International Scholars Journals

African Journal of Dairy Farming and Milk Production ISSN 2375-1258 Vol. 8 (3), pp. 001-003, March, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Blood profiles from wild populations of green sea turtles in Taiwan

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Accepted 08 December, 2019

Blood samples were obtained from twenty seven clinically healthy bycatched green turtles (*Chelonia mydas*). The packed cell volume (PCV) and 14 plasma biochemistry parameters were determined from these samples. The PCV values were lower in sub-adult green turtles than in the adults while the plasma AST concentration was higher in the sub -adults than in the adults. These variations may be due to the age of the turtle and the period of the year when the samples were taken. The results of this study provide the first plasma biochemistry baseline profiles for free-ranging green sea turtles in Taiwan.

Key words: Green sea turtle, plasma biochemistry baseline, Taiwan.

INTRODUCTION

There are five species of sea turtles in Taiwan, namely green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricate*), loggerhead turtle (*Caretta caretta*), olive ridley turtle (*Lepidochelys olivacea*) and leatherback turtle (*Dermochelys coriacea*) (Chen and Cheng, 1995; Cheng et al., 2006). All of them are listed as endangered species internationally (International Union for the Conservation of Nature and Natural Resources, 2009) and they face various anthropogenic threats in the coastal waters including pollutants, inciden-tal capture and strandings (Cheng and Chen, 1997; Godley et al., 1999; Mascarenhas et al., 2004). In Taiwan, many live stranded turtles have been sent to nearby aquariums for rehabilitation followed by release based on observed growth and weight gain (Fong, 2008). Plasma biochemistry would complement clinical evalua-tion of rehabilitated turtles but to date normal values for sea turtles in Taiwan are lacking. Given that blood chemistry profiles vary depending on species, geographic location, age, gender, season, food availability, environmental conditions, disease and activity levels (Arthur et al., 2008; Deem et al., 2009; George, 1997; Jon, 2003; Lutz and Dunbar-Cooper, 1987; Whiting et al., 2007), it is important to establish plasma biochemistry baseline profiles on a site, regional and population basis (Aguire and Balazs, 2000; Bolten and Bjorndal, 1992).

The purpose of this study was, therefore, to establish the plasma biochemistry baseline profiles for free-ranging green sea turtles in Taiwan.

MATERIALS AND METHODS

Blood sample collection

Green sea turtles (n = 30) were sampled from a set-net fishing ground in I-Lan County, Northeast Taiwan from March 2007 to November 2008. Turtles from the by catch sample were held for a maximum of 2 h on board the shipping vessel. The turtles were aged according to the curved carapace length (CCL) which was measured to the nearest 0.1 cm. Turtles with CCL values > 85 cm were recorded as adults (Limpus and Chaloupka, 1997; Hamann et al., 2006). Only clinically healthy turtles (n = 27) were included in the study. They were defined as animals in good physical condition (that is, not emaciated and free from lesions, epibionts and injuries), demonstrating agility in swimming and with normal reflexes (Arthur et al., 2008). Blood (7 ml) was collected from the dorsal cervical sinus of each turtle with a 21 G or 22 G sterilized needle (Owens and Ruiz, 1980) and stored in two 4 ml lithium-heparin vacuum anti-coagulant tubes (BD, Vacutainer®, USA). Blood samples were kept cold in an ice-filled plastic cooler and transported back to the labo-ratory within two hours. In the laboratory, the packed cell volume (PCV) was determined by using a micro-hematocrit centrifuge (Hsiangtai Machinery Industry Co., Model H-240).

The remaining heparinized blood was centrifuged at 128 x*g* for 10 min and plasma was then pipetted into micro-centrifuge tubes (PLASTIBRAND®) and stored in a freezer (-20℃) until analysis.

Sample analysis

Plasma biochemistry was analyzed with a COBAS Mira analyzer

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Table 1. The curved carapace length (CCL) of bycatched green turtles from Don-Ou, I-Lan County northeastern Taiwan. The unit is cm.

	Sub-adult	Adults
Mean (s. d.)	77.6 (4.48)	94.2 (7.2)
Range	70 - 83.5	86 - 107.3
Replicate	13	17

Table 2. The plasma biochemistry data for pooled bycatch adults and pooled bycatch immature green turtles of I-lan County collected from 2007 to 2008.

Parameters	All samples			CCL 85 cm (sub-adult)			CCL 85 cm (adults)		
	Mean (s. d.)	Interval*	n	Mean (s. d.)	Interval*	n	Mean (s. d.)	Interval*	n
CCL (cm)	86.5 (9.5)	105.775	27	78.1 (4.3)	83.45	12	93.3 (6.7)	106.73	15
PCV (%)	29.1 (2.6)	33.6	26	27.8 (2.04)	30	10	30.1 (2.6)	35.55	13
TP (g/dl)	4.78 (0.88)	6.026	27	5.15 (0.99)	7.263	12	4.49 (0.68)	5.88	15
Albumin (g/dl)	2.32 (0.4)	3.156	26	2.46 (0.41)	3.153	12	2.32 (0.62)	3.842	15
Globulin (g/dl)	2.48 (0.8)	3.832	26	2.68 (0.99)	4.669	12	2.16 (0.82)	3.32	15
A/G ratio	1.03 (0.35)	2.09	26	1.03 (0.4)	1.6	12	2.82 (6.96)	21.357	15
AST (U/l)	142.8 (53.2)	274.04	27	174.4 (46.7)	253.69	12	117.6 (44.9)	195.18	15
ALT (U/l)	11.2 (16.7)	39.4	27	9.03 (12.76)	39	12	13 (19.6)	65.775	15
ALP (U/l)	29.4 (25.2)	87	27	33.86 (29.57)	92.58	12	25.9 (21.4)	77.5	15
LDH (U/l)	246.5 (200)	1337.7	27	292.1 (199)	1461.9	12	249.9 (308.9)	1059.5	15
Glucose (mg/dl)	108.5 (17.7)	120.685	27	105.9 (16.6)	130.41	12	109.7 (18.9)	136.03	15
Cholesterol (mg/dl)	194.4 (85.8)	366.3	27	178.2 (39.9)	238.3	12	207.5 (109.6)	449.25	15
Triglyceride	49.07 (34.5)	699.45	27	50.98 (43.03)	161.23	12	47.5 (27.34)	117.2	15
Calcuim (mg/dl)	8.87 (1.62)	11.551	27	8.58 (0.76)	9.606	12	9.1 (2.1)	13.845	15
Magnesium (mg/dl)	4.39 (2.28)	8.895	27	4.3 (2.13)	8.64	12	4.46 (2.47)	91.5	15
BUN (mg/dl)	16.65 (9.9)	34.415	27	14.28 (9.76)	34.1	12	18.55 (9.9)	35.65	15
Creatinine (mg/dl)	0.3 (0.1)	0.485	27	0.29 (0.09)	0.453	12	0.31 (0.1)	0.557	15
Uric acid (mg/dl)	1.38 (0.47)	2.28	27	1.45 (0.38)	1.89	12	1.34 (0.54)	24.5	15

* Interval: 95% confidence interval of mean.

(Roche Scientifics). The following fourteen commercial kits were used: total protein (TP), albumin, amino transferase (AST, GOT), alanine amino transferase (ALT, GPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), glucose, cholesterol, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, calcium and magnesium. The instrument was calibrated with Roche standards prior to each analysis.

Statistical analyses

A Student t-test was used to determine the differences between sub-adults and adults of each plasma biochemistry value. An arcsin transformation was used to transfer the percentage of the PCV values before the student t-test was performed.

RESULTS

Among the 27 clinically healthy green turtles, 12 were subadults and 15 were adults. The size distribution of the turtles is listed in Table 1. Statistical analyses showed that the PCV values were lower in subadult turtles than in adults (combining both sexes; P = 0.029). Amino-transferase (AST) concentrations were higher in the subadult than in the adult turtles (P = 0.004) . The plasma biochemistry profile of the clinically healthy green turtles in Taiwan is presented with maturity data in Table 2.

DISCUSSION

In this study, the PCV values were lower in subadult turtles than in adults. PCV values have previously been found to increase significantly with age in loggerhead sea turtles (Kakizoe et al., 2007). In contrast, AST concentrations were higher in the subadults' green turtles than in the adults. Hamann et al. (2006) reported that plasma AST concentration may increase due to muscle or hepatic damage, rather than as a function of body size.

However, all the turtles examined in this study were clinically healthy. Anderson et al. (1996) found that snapping turtles in New Guinea had higher AST concentrations when they were reared at higher temperatures. Most of the subadults in this study were sampled during the summer and autumn, when water temperatures were higher and so the plasma AST concentration may have been higher in the subadults than in the adults because of this difference in the sampling period. Arthur et al. (2008) found that plasma triglycerides and cholesterol concentrations were all significantly correlated with CCL size in Australian green turtles. No such relationships were found in this study possibly because the sample size in this study was not large enough to detect such differences.

We have constructed the first plasma biochemistry baseline profiles for free-ranging green sea turtles in Taiwan. Although most of the samples have been obtained from subadult and adult females, they still represent the normal blood chemistry of a significant portion of the sea turtle populations in the vicinity of Taiwan. By-catch sea turtles from the coastal fisheries are frequently reported in Taiwan (Cheng, unpublished data). Results of this study can now serve as a baseline reference profile for the evaluation of healthy by catch and rehabilitated sea turtles. Comparison of the blood chemistry of unhealthy or injured turtles with these profiles can guide recommendations for rehabilitation and the design of therapies for this species of turtles.

ACKNOWLEDGMENTS

The authors thank the *Don-Au* fishing crew of I-Lan County for their assistance with blood extractions. The authors also thank Ms. Shu, J -W, Mr. Chang, C-M, Mr. Chen, J-L, and Ms. Wang, T-J for their work in the field. This study was supported by a grant from the Forestry Department, Council of Agriculture, ROC (Grant No. 97 Forest - 02.1 - conserv-50).

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