45.22 ± 1.7[b]	40.26 ± 1.6[b]	38.2 ± 2.0[b]	35.9 ± 1.23
Viscosity	3.4 ± 0.05	3.6 ± 0.02[b]	4.0 ± 0.04[b]	4.56 ± 0.07[b]	3.9 ± 0.03[b]	3.8 ± 0.02[b]	3.5 ± 0.04

Subclass means within a given parameter superscribed by letter "b" differ significantly (P≤0.05) from respective control mean value; n = number of sheep

The pattern of fluid losses from different compartments showed that intracellular fluid volume compartment resisted the loss in volume due to its balancing by the extra cellular fluid compartment. The observation was based on the fact that plasma volume, which was a part of ECF, was most affected in the present study. This indicated that water movement was from the plasma volume to the ICF compartment in order to prevent the marked rise in osmolality (KATARIA et al., 2003). NOSE et al. (1988) also noted the movement of water from extra vascular to intracellular spaces as a part of compensatory mechanism. This compensatory reaction was also seen in the form of increased PCV and viscosity of blood to a greater degree. Fifty percent loss in plasma volume was a potent stimulus for a threefold rise in aldosterone concentration. These physiological mechanisms were probably elicited as a part of compensatory reactions in the body. High rise of cortisol probably also helped the animal in regulation of fluid balance.

It was clear that inter compartmental movement of water was governed by mechanisms involved in the maintenance of osmolal environment of the cell. It was based on the fact that the minimum comparative loss was noticed in ICF during the 6th day of dehydration, which was comparable to an earlier similar study in camels (KATARIA et al., 2003). This probably helped the animals to maintain ionic balance in the cell which was crucial in the condition of dehydration, and prevented the cells from osmotic damage. On percent body mass basis the value of ICF increased during dehydration. It also supported the finding that water loss from the ICF compartment was a slower process that the overall body water loss which tended to decrease the body mass. The pattern of change in ISF compartment followed that in ECF, as the former was a part of the latter.

Immediately after rehydration the effect of water influx on the water compartments was evident through the replenishment of the water losses. At hour ½ of rehydration the highest gain in blood volume was also reflected in the mean values of other parameters like PCV, viscosity and aldosterone, all of which showed marked dilution effects. The low gain in RCV was advantageous to animals bearing in mind that hypotonicity produced by the water influx could have otherwise damaged the erythrocytes due to the fact that RBCs of sheep are less efficient in retaining water than that of a camel (KATARIA et al., 2003). The magnitude of replenishment in ICF compartment was also lower, probably because the movement of water from outer spaces takes time to move inside the cell. This favours the slow changes in the ionic concentration helping in maintenance of ionic equilibria.

Cortisol and aldosterone. Increase in cortisol showed that dehydration was a stress to animals and even after rehydration recovery took some time. On hour 72, raised cortisol level pointed towards mild tissue damage coupled with higher CK activity since a 6% loss in the body mass could not be replenished with water intake. The mean values of blood indices clearly reflected haemoconcentration during dehydration and haemodilution immediately after drinking. However, other parameters analyzed showed that reactions

other than haemoconcentration also governed the physiological changes taking place during dehydration. Low blood volume during dehydration phase was a potent stimulus for increased aldosterone secretion which in turn helped the body to retain water.

Although following the rehydration PCV and viscosity showed normalcy at hour 72, the plasma volume recovery was only 89%. The serum aldosterone showed normalcy at hour 72, which points to the plasma volume being recovered to such a level so as to eliminate the stimulatory drive for more aldosterone secretion. The increased extra cellular fluid volume inhibits secretion of aldosterone.

γ-glutamyl transferase. The increase in γ-glutamyl transferase activity during dehydration period pointed towards an increase in renal metabolism, probably in order to increase tubular reabsorptive functions. This is associated with glutathione metabolism the greatest amount of which is present in the renal tubules (KANEKO et al., 1999). The most important reabsorption is that of sodium from distal part of nephron under the influence of raised aldosterone (KATARIA et al., 2000).

Creatine kinase and creatinine. The increased activity of creatine kinase, which is a rather sensitive indicator of muscle damage, did point to a certain level of such damage. (YILMAZ et al., 2006). In the present study cortisol concentration increased during dehydration, which was related to a mild muscle wasting. This was also reflected in the concentrations of creatinine which were significantly ($P \leq 0.05$) higher during dehydration period. The possible reason for high cortisol was the stress to animals. The higher cortisol level substantiated the significance of compensating for the energy crisis during physical stress, bearing in mind that cortisol increases glucose supply due to glucogenolytic and gluconeogenetic properties (KATARIA et al., 2000).

*Alkaline phosphatase***.** Glucocorticoids are well known inducers of alkaline phosphatase (KANEKO et al., 1999). This could be the possible reason of increased alkaline phosphatase activity during dehydration. These results supported the fact that during dehydration weight loss was not merely due to water deficit but some tissue damage was also there.

Conclusion

It was concluded that six days of dehydration provoked physiological mechanisms in the body in a manner that helped the animals to survive. Although dehydration was a stress to the animals the changes brought about by six days of dehydration were reversible.

Water compartmental management helped to maintain the *milieu interior* as shown by the balancing of ICF compartment by ECF. The endocrine responses like cortisol and aldosterone helped to struggle through dehydration stress. Time taken for the recovery and the higher cortisol indicated tissue damage, although of a lesser degree. Blood

indices analyzed showed influence of inter compartment water movement. However, the investigation of dehydration recovery should also include the analyses of parameters like cortisol, creatinine and creatine kinase because the present study showed that although the packed cell volume value returned to normal at hour 72, the cortisol value did not attain normalcy, thereby demonstrating that the animals did not recover completely from dehydration stress.

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SAŽETAK

U svrhu istraživanja prometa vode između pojedinih odjeljaka tjelesnih tekućina u ovce Marwari ustanovljene su različitosti volumena tjelesnih tekućina za vrijeme dehidracije, rehidracije i u kontrolnom razdoblju. Kontrolna srednja vrijednost ukupne vode u tijelu iznosila je 20,0 ± 0,8 L, izvanstanične tekućine 1,80 ± 0,1 L, volumena plazme 2,8 ± 0,20 L, volumena krvi 0,56 ± 0,02 L, volumena crvenih krvnih stanica 9,3 ± 0,4 L, volumena stanične tekućine 11,0 ± 0,4 L i volumena intersticijske tekućine 7,5 ± 0,3 L. Tako se 64,26% ukupne tjelesne mase odnosilo na ukupnu vodu u tijelu, 5,78% na izvanstaničnu tekućinu, 8,99% na volumen plazme, 1,79% na volumen krvi, 29,88% na masu vode u crvenim krvnim stanicama, 35,34% na volumen stanične tekućine i 24,10% na volumen intersticijske tekućine. Značajno ($P \leq 0,05$) smanjenje volumena svih tjelesnih tekućina bilo je ustanovljeno nastupom dehidracije. Najviše se smanjio volumen plazme (50%), dok je gubitak stanične tekućine bio najmanji. Dinamika vraćanja tjelesnih tekućina nakon rehidracije također je bila različita. Odmah nakon rehidracije, najveći porast ustanovljen je u volumenu krvi, a sporiji porast zabilježen je za volumen stanične tekućine i tekućine u crvenim krvnim stanicama. Smanjeni volumen plazme za vrijeme dehidracije bio je u korelaciji sa značajnim povećanjem ($P \leq 0,05$) razine serumskoga aldosterona. Istodobno je dehidracija kao stres značajno ($P \leq 0,05$) utjecala na povećanje razine serumskog kortizola popraćene povećanjem serumskoga kreatinina i povećanjem aktivnosti kreatinin kinaze, γ-glutamil transferaze i alkalne fosfataze.

Ključne riječi: aldosteron, kortizol, dehidracija, rehidracija, tjelesne tekućine