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Reference values for biochemical analytes in feral sheep from Socorro Island, Revillagigedo Archipelago, Mexico

[*Valores de referência para analitos bioquímicos em carneiros selvagens na Ilha Socorro, Arquipélago de Revillagigedo, México*]

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ABSTRACT

To establish reference values for biochemical analytes related to freshwater shortage adaptation, a total of 376 blood samples were collected from feral sheep at Socorro Island, Revillagigedo Archipelago. Year-round variation was assessed by sampling at the beginning of each season defined by the March equinox, June solstice, September equinox, and December solstice. The resulting data set was analyzed using Gaussian distribution and descriptive statistics. Confidence intervals of 95% were established. Analysis of variance was used to compare the mean values of each season. Total cholesterol, triglycerides, urea, albumin, total protein, sodium ion, anion gap, creatine kinase, arginine vasopressin, and aldosterone showed concentrations above the reference range for domestic sheep. Triglycerides, urea, albumin, sodium ion, and aldosterone showed concentrations within the reference range for domestic goats. Most biochemical analytes showed differences ($P<0.05$) between seasons, with the highest values occurring during winter, and the lowest during spring. Results could help improve the accuracy of metabolic profiles used as a tool for evaluating dehydration indicators, and to describe the physiological mechanisms employed by feral sheep to cope with seasonal availability of freshwater.

Keywords: blood chemistry, metabolic profile, feral sheep

RESUMO

Para estabelecer valores de referência para analitos bioquímicos relacionados à adaptação da escassez de água doce, um total de 376 amostras de sangue foram coletadas de carneiros selvagens na ilha de Socorro, no arquipélago de Revillagigedo. A variação durante todo o ano foi avaliada por amostragem no início de cada estação definida pelo equinócio de março, solstício de junho, equinócio de setembro e solstício de dezembro. O conjunto de dados resultante foi analisado usando distribuição Gaussiana e estatística descritiva. Intervalos de confiança de 95% foram estabelecidos. A análise de variância foi usada para comparar os valores médios de cada estação. O colesterol total, triglicerídeos, ureia, albumina, proteína total, íon sódio, hiato aniônico, creatina quinase, arginina vasopressina e aldosterona apresentaram concentrações acima do intervalo de referência para carneiros domésticos. Triglicerídeos, ureia, albumina, íon sódio e aldosterona apresentaram concentrações dentro da faixa de referência para cabras domésticas. A maioria dos analitos bioquímicos apresentou diferenças ($P<0,05$) entre as estações, com os maiores valores ocorrendo no inverno e os menores na primavera. Os resultados podem ajudar a melhorar a precisão dos perfis metabólicos usados como uma ferramenta para avaliar os indicadores de desidratação e descrever os mecanismos fisiológicos empregados pelas carneiros selvagens para lidar com a disponibilidade sazonal de água doce.

Palavras-chave: bioquímica sanguínea, perfil metabólico, carneiros selvagens

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INTRODUCTION

The limited availability of water for livestock has been a growing problem in some parts of the world, with droughts becoming more widespread and worsening due to climate change (Bernabucci, 2019). Consequently, it has been difficult for livestock in drought-prone areas to meet their food and fresh water needs for optimal health and production (Rust, 2019). Fortunately, small ruminants show several adaptive physiological mechanisms that may help them overcome unfavorable environmental conditions (Berihulay *et al.*, 2019). A remarkable example of these adaptive mechanisms started with the introduction and abandonment of Merino sheep by Australian settlers to the Socorro Island of the Revillagigedo Archipelago in 1869 (Salas de León *et al.*, 2015).

In the absence of handling, sheep became feral, adapting to the food and freshwater scarcity (Hernández *et al.*, 2017), characteristic of volcanic islands affected by tropical storms (Ortiz *et al.*, 2016). The prolonged isolation allowed the sheep population to develop traits adapted to unfavorable environmental conditions (Pickering *et al.*, 2013). This chain of events provided unique traits and exceptional scientific value to the flock (Newsome, 2014). In spite of this, the Chamber of Deputies in Mexico approved the extermination of the feral sheep on the island (Ortiz *et al.*, 2016). This measure was instrumented after findings by Evans III *et al.* (2015) and Ortiz *et al.* (2016).

These authors argued that the feral sheep of the Socorro Island contributed to deforestation by overgrazing, in addition to compacting and eroding the soil of this unique ecosystem. As a result, the Townsend's Shearwater (*Puffinus auricularis*) and the Socorro dove (*Zenaida graysoni*), endemic birds of the island, were virtually pushed to extinction. To preserve the valuable germplasm of Socorro island sheep population, the Faculty of Veterinary Medicine of the University of Colima and the National Council of Science and Technology carried out a program of extraction and recovery (Hernández *et al.*, 2017). Regarding farm animals' endemic breeds and feral populations, several countries implement governmental programs to preserve, characterize, and assess their unique features (Ciani *et al.*, 2015).

A thorough understanding of the adaptive physiological mechanisms in relation to limited water availability should be the starting point of all efforts to improve the resilience of domestic sheep to climate change. Thus, the aim of this study was to establish reference values for different biochemical analytes in feral sheep from Socorro Island, Revillagigedo Archipelago, Mexico. Understanding the physiological mechanisms associated to these values, would help researchers select animals that maintain their productive ability under environmental stressors such as drought.

MATERIALS AND METHODS

This experiment followed institutional and national guidelines for the care and use of animals. All experimental procedures were approved by the Committee of Ethical Review at Colima University (protocol approval number: 021/19). This study analyzed 376 blood serums obtained from the jugular vein of 94 feral sheep from Socorro Island, Revillagigedo Archipelago, Mexico. This island is located in the Pacific Ocean, 540 km south of Cabo San Lucas in Baja California Sur and 720 km west of Manzanillo, Colima. (Figure 1).

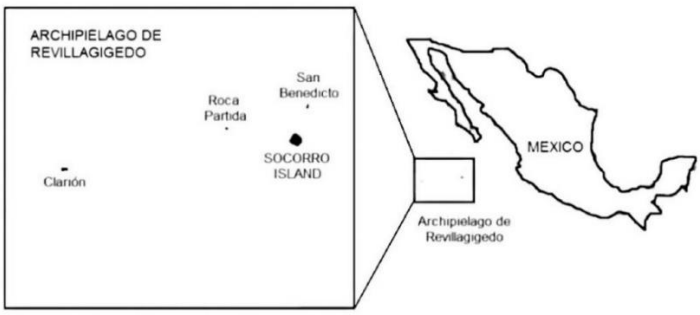

Figure 1. Location of Socorro Island, Revillagigedo Archipelago, 720 km west of Manzanillo, Colima

Currently, the livestock inventory is located on the animal husbandry experimental unit in the University of Colima Faculty of Veterinary Medicine at Tecoman, Colima, Mexico (18° 56′ 53″ N; 103° 53′ 50″ W). Climate is warm, sub-humid (Köppen Cfb) with summer rains (Peel *et al.*, 2007). The average temperature is 26°C with a pluvial precipitation of 750mm/year (INEGI, 2020). Sampling was done according to the methodology proposed and described by Payne *et al.* (1974) for the Compton metabolic profile. The total livestock inventory (94 feral sheep: 84 females and 10 males with an initial body weight [average ± S.D.] of 45 ± 4kg and 60 ± 6kg, respectively) was used (Figure 2).

Figure 2. Feral sheep (*Ovis aries*) from Socorro Island, Revillagigedo Archipelago, Mexico.

To assess the annual metabolic pattern of analytes, samples were drawn from the same animals on the first day of each season of the year (solstice or equinox). Feeding conditions of sheep in the island were maintained at the experimental housing unit. Animals were able to graze *Conocarpus* scrub, *Croton masonii* scrub, and *Pteridium-Dononaea* scrub, introduced from Socorro Island (Flores *et al.*, 2009). Water was available *ad libitum*. Before the spring sampling, the herd was dewormed with Ivermectin (1mL/25kg of live weight subcutaneously) followed by immunizations against *Clostridium*, *Pasteurella multocida* and *Mannheimia haemolytica* (BOBACT 8. SAGARPA B-0273-111; Intervet., Mexico City, Mexico) 2.5mL/animal subcutaneously).

Blood samples were collected by puncture of the jugular vein before morning feeding. To analyze energy, protein, mineral, and enzyme profiles, 8.5mL of blood were collected into vacuum tubes with clot activator and serum separator gel (BD Vacutainer 367988; Becton-Dickinson Co., Franklin Lakes, United States). To analyze the hormonal profile, additional samples of 5mL of blood were collected into vacuum tubes with EDTA-K_3 (BD Vacutainer 366352; Becton-Dickinson Co., Franklin Lakes, United States). To collect serum, blood samples were centrifuged at 1500 x *g* for 10min as described in Van Saun (2010) by using a portable centrifuge (Porta-Spin C828; UNICO., Dayton, United States).

Serum samples were separated using 1.5mL tubes with lid (Tubes Safe-Lock 3810X; Eppendorf., Madrid, Spain) and transported at 4°C in a portable cooler (Thermoelectric Cooler Car/Home M5644-710; Coleman Company., Kansas, United States) to the clinical laboratories at the Autonomous Metropolitan University and the University of Colima, where they were frozen at -20°C until analysis. The concentration of each analyte was determined with a UV-Vis double beam spectrophotometer (Biochemistry Analyzer; KONTROLab., Guidonia, Italy) and the hormones were determined with a gamma counter (PC-RIA MAS; Stretec., Germany). Biochemical analytes, analytical method for each parameter, units in which the results were expressed, and corresponding commercial reagents, are described in Table 1.

Table 1. Biochemical analytes, units, analytical methods, and corresponding commercial reagents

Analyte*	Unit	Method	Reagent
		Energy profile	
Glucose (GLU)	mM	Colorimetric. Trinder[a]	1001190[1]
Total cholesterol (COL-T)	mM	Colorimetric. Liquid[b]	41020[1]
Triglycerides (TAG)	mM	Colorimetric. Liquid[c]	41032[1]
β-hydroxybutyrate (β-HBA)	mM	Enzymatic[d]	RB1007[2]
		Protein profile	
Urea	mM	Enzymatic[e]	1001333[1]
Albumin (ALB)	g/dL	Colorimetric. Bromocresol green	1001020[1]
Globulin (GLOB)	g/dL	(PROT-T) – (ALB)	Difference
Total protein (PROT-T)	g/dL	Colorimetric. Biuret	1001291[1]
		Mineral profile	
Calcium ion (Ca^{2+})	mM	Colorimetric. Arsenazo III	CA2391[2]
Inorganic phosphate (P_i)	mM	Colorimetric. Phosphomolybdate	1001155[1]
Sodium ion (Na^+)	mM	Enzymatic. Galactosidase	1001385[1]
Potassium ion (K^+)	mM	Enzymatic[f]	1001395[1]
Magnesium ion (Mg^{2+})	mM	Colorimetric. Xylidyl Blue	1001286[1]
Chlorine ion (Cl^-)	mM	Colorimetric. Mercuric Thiocyanate	1001360[1]
Carbon dioxide (CO_2)	mM	Enzymatic[g]	CD127[2]
Hydrogen carbonate ion (HCO_3^-)	mM	Enzymatic by CO_2 total and gas dissolved	99852[3]
Anion gap	mM	$[(Na^+ + K^+) – (Cl^- + HCO_3^-)]$	Difference
		Enzyme profile	
Alanine aminotransferase (ALT)	U/L	Enzymatic[h]	41274[1]
Aspartate aminotransferase (AST)	U/L	Enzymatic[i]	41264[1]
Creatine kinase (CK)	U/L	Enzymatic[j]	41250[1]
γ-glutamyl transpeptidase (γ-GT)	U/L	Enzymatic[k]	41292[1]
		Hormone profile	
Arginine vasopressin (AVP)	pg/mL	Radioimmunoassay[l]	KIPERB319[4]
Aldosterone (Aldo)	pg/mL	Radioimmunoassay[m]	KIPZ0100[4]

*Official abbreviation of the International Union of Pure and Applied Chemistry (IUPAC); [a]Glucose Oxidase-Peroxidase; [b]Cholesterol Oxidase-Peroxidase; [c]Glycerol Phosphate Dehydrogenase-Peroxidase; [d]β-hydroxybutyrate Dehydrogenase; [e]Urease-Glutamate Dehydrogenase; [f]Phosphoenolpyruvate-Lactate Dehydrogenase; [g]Phosphoenolpyruvate Carboxylase-Malate Dehydrogenase; [h]Glutamate-Piruvate NADH; [i]Glutamate-Oxalacetate NADH; [j]Phosphocreatine NADPH; [k]γ-glutamyl group-Glycylglycine 2-nitro-5-aminobenzoic; [l]sensitivity 20pmol/L and intra-assay and inter-assay coefficient of variations were 5.6% and 6.1% respectively; [m]sensitivity 6pg/mL and intra-assay and inter-assay coefficient of variations were 9.5% and 10.4% respectively; [1]Spinreact., Girona, Spain; [2]*Randox* Laboratories., Northern Ireland, United Kingdom; [3]Biolabo Laboratory., Grandcamp-Maisy, France; [4]DIAsource ImmunoAssays., Ottignies-Louvain-la-Neuve, Belgium.

The precision and reliability of the techniques were controlled using lyophilized control serum (SPINTROL NORMAL 1002100; Spinreact., Girona, Spain) and Assayed Multi-Sera AL 1027 (Randox Laboratories., Northern Ireland, United Kingdom). Hemolysis of serum was recorded on a qualitative scale of 0 (none) to 3 (dark). Samples showing hemolysis scores of 2 and above constituted less than 1% of all samples and did not introduce a significant bias in any of the tested models; thus, the influence of serum hemolysis was ignored.

The statistical procedure used to calculate 95% confidence intervals for the different biochemical analytes follows the recommendation of the International Federation of Clinical Chemistry (Solberg, 1987). The data were described by means and SD and they were tested for normal distribution (Shapiro-Wilk test). The comparison between seasons was assessed by Analysis of Variance (SPSS…, 2013). A multiple comparison test of Tukey was performed when the effect of season was found to be significant ($P<0.05$).

RESULTS

The descriptive statistics for glucose (GLU), total cholesterol (COL-T), triglycerides (TAG), β-hydroxybutyrate (β-HBA), urea, albumin (ALB), globulin (GLOB), total protein (PROT-T), calcium ion (Ca^{2+}), inorganic phosphate (P_i),

sodium ion (Na^+), potassium ion (K^+), magnesium ion (Mg^{2+}), chloride ion (Cl^-), carbon dioxide (CO_2), hydrogen carbonate ion (HCO_3^-), anion gap, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), γ-glutamyl transpeptidase (γ-GT), arginine vasopressin or antidiuretic hormone (AVP), and aldosterone (Aldo), determined from 376 blood serums of 94 feral sheep from Socorro Island, Revillagigedo Archipelago, and its respective international reference range values for small ruminants (sheep and goat), are shown in Table 2.

The energy profile: COL-T and TAG showed concentrations above the reference range for sheep. The TAG results were within the reference value for goats. The protein profile: urea, ALB, and PROT-T showed higher concentrations than the reference range for sheep. The urea and ALB levels were within the reference value for goats. The mineral profile: Na^+ and anion gap concentrations were above the reference range for sheep. Na^+ measures were within the reference value for goats. The enzyme profile: CK concentration was slightly above the reference range for sheep, and the hormone profile: AVP and Aldo concentrations were above the reference range for sheep. The Aldo showed levels within the reference value for goats (Table 2).

Table 2. Mean ± standard deviation, reference range value, and confidence interval for different biochemical analytes of feral sheep from Socorro Island, Revillagigedo Archipelago, by the annual metabolic scheme. Combined males and females *n* = 376 blood serums

Analyte	Mean ± standard deviation	Reference range for sheep	Reference range for goat	Confidence interval of 95%
Energy profile				
Glucose (mM)	3.76 ± 0.25	3.80 ± 0.33†	3.49 ± 0.39†	3.73 - 3.78
Total cholesterol (mM)**	2.14 ± 0.12	1.66 ± 0.31†	1.55 ± 0.67†	2.13 - 2.15
Triglycerides (mM)*	0.39 ± 0.01	0.33 ± 0.01§	0.20 ± 0.09¥	0.39 - 0.39
β-hydroxybutyrate (mM)	0.59 ± 0.01	0.55 ± 0.04†	0.50 ± 0.14¥	0.59 - 0.59
Protein profile				
Urea (mM)**	8.63 ± 0.39	7.70 ± 0.43§	9.60 ± 3.02¥	8.59 - 8.67
Albumin (g/dL)**	3.53 ± 0.39	2.70 ± 0.11†	3.30 ± 0.33†	3.49 - 3.57
Globulin (g/dL)	5.19 ± 0.09	4.40 ± 0.73†	3.60 ± 0.50†	5.18 - 5.20
Total protein (g/dL)*	8.73 ± 0.34	7.20 ± 0.52†	6.90 ± 0.48†	8.69 - 8.76
Mineral profile				
Calcium ion (mM)	3.06 ± 0.12	3.04 ± 0.07†	2.58 ± 0.18†	3.05 - 3.08
Inorganic phosphate (mM)	2.12 ± 0.16	2.07 ± 0.06†	4.62 ± 0.25†	2.10 - 2.14
Sodium ion (mM)**	162.9 ± 11.4	141.5 ± 4.1†	150.0 ± 3.1†	161.7 - 164.1
Potassium ion (mM)	4.32 ± 0.20	4.23 ± 0.5§	4.40 ± 0.56¥	4.30 - 4.34
Magnesium ion (mM)	1.18 ± 0.12	1.03 ± 0.12†	1.32 ± 0.14†	1.17 - 1.19
Chlorine ion (mM)	99.04 ± 2.70	99.0 ± 4.0†	105.1 ± 2.9†	98.7 - 99.3
Carbon dioxide (mM)	26.79 ± 1.27	26.20 ± 1.0†	27.40 ± 1.40†	26.66 - 26.92
Hydrogen carbonate ion (mM)	23.29 ± 1.24	22.50 ± 2.50†	26.53 ± 2.66∞	23.17 - 23.42
Anion gap (mM)*	44.92 ± 11.83	24.23 ± 3.90º	22.87 ± 3.70º	43.72 - 46.11
Enzyme profile				
Alanine aminotransferase (U/L)	26.77 ± 2.10	24.13 ± 1.0§	12.0 ± 6.0†	26.56 - 26.98
Aspartate aminotransferase (U/L)	79.87 ± 2.11	80.92 ± 4.0§	85.1 ± 28.02¥	79.65 - 80.08
Creatine kinase (U/L)*	13.07 ± 0.26	10.3 ± 1.6†	4.50 ± 2.8†	13.04 - 13.10
γ-glutamyl transpeptidase (U/L)	35.88 ± 1.42	33.50 ± 4.30†	38.0 ± 13.0†	35.73 - 36.02
Hormone profile				
Arginine vasopressin (pg/mL)*	8.29 ± 0.40	3.30 ± 1.37æ	13.64 ± 1.72£	8.25 - 8.34
Aldosterone (pg/mL)**	27.86 ± 1.03	20.10 ± 2.21¶	30.10 ± 2.21æ	27.75 - 27.96

†(Kaneko *et al.*, 2008); §(Lotfollahzadeh *et al.*, 2016); ¥(Soares *et al.*, 2018); ∞(Antunović *et al.*, 2019); æ(Mengistu *et al.*, 2016); £(Kaliber *et al.*, 2016); ¶(Kataria and Kataria, 2007); ºInternational reference range (sodium ion + potassium ion) – (chlorine ion + hydrogen carbonate ion)]; *Differences with the reference range for sheep.

As shown in Table 3, except for GLU, TAG, Na^+, CO_2, and γ-GT, the serum concentration of all other biochemical analytes showed differences (P<0.05), between seasons. Higher values correspond to the first day of winter (December solstice), and lowest values to the first day of spring (March equinox).

Table 3. Comparison of different biochemical analytes of feral sheep from Socorro Island, Revillagigedo Archipelago. Combined males and females n = 94 blood serums/group

Analyte*	March equinox	June solstice	September equinox	December solstice
Energy profile				
Glucose (mM)	3.75 ± 0.24[a]	3.76 ± 0.25[a]	3.75 ± 0.24[a]	3.76 ± 0.26[a]
Total cholesterol (mM)	2.08 ± 0.11[a]	2.13 ± 0.11[b]	2.13 ± 0.11[b]	2.22 ± 0.11[c]
Triglycerides (mM)	0.39 ± 0.01[a]	0.39 ± 0.01[a]	0.39 ± 0.02[a]	0.39 ± 0.01[a]
β-hydroxybutyrate (mM)	0.58 ± 0.012[a]	0.58 ± 0.011[b]	0.59 ± 0.012[b]	0.60 ± 0.011[c]
Protein profile				
Urea (mM)	8.15 ± 0.18[a]	8.51 ± 0.14[b]	8.72 ± 0.12[c]	9.15 ± 0.17[d]
Albumin (g/dL)	3.04 ± 0.16[a]	3.42 ± 0.10[b]	3.63 ± 0.08[c]	4.04 ± 0.22[d]
Globulin (g/dL)	5.25 ± 0.06[a]	5.19 ± 0.04[b]	5.21 ± 0.04[b]	5.11 ± 0.11[c]
Total protein (g/dL)	8.30 ± 0.15[a]	8.62 ± 0.14[b]	8.84 ± 0.12[c]	9.16 ± 0.14[d]
Mineral profile				
Calcium ion (mM)	2.90 ± 0.05[a]	3.02 ± 0.03[b]	3.11 ± 0.02[c]	3.22 ± 0.05[d]
Inorganic phosphate (mM)	2.06 ± 0.11[a]	2.11 ± 0.11[b]	2.12 ± 0.11[b]	2.21 ± 0.11[c]
Sodium ion (mM)	161.28 ± 11.53[a]	163.18 ± 11.24[a]	162.32 ± 12.01[a]	164.98 ± 11.04[a]
Potassium ion (mM)	4.10 ± 0.15[a]	4.32 ± 0.11[b]	4.33 ± 0.12[b]	4.54 ± 0.13[c]
Magnesium ion (mM)	1.14 ± 0.12[a]	1.18 ± 0.11[a]	1.18 ± 0.12[a]	1.23 ± 0.11[b]
Chlorine ion (mM)	95.77 ± 2.07[a]	98.40 ± 0.50[b]	99.66 ± 0.53[c]	102.34 ± 1.41[d]
Carbon dioxide (mM)	26.79 ± 1.29[a]	26.85 ± 1.25[a]	26.70 ± 1.34[a]	26.83 ± 1.23[a]
Hydrogen carbonate ion (mM)	21.72 ± 0.62[a]	22.87 ± 0.21[b]	23.69 ± 0.19[c]	24.90 ± 0.57[d]
Anion gap (mM)	47.88 ± 11.81[a]	46.22 ±11.32[ab]	43.30 ± 12.24[b]	42.28 ± 11.25[b]
Enzyme profile				
Alanine aminotransferase (U/L)	26.93 ± 1.95[a]	27.23 ± 2.33[a]	26.98 ± 2.13[a]	25.96 ± 1.76[b]
Aspartate aminotransferase (U/L)	80.09 ± 1.95[a]	80.32 ± 2.32[a]	80.09 ± 2.13[a]	78.97 ± 1.79[b]
Creatine kinase (U/L)	12.72 ± 0.09[a]	13.01 ± 0.04[b]	13.14 ± 0.05[c]	13.42 ± 0.10[d]
γ-glutamyl transpeptidase (U/L)	35.94 ± 1.45[a]	35.87 ± 1.42[a]	35.87 ± 1.41[a]	35.84 ± 1.43[a]
Hormone profile				
Arginine vasopressin (pg/mL)	8.13 ± 0.39[a]	8.28 ± 0.37[b]	8.29 ± 0.39[b]	8.47 ± 0.37[c]
Aldosterone (pg/mL)	26.78 ± 0.68[a]	27.59 ± 0.63[b]	28.09 ± 0.64[c]	28.96 ± 0.69[d]

Significant differences were obtained between seasons indicated with different letters; (P<0.05); all data are presented means ± SD.

DISCUSSION

Serum volume decreases when small ruminants become dehydrated during water restriction. This decrease can be explained by water uptake in tissue cells (Kenneth, 2011). Hyperosmolality due to increased solute concentrations is commonly detected in water-restricted animals (Pratt *et al.*, 2016). Reduced serum volume also increases concentrations of certain biochemical analytes, compelling animals to activate physiological mechanisms to cope with water restrictions and dehydration. In this sense, increased concentrations of biochemical analytes such as COL-T, TAG, urea, ALB, PROT-T, Na^+, CK, AVP, and Aldo in the blood of small ruminants have been considered as indicators of dehydration.

Under restricted water regimes, Jaber *et al.* (2004) and Ghanem *et al.* (2008) reported that serum COL-T concentration consistently increased in Awassi sheep (1.90 vs. 2.14 and 1.61 vs. 2.06mM, respectively). The serum COL-T concentration also increased (47 vs. 51, 54 and 62mg/100mL with 41, 31 and 21 d, respectively) when water intake in goats (75% German fawn and 25% Hair goat) was restricted (Kaliber *et al.*, 2016). Vosooghi-Postindoz *et al.* (2018) found that the serum TAG concentration tended to

increase when Baluchi lambs with restricted water intake were compared with groups that had free access to water (respectively 33.50mg/dL vs. 30.50mg/dL). Our findings agree with these studies, as increased serum COL-T and TAG concentrations can be attributed to reduced serum volume.

Urea is a detoxifying nitrogenous compound, synthesized from ammonium ions (NH_4^+) by periportal hepatocytes, and excreted by the renal tubules to dispose of excess dietary nitrogen (Weiner *et al.*, 2015), or recycled via salivary secretion (Jaber *et al.*, 2004). Serum urea concentration is a good indicator of the energy intake of sheep, as it indicates the existing synchronization rate between fermentable carbohydrates and rumen degradable protein (RDP) (García *et al.*, 2017). CK, another nitrogenous compound, is produced by myocytes and excreted by renal tubules in proportion to the muscular mass and the rate of early endogenous proteolysis in the animal (Caldeira *et al.*, 2007).

However, under restricted water regimes, the renal function is altered with slower glomerular filtration and higher urea reabsorption rates. These can lead to increased serum concentrations of urea and CK (Weiner *et al.*, 2015). Physiological responses of water restricted Lacaune sheep showed increased serum CK and PROT-T concentrations (Casamassima *et al.*, 2016). Jaber *et al.* (2004) informed that serum urea and CK concentrations consistently increased in water deprived Awassi sheep. In another study (Abdelatif *et al.*, 2010), water-deprived Nubian goats also showed increased osmolality and serum concentrations of PROT-T, ALB, CK, and urea, but did not affect serum GLU concentration, as we found in our sheep.

Hormonal based response mechanisms to water deprivation consider AVP and Aldo as the key biochemical analytes in promoting water reabsorption at renal tubules, decreasing urine volume and water absorption at gastrointestinal level (Kenneth, 2011; Rotondo *et al.*, 2016), as well as decreasing hyperosmolality as blood vessels refill with water (Ames *et al.*, 2019). In water restricted regimes, serum AVP concentration sharply increases. Physiological responses of sheep and goat with 2 weeks of water restriction at 60 (Katahdin sheep), 50 (Boer goats), and 40 % (Spanish goats) of recommended water intake, showed increased serum AVP concentrations (2.12 vs. 6.40, 7.22, and 7.06pg/mL, respectively) (Mengistu *et al.*, 2016).

In another study where 2 L, 3 L and 4 Ld^{-1} of water were restricted during 3 wk., increased serum AVP concentrations (3.47 vs. 12.9pg/mL) were followed by a subsequent descent (12.9 vs. 17.4, 16.1, and 14.6pg/mL). This drop in serum AVP concentration over time suggests that animals adapted to drinking water shortage, as this hormonal response was correlated to reductions in plasma osmolality and greater water conservation in goats (75% German fawn and 25% Hair goat).

Finally, the mineralo corticoid Aldo is responsible for water conservation and Na^+ reabsorption by renal tubules (Ames *et al.*, 2019). In this regard, serum Aldo concentrations in Marwari sheep deprived of water increased from 18.1pg/mL to 24.0, 35.0, and 55.0pg/mL during 2, 4, and 6 d of water restriction, respectively. A subsequent descent to a level of 18.8pg/mL after 72h of rehydration was also found (Kataria and Kataria, 2007). Climate change will compel small ruminants to become more resilient to rising environmental temperatures, and expansion of droughts (Rust, 2019). One way to attain this resilience is by selective breeding and crossbreeding programs. Therefore, populations of each species that are most resilient to warmer and dryer conditions need to be identified. Within these populations, feral sheep from Socorro Island have shown a clear seasonal variability of several biochemical analytes that indicate an adequate physiological adaptation to restricted water regimes.

CONCLUSIONS

The proposed approach of establishing reference values for different biochemical analytes in feral sheep from Socorro Island proved useful to improve the accuracy of biochemical profiling as a tool for assessment of dehydration. Calculated confidence intervals could be used at herd level to detect physiological mechanisms to cope with water restriction. Variability of reference values also opens up the possibility of selective breeding and crossbreeding programs within populations resilient to rising environmental temperatures and expanding droughts.

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