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BLOOD BIOCHEMISTRY PROFILE OF GREEN (*Chelonia mydas)* SEA TURTLE REHABILITATED IN TURTLE CONSERVATION AND EDUCATION CENTER (TCEC) SERANGAN

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Abstract: **Indonesia's marine waters become a habitat for six types of sea turtles from seven species of turtles in the world. Green sea turtles (*Chelonia mydas*) are in the highest number and spread in almost all Indonesian waters. Although there are many types of turtles in Indonesia, this is inversely proportional to the population. It is estimated that there are 100,000 green sea turtles killed around the IndoAustralia islands every year. Plasma chemical measurement is one that is commonly used as a diagnostic technique to determine the health status of individual animals, but until now information about the value of the chemical parameters of green turtle blood in captivity in Indonesia, especially Bali, is unknown. The purpose of this study was to obtain the values of transaminase enzyme activity (AST & ALT) and Creatinine levels as parameters of turtle health status at the Turtle Conservation and Education Center (TCEC), Serangan, Bali, Indonesia. The sample from this study was serum from 10 turtles with three different age groups. Each sample was tested for AST, ALT and creatinine levels using the iCubio iChem-535Vet machine. Data obtained from AST, ALT and blood creatinine samples and age will be analyzed descriptively in a mean ± deviation form, the relationship between body condition index (BCI) and the results of blood biochemical parameters analysis was analyzed by the Spearman correlation test assisted with IBM SPSS version 21. The average value for juvenile green turtle is AST 148.2 U / L, ALT 16.94 U / L, Creatinine 0.42 mg / dL; subadult AST 188 U / L, ALT 10.03 U / L, Creatinine 0.80 mg / dL; adult AST 176.5 U / L, ALT 14.50 U / L, Creatinine 1.05 mg / dL.**

Keywords: **AST, ALT, Creatinine, Green sea turtle.**

1. INTRODUCTION

Indonesia's marine waters become a habitat for six types of sea turtles from seven species of turtles in the world. Two types, namely Green turtles / Green turtles (Chelonia mydas) and Hawksbill / Hawksbill turtles (Eretmochelys imbricata) are in the highest number and spread throughout the region in Indonesia. More than 35,000 Green turtles and 28,000 Hawksbill turtles lay eggs on more than 150 nesting beaches that have been identified in Indonesia (Adnyana and Hitipeuw, 2009). Although there are many types of sea turtles in Indonesia, this is inversely proportional to the population. The turtle population in Indonesia is increasing, according to Karnan (2008) it is estimated that there are 100,000 green turtles killed around the IndoAustralia islands every year.

In Indonesia, the decline has occurred ten times since the 1940s, and more than half in FrenchPolynesia. The SSC Marine Turtle Specialist Group has rated this species as "Endangered". Recovery can occur if conservation is carried out with strict management. This decline in population is because turtles are animals whose lives are susceptible to disturbances such as coastal habitat damage, climate change, illegal fishing, and disease. Diseases that often infect turtles besides due to environmental pollution, are also caused by bacteria and viruses (Dermawan et al., 2009).

Study of clinical parameters is an important knowledge in evaluating the health status of turtles, because it is to determine the function changes of organisms and their environment (Swimmer, 2000). Early changes in organ function can be identified through changes in blood components, before clinical signs appear. Therefore, blood tests are considered very useful and non-invasive in determining turtle health status, because blood characteristics reflect not only physiological conditions but also ecological differences and characteristics for each species, this makes it possible to detect changes in metabolic activities of animals (Perpiñán, 2017; Espinoza-Romo, 2018).

Plasma chemical measurement is one that is commonly used as a diagnostic technique to determine the health status of individual animals (Swimmer, 2000). To see the health status of liver function, it can be seen through an examination of the activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), while for kidney function by determining creatinine levels. However, until now information about the value of the chemical parameters of green turtle blood in captivity in the Indonesian region, especially Bali, is not published. So that the condition of turtles that have been rehabilitated in captivity can not be measured in their health status. Thus, determining the activity of AST, ALT, and creatinine levels as a reference is very necessary.

2. MATERIAL AND METHODS

Research Object

The object of this research is green turtle (Chelonia mydas), as many as 10 green turtles with details of 5 juveniles (adolescents), 3 subadults (almost adults), and 2 adults (adults). The turtles used as samples are sea turtles at the Turtle Conservation and Education Center (TCEC), Denpasar, Bali.

Research Materials and Equipment

The materials used in this study were: turtle blood serum, Reiged reagent and antiseptic. The equipment used in this study include: syringe and needle, handscoon, measuring tape, meter, blood storage tube without anticoagulant (no additive hematology tube), ice box (cool box), ice cube, eppendorf tube, micropipette, centrifuge machine, iCubio iChem-535Vet machine.

Research Design

The study design used was an observational analytic method with a cross sectional approach, where data on biochemical analysis of turtle blood (AST, ALT, and creatinine) and age were rehabilitated at the Turtle Conservation and Education Center (TCEC), taken at the same time Sumertajaya, 2000).

Sampling

The age group of turtles is done by doing morphometry. The turtle morphometry measured included Straight Carapace Length (SCL), Straight Carapace Width (SCW), Curved Carapace Length (CCL), and Curved Carapace Width (CCW). The tool used to measure morphometry is the measuring tape and meter. Measurements of body condition index (BCI = body mass x 10000 / SCL3) were calculated to evaluate relative "obesity". This index is used as an indirect predictor of nutritional status and / or animal health conditions (Bjorndal et al., 2000).

Before performing venipuncture, a physical examination is carried out first to make sure the turtle is in a healthy condition, so that the results reflected by the blood biochemical examination are more maximal. Examination begins with the skin, for signs of excessive desquamation, decubitus, abscesses, wounds, scars, and the presence of barnacles. Next, it checks the position, shape, size, symmetry, color, and proportion of the head and skull as a whole, with the aim of determining injury, trauma, or pain. The conjunctiva, sclera, cornea, iris, and pupils are examined using a flashlight. Please note, turtle eyeballs are protected by keratinized and movable dorsal and ventral eyelids, then there is also a secondary eyelid on keratinized and immobile lateral canthus (Wyneken, 2004). This step needs to be considered, because fibropapillomatosis disease manifests in these areas including the maxilla & mandibular area (Work & Balazs, 1999). Furthermore, the neck part is inspected, flipper is considered carefully to avoid fractures, inflammation and so on. The last

is carapace and plastron examination, whether abnormal keratinization, density and flexibility, fracture, ulceration, barnacles, and so on are found.

The venipuncture location must be cleaned and prepared aseptically before the blood collection. Based on patient size 21-25 measuring needles on 1-3 cc syringes are used for venipuncture (Stahl, 2006). The dorsal cervical sinus vein is the most common venipuncture site in turtles. Depending on the size of the animal, the sinus is found at a depth of 0.5 cm to 3 cm at the dorsolateral neck. The turtle must be restrained at the sternal recumbency position and the head extended and slightly flexed to the top of the table end. The needle can be inserted perpendicular to the neck or towards the tail (figure 4) (Perpiñán, 2017).

After blood is aspirated into the syringe, transfer blood to the hematology tube, then let stand for 20 minutes until the blood clots, then centrifuge at 3000 rpm for 15 minutes, after separate serum is immediately aspirated using a syringe and stored in a new hematological tube. Save the hematology tube containing the serum in the cool box. Then the serum was homogenized with reagents and examined using an iCubio iChem-535Vet machine.

The iChem-535Vet Semi-automatic Biochemistry Analyzer is a semi-automatic machine for conducting blood biochemical examinations, which still use reagents homogenized with serum during their inspection. The iChem-535Vet series is a machine designed for use by veterinarians. The way to use this machine is after the power is turned on, wait 10 minutes for the Preheating time, after the preheating has finished incubating (37oC) the reagent that has been homogenized with the incubator serum on the kuranng device for more than one minute (the amount of reagent + serum attached). After a serum temperature of 37oC, select Service> Zero Set> Aquades Aspiration (press the aspirate button in front)> Note the number that comes out in zero settings. Next, select Flowcell> Select parameter> Zero set> Aspiration aquades> Match the Prime Wave with the number Zero setting on the screen, if it differs slightly then the test can continue. After matching the numbers, select Test> Aspiration of the incubated sample> continue to the next sample with the same parameters. If it's finished / will change to another parameter, select Clean> Aspiration of cleaning fluid (Aquades + Bayclean 10: 1)> Clean again with pure Aquades.

Data Analysis

Data obtained from AST, ALT and creatinine values are presented in the form of a mean ± standard deviation. The analysis was carried out descriptively and compared with existing references. The relationship between body condition index (BCI) and the results of examination of blood biochemical parameters was analyzed by the Spearmen correlation test (Sampurna and Nindhia, 2017).

Location and Time of Research

The study was conducted in March-April 2019 Turtle Conservation and Education Center (TCEC), Serangan Island, Denpasar Selatan District, Denpasar City, Bali Province.

3. RESULT AND DISCUSSION

Result

The results of physical examination on 10 green turtles that joined the object of the study showed no fibropapiloma, tumors, barnacles, excessive desquamation, decubitus, wounds, scars, fractures or movement abnormalities.

Morphometric results and Body Condition Index (BCI) in Table 1. The results of blood biochemical examination in the form of a mean ± standard deviation of each age are presented in Table 2.

Table 1. Green turtle morphometry data at TCEC

Parameter	mean±SD *Juvenile* (n=5)	mean±SD *Sub adult* (n=3)	mean±SD *Adult* (n=2)
CCL (cm)	47.40±10.52	75.67±5.03	96±1.41
CCW (cm)	43.40±9.96	70±3.46	86±1.41
SCL (cm)	44.80±10.14	71.10±4.25	91.85±1.90
SCW (cm)	38.40±7.30	56.33±2.08	69.25±0.35
Berat (kg)	13.94±6.42	42.66±3.51	98.95±2.75
BCI	1.49±0.25	1.18±0.11	1.27±0.12

The 10 green turtles sampled in this study had a body condition index (BCI) ranging from 1.25 - 1.80 for 5 juvenile turtles (adolescents), 1.06 - 1.26 for 3 subadult turtles (almost adults), and 1.19 - 1.36 for adult turtles.

Table 2. The biochemical results of green turtle blood at TCEC

Parameter	mean±SD Juvenile (n=5)	mean±SD *Subadult* (n=3)	mean±SD *Adult* (n=2)	Reference
AST (U/L)	148.20±30.06	188±9.5	176.50±19.09	74,1 – 244,6*
ALT (U/L)	16.94±7.37	10.03±5.91	14.50±7.91	0.0-50.0**
Kreatinin (mg/dL)	0.42±0.25	0.80±0.02	1.05±0.49	0.4-0.9***

* Physiological value of AST in green turtles (Flint, 2010)

** Physiological value of ALT in green turtles (Aguirre and Balazs, 2000)

*** Physiological value of creatinine in green turtles (Resendiz and Lara, 2018)

4. DISCUSSION

Based on these categories, the results showed that juvenile green turtles with a range of BCI 1.25 - 1.80 can be categorized very good, for subadult turtles (almost adults) with a range of BCI 1.06 - 1.26 can be categorized as normal to very good, and for turtles adult) with BCI 1.19 - 1.36 it can be categorized as robust to very good. Thus, the turtle that was the sample in this study was very healthy.

The results of AST examination showed an average value of 148.20 ± 30.06 U / L for juvenile green turtles (adolescents), 188 ± 9.5 U / L for subadult green (almost adults), and 176.50 ± 19.09 U / L for adult (adult) green turtles. This value is entirely within the normal range of the average published by Flint (2010), which is 74.1 - 244.6 U / L. So that it can be ascertained that green turtles do not experience disease in the muscle, lung, and kidney tissue, as explained by Campbell (2014) that AST is one marker to determine damage to muscle tissue, lungs, and kidneys.

The results of ALT examination showed an average value of 16.94 ± 7.37 U / L for juvenile green turtles (adolescents), 10.03 ± 5.91 U / L for subadult green turtles (almost adults), and 14.50 ± 7.91 U / L for adult turtles. This value is all in the normal range according to Aguirre and Balazs (2000), namely 0.0-50.0 U / L. So that it can be ascertained that the liver in green turtles that are rehabilitated at the Turtle Conservation and Education Center (TCEC) attacks in healthy conditions. This is reinforced by the results of AST values that are in the normal range.

Creatinine levels from the results of the examination showed an average value of 0.42 ± 0.25 mg / dL for juvenile green turtles (adolescents), 0.80 ± 0.02 mg / dL for subadult green turtles (almost adults), and 1.05 ± 0.49 mg / dL for green turtles adult (adult) The value of juvenile and subadult green turtles tends to be the same as the average according to Resendiz and Lara (2018), namely 0.4-0.9 mg / dL. Unlike adult green turtles, where A1 turtles have a value of 1.40 mg / dL, which means that the turtle creatinine value exceeds the threshold stated by Resendiz and Lara (2018). It should also be noted, the value mentioned by Resendiz and Lara (2018) is the value for juvenile and subadult green turtles. It is possible for adult green turtles to have a higher creatinine value, because according to Latimer (2011) variations in creatinine concentration are influenced by muscle mass and sex (higher males), and Barger and Macneill (2015) added that creatinine production is comparable to muscle mass. As presented, the weight of green turtles A1 far above juvenile and subadult green turtles, also identified that green turtle A1 is a male sex. The A1 turtle is most likely not experiencing any impairment of kidney disease which can increase creatinine levels, because based on the AST values examined it is still in the normal range of 176.50 ± 19.09 U / L.

5. CONCLUSION

The results of rehabilitated green turtle biochemistry tests at the Turtle Conservation and Education Center (TCEC) Attack showed normal values.

1. AST value of juvenile green turtles (adolescents) 104-185 U / L, subadult green turtles (almost adults) 179-198 U / L, adult green turtles 163-190 U / L. N
2. ALT value of juvenile green turtles (adolescents) 12.08-30 U / L, subadult green turtles (almost adults) 5.30-16.66 U / L, green turtles adult 8.90-20.10 U / L.
3. Creatinine levels for juvenile green turtles (adolescents) 0.40-0.76 mg / dL, subadult green turtles (almost adults) 0.77-0.82 mg / dL, green turtles adult (adults) 0.70-1 , 40 mg / dL.

REFERENCES

[1] Adnyana, IBW dan Hitipeuw C. 2009. *Panduan Melakukan Pemantauan Populasi Penyu di Pantai Peneluran di Indonesia*. Jakarta: WWF-Indonesia

[2] Aguirre, A dan Balazs GH. 2000. Blood Biochemistry Values of Green Turtles (*Chelonia mydas*) with and without fibropapillomatosis. *Comparative Haematology International* 10:132-137

[3] Barger AM dan Macneil AL. 2015. *Clinical Pathology and Laboratory Techniques for Veterinary Technicians*. Wiley: Oxford

[4] Bjorndal KA, Bolten AB, Chaloupka MY. 2000. Green Turtle Somatic Growth Model: Evidence for Density Dependence. *Ecol Appl* 10:269-282

[5] Dermawan A, Nuitja INS, Soedharma D, Halim MH, Kusrini MD, Lubis SB, Alhanif R, Khazali M, Murdiah M, Wahjuhardini PL, Setiabudiningsih, Mashar A. 2009. *Pedoman Teknis Pengelolaan Konservasi Penyu*. Jakarta: Direktorat Konservasi dan Taman Laut

[6] Espinoza-Romo BA, Sainz-HernaÂndez JC, Ley-QuiñoÂnez CP, Hart CE, Leal Moreno R, Aguirre AA. 2018. Blood biochemistry of olive ridley (Lepidochelys olivacea) sea turtles foraging in northern Sinaloa, Mexico. *PLoS ONE* 13(7): e0199825. https://doi.org/10.1371/journal.pone.0199825

[7] Flint M, Morton JM, Limpus CJ, Patterson-Kane JC, Murray PJ, Mills PC. 2010. Development and Application of Biochemical and Haematological Reference Intervals to Identify Unhealthy Green Sea Turtles (Chelonia mydas). *The Veterinary Journal* 185. doi: 10.1016/j.tvjl.2009.06.011

[8] *Karnan. 2008. Penyu Hijau: Status dan Konservasinya. Jurnal Pijar MIPA 3(1)*

[9] Latimer KS. 2011. *Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology*. UK: Wiley-Blackwell

[10] Mattjik AA, dan Sumertajaya IM. 2000. *Perancangan Percobaan dengan Aplikasi SAS dan Minitab Jilid I*. Edisi Kedua. Bogor: IPB-Press

[11] Perpiñán D. 2017. Chelonian Haematology 1. Collection and Handling of Samples. *In Practice* 39. doi: 10.1136/inp.j1692

[12] Resendiz E, Lara MM. 2018. *Health Assesments in Free-Ranging Sea Turtles: Perspective of Animal Welfare in Wildlife*. Intechopen: Mexico

[13] Sampurna IP, Nindhia TS. 2017. *Aplikasi SPSS dalam Biostatistika*. Denpasar: Universitas Udayana

[14] Stahl SJ. 2006. *Reptile Hematology and Serum Chemistry*. The North American Veterinary Conference. Orlando, Florida

[15] Swimmer JY. 2000. Biochemical Responses to Fibropapilloma and Captivity in The Green Turtle. *Journal of Wildlife Disease* 36(1)

[16] Work T, Balazs GH. 1999. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. *Journal of Wildlife Diseases* 35:804-807

[17] Wyneken J. 2004. *The Anatomy of Sea Turtles*. NOAA Technical Memorandum NMFS-SEFSC: U.S.