academicJournals

Vol. 8(11), pp. 176-182, November 2016
DOI: 10.5897/JVMAH2016.0517
Article Number: 79BBF9661210
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Hematology and serum biochemistry of farmed bullfrog, *Lithobates catesbeianus* during the active and hibernating periods

Fei Peng, Rui Zhang, Xue Zhu, Huan Wang and Shengzhou Zhang*

Anhui Normal University, China.

Received 24 August, 2016: Accepted 17 October, 2016

The Bullfrog *Lithobates catesbeianus* is a species of economic important edible frog and often chosen as experimental animal model. Although, some studies in the literatures have reported the parameters of hematology and serum biochemistry in several Rana species, a comprehensive profile of hematology and biochemistry in farmed bullfrogs was very limited, especially during the hibernating period. 140 apparently healthy farmed bullfrogs (70 males and 70 females) were used during the active and hibernating periods to determine the hematology and serum biochemistry parameters including morphometry of erythrocytes, PCV, HGB, MCV, MCH, MCHC, RBC count, WBC count, differential leukocyte count, glucose (Glu), triglycerides (TG), cholesterol (Cho), blood urea nitrogen (Bun), uric acid (UA), creatine (Cre), total protein (TP), albumin (Alb), globulin (Glo), γ-glutam (GGT), total bilirubin (TB), alkaline phosphatase (Alp), a-amylase (Amy), CK, AST, ALT, LDH, K, Na, Cl and nCa (that is, the ionized calcium level when pH=7.4). Differences between sexes showed that male bullfrogs possessed a statistically higher LDH activity level, and statistically lower levels of Cre, Na and Ca concentrations. Additionally, it was noted that bullfrogs during the active period had significantly lower values for HGB, PCV, MCV, ALT, Glo, Bun, Na, Cl and surface areas and volumes of RBCs and their nuclei, and significantly higher values for WBCs counts and Cre than the hibernating ones. These baseline data could be used for health monitoring and disease diagnostics in bullfrogs artificial farming and serve as general reference values for future studies on the physiology of this species.

Key words: Bullfrogs, hibernation, hematology, serum biochemistry.

INTRODUCTION

Hematology and serum biochemistry parameters, as measured in peripheral blood, are useful in assessing the health and nutritional status of humans and animals (Artacho et al., 2007; Allender and Fry, 2008). The combination of many parameters is required to identify anemia, inflammatory diseases, parasitemia,

*Corresponding author. E-mail: szzhang@mail.ahnu.edu.cn.

hematopoietic disorders and homeostatic alterations (Campbel et al., 2007; Vasaruchapong et al., 2013). The measurements of erythrocyte dimension can be used for studies on environmental, seasonal, or altitudinal acclimatization and relationship between exchange of gas with tissues and metabolic rate (Ruiz et al., 1989; Pagés et al., 1992). The number of erythrocytes, HGB and PCV reflect the efficiency of oxygen carrying capacity and nutritional status. Leukocytes are involved in the defense of the organism under several conditions, such as stress (Silveira-Coffigni et al., 2004), inflammation (Martins et al., 2006) and parasitism (Azevedo et al., 2006), the number of which could be used as an indicator for infectious diseases (Pessier, 2007; Jamalzadeh et al., 2009). The serum biochemical analyses provide information on internal organs, proteins, electrolytes, and nutritional and metabolic parameters (Newman et al., 1997).

So far, most studies on hematology and serum biochemistry focused on fishes, reptiles and mammals when compared with amphibians. Some limited previous studies have reported on different hematology and serum biochemistry parameters of several amphibian species, including *Rana esculenta* (Sinha, 1983), rhacophorid frog (Mahapatra et al., 2012) and dubois's Tree Frog *Polypedates teraiensis* (Das and Mahapatra, 2014). Hematology and serum biochemistry parameters vary under different pathological, physiological, ecological and environmental conditions in animal population (Llacuna et al., 1996; Sarasola et al., 2004; Seaman et al., 2005). Amphibians are considered to be sensitive animals and show physiological variables to acute environmental changes (Carey, 2005). Two characteristic phases can be distinguished in the annual life cycle, that is, periods of activity and hibernation. Up to date, few documents were found on hematology and serum biochemistry parameters in amphibians at hibernating state.

Bullfrogs, *Lithobates catesbeianus*, being widely farmed over the world, is a species of economic important edible frog for farmers and often chosen as experimental animal model. Cathers et al. (1997) reported on hematology and serum biochemistry parameters of anesthetized American bullfrogs, *Rana catesbeiana*. However, no studies have been published in the literature on the hematology and serum biochemistry reference ranges during the active and hibernating periods for farmed bullfrogs. A relevant knowledge of bullfrog blood physiology is getting increasingly imperative due to diagnostic demand with the increase of breeding industry. It would also help to ensure food safety for eaters.

The aim of this study was to establish the hematology and serum biochemistry reference ranges of farmed bullfrogs during the active period (in May) and the hibernating period (in January). These baseline data could be used for health monitoring and disease diagnostics in bullfrogs artificial farming and serve as general reference values for future studies on the physiology of this species and other anuran species.

MATERIALS AND METHODS

Animals

140 farmed bullfrogs (35 males and 35 females each period) with mean weight 0.39±0.10 kg, ranging from 0.28 to 0.55 kg, were purchased from local raising households in Wuhu City, Anhui Province, China in January and May, respectively. Wuhu is in the southern region of China, with subtropical monsoon climate. These local bullfrogs would turn into the state of hibernation, that is, they neither eat nor move and breathe mainly through the skin when environmental temperature continuously fall below 10°C in January.

Sample collection

The blood samples of 140 bullfrogs were obtained using cardiocentesis. Prior to blood collection, the hibernating bullfrogs were housed in the terrarium for a 20-day continuous hibernation to ensure that they are still in the hibernating state, and the blood samples of active bullfrogs were collected in the morning over 12 h after a last feeding in May. Tubes coated with K_2-EDTA were used to collect samples for hematologic analysis and serum separation tubes without anticoagulant were used to collect samples for serum biochemistry and electrolyte parameters. All experiments were performed in compliance with national and provincial guidelines and in accordance with the Guide for the Care and Use of Laboratory Animals.

Analysis of hematology and biochemistry parameters

The serum biochemistry parameters including activities of CK, ALT, AST, GGT, LDH, Alp, Amy and concentrations of Cre, UA, Bun, TP, Alb, Glo, GLU, TB, Cho and TG were measured on serum samples by using an autoanalyzer (KHB 450, Shanghai, China). And the concentrations of K, Na, Cl and Ca in serum were determined using an electrolyte analyzer (IMS-972，HORRON, Shenzhen, China).

Total number of erythrocytes (RBC) and leukocytes (WBC) were analyzed in Neubauer Chamber, with dilution being performed by standard Hayem's solution for RBCs and Turk's solution for WBCs (Parida et al., 2011). The HGB concentration was determined by using an automated hematology analyzer (BC-3000plus, Mindray, Shenzhen, China). The PVC was examined by the microhematocrit method (Parida et al., 2011). The tubes were spun in a microhematocrit centrifuge (TDL-50B, Anke, Anting Scientific Instrument, Shanghai, China) for 5 min at 12000 rpm and the PVC was calculated with the total blood level divided by the blood cell level. The MCH, MCV and MCHC were calculated according to standard formulas (Campbell and Ellis, 2007).

In the laboratory, blood smears were stained with Wright Stain Solution (Tianda Diagnostic reagents co., LTD. Hefei, China) for the differential leukocyte count and then examined under a microscope (BM2000, Jiangnan Yongxin Co., LTD. Nanjing, China). The percentages of different leukocytes were determined after counting a total of 100 white blood cells.

The sizes of RBCs and their nuclei were acquired by measuring their long and short axes (major and minor axes) under a light microscope with an ocular micrometer (ERMA, Japan). Twenty to thirty cells from each blood slide of frogs (n=72) were selected for measurement of the major and minor diameters of erythrocytes and their nuclei (Benfey et al., 1984). The cell surface area, nuclear area and their volume were calculated using the below formula suggested by Lemoine and Smith (1980):

$S=a\times b\times \pi/4$ and $V= (a/2) \times (b/2)\ 2\pi\times 4/3$

Where a is the major and b is the minor axis of the cell or nucleus.

Statistical analysis

Hematology and serum biochemistry data resulting from our study were presented as means and standard deviation (SD) via the software SPASS19 for windows. The results were compared among periods in each sex and among sexes within each period. Significant differences were determined using an independent sample t test model. Results were considered significant at $p<0.05$.

RESULTS

In this study, no parasites were observed, no hemolysis occurred and no chyle blood serum appeared. Values obtained are expressed as mean±SD (ranges). Morphometry parameters of RBCs and their nuclei, hematology and biochemistry values of bullfrogs during the active and hibernating periods are summarized in Tables 1, 2 and 3, respectively.

Morphometry parameters of erythrocytes in bullfrogs during the active and hibernating periods

The morphometry parameters of erythrocytes are shown in Table 1, major and minor axes of erythrocytes and their nuclei were measured values, while surface area and volume of erythrocytes and their nuclei were calculated values. No significant differences were found in erythrocyte morphometry parameters between the two sexes. But the male bullfrogs tended to have lower values for cell minor axis, cell surface area, cell volume, nuclear minor axis, nuclear major axis, nuclear surface area and nuclear volume, than the female ones. The bullfrogs had significantly lower values for morphometry parameters during the active period than those during the hibernating period, particularly for surface area and volume of red blood cells and their nuclei, which were significantly increased during the hibernating period ($p<0.05$).

Hematology parameters of bullfrogs during the active and hibernating periods

The hematology parameters are shown in Table 2. The male bullfrogs had higher values of RBC count, neutrophil percentage and eosinophil percentage, but lower values of PCV and MCV than the females without a statistical significance. The total number of WBCs were found to be significantly higher during the active period than those during the hibernating period ($p<0.05$). However, the values of hemoglobin concentration, MCV and PCV percentage were statistically lower during the active period than those during the hibernating period ($p<0.05$). In addition, the values of MCH and RBC during the active period were lower than those during the hibernating period, but no statistical significance.

Morphologically, leucocytes of bullfrogs could be classified into the following five types: monocytes, lymphocytes, neutrophils, basophils, eosinophils, the first two were agranulocytes while the rest were granulocytes. The percentage of lymphocyte was observed to be the highest in bullfrogs, and the percentage of basophil was the lowest. The percentage of monocyte was close to that of neutrophil. It was found that males possessed slightly higher percentage of eosinophil than females. The percentage of the neutrophil and the N/L ratio during the active period were lower than those during the hibernating period, but not statistically significant.

Serum biochemistry parameters of bullfrogs during the active and hibernating periods

The serum biochemistry parameters are shown in Table 3. The male bullfrogs had significantly higher levels of LDH activity, and significantly lower levels of Cre, Na and Ca concentrations than the females ($p<0.05$). Additionally, the males tended to have high activity levels of AST, ALP, ALT, Amy, TB, but low GLU, TG, Cho and K concentration levels versus those for females ($p > 0.05$). The values of ALT, Glo, Bun, Na and Cl were significantly lower in both sexes during the active period than those during the hibernating period ($p<0.05$). Conversely, the Cre value of bullfrogs was statistically higher during the active period than those during the hibernating period in two sexes ($p<0.05$). In addition, the values of Amy, TP and Glu were found to tend to decrease during the active period ($p>0.05$). However, the values of GGT and Cho were found to increase during the active period versus those during the hibernating period ($p>0.05$).

DISCUSSION

Hematology and serum biochemistry are important tools for assessing health status in human and animals. It is well known that many hematology and serum parameters vary with sex, age, season, and physiological state (Boily et al., 2006). This study may be the first to present hematology and biochemistry reference ranges for farmed bullfrog and comparison between the active period (in May) and the hibernating period (in January).

Non-mammalian erythrocyte such as in fishes, amphibians, reptiles and birds is nucleated, flattened and ellipsoidal (Rowley and Ratcliffe, 1988). This study showed that the shape of RBCs was elongated or oval or elliptical in bullfrogs. The measurement of erythrocyte dimensions is often an important component of standard hematologic survey in amphibians (Hartman and Lessler, 1963). There was a relationship between cell size and metabolic rate. The cells with the smaller surface have lower metabolic cost per unit of cell mass (Olmo et al., 1989). In this study, the measurements of erythrocytes and their nuclei in bullfrogs during the active period were lower than those during the hibernating period, which were in agreement with the results observed for *Rana Esculenta* (Sinha, 1983), it was inferred that decrease in size of erythrocytes during the active period might be a physiological adaptation required for quicker blood circulation due to increased physical activity. Prosser (1973) reported that a higher MCH value is due to larger size of the RBCs. It was found that bullfrogs had higher values of MCH, RBCs counts, PCV percentage and HGB concentration during hibernation. In our opinion, an increase in MCH, RBCs count, PCV percentage, hemoglobin concentration and erythrocyte dimensions during hibernation may be explained by the “respiratory compensation” mechanism. According to Guijarro et al. (2003), respiratory compensation is necessary for fish to keep high oxygen availability to tissues in low oxygen condition. Likewise, this theory is applied to frog species. As known, the bullfrogs during hibernation breathed oxygen by low efficient way, that is, through the skin. Therefore, bullfrogs were trying to supply a more demand

Table 1. Morphometry parameters of erythrocytes in bullfrogs during the active and hibernating periods.

Parameters	Males		Females	
	Active period	Hibernation	Active period	Hibernation
Cell minor axis (um)	15.2±1.02(14.1-16.7)	16.3±1.36(15.0-17.9)	15.7±1.87(13.6-17.4)	17.0±2.27(16.3-19.9)
Cell major axis (um)	25.7±2.67(22.8-28.4)	26.0±4.91(23.4-30.1)	25.6±2.73(22.9-28.4)	26.2±2.90(22.5-28.6)
Cell surface area (um^2) [a]	306.7±34.85(256.3-330.9)	332.9±69.72(300.6-416.1)	315.5±39.83(272.9-355.5)	349.6±47.50(309.1-404.2)
Cell volume (um^3) [a]	817.7±49.73(762.4-869.0)	887.8±260.41(701.6-1199.7)	841.4±47.15(790.2-891.4)	932.3±126.90(824.3-1077.6)
Nuclear minor axis (um)	4.2±0.97(3.4-5.2)	5.9±1.02(4.8-7.0)	4.3±0.93(3.3-5.6)	6.8±1.18(5.1-7.9)
Nuclear major axis (um)	8.3±0.16(7.4-8.8)	9.4±1.60(8.1-10.7)	8.7±1.24(7.3-9.9)	10.0±2.06(9.1-13.3)
Nuclear surface area (um^2) [a]	27.4±8.76(19.0-35.3)	43.5±7.01(38.2-51.6)	29.4±9.88(20.0-38.7)	53.4±9.80(46.2-63.8)
Nuclear volume (um^3) [a]	72.9±2.10(69.3-74.1)	116.1±18.40(100.2-133.6)	78.3±2.42(67.9-80.4)	142.3±18.11(120.2-163.7)

[a] Significant difference($P < 0.05$) according to different periods.

Table 2. Hematology parameters of bullfrogs during the active and hibernating periods.

Hematology Parameters	Males		Females	
	Active period	Hibernation	Active period	Hibernation
RBC($\times 10^{12}$/L)	0.25±0.09(0.19-0.36)	0.34±0.14(0.16-0.51)	0.24±0.05(0.18-0.33)	0.32±0.07(0.26-0.40)
WBC($\times 10^9$/L) [a]	9.93±0.81(6.25-12.40)	2.41±0.79(1.71-3.50)	9.03±3.06(6.87-13.10)	2.43±1.03(2.00-3.63)
HGB(g/L) [a]	73.0±7.55(65.0-81.0)	105.0±13.1(93.0-119.0)	60.7±21.76(38.0-88.0)	106.0±14.7(95.0-123.0)
PCV(%)[a]	20.24±2.00(18.22-23.41)	29.78±6.40(22.90-38.13)	20.32±5.81(16.24-25.11)	29.91±2.40(26.02-35.56)
MCV($\times 10^3$fl)[a]	809.60±88.91(732.14-848.77)	875.88±67.32(832.31-957.07)	846.66±41.33(807.21-889.76)	934.68±93.82(874.20-1029.35)
MCH(pg)	290.00±74.10(226.02-335.12)	310.82±36.94(189.22-377.14)	252.92±81.78(193.30-309.28)	331.25±45.50(290.00-377.43)
MCHC(g/L)	360.67±40.64(318.7-407.8)	352.78±44.94(310.2-387.4)	300.72±51.43(253.7-320.2)	354.40±37.68(311.3-400.1)
Neutrophil(%)	23.09±10.19(10.72-31.10)	29.33±18.4(17.50-55.21)	20.63±6.72(12.93-25.08)	29.21±6.50(24.26-36.59)
Lymphocyte (%)	41.86±14.46(27.50-56.41)	39.66±9.10(29.52-62.11)	52.87±9.23(44.61-62.50)	39.43±5.20(36.58-45.83)
Monocyte(%)	25.31±8.18(17.86-35.00)	25.07±12.40(17.31-40.50)	22.28±4.39(18.00-26.79)	25.33±5.12(20.83-30.77)
Eosinophil(%)	6.43±3.90(2.36-10.17)	4.61±1.13(1.19-5.43)	4.59±0.69(4.00-5.36)	3.88±2.11(2.44-6.41)
Basophil(%)	3.30±1.52(1.56-4.36)	1.17±0.18(0-2.93)	2.73±1.45(1.79-4.41)	2.23±0.91(0-3.17)
N/L ratio	0.53±0.41(0.24-1.18)	0.74±0.09(0.59-0.89)	0.39±0.17(0.22-0.63)	0.74±0.04(0.67-0.81)

N/L ratio: Neutrophil/lymphocyte ratio. [a] Significant ($P < 0.05$) difference according to different periods.

for tissue oxygen content through this mechanism. Leukocytes are cells that are directly associated with specific and unspecific immunological responses (Iwama and Nakanishi, 1996). In this study, leukocyte count was significantly more during the active period than those during the

Table 3. Serum biochemistry parameters of bullfrogs during the active and hibernating periods.

Biochemistry parameters	Males		Females	
	Active period	Hibernation	Active period	Hibernation
CK(U/L)	584.0±176.17(215.0-801.0)	414.3±135.21(257.5-499.1)	403.5±144.64(243.0-592.0)	508.5±161.23(183.3-702.2)
Amy(U/L)	907.2±314.30(612.7-1359.0)	1099.7±177.50(902.1-1407.0)	901.8±296.41(577.8-1278.3)	1057.3±432.31(462.7-1523.2)
LDH(U/L) [b]	205.8±80.72(101.4-312.6)	281.5±62.32(117.4-677.1)	161.5±76.27(71.9-291.0)	152.6±24.70(92.4-177.6)
ALT(U/L) [a]	14.5±9.11(5.7-26.3)	41.3±9.13(11.1-52.8)	14.1±8.22(3.6-29.4)	38.9±11.01(27.6-49.1)
AST(U/L)	111.5±70.00(30.3-191.7)	118.3±20.13(33.7-217.0)	101.2±66.50(29.0-181.3)	100.0±42.16(64.7-155.2)
GGT(U/L)	5.09±2.16(3.01-8.43)	4.09±2.60(2.10-7.43)	5.25±2.87(2.04-9.42)	4.01±0.51(2.90-5.91)
ALP(U/L)	18.40±8.79(8.93-31.00)	18.23±6.80(13.0-26.30)	17.75±10.09(3.87-28.41)	17.70±5.11(8.40-20.42)
TP(g/L)	29.21±6.36(19.32-37.53)	34.42±5.40(30.11-40.60)	29.07±9.65(19.31-38.70)	36.06±8.56(27.50-43.03)
Alb(g/L)	19.82±4.40(14.70-25.31)	16.83±2.33(15.12-19.53)	17.73±8.61(10.40-17.73)	17.96±4.01(13.30-20.60)
Glo(g/L) [a]	9.38±0.27(9.10-9.69)	17.62±2.99(14.90-21.11)	9.34±0.31(8.96-9.69)	18.10±3.98(15.21-24.42)
A/G	1.99±0.60(1.04-2.16)	0.93±0.06(0.88-1.10)	1.90±0.28(1.50-2.25)	0.96±0.04(0.80-1.06)
TB(umol/L)	0.93±0.31(0.70-1.99)	0.99±0.25(0.79-2.06)	0.92±0.39(0.66-1.97)	0.90±0.30(0.63-1.62)
Bun(mmol/L) [a]	4.14±0.55(2.70-4.74)	7.48±1.99(5.30-9.21)	4.82±2.21(3.02-7.03)	7.08±2.01(5.13-9.94)
Cre(umol/L) [a b]	18.56±8.96(10.01-30.14)	9.66±2.21(3.80-12.17)	27.51±12.89(12.02-40.17)	15.76±3.27(4.32-24.03)
UA(umol/L)	NO	7.33±3.32(4.00-12.03)	NO	7.23±3.05(4.06-19.84)
GLU(mmol/L)	1.99±0.18(1.12-2.20)	2.21±0.92(1.41-2.97)	2.00±0.21(1.19-2.55)	2.23±1.00(1.67-2.99)
TG(mmol/L)	0.14±0.06(0.05-0.20)	0.13±0.04(0.09-0.19)	0.15±0.06(0.11-0.24)	0.16±0.05(0.10-0.29)
Cho(mmol/L)	1.25±0.15(1.06-1.39)	1.16±0.28(0.77-1.38)	1.36±0.13(1.00-1.74)	1.20±0.87(0.98-1.55)
K(mmol/L)	6.13±0.34(5.17-6.58)	6.01±0.41(4.77-6.39)	6.43±0.52(5.91-7.12)	6.37±1.90(4.18-7.56)
Na(mmol/L) [a b]	108.7±3.80(102.0-110.6)	121.4±17.66(109.5-140.7)	116.8±8.24(108.5-121.9)	136.0±30.52(105.2-181.0)
Cl(mmol/L) [a]	54.6±6.43(41.6-57.8)	75.1±22.84(61.2-101.4)	52.3±6.27(42.7-58.3)	80.9±27.01(49.9-99.7)
nCa(mmol/L) [b]	0.89±0.09(0.86-0.99)	0.87±0.17(0.71-1.05)	0.99±0.08(0.89-1.07)	0.96±0.23(0.81-1.35)
Pb(ug/L)	18.0±4.08(12.03-21.10)	17.1±4.12(14.97-20.78)	17.8±4.00(15.38-20.01)	17.6±3.98(16.11-20.54)

A/G: Albumin/globulin ratio. NO: not detectable. [a] Significant ($P < 0.05$) difference according to different periods. [b] Significant ($P < 0.05$) difference according to sex.

hibernating period, and was less than those reported earlier in wild-caught *Xenopus laevis* by Wilson et al. (2011). The levels of leukocyte in the whole blood vary depending on environmental quality (Lea Master et al., 1990), nutritional state (Barros et al., 2002), the presence of infectious agents (Martins et al., 2008) and parasitism (Martins et al., 2004). This study showed that lymphocytes in bullfrogs were the most predominant cells as compared to the other types of leukocytes. This result was consistent with data presented for Wild Caught Dubois's Tree Frog (Das and Mahapatra, 2014) and for anesthetized American bullfrogs (Tama Cathers et al., 1997), suggesting that lymphocytes are the major cells involved in the immunological responses in frogs. In contrast, the percentage of basophil was the lowest in the present study on bullfrog, which was consistent with dubois's Tree Frog (Das and Mahapatra, 2014). The percentage of basophil depends on species and possibly on season, geographic region and age of the animal or may be associated with blood parasites or viral infection (Vasaruchapong et al., 2013).

As far as we know, no information is available on the serum biochemistry parameters of frogs in winter. Serum hepatic enzyme tests in animals are used to indicate hepatocellular injury or repair (Kasamatsu et al., 2012). ALT was produced by the liver, being more liver-specific in human and animals. In some animals with hepatic disease, the level of serum ALT activity would get higher than those in normal conditions. This study showed that serum ALT activity level during hibernation was significantly increased, which was consistent with the report for captive finless porpoises by Kasamatsu et al. (2012). This change probably suggested that there was hepatocellular injury in bullfrogs during hibernation. As known, Glo was also often used as an index of hepatic disorder. Serum Glo concentration of bullfrogs was strikingly increased during hibernation, which was likely due to promoting immune defense under the hibernating state.

Cre, being a break-down product in muscle, is usually produced at a very constant rate depending on muscle mass (Di Wu et al., 2014). There was a slow catabolism of Cre at a rate directly proportional to muscle mass (Kasamatsu et al., 2012). In the present study, the value of Cre concentration was found to be markedly increased during the active period, which was in general agreement with previous report for captive finless porpoises (Kasamatsu et al., 2012). Additionally, male bullfrogs had a statistically higher value of Cre in comparison with female bullfrogs, which may be attributed to variations in physical activity intensity, and increases in renal blood flow and glomerular filtration rate during the active period. The Cre concentration level in bullfrogs was higher than those in dubois's tree frog (Das and Mahapatra, 2014), which might be due to species specificity and variations in the muscle mass.

Besides Cre, Bun was also an important indicator of kidney disease. The Bun concentration level reported here for bullfrogs during the active period was notably lower than those during the hibernating period, but higher than those in wild-caught *Xenopus laevis* (Sabrina Wilson et al., 2011). The significant difference in Bun concentration level between bullfrogs and *X. laevis* was not in agreement with the conclusion drawn previously by Wilson et al. (2011), who observed no statistical difference in Bun concentration level between bullfrogs and *X. laevis.*

LDH is a cytosolic enzyme, which is originally present in all the tissues involved in glycolysis, especially in cardiac tissue. Consequently, detection of raised concentration of this enzyme released into the blood stream from the damaged tissue has become a definitive diagnostic and prognostic criterion for various disorders and diseases (Saravanan et al., 2012). In this study, the value of serum LDH activity was notably lower when compared with those of wild-caught *Xenopus laevis* by Wilson et al. (2011). The level of LDH activity in male bullfrogs was statistically higher than those in females, which was in accordance with those previously obtained for captive finless porpoises by Kasamatsu et al. (2012). So far, the mechanism of serum LDH activity change between sexes was not illustrated in amphibians in the literatures.

Serum electrolyte values were important basic information for clinicians and researchers to assess the health status of animals. In this study, male bullfrogs had lower values of serum Na and Cl concentrations than those of female bullfrogs, which coincided with the results obtained earlier for wild-caught *X. laevis* (Wilson et al., 2011), and for anesthetized American bullfrogs (Cathers et al., 1997). In addition, it was found that serum Na and Cl concentrations significantly increased in bullfrogs during the hibernating period.

In summary, hematology and serum biochemistry parameters are effective at identifying the changes of body function prior to a sign of clinical abnormality. As far as we know, this study provided the first set of reference ranges of hematology and serum biochemistry for healthy farmed bullfrogs according to their sexes and two different periods. These baseline data could be used for future studies on its physiology and assessments for health monitoring and disease diagnostics. It is believed that these baseline data could also serve as general reference values for future investigations involving this species and other anuran species.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations:

RBC, Red blood cell; **WBC,** white blood cell; **HGB,** hemoglobin; **PCV,** packed cell volume; **MCV,** mean corpuscular volume; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **ALP,** alkaline phosphatase; **Amy,** a-amylase; **ALT,** alanine aminotransferase; **AST,** aspartate aminotransferase; **Cho,** cholesterol; **Cre,** creatine; **Glu,** glucose; **TG,** triglycerides; **TB,** total bilirubin; **UA,** uric acid; **Bun,** blood urea nitrogen; **GGT,** γ-glutam; **LDH,** lactate dehydrogenase; **CK,** creatine kinase; **TP,** total protein; **Alb,** albumin; **Glo,** globulin.

ACKNOWLEDGEMENTS

The authors are thankful to Wuhu Zhengyu Medical Laboratory for help with determination of serum biochemistry and HGB concentration values. This work was supported by Natural Science Foundation of Anhui Province (Grant No. 11040606M75).

REFERENCES

Allender MC, Fry MM (2008). Amphibian hematology. Vet. Clin. North Am. Exot. Anim. Pract. 11(3):463-480.

Artacho P, Soto-Gamboa M, Verdugo C, Nespolo RF (2007). Using haematological parameters toinfer the health and nutritional status of an endangered black-necked swan population. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 147:1060-1066.

Barros MM, Pezzato LE, Kleemann GK, Hisano H, Rosa GJM (2002). Níveis de vitamina C e ferro para tilapia do Nilo (Oreochromis niloticus). Rev. Bras. Zootec. 3:2149-2156.

Benfey TJ, Sutterlin AM, Thompson RJ (1984). Use of erythrocyte measurements to identify triploid salmonids. Can. J. Fish Aquat. Sci. 41:980-984.

Boily F, Beaudoin S, Measures LN (2006). Hematology and serum chemistry of Harp (Phoca groenlandica) and Hooded seals (Cystoohora cristata) during the breeding season, in the Gulf of St. Lawrence, Canada. J. Wildl. Dis. 42:115-132.

Campbell TW, Ellis WC (2007). Avian and exotic animal haematology and cytology. 3rd edition. USA: Blackwell Publishing. pp. 51-81.

Carey C (2005). How physiological methods and concepts can be useful in conservation biology. Integrative Comp. Biol. 45:4-11.

Cathers T, Lewbart GA, Correa M, Stevens JB (1997). Serum chemistry and hematology values for anesthetized American bullfrogs(*Rana catesbeiana*). J. Zoo Wildl. Med. 28:171-174.

Das M, Mahapatra PK (2014). Hematology of Wild Caught Dubois's Tree Frog Polypedates teraiensis, Dubois, 1986 (Anura: Rhacophoridae). Sci. World J. 49:1415.

Guijarro AI, Lopez-Pation MA, Pinillos ML, Isorna E, Depedro N, Alonso-Gomez AL, Alonso-Bedate M, Delgado MJ (2003). Seasonal changes in haematology and metabolic resources in the tench. J. Fish Biol. 62:803-815.

Hartman FA, Lessler MA (1963). Erythrocyte measurements in birds. Aukland 80:467-473.

Iwama G, Nakanishi T (1996). The immune system. Califórnia: Acad. Press. 380p.

Jamalzadeh HR, Keyvan A, Ghomi MR, Gherardi F (2009). Comparison of blood indices in healthy and fungal infected Caspian salmon (Salmo trutta caspius). Afr. J. Biotechnol. 8:319-322.

Kasamatsu M, Hasegawa K, Wakabayashi I, Seko A, Furuta M (2012). Hematology and Serum Biochemistry Values in Five Captive Finless Porpoises (Neophocaena phocaenoides). J. Vet. Med. Sci. 74:1319-1322.

Lea Master BR, Brock JA, Fujioka RS, Nakamura RM (1990). Hematologic and blood chemistry values for Sarotherodon melanotheron and a red hybrid tilapia in freshwater and seawater. Comp. Biochem. Physiol. 97:525-529.

Lemoine LH, Smith TL (1980). Polyploidy induced in brook trout by cold shock. Trans. Am. Fish Soc. 109:626-631.

Llacuna S, Gorriz A, Riera M, Nadal J (1996). Effects of air pollution on hematological parameters in passerine birds. Arch. Environ. Contam. Toxicol. 31:148-152.

Mahapatra BB, Das M, Dutta SK, Mahapatra PK (2012). Hematology of Indian rhacophorid tree frog Polypedates maculatus Gray, 1833 (Anura: Rhacophoridae). Comp. Clin. Pathol. 21:453-460.

Martins ML, Moraes FR, Fujimoto RY, Onaka EM, Bozzo FR, Moraes JRE (2006). Carrageenin induced inflammation in Piaractus mesopotamicus (Osteichthyes: Characidae) cultured in Brazil. Bol. Inst. Pesca. 32:31-39.

Martins ML, Mourino JL, Amaral GV, Vieira FN, Dotta G, Jatoba AM, Pedrotti FS, Jeronimo GT, Buglione-neto CC, Pereira JG (2008). Haematological changes in Nile tilapia experimentally infected with Enterococcus. Braz. J. Biol. 68:657-661.

Martins ML, Tavares-Dias M, Fujimoto RY, Onaka EM, Nomura DT (2004). Haematological alterations of Leporinus macrocephalus (Osteichthyes: Anostomidae) naturally infected by Goezia leporini (Nematoda: Anisakidae) in fish pond. Arq. Bras. Med. Vet. Zootec. 6(5):640-646.

Newman SH, Piatt JF, White J (1997). Hematological and plasma biochemical reference ranges of Alaskan seabirds: their ecological significance and clinical importance. Waterbirds 20:492-504.

Olmo E, Capriglione T, Odierna G (1989). Genome size evolution in vertebrates: trends and constraints. Comp. Biochem. Physiol. B. 92:447-453.

Pagés T, Peinado V I, Viscor G (1992). Seasonal changes in hematology and blood chemistry of the freshwater turtle *Mauremys caspica* leprosa. Comp. Biochem. Physiol. A. 103:275-278.

Parida SP, Dutta SK, Pal A (2011). Hematological and plasma biochemistry in *Psammophilus blanfordanus* (Sauria: Agamidae). Comp. Clin. Pathol. 21:1387-1394.

Pessier AP (2007). Cytologic Diagnosis of Disease in Amphibians. Vet. Clin. North Am. Exot. Anim. Pract. 10(1):187-206.

Prosser, C. L. (1973). Respiratory functions of blood. Comparative animal physiology. Saunders, Philadelphia. pp. 317-361.

Rowley AF, Ratcliffe NA (1988). Vertebrate blood cells. Cambridge University Press, Cambridge, UK. pp. 257-336.

Sarasola JH, Negro JJ, Travaini A (2004). Nutritional condition and serum biochemistry for free-living Swainson's Hawks wintering in central Argentina. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 137:697-701.

Saravanan R, Shanmugam A, Rajkumar D (2012). Preventive effect of glycosaminoglycans from Amussium pleuronectus (Linne) on biomolecules, lactatdehydrogenase-isoenzyme and electrocardiographic patterns in isoproterenol-induced myocardial infarction in Wistar rats. Indian J. Pharmacol. 44:602-606.

Seaman DA, Guqlielmo CG, Williams TD (2005). Effects of physiological state, mass change and diet on plasma metabolite profiles in the western sandpiper Calidris mauri. J. Exp. Biol. 208:761-769.

Silveira- Coffigni R, Prieto-Trujillo A, Ascencio-Valle F (2004). Effects of different stressors in haematological variables in cultured Oreochromis aureus S. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 139:245-250.

Sinha RC (1983). Haematological studies on prewintering and wintering frog, Rana Esculenta. Comp. Biochem. Physiol. A. Comp. Physiol. 74:311-314.

Vasaruchapong T, Disarapong P, Chulasugandha P, Khow O, Chanhome L, Chiobamroongkiat M, Chaiyabutr N, Sitprija V (2013) Comparative studies on hematological and plasma biochemical parameters in different types of venomous snakes in Thailand. Comp. Clin. Pathol. 23(4):955-959.

Wilson S, Felt S, Torreilles S, Howard A, Behan C, Moorhead R, Green S, (2011). Serum Clinical Biochemical and Hematologic Reference Ranges of Laboratory-Reared and Wild-Caught Xenopus laevis. J. Am. Assoc. Lab. Anim. Sci. 50:635-640.

Wu D, Yi Y, Sun F, Zhou L, Yang F (2014). Effects of Age and Sex on the Hematology and Blood Chemistry of Tibetan Macaques (*Macaca thibetana*). J. Am. Assoc. Lab. Anim. Sci. 53:12.