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Comparison of Different Physiological Parameters for the Assessment of Hydration Status in Turtles of the Genus *Trachemys*

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Abstract

Because of their anatomical and physiological characteristics, and the limited published research on the subject, it is difficult to objectively determine the hydration status of reptiles and consequently provide appropriate fluid therapy. Various clinical and laboratory parameters have been used as indicators of chelonian hydration status, such as the presence of saliva strands or hematocrit, but their correlation with actual hydration status has not been verified. The objective of this work was to determine whether there is a significant change in various physiological parameters in *Trachemys* turtles exposed to a dehydration period. Fifteen *Trachemys* spp. turtles were studied. A physical examination was performed on day 1, including skin turgor test (SK), eyeball position (EY), tear production (Schirmer tear test (STT)), mucosal moisture (MM), cornea moisture (MC), and body weight (BW). Also, blood samples were collected to measure total solids (TS), uric acid (UA), total protein (TP), serum osmolarity (OS), and hematocrit (Hct). Water intake was completely withheld for 10 days, and sampling and physical examination were repeated. The OS, TS, TP, SK, EY, and BW differed significantly between days 1 and 10 ($P < 0.05$). Using these analytes in sick and dehydrated *Trachemys* spp. turtles will allow development of a more precise scale for hydration status in these reptiles. By contrast, UA, Hct, STT, MM, and MC did not differ significantly between the start and end of the study, indicating that these analytes may be less reliable indicators of hydration status for these species.

Keywords: Blood analyte, hydration status, osmolarity, reptile, turtle, *Trachemys*

Introduction

When turtles live in inappropriate conditions, or suffer from pathological processes, a hydration imbalance can occur. Given their particular anatomical and physiological characteristics, it is difficult to objectively determine their hydration status and therefore administer appropriate and timely therapy (Martinez-Jimenez and Hernandez-Divers, 2007). Various characteristic clinical signs have been cited as indicators of hydration status, such as the degree of corneal moisture (MC), oral cavity or cloacal mucosa moisture, and the position of the eyeball (EY) and the skin turgor test (SK), but it has not been documented that all of these indicators actually reflect the physiological hydration status in reptiles (Hernandez-Divers, 2003; Martinez-Jimenez and Hernandez-Divers, 2007). Some blood analytes can be indicators of hydration status, for example, hematocrit (Hct), total protein (TP), or uric acid (UA), but these factors can be influenced by other variables, such as concomitant disease and malnutrition (Hernandez-Divers, 2003).

Serum osmolarity (OS) is a measurement that describes the ratio between dissolved substances and solvents in serum. Unlike the above-mentioned analytes, it is less affected by concomitant diseases and malnutrition, making it more accurate for evaluating hydration (Armstrong, 2007). Peterson and Greenshields (2001) reported the OS values of hydrated (mean, 275 mOsm/L) and dehydrated (mean 402.1 mOsm/L) red-eared sliders (*Trachemys scripta elegans*), but did not describe whether these were clinically consistent with the physiological changes reported in the literature.

Identifying which clinical sings and physiological parameters are a more precise reflection of the serological analytes related with the hydration status of turtles would be useful to make evidence-based diagnostic and therapeutic decisions. It is not known whether the signs reported in the literature as dehydration in both the physical examination (mucosal moisture [MM], SK, EY, MC, tear production (Schirmer tear test [STT]), change in body weight [BW]) and laboratory analytes (UA, Hct, total solids [TS], TP, and OS) actually differ between hydrated and dehydrated turtles. The aim of this study was therefore to determine whether these physiological parameters (clinical signs and serological analytes) changed significantly in slider turtles (*Trachemys* spp.) exposed to a period of dehydration.

Materials and Methods

Study subjects: Twenty-two slider turtles belonging to the Zoologico Moroleón Areas Verdes in the city of Moroleón, Guanajuato, Mexico, were included. The animals belonged to the park: they were voluntarily surrendered at different times by caretakers who were unable to care for them, but all had been at the zoo for at least 6 months. The average weight ± SD of the animals was 1.07 ± 0.6 kg, and the age was unknown. The experimental protocol was approved by the Internal Committee for the Care and Use of Animals, Faculty of Veterinary Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico, under number 726.

Intervention: At the beginning of the study, the turtles were removed from their pond and placed in plastic tubs with a slightly moist black soil substrate with leaf litter and tree bark. They were kept at an ambient temperature of 18–25°C (64–77°F). The animals were fed green leaves (lettuce, coriander, basil, cucumber, among others) and tilapia fillet *ad libitum* on a plastic plate three times a week; however, they refused to eat during the entire study period. On day 1, turtles were considered normally hydrated. Each turtle was subjected to a physical examination, and blood samples were collected. The turtles were kept without access to water for 10 days during the experimental period.

For blood collection, two veterinarians collected 2-ml blood samples from the subcaparacial sinus by using a 22-ga needle. If a clear fluid was observed or the color of the blood changed, the sampling was interrupted, the blood was discarded, and a new sample was taken to avoid lymph contamination. Turtles from which samples could not be obtained were excluded. In total, 0.3 ml of blood was placed in a tube containing heparin (BD Microtainer® Blood Collection with lithium heparin, Becton Dickinson and Company, Franklin Lakes, NJ, USA) and 1.7 ml into a red clot blood tube (BD Vacutainer® Tube, Becton Dickinson and Company). After 10 days without access to water, each individual was again physically restrained and the physical examination and blood sampling were repeated according to the same protocol as on day 1. The turtles then received fluid therapy with 10 ml/kg Hartman's solution (HT®, 0.600 g of NaCl, 0.030 g of KCl, 0.020 g of CaCl, 0.310 g of sodium lactate, and injectable water to a quantity of 100 ml, PISA Salud Animal, Guadalajara, Jalisco, Mexico) in a single intracelomic bolus (Petritz and Son, 2019), and they were reintroduced into their pond.

Physical examination: Based on the results of the general physical examination, any turtles with alterations suggestive of disease were excluded from the study. All of the physical examination variables were evaluated by two veterinarians considering the recommendations of Divers (2019). The SK in the lateral femoral area was recorded in seconds. The EY was recorded as normal if the eyes were in their natural anatomical location with a rounded shape that slightly protruded over the orbital fossa, or they were recorded as sunken when they retracted slightly into the orbital fossa and a slight shadow was observed around the eyeball. The MC was categorized as moist (shiny) or dry (opaque). The STT of each eye was measured with Schirmer strips cut in half, measured in millimeters per minute (Somma *et al.*, 2015). The oral mucosa was evaluated when the turtle opened the mouth voluntarily or with the aid of a thin plastic card, and MM was classified as moist (with the presence of saliva strands) or dry.

Blood evaluation: Blood samples were transported under refrigeration at 4°C (32°F) and analyzed the same day. The TS (g/dl), UA (μmol/L), TP (g/L), and Hct (%) were evaluated 3 h later at the COCAN Veterinary Clinical Analysis Laboratory (Chapultepec Oriente, Morelia, Mich., México). For colorimetry, the equipment used was a FUJI DRI-CHEM NX500i dry chemistry analyzer (Fujifilm Corporation Minato, Tokio, Japan). The OS (mOsm/kg) was measured within 8 h at the Physiology Laboratory of the Medicine College of the National University of Mexico (UNAM) by using an Osmette^{TM} A Osmometer (model 5002, Precision Systems Inc., Natick, MA, USA) (via freezing point depression method).

Statistical analysis: The normality of the variables was assessed using the Shapiro–Wilk test. The variables evaluated during the physical exam and blood analysis on days 1 and 10 are described using the mean or median and SD, depending on whether their distribution was normal or not. The difference in variables OS, Hct, TP, TS, and STT in the left eye, and SK between days 1 and 10, were compared by Student's *t*-test for related samples when they met the assumptions of the test. Only STT in the right eye, UA, and BW were compared with Wilcoxon's signed rank test. Differences in MM, EY, and MC were analyzed using the McNemar test. The data were processed in Excel (Microsoft 365, 2023), and the statistical analyses were performed in SPSS 25 (IBM SPSS software, 2023) at a significance level of 0.05.

Results

The results obtained from the physical examination and blood analyses of 15 slider turtles are included in this study; the other 7 turtles were excluded due to insufficient sample and/or a gross evident lymph contamination.

The variables that differed significantly ($P < 0.05$) between days 1 and 10 on physical examination were SK, EY, and BW. Blood analytes that differed included OS, TS, and TP

Table 1. Physical and blood parameters evaluated in captive slider turtles (*Trachemys* spp.) before and after 10 days of water deprivation. Values with normal distribution are reported as mean ± SD and 95% CI, and values without normal distribution are reported as median and 95% CI.

			Hydrated turtles (day 1)		Dehydrated turtles (day 10)		
Variable	**Nd1/Nd10**	**Unit**	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**	***P* value**
Osmolarity	15/15	mOsm/kg	291.53 ± 15.23	283.10–299.97	369.33 ± 59.36	336.46–402.21	0.0001*
Uric acid	15/15	μmol/L	138.47 ± 49.03	111.31–165.62	233.73 ± 188.06	NA	NA
Total protein	15/15	g/L	58.53 ± 14.10	50.72–66.34	71.13 ± 20.76	59.64–82.63	0.023*
Total solids	15/15	g/dl	6.87 ± 1.42	6.08–7.66	8.81 ± 2	7.70–9.92	0.002*
Hematocrit	15/15	%	27.95 ± 7.62	23.72–32.17	29.63 ± 8.26	25.05–34.21	0.532
Right-eye tear production	13/15	mm/min	7 ± 2.70	5.36–8.64	9.20 ± 5.32	NA	NA
Variable	**Nd1/Nd10**	**Unit**	**Median**	**95% CI**	**Median**	**95% CI**	***P* value**
Skin turgor test	15/15	sec	2	1.5–2	3	2.5–3.5	0.012*
Weight	15/15	kg	1	0.78–1.16	0.88	0.68–1	0.001*
Left-eye tear production	13/15	mm/min	6	5–7.5	7	5.5–9.5	0.513
Right-eye tear production	13/15	mm/min	7	NA	7	6–12.5	0.414
Uric acid	15/15	μmol/L	143	NA	31	103–350	0.191

CI, confidence interval; Nd1, number of individuals on day 1; Nd10, number of individuals day 10; NA, not applicable.
Asterisk (*) indicates statistically significant. *P* values are for the variable mean difference.

(Tables 1–2). The rest of the parameters evaluated showed no statistically significant differences.

The mean, SD, and 95% confidence intervals (CIs) were calculated for blood values OS, UA, TP, Hct, and TS and STT of the right eye. These values are reported before and after dehydration. For the variables BW, SK, and STT of the left eye, the median was calculated and the 95% CI of the median was obtained if it did not comply with normality (Table 1). For the variables EY, MM, and MC, the mode was determined for days 1 and 10 and the results are presented in Table 2 as the mode before and after the dehydration period as a percentage of this mode.

Discussion

The hydration status of a reptile can be assessed by two components: a clinical (subjective) component and an objective component (through laboratory tests; Perry and Mitchell, 2019). The OS is one of the most objective parameters (Armstrong, 2007); for this reason, it was used as the gold standard in this study. The OS increased between day 1 and day 10, indicating that the turtles were dehydrated. This result is consistent with that of Peterson and Greenshields (2001), who evaluated dehydration in turtles.

Acute weight loss (days to weeks) can be a sign of dehydration (Friend, 2000; Zubac *et al.*, 2016) and is consistent with our finding of BW differences between day 1 and day 10. This loss in BW is mainly due to the loss of body fluids. Herein, the average weight at the beginning of the experiment was 1,070 g and the average weight on day 10 was 960 g, so an average dehydration of 10% was estimated. The body of a reptile consists of 60–80% water (Parkinson, 2023). In the turtles included in this study, part of the BW loss could be due to the loss of body fat, but the lack of food intake of the turtles was not considered relevant because the mobilization of fat reserves in reptiles can take 15–60 days (Oliveira *et al.*, 2013), a period that was not exceeded during the study. Weight loss may be a relevant factor for evaluating changes in hydration in short periods (up to 10 days), but it would be worth evaluating whether weight gain following fluid therapy can be a tool for assessing hydration status in reptiles as it is in mammals (Hansen and DeFrancesco, 2002).

Regarding the EY, 93% of the specimens had a normal position on day 1, whereas on day 10, only 20% had an eyeball in a normal position and 80% had a sunken eyeball. In humans, the sinking of the eyeball has been associated with dehydration, with moderate-to-good validity (McGee *et al.*, 1999; McGarvey *et al.*, 2010), and is consistent with

Table 2. Eyeball position and corneal and mucous membranes values in captive slider turtles (*Trachemys* spp.) before and after 10 days of water deprivation.

		Hydrated turtles (day 1)		Dehydrated turtles (day 10)		
Variable	***n* (d1/d10)**	**Result**	**%**	**Result**	**%**	***P* value**
Moisture of the mucous membranes	15/15	Moist	67	Moist	80	0.727
Corneal moisture	15/14	Wet cornea	100	Wet cornea	85	0.500
Eyeball position	15/15	Normal position	93	Sunken eyeball	80	0.001*

n, number of individuals tested; d1, day 1; d10, day 10.
Asterisk (*) indicates statistically significant.

the results of the present study. It is important to consider the influence of body condition and fat reserves on EY (Winer *et al*., 2018), because although this has not been demonstrated, it is possible that hydrated specimens may exhibit sunken eyes when body condition is low. This factor should always be considered when using this characteristic to assess hydration.

The variable SK was greater in the dehydrated state (day 10) than in the hydrated basal state (day 1). This decrease in elasticity is due to the loss of fluid in the cells. Although there are reports that this indicator has no predictive value for dehydration in some species, such as horses (Pritchard *et al*., 2008), in others, such as dogs, SK is a good indicator of dehydration, even mild dehydration of 3% (Goucher *et al*., 2019). This seems to be consistent with the results of this study.

In the turtles evaluated for this study, MM remained without changes, that is, the mucosa remained moist from day 1 to day 10, in most specimens, in addition to the presence of saliva threads both at the beginning and at the end of the study. Although there are reports of the value of this parameter in humans (Fukushima *et al*., 2019), there is little evidence of the usefulness of MM in assessing hydration in other animals. Pritchard *et al*. (2008) found no changes in MM of dehydrated horses; however, they attributed this to the design of their experiment, so it was not confirmatory. The results of the present study support the conclusion that MM is not an indicator of hydration status in turtles, nor is the presence or absence of saliva strands. This may be due to several factors; for example, the degree of dehydration of the turtles in the present study may not have been sufficient to visibly affect saliva production.

There were no statistical differences in the mode value of MC and STT between day 1 and day 10 in the turtles studied. Although these variables are used to evaluate hydration status in humans (Walsh *et al*., 2012, Sherwin *et al*., 2015), they do not appear to change between hydrated and dehydrated turtles.

Blood analysis (OS, UA, TS, TP, and Hct): Considering the relationship between the analytes used, it is important to analyze their results comparatively. Both TS and TP increased from day 1 to day 10. TS is one of the blood indicators used to assess dehydration, and its fluctuation is related to the state of hydration. However, hydration in animals is also strongly influenced by TP (Allison, 2022), so it is to be expected that they behave similarly. TP consists mainly of albumin and globulins, increases in which may be related to dehydration, although other factors, such as inflammation, infection, hepatic disease, and neoplastic diseases, may also influence its fluctuation (Allison, 2022). The results of this study indicate that elevated TP and TS may be indicators of dehydration in turtles. Because a complete blood count was not performed in this study, it is not possible to know whether the increases in TP and TS were partly influenced by an inflammatory process reflected in hematology.

The Hct can fluctuate relatively or absolutely; it can decrease with overhydration and rise relatively with dehydration. An absolute decrease may be due to the destruction, loss, or lack of production of red blood cells. An absolute increase in Hct is very rare and is associated with neoplastic processes (Thrall, 2022). The turtles in the present study were considered healthy specimens based on physical examination; however, the lack of additional paraclinical tests is a limitation of the present study. Because of this limitation, it cannot be ruled out that the Hct of some animals was influenced by subclinical disease, confounding any possible influence of dehydration.

UA is another analyte that can be altered depending on the state of hydration; however, other factors that can influence UA levels are food content, kidney disease, and gout (Selleri and Hernandez-Divers, 2006). Because the turtles refused to consume food during the experimental period, dietary UA elevation was not considered as a possible influence. Researchers have shown that freshwater turtles produce urea as their main waste product (45–95% of all nitrogenous waste; Holz, 2020), so urea may be a more sensitive metabolite than UA in this species for assessing hydration. Because of the design of this study, urea was not measured, representing an important limitation that should be considered in future studies. Because no significant difference was observed in UA between day 1 and day 10, this parameter is unreliable for evaluating hydration status in slider turtles; however, there is not enough information to confirm these findings physiologically because these values did not change between the groups.

The subcarapacial sinus was selected for blood collection in this study because the turtles were highly stressed when the jugular was tried. In addition, the required blood volume could not be obtained from the jugular vein in all turtles. To standardize the process, we therefore decided to use the subcarapacial sinus. This could represent a limitation of the study because of the risk of lymph contamination. Mild lymph contamination can cause an elevation in blood UA, whereas severe contamination could lead to low protein levels (Glassman *et al*., 2022). Although it is possible that some degree of lymph contamination occurred and was not noticed, there was no clear evidence of this based on the UA and TP levels that were measured.

Based on the results observed in this study, OS, TS, TP, BW, SK, and EY were demonstrated to reflect the chelonian hydration more objectively than other measures. However, several of the parameters routinely used to assess hydration (UA, Hct, STT, MM, and MC) showed no differences between hydration statuses in our study. Although more information is needed to determine the accuracy of these parameters as hydration indicators in slider turtles, they are good starting points for improved evaluation of hydration status in reptiles.

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