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ORIGINAL ARTICLE

Alternative therapy improves hematological profiles of captive snakes from northeastern Brazil

Efeitos positivos de uma terapia alternativa nos perfis hematológicos de serpentes cativas no nordeste brasileiro

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Abstract

Objective: Evaluate the effects of alternative therapy on the hematological profiles of different families of captive snakes. **Methodology:** Captive snakes at NUROF-UFC were submitted to a clinical and hematological evaluation before and after applying an alternative treatment, including systematic sunbathing and hydration by soaking bathes twice a week for five weeks. The biometric, clinical, and hematological data were compared by multivariate analysis of variance and investigated for possible causal relationships by general linear models. **Results**: A significant difference was observed between erythrograms and global leukograms in the three families of snakes evaluated before and after treatment. The significant reduction in the heterophil: lymphocyte ratio in the Family Colubridae after treatment was noteworthy. Discussion: The results were most likely due to stress level reduction by improving thermoregulation and conversion of vitamin D during sunbathing, oral rehydration and refreshing in soaking baths, and general metabolic rates due to physical exercise. **Conclusion**: The results confirm the initial hypothesis, assuming that a simple but systematic treatment that included sunlight exposure and immersion hydration was efficient in reducing stress rates.

Keywords: Reptiles; Hematology; Sunbathing; Hydration.

Resumo

Objetivo: Avaliar a influência de banhos de sol e de imersão em água nos perfis hematológicos de serpentes cativas, antes e após a aplicação deste tratamento alternativo. **Metodologia**: Serpentes cativas no NUROF-UFC foram submetidas à avaliação clínica e hematológica, depois submetidas ao tratamento alternativo por cinco semanas, sendo novamente avaliadas após. Os dados obtidos foram submetidos à análise estatística multivariada (NPMANOVA e GLM) para investigação de possíveis relações causais entre o tratamento e os perfis hematológicos. **Resultados:** Foi observada diferença significativa entre os eritrogramas e leucogramas nas três famílias de serpentes avaliadas antes e após o tratamento. Ressalta-se a redução significativa na razão heterófilos:linfócitos na Família Colubridae após o tratamento. **Discussão**: A diferença estatística deveu-se provavelmente à redução nos níveis de estresse, possibilitada por aquecimento e aumento de conversão da vitamina D durante a exposição solar; reidratação oral e refrescância nos banhos de imersão, e ao recondicionamento pelo exercício físico. **Conclusão**: Os resultados confirmaram a hipótese inicial, admitindo que um tratamento alternativo, simples mas aplicado sistematicamente, que incluiu exposição à luz solar e hidratação por imersão em água foi eficiente em reduzir as taxas de estresse em serpentes cativas.

Palavras-Chave: Répteis; Hematologia; Banho de sol; Hidratação.

INTRODUCTION

Snakes are the second most diverse group inside de Class of Reptiles, with 3.956 species[1]. They are scaled, fusiform, and limbless, with a vast diversity of shapes, color patterns, sizes, and surface textures, contributing to different behaviors, physiology, and ecology[2].

Brazil comprises one of the greatest biodiversity worldwide, with approximately 417 ophidian species distributed across ten families[1]. Some of the main Brazilian species are *Boa constrictor*, *Epicrates assisi, Philodryas nattereri, and Crotalus durissus*. They have great ecological importance, as they can control urban pests, serve as prey for other animals and act as bioindicators[3]. In addition, they represent essential experimental species for carrying out toxicological and pharmacological studies about their venom, antivenom production, therapeutic molecules, and scientific divulgation[4,5].

Herpetological medicine evaluates their general condition, diagnoses diseases, improves husbandry, and can identify and mitigate stress both in captivity and in native environments[6]. Physical examinations associated with clinical hematology are important tools to determine the health status of snakes when analyzing their response to diseases and treatments[7].

Changes in the numbers and morphology of blood cells are fundamental aspects of hematological interpretation, as

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they may indicate pathological conditions[8]. The heterophil/lymphocyte ratio (H:L) is considered a hematological stress marker, as well as leukocytosis[9] and blood glucose index[10].

Environmental enrichment is the foremost tool to ameliorate the welfare of captive individuals, as it simulates native conditions of the specific natural habitat[11]. Alternative therapies or husbandry correction are also important to accurate physiological functioning[12]. Although there is some information about this topic[12,13], papers that measure the benefit of alternative treatments as stress reducers in captivity are still scarce.

The objectives of the current work were to compare hematological markers before and after applying an alternative therapy in captive Brazilian snakes, verifying it as a possible stress reliever.

METHODS

Ethics

The present study was approved by the Committee for Ethics in the Use of Animals (CEUA) of the Federal University of Ceará (protocol # 4021011220). The experiments accorded with the rules and resolutions of the National Council of Controlling in Animal Experimentation (CONCEA).

Study area and experimental period

The experiment was carried out at the Regional Nucleus of Ophiology of the Federal University of Ceará (NUROF - UFC), located in Fortaleza (3° 43' 02" S and 38º 32' 35" W), state of Ceará, northeastern Brazil. Its phytogeographic domain is the Caatinga, with average annual temperature and pluviometry of 26.7 ºC and 1042 mm, respectively[14].

The study was divided into three stages: (1) Pre-treatment clinical evaluation and blood collection (Feb-Sep/2021); (2) Treatment application (Sep-Oct/2021); and (3) Post-treatment evaluation and blood collections (Nov/2021).

Sampling

The sample comprised snakes from three families, which were kept in NUROF-UFC for different purposes. Boidae snakes have been used for teaching[15]; Colubridae and Viperidae have been used for prior experimental venom studies[16]. All the snakes were originally wildlife ones rescued from urban buildings next to nature fragments or collected for scientific purposes.

Treatment

The experimental treatment lasted five weeks, comprising sunbathing (8 to 24 minutes, depending on the species), followed by soaking baths in tap water (60 minutes)[17] twice a week for all species. The snakes were exposed to the sunlight according to their specific active period[18].

Sunbathing took place in the frontal external area of the NUROF-UFC building. This environment contained a loft made of stacked logs, native ornamental vegetation, and shaded areas on earth substrate to provide an enriched environment as close as possible to the natural habitat of the sampled snakes. Individuals could remain in the sunlight or move to shaded areas during sunbathing. After sunbathing, the snakes were put in 45-liter buckets with tap water at around 30°C, enough to immerse the whole body[17].

Biometrics and Clinical Evaluation

The snakes were physically restrained by herpetological hooks (Boidae and Colubridae) or contention tubes (Viperidae). Snout-vent length (SVL) and body mass were measured with a standard measuring tape and a digital scale (15kg, ± 5g), respectively. The clinical criteria comprised tongue darting, suspension reflex, muscle tonus, head balance, body posture, and typical gait. Blood samples were obtained by caudal coccygeal (Colubridae and Viperidae) or dorsal vertebral venipuncture (Boidae) using heparinized syringes[9]. A clinical examination was performed. Divers and Stahl (2019)[6] contain the restraint and examination protocols.

Hematological processing

All the samples were processed at the Academic Laboratory of Wildlife Pathology (LAPS-UFC). Hematological parameters were composed of hematocrit (Ht), the concentration of blood hemoglobin (Hb), total erythrocyte count (CTE), and total leukocyte count (CTL). The Ht was obtained by centrifugation[19], and Hb was measured using a *Mission Digital Hb Hemoglobin Testing Meter®* electroenzymatic method. CTE and CTL were counted at a 1:200 ratio of whole blood to Natt-Herrick solution in the Neubauer chamber, according to Campbell (2015)[9]. Blood smears were made and stained with Panótico Rápido (hematology quick stain) and Wright[20] to perform the differential leukocyte count and the estimation of global leukometry at 400x, including counting on heterophils (Het), eosinophils (Eos), basophils (Bas), monocytes (Mon), lymphocytes (Linf) and thrombocytes (Thrb).

Biochemical analysis

Blood glucose index was measured with one drop of the total blood in the *Accu Check Guide®* portable glucose meter electroenzymatic method seven days post-feeding. Total plasmatic proteins (PPT) were measured by a clinical analogic refractometer.

Statistical analysis

Analyses were performed using R v. 4.1.2[21]. The R Basic package was used to run descriptive statistics, and the *R* Vegan package was applied for inferential multivariate analysis, including

Nonparametric Permutation Multivariate Analysis of Variance (NPMANOVA), which investigated possible causal relationships between the matrix of independent categorical variables (species, families, treatment application) and the matrix of dependent numeric variables (Ht, Hb, VCM, HCM, CHCM, CTE, GLIC, PPT, CTL, Het, Eos, Bas, Linf, Mono, Thrb), looking for a possible significant difference in the set of parameters evaluated before and after treatment. Generalized linear models (GLM) were used to investigate possible influences of the treatment in the H/L ratio.

RESULTS

At the start, 45 snakes belonging to three families were selected, with different permanency times in captivity. After the three phases of the experiment, 29 animals were included in the paired multivariate statistical analysis (Table 1). Sixteen snakes failed to acquire the complete paired data (sample processing failure, pregnancy as a limitation to be restrained, returned to native habitat, etc.), so they were excluded afterward.

Table 1. Alternative therapy on hematological profiles in captive snakes: sample characterization and biometric means ± standard deviation

FAMILY	SEX	N	SVL mean (cm)	BODY MASS mean (kg)
Boidae	Female	7	120,7 ± 21,1	1,361 ± 1,60
	Male	8	112,1 ± 4,6	0,779 ± 0,27
SUBTOTAL		15	116,1 ± 14,9	1,051 ± 1,11
Colubridae	Female	7	107,1 ± 10,6	0,376 ± 0,12
	Male	3	80,5 ± 10,1	0,445 ± 0,25
SUBTOTAL		10	99,1 ± 16,2	0,397 ± 0,15
Viperidae	Female	2	111,4 ± 11,9	1,130 ± 0,03
	Male	2	113,5 ± 4,9	1,175 ± 0,18
SUBTOTAL		4	112,5 ± 7,5	1,153 ± 0,11
TOTAL		**29**	**109,7 ± 16,2**	**0,839 ± 0,85**

Boidae snakes included three species, namely Boa constrictor (n=13), *Epicrates cenchria* (n=1), and *Corallus hortulanus* (n=1); Colubridae snakes included *Philodryas nattereri* (n=9) and *P. olfersii* (n=1), and Viperidae snakes counted only *Crotalus durissus cascavella* (n=4). The snakes and procedures are presented in Figure 1.

The captivity permanency lasted between 9 days and 240 months (median 101 months), 42 to 240 months for Boidae, nine days to 180 months for Colubridae, and nine days to 131 months for Viperidae.

During the pre-treatment examination, 10,3% (3/29) of the sample showed any clinical alteration, such as reduced balance/erratic strike (1/3); reduced olfactory reflex/reduced darting/mild multifocal scalar dermatitis/bilateral cataract/increased peripheral vascular resistance, with difficult blood venipuncture (1/3); and skin fragility/increased coagulation time (1/3). After the treatment, the chronic alterations remained; however, it was observed mild improvement in altered reflexes. Another specimen had caudal swelling.

For safety reasons, Viperidae snakes were subjected to indirect sunbathing simultaneously with the soaking baths inside the 45-liter buckets placed under the internal skylight in the building roof. In the course of the sunbathing, the studied snakes showed constant climbing behavior and active gait, specially Colubridae. It demonstrated the positive effect of the alternative therapy on their behavior once they are diurnal species, active-seeking predators with high metabolic rates[22].

Most reptiles tolerate blood collection up to 1% of body weight[9]; however, in practice, only 0,2 - 0,3 mL are enough for routine hematological studies. For this reason, the collected blood volume did not exceed 0,5 mL. Nevertheless, the over-blood ensured a safe margin for suitable analysis once blood could usually leak during the centrifugation.

The mean hematological values by snake species are described in Table 2.

Although intuitively it is expected to compare the observed blood values with the textbook reference ones, this is not a good idea since these blood parameters are highly correlated with geographic and environmental factors. The paired results by snake families are shown in Figure 2.

NPMANOVA indicated a significant difference in global blood counts among families after treatment (F=3,3283 and p=0,039), specifically between Boidae and Colubridae (F=3,9335 and p=0,013) at the Bonferroni PostHoc Test. Based on the parameters "CTE", "Het", "Eos", "Bas", "Mono", "Lymph", and "H:L ratio", the first analysis showed marginal significance (F=2,3108 and p=0,052), interpreted as a trend toward significance after treatment. Excluding individual leukocyte counting and considering only the "CTL" and "H:L ratio" matrix, the new model showed the statistical difference of the studied markers after the treatment (F=3,336 and p=0,042), particularly between Boidae and Colubridae (F=3,9475 and p=0,025) at the Bonferroni PostHoc Test.

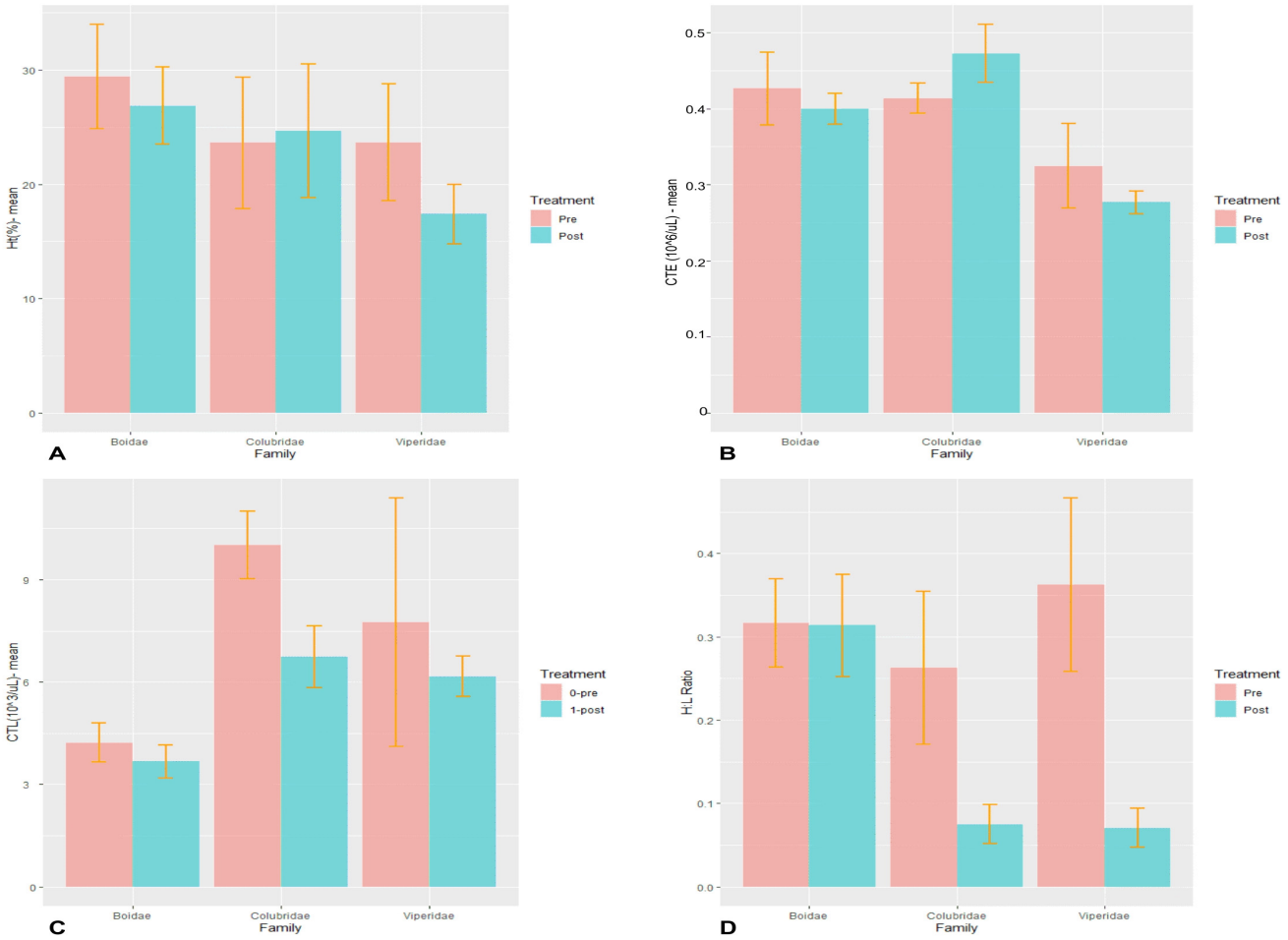
Figure 1. Alternative therapy on hematological profiles in captive snakes: A. Handling and restraining B. constrictor; B. Blood collection from the dorsal vein in B. constrictor; C. P. nattereri exposed to sunlight and performing physical exercise; D. C.d. cascavella soaking bath; D. Blood collection from caudal venopunction in C.d.cascavella; F. C.d. cascavella intubation restraint.

Table 2. Alternative therapy on hematological profiles in captive snakes: Mean hematological values per evaluated snake family previous to alternative therapy application.

Variables	Family		
	Boidae	Colubridae	Viperidae
Ht (%)	28,3 (15,0-36,0)	21,8 (5,0-32,0)	23,7 (20,0-29,5)
Hb (g/dL)	7,9 (4,3-11,8)	9,8 (5,0-16,3)	12,6 (9,3-17,0)
VCM (fL)	74,3 (43,0-114,3)	57,5 (40,4-74,7)	78,9 (46,0-113,9)
HCM (pg)	21,3 (5,8-38,2)	32,8 (12,7-121,7)	42,7 (21,4-65,6)
CHCM (g/dL)	2,8 (1,3-4,4)	5,9 (2,0-20,2)	5,2 (4,7-5,8)
CTE (10^6/uL)	0,39 (0,17-0,74)	0,38 (0,08-0,51)	0,3 (0,23-0,43)
CTL(10^3/uL)	3,95 (1,71-7,1)	9,6 (5,4-13,44)	7,07 (3,80-15,01)
PPT (g/dL)	5,92 (3,00-9,00)	6,69 (4,10-9,20)	4,47 (4,00-4,80)
GLIC (mg/dL)	25,27 (18,00-39,00)	53,33 (30,00-88,00)	42,50 (36,00-51,00)
HET (%)	15,84 (2,97-34,70)	12,83 (0,00-30,55)	21,29 (4,67-29,62)
EOS (%)	11,66 (0,00-31,10)	8,05 (0,00-30,55)	6,02 (1,86-9,73)
BAS (%)	1,89 (0,00-9,25)	0,75 (0,00-4,90)	0,78 (0,00-3,14)
MONO (%)	15,02 (2,94-38,00)	6,77 (0,00-23,52)	6,58 (3,70-12,59)
LINF (%)	55,61 (35,0-77,6)	71,58 (35,18-100,00)	65,31 (51,18-87,85)
THRB (%)	174,70 (66,00-2720,0)	42,00 0,00-147,00)	95,75 (32,00-182,00)

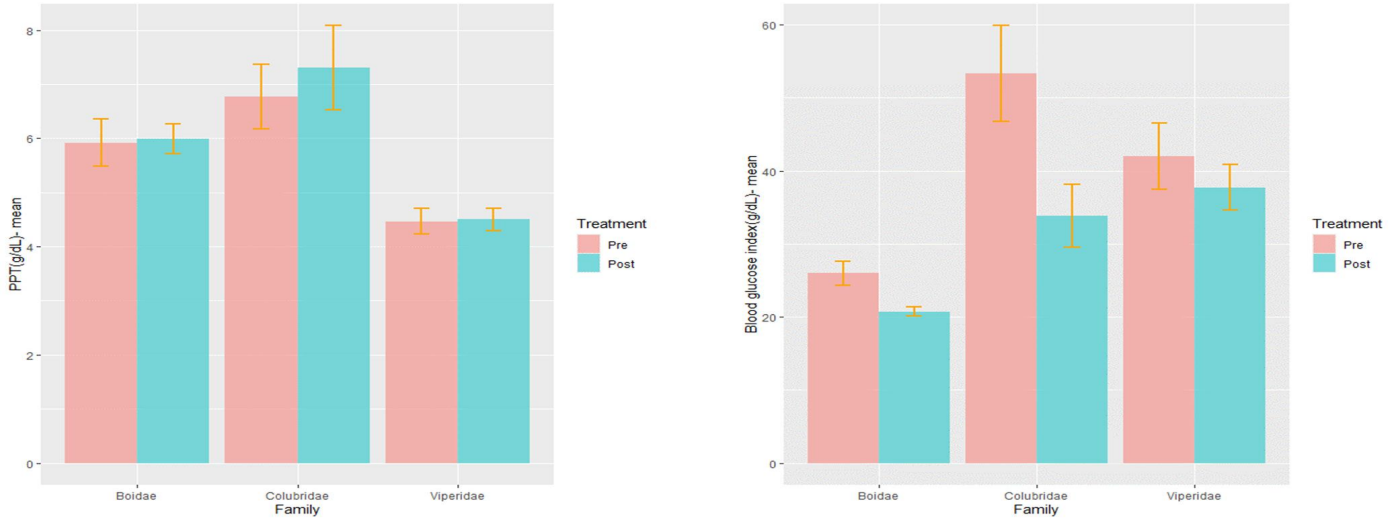
Ht. Hematocrit; Hb. Hemoglobin; VCM. Mean corpuscular volume; HCM. Mean corpuscular hemoglobin; CHCM. Mean corpuscular hemoglobin concentration, CTE. Total erythrocyte count, CTL. Total leukocyte count, PPT. Total plasma proteins, GLIC. Glucose, HET. Heterophils, EOS. Eosinophils, BAS. Basophils, MONO. Monocytes, LINF. Lymphocytes, THRB. Thrombocytes.

Figure 2. Mean blood cell countings, with standard error, before and after applying alternative therapy in the studied snake families. A. Hematocrit; B. Total erythrocyte counting; C. Total leukocyte counting; D. H:L ratio.



The biochemical analyses matrix (PPT + blood glucose index model) showed no significant difference before and after treatment within families (F=2,5633, R2=0.04652, p= 0.073). The GLM results showed that the blood glucose index depended on the feeding interval only in the Colubridae family (t= -2,580 and p=0,0134). Singly, the blood glucose index was significantly influenced by treatment (t=2,420 and p=0,01946), showing a significative mean reduction of 36% (Figure 3).

Figure 3. Alternative therapy on hematological profiles in captive snakes: Mean plasma biochemistry analyses, with standard error, before and after applying alternative therapy in the studied snake families. A. Total Plasma Proteins; B. Blood glucose index.



The Biometrics matrix (SVL + body mass) showed no significant difference after treatment (F = 0,0675, R2 = 0,00189, p = 0,948).

According to the presented analysis and data in Figures 2 and 3, Colubridae had an increased CTE, in addition to a reduced CTL, blood glucose index, and H:L ratio, which strongly suggested ameliorating in general physiology with a reduction of stress.

DISCUSSION

Ht had a slight overall reduction, indicating improved hydration levels, possibly by oral intake and cloacal absorption during the soaking baths. The cloacal reabsorption function has already been demonstrated in crocodilians[23], snakes[24], and terrestrial lizards[25]. Boidae and Viperidae had their Ht reduced after the treatment, possibly due to rehydration, but with no statistical significance.

The difference between Colubridae and Boidae paired blood counts was probably due to the rehydration and reduction in stress levels by the routine enrichment. The treatment relieved stress, providing wide space for movement, physical exercise, and practicing the typical behaviors of each species. Divers and Stahl (2019)[6] indicate soaking baths to stimulate water intake, excretion, and ecdysis. The benefits of hydrotherapy have been predominantly studied in humans, dogs, and horses, denoting protective effects on cardiovascular health, increases in blood count parameters and tissue oxygenation, reduction in cortisol levels, and pain control[12]; however, applying this knowledge, the Melbourne Zoo instituted bathing and swimming for snakes to prevent diseases resulting from a sedentary lifestyle[13].

Although the overall leukogram showed only a tendency to be different within families after the treatment, it was possible to observe a reduction in general and specific numbers of leukocytes. It is supposed to be associated with reducing acute and chronic stress levels for captivity and husbandry factors[6]. As expected, all families showed a reduction in leukocyte numbers, specially Colubridae, which seemed to be more stress-susceptible. According to Campbell (2015)[9], the high H:L ratio reveals increased cortisol and glucocorticoid blood circulation, indicating high-stress levels and low welfare. Decreased heterophils and increased lymphocytes were observed in all studied families corroborating the hypothesis that stress was being alleviated with treatment. There was a greater emphasis on the H:L ratio in Colubridae, reinforcing once more that these individuals are more susceptible to the maladaptive syndrome, which was supported by the statistical analysis. Although H:L was reduced in Viperidae, it showed no significance, most likely due to the low sample size. Monocytosis was observed in all families after the treatment and may be related to the positive regulation of monocyte colonies by hydroxylation of vitamin D3 in bioactive calcitriol (1,25-hydroxy-cholecalciferol) induced by UVB radiation[26]. Eosinophilia is frequently associated with allergies or parasite infections[9]; however, it was not observed in Colubridae. The observed lower numbers of eosinophils and basophils suggest a response to globally reduced leukocyte counts. Suggested basophilia pre-treatment may be seasonal or related to histamine release[27] once histamine renewal is associated with acute and chronic stress[28].

The Colubridae's high metabolic rates corroborate the blood glucose index to be significantly dependent on the feeding interval only in the studied snakes of this family[22]. A possible reason is a stress-induced hyperglycemia, once cortisol and catecholamines disturb glucose homeostasis, causing insulin resistance and increased gluconeogenesis[29].

CONCLUSIONS

The results confirm the initial hypothesis, assuming that a simple but systematic treatment that included sunlight exposure and immersion baths was efficient in improving multiple hematological parameters, reducing stress rates, especially chronic stress in Colubridae snakes, and enhancing the general physiological status.

Global blood cell countings and glucose index were demonstrated to be suitable markers to quantify the alternative therapy benefits. The Colubridae's high metabolism rates were also reflected in the blood glucose index feeding-interval-dependent, not withstand reaching a one-third reduction after treatment. The significant reduction in the H:L ratio confirmed positive regulation of homeostasis secondary to therapy application.

These results brought future perspectives on the low-cost improvement of welfare in captive snakes in northeastern Brazil.

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