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Full Length Article

Hematological and Plasma Biochemical Parameters of Chinese Soft-Shelled Turtle during Hibernation and Non-Hibernation

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Abstract

Hibernation is a natural process in many mammals to maximize survival during extreme environmental conditions. The ability of hibernating animals to recover from hibernation with no obvious sign of injury makes them excellent model in natural protecting mechanism, which has great interest in medical research. Hematologic and biochemical analysis are an important tool which are used for the assessment of the health, physiological, behavioral, reproductive status and diagnosis for the disease for clinical and laboratorial point of view. The main objective of current study was to identify the hematological and plasma biochemical values of Chinese soft-shelled turtle during hibernation and non-hibernation. These results showed the hematological values of hemoglobin (Hb), red blood cells (RBCs), packed cell volume (PCV), heterophils, lymphocytes, basophils, and mean corpuscular volume (MCV), and plasma biochemical values of aspartate aminotransferase (AST), calcium, phosphorus, glucose, total protein, uric acid, creatinine, triglyceride, cholesterol, potassium and chloride were significantly increased during non-hibernation as compared to hibernation. However, the hematological values of white blood cells (WBCs) and monocytes, and plasma biochemical values of blood urea nitrogen (BUN) and sodium level were significantly increased during hibernation as compared to non-hibernation. This information can serve as baseline reference data for future assessment study during hibernator and non-hibernator behavior of turtle.

Keywords: Hematology; Plasma biochemistry; Hibernation; Non-hibernation; Chinese soft-shelled turtle

Introduction

Chinese soft-shelled turtles (*Pelodiscus sinensis)* are an important aquatic reptile and are highly valued cultured animal species in Asian countries, mostly in China (Yin *et al.* 2005; Yousaf and Mahmood 2018). This species plays an essential role in nutrition and pharmacological values (Ahmed *et al.* 2017). It has been also considered a product with medical benefits in Chinese tradition for a long time (Zou *et al.* 2011). The bile and gallbladder are added to wine, they have the effect for to remove heat, detoxifying and stopping cough (Teng *et al.* 2012). In recent decades the population have sharply reduced due to unregulated harvesting and used as food consumption (Que *et al.* 2007). However, its estimate population is 300 million (Haitao *et al.* 2008). The life cycle of *P. sinensis* belongs to two periods, hibernation and non-hibernation. Hibernation is an adaptation survival approach that occur at physiological, behavioral and molecular levels to survive life during winter months when resources are insufficient or unpredictable (Pan 2018). It is generally associated with a profound reduction in core body temperature, metabolic rate and other different physiological parameters (Heldmaier *et al.* 2004).

Blood is the connective tissue in which all the blood cells (RBCs, WBCs and platelets) are suspended in the plasma. Plasma is the fluid portion of the blood whereas when remove the fibrinogen from the plasma the fluid is called as Serum (Bolten *et al.* 1992). The research has indicated that, according to the morphological variation of blood cells among their order even in the family members made them heterogeneous groups of vertebrates. The cells which are identified in the turtles are: erythrocytes, leucocytes (heterophils, basophil and eosinophil) and thrombocytes (Arıkan and Çiçek 2010). Health monitoring different physiological processes and diagnosis of disease of animals is widely analyzed by hematological examination (Christopher *et al.* 1999; Campbell and Ellis 2007; Tumkiratiwong *et al.* 2012; Lisičić *et al.* 2013). Moreover, hematological studies are also used for the determination of the taxonomical relationship of species (Troiano *et al.* 1997, 2000). Hematological examination is widely used in many wild species of animals especially for those which are threatened or endangered and which might play important role in evaluating ecosystem and health (Tamukai *et al.*

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2011). Mentally, physiologically and reproductively animals are highly sensitive to their environment so they have complex and sensitive response against stress to protect from any change in the environment otherwise it is harmful to the animals (Kumar-Velmurugan *et al.* 2012). Unlike most of mammals, the blood of reptiles has nucleated RBC, nucleated platelets, neutrophils, eosinophils, basophils, monocytes and lymphocytes. Hematological study is used to identify these hematological disorders like anemia, hemopoietic disorder, hemostatic alteration and bacterial, viral and parasitic infection (Campbell 2006). Studies on reptile's hematology are started from 1842 (Knotková *et al.* 2002) but now, there is increase in the interest in the reptile's hematology, which work as a tool for their trading, conservation and for clinical pathological research of the reptiles (Gulliver, 1842; Hartman and Lessler 1964; Hutchison and Szarski 1965; Szarski and Czopek 1966; Uğurtaş *et al.* 2003; Arıkan *et al.* 2004; Campbell 2006; Campbell 2006) However, the hematology of Chinese soft-shelled turtle during different season remains unclear.

The physiological status of an animals is identified by biochemical profiles of blood. The environmental condition, age, gender, nutrition, use of anesthetics and physiological status such as dehydration and estrus are the major factor which affect biochemical values in plasma (Dessauer 1970; Samour *et al.* 1986; Lawrence 1987; Campbell 2006; Chung *et al.* 2009). Many studies have been conducted on hematological values in turtles, mainly marine (Bolten and Bjorndal 1992) and terrestrial turtles (Dickinson *et al.* 2002) and very few in freshwater turtles (Brenner *et al.* 2002; Omonona *et al.* 2011). Limited research studies have been reported in Chinese soft-shelled turtle; conservation and nutrition (Liu *et al.* 2015). No information exists on the hematology and plasma biochemistry of the Chinese soft-shelled turtle. The objective of this study was to identify the accurate values of the hematology and blood biochemical values of *P. sinensis* during hibernation and non-hibernation, which should be beneficial for clinical and laboratorial point of view.

Materials and Methods

Animals

All techniques with the Chinese soft-shelled turtles (*P. sinensis)* were conducted according to Animal Research Institute Committee guidelines of Nanjing Agricultural University, China. Total (20) mature *P. sinensis*, age of (4–5) years old were selected for current research, and turtles were brought from aquatic pond in Nanjing, Jiangsu Province of China during hibernation, month of March and February (n = 10) and non-hibernation, the month of August and September (n = 10). All adult *P. sinensis* (mean ± SD) body weight 1.45 ± 0.10 kg. Before sampling, the temperature was noted, during the hibernation (4 to 8°C) and non-hibernation (20 to 25°C).

Hematological and plasma chemical assays

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (20 mg/animal) and 1.5 mL of blood was being collected by jugular vein by using syringe with needle of 29 guage ×12.7 mm. Two tubes were taken for the blood collection; lithium heparin tube and silica gel tube (Serum Separating Tubes), 0.75 mL of blood was collected in each tube, and the blood which was placed in the lithium heparin tube was used for the hematological assay while the blood which was placed in the Silica Gel (Serum Separating tube) was used for the separation of the serum from the whole blood for the biochemical assay. Hemocytometer and Natt and Herrick's solution were used for the counting of RBC and WBCs (Campbell 2006). To determine the packed cell volume (PCV), blood was filled with micro-hematocrit tube having ammonium heparin and centrifuged at 15,000/g for 3 min. The cyanmethomoglobin method used for Hb. An autoanalyzer was used for the biochemical assay (Cobas Integra 800; Roche, Mannheim, Germany).

Statistical analysis

Statistically analyzed with GraphPad Prism 7.0 software and presented by Origin Pro 2018. The results are shown as the mean ± SEM. The statistical significance difference between two groups was analyzed by student *t*-test (two-tailed analysis) ($P < 0.05$).

Results

Hematological assay

The mean values for the hematological values of *P. sinensis* during hibernation and non-hibernation are given in (Table 1 and 2). During non-hibernation, the Hb, RBCs, PCV, heterophils, lymphocytes, basophils, and MCV levels were significantly higher as compared to hibernation. However, the value of WBCs and monocytes count was significantly higher in hibernation than non-hibernation. There was no significant difference in the eosinophils values.

Biochemical assay

During the non-hibernation, the AST, calcium, phosphorus, glucose, total protein, uric acid, creatinine, triglyceride, cholesterol, potassium, and chloride values were significantly higher than hibernation. However, the value of BUN and sodium was significantly higher during hibernation compared with non-hibernation. There was no significant difference in the values of LDH and CK during hibernation and non-hibernation. The serum biochemical values of the *P. sinensis* during hibernation and non-hibernation are given in (Table 3–4).

Table 1: Hematological values of *P. sinensis* in hibernation and non-hibernation

Parameters	Hibernation	Non-hibernation
Hb (g/dL)	2.8-3.2	3.1-3.9
RBCs (10^6/μL)	0.40-0.60	0.66-0.78
WBCs (μL)	14240-16950	12299-13988
PCV (%)	14-18	18-21
Heterophils (μL)	42-60	55-65
Lymphocytes (μL)	18-22	20-28
Basophils (μL)	10-13	12-18
Eosinophils (μL)	0-1	0-1
Monocytes (μL)	6-8	2-8
MCV (fL)	170-204	600-525

Hb-hemoglobin, RBCs- red blood cells, WBCs- white blood cells, PCV- packed cell volume or hematocrit, MCV-mean corpuscular volume

Table 2: seasonal difference in hematological values of *P. sinensis* in hibernation and non-hibernation

Parameters	Hibernation	Non-hibernation
Hb (g/dL)	3.02 ± 0.0442	3.36 ± 0.113*
RBCs (10^6/μL)	0.500 ± 0.044	0.716 ± 0.024**
WBCs (μL)	15500 ± 430**	13500 ± 323
PCV (%)	15.8 ± 0.917	19.6 ± 0.678*
Heterophils (μL)	48.0 ± 3.16	60.0 ± 1.70*
Lymphocytes (μL)	19.2 ± 1.02	24.8 ± 1.62*
Basophils (μL)	11.4 ± 0.60	15.2 ± 1.02*
Eosinophils (μL)	0.800 ± 0.20	0.800 ± 0.20
Monocytes (μL)	7.20 ± 0.49*	4.00 ± 1.10
MCV (fL)	507 ± 27.1	601 ± 27.6*

Values are mean ± SEM (n = 10) (*) high significance value

Table 3: Biochemical values of *P. sinensis* in hibernation and non-hibernation

Parameter	Hibernation	Non-Hibernation
AST (U/L)	78-130	110-130
BUN (mg/dL)	8-14	6-11
LDH (IU/L)	1,383-1,400	1,395-1,490
Calcium (mg/dL)	8.1-10.2	11.2-15.3
Phosphorus (mg/dL)	2.8-3.0	3.8-5.1
Glucose (mg/dL)	72-90	96-118
Total protein (g/dL)	3.0-3.8	4.1-4.6
CK (U/L)	350-388	359-402
Uric acid (mg/dL)	0.6-0.9	0.8-1.2
Creatinine (mg/dL)	0.09-0.1	0.19-0.2
Triglyceride (mg/dL)	170-185	190-206
Cholesterol	120-127	130-140
Sodium (mmol/L)	126-132	112-120
Potassium (mmol/L)	3.0-3.9	4.0-4.8
Chloride (mmol/L)	92-102	101-118

AST, aspartate aminotransferase, BUN-blood urea nitrogen, LDH-lactate dehydrogenase, CK-creatine kinase

Discussion

Chinese soft-shelled turtles are one of the aquatic animals that belongs to the reptilia class. Hibernation period of this species starts from the early days of December to March (Yoshii and Mizushima 2017) and non-hibernate (reproductive phase) from May to October (Hu *et al*. 2016). Therefore, we selected those months in which the both periods are at the peak of their activity to investigate the hematological and biochemical parameters in the blood in these two periods.

Table 4: seasonal difference in biochemical values of *P. sinensis* in hibernation and non-hibernation

Parameter	Hibernation	Non-Hibernation
AST (U/L)	93.6 ± 9.43	120 ± 4.49*
BUN (mg/dL)	12.4 ± 1.17*	8.60 ± 0.87
LDH (IU/L)	1390 ± 3.17	1430 ± 18.7
Calcium (mg/dL)	9.58 ± 0.389	12.6 ± 0.733**
Phosphorus (mg/dL)	2.916 ± 0.03816	4.300 ± 0.3066**
Glucose (mg/dL)	86.00 ± 3.521	111.0 ± 4.050**
Total protein (g/dL)	3.640 ± 0.160	4.220 ± 0.096*
CK (U/L)	368 ± 8.45	377 ± 7.55
Uric acid (mg/dL)	0.840 ± 0.060	1.12 ± 0.080**
Creatinine (mg/dL)	0.0967 ± 0.002	0.158 ± 0.014**
Triglyceride (mg/dL)	180 ± 2.77	196 ± 3.92*
Cholesterol	125 ± 1.28	132 ± 1.94*
Sodium (mmol/L)	130 ± 2.14**	118 ± 1.47
Potassium (mmol/L)	3.50 ± 0.173	4.24 ± 0.160*
Chloride (mmol/L)	98.4 ± 1.83	113 ± 3.06**

Values are mean ± SEM (n = 05) (*) high significance value

Hematological and biochemical parameters are very useful tools in measuring the physiological status of turtle because they may provide information for diagnosis and prognosis of diseases. Such tools have been used physiological disturbance indicators of diseases (Tavares-Dias *et al.* 2008). In present study the level of AST, glucose, total protein and cholesterol was significantly increased during non-hibernation as compared to hibernation, which may due to their high reproductive activity and egg production during this period (Cheng *et al.* 2010). During the late hibernation period, the turtles are prepared for reproduction, and during mating stresses and tissue injuries may also cause the increase level of AST, glucose (Zaias *et al.* 2006), calcium, cholesterol, and triglycerides (Christopher *et al.* 1999). Moreover, increased AST, creatinine and CK level in the non-hibernation period, it may show that in this period there is increased above defined activities and having higher metabolic activities (Dickinson *et al.* 2002). Hibernation is energy saving program of reptiles, they produce and store nitrogenous waste in the bladder. During hibernation BUN values were increased, which cause increase in the osmolality result in increase in prevention of water loss. After the hibernation period there is decreased the BUN level along with increase in the water intake (Dessauer 1970; Christopher *et al.* 1994; Chung *et al.* 2009). The concentration of phosphorous and uric acid in summer is increased due to increased food consumption and high level of nitrogenous waste being discharged during hot seasons (Campbell 2006). Reptiles absorb dietary sodium through intestine and excrete through kidney according to their requirement of the body. In reptiles, sodium and potassium play an important role in the activation of angiotensin-rennin system for the osmoregulation (Campbell 2006). In present study the sodium level increased during hibernation. sodium level enhanced in winter because turtle live less time in water and more time on cold land. Potassium concentration can be affected by intestinal potassium loss and renal secretion (Campbell 2006). The significant lower biochemical and

glucose concentration during hibernation may have been due to low activity and metabolic rate.

In the present study the level of Hb, RBCs, PCV, heterophils, lymphocytes, basophils, and MCV was significantly higher during non-hibernation as compared to hibernation. However, the values of WBCs and monocytes count were significantly higher during hibernation compared with non-hibernation. The enhanced level of heterophils during the non-hibernation (summer) which may be due to increase in the physical activity, surviving, feeding, reproductive behavior, and fighting which may cause bruises and inflammation, result in increase in the heterophils count (Campbell 2006). At high temperature environment the values of RBCs, heterophils and lymphocytes are significantly increased. But, the values of WBCs and monocytes are decreased (Yu *et al.* 2013). Previously have been reported that the increase in the plasma enzyme in the reflection of metabolic activity (Christopher *et al.* 1999). During hibernation of Chinese soft-shelled the physiological parameters such as, respiratory, heart rate and temperature, and metabolic rate is reduced as compared to non-hibernation (Vistro *et al.* 2019).

Conclusion

This study revealed that 20 healthy different turtles blood showed significant difference in hematological and biochemical values during hibernation and non-hibernation. The hematological and blood biochemical data can be used for evaluating the health status and identification of hibernating and non-hibernating behavior of Chinese soft-shelled turtle. Further studies are required to identify whether other factors-age, sex and captivity.

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References

Ahmed N, P Yang, Y Huang, H Chen, T Lui, L Wang, F Nabi, Y Liu, Q. Chen (2017). Entosis acts as a novel way within Sertoli cells to eliminate spermatozoa in seminiferous tubule. *Front Physiol* 8:1–11

Arıkan H, K Çiçek (2010). Morphology of peripheral blood cells from various species of Turkish Herpetofauna. *Acta Herpetol* 5:179–198

Arıkan H, Y Kumlutaş, O Türkozan, İ Baran, Ç Ilgaz (2004). The morphology and size of blood cells of some viperid snakes from Turkey. *Amphibia–Reptilia* 25:465–470

Bolten AB, KA Bjorndal (1992). Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the southern Bahamas: size-specific and sex-specific relationships. *J Wildlife Dis* 28:407–413

Bolten AB, ER Jacobson, KA Bjorndal (1992). Effects of anticoagulant and autoanalyzer on blood biochemical values of loggerhead sea turtles. *Vet Res* 53:1–4

Brenner D, G Lewbart, M Stebbins, DW Herman (2002). Health survey of wild and captive bog turtles (*Clemmys muhlenbergii*) in North Carolina and Virginia. *J Zoo Wildlife Med* 33:311–317

Campbell TW (2006). Clinical pathology in reptiles. *In: Reptile Medicine and Surgery*, 2nd edn, Mader DR (ed), pp: 453–470. WB Saunders, Co., Philadelphia, Pennsylvania, USA

Campbell TW, C Ellis (2007). *Avian and Exotic Animal Hematology and Cytology*, 3rd edn. Blackwell Publication, Ames, Iowa, USA

Cheng YY, TY Chen, PH Yu, CH Chi (2010). Observations on the female reproductive cycles of captive Asian yellow pond turtles (Mauremys mutica) with radiography and ultrasonography. *Zoo Biol* 29:50–58

Christopher MM, KH Berry, IR Wallis, KA Nagy, BT Henen, CC Peterson (1999). Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert. *J Wildlife Dis* 35:212–238

Christopher MM, R Brigmon, E Jacobson (1994). Seasonal alterations in plasma β-hydroxybutyrate and related biochemical parameters in the desert tortoise (*Gopherus agassizii*). *Compar Biochem Physiol A Physiol* 108:303–310

Chung CS, CH Cheng, SC Chin, AH Lee, CH Chi (2009). Morphologic and cytochemical characteristics of Asian yellow pond turtle (*Ocadia sinensis*) blood cells and their hematologic and plasma biochemical reference values. *J Zoo Wildlife Med* 40:76–85

Dessauer HC (1970). Blood chemistry of reptiles: physiological and evolutionary aspects. *Biol Rept* 3:1–72

Dickinson VM, JL Jarchow, MH Trueblood (2002). Hematology and plasma biochemistry reference range values for free-ranging desert tortoises in Arizona. *J Wildlife Dis* 38:143–153

Gulliver G (1842). On the blood corpuscles of the British ophidians, reptiles and other oviparous vertebrates. *Proc Zool Soc Lond* 10:108–111

Haitao S, JF Parham, F Zhiyong, H Meiling, Y Feng (2008). Short communication evidence for the massive scale of turtle farming in China. *Oryx* 42:147–150

Hartman F, M Lessler (1964). Erythrocyte measurements in fishes amphibia, and reptiles. *Biol Bull* 126:83–88

Heldmaier G, S Ortmann, R Elvert (2004). Natural hypometabolism during hibernation and daily torpor in mammals. *Res Physiol Neurobiol* 141:317–329

Hu L, Q Li, P Yang, JA Gandahi, TS Arain, Y Le, Y Liu, Q Chen (2016). Expression of TLR2/4 on epididymal spermatozoa of the Chinese soft-shelled turtle Pelodiscus sinensis during the hibernation season. *Anat Record* 299:1578–1584

Hutchison VH, H Szarski (1965). Number of erythrocytes in some amphibians and reptiles. *Copeia* 1965:373–375

Knotková Z, J Doubek, Z Knotek, P Hájková (2002). Blood cell morphology and plasma biochemistry in Russian tortoises (*Agrionemys horsfieldi*). *Acta Vet Brno* 71:191–198

Kumar-Velmurugan B, F Jiang, HY Shih, DN Lee, CF Weng (2012). Respiratory burst activity in head kidney and spleen leukocytes of tilapia (*Oreochromis mossambicus*) under acute osmotic stress. *Zoo Stud* 51:1290–1297

Lawrence K (1987). Seasonal variation in blood biochemistry of long term captive Mediterranean tortoises (*Testudo graeca* and *T. hermanni*). *Res Vet Sci* 43:379–383

Lisičić D, D Đikić, V Benković, AH Knežević, N Oršolić, Z Tadić (2013). Biochemical and hematological profiles of a wild population of the nose-horned viper *Vipera ammodytes* (Serpentes: Viperidae) during autumn, with a morphological assessment of blood cells. *Zoo Stud* 52:1–9

Liu L, Z Cao, F Lin, XP Ye, Y Xu (2015). Partial sequence of a novel virus isolated from Pelodiscus sinensis hemorrhagic disease. *Intervirology* 58:197–204

Omonona AO, SG Olukole, FA Fushe (2011). Haematology and serum biochemical parameters in freeranging African side neck turtle (*Pelusios sinuatus*) in Ibadan, Nigeria. *Acta Herpetol* 6:1–8

Pan M (2018). Hibernation induction in non-hibernating species. *Biosci Horiz Intl J Student Res* 11:1–10

Que Y, B Zhu, H Rosenthal, J Chang (2007). Isolation and characterization of microsatellites in Chinese soft-shelled turtle, *Pelodiscus sinensis*. *Mol Ecol Notes* 7:1265–1267

Samour J, C Hawkey, S Pugsley, D Ball (1986). Clinical and pathological findings related to malnutrition and husbandry in captive giant tortoises (*Geochelone species*). *Vet Rec* 118:299–302

Szarski H, G Czopek (1966). Erythrocyte diameter in some amphibians and reptiles. *Bull Acad Pol Sci Ser Sci Biol* 14:433

Tamukai K, Y Takami, Y Akabane, Y Kanazawa, Y Une (2011). Plasma biochemical reference values in clinically healthy captive bearded dragons (*Pogona vitticeps*) and the effects of sex and season. *Vet Clin Pathol* 40:368–373

Tavares-Dias M, AA Oliveira-Júnior, JL Marcon (2008). Methodological limitations of counting total leukocytes and thrombocytes in reptiles (Amazon turtle, *Podocnemis expansa*): an analysis and discussion. *Acta Amazon* 38:351–356

Teng Y, SL Zhang, CL Wang (2012). *The Technology Study on the Series Products of Trionyx sinensis*. Acad Period of Farm Products Process, China

Troiano JC, JC Vidal, EF Gould, J Heker, J Gould, AE Vogt, A De Roodt (2000). Hematological values of some Bothrops species (Ophidia-Crotalidae) in captivity. *J Venom Anim Toxins* 6:194–204

Troiano JC, JC Vidal, J Gould, E Gould (1997). Haematological reference intervals of the South American rattlesnake (*Crotalus durissus terrificus*, Laurenti, 1768) in captivity. *Compar Haematol Intl* 7:109–112

Tumkiratiwong P, W Meesuk, L Chanhome, A Aowphol (2012). Reproductive patterns of captive male and female monocled cobra, *Naja kaouthia* (Lesson, 1831). *Zoo Stud* 51:692–700

Uğurtaş IH, M Sevinç, HS YıldIrımhan (2003). Erythrocyte size and morphology of some tortoises and turtles from Turkey. *Zoo Stud* 42:173–178

Vistro WA, Y Liu, M Xu, P Yang, A Haseeb, Y Huang, Q Chen (2019). Mitochondria-rich cells: A novel type of concealed cell in the small intestine of chinese soft-shelled turtles (*Pelodiscus sinensis*). *Animals* 9:1–12

Yin J, Y Tezuka, L Shi, JY Ueda, K Matsushige, S Kadota (2005). A combination of soft-shell turtle powder and essential oil of a unicellular chorophyte prevents bone loss and decreased bone strength in ovariectomized rats. *Biol Pharm Bull* 28:275–279

Yoshii SR, N Mizushima (2017). Monitoring and measuring autophagy. *Intl J Mol Sci* 18:1–13

Yousaf, S. and T. Mahmood, 2018. Feeding ecology of four freshwater turtle species in Pothwar plateau and its potential implication in turtle farming in Pakistan. *Intl J Agric Biol* 20:723−729

Yu PH, PY Yang, YS Chiu, CH Chi (2013). Hematologic and plasma biochemical reference values of the yellow pond turtle Mauremys mutica and the effects of sex and season. *Zoo Stud* 52:1–6

Zaias J, T Norton, A Fickel, J Spratt, NH Altman, C. Cray (2006). Biochemical and hematologic values for 18 clinically healthy radiated tortoises (*Geochelone radiata*) on St. Catherines Island, Georgia. *Vet Clin Pathol* 35:321–325

Zou Y, Q Ai, K Mai (2011). Ontogenic development of digestive enzyme activities in juvenile soft-shelled turtle (*Pelodiscus sinensis*) under cultured conditions. *Front Agric Chin* 5:624–630