Evaluation of hematological and urine parameters of Anoa (Bubalus Spp.) at Environment and Forestry Research and Development Institute of Manado (BP2LHK)

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Abstract. Anoa (Bubalus spp.) is an endemic species found only on Sulawesi Island, Indonesia. The lack of intact review evaluating the physiological condition leads to difficulties in the conservation efforts of this endangered species. This study aimed to examine the hematological and urine parameters of Anoa at the Environment and Forestry Research and Development Institute of Manado (BP2LHK). Four Anoa were used in this study. The physiological conditions were recorded during the study period, while hematological and urine parameters were only examined once and twice, respectively, due to limited access to the animals. The level of hemoglobin was 17.18 ± 0.8 g/dL, packed cell volume of 45.22 ± 3.4 %, total erythrocyte count was 10.46 ± 0.5 ×10^6/µl and total leukocyte count was 5.65 ± 1.0 ×10^3/µl. The mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was 43.38 ± 3.5 fl, 16.48 ± 0.7 gl, and 38.28 ± 1.3 g/dL, respectively. The urine leukocytes were 0-15 leuko/µl, urobilinogen level was 3.50 µmol/L, protein value was 39.50 ± 10.3 mg/dL, and urine pH and urine density were 9.00 and 1.00 g/mL, respectively. The urine sedimentation analysis showed the formation of the urinary crystal (amorphous crystal, calcium carbonate, and struvite). We conclude that hematological and urine parameters were not different among Anoa in this study.

1. Introduction

Anoa (Bubalus spp.) is endemic species from Indonesia which is only found on Sulawesi Island and Buton Island. There are two species of Anoa, namely lowland Anoa (Bubalus depressicornis) and mountain Anoa (Bubalus quarlesi) [1,2]. These animals become endangered because their population is decreasing in nature. The main cause of the decline in population due to illegal hunting, forest diversion becomes agricultural and residential areas [3] and hunting by the local community for meat and leather [4]. Diminishing population causes them to live in small populations (sub-populations) making it difficult to mate and trigger inbreeding [5].
At present, the existence of Anoa is increasingly threatened with extinction, therefore the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITIES) in 2016 classified them in Appendix 1, while the International Union For Conservation of Nature And Natural Resources (IUCN) classified in endangered species. Various conservation programs both ex-situ and in-situ are carried out by the Government of Indonesia, non-governmental organizations (NGOs), and local communities in Sulawesi. This is intended to prevent them from being threatened with extinction due to the dramatic decline in population numbers in the last few years [3]. One of the most obvious programs is Anoa Conservation Action Plan 2013-2022 by the Ministry of Environment and Forestry, which includes in-situ and ex-situ conservation programs [6]. The ex-situ program that has been successfully implemented is the captive breeding called Anoa Breeding Center (ABC) at Environment and Forestry Research and Development Institute of Manado (BP2LHK) which has given birth to three Anoas from previously recorded only seven in 2016 [7].

The medical examination is an effort to support the success of the captive breeding program of wildlife because an understanding of healthy animals is different than the sick one, so we need an understanding of how tests to support the diagnosis [8]. Study on Anoa in veterinary is still limited, therefore, there is no value control on the physiological status of these animals. Researches related that had been reported such as ectoparasite [9], reproduction [10,11], and hematological evaluation [12,13]. This study aimed to know the hematology profiles and urine parameters of Anoa in ABC BP2LHK Manado. This research is expected to upgrade the knowledge of the health status of these animals and assist veterinarians in decision diagnoses.

2. Material and method

Five Anoas which consist of two male and three female with a range of 2.5-7 years. All of them that examined were bred at ABC BP2LHK Manado with the same breeding treatment. All of them were sampled for urine examination, while for hematological examination, rectal temperature and respiration rate were carried out in four Anoas.

Sampling was done in the morning by taking 3 ml of blood in the jugular vein and then stored in an Ethylene Diamine Tetra Acid (EDTA) tube. Blood samples were examined for hematology using a hematology analyzer (Sysmex Xs-800i). While the urine sampling was conducted by collecting the specimen in a baker container then examining the urine chemistry with dipstick test (VerifyTM), microscopic examination (Olympus CX21), and organoleptic. Observation of rectal temperature and respiration rate is also carried out as additional references in considering the health status of Anoa were carried out every day for two consecutive weeks.

The hematological examination was performed to observe hemoglobin (Hb), total erythrocytes and leukocytes, and packed cell volume (PCV), while the erythrocyte index included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using existing standard calculations [14]. Chemical urine tests are performed to observe leukocytes, nitrites, urobilinogen, protein, pH, weight, type, ketones, bilirubin, and glucose. Microscopic examination to observe urine sediment and organoleptic for color and aroma.

3. Result and discussion

3.1. Hematologic examination

3.1.1. Hemoglobin. The hematological examination was carried out to see the blood profile of Anoa's health conditions at ABC BP2LHK Manado. However, there is no control value for normal blood profile of them, thus, the results obtained will be compared with the blood profile from Anoa which was bred at Liepzig Zoo, Germany [12], and several zoos in Indonesia [13]. Hematology evaluation can be seen in Table 1.
Table 1. Blood profiles of Anoa (Bubalus spp.) at ABC BP2LHK Manado.

| Blood samples | Hemoglobin (g/dL) | PCV (%) | Erythrocytes (µl) | Leukocytes (µl) |
|---------------|-------------------|---------|-------------------|-----------------|
| Anoa A (♂)   | 18.5              | 47.6    | 11.96 × 10^6      | 3.5 × 10^7      |
| Anoa B (♀)   | 18.6              | 54.3    | 10.08 × 10^6      | 4.8 × 10^3      |
| Anoa C (♂)   | 16.1              | 40.2    | 9.93 × 10^6       | 8.2 × 10^3      |
| Anoa D (♀)   | 15.5              | 38.8    | 9.87 × 10^6       | 6.1 × 10^3      |
| Mean value   | 17.18 ± 0.8       | 45.22 ± 3.4 | 10.46 ± 0.5 × 10^6 | 5.65 ± 1.0 × 10^3 |

The results of the hematological examination can be seen in Table 1. The hemoglobin levels obtained were lower when compared to Anoa that had been reported at Leipzig Zoo that was (8.91-12.81 g / dl) [12] and higher than reported at Ragunan Zoo and Surabaya Zoo that were (13.4 ± 1.6 g / dl) and (13 ± 2 g / dl) respectively [13]. However, the difference in hemoglobin values of each Anoa in ABC Manado is not much different, this is possible because the treatment and nutritional control are given similar for each one. Hemoglobin is a protein that functions in the process of transporting oxygen and carbon dioxide from the lungs to the other tissues. Hemoglobin levels in the blood could be a reference for measuring the state of anemia in an individual animal. High levels of hemoglobin affect the high number of erythrocytes in circulation because hemoglobin is an essential component of its filling [15]. The high level of hemoglobin in Anoa at ABC BP2LHK Manado may be due to the tendency to consume more green grass feeds such as Australian grass (*Paspalum dilatatum*), Napier grass (*Pennisetum purpureum*), and corn stalks. High crude fiber which contains protein and low concentrates consequence in an increase in the number of erythrocytes that will be followed by increasing hemoglobin levels [16].

3.1.2. Packed cell volume (PCV). Packed cell volume (PCV) is the volume of all erythrocytes in 100 ml of blood and is also called blood volume percentage which is determined by venous/capillary blood. Level PCV varies widely depending on the type of species, in large animals, ranging from 30% to 40% [14]. The examination results showed that the PCV levels of Anoa at ABC did not show a significant dissimilarity when compared to Anoa in Leipzig Zoo that was (33.0 - 44.0%) [12] and several zoos in Indonesia such as Ragunan Zoo and Surabaya Zoo that was (46 ± 37%) and (41 ± 6.6%) respectively [13]. The fluctuation result value of Anoa at ABC BP2LHK Manado may be influenced by environmental conditions around the cage which can reach a temperature of 33°C. Stress is a consequence of environmental changes that could affect physiological changes that have following an impact on PCV levels. High PCV levels occur due to increased erythrocyte production and decreased blood plasma [17].

3.1.3. Erythrocytes and Leukocytes. Erythrocytes have a function in transporting hemoglobin which carries oxygen from the lungs to all tissues [14,15]. The results study of erythrocytes level on ABC BP2LHK Manado was higher than reported at Leipzig Zoo that was (3.87 × 10^6 - 7.24 × 10^6 / µl) [12]. The result that tended to be lower were also reported at the Surabaya Zoo and Ragunan Zoo that was (7.58 ± 1.15) and (9.91 ± 2.4) respectively [13]. These indicate a correlation with PCV and hemoglobin results that have been described, erythrocyte levels tend to correlate with changes in PCV levels. Several studies explained that different erythrocyte levels can be determined by climate, age, genetic variation [18], nutrition, and breeding patterns [19].

Leukocytes are makeup 1% of the total blood in the body that has important functions in the immune system. The study showed that Anoa at ABC Manado was higher than reported at Leipzig Zoo, which was around (2.89 × 10^3-5.24 × 10^3 / µl) [12] and some in Indonesia such as Ragunan Zoo and Surabaya Zoo that was (3.83 ± 0.92 / µl) and (6.9 + 3.17 / µl) respectively [13]. The variation of leukocyte value of each individual is influenced by age, nutrition, infection, inflammation, and level of stress. In stressful conditions, there will increase cortisol to encourage neutrophils and reduce lymphocytes. Cortisol causes lymphopenia, eosinopenia, and basopenia by releasing lymphocyte cells
from the lungs and spleen and reducing lymphocyte mitosis from bone marrow [20]. Inflammation or infection are affected by the invasion of parasites stimulate releasing neutrophils [21]. Previous research was explained the number of parasites invasion around the ABC BP2LHK Manado cage [9].

3.2. Erythrocyte index
Erythrocyte index purpose for observing the anemia condition of animals. The results study of the erythrocyte index in Anoa will be compared to other at Taman Safari Indonesia and Kanazawa Zoological Garden of Yokohama, Japan [13]. Erythrocyte index at Anoa Breeding Center BP2LHK Manado compared with values for Anoa (Bubalus spp.) in Taman Safari Indonesia and Kanazawa Zoological Garden are shown in table 2.

**Table 2.** Erythrocyte index at Anoa Breeding Center BP2LHK Manado compared with values for Anoa (Bubalus spp.) in Taman Safari Indonesia and Kanazawa Zoological Garden, Japan.

| Erythrocyte index | Anoa A | Anoa B | Anoa C | Anoa D | Mean value | Taman Safari Indonesia* | Kanazawa Zoological Garden* |
|-------------------|--------|--------|--------|--------|------------|--------------------------|-----------------------------|
| MCH (gl)          | 15.5   | 18.5   | 16.2   | 15.7   | 16.48 ± 3.5 | 21.0                     | 14.23                       |
| MCV (fl)          | 39.8   | 53.9   | 40.5   | 39.3   | 43.38 ± 0.7 | 68.0                     | 36.46                       |
| MCHC (g/dL)       | 38.9   | 34.3   | 40.0   | 39.9   | 38.28 ± 1.3 | 30.5                     | 38.77                       |

*Sources: [13]

The levels of MCH and MCV showed lower yields compared to Anoa in Taman Safari Indonesia but higher than reported in Kanazawa Zoological Garden [13]. Normal value in MCH indicates average hemoglobin mass in red blood cells also normally and vice versa. The MCV value shows the size of the erythrocytes obtained from simple arithmetic between PCV and other erythrocytes. Highly value represents a large size of erythrocytes or macrocytic. On the other hand, low MCV indicates the size of small or microcytic red blood cells. Normal MCV size indicates normal or normocytic red blood cell size [14].

MCHC measure of hemoglobin concentration in erythrocytes showed the difference is not too significant compared have been reported [13]. Abnormality of extremely high MCHC values is unlikely as is the case with hyperchromic red blood cells. The condition of erythrocytes may not contain a high hemoglobin concentration due to the maximum capacity of the amount of hemoglobin that can be contained in erythrocytes.

3.3. Physiological conditions: respiration rate and rectal temperature
Physiological conditions can be used as an indicator of animal health. Respiration rate is a representation of the requirements for the disposal of gas metabolism and body heat. Factors that can affect the physiological changes of an individual are heat stress that is obtained from circumstance and environmental temperatures that tend to be unstable, this can also be a factor in the occurrence of stress in animals [22]. Heat stress is indicated by raising body temperature, frequency of respiration, drinking and consumption pattern, decreased appetite, and increased catabolism.

**Table 3.** Observation of the respiration rate of Anoa at ABC BP2LHK Manado compared to buffalo (Bubalus bubalis).

|                      | Respiration rate (times/minute) | Buffalo (Bubalus bubalis) |
|----------------------|--------------------------------|---------------------------|
|                      | morning | noon | afternoon | Markvichitr (2006) [23] | Fahimuddin (1975) [24] |
| Anoa A               | 56-80   | 56-80| 56-80     | 25.6 – 29.4 /minute     | 20-25 /minute          |
| Anoa B               | 52-76   | 50-76| 50-76     |                         | 16/ minute             |
| Anoa C               | 46-80   | 44-80| 60-84     |                         |                         |
| Anoa D               | 40-76   | 52-80| 56-84     |                         |                         |
Table 4. Observation of rectal temperature of Anoa at ABC BP2LHK Manado compared to buffalo (*B. bubalis*).

| Rectal temperature (°C) | Male | Female |
|-------------------------|------|--------|
| Anoa A                  | 37.4 -38.4 |         |
| Anoa B                  | 37.0 -38.1 |         |
| Anoa C                  | 37.1 -38.8 |         |
| Anoa D                  | 37.2 -39.1 |         |

Increasing the respiratory rate is an adaptation effort in releasing body heat into the environment through heat stress. The results of observations of respiration in Anoa at ABC BP2LHK Manado can be seen in Table 3, and rectal temperature in Table 4. The results showed a significantly different value when compared to the buffalo that had been reported [22,23]. Respiratory rate is a normal physiological response to adjusting the environmental changes, therefore it is essential to know the normal indicators when the animal feels comfortable or not [25]. Meanwhile, the increasing rectal temperature might occur when the body cannot regulate the heat balance to increase respiration rate and heart rate [26].

The correlation between changing respiration rate and rectal temperature of Anoa at ABC BP2LHK Manado is influenced by the temperature around the cage which tends to follow the body's response. Low temperature contains low oxygen content in the air, the body should increase frequency to optimize oxygen uptake. Increasing respiration will correlate with the cardiovascular system in the body which is marked by high body temperature. The environmental temperature will also respond thermoregulation system to maintain constant.

3.4. Urine examination

3.4.1. Urine chemistry. Urine examination is performed for diagnostic purposes due to plays an important role in therapy [27]. Properly examine might be seen that there are normal substances in deviant levels and changing substances contained in urine [28]. The urinary test conducted in this study included chemistry with the dipstick test which can be seen in Table 5, while microscopic examination and organoleptic are seen in Table 6. The results are compared with reference values that have been reported [29,30], which showed that were not much different on the examination of leukocytes, urobilinogen, and specific gravity, however, the pH test showed highly. Increase leukocytes in urine may indicate an infection of the urinary and reproductive tract [31,32], while high urobilinogen levels may be caused by bile duct obstruction and post hepatic disorders [33]. High pH levels of Anoa at ABC BP2LKH may be caused by dietary patterns of food consumption such as vegetables, fruits, and grasses as explained by several researchers [34]. In general, Anoa in ABC Manado showed no indication of urinary tract disturbances based on anamnesis and general observations. Other parameters such as nitrates, ketones, blood, and glucose also did not show abnormalities. Therefore, feed variation and consumption patterns may be the main factors for differences in urine chemistry results.

Table 5. Chemical examination of urine on Anoa at ABC BP2LHK Manado.

| Parameter     | Result                  | Control value* |
|---------------|-------------------------|----------------|
| Leukocytes    | 0-15 leuko/µl           | 0 – 25 Leuko/µl|
| Urobilinogen  | 3.50 µmol/L             | 3.5 – 17 µmol/L|
| Protein       | 39.50 ± 10.3 mg/dL      | Negative       |
| pH            | 9.00                    | 7.4 – 8.4      |
| Specific gravity | 1.00 g/mL              | 1.015 – 1.030 g/ml |

* [29,30]
Table 6. Results of urine sedimentation and organoleptic examination of Anoa at ACB BP2LHK Manado.

| Week  | Sediment                | Turbidity | Colour   | Odor                |
|-------|-------------------------|-----------|----------|---------------------|
| Week-1| Struvite                | Clear     | Yellow   | Specific flavorful   |
|       | Calcium carbonate       |           | Light yellow |                    |
|       | Amorphous phosphate     |           | Light yellow |                    |
| Week-2| Struvite                | Clear     | Yellow   | Specific flavorful   |
|       | Mucus urine             |           |          |                     |
|       | Amorphous phosphate     |           |          |                     |

3.4.2. Urine sedimentation. Microscopic observation in Table 6, shows the overall data of urine containing crystal-type sediments and mucus. The types of crystals found included struvite, calcium carbonate, and amorphous phosphates which is normal sediment found in urine that has a pH over 7 or in alkaline conditions [35]. On the other hand, when urine is in an acidic state, calcium oxalate, cysteine, sodium urate, and xanthin deposits will form [36]. Urine sedimentation of Anoa at ABC BP2LHK Manado are shown in figure 1.

![Figure 1](image-url)

Figure 1. Urine sedimentation of Anoa at ABC BP2LHK Manado, magnification 40X. (a) Amorphous phosphate, (b) calcium carbonate, (c) struvite, (d) urine mucus.

Struvite (MgNH₄PO₄·6H₂O) has clinical benefits due to its form of spontaneously in alkaline urine. However, it needs to be a concern when crystals are found in large quantities because it possible for them to feed in low calcium and phosphorus (Ca: P). It got worse when there are excess urease-producing bacteria [37]. Both calcium carbonate (CaCO₃) and amorphous phosphate are formed due to the high feed intake of calcium and phosphate that affect pH conditions of more than 7.5 [38]. Finally, these conditions will consequence in hypercalciuria and hyperphosphaturia which accelerate the formation of urine crystals [39].

Prevention crystalluria is to adjust the feed intake given according to the nutrients needed. Common strategies include using high-quality concentrates, ad-libitum drinking water, applying acidifiers such as 4% NaCl, 10% acetic acid to lowering pH that is too alkaline and prevents some
crystalline triggers such as sorghum. In particular, an optimizing ratio of Ca/P into (2:1). Ammonium and phosphate in the urine are obtained from feed protein therefore using concentrate should not be excessive. Amorphous phosphate is also prevented by low-phosphate feed while calcium carbonate is prevented by low-calcium feed [40].

The microscopic evaluation shows that all the samples are in normal condition. The occurrence of crystalluria, in this case, is not pathological because it formed spontaneously in alkaline urine in small amounts. Other sedimentary elements such as erythrocytes, leukocytes, epithelial cells, cylinders (casts), bacteria, fungi, and parasites were not found in the urine sample. The difference in crystal types in the first and second weeks might due to feeding variations. Anoa at ABC BP2LHK were fed with Australian grass and Napier grass. The ratio Ca/P content of Anoa feed ingredients that have been reported that ranges from 0.27-0.44% and 0.14-0.49% [6].

4. Conclusion
Conservation programs have been done by the Indonesian Government, NGOs, private companies, and local communities for saving Anoa from extinction threatening. However, it still has many obstacles due to limited data on health management in Anoa conservation, making it difficult to determine health status. Hematologic and urine evaluations that have been carried out in this study are expected to provide an overview of the health status and management of Anoa in conservation.

In summary, hematologic and urine evaluations showed varied results for all Anoa at ABC BP2LHK Manado. Hematology showed lower hemoglobin yields when compared with Anoa reported in other conservations, whereas erythrocytes and leucocytes showed higher levels. Erythrocytes also affected the results of the erythrocyte index such as different MCV, MCH, and MCHC. Urine examination also showed pH value that tended to be higher when compared to the control value, while the other urine chemistry results did not show a significant difference. An alkaline pH level can stimulate the formation of urine crystals such as amorphous phosphate, calcium carbonate, and struvite, as had been found on microscopic examination. Differential results on the hematological and chemical examinations of urine, presumably due to stress of shifting environmental conditions in the cage, dietary and consumption patterns, and different maintenance management from others captive that has been reported.

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