

**PROFILE OF CONSTITUENTS ENZYMATIC SERUM IN TORTOISES *Chelonoidis carbonaria* (REPTILIA, TESTUDINIDAE) HELD IN CAPTIVITY**

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**SUMMARY:** The species *Chelonoidis carbonaria* was first described by Spix in 1824, popularly known as jabuti-piranga or tortoise-legged-red, it is a terrestrial animal found in various regions of South America. The objective of measuring the variations in blood serum enzyme constituents in *Chelonoidis carbonaria* captive using analytical methods routinely used. The animals are part of the breeding Study and Research Laboratory in wild animals UFU were subjected to manual restraint for blood collection, where they were collected, approximately 4 ml of venous blood of each animal in 5 mL disposable syringe with hypodermic needle disposable 27G after previous antiseptic site punctured with 70% alcohol. Constituents Enzymatic Serum were analyzed: Alanine aminotransferase; Aspartate aminotransferase; Total creatine kinase; Creatine kinase MB; Alkaline phosphatase (ALP); Gamma glutamyl transferase (GGT); Lactate dehydrogenase (LDH). The values for assessing blood serum enzyme constituents in *Chelonoidis carbonaria*, generally show no significant differences in values observed in other species of Testudines.

**Keywords:** Biochemistry, Blood, Enzymes, Metabolic Profile, Testudines.

**PERFIL ENZIMÁTICO SÉRICO DE JABUTIS *Chelonoidis carbonaria* (REPTILIA, TESTUDINIDAE) MANTIDOS EM CATIVEIRO**

**RESUMO:** A espécie *C. carbonaria*, conhecida popularmente como jabuti-piranga ou jabuti-de-patas-vermelhas é um animal terrestre encontrado em diversas regiões da América do Sul. Objetivou-se mensurar as variações dos constituintes enzimáticos séricos em *Chelonoidis carbonaria* em cativeiro utilizando métodos analíticos rotineiros. Os animais fazem parte do plantel do Laboratório de Estudo e Pesquisa em Animais Silvestres (LAPAS) da Faculdade de Medicina Veterinária (FAMEV), Universidade Federal de Uberlândia (UFU) e foram submetidos à contenção manual para colheita do sangue, onde foram colhidos, aproximadamente 4 mL de sangue venoso de cada animal. Foram analisadas as enzimas: Alanina aminotransferase (ALT); Aspartato aminotransferase (AST); Creatina quinase total (CK); Creatina quinase MB (CK-MB); Fosfatase alcalina (ALP); Gamma-glutamiltransferase (GGT); Lactato Desidrogenase (LDH). Os resultados obtidos para os constituintes enzimáticos séricos não têm médias aritméticas, desvios padrão e amplitude de variação. Os testes de comparação entre os valores entre macho e fêmea apresentaram  $p > 0,05$ , portanto, não há evidências de diferenças significativas entre os dois grupos estabelecendo-se assim, intervalos de confiança único. Os valores encontrados para avaliação dos constituintes enzimáticos séricos em *Chelonoidis carbonaria*, de forma geral, não apresentam significativas diferenças dos valores observados em outras espécies de Testudines.

**Palavras-chave:** Bioquímica Sanguínea, Enzimas, Perfil Metabólico, Testudines.

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## 1 INTRODUCTION

The *Chelonoidis* gender is represented by four species from South America, which are: *Chelonoidis carbonaria*, *Chelonoidis denticulata*, *Chelonoidis chilensis* and *Chelonoidis nigra*, with the last one being exclusive from Galápagos Island. In the Brazilian territory, the *Chelonoides carbonaria* species can be found in the northeast, southeast and south, and in the savanna areas, while the *Chelonoides denticulata* lives in areas with dense forests, which are most found in the northern region of Brazil (FLOSE, 2001; LE *et al.*, 2006).

These animals present a strong shell, relatively long convex from gray to brown or black, with symmetric, red, yellow and live colored drawings. The scales from the head and the paw are red, their cravats presents the same dark coloration, being concave on the males, higher than the ones presented on the females, with an average of 30.4cm, while the females have 28.9cm. They present a sexual maturity between 5 and 7 years, put 15 to 20 eggs by posture, and their reproduction period occurs on spring/summer (BARROS *et al.*, 2012).

The biochemical composition from the sanguine constituents of wild animals provide essential information about the health of these animals, as well as about their nutritional features, admitting, this way, an appreciation of the metabolic state of the animal tissues, supporting on the prediction of diseases, on the diagnoses, staging, monitoring, on the evaluation of the handling and adaptation of these captive animals (FLOSE, 2001).

The enzyme's analysis implies on the measurement of its catalytic activities, because they catalyze biochemical reactions converting a substrate in a product and the results expressed in terms of quantity about a present activity in a certain sample volume (THRALL, 2007). The enzymatic profile evaluations most used on the veterinary clinic are the alanina-aminotransferase (ALT) - , amylase, aspartate- aminotransferase (AST), lactate dehydrogenase (LDH), Creatino kinase MB (CK-MB), alkaline phosphatase (ALP), gamma- glutamyltransferase (GGT), total creatine kinase (CK), among others (GONZÁLEZ; SILVA, 2017).

Bearing in mind the influence of data over the serum enzymatic constituents in Testudines from the Brazilian fauna and the growing demand for the evaluation of the healthy state in *C. carbonaria*, it's needed to study the complementary diagnoses mean, where the hematological evaluation has a great highlight.

In this context, it was aimed the measurement of the serum enzymatic constituents variations in captive *Chelonoidis carbonaria*, using ordinary analytical methods, adding subsidies for the development of the clinical specie's pathology under discussion.

## 2 MATERIALS AND METHODS

For the study performance, 25 *C. carbonaria* models were used, being 15 males and 10 females, belonging to the Wild Animals Research Laboratory (LAPAS) roster, from the Veterinary Medicine College (FAMEVA), Uberlândia University (UFU), deriving from rescues and/or apprehensions made by the Environmental Military Police, or through donations. In the experimental group, it was only used specimens from the roster whose curvilinear length of the shell (CCC) was higher or equal to 25cm, so the sexually dimorphical features were glimpsed, since animals whose CCC presented are below 25cm don't have clear features (LEVINE; SCHAFFER, 1992). The experiment was carried out between May of June 2016. The climate in the city of Uberlândia is dry at this time of the year and the temperature varies from 15° to 26° C.

The animals were found housed in masonry enclosures, chlorinated water at will, maximum density of occupation of 10 animals/4m<sup>2</sup>, with solarium and shaded place, and daily feed with greenery, fruits, vegetables and commercial ration of canine with at least 18% of raw protein, three times a week.

This research is authorized by Chico Mendes Biodiversity Preservation Institute (ICMBio), by the system of Biodiversity Authorization and Information (SISBio) n° 46723-2 and approval by the Commission of Ethics on the Use in Animals (CEUA) of Federal University of Uberlândia (UFU), according to the protocol n° 035/15.

Before the harvest, all animals were physically evaluated, where it was observed: the presence of ectoparasites, corporal condition, tumors and cutaneous lesions. It was selected animals considered healthy, without deformities in the shell and/or sexual dimorphism was defined (LEVINE; SCHAFFER, 1992).

The red-foot tortoises were manually contained for blood collection. Nearly 4 mL of venous blood was collected in each animal, with a disposable syringe of 5 mL with a disposable hypodermic needle 27G after a prior antiseptis of the punctured spot with 70% alcohol. The harvests were primarily made through a puncture of the caudal sub-shell vein. When this via wasn't easily accessed, it was opted the sub-shell cranial vein (CAMPBELL, 1996), respecting the maximum corresponding volume of 10% of the total blood volume in each animal (5 to 8% of its living weight) (CAMPBELL, 1996).

The samples were wrapped in silicone tubes without anticoagulant (Vacutainer Becton Dickson), identified immediately after the blood clot retraction, to sediment the elements configured on the blood and acquisition of serum. The samples were submitted to centrifugation (720g), during five minutes (ALMOSNY; MONTEIRO, 2007), in a centrifuge Excelsa Baby (FANEM, model 208N). The serum was separated by aspiration in aliquot, secured in microtubes 05ml, type *Eppendorf*, and refrigerated in temperatures of 2 to 8°C, by a period not above 8

hours. Up next, they were forwarded in isothermal boxes with ice to the Clinical Analysis Laboratory of Patos de Minas University, where they were processed.

The *Chelonoidis Carbonaria* blood samples analysis were performed in an automatic analyzer FLEXOR XL (Vital scientific/Elitech), using reagents from the brand Elitech Clinical Systems, following the manufacturer's recommendations. At a temperature of 37°C, after a previous calibration of the equipment using a multiparametrical calibrator (Elitech Elical II) and benchmarking with a control serum (Control Lab). It was determined, from each sample, the serum values described on Board 1, with respective methodologies utilized to determine their serum levels.

It was performed the data analysis, also considering each gender according with what was proposed by (VIEIRA *et al.*, 2002) and (AYRES, 2007), and using the BioEstat program (VIEIRA *et al.*, 2002), and on the Action app® which uses the program R®

### 3 RESULTS AND DISCUSSION

The results obtained for serum enzymatic constituents in *Chelonoidis carbonaria* are disposed on Table 2, containing arithmetic averages, standard deviations and range of variation. The comparison tests between the male and female values were considered  $p > 0,05$  (table 1), therefore, there isn't evidences about significant differences between the two groups, establishing, thus, unique trust intervals.

**Board 1** - Serum enzymatic constituents, with the respective methodologies used for biochemical analysis.

BIOCHEMICAL CONSTITUENT	METHODOLOGY
Alanine aminotransferase (ALT) (EC 2.6.1.2) <sup>1</sup>	Kinetic UV-IFCC without pyridoxal phosphate
$\alpha$ -Amylase (EC 3.2.1.1) <sup>1</sup>	Enzymatic kinetic, substrate: CNP-G3(2-cloro-4-nitrofenol- $\alpha$ -maltotrioxide)
Aspartate aminotransferase (AST) (EC 2.6.1.1) <sup>1</sup>	Kinetic UV-IFCC without pyridoxal phosphate
Total Creatine kinase (CK) (EC 2.7.3.2) <sup>1</sup>	Kinetic UV-IFCC
Creatine kinase MB (CK-MB) (EC 2.7.3.2) <sup>1</sup>	Kinetic UV-IFCC Immune-inhibition
Alkaline phosphatase (ALP) (EC 3.1.3.1) <sup>1</sup>	Enzymatic kinetic, based on the DGKC and SCE methods
Gamma- glutamyltransferase (GGT) (EC 2.3.2.2) <sup>1</sup>	Modified Szasz IFCC
Lactate dehydrogenase (LDH) (EC 1.1.1.28) <sup>1</sup>	Kinetic UV-IFCC (substrate: lactate)

UV = ultraviolet.; IFCC = International federation of clinical chemistry; <sup>1</sup> Nomenclature according with the IUPAC-IUBMB (Committee of the International Union of Biochemistry and Molecular Biology) (IUBMB, 2020).

The use of enzymes to diagnose is commonly used, since the clinic value resulting from

the study about the enzymatic profile, from the measurement of several enzymes present on the animals' bloodstream (LITZGUS; HOPKINS, 2003).

About the enzymes (table 2), it was observed levels of alkaline phosphatase (ALP) of *C. carbonaria* of 72,04 ( $\pm 35,67$ ) U/L. The normal ALP values in reptiles are variable and depend on the species and age group, because the serum activity of this enzyme is higher in younger animals, in corporal growth, comparing with adult animals (MARTÍNEZ-SILVESTRE *et al.*, 2013).

**Table 1** – Averages per gender, statistical tests and p-value from the captive *Chelonoidis carbonaria* sanguine biochemical constituents (n=25), Uberlândia-MG, 2015

Biochemical Constituent	Average by sex		Test	p-value
	Male	Female		
Amylase (U/L)	4,20	3,10	Wilcoxon	0,248
ALP (U/L)	76,06	84,60	Wilcoxon	0,677
LDH (U/L)	167,90	215,80	Wilcoxon	0,304
AST (U/L)	90,33	87,50	Wilcoxon	1
ALT (U/L)	10,13	6,80	Wilcoxon	0,887
CK (U/L)	1157,30	1567,10	Wilcoxon	0,368
CK-MB (U/L)	1951,53	2650,90	Wilcoxon	0,237

ALP - Alkaline phosphatase. LDH - Lactate dehydrogenase. AST - aspartate aminotransferase. ALT - Alanine aminotransferase. CK-MB - Creatine kinase MB. CK - Total creatine kinase. U/L: Unity per liter.

**Table 2** – Averages, standard deviations, variations range and trust intervals of 95% for the demographic average of captive *Chelonoidis carbonaria* serum enzymes (n=25), Uberlândia-MG, 2015

Enzymatic constituent	Unity	Average	Standard deviation	Variation range		IC 95%	
				Minimal	Maximum	Inferior limit	Superior limit
Amylase	U/L	3,50	2,10	1,00	8,00	2,70	4,20
ALP	U/L	72,04	35,67	29,00	155,00	57,80	83,30
LDH	U/L	187,10	105,90	47,00	421,00	143,30	230,80
AST	U/L	71,05	36,06	18,00	150,00	55,05	87,03
ALT	U/L	4,80	2,57	2,00	10,00	3,70	5,70
CK	U/L	1076,00	913,00	99,00	3126,00	743,40	1391,20
CK-MB	U/L	1837,00	1815,90	189,00	5814,00	1140,70	2440,60

ALP - Alkaline phosphatase. LDH - Lactate dehydrogenase. AST - aspartate aminotransferase. ALT - Alanine aminotransferase. CK-MB - Creatine kinase MB. CK - Total creatine kinase. IC 95% - Trust interval of 95% for the demographic average

U/L: Unity per liter.

The ALP levels 73,00 ( $\pm 40$ ) U/L in *C. carbonaria* (CARPENTER, 2005) compares to what was found on the current study: a near interval for *Phrynosoma geoffroanus* (Cágado-de-barbicha - Schweigger, 1812) (21 a 97) U/L (GRADELA, *et al.*, 2017). However, inferior values were found in demographics of *C. carbonaria* e *C. denticulata* (Yellow-footed tortoise) 41,73 ( $\pm 22,35$ ) U/L (SANTOS, *et al.*, 2011).

ALP levels superior to the ones presented were in *Podocnemis expansa* 131,13 ( $\pm 59,96$ ) U/L (SANTOS, *et al.*, 2005), also in *Podocnemis expansa* 107,0 ( $\pm 45,72$ ) U/L (MUNDIM, 1999), in *Geochelone spp.* 111,0 $\pm$ 46,0 U/L (SAMOUR, 1986) for other tortoises from the *Chelonoides* gender *C. elegans*: 173,0 ( $\pm 108,0$ ) U/L and *C. radiata*: 93,00 (72-100) U/L (CARPENTER, 2005).

The Lactate dehydrogenase levels (LDH) for *C. carbonaria* were 187,10 ( $\pm 105,90$ ) U/L. (CARPENTER, 2005) described averages for tortoises of the *Chelonoides* gender (*C. carbonaria* 428 ( $\pm 228$ ) U/L; *C. elegans*: 667 ( $\pm 297$ ) U/L and *C. radiata*: 402 (213-592) U/L), being superior than the value found on the current study. Superior averages from other Testudines species were described in *Testudo radiata*. However, the average found for *C. carbonaria* finds itself on the intervals referred in *Gopherus polyphemus* (17,8 a 909,9 U/L) (TAYLOR e JACOBSON, 1992) in *Phrynops geoffroanus* (68 a 356) U/L (GRADELA, *et al.*, 2017).

The serum levels from LDH superior to 700 U/L are associated in reptiles, unspecific tissue lesions, estomatites, gastrointestinal obstructions, cloacal prolapse, renal damage, among other; in LDH levels superior to 1000 U/L, it may be related to damages on the hepatic and muscular tissues (WAGNER; WETZEL, 1999).

The aspartate aminotransferase values (AST) in *C. carbonaria* 71,05 ( $\pm 36,06$ ) U/L were inferior to the ones 214,0 ( $\pm 152$ ) U/L (CARPENTER, 2005), for the same species, 100,56 $\pm$ 14,26 U/L (SANTOS, *et al.*, 2011), demographic of tortoises from the same species and gender. In studies made with *Podocnemis expansa*, superior averages were 194,56 ( $\pm 154,27$ ) U/L (SANTOS, *et al.*, 2005) and 223,0 ( $\pm 129,61$ ) U/L, (CAMPBELL, 1996, FONSECA *et al.*, 2016) in *Malacochersus tornieri* 157,0 U/L (22). Similar results in *Chelonoidis radiata* (Madagascar tortoise) 73,00 (42-134) U/L (CARPENTER, 2005) and in *Testudo radiata* 42,0 a 134,0 U/L (MARKS e CINTINO, 1990). Interval inferior to the AST activity was found for *Phrynops geoffroanus* (15 to 45) U/L (GRADELA, *et al.*, 2017).

Usually, the AST activity on the serum or plasma of healthy reptiles is inferior to 250 U/L (MARTÍNEZ-SILVESTRE *et al.*, 2013). The increase of the AST serum activities may suggest a hepatobiliary disease, but it must be considered that the hepatic tissue of some reptiles presents few activities from this enzyme and, therefore, is not a reliable enzyme to detect injuries on the hepatic tissue (CAMPBELL, 2006). Furthermore, it's an enzyme present in a lot of corporal tissues, not being its specific organ determination, although the ALT concentration are especially significant in the liver, kidneys and in the heart (WAGNER; WETZEL, 1999).

As for the alanine- aminotransferase values (ALT) found for *C. carbonaria* were of 4,80 ( $\pm 2,57$ ) U/L and it's close to the averages in (*C. carbonaria* and *C. denticulata*) 3,67 ( $\pm 3,21$ )

U/L (SANTOS, *et al.*, 2011), in *Podocnemis expansa* 4,04 ( $\pm 2,99$ ) U/L (SANTOS, *et al.*, 2005). Superior averages were in *Podocnemis expansa* 5,93 ( $\pm 6,47$ ) U/L (FONSECA, *et al.*, 2016), and *Geochelone spp.* 7,0 ( $\pm 9,0$ ) U/L (SAMOUR, 1986; CARPENTER, 2005), *C. elegans*: (8) ( $\pm 10$ ) U/L, *C. radiata*: 9,0 ( $\pm 11$ ) U/L. and *C. carbonaria* 9,0 ( $\pm 5$ ) U/L and in *Phrynops geoffroanus* (11 to 39) U/L (GRADELA, *et al.*, 2017), but they're found within the values established for reptiles, which is inferior to 20 U/L (MARTÍNEZ-SILVESTRE *et al.*, 2013).

In *C. carbonaria*, the average of amylase found in the sanguine serum was of 3,50 ( $\pm 2,10$ ) U/L. Discrepant results, with markedly superior averages for *Phrynops geoffroanus* (193 a 794) U/L (GRADELA, *et al.*, 2017). In reptiles, the amylase is used on the diagnoses of pancreatic lesions (25). However, it's important to emphasize that no value referring to this enzyme, for the Chelonoides gender, was found in literature.

The creatine kinase (CK) average values observed in this work for red-footed tortoise 1076,00 ( $\pm 913,00$ ) U/L, were higher than the ones in *C. carbonaria* 754 ( $\pm 599,0$ ) U/L (CARPENTER, 2005), and *C. radiata* 723 ( $\pm 437$ ) U/L, on the next averages established by the author for *C. elegans* 1099 ( $\pm 1724$ ) U/L. CK averages were described to the Testudines genders in *Gopherus Polyphemus*, where they established an interval of 32 to 628 U/L (TAYLOR e JACOBSON, 1992), and for *Phrynops geoffroanus* 281 to 888 U/L (GRADELA, *et al.*, 2017), in *Malacochersus tornieri* in natural habitat (80 a 2155) U/L (RAPHAEL, *et al.*, 1994). It was observed that the referred authors described different intervals than the intervals described in this study. In relation to the creatine kinase MB isoenzyme (CK-MB), it was observed averages of 1837,00 ( $\pm 1815,90$ ) U/L in *C. carbonaria*.

It's possible to observe that there were great variations from the standard deviations for CK-MB. Due that, the correlation of the results obtained with the study about the animals' health is diffculted. Furthermore, the CK and CK-MB enzymatic activity in Testudines seems to be insufficient studied yet; no value referring to CK-MB in Testudines was found in literature.

Is CK dosage in Testudines, also found high standard deviations (CARPENTER, 2005; TAYLOR; JACOBSON, 1992; RAFAEL, *et al.*, 1994). Drastic variations from the CK levels from animal to animal are expected for reptiles (WAGNER; WETZEL, 1999).

Considered an enzyme specifically muscular, its serum quantification is a key diagnostic tool to evaluate injuries in the skeletal and cardiac muscular cells, such as in pathological processes, such as: synthetic infections that affects the muscles, tissue necrosis, hepatic diseases; in these cases, they can present a high elevated activity. Increments might occur, yet, in the outcome from muscular lesions with the sanguine collection, as well as in animals that suffer convulsions or due the stress during their capture and immobilization, in gestational and *Estasi follicular* processes (MARTÍNEZ-SILVESTRE *et al.*, 2013).

The serum measurement of a specific muscular enzyme, such as the CK, devotedness contributes for the clinical differentiation of damages on the muscular hepatic tissue. Bearing in mind that the AST, LDH and ALP plasmatic activity are not organ-specific for the muscle, with the ability to increase in hepatobiliary diseases. Thus, the increase of the LDH, AST and ALP serum activity with normal levels of CK may indicate a hepatic lesion, while their simultaneous increase might suggest pathological states where both tissues, muscular and hepatic, are lesioned, like it occurs in traumas and septicemias (CAMPBELL, 1996, CAMPBELL, 2006). However, it's necessary to consider that to diagnose a hepatic disease in reptiles is complex and that besides the biochemical trials, the utilization of other diagnostic methodologies, such as examination of images and the liver *histopatologios* biopsy, are necessary (HERNANDEZ-DIVERS; COOPER, 2000). It stands out albumin is very precise and sensitive and that, therefore, it should be the method of choice for measurement of serum albumin (PRICE, 1979).

The gamma- glutamyltransferase (GGT) levels in the current study couldn't be punctually quantified by the methodology and used biochemical analyzer. To measure the GGT levels, using serum from the 25 *C. carbonaria* studied specimens, the values issued by the equipment were inferior to 1,00 U/L, not demonstrating the value quantitatively obtained for each dosage.

According to the reagent manufacturer's guidelines (Elitech), used to determine the GGT levels in this experiment, it the linear reagent in intervals of 15 to 1000 U/L, and the detection limit is 2 U/L. Probably due this, the GGT levels in *C. carbonaria* couldn't be detected.

An enzymatic activity too low for GGT was 0,60 ( $\pm 0,84$ ) U/L (SANTOS, *et al*, 2005), and 0,79 $\pm$ 1,12 U/L (MUNDIM, 1999) when evaluating the sanguine biochemical constituents from *Podocnemis expansa* (Amazon tortoise). In the *C. carbonaria* and *C. denticulata* red-footed-tortoise demographics, (SANTOS, *et al.*, 2011) also described low GGT averages of 1,31 ( $\pm 2,70$ ) U/L.

The GGT enzymatic activity quantification in reptiles isn't a very sensible test, and its diagnostic utilization is controversial: the reptiles' hepatic and renal tissues present an activity of these enzymes, but the GGT levels doesn't increase in pathologies that may attack these organs (9).

The differences occurred among the serum biochemical constituents' levels from the *C. carbonaria* found in this study, when confronted with the data referred in the literature for other Testudines demographics probably derive from variability factors, such as biological rhythms and constitutional factors, such as gender, age, species, even with a taxonomic proximity from the outcome of genetic variety and external factors, such as the handling system and stress during the manipulation of animals (SANTOS, *et al*, 2005).

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The diets between captive and free Testudines might affect the values of several biochemical sanguine components. Furthermore, there's a higher food availability for captive animals, and they're normally less physical active (CHRISTOPHER, 1999).

Other external factor to be considered is the environmental temperature. Since the Testudines are ectothermic animals, the environmental temperature influents their metabolism, therefore an increase on the temperature results in an increase of food, in the digestion metabolism and on the digestive efficiency, consequently altering the serum concentrations from the sanguine biochemical constituents (ZIMMERMAN; TRACY, 1989; LITZGUS; HOPKINS, 2003). Besides, there might be factors inherent to the differences in the experimental and methodological planning. Such variability factors might be also correlated with a great range of variation that some evaluated constituents presented, a fact that can also be observed on the results of the discussed researches.

#### 4 CONCLUSIONS

The values found to evaluate the serum enzymatic constituents in *Chelonoidis carbonaria*, in a general way, doesn't presented significant differences of the observed values in other Testudines species. There weren't any significant differences between the male and female averages for serum enzymatic constituents. The use of analytical methodologies and dedicated reagents for biochemical exams seems to be proper to determine the serum enzymatic constituent profiles. However, the methodological technique uses to determine the gamma-glutamyltransferase enzyme level (GGT) showed to be inadequate and the obtained values for creatine kinase (CK) and creatine kinase MB (CK-MB) enzymes presented great variations from the standard deviations. The results obtained in this study might contribute to a better comprehension about the serum biochemical parameter of these tortoises, as well as to ease their interpretation. They might also subsidize other studies that search reference values for *C. carbonaria*.

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