COMPARISONS OF PLASMA BIOCHEMISTRIES OF NESTING AND CAPTIVE GREEN TURTLES WITH THE BASELINE DATA IN TAIWAN

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ABSTRACT: Plasma biochemistries are commonly used to assess the health condition of animals, including sea turtle. However, these values can be influenced by both physiology and environmental factors, and affect the accuracy of health assessments. Therefore, a reliable and sizable database of plasma biochemistries for the clinically normal turtle is essential. We have established the baseline plasma biochemistries for the free-ranging green turtles in Taiwan. This provides us a better tool to evaluate the health conditions of rehabilitated turtles. Yet, there are several questions remained unanswered. Is the triglyceride concentration the only plasma biochemistries that fluctuate during the nesting activities of gravid turtles? Can the rehabilitated turtles release back to the wild based on the plasma biochemistry evaluation? How long should they be kept in the rehabilitation center? In this study, the plasma biochemistries of nesting females and long-term captive green turtles are compared with the baseline values. We also try to identify the key parameters to assess the health condition of sea turtles, both in the wild and in captivity.

Keywords: Healthy assessment, Blood parameters, Rehabilitation, Green turtle.

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 or marine research center for rehabilitation followed by release based on observed growth and weight gain (Fong, 2008). Plasma biochemistry would complement clinical evaluation of rehabilitated turtles but to date normal values for sea turtles in Taiwan are lacking. The blood or plasma 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). Because of the differences in environment and diet between free-ranging and rehabilitated animals, the blood profils or reference intervals generated from the wild may not useful for assessing the the health status of individual rehabilitated animals (Greig et al., 2010).

The objectives of this study were to to determine the effect of age class, nesting behavior, captive environment and body condition on blood variables from free-ranging green sea turtles.

MATERIALS AND METHODS

Blood sample collection

Nesting female green turtles (n=20) were sampled from Lanyu and Penghu islands since June to September of 2006 to 2010. Captive or rehabilitated turtles (n=24) were sampled from Penghu Marine Biology Research Center since June 2006 to December 2010. 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 nesting female turtles 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 anticoagulant tubes (BD, Vacutainer®, USA). Blood samples were kept cold in an ice-filled plastic cooler and transported back to the laboratory 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°C) until analysis.

Sample analysis

Plasma biochemistry was analyzed with a COBAS Mira analyzer (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 chemistry analyzer was designed for human use and were calibrated with Roche standards prior to each analysis.

Statistical analyses

The reference range of plasma biochemistry for green sea turtles is based on previous study of Fong (2008) and Fong et. al. (2010). An Independent sample T-test was used to determine the difference of each plasma biochemistry value between free-ranging adult females and nesting females. Use one-way ANOVA and Tukey's Multiple Comparison Test to compare the difference between clinically health captive turtles, clinically unhealthy turtles and the reference range which base on clinically healthy turtles. Discriminant function analysis were used to clarify the effects of sickness and captivity in those three sets.

RESULTS

The morphology data and the range of plasma biochemistry values among the 20 clinically healthy nesting green turtles is listed in Table 1. The partial data of ALT are lower than the detective limit (3 U/L) of analyzer. Glucose, Triglyceride, Magnesium, BUN and Uric acid (the *p* values of last two variables are <0.001) are significantly difference between nesting female and free-ranging female adults. Among the 24 captive green turtles, 5 were classified as clinically unhealthy or sick and 19 were clinically normal. The size distribution of captive green turtles is shown in Fig 1. Most of them are subadult, then adult females. The plasma biochemistry values of sick captive, clinically normal and wild healthy green turtles were summarized in Table 2. Six variables are significantly difference between each group, which are Albumin, A/G ratio, AST, LDH, Magnesium and BUN. Prediction of turtle group based on Discriminant function analysis classification (Fig. 2) were 95.6% correct for wild healthy (n=31), captive clinically healthy (n=19) and captive clinically unhealthy or sick turtles (n=5). The Eigenvalues of function 1 and function 2 are 2.792 and 0.497, respectively.

TABLE 1. The plasma chemistry values of nesting female green turtles (n=27) around Lanyu island and Penghu island, Taiwan, 2006-2010.

Parameters	Nesting Female green turtles Mean (SD)	Range	N
CCL	104.16 (5.00)	95.0 - 115.0	18
PCV(%)	27.76 (3.76)	21.5 - 35.0	20
TP(g/dl)	4.02 (0.68)	2.8 - 5.2	20
Albumin(g/dl)	2.21 (0.22)	1.8 - 2.6	20
Globulin(g/dl)	1.82 (0.50)	0.9 - 2.6	20
A/G ratio	1.31 (0.37)	1.0 - 2.4	20
AST(GOT)(U/L)	83.16 (31.11)	52.0 - 205.0	20
ALT(GPT)(U/L)	5.60 (1.96)	3.0 - 9.0	5
ALP(U/L)	25.35 (4.41)	20.0 - 36.0	17
LDH(U/L)	597.42 (325.74)	237.0 - 1346.0	20
CK(U/L)[a]	957.21 (1007.89)	164.0 - 4452.0	20
Glucose(mg/dl)*	82.11 (27.84)	36.0 - 151.0	20
Cholesterol(mg/dl)	252.74 (43.72)	172.0 - 324.0	20
Triglyceride(mg/dl)*	702.00 (339.21)	122.0 - 1323.0	20
Calcium(mg/dl)	10.87 (3.43)	3.1 - 17.4	20
Magnesium(mg/dl)*	7.62 (1.49)	5.1 - 11.2	20
BUN (mg/dl)**	3.81 (1.24)	2.0 - 6.5	20
Creatinine(mg/dl)	0.34 (0.11)	0.2 - 0.7	20
Uric acid(mg/dl)**	0.71 (0.21)	0.5 - 1.1	20

a. No data of CK values in free-ranging female adults.
* $p < 0.05$; ** $p < 0.001$.

TABLE 2. The plasma chemistry parameters of clinically unhealthy or sick captive, normal captive green turtles and wild healthy green turtles. The captive green turtles are rehabilitated in the Penghu Marine Research center, Taiwan, 2006-2010.

Parameters	Sick captive green turtles			Normal captive green turtles			Wild healthy green turtles		
	Mean (SD)	Range	N	Mean (SD)	Range	N	Mean (SD)	Range	N
CCL (cm)	78.6 (10.5)	62.5-87.65	5	56 (15.5)	30.6-96.3	19	84.8 (10.1)	70-107.3	31
TP (g/dl)	5.1 (0.5)	4.34-5.60	5	4.9 (0.9)	3.06-6.91	18	4.62 (0.95)	2.8-7.41	31
Albumin (g/dl)*	3.4 (0.4) [a]	2.81-3.82	5	3.0 (0.8) [a]	1.61-4.84	18	2.29 (0.38) [b]	1.44-3.16	30
Globulin (g/dl)	1.7 (0.7)	0.66-2.44	5	2.0 (0.5)	0.99-3.14	18	2.34 (0.86)	0.9-4.76	30
A/G ratio*	3.1 (2.4)[a]	1.18-7.20	5	1.7 (0.6)[b]	0.68-2.73	18	1.1 (0.4)[b]	0.51-2.11	30
AST (U/L)*	181.4 (99.6)[a]	102.2-351.1	5	238.1 (157.1)[a]	61.55-593.7	19	137.7 (55.8)[b]	32.4-254.3	31
ALT (U/L)	19.1 (19.9)	0.9-52.4	5	30.1 (57.7)	2.9-245.6	18	10.5 (15.7)	0.3-79.7	31
ALP (U/L)	19.1 (8.1)	11.2-28.8	5	28.3 (25.8)	6.3-111.5	19	28.8 (23.5)	2-93.2	31
LDH (U/L)**	81.3 (36.1)	51.7-143.9	5	159.3 (131.6)	24.1-481.3	19	277.2 (210.2)	34.5-698	31
Glucose (mg/dl)	98.9 (15)	82.7-118.8	5	111.6 (14)	87.8-143.6	19	108 (17.7)	36-137.9	27
Cholesterol (mg/dl)	217.7 (56.4)	128-279	5	177.1 (58.7)	87-341	19	199 (81)	94-476	31
Triglyceride (mg/dl)	128.8 (18.9)	111.8-154	5	105.4 (55.9)	33.5-203.1	19	49.1 (34.5)	17.7-170.1	27
Calcium (mg/dl)	9.6 (0.6)	8.64-10.31	5	8.6 (0.9)	7.3-10.6	19	8.66 (1.87)	3.1-14.6	31
Magnesium (mg/dl)*	3.3 (0.1)[a]	3.05-3.45	5	3.1 (0.4)[a]	2.54-3.53	19	4.39 (2.28)[b]	2.36-9.3	27
BUN (mg/dl)*	51.3 (21.3)[a]	30.2-80.8	5	62.3 (52)[a]	23.6-264.7	19	16.65 (9.9)[b]	5-36.6	27
Creatinine (mg/dl)	0.3 (0.1)	0.17-0.34	5	0.3 (0.1)	0.20-0.53	19	0.30 (0.1)	0.12-0.6	31
Uric acid (mg/dl)	1.6 (0.4)	0.9-2.1	5	1.4 (0.6)	0.5-2.4	19	1.38 (0.47)	0.5-2.5	27

* $p<0.05$
** $p < 0.05$, but no difference between each group.

FIG 1. The size distribution of captive green turtles during rehabilitation in Penghu Marine Research center.

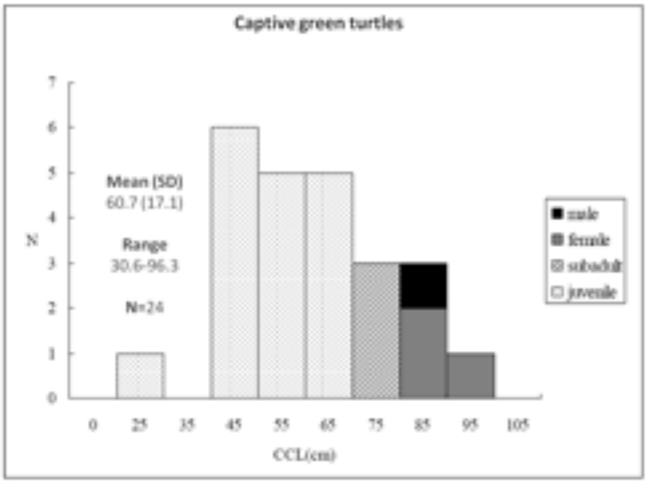
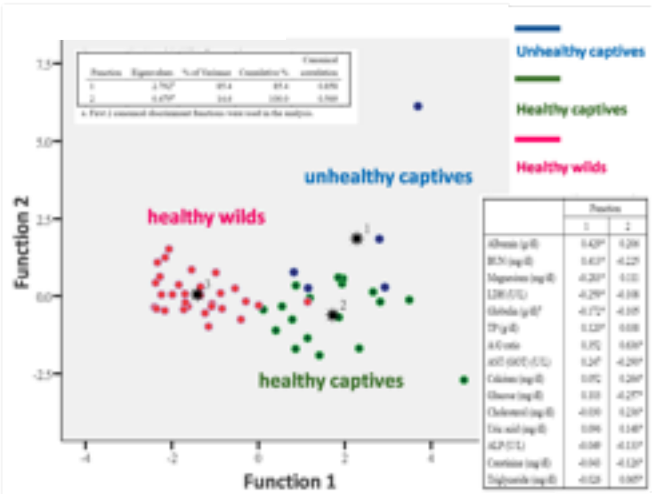


FIG 2. Discriminant function analysis of unhealthy captives(n=5), healthy captives(n=19) and healthy wild green turtles(n=31).



DISCUSSION

Nesting behavior

The significant difference parameters between nesting female and free-ranging female adults includes glucose, triglyceride, magnesium, BUN and uric acid are related to the nutritional status. The nesting female adults have higher level of triglyceride and magnesium. There are several physiological and behavioral mechanisms about utilisation of somatic energy during the breeding season of sea turtles. For example,during the inter-nesting period, female *C. mydas* are hypoactive and thus presumably hypometabolic (Hays et al.1999). Hamann et al. (2002) found that plasma triglyceride concentration increased in vitellogenic female *C. mydas* in the 6 months preceding a breeding season. Similar results was found by Santoro and Meneses (2007), the concentration of albumin, calcium, cholesterol, magnesium, uric acid and BUN are significant difference between nesting female and reproductive males in the species *Lepidochelys olivacea.* Deem et al. (2009) found that loggerhead turtle (*Caretta caretta*) from the forage habitat have the highest glucose and the lowest BUN level than nesting female and stranding turtles which were caused by the different foraging status. The elevation of BUN concentration are related to the high protein diet or digestion of muscle tissues (Lawrence, 1987). When the food of sea turtles have higher protein constitution, the BUN values in the blood will increased(Whiting et al., 2007). In captive reptiles, the values of uric acid will increased after feeding (Maixner et al. 1987). According to the previous studies, our results from nesting females suggest that they were under fasting during nesting period.

Vitellogenesis and egg production effected the plasma biochemistry in many reptiles, such as *Ocadia sinensis* and *Lepidochelys olivacea.* The study of Chinese stripe-necked turtle have shown that reproductive cycle would effect the BUN and UA levels which are higher in female with eggs (Jon, 2003). The vitellogenesis and nesting behavior may elevated the level of calcium, cholesterol and triglyceride in the plasma (Christopher et al., 1999;Hamann et al., 2002). However, there is no significant difference of cholesterol values in this study. But the mean of cholesterol in nesting female (299.8±18.8 mg/dl) are higher than female adults (191.5±110.9 mg/dl). Santoro and Meneses(2007) found that nesting female have higher level of magnesium than reproductive male olive ridley and other female adult which may related to the vitellogenesis. The composi-tion of food items also effect the magnesium concentration, for example, *Caiman latirostris* and *Caiman yacare* which captive in the zoo have lower concentrations of calcium, inorganic phosphate, copper, sodium, and magnesium than those in the alligator farm. Therefore, the concentration of these ions could represent as the indicator of mineral deficiency (Coppo et al., 2006). The magnesium of yolk plays an important role during the development of embryo (Packard and Packard, 1989), this demand may lead nesting female tend to intake more nutrition and minerals, then reflected in the plasma with higher concentration of magnesium.

Rehabilitation

The captive green turtles have higher albumin , AST and BUN level, where as the free-ranging green turtles have higher concentration of magnesium. The main reason is that abundant food and high protein diets are supplied during the rehabilitation. Similar result were found in study of Varela (1997) and Swimmer (2000) that clinically healthy captive green turtles have higher protein concentrations in Miami seaquarium in Florida and Ocean World in California. Continues of over-feeding would cause the metabolic abnormal, especially in the delivery of fatty acid and decomposition of protein, or increased the level of UA (Maixner, 1987). However, the formation and excretion of nitrogenous wastes in sea turtles including UA, BUN and ammonia, which cause the variation of UA level is less significant (Khalil, 1947;Moyle, 1949;Campbell, 2004). We found that the level of BUN increase rapidly, even under a short period of rehabilitation. The lower level of magnesium in captive green turtles indicated that increasing the diversity of food items and supplement of vitamins and minerals are need during the rehabilitation.

According the study of Norton et al. (1990) and Varela (1997), the sea turtle with fibropapillomatosis have higher level of AST enzyme. Moreover, the dessert tortoises (*Xerobates agassizii*) with chronic upper respiratory tract disease (URTD) infection have elevated AST levels (Jacobson et al., 1991);however, Swimmer(2000) found no difference of ALT levels between health sea turtle and turtles with fibropapillomatosis in both wild population and captive environment. In this study, there is no significant difference of ALT levels between both groups.

The sick green turtles have higher albumin and A/G ration than clinically healthy turtles in captivity. In the animals with anemia, malnutrition, parasite infection or enteritis would caused the decrease of albumin concentrations (Wilkinson, 2003). The concentration of globulin reflect the immune status of animals, the level will increased under chronically infection (Campbell, 2004). In contrast, the hawaii green turtle with severely tumor infection have lower globulin levels which may caused by immunosuppression (Work et al., 2001). The abundant of globulin related to the stress reaction, especially CBG (corticosteroid-binding globulin), CBG will decrease when animals under stress. The decreasing of CBG induce the level of cortisol increase, which could mobile more actively to stress than globulin (Breuner et al., 2006). The stress of rehabilitation environment might be the reason of lower globulin in this study.

Base on discriminant function analysis, function 1 could predict 85% of each turtle group. The major factors are similar with the results of ANOVA test. Besides the lactate dehydrogenase (LDH), most of the variables have strong connection of nutrition status. Therefore, when conducting the health assessment of the captive turtles, minor adjustment of reference range is allowed to avoid the misestimate.

In this study, we have applied the baseline profile for free-ranging green turtles in Taiwan to the captive green turtles and nesting female population. However, the reference range for captive normal turtles is need as well. The suggestions for sea turtle husbandry have been made, like the diversity of food items, avoiding prolong maintenance, and over feeding.

ACKNOWLEDGMENTS

The authors thank to Mr. Chen, H-C, Mr. Tsung, T-C and lab mate from Dr. Cheng 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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