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HEMOLYMPH BIOCHEMISTRY REFERENCE RANGES FOR WILD-CAUGHT GOLIATH BIRDEATER SPIDERS (*THERAPHOSA BLONDI*) AND CHILEAN ROSE SPIDERS (*GRAMMOSTOLA ROSEA*)

Trevor T. Zachariah, D.V.M., Mark A. Mitchell, D.V.M., Ph.D., Clare M. Guichard, and Rimme S. Singh, B.A.

Abstract: Theraphosid spiders have become increasingly popular for private and public uses in the United States. However, little is known about their physiology from a medical standpoint. This study represents the first attempt to establish reference hemolymph values for two common species of theraphosids, the goliath birdeater spider (*Theraphosa blondi*) and the Chilean rose spider (*Grammostola rosea*). Eleven *T. blondi* and twelve *G. rosea*, all wild-caught subadults, were obtained after importation and hemolymph was collected for biochemical analysis. After 8 wk of captivity, hemolymph was again collected from the spiders and analyzed. The biochemical analytes measured in the study included aspartate transferase, creatine kinase, glucose, total protein, albumin, uric acid, blood urea nitrogen, phosphorous, calcium, potassium, and sodium. The osmolality of the hemolymph was estimated for each spider using two different formulae. There were significant differences in body weight, sodium, potassium, and osmolality between the sampling times for both species. There were also significant differences in creatine kinase, calcium, total protein, and blood urea nitrogen between sampling periods for *T. blondi*. The results of this study suggest that serial hemolymph samples may be used to assess the hydration status of theraphosid spiders. In addition, the differences in hemolymph analytes between spiders suggest that there may be differences between species that should be addressed in future studies.

Key words: Biochemistry, *Grammostola rosea*, hemolymph, spider, Theraphosidae, *Theraphosa blondi*.

INTRODUCTION

Invertebrates comprise the majority of the world's fauna, and are popular as research, display, and pet animals. In the pet trade, spiders represent a significant portion of the invertebrate specimens available for sale, and most of these are from the family Theraphosidae. It has been estimated that more than 100,000 theraphosid spiders are imported into the United States each year, and approximately the same number are bred in captivity (Breene, pers. comm.). Currently, there are 22 species of theraphosid spiders listed with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II.[15] The listing of these invertebrates under CITES protection suggests that there are natural and/or unnatural influences that can affect the long-term survival of these spiders. To protect the future of these theraphosids, research to increase our understanding of their physiologic functions is required.

Reference values for blood biochemical and gas constituents have long been established for domestic species, but only a few limited attempts have been made for spiders of any taxonomic group.[1,2,5,7,12,13] These previous attempts focused on analyzing different organic and inorganic hemolymph constituents using relatively slow, technically involved procedures with equipment that would not be found in the average veterinary clinic or zoological hospital. The primary objective of this research was to establish reference ranges for the biochemical components of goliath birdeater spider (*Theraphosa blondi*) and Chilean rose spider (*Grammostola rosea*) hemolymph using a standard analytical machine that could be commonly found in general veterinary practice. The secondary objective of this study was to compare paired hemolymph samples from spiders post-import and after 8 wk of captivity to assess the effect of captivity on biochemical parameters.

The specific biological hypotheses tested in this study were to determine if significant differences existed in body weight, sodium (Na), and total protein (TP) between the paired hemolymph samples and if there were differences in the biochemical analytes between male and female *T. blondi*.

MATERIALS AND METHODS

Theraphosa blondi

Eleven (six male and five female) wild-caught, subadult *T. blondi* were obtained from an invertebrate importer in Florida (LASCO, Naples, Florida

From the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana 70803, USA. Correspondence should be directed to Dr. Zachariah (zachariahdvm@yahoo.com).

34119, USA). The spiders were housed individually in rectangular, 5.7-L plastic storage containers with lids. A 50:50 mixture of potting soil and vermiculite was used for the substrate. The spiders were provided a 12-hr light cycle; half of a plastic flower pot served as a hiding space. The temperature and humidity in the enclosures were maintained at approximately 23.9°C (75°F) and 80%, respectively.

Each spider was placed into a square, 3-L plastic storage container that was modified into a gas anesthetic chamber. The container was modified by drilling a hole in one side and inserting an endotracheal tube adapter. Each spider was anesthetized with 5% isoflurane[10] (Isoflo, Abbott Laboratories, North Chicago, Illinois 60064, USA) at a flow rate of 1 L/min oxygen. Once the spider had lost its ability to right itself, it was removed from the anesthetic chamber and weighed. A 26-ga, ¾-inch needle fastened to a 3-ml syringe was used to collect an intracardiac hemolymph sample.[16] A volume of 0.5 ml was collected from each individual by inserting the needle at approximately a 45° angle through the exoskeleton at the midpoint of the dorsal midline of the opisthosoma. This method was used to facilitate the rapid collection of a relatively large amount of hemolymph. Hemolymphstasis was accomplished by applying a small amount of Nexaband glue (Veterinary Products Laboratories, Phoenix, Arizona 85067, USA) to the collection site. The spiders were recovered in 100% oxygen, and recovery from anesthesia was uneventful in all spiders.

Each sample was placed in a lithium heparin Microtainer tube (Becton Dickinson, Franklin Lakes, New Jersey 07417, USA) and centrifuged for 10 min at 1,411 *g*. After centrifugation, the supernatant was removed and analyzed using an avian/reptile rotor for the VetScan Analyzer (Abaxis Inc., Union City, California 94587, USA). Results were collected for the following biochemical analytes: aspartate transferase (AST), creatine kinase (CK), glucose (GLU), TP, albumin, uric acid (UA), blood urea nitrogen (BUN), phosphorous, calcium (Ca), potassium (K), and Na. All samples were analyzed within 30 min of sample collection. Na levels for the VetScan Analyzer have a maximum measurable limit of 180 mmol/L. Because Na levels for these spiders were often greater than that level, an aliquot of each sample was tested using the Olympus AU600 Clinical Chemistry Analyzer (Olympus America Inc., Melville, New York 11747, USA). An estimated osmolality was calculated for each spider using the following formulae:

Formula 1.[17] $(NA + K) \times 2 + BUN + GLU = \text{milliosmoles/L}$

Formula 2.[3] $1.86 \times (NA + K) + (GLU/18) + (BUN/2.8) + 9 = \text{milliosmoles/L}$

The spiders were held under the conditions described earlier for an additional 8 wk. The spiders had constant access to chlorinated tap water and were fed five adult crickets weekly. The crickets had access to a high-Ca cricket food and water source (High Calcium Cricket Diet and Cricket Quencher Calcium, Fluker's Farms, Port Allen, Louisiana 70767, USA) until being offered to the spiders. A second hemolymph sample was collected 8 wk after the initial sample. Sample collection, handling, and analysis were repeated as described, with the exception that immediately after sample collection, 0.15 ml of the hemolymph sample was analyzed using a CG8+ blood gas cartridge with the i-STAT analyzer (Abbott Laboratories, North Chicago, Illinois 60064, USA). Results were collected for the following additional hemolymph values: total carbon dioxide, ionized Ca, pH, partial pressure of carbon dioxide, partial pressure of oxygen, bicarbonate, and oxygen saturation.

Grammostola rosea

Twelve wild-caught *G. rosea* were obtained from a vendor at a reptile show in Mandeville, Louisiana. The spiders used for this study were all subadults of unknown gender. The spiders were housed in the same type of containers described for *T. blondi*. Bed-A-Beast ground coconut hull was used as the substrate (Pet-Tech Products LLC, Van Nuys, California 91406, USA). The temperature and humidity in the enclosures were maintained at approximately 23.9°C (75°F) and 70%, respectively. The spiders were provided a 12-hr light cycle; half of a plastic flower pot was provided as a hiding space.

The spiders were anesthetized using the same technique described for *T. blondi*. Hemolymph samples were collected using a 30-U insulin syringe fitted with a 30-ga needle. A total of 0.15 ml hemolymph was collected from each individual. Because of the spiders' small size, a bolus of 0.5 ml 0.9% saline (Hospira Inc., Lake Forest, Illinois 60045, USA) was administered into the opisthosoma after the hemolymph was collected. Recovery from anesthesia was uneventful for all spiders.

After the initial hemolymph collection, the spiders were provided constant access to water and were fed three adult crickets weekly for 8 wk. The

Table 1. Biochemical values for normally distributed data between sampling times for goliath birdeater spiders (*Theraphosa blondi*).

	Mean	Standard deviation	95% Confidence interval	Minimum–maximum
Calcium (mg/dl)				
Time 1	13.9	2.5	11.9–15.8	8.9–17.1
Time 2	11.9	1.7	10.6–13.2	8.8–14.4
Potassium (mmol/L)				
Time 1	2.4	0.2	2.3–2.6	2.1–2.7
Time 2	2.0	0.4	1.8–2.3	1.6–2.8
Sodium (mmol/L)				
Time 1	221.0	14.6	209.0–232.0	206.0–253.0
Time 2	190.0	14.3	178.7–200.6	170.0–218.0
Osmolality (milliosmoles/L), formula 1				
Time 1	450.6	29.1	428.3–473.0	420.2–514.5
Time 2	385.0	27.5	363.9–406.2	346.2–442.2
Osmolality (milliosmoles/L), formula 2				
Time 1	428.5	27.0	407.7–449.3	400.1–487.7
Time 2	368.7	25.0	349.1–388.4	331.2–420.4

Table 2. Biochemical values for non-normally distributed data between sampling times for goliath birdeater spiders (*Theraphosa blondi*).

	Median	10–90% Quantiles	Minimum–maximum
Weight (g)			
Time 1	32.0	25.0–35.0	25.0–53.0
Time 2	33.5	28.0–41.0	27.8–57.2
Total protein (g/dl)			
Time 1	5.6	0.8–6.0	0.8–6.5
Time 2	4.1	0.8–4.7	0.8–5.0
Blood urea nitrogen (mg/dl)			
Time 1	8.0	3.0–10.8	3.0–20.0
Time 2	3.0	1.0–6.8	1.0–23.0
Creatine kinase (U/L)			
Time 1	23.0	5.0–82.2	3.0–83.0
Time 2	17.0	2.4–28.8	2.0–29.0

crickets were maintained using the same technique described previously. A second hemolymph sample was collected 8 wk after the initial sample to determine if the biochemistries differed from the time of import. Sample collection, handling, and analysis were repeated as described. Hemolymph gas analysis was not performed in this species.

The distribution of each hemolymph biochemical parameter was evaluated for normality using the Shapiro–Wilk test, skewness, and kurtosis. Levene's test was used to assess the homogenicity of the data. The mean, standard deviation (SD), 95% confidence intervals (95% CI), and minimum-maximum values are reported for normally distributed data. The median, 10–90% quantiles, and minimum-maximum are reported for non-normally distributed data. For purposes of analysis, all non-normally distributed data was $\log_{10}$ transformed. A paired *t*-test was used to determine if there were differences in the biochemical parameters of *G. rosea* and *T. blondi* between the samples collected after import (time 1) and those collected 8 wk later (time 2). To assess gender (between subject) variability for *T. blondi*, an unpaired *t*-test was performed on the difference scores for the two groups. An unpaired *t*-test was also used to compare the mean differences for the two different osmolality measurements. A Student's *t*-test was used to compare hemolymph gases between genders for *T. blondi*. When there was no difference between the paired samples, the arithmetic mean was used to calculate the final reference interval. A power analysis was performed when no difference was found to assess the potential for a type II error. For those cases where no difference was found, the highest observed power was 0.62. Significance testing was set at $P \leq 0.05$. The statistical analysis was performed using a commercial software package (SPSS 11.0, SPSS Inc., Chicago, Illinois 60606, USA).

RESULTS

For *T. blondi*, there were significant differences in body weight ($P = 0.002$), TP ($P = 0.007$), BUN ($P = 0.04$), CK ($P = 0.049$), Ca ($P = 0.02$), K ($P = 0.03$), Na ($P = 0.007$), and osmolality ($P = 0.0001$) between sampling times (Tables 1 and 2). There was also a significant difference in the osmolality between the two different estimation methods for the first ($P = 0.001$; mean difference: 22.0 $\pm$ 2.0) and second ($P = 0.001$; mean difference: 16.3 $\pm$ 4.9) hemolymph samples. There were no significant differences between sampling times for any of the other hemolymph biochemistries in *T. blondi* (Tables 3 and 4). There were also no significant differences between gender for any of the hemolymph biochemistries or gases in *T. blondi*.

For *G. rosea* there were significant differences in body weight ($P = 0.001$), K ($P = 0.01$), Na ($P = 0.04$), and osmolality ($P = 0.006$) between sampling times (Table 5). There was also a significant difference in the osmolality between the two different estimation methods for the first hemolymph sample ($P = 0.001$; mean difference: 29.3 $\pm$ 12.6).

Table 3. Reference intervals for normally distributed biochemical values and hemolymph gases for goliath birdeater spiders (*Theraphosa blondi*).

	Mean	SD	95% CI	Minimum–maximum
Aspartate transferase (U/L)	4.4	1.7	3.3–5.4	2.0–7.5
Glucose (mg/dl)	18.6	2.1	17.1–20.1	14–31.5
Phosphorous (mg/dl)	2.0	0.6	1.6–2.4	1.1–3.1
pH	7.44	0.05	7.41–7.47	7.38–7.53
Partial pressure of carbon dioxide (mmHg)	20.3	3.9	17.7–22.9	15.0–28.8
Bicarbonate (mmol/L)	13.8	2.7	12.0–15.6	9.2–17.6
Total carbon dioxide (mmol/L)	14.5	2.6	12.7–16.3	10.0–18.0

There were no significant differences between sampling times for any of the other hemolymph biochemistries in this species (Tables 6 and 7).

DISCUSSION

All the spiders survived the anesthesia and hemolymph sampling procedures. Fluid therapy was administered to all 12 *G. rosea* to compensate for hemolymph losses as a result of sample collection. In order to obtain adequate sample sizes, we had to collect between 1% and 2% (1–2 ml/100 g body weight) hemolymph by body weight. In vertebrates, it is recommended that no more than 1 ml of blood be collected per 100 g body weight. The level of hypovolemia that can be tolerated by theraphosid spiders is not known, but because of their small size, the lack of hydrostatic pressure could have had potentially debilitating effects. The fluid therapy was well tolerated by all 12 spiders of this species. Saline (0.9%, 308 milliosmoles/L) was chosen as the replacement fluid because it is a commercial product with an osmolality closest to the hemolymph osmolality of *Eurypelma californicum* (477 milliosmoles/L)[13] (now classified in the genus *Avicularia*),[11] the only previous value reported for a theraphosid spider. In retrospect, another fluid may have been preferred because of the high Na levels found at the time of presentation.

The average hemolymph osmolalities of *G. rosea* at time 1 and time 2 were 489 milliosmoles/L and 351 milliosmoles/L, respectively, indicating a 28.2% difference. The average hemolymph osmolalities of *T. blondi* at time 1 and time 2 were 451 milliosmoles/L and 386 milliosmoles/L, respectively, indicating a 14.4% difference. These changes may be a direct reflection of the hydration status of the spiders, as they would be in vertebrates,[3] or may be because of metabolic changes associated with improved nutrition between sampling times. The osmolalities recorded in these spiders were much lower under proper husbandry conditions (time 2) than those recorded for the theraphosid spider *E. californicum* (477 milliosmoles/L)[13] and those recorded for two species of araneid spiders (536 and 508 milliosmoles/L).[2] However, the *G. rosea* and *T. blondi* osmolality values are within the range of 320–512 milliosmoles/L recorded for six species of lycosid spiders.[12] There appears to be significant variation in the hemolymph osmolality of these invertebrates, and future studies to elucidate the importance of these differences are needed. Between the two sampling times, Na levels decreased for both species (28.3% for *G. rosea* and 14.0% for *T. blondi*), and the BUN and TP levels decreased (62.5% and 26.8%, respectively) for *T. blondi*. Na, BUN, and TP levels in vertebrates can be adversely affected by hydration status, and this may also be true for theraphosid spiders. The VetScan Analyzer does not measure chloride, but we would predict that chloride levels would decrease between sampling times as well.

Assessing the hydration status of a theraphosid

Table 4. Reference intervals for non-normally distributed biochemical values and hemolymph gases for goliath birdeater spiders (*Theraphosa blondi*).

	Median	10–90% Quantiles	Minimum–maximum
Albumin (g/dl)	0.0	0.0–0.15	0.0–0.2
Uric acid (mg/dl)	0.2	0.10–0.32	0.10–0.35
Oxygen saturation (%)	100.0	99.0–100.0	99.0–100.0
Partial pressure of oxygen (mmHg)	200.0	147.8–348.0	147.0–372.0
Ionized calcium (mmol/L)	1.8	1.67–2.27	1.66–2.34

Table 5. Biochemical values for normally distributed data between sampling times for Chilean rose spiders (*Grammostola rosea*).

	Mean	Standard deviation	95% Confidence interval	Minimum–maximum
Weight (g)				
Time 1	10.1	1.2	9.3–10.9	8.0–12.0
Time 2	12.9	2.2	11.5–14.3	9.8–15.8
Potassium (mmol/L)				
Time 1	2.7	0.6	1.7–2.4	1.8–3.8
Time 2	2.0	0.5	1.7–2.4	1.1–2.8
Sodium (mmol/L)				
Time 1	241.1	25.4	219.8–262.4	201.0–276.0
Time 2	172.8	33.3	137.9–207.8	115.0–206.0
Osmolality (milliosmoles/L), formula 1				
Time 1	496.8	59.0	442.3–551.4	410.5–585.5
Time 2	366.0	88.0	273.6–458.4	236.3–495.7
Osmolality (milliosmoles/L), formula 2				
Time 1	467.6	49.1	422.2–513.0	391.0–528.5
Time 2	335.5	61.9	270.6–400.5	228.8–397.7

Table 7. Reference intervals for non-normally distributed biochemical values for Chilean rose spiders (*Grammostola rosea*).

	Median	10–90% Quantiles	Minimum–maximum
Aspartate transferase (U/L)	5.7	2.1–34.7	2.0–41.5
Glucose (mg/dl)	17.5	13.9–27.8	13.5–30.5
Total protein (g/dl)	6.4	3.7–7.2	3.2–7.3
Albumin (g/dl)	0.02	0.0–0.15	0.0–0.5
Uric acid (mg/dl)	0.12	0.0–0.3	0.0–0.7
Blood urea nitrogen (mg/dl)	1.0	0.5–1.4	0.5–2.0

spider is difficult. Extreme dehydration or acute hemolymph loss can lead to decreased hydrostatic pressure, subsequent inability to move, and a shrunken opisthosoma. For mild cases of dehydration, the techniques used to assess hydration in vertebrates (e.g., skin turgor, mucus membrane evaluation, globe position in the orbit, urine production) are not possible in theraphosid spiders. The best measure of dehydration in invertebrates is likely the evaluation of serial body weights and hemolymph electrolyte levels. We suspected dehydration in these animals because of the changes observed in the body weights between sampling periods and alterations noted in Na in both species and the TP and BUN in *T. blondi*. *Grammostola rosea* and *T. blondi* experienced 27.7% and 4.7% increases in body weight between the sampling periods, respectively.

Both *G. rosea* and *T. blondi* had measurable levels of AST and CK. This is not surprising considering that spiders have striated skeletal muscle,[4] which is a source of these enzymes in vertebrates. In vertebrates, CK blood levels rise when muscle cells leak, are inflamed, or are damaged.[9] Aspartate transferase levels increase when muscle cells are inflamed or necrotic.[18] The same physiologic mechanisms observed in vertebrates may also occur in theraphosid spiders. In this study, CK levels in *T. blondi* were significantly lower in the second samples. Although statistically significant, the difference in CK between sampling periods (26.1%) may not be biologically important.

In addition to being found in muscle cells, AST is also produced by the hepatocytes of vertebrate species. Levels of this enzyme can be detected when damage occurs in hepatocytes. The hepatopancreas of theraphosid spiders serves many of the same functions as the vertebrate liver. If AST is produced in the hepatopancreas of theraphosid spiders, it may be useful in assessing damage to that organ.

The Ca values for the spiders in the study are similar to those reported for *E. californicum* (3.94 mmol/L, or 15.8 mg/dl),[13] and greater than those of six lycosid spider species (1.9–5.4 mg/dl).[12] Interestingly, the Ca levels in *G. rosea* were 23.7% higher than in *T. blondi*. This difference may be related to species differences. Theraphosid spiders require Ca to ensure striated muscle function and presumably for various other physiologic processes,

Table 6. Reference intervals for normally distributed biochemical values for Chilean rose spiders (*Grammostola rosea*).

	Mean	Standard deviation	95% Confidence interval	Minimum–maximum
Creatine kinase (U/L)	25.7	7.5	20.9–30.5	8.0–36.0
Phosphorus (mg/dl)	1.2	0.4	1.0–1.5	0.7–2.1
Calcium (mg/dl)	16.9	1.8	15.7–18.1	13.3–19.9

such as gated cell membrane channels. The ionized Ca levels in *T. blondi* were higher than those reported in vertebrates. Total Ca comprises both ionized and un-ionized Ca. In vertebrates, un-ionized Ca is generally bound to albumin. The spiders in this study had negligible albumin levels in their hemolymph. The absence of albumin may account for the higher levels of ionized Ca in the spiders.

Despite its low hemolymph concentration, hemocyanin is the predominant organic substance in hemolymph, comprising 80% of the protein molecules.[4] The composition of the other 20% is not known. The TP values found in the study were greater than those found for two araneid species (3.3 g/L, or 0.33 g/dl)[2] and six lycosid species (0.6–2.9 g/L, or 0.06–0.29 g/dl),[12] but similar to the TP values for *E. californicum* (56.9 g/L, or 5.69 g/dl)[13] and a salticid species (48 g/L, or 4.8 g/dl).[7] The minimal level of albumin measured in the hemolymph of these theraphosid spiders is not surprising. Theraphosid spiders possess a semi-open circulatory system. The heart pumps hemolymph through arteries and capillaries to bathe the tissues, but there is an open venous return system. Therefore, oncotic pressure within the vasculature probably does not need to be maintained.

The hemolymph pH of *T. blondi* (Table 3) was similar to that found in *E. californicum* (7.49),[1] but higher than that described for *Dugesiella hentzi* (7.25–7.35)[14] (now classified in the genus *Aphonopelma*).[11] The hemolymph pH of *T. blondi* was also found to be lower than that reported for other spiders, including *E. californicum* (7.81),[13] *Dugesiella californica* (7.9),[6] and a salticid spider (8.1–8.3).[7] Interestingly, the range of pH for *T. blondi* (Table 4) was relatively narrow (7.38–7.53). To better understand the pH tolerance of these animals, studies should be pursued to assess morbidity and mortality when pH values increase or decrease beyond this range. The value of partial pressure of oxygen for *T. blondi* was higher than that reported for *E. californicum* (28 mmHg).[1] Whether the measured values in this study are indicative of the true normal state for *T. blondi* is unknown, because the animals were sampled after having been anesthetized with pure oxygen and isoflurane. This could have resulted in an artificial elevation in oxygen saturation and partial pressure of oxygen, and reduction in partial pressure of carbon dioxide and total carbon dioxide. This may also have affected an increase in pH.

Both *G. rosea* and *T. blondi* had circulating levels of BUN and UA in their hemolymph. Similar to terrestrial reptiles, theraphosid spiders excrete crystallized UA, along with guanine, adenine, and hypoxanthine, for conservation of water.[4] The role of BUN is probably to deliver urea to the excretory organs, the Malpighian tubules of the hindgut, for processing into UA. The UA levels in these spiders were barely detectable. The BUN levels were consistent with those reported in vertebrate species. In vertebrates, UA and BUN can be used as predictors for hydration. In spiders, this may not be the case for UA, but may be possible for BUN.

Theraphosa blondi and *G. rosea* both had measurable levels of GLU present in their hemolymph. Levels were higher than those found for *E. californicum* (0.12 g/L, or 12.0 mg/dl)[13] and *D. hentzi* (0.05 g/L, or 5.0 mg/dl).[14] GLU is the primary energy source for insect and vertebrate tissues, with both groups possessing a similar glycolysis process.[8] GLU is transported through the hemolymph in the form of trehalose, a disaccharide,[8] and presumably, this is also the case in theraphosid spiders.

The K levels in *G. rosea* and *T. blondi* were similar to those reported for *E. californicum* (1.9 mmol/L),[13] and greater than those reported for six lycosid spider species (4.3–9.6 mg/L, or 0.11–0.25 mmol/L).[12] K in *T. blondi* decreased significantly between sampling time 1 and time 2. Causes of hypokalemia in vertebrate species include loss, translocation from extracellular to intracellular fluid, and decreased intake.[3] Potassium levels are not affected by hydration status.[3] If during the importation, these animals had not been offered food over an extended period of time, it is possible that a shift in K from the intracellular to extracellular space occurred to prevent hypokalemia, similar to what occurs in starving vertebrates. Thus, a decrease in K levels from time 1 to time 2 should occur due to improved husbandry, and did in this present study.

CONCLUSIONS

It is possible to show that alterations in hemolymph biochemistries can occur over time in theraphosid spiders. To ascertain the true homeostatic state of a spider, serial samples may be required. Further study to correlate hemolymph and tissue levels of the biochemical analytes are needed. The differences found between the two species also suggest that hemolymph biochemistry references may be required on a species-by-species basis.

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