Hematological and Biochemical Parameters in Prepubescent Water Buffaloes (*Bubalus bubalis*)

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Abstract: Water buffaloes are bred in Europe, Australia, North America, South America, and some African countries. European water buffaloes are all of the river type and are considered to be of the same breed named the Mediterranean water buffalo. This species plays a key role in milk production in Italy. Buffalo breeding for milk production has represented an important economic value in recent years, even in Italian regions where this activity was not historically rooted. Therefore, it is very important to monitor animal health and welfare by performing periodical clinical examinations combined with different hematological and biochemical tests. However, there are few studies regarding these aspects, as well as research aimed at establishing the reference ranges for species. This study aims to define hematological and biochemical parameters in a population of healthy water buffalo in controlled conditions for over a year in Italy.

Keywords: Water buffalo, *Bubalus bubalis*, hematology, biochemical parameters, prepubescent water buffalo.

1. INTRODUCTION

The domestic water buffalo (*Bubalus bubalis*) represents an important source of milk in many European countries, especially in Italy. Buffaloes' milk and milk products are particularly appreciated by consumers [1]. In 2018, buffalo produced 127 million tons of milk worldwide; in Europe, production was estimated at 390,000 tons, of which 378,000 tons have been produced in Italy [2]. From 2015 to 2019, the buffalo population in Italy increased by 7% [3-5]. The buffalo breeding, aimed at milk production, represents an important economic resource, even in regions where this activity was not historically rooted. The buffalo mozzarella, recognized under the EU's Protected Designation of Origin (PDO) scheme, is estimated at a sales value of 766 million Euros [6]. Proper management of these animals would produce individual water buffaloes with high genetic and health quality. In this context, studies are encouraged to establish reference values for hematological and biochemical parameters in this species. This is justified by the need to investigate possible parameter variations, such as health, nutrition, and breeding systems. These data may be used in clinical practice, as well as a tool for assessing the health and welfare of these animals. Few studies are available in the international literature aimed at determining the hematological and biochemical parameters of water buffaloes [7]. The study aimed to investigate hematological and biochemical parameters in prepubescent water buffaloes to establish reference values for functional health and welfare evaluations.

2. MATERIALS AND METHODS

2.1. Animals under Study

Twenty-six buffaloes, 4-6 months old (15 females and 11 males), were enrolled in the study (Table **1**). All individuals were born in captivity and were used to frequent handling. Clinical evaluation was performed by general inspection, palpation, and the measurement of heart and respiratory functions. Cardiac and pulmonary functions were measured by hearing accurately to identify sounds of the heart and lungs, using a stethoscope (Littmann® Classic II, 3M™ USA) at the apex of the heart and on the whole lung area. The animals were preliminarily subjected to serological tests for antibodies against Brucellosis, Chlamydiosis (*Chlamydia psittaci*), Parainfluenza 3, Bovine Viral Diarrhea (BVD), Q fever (*Coxiella burnetii*) and *Yersinia enterocolitica* O:9. The trial was carried out in the premises of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", IZS-Teramo, Italy) where it is a duly authorized premise pursuant to Italian law (D. Lgs. 26/2014) regulating the use of animals in experimental conditions (Figure **1**). The animals were housed in standard rearing environments, with ad libitum feeding and drinking water. The buffaloes were fed three times a day with a mix of complete feed for buffaloes, in addition to hay (mixed first and second cut), always available ad libitum (Table **2**).

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Table 1: Buffaloes Subjected to Blood Sampling and their Gender, Age at the beginning of the Observation, and Age at the End of the Study Observation

ID	Gender	Date of Birth	Months of age at the beginning of observation	Months of age at the end of observation
*609	F	27/12/2019	7	19
*610	M	28/12/2019	7	19
*612	F	01/01/2020	7	19
*613	F	02/01/2020	7	19
*615	M	02/01/2020	7	19
*616	M	04/01/2020	7	19
*617	F	05/01/2020	7	19
*618	F	06/01/2020	7	19
*619	F	07/01/2020	7	19
*621	F	07/01/2020	7	19
*622	M	08/01/2020	7	19
*623	M	08/01/2020	7	19
*624	F	10/01/2020	7	19
*626	F	11/01/2020	7	19
*627	M	11/01/2020	7	19
*628	F	13/01/2020	7	19
*629	F	16/01/2020	7	19
*630	M	16/01/2020	7	19
*631	F	17/01/2020	7	19
*632	M	19/01/2020	7	19
*634	M	22/01/2020	7	19
*637	F	25/01/2020	7	19
*641	F	02/02/2020	7	19
*644	M	07/02/2020	7	19
*649	M	17/02/2020	7	19
*653	F	24/02/2020	7	19

Figure 1: Image of the water buffaloes enrolled in the study standing on the premises where the observation was carried out.

Table 2: Nutritional Value of Feed Administered to the Buffaloes under Observation

Crude protein	20.5%
Crude fats	4.3%
Crude fiber	8.6%
Crude ash	7.9%
Sodium	0.4%

2.2. Sampling Methods and Frequency

Animals were observed for 12 months (August 2020-August 2021). Blood samples were taken from the jugular vein using sterile syringes and needles, using the Vacutainer TM system (VACUETTE® Multiple Use Drawing Needles 20G, Greiner Bio-One International GmbH, Cassina de Pecchi, Italy). Starting from day T0, samples were collected from all animals twice a week for the first 8 weeks (T0-T58); from week

9 to 16 (T62-T111), the samples were taken weekly. From week 17 (T125) to the end of the observations, blood samples were taken every 2 weeks. For biochemical analysis, blood was collected in vacuum tubes without anticoagulant, left at room temperature for clot retraction, and centrifuged at 863 rcf (relative centrifugal force) for 4 minutes. The sera collected were frozen at -20 °C until analysis. For the haemogram, blood was taken on a weekly basis from T0 until the 16th week after, then every 2 weeks until the end of observation.

2.3. Hematological Analyses

Blood counts were performed on blood samples collected in EDTA vacutainer tubes (VACUETTE® EDTA) and dispatched to the laboratory within 24 hours after collection, stored at 4 °C ± 2 °C. Hematological analyses were performed at the IZS Teramo branch laboratory of Pescara, Italy, using commercial kits (Advia, Siemens, Bayer Diagnostics, NY, USA) and an ADVIA 120 hematology system (Siemens, Bayer Diagnostics, NY, USA), equipped with specific software for veterinary use. The following values were determined: red blood cell count (RBC), volume distribution width (RDW), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), hemoglobin distribution width (HDW), mean corpuscular hemoglobin (MCH), hematocrit (HCT), mean corpuscular volume (MCV), white blood cells count (WBC), lymphocytes, neutrophils, basophils, eosinophils, monocytes. Hematological parameters were measured according to Feldman B.F. *et al.* [8].

2.4. Biochemical Analyses

Biochemical analyses were performed on blood samples collected in vacutainer tubes without anticoagulant and dispatched to the laboratory within 24 hours after collection. Biochemical analyses were carried out at the IZS-Teramo branch laboratory of Pescara (Italy) and performed using commercial kits (Quantilab Kits, Werfen Company, Milano, Italy) and an ILAB 650 automated system (Instrumentation Laboratory, MA, USA). The following values were determined: glucose, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin, total protein, albumin, calcium, cholesterol, triglycerides, amylase, alkaline phosphatase (ALP), aspartate-transaminase (GOT/AST), alanine - aminotransferase (GPT/ALT) and gamma – glutamyl-transferase (GGT). Biochemical parameters were measured according to Sodikoff C.H. [9].

2.5. Statistical Analysis

The software used for statistical calculation was SPSS version 20 (SPSS for Windows, version 20.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to summarize the data, including median, 2.5, and 97.5 percentiles and minimal-maximum values.

Table 3: Hematological Parameters

HAEMATOLOGIC AL VALUES	Reference UNIT	Median	Percentile 2.5	Percentile 97.5	Minimum	Maximum
Red Blood Cells (RBC)	(x 106/µL)	7.1	5.9	8.2	5.4	8.9
Red Blood Cells Distribution Width (RDW)	(%)	19.1	17.3	21.5	16.4	23.6
Haemoglobin (HGB)	(g/dL)	12.0	9.9	14.5	9.0	16.2
Mean Corpuscolar Haemoglobin Concentration (MCHC)	(g/dL)	41.7	39.9	43.8	38.5	44.6
Haemoglobin Distribution width (HDW)	(g/dL)	3.0	2.4	5.3	2.2	7.0
Mean Corpuscolar	(pg)	17.0	14.8	19.4	14.3	20.3
Hemoglobin (MCH)						
Haematocrit (HCT)	(%)	28.7	23.5	35.1	22.1	40.6
Mean Cells Volume (MCV)	(fL)	40.8	34.9	47.7	33.9	50.1
White Blood Cells (WBC)	(x 103/µl)	12.4	8.0	16.9	6.7	19.7
Lymphocytes	(%)	61.4	50.4	72.1	43.6	82.3
Neutrophils	(%)	32.5	21.0	43.4	11.9	50.3
Basophils	(%)	1.3	0.9	2.2	0.7	4.0
Eosinophils	(%)	1.8	0.7	4.9	0.3	9.8
Monocytes	(%)	2.0	0.9	4.9	0.6	10.7

Table 4: Biochemical Parameters

BIOCHEMICAL VALUES	Reference UNIT	Median	Percentile 2.5	Percentile 97.5	Minimum	Maximum
Glucose	(mg/dL)	61.0	37.0	85.0	2.0	95.0
BUN	(mg/dL)	21.0	10.0	33.0	6.0	47.0
Creatinine	(mg/dL)	1.2	0.8	1.9	0.5	2.5
Uric Acid	(mg/dL)	0.8	0.4	1.2	0.3	1.5
Total Bilirubin	(mg/dL)	0.1	0.0	0.3	0.0	0.4
Total Protein	(g/dL)	6.2	5.6	6.8	5.2	7.5
Albumin	(g/dL)	3.1	2.7	3.7	2.5	3.9
Calcium	(mg/dL)	10.3	9.5	11.5	9.2	12.3
Cholesterol	(mg/dL)	76.0	55.0	100.2	44.0	122.0
Triglycerids	(mg/dL)	33.0	17.0	59.8	12.0	73.0
Amylase	(IU/L)	3.0	0.0	0.3	0.0	0.4
ALP	(IU/L)	198.0	107.0	468.2	77.0	574.0
GOT (AST)	(IU/L)	153.0	111.8	263.0	94.0	528.0
GPT ALT	(IU/L)	62.0	42.0	80.2	30.0	96.0
GGT	(IU/L)	21.6	13.6	30.6	9.4	38.5

3. RESULTS

3.1. Hematological Analyses

Among the parameters considered, the 12-month observation did not show significant changes in the study period (August 2020-August 2021). Hematological tests carried out during the study did not highlight important changes during the observation period. Results are reported in Table **3**.

3.2. Biochemical Analyses

Among the parameters considered at the first observation, no significant changes in the 12-month study period were observed (August 2020-August 2021). Biochemical tests performed during the study do not seem to highlight important changes related to particular stressful situations, both infectious and environmental. There were no other statistically significant differences between males and females for any other parameters considered. Results are reported in Table **4**.

4. DISCUSSION

The hematological and biochemical parameters found in this study for water buffaloes are in accordance with those already investigated by several authors [8-12].

As regards the relation with age, Ciaramella *et al.* observed lower levels of red and white blood cell count from older to younger animals; in this case, this is due to a reduced hematopoietic action by hormones, which decreases with age. In fact, in our study, we did not observe significant changes in these values just because we focused on prepubescent buffaloes [14].

As regards the effects of gender on hematological values, our results are in accordance with Patel *et al.* study, which demonstrated no significant differences between males and females in young buffaloes [16].

Furthermore, Patel *et al.* observed a significant variation of glucose, total protein, total cholesterol, creatinine, and BUN levels in relation to age and gender and/or physiological status in adult buffaloes; however, in the case of young buffaloes, the authors found a non-significant variation of this values between males and females [15].

The twenty-six buffaloes chosen for our study were 4-6 months old at the beginning of the observation (lasted for one year), so the study's results are focused on prepubescent animals. According to previous studies [13-15], it was observed that, in the case of prepubescent animals, the variations are not significant between males and females.

Moreover, water buffaloes (like other animal species) generally have almost the same metabolism

between males and females until sexual maturity. Therefore, this study did not compare the results by gender and age. Pregnancy and lactation are the two most important stages in the life of dairy animals that affect metabolism, resulting in the alteration of the hematological parameters [16, 17]. However, these aspects were not investigated, given the age of the animals under study.

Among the parameters considered, the 12-month observation (August 2020-August 2021) did not show significant changes in the considered parameters during the study period. In Italy, there is temperate weather, so this may explain the absence of significant changes across the different seasons. A one-year monitoring period has allowed a broad observation of the buffaloes' blood parameters, which made it possible to evaluate various external factors, possibly conditioning the metabolism of the animals. To the best of our knowledge, this is the first study that considered such long monitoring to identify the trend of blood values over time in buffaloes.

The values identified by this study for RBC, RDW, HGB, MCHC, HDW, MCH, HCT, MCV, WBC, Lymphocytes, Neutrophils, Basophils, Eosinophils and Monocytes are in accordance with previous reports [14]. The levels identified for Glucose, BUN, Creatinine, Uric Acid, Total Bilirubin, Total Protein, Albumin, Calcium, Cholesterol, Triglycerides, Amylase, ALP, GOT (AST), GPT (ALT), GGT do not vary significantly between male and female calves, in agreement with the observations of other studies [13]. All the parameters remained stable during the one-year time observation. As previously reported, some serum enzyme values after the exercise could rise [18]. Also, in our study, the value of transaminases (GOT (AST), GPT (ALT), and GGT) are higher than previously reported, and this could be related to the free husbandry practice for buffaloes on the farm. This condition allows the animals to walk and even run, generating a muscular activity that may determine a physiological increase in these enzymes in the blood.

5. CONCLUSIONS

The findings of this study serve as a valuable reference tool for understanding the health and welfare of prepubescent water buffaloes. It is important to acknowledge that these markers were assessed under specific conditions, and further evaluation under varying circumstances, including different nutritional and climatic stresses, is essential. These factors are crucial in an animal's ability to adapt to its captive environment. Additionally, variables such as age, nutrition, management conditions, and housing can significantly influence the hematological and biochemical parameters of water buffaloes.

In conclusion, this study underscores the need for continued research to expand our understanding of water buffalo health and well-being. Future investigations should encompass a wider range of conditions and variables to promote the establishment of optimal welfare standards and facilitate a more comprehensive interpretation of hematological and biochemical values in water buffaloes. Such endeavors will contribute to the overall betterment of their care and management.

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