WILDLIFE

Increased BUN and glucose in a group of San Cristóbal galapagos tortoises (*Chelonoidis chathamensis*)

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SUMMARY

Veterinary health examinations were performed on 19 captive tortoises in seminatural conditions at the 'Galapaguera of Cerro Colorado' and at the 'Otoy Ranch' on San Cristóbal Island, Galápagos. No abnormalities were found during physical examination, but there were significant increases in glucose and blood urea nitrogen concentrations in the tortoises sampled at the Otoy Ranch ($p = 0.001$ and $p < 0.001$, respectively). The glucose concentration in tortoises at the Otoy Ranch was also increased compared with health assessments conducted 1 year previously. It is suspected that diet is responsible for these differences, due to the fact that in the wild, Galapagos tortoises consume large quantities of low energy forage and previous studies have correlated a captive diet with excess dietary protein to a shortened life expectancy. These peripheral blood chemistry abnormalities constitute a need for future research in the nutrition of the Galapagos tortoise to help ensure the health and survival of these species.

BACKGROUND

The San Cristóbal Galapagos tortoise, *Chelonoidis chathamensis* is an endangered species of chelonian endemic to San Cristóbal Island, the oldest island of the Galapagos Archipelago.[1 2] As the largest species of chelonian in the world, Galápagos tortoises are commonly displayed in zoological facilities, yet are endangered due to previous human exploitation and habitat degradation.[3] A correct and balanced diet is an important component of maintaining reptiles in captivity and encouraging reproduction at breeding facilities, such as the Galapaguera of Cerro Colorado, to help recover wild populations.[4 5]

Several studies of Galapagos tortoises have mainly focused on their ecology and behaviour, but little is known about how nutrition impacts their health status in captivity. Previous studies have established normal blood biochemistry values in San Cristóbal Galapagos tortoises and the effects of different diets on morphometric parameters in Galapagos tortoises have been described.[6–8] This case report provides the first evidence of peripheral blood chemistry differences in Galapagos tortoises housed at two locations suspected to be caused by diet. Although clinicopathological values may be influenced by external factors such as stress, environment and feeding, the results of these health assessments underscore the need for continued research to help guide diet selection and husbandry of captive populations of Galapagos tortoises.

CASE PRESENTATION

As part of a population health assessment of San Cristóbal Galapagos tortoise populations authorised by the Galapagos National Park (PNG), veterinary health examinations were performed on captive tortoises at the Galapaguera of Cerro Colorado and at the Otoy Ranch in March 2017 and March 2018. Both sanctuaries are located on the northeast side of San Cristóbal island, approximately 1.2 km apart from one another. Veterinary health examinations were performed on each animal in accordance with the ethics and animal handling protocols of Galapagos Science Centre and the PNG.

The Galapaguera of Cerro Colorado is a 12 hectares captive Galapagos tortoise breeding facility and sanctuary that houses approximately 140 free-ranging tortoises. While the tortoises housed at Otoy Ranch live freely on an approximately 10 hectare area. Tortoises at the Galapaguera facility are allowed free range over the 12 hectares which contains a vast quantity of vegetation native to San Cristóbal Island and are supplemented with 'Otoy' (*Xanthosoma sagitifolium*) and 'Porotillo' (*Erythrina smithiana*). The supply of the food in the Galapaguera of Cerro Colorado was made every 2 days, the amount of food administered (mix of 'otoy' and 'porotillo') was 5 kg/tortoise, for all groups. Tortoises housed at the Otoy Ranch are also allowed free range over the facility; however, the environment is largely composed of open areas with sparsely dispersed native plants and introduced trees. At the Otoy Ranch, tortoises are fed a mixture of approximately 5 kg/tortoise, composed of Otoy (*Xanthosoma sagitifolium*) and elephant grass (*Pennisetum purpureum*) every other day. The tortoises in both sites also have access to opuntia cactus (*Opuntia echios*) which grows wildly in these places. During the health assessments, authors noted multiple fruit plants introduced: passion fruit (*Passiflora edulis*), banana (*Musa paradisiaca*), guava (*Psidium guajava*), plum (*Spondias movie*) and orange (*Citrus aurantium*) at the Otoy Ranch. According to PNG officials, it is possible that the tortoises can also access these resources.

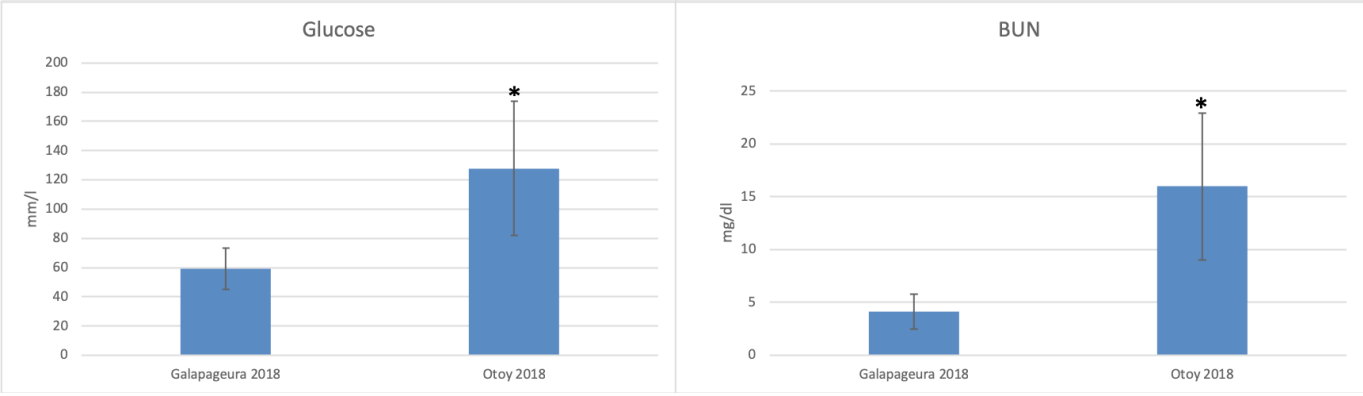

Figure 1 Average peripheral blood glucose and BUN of the tortoises sampled at the Galapaguera of Cerro Colorado and at the Otoy Ranch in March 2018. BUN, blood urea nitrogen.

INVESTIGATIONS

A total of 19 tortoises were sampled in March 2018. Of these tortoises, 12 were sampled at the Galapaguera of Cerro Colorado previously in March 2017. After the health assessments, five tortoises were transferred to the Otoy Ranch. During the health assessments conducted in 2018, seven tortoises were sampled at the Galapageura facility and 12 tortoises at the Otoy Ranch.

A thorough physical examination was performed on each tortoise that included body temperature, morphometric measurements, heart and respiratory rates. Morphometric measurements were taken using a flexible 100 cm measuring tape. These included curved carapace length, curved carapace width, plastron length and shell depth as previously described.[7] Physical examinations on tortoises at both facilities revealed small abrasions (1–3 cm) on the plastron, forelimbs and hindlimbs of three tortoises with neither abnormalities nor differences in body temperature, heart or respiratory rate.

Approximately 2.5 mL of blood was obtained from either the brachial sinus or dorsal coccygeal vein using a heparinised (Heparin Sodium USP, 1000 units/mL; APP Pharmaceuticals, LLC, Schaumburg, IL, USA) 3.0 mL syringe with a 1.0″ 22-gauge needle. Within 10 min of sample collection, blood was loaded onto an iSTAT portable clinical analyzer (Abbot Point of Care, Princeton, NJ, USA) to obtain biochemistry, blood gas and electrolyte values using Chem8 cartridges (Abbot Point of Care). The following parameters were recorded: sodium (Na), potassium (K), chloride (Cl), ionised calcium (iCa), Total CO_2, glucose, blood urea nitrogen (BUN), haematocrit, haemoglobin, anion gap and lactate. Blood lactate was determined using a portable Lactate Plus analyzer (Nova Biomedical, Waltham, MA, USA).

An analysis of variance (ANOVA) was used to detect significant differences in morphometric and biochemistry parameters between the tortoises at the two facilities and a repeated measures ANOVA was conducted to detect significant differences in biochemistry parameters between tortoises in the 2017 and 2018 health assessments. Due to the large number of comparisons carried out concurrently, the Holm-Bonferroni correction was applied to determine each p value cut-off.

Evaluation of biochemistry parameters revealed that tortoises housed at the Otoy Ranch had increases in glucose and BUN concentrations compared with the tortoises at the Galapaguera of Cerro Colorado (figure 1). There were neither outward clinical signs nor abnormalities noted during physical examination of the tortoises at both facilities; however, glucose and BUN concentrations were significantly increased compared with tortoises at the Cerro Colorado facility ($p=0.001$ and $p<0.001$, respectively).

In comparison to previously established normal reference ranges for this species, the tortoises at the Otoy Ranch had increased glucose and BUN. A 95% CI composed of the healthy adult San Cristóbal Galapagos tortoises sampled in 2017 demonstrates that the average glucose and BUN of the tortoises sampled at the Otoy Ranch falls outside these reference ranges (table 1).

Analysis of the five tortoises which were housed at the Galapaguera of Cerro Colorado in March 2017 and then transferred to Otoy Ranch demonstrated significantly increased glucose concentrations compared with their previous health assessments in March 2017 ($p=0.027$) (figure 2). Similarly, BUN was also increased compared with 1 year previously; however, this difference was not statistically significant ($p=0.096$).

DIFFERENTIAL DIAGNOSIS

Hyperglycaemia in reptiles is often a result of iatrogenic delivery of excessive glucose; however, the stress of capture and restraint or preprandial/postprandial samples may affect glucose concentration.[9] Given that all of the tortoises in this study were captured and restrained in the same manner and were not fed prior to blood collection, diet was suspected as the leading cause for the increased glucose in animals at the Otoy Ranch. Elevated BUN could include renal compromise, muscle catabolism, dehydration or increased protein consumption.[9 10] On the basis of physical examination and haematology, there was no evidence of renal compromise and all tortoises were euhydrated with no evidence of muscle catabolism nor increased protein consumption. Therefore, based on the current body of knowledge for managing this species in captivity, the significant differences in glucose and BUN between the two facilities were suspected to be caused by the differences in diet fed at the two locations.

OUTCOME AND FOLLOW-UP

The authors recommended adjusting the diet to ensure that there is no access to sources of excess protein and that they limit the tortoises' accessibility to high glucose fruits. In order to provide more opportunity for natural foraging behaviour, we suggested that the Otoy Ranch incorporate a greater quantity of low energy forage, similar to the natural diet of wild

Table 1 Blood chemistry and blood gas parameters collected from Galapagos tortoises (*Chelonoidis chathamensis*) at the Galapaguera of Cerro Colorado in 2018, the Otoy Ranch in 2018, and at the Galapaguera of Cerro Colorado in 2017

Blood chemistry parameter	Sample	N	Mean	SD	Min	Max
Na (mmol/L)	Galapaguera in 2018	7	129.4	4.9	121	136
	Otoy Ranch in 2018	12	125.7	3.4	119	131
	Galapaguera in 2017	23	130.1	3.4	123	137
K (mmol/L)	Galapaguera in 2018	7	5.1	0.8	3.7	6.0
	Otoy Ranch in 2018	12	6.1	1.2	3.6	7.9
	Galapaguera in 2017	23	4.4	0.5	2.8	5.3
Cl (mmol/L)	Galapaguera in 2018	7	97.8	4.7	94	106
	Otoy Ranch in 2018	12	95.6	3.9	88	102
	Galapaguera in 2017	23	99.9	3.9	92	106
iCa (mmol/L)	Galapaguera in 2018	7	1.4	0.1	1.2	1.6
	Otoy Ranch in 2018	12	1.5	0.1	1.3	1.7
	Galapaguera in 2017	23	1.4	0.2	1.0	1.6
TCO_2 (mm Hg)	Galapaguera in 2018	7	28	2.2	26	31
	Otoy Ranch in 2018	12	29	2.4	23	32
	Galapaguera in 2017	23	25.2	4.6	15	38
Glucose (mmol/L)	Galapaguera in 2018	7	59.1	14.2	42	88
	Otoy Ranch in 2018	12	127.9	46	40	190
	Galapaguera in 2017	23	46.4	11.5	21	68
BUN (mg/dL)	Galapaguera in 2018	7	4.1	1.7	3	7
	Otoy Ranch in 2018	12	16.8	4.7	6	26
	Galapaguera in 2017	23	11	6.3	3	26
HCT (%)	Galapaguera in 2018	7	16.7	2	15	20
	Otoy Ranch in 2018	12	19	3.1	6.1	9.2
	Galapaguera in 2017	23	18.4	3.0	15	25
Hb (g/dL)	Galapaguera in 2018	7	6.1	0.6	5.4	6.8
	Otoy Ranch in 2018	12	6.8	0.9	6.1	9.2
	Galapaguera in 2017	23	6.5	0.9	5.1	8.5
Anion gap (mmol/L)	Galapaguera in 2018	7	9.1	3.8	2	13
	Otoy Ranch in 2018	12	8.3	1.8	5	10
	Galapaguera in 2017	23	9.8	3.4	3	19
Lactate (mmol/L)	Galapaguera in 2018	7	2.4	1.3	0.8	5.1
	Otoy Ranch in 2018	12	2.2	1	0.8	4.3
	Galapaguera in 2017	23	2.4	1.1	0.8	6.1
PCV (%)	Galapaguera in 2018	7	17	3	12	20
	Otoy Ranch in 2018	12	20	5.5	9	26
	Galapaguera in 2017	23	19	5.9	5	27
Total protein (mg/dL)	Galapaguera in 2018	7	6.5	1.9	4.5	9.5
	Otoy Ranch in 2018	12	6.9	2	3.6	9.6
	Galapaguera in 2017	23	5.2	1.5	1.2	7.6

BUN, blood urea nitrogen; HCT, haematocrit; Hb, haemoglobin; PCV, packed cell volume.

Galápagos tortoises and the tortoises at the Galapaguera of Cerro Colorado. Repeat health assessments, including physical examination, morphometric measurements and peripheral blood chemistry analysis are planned at both facilities in 1 year.

DISCUSSION

Breeding centres such as the Galapaguera of Cerro Colorado were developed in order to encourage captive reproduction of the endangered San Cristóbal Galapagos tortoise and then

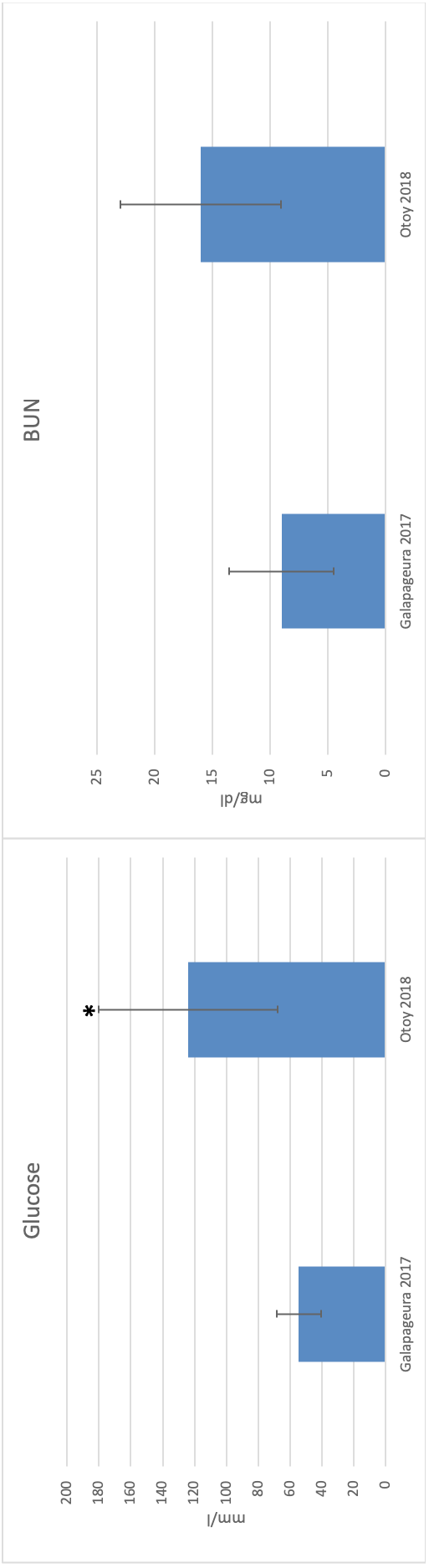

Figure 2 Average peripheral blood glucose and BUN conducted at the Galapaguera of Cerro Colorado in 2017 compared with the same tortoises at the Otoy Ranch assessed in 2018. BUN, blood urea nitrogen.

release them in their areas of origin to recover the wild populations of these reptiles. Since much of the success in maintaining and encouraging reproduction in captive reptiles relies on a correct and balanced diet, correlating peripheral blood chemistry abnormalities to differences in diet and husbandry are important to provide quantitative data to underscore the importance of providing a diet in captivity that mimics the natural diet.[8]

Galapagos tortoises have a similar digestive efficacy as mammalian hindgut fermenters.[4] In the wild, Galapagos tortoises consume large quantities of low energy forage, and occasionally the fruits or pads of the opuntia cactus (*Opuntia echios*).[11] Furthermore, comparative studies that evaluated the growth of juvenile Galapagos tortoises in their natural habitat versus captive conditions showed that a low-fibre, high-protein diet was responsible for inappropriate growth rates in captive tortoises, which were approximately twice the size and 10 times heavier than tortoises in their natural habitat. Excess dietary protein was also associated with earlier sexual maturity and a shorter life expectancy.[6]

Since no studies have been conducted to determine the nutritional values of otoy, it is difficult to compare this diet with that of plants consumed by tortoises in captivity.[8] This study sought to minimise external factors which may have altered clinicopathological findings by standardising the methods of restraint and performing all sampling and processing concurrently. There was no evidence of renal compromise, muscle catabolism, dehydration or increased protein consumption which would have explained the increase in BUN and animals were not fed prior to sample collection. Therefore, the authors suspect that the differences in diet between the facilities were most likely responsible for the changes in serum biochemistry. Given that two sampling sites were used for blood collection, it is possible that the difference in sampling sites may have affected biochemistry results due to lymph contamination; however, previous work in chelonians has demonstrated that lymph contamination does not significantly affect glucose and BUN values.[12]

The results of this study and previous work indicate that a low energy forage should be considered as a staple in the diet of Galapagos tortoises. Tortoises at the Otoy Ranch were observed eating fruit found growing in their habitat along with insects consuming the fruit. While the results of this case report do not definitively provide evidence for diet being the sole factor responsible for these clinicopathological changes, given our current knowledge of the species, the clinical presentation of the tortoises in this study and experimental method of sampling, diet should be considered a significant contributing factor to the changes in serum biochemistry. Therefore, the increased glucose and BUN concentrations in the tortoises at the Otoy Ranch during these health assessments constitutes the need for future research in the field of nutrition of Galapagos tortoises in captivity.

Although the long-term consequences of increased glucose and BUN concentrations in this species are unknown, implementing a new and efficient nutrition programme that more closely resembles the natural foraging of wild tortoises may help to prevent the development of future health complications and encourage reproduction in captivity.

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REFERENCES

1. Caccone A, Cayot LJ, Gibbs JP, *et al*. The IUCN red list of threatened species 2017. *Chelonoidis chathamensis* 2017.
2. Poulakakis N, Russello M, Geist D, *et al*. Unravelling the peculiarities of island life: vicariance, dispersal and the diversification of the extinct and extant giant Galápagos tortoises. *Mol Ecol* 2012;21:160–73.
3. Hamann O, recovery Ovegetation. Goats and giant tortoises on Pinta Island, Galapagos, Ecuador. *Biodiversity and Conservation* 1993;2:138–51.
4. Hatt J-M, Clauss M, Gisler R, *et al*. Fiber digestibility in juvenile Galapagos tortoises (*Geochelone nigra*) and implications for the development of captive animals. *Zoo Biol* 2005;24:185–91.
5. Parham JF. (2008) Rediscovery of an "extinct" Galápagos tortoise. *Proc Natl Acad Sci USA* 2008;105:15227–8.
6. Furrer SC, Hatt JM, Snell H, *et al*. Comparative study on the growth of juvenile Galapagos giant tortoises (*Geochelone nigra* at the Charles Darwin Research Station (Galapagos Islands, Ecuador) and Zoo Zurich (Zurich, Switzerland). *Zoo Biol* 2004;23:177–83.
7. Lewbart GA, Griffioen JA, Savo A, *et al*. Biochemistry and hematology parameters of the San Cristóbal Galápagos tortoise (*Chelonoidis chathamensis*). *Conserv Physiol* 2018;16.
8. Puga-Torres BH, Morales-Arciniega SM, Núñez LFN, *et al*. Comparison of the Effect of Three Food Diets Provided to Giant Tortoises (*Chelonoidis guntheri* and *Chelonoidis vicina*) Bred in Captivity at Isabela Island – Galápagos. *J Vet Sci Ani Husb* 2017;5.
9. Campbell TW. Clinical pathology of reptiles. In: Mader DR, ed. *Reptile medicine and surgery*. 2nd edn. St. Louis, Missouri: Saunders/Elsevier, 2006: 453–70.
10. Temporal PCC. Population, and sexual variation in hematocrit of free-living desert tortoises: correlational tests of causal hypotheses. *Can J Zool* 2002;80:461–70.
11. Hairston C, Burchfield PM. Management and reproduction of the Galapagos tortoise *Geochelone elephantopus* at the Gladys Porter Zoo. *Int Zoo Yearb* 1989;28:70–7.
12. Crawshaw GJ, Holz P. Comparison of plasma biochemical values in blood and blood-lymph mixtures from red-eared sliders, *Trachemys scripta elegans*. *Bulletin of the Association of Reptilian and Amphibian Veterinarians* 1996;6:7–9.