

Morphological and biochemical parameters of blood and peroxidation state in water buffalo transition period

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Received: 09.03.2021. Accepted 14.04.2021

The paper presents the study of morphological and biochemical parameters of blood and the state of peroxidation in female water buffaloes during the transition period in the conditions of Western Ukraine. Ten pregnant buffalo cows aged 2–2.5 years with a live weight of 480–530 kg, kept in the subsidiary "Play", Klymets Village, Lviv region, have been selected for the research. At the beginning of the study, the gestation period was 8–8.5 months, and milk productivity at the beginning of lactation made up 12–18 L per day. The analysis of the morphological parameters of the cows' blood showed that all indicators were within the reference range. After calving, the number of erythrocytes and leukocytes decreased significantly, while the number of platelets increased. The changes in the leukogram indicate the development of an inflammatory post-calving process. Calving itself led to a rise in the intensity of catabolic processes in the cows' bodies, but these changes were within physiological limits, which justifies the high adaptability of buffaloes. A significant increase in lactate dehydrogenase and creatine kinase activity alongside a decreased activity of other indicator enzymes has been established. An essential area of the research conducted was the identification of the interrelation between metabolites. In the dry spell, the highest degree ($r > 0.9$) of the correlation was found between glucose and bilirubin. Also, a relatively high negative correlation was registered between the concentration rates of total protein. A high level of positive correlation was observed between Potassium concentration and creatine kinase (CK) activity. After calving, the relationships between the metabolites changed significantly; thus, Potassium and urea's highest degree of negative correlation was found. A high degree of positive correlation was found between the concentration rates of cholesterol and urea. A study of the lipid peroxidation intensity in the buffaloes' blood during the transition period showed that the concentration of both metabolites under research increased in the postpartum period: lipid hydroperoxides content by 80%, whereas malonic dialdehyde, increased by 2.2 times. Since there was a progressive accumulation of malonic dialdehyde in the blood, these changes may indicate a certain intensity of the enzymatic reserves of the antioxidant system in cow-buffaloes in the postpartum period.

Keywords: water buffalo cows, morphological, biochemical parameters of blood, lipid peroxidation.

Introduction

In the recent past, the breeding of water buffaloes (*Bubalus bubalis*, Linnaeus, 1758) in Ukraine has been a part of the traditional animal husbandry of Western Ukraine and its South. However, during 1990–2000, this industry declined somewhat, and only in 2007–2009, the introduction of this species of animals and their breeding as a separate branch of animal husbandry began. Due to its chemical composition, buffalo milk is widely used to manufacture fermented milk and fat milk products (Perišić et al., 2015). Despite some toughness, the meat of these animals is of high quality, including low levels of triglycerides, cholesterol, ω -3, and ω -6 fatty acids (Naveena & Kiran, 2014).

Reintroduction of buffaloes, in addition to the use of milk and meat, plays a vital role in the formation of the ecosystem, changing the landscape of the river banks and pastures and increasing the diversity of trophic chains; buffalo products are used as fertilizers for organic agricultural production (Naveena & Kiran, 2014).

In the lives of buffaloes and other ruminants, the so-called transition period, which includes the time during the three weeks before and after calving, is essential. After calving, significant energy needs characterize this period, the rapid use of carbohydrate reserves to synthesize colostrum components, and muscle contractions. During the transition period, ketosis and lipidosis of the liver are frequent pathological phenomena (Marutsova et al., 2019). After calving, the cow's body experiences metabolic, energy, and oxidative stress, which results in the development of systemic inflammation, or cell cytolysis. These phenomena significantly change the morphological and biochemical profile of blood. High energy needs a short calving time cause pathological changes in oxidative phosphorylation and accumulation of reactive oxygen intermediates (ROIs), initiating lipid peroxidation (LPO) (Colakoglu et al., 2017; Urh et al., 2019). LPO processes lead to disruption of the structure of the lipid

part of cell membranes, the release of indicator enzymes into the blood, thus enhancing metabolic disorders caused by physiological changes in female buffaloes during the transition period (Pilarczyk et al., 2012).

The research aims to study the morphological and biochemical composition of blood and lipid peroxidation in buffalo cows before and after calving.

Materials and methods

Experimental studies were conducted in the educational and scientific clinical and diagnostic laboratory of Polissya National University. Ten pregnant cow-buffaloes kept in the subsidiary "Play", Klymets Village, Lviv region, has been selected for the research. The buffaloes were brought to the farm in 2019 as heifers. The animals aged 2–2.5 years had a live weight of 480–530 kg and, at the beginning of the study, the gestation period made up 8–8.5 months. Milk productivity at the beginning of lactation was 12–18 l per day. The free-range animals were kept untied in a stable. During the research, the diet of buffaloes included hay, straw, haylage, concentrates; the total weight of the diet made up 28–31 kg per head, 8.5 kg of which was concentrated feed. Before starting the study, the animals were examined according to a standard protocol for the presence of parasitism and infectious diseases. Blood samples were taken from the jugular vein before feeding in the morning. Blood was collected into vacuum tubes following the rules of asepsis and antiseptics. The whole blood was stabilized with EDTA. Blood was collected into separate tubes to obtain serum, samples were cooled to 4 °C and then frozen. Blood was collected during the dry spell, two weeks before and after calving.

Blood biochemical parameters were determined using a semi-automatic biochemical analyzer Chem 7 (Erba, Czech Republic) with a line of reagents from DAC (Republic of Moldova). Morphological blood parameters were determined using an automatic hematological analyzer, Abacus vet 5 (Diatron, Austria). The GPL concentration was determined using a Mapoda UV-1800 spectrophotometer (Shanghai Mapoda Instruments Co., Ltd, China) (wavelength $\lambda = 232$ nm, UV spectra). The plasma lipid fraction was being extracted in isopropanol-heptane (1:1) for one hour, the mixture received was acidified with hydrochloric acid, and distilled heptane was added. The concentration of MDA (TBA-active products) was also spectrophotometrically (wavelength $\lambda = 550$ nm) determined in plasma. A mixture of plasma samples and orthophosphate and thiobarbituric acids (0.5%), which was preliminarily incubated in a water bath, was cooled, then n-butane was added, and all this was centrifuged. The results were statistically processed using software Statistica 13.3; the data were checked for normal distribution using the Distribution Fitting module. The research presupposed establishing the arithmetic mean and its standard error. As far as in the studies $n = 10$, the calculations were performed according to the formulas for a small sample. To assess the difference between the arithmetic means before and after calving, Student's t-test was determined for a significance level of 0.05. Also, the Pearson correlation coefficient (r) was determined.

Results

Analysis of morphological parameters of the blood of female buffaloes showed that the concentration of hemoglobin after calving was higher than in the dry period ($p > 0.05$) (Table 1).

The number of erythrocytes in cows decreased after calving by 25% ($p < 0.01$). The number of platelets in the blood of the animals under research increased compared to that in the dry period by 9.7% ($p > 0.05$). As for leukocytes, their total number decreased slightly ($p > 0.05$) after calving. Turning to the leukocyte fractions, after calving, the most significant differences were observed in the number of lymphocytes, which increased 1.2 times compared to the dry period ($p < 0.01$) neutrophils, eosinophils and monocytes decreased. Basophils in the blood of buffaloes were not detected before as well as after calving.

Table 1. Morphological parameters of buffalo blood in the dry and postpartum period ($M \pm m$, $n=10$)

Indicator	Dry period	Postpartum period
Hemoglobin, g/l	116.7±5.56	123.8±4.04
Erythrocytes, T/l	8.8±0.37	6.6±0.35**
Platelets, T/l	140.4±7.90	155.5±9.07
Leukocytes, G/l	10.9±0.49	9.7±0.78
Lymphocytes, %	51.2±2.25	60.6±1.64**
Segmented neutrophils, %	41.7±2.69	34.2±1.70**
Band neutrophils, %	0.8±0.23	0.6±0.28
Eosinophils, %	2.9±0.25	2.0±0.22
Monocytes, %	3.4±0.19	2.6±0.24**
Basophils, %	0	0

Note: *, ** – the difference between indicators before and after calving is statistically significant at the level $p < 0.05$ and $p < 0.01$ respectively.

Calving is a complex physiological process that leads to significant changes in the metabolism of the cow's body (Bomko et al., 2018; Slivinska et al., 2019; Kulyaba et al., 2019; Borshch et al., 2020; Roman et al., 2020; Grymak et al., 2020). Biochemical parameters of serum of buffaloes before and after calving are given in Table 2. The concentration of glucose and total protein in the blood serum of cows after calving decreased ($p < 0.05$), while the concentration of protein metabolism, creatinine ($p < 0.05$), and urea ($p > 0.05$) increased compared to those in the dry period. The concentration of cholesterol in the serum of the animals under research did not change but tended to increase in the postpartum period ($p > 0.05$).

The activity of indicator enzymes in blood serum changed in different ways – for example, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) decreased after calving, while the activity of lactate dehydrogenase (LDH) and creatine kinase (CK) – increased ($p < 0.01$). Calcium and inorganic phosphorus rate in the blood increased after calving ($p < 0.05$).

Table 2. Biochemical parameters of buffalo serum in the dry and postpartum period (M±m, n=10)

Indicator	Dry period	Postpartum period
Glucose, mmol/l	4.2±0.05	3.5±0.16*
Total protein, g/l	75.3±1.88	67.7±2.15**
Total bilirubin, µmol/l	8.6±0.16	10.0±0.31**
Creatinine, µmol/l	84.5±2.26	123.7±6.84**
Urea, mmol/l	3.9±0.07	4.2±0.19
Cholesterol, mmol/l	5.3±0.08	5.47±0.18
ALT, U/l	100.2±2.5	82.98±2.5**
AST, U/l	56.5±2.19	53.52±2.55
LDH, U/l	802,8±55,33	1553.4±0.90**
ALP, U/l	131.0±0.96	120.8±1.50**
CK, U/l	43.0±6.00	85.6±3.79**
Calcium, mmol/l	2.45±0.02	2.64±0.030**
Inorganic phosphorus, mmol/l	0.99±0.05	1.41±0.09**
K, mmol/l	4.61±0.12	4.13±0.25

Note: *, ** - the difference between indicators before and after calving is statistically significant at the level $p<0.05$ and $p<0.01$, respectively.

The concentration of Potassium (K) tended to decrease after calving ($p>0.05$). A significant step in understanding the biochemical changes in cow-buffaloes during the transition period is establishing a relation between different metabolites. The correlation analysis held showed that the highest degree of negative correlation was found between glucose and bilirubin ($p<0.05$), between ALP activity and total protein concentration ($p<0.05$), whereas a positive correlation was observed between Potassium concentration and CK activity ($p>0.05$) (Table 3).

Table 3. The coefficient of correlation between biochemical parameters of buffalo blood in the dry spell

Indicator	Glucose	Total protein	Bilirubin	Creatinine	Urea	ALT	AST	LDH	ALP	Cholesterol	CK	Calcium	Inorganic phosphorus
Glucose													
Total Protein	-0.55**												
Bilirubin	-0.96****	-0.04*											
Creatinine	0.33*	0.89***	-0.42*										
Urea	0.2*	-0.11*	-0.31*	0.17*									
ALT	0.33*	-0.06*	-0.31*	0.25*	0.85***								
AST	0.27*	-0.86***	-0.08*	-0.67**	0.08*	0.31*							
LDH	0.68**	0.59**	-0.64**	0.84***	0.12*	0.42*	-0.18*						
ALP	-0.06*	-0.98****	0.14*	-0.88***	0.26*	0.21*	0.84***	-0.61**					
Cholesterol	-0.8***	0.39*	0.68**	0.15*	0.3*	0.15*	-0.5**	-0.29*	-0.2*				
CK	0.14*	0.58**	-0.3*	0.75***	0.74***	0.64**	-0.52**	0.5**	-0.46*	0.48*			
Calcium	-0.5**	-0.75***	0.53**	-0.81***	0.33*	0.22*	0.56**	-0.77***	0.87***	0.31*	-0.25*		
Inorganic phosphorus	0.46*	0.15*	-0.43*	0.14*	-0.71***	-0.66**	0.12*	0.27*	-0.36*	-0.73***	-0.46*	-0.7***	
Potassium	0.17*	0.33*	-0.29*	0.56**	0.89***	0.85***	0.22*	0.43*	-0.17*	0.44*	0.94****	-0.003*	-0.65**

Note: 0,nn * - very weak or weak correlation, $r<0.5$; 0,nn** - medium strength correlation, $r = 0.5-0.7$; 0,nn*** - strong correlation, $r = 0.7-0.9$; 0,nn**** - very strong correlation, $r>0.9$

A significant positive correlation was found between the following indicators: creatinine and total protein concentration ($p>0.05$), ALT activity and urea concentration ($p>0.05$), LDH activity and creatinine concentration ($p>0.05$), ALP activity and AST ($p>0.05$), CK activity and creatinine concentration and urea concentration ($p<0.05$), between ALP activity and Calcium

concentration ($p < 0.05$), between Potassium concentration and urea rate and ALT activity ($p > 0.05$). A strong negative correlation was established between the following indicators: ALT activity and total protein concentration ($p > 0.05$), ALP activity and creatinine concentrations ($p > 0.05$), cholesterol and glucose rates ($p > 0.05$). The level of Calcium was negatively correlated with the content of total protein and creatinine ($p < 0.05$). The concentration of inorganic phosphorus had a strong negative correlation with the concentration of urea ($p < 0.05$) and cholesterol and Calcium ($p > 0.05$). After calving, the degree of connection and their direction changed (Table 4).

Table 4. The coefficient of correlation between biochemical parameters of buffalo blood in the postpartum period

Indicator	Glucose	Total protein	Bilirubin	Creatinine	Urea	ALT	AST	LDH	ALP	Cholesterol	CK	Calcium	Inorganic phosphorus
Glucose	-												
Total protein	0.38*												
Bilirubin	0.48*	-0.3*											
Creatinine	0.001*	0.3*	-0.8***										
Urea	-0.08*	0.3*	0.63**	-0.37*									
ALT	-0.22*	0.52**	-0.42*	0.04*	-0.4*								
AST	0.63**	-0.04*	-0.37*	0.67**	-0.62**	0.22*							
LDH	0.24*	-0.75***	0.09*	-0.4*	-0.66**	0.17*	0.13*						
ALP	0.16*	0.12*	-0.3*	0.79***	0.15*	-0.53**	0.40*	0.62**					
Cholesterol	-0.16*	0.34*	0.62**	-0.42*	0.99****	-0.4*	-0.68**	-0.64**	0.07*				
CK	-0.3*	0.13*	0.21*	-0.72***	-0.06*	0.65**	-0.45*	0.42*	-0.97****	0.04*			
Calcium	0.14*	-0.51**	0.86***	-0.8***	0.61**	-0.64**	0.65**	0.17*	-0.26*	0.61**	0.15*		
Inorganic phosphorus	0.3*	-0.44*	-0.52**	0.72***	-0.67**	-0.22*	0.75***	0.25*	0.57**	-0.74**	-0.69**	-0.44*	
Potassium	0.25*	-0.41*	-0.61**	0.54**	-0.94****	0.2*	0.75***	0.55**	0.14*	-0.97****	-0.26*	-0.58**	0.88***

Note: 0,nn* – very weak or weak correlation, $r < 0.5$; 0,nn** – medium strength correlation, $r = 0.5-0.7$; 0,nn*** – strong correlation, $r = 0.7-0.9$; 0,nn**** – very strong correlation, $r > 0.9$

Table 5. Indicators of lipid peroxidation of buffalo blood in the dry and postpartum period ($M \pm m$, $n=10$)

Indicator	Dry period	Postpartum period
HPL, units	2,64±0,19	4,75±0,39*
MDA, $\mu\text{mol/l}$	1,38±0,16	2,99±0,31**

Note: *, ** – the difference between indicators before and after calving is statistically significant at the level $p < 0.05$ and $p < 0.01$, respectively.

A similar pattern was found for the concentration of MDA (after calving, the concentration of this metabolite was almost twice as high as during pregnancy). The highest positive correlation was found between the concentration of cholesterol and urea ($p < 0.05$), a significant negative correlation was observed between the concentrations of Potassium and urea ($p > 0.05$) and Potassium and cholesterol ($p > 0.05$), the activity of ALP and CK ($p > 0.05$). Turning to a strong correlation, a positive direction was found between the following metabolites: creatinine and inorganic phosphorus concentrations ($p > 0.05$), CK activity, between AST activity ($p > 0.05$) and inorganic phosphorus and Potassium concentrations ($p > 0.05$), between the concentrations of Potassium and inorganic phosphorus ($p > 0.05$). A high degree of negative correlation was found between total protein and LDH activity ($p < 0.05$), between concentrations of bilirubin and creatinine ($p > 0.05$), creatinine and CK activity ($p > 0.05$), Calcium and creatinine ($p > 0.05$). Lipid hydroperoxides are the primary product of LPO (Martyschuk et al., 2016; Gutyj et al., 2016; 2017;

Slivinska et al., 2020), their concentration in the blood of female buffaloes after calving was significantly higher than that at the end of the gestation period ($p < 0.05$) (Table 5).

Discussion

The analysis of morphological parameters of the cows' blood showed that all indicators were within the reference range (Abd Ellah et al., 2013, Abdulkareem, 2013). After calving, the number of erythrocytes decreased significantly, while the concentration of hemoglobin increased. Such changes can be caused by blood loss during calving and a compensatory increase in hemoglobin synthesis. This statement is partially confirmed by the results of studies of the number of platelets that increased after calving ($p > 0.05$), which indicates the development of the inflammatory process (Marini, 2016). Some authors (Ashmawy, 2015) claim that the postpartum period is characterized by developing an inflammatory process characterized by an increase in the number of leukocytes (Sundrum, 2015). The data obtained in the result of this research differ slightly from those in the literature, as the number of leukocytes after calving decreased slightly; such changes might be due to the release of some leukocytes to tissues uterus particular, where the inflammatory process develops. The after calving decrease in the proportion of neutrophils in the blood of cows alongside a sharp increase of lymphocytes indicates a chronic inflammatory process in the postpartum period. Similar changes have been described by Abdulkareem, 2013, Pathan, 2015, who believe that the development of chronic inflammation characterizes the postpartum period in buffaloes. The analysis of the biochemical composition of the cow-buffaloes in the transition period showed a significant decrease in glucose concentration after calving, which is typical of this physiological period. Glucose is an energy source used for muscle contractions during calving and synthesizing colostrum components (Morales et al., 2016; Marutsova et al., 2019). Decreased glucose concentration during this period can lead to the use of fatty acids as an energy source and ketosis development (Sundrum, 2015; Fiore et al., 2017; Delfino et al., 2018). However, the glucose concentration was within the reference range, which indicates a high level of compensatory mechanisms of glycogenesis and gluconeogenesis in cow-buffaloes in the transition period. The concentration of total serum protein in the postpartum period also decreased, which is explained by the use of separate fractions of serum proteins to synthesize colostrum and milk (Perišić et al., 2015; Agudelo-Gómez et al., 2016). In the postpartum period, the concentration of nitrogen metabolism products (creatinine and urea) increased, which indicates the growth in catabolic processes in this period (Sundrum, 2015). The intensity of metabolic processes primarily affected liver functions, particularly the increase in bilirubin concentration; however, the activity of the indicator enzymes ALT and AST in the blood of the cows after calving decreased, which differs from the existing data (Ashmawy, 2015). The differences obtained maybe since most of the previously described studies presented in the literature describe the period immediately after birth (Couch et al., 2017); as for this research, it relates to the period of two weeks after calving.

A study of serum LDH activity in buffalo cows showed that in the postpartum period, it almost doubled compared to that in the dry spell; such changes result from not only the growth of cell cytolysis but also from the increase in absolute values of the activity of this enzyme, associated with rising aerobic metabolism of glucose amid its reduction and the need for significant amounts of energy (Helmy et al., 2019). ALP activity in the dry period was higher than in the postpartum one due to increased activity of the placental isoenzyme before calving, as there is a distraction of the integrity of the placenta and its preparation for separation from the uterus (Abdulkareem, 2013). After calving, the activity of CK in the blood of cows doubled compared to the dry period. This enzyme catalyzes the formation of creatine phosphate, one of the essential macroergs of muscle tissue. According to Sattler & Füll (2004), irritation of the uterine myometrium caused by calving, endometritis, or other factors causes an increase in the activity of this enzyme, in contrast to other indicator enzymes. Thus, the increase in CK activity may result from intensive cretin phosphate synthesis in the muscles and processes occurring in the uterus in the postpartum period. The concentration of cholesterol in the blood of cows before and after calving did not differ, but in the postpartum period, this figure increased slightly, which contradicts other studies (Abd Ellah et al., 2013; Jerome et al., 2016). Some authors believe that the reduction in cholesterol levels in females in the postpartum period is associated with its use for the synthesis of steroid hormones (Ashmawy, 2015). However, it is known that cholesterol is used to stabilize cell membranes, the synthesis of components of bile and cholecalciferol; thus, changes in the concentration of this metabolite in the blood of buffaloes require further study. Calcium and phosphorus concentrations in the blood of the studied animals increased after calving compared to that in the dry period. As for inorganic phosphorus, after calving, its concentration naturally increased due to the decomposition of replete with phosphorylated compounds (Couch et al., 2017). The concentration of Calcium in the blood of female buffaloes after calving increased significantly, which contradicts the data presented in other studies (Delfino et al., 2018). In contrast to hypocalcemia, which is common in cows in the postpartum period, such changes may indicate significant adaptive reserves for this element in buffaloes.

An important area of research was the correlation between metabolites. In the dry period, the highest degree ($r > 0.9$) of the correlation was found between glucose and bilirubin, which may be explained by a decrease in the formation of glucuronic acid from glucose required for conjugation of bilirubin and the development of destructive phenomena in the liver parenchyma caused by different detoxicating processes at the end of pregnancy (Wang et al., 2006). An important area of research was the correlation between metabolites. In the dry period, the highest degree ($r > 0.9$) of the correlation was found between glucose and bilirubin, which may be explained by a decrease in the formation of glucuronic acid from glucose required for conjugation of bilirubin and the development of destructive phenomena in the liver parenchyma caused by different detoxicating processes at the end of pregnancy (Wang et al., 2006). Also, a reasonably strong correlation was found between the concentrations of total protein. Such changes may indicate stagnation in the liver, which led to a decrease in the synthesis of serum proteins by this organ. A high level of positive correlation was found between the concentration of K and the activity of CK; such integration is probably caused by the release of both Potassium and enzyme from the cells of the myometrium into the blood before calving. After calving, the correlation between metabolites underwent significant changes. Thus, the highest degree of negative correlation was found between Potassium and urea; such changes may result from Potassium retention in tissues and,

consequently, decreased blood levels and increased protein metabolism and urea synthesis. Similar changes were recorded in the studies of Fadlalla et al., 2020. The authors mentioned a negative correlation between Calcium and Potassium concentrations in the blood of lactating cows. Buffalo-cows also had a negative correlation between these indicators, but its rate was moderate. A strong positive correlation between cholesterol and urea concentrations, an increase in cholesterol concentration may be possible due to its need to synthesize steroid hormones and muscle cell membranes that were disrupted during calving (Abdulkareem et al., 2013).

Calving and the postpartum period require significant energy expenditure, increase in the activity of oxidative processes aimed at obtaining energy by oxidative phosphorylation (Sundrum, 2015). However, the stress caused by the calving process leads to disruption of oxidation and phosphorylation and, consequently, the formation of ROIs. One of the most active ROIs is the superoxide radical, which initiates lipid peroxidation and products of this reaction – GPL and MDA (Colakoglu et al., 2017; Urh et al., 2019). A study of the LPO intensity in the blood of cow-buffaloes during the transition period showed that the concentration of both studied metabolites increased in the postpartum period: GPL rate dropped by 80%, MDA decreased 2.2 times. As there was a progressive accumulation of MDA in the blood, these changes may indicate a certain tension of the enzymatic reserves of the antioxidant system in buffaloes in the postpartum period (Pilarczyk et al., 2012). Similar changes have been found in other studies (Megahed et al., 2008; Omid et al., 2017).

Conclusion

Thus, the study of the morphological, biochemical composition of the blood of buffalo cows during the transition period revealed that there was a decrease in the number of erythrocytes in the postpartum period in the blood of animals leukocytes and an increase in the number of platelets. The changes in the leukogram indicate the development of a post-calving inflammatory process. Calving caused a rise in the intensity of catabolic processes in the body of cow-buffaloes, but these changes were within physiological limits, which indicates the high adaptability of buffaloes. There was a significant increase in lactate dehydrogenase and creatine kinase activity alongside a decrease in the activity of other indicator enzymes.

Calving leads to an increase in the intensity of lipid peroxidation and the accumulation in the blood of lipid hydroperoxides and malonic dialdehyde.

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Citation:

Hryshchuk, H., Chala, I., Yevtukh, L., Pinsky, O., Revunets, A., Kovalchuk, Yu., Karpiuk, V., Kovalov, P., Kovalova, L., Zakharin, V., Veremchuk, Ya., Pobirskyi, M. (2021). Morphological and biochemical parameters of blood and peroxidation state in water buffalo transition period. *Ukrainian Journal of Ecology*, 11 (2), 261-267.



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