HEMATOLOGICAL CHANGES IN BUFFALO (*BUBALUS BUBALIS*) WHOLE BLOOD STORED IN CPDA-1 OR CPD/SAG-M PLASTIC BAGS

Osvaldo Gato Nunes Neto[1], Suelen Andrade Ricarte[1], Rejane Santos Sousa[2], Juliana Machado Portela[1], Adriana Caroprezo Morini[1], Kedson Alessandri Lobo Neves[1], Helder Ribeiro Batista[1], Francisco Leonardo Costa Oliveira[2], Enrico Lippi Ortolani[2], Raimundo Alves Barreto Junior[3] and Antonio Humberto Hamad Minervino[1,*]

ABSTRACT

The present study aimed to evaluate the hematology of buffalo blood stored in plastic bags containing either CPDA-1 or CPD/SAG-M. Ten healthy adult male buffaloes were used. In total, 900 g of blood was collected from each specimen, 450 g of which was packed in a bag containing CPDA-1; the other 450 g was packed in a bag containing CPD/SAG-M. The bags were then stored at 2°C to 6°C for 42 days. The blood samples were evaluated 7 times during this period: day 0 (D0; immediately after collection), D7 (7 days after collection), D14 (14 days after collection), D21 (21 day after collection), D28 (28 days after collection), D35 (35 days after collection) and D42 (42 days after collection). At each time point, the following hematological parameters were analyzed: globular volume, red blood cell count, mean corpuscular volume, degree of hemolysis, number of leukocytes, and plasma hemoglobin. During storage, the number of red blood cells and leukocytes decreased, whereas plasma hemoglobin values and mean corpuscular volume increased ($P<0.05$). Buffalo whole blood packed in bags containing either CPDA-1 or CPD/SAG-M underwent hematological changes during storage; however, the blood remained viable for transfusion when stored for up to 42 days at 2 to 6°C. No clinically important differences were observed between CPDA-1 and CPD/SAG-M in terms of the conservation of total buffalo blood. Therefore, both types of bag were indicated for use in this species.

Keywords: *Bubalus bubalis*, buffalo, hematology, hemolysis, transfusion, conservation

INTRODUCTION

Transfusion of whole blood is carried out in animals to re-establish their capacity for transport and diffusion of oxygen to tissues after significant reductions in hemoglobin values, such as occur during severe anemia or acute blood loss (Purdy *et al*., 2014). Transfusion blood should be stored in plastic bags. However, during storage, blood tissue becomes increasingly damaged. Such changes are known collectively as storage lesions (Salaria *et al*., 2014).

[1]Laboratory of Animal Health (LARSANA), Federal University of Western Pará (UFOPA), Rua Vera Paz, Santarém, Brazil,*E-mail: ah.minervino@gmail.com

[2]Department of Clinical Science, College of Veterinary Medicine and Animal Science, University of Sao Paulo (FMVZ/USP), Cidade Universitária, São Paulo, Brazil

[3]Department of Animal Science, Federal Rural University of the Semiarid Region (UFERSA), Costa e Silva, Mossoró, Brazil

Storage lesions are characterized by metabolic, biochemical, molecular, and morphological changes in blood components, as well as by the accumulation of bioreactive substances that are potentially harmful and may have adverse clinical effects during blood transfusion, especially in critically ill patients (Aubron, 2013). If technicians fail to monitor these changes throughout the preservation period, stored blood may become unusable or less effective. Studies have shown that the tissue of each animal species presents different behaviors when stored in plastic bags; specifically, various investigations have evaluated storage lesions in sheep (Sousa *et al*., 2013), donkeys (Barros, 2011), and goats (Tavares, 2013).

Buffaloes are important economic animals in the Amazon region, especially in Pará State, which has the largest herd in Brazil (Vale *et al*., 2014). Considerable economic losses are incurred as a result of herd mortality due to blood loss, which can be caused by surgical procedures and hemolytic diseases such as hemoparasites (Silva *et al*., 2014). In such cases, to replace blood volume, transfusion of whole blood, or its components, should be carried out (Sousa *et al*., 2012). However, no reports have been published regarding the storage lesions that occur during the conservation of buffalo blood; consequently, there are no indices that allow a safe transfusion procedure in buffaloes. The objective of the present study was to evaluate hematological alterations in buffalo blood stored for 42 days in plastic bags containing either CPDA-1 or CPD/SAG-M.

MATERIALS AND METHODS

This project was approved by the Commission of Ethics in the Use of Animals of the Federal University of Western Pará (process number: 07006/2013). Ten healthy cross-breed male buffaloes were used in the present study. The buffaloes were aged 2.5 years and weighed an average of 416 kg (SD ± 18.1 kg). They belonged to a commercial farm, where they were submitted to semi-intensive management, receiving forage-based feeding, mineral supplementation, and free access to water. Prior to the experiment, the buffaloes received a moxidectin-based endectocide. The animals selected were clinically evaluated and submitted to laboratory tests to ensure that they were physiologically normal and free of hemoparasites.

Two bags of whole blood-totaling 900 g were collected from each animal; 450 g of the blood was packed in a sterile bag containing the preservative CPDA-1, which is composed of citrate, phosphate, dextrose, and adenine. The other 450 g were packed in a sterile bag containing CPD/SAG-M, wherein the primary bag contains a preservative solution composed of citrate, phosphate, and dextrose, and a satellite bag contains a solution of sodium chloride, adenine, glucose, and mannitol. In the CPD. SAG-M bags, the additive solution in the satellite bag was transferred to the main bag immediately before blood collection. Whole blood was then collected. To determine how much blood was collected, the bags were weighed on a precision scale. They were then stored immediately after collection in a temperature-controlled refrigerator (2°C to 6°C, mean: 3.7°C±0.5°C) for 42 days; they were homogenized manually on alternate days during this period. The blood was collected in a containment trunk without any anesthetic, and the order of the type of bag used was alternated throughout these collections.

Hematological evaluations of the whole

blood were carried out at seven different time points: immediately after collection (D0), 7 days after collection (D7), 14 days after collection (D14), 21 days after collection (D21), 28 days after collection (D28), 35 days after collection (D35) and 42 days after collection (D42). At each time point, the following parameters were measured using 3 ml of whole blood taken from the pocket of each bag: erythrocyte count, total leukocyte count, and globular volume. The erythrocyte and leukocyte counts were determined by macrodilution using the manual method, which involves a Neubauer chamber and follows the procedures of Hendrix (2005). Globular volume was measured by centrifugation for microhematocrit using capillary tubes 75 mm in length. Samples were centrifuged at 1000 × *g* for 5 minutes and read in a microhematocrit table (Kerr, 2003). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the equations introduced by Jain (1993). The total hemoglobin concentration was determined using the cyanometahemoglobin method, whereby 10 µl of whole blood were diluted in 2.5 ml of Drabkin's solution, and a subsequent spectrophotometer reading was performed at 540 nm, as described by Hendrix (2005).

To guarantee that no contaminations could interfere with the results, microbiological analyses were performed at all experimental times using the three-phase Hemobac Triphasic system (Hemobac Trifásico; Probac, São Paulo, SP, Brazil), which uses chocolate agar, sabouraud agar, and MacConkey agar. The collected samples were kept in a greenhouse (Sterilifer digital SX1.1; Sterilifer, Diadema, SP, Brazil) for 7 days at 35°C. During microbiological examination, all procedures were performed according to the manufacturers' instructions.

Initially, data were analyzed using the Kolmogorov-Smirnov test to verify their distribution. Data that presented a normal distribution were analyzed using two-way repeated measure analysis of variance, followed by Dunnett's means-comparison test, which evaluates the effect of time (different days of evaluation). Sidak's test was then used to compare the results between the types of bag (CPDA-1 or CPD/SAG-M). GraphPad Prism® statistical software was used to perform the analyses; P-values <0.05 were considered significant.

RESULTS AND DISCUSSION

Two bags, collected from the same animal (buffalo 2), already yielded growth of microorganisms at D7 and maintained this result throughout the other time points. It was considered likely that contamination had occurred at the time of collection, because buffalo 2 was particularly aggressive and difficult to restrain. For this reason, these bags were removed from the study, and all analyses were performed using only 9 animals and 18 blood bags. At none of the experimental time points were any microbiological changes observed in the blood conserved in the remaining 18 plastic bags, and no microorganism growth occurred in any of the culture media used, suggesting that the results were reliable.

Table 1 presents the results of the hematological analyses pertaining to red blood cells, globular volume, and total hemoglobin. The number of red blood cells decreased during the 42 days of storage with differences ($P<0.05$) from D7 onwards in both the CPDA-1 and CPD/SAG-M bags but without differences ($P>0.05$) among bag types during the experimental period. Reductions

in red cells count are a common finding in studies on blood conservation (Cancellas, 2014; Erve *et al*., 2014; Obrador, 2014). This phenomenon is attributed to hemolysis that occurs throughout the shelf life of the blood and is a result of its natural aging (Hees, 2014). At the end of the present experiment, the red blood cell was 80% of the baseline count, confirming that these cells are preserved in these bags. In a similar experiment, Barros (2011) analyzed the preservation of donkey blood and demonstrated that, after 42 days stored, 87% of erythrocytes survived in CPDA-1 bags, whereas 80% survived in CPD/SAG-M bags.

The globular volume did not change throughout the study period in the present experiment ($P>0.05$). However, at all experimental time points, globular volume differed between the bag types ($P<0.05$), being higher in CPDA-1 bags than in CPD/SAG-M bags. Tavares (2013) reported the same discrepancy after analyzing blood conservation in goats using the same bags. Therefore, the differences between the globular volumes were predicted; they occur because the CPD/SAG-M bags contain greater amounts of diluents. Despite the reduction in erythrocytes, the globular volume remains stable because the MCV increases.

Regarding total hemoglobin, there was no significant difference between the bag types ($P>0.05$) at any of the time points evaluated. Bertoletti (2011) found a similar result. During the 42 days of the present experiment, the hemoglobin values differed between the two bag types; the CPDA-1 bags had higher values than the CPD/SAG-M bags. Tavares (2013); Barros (2011), who performed experiments on goats and donkeys, respectively, also reported differences between the bags in terms of hemoglobin concentration. The different hemoglobin concentrations are due to the greater amount of diluents found in the CPD/SAG-M bags. The same two researchers described reductions in hemoglobin concentration over the duration of storage, contradicting the findings of the present experiment. These data suggest that buffalo hemoglobin is less sensitive to the denaturation process during the conservation time applied in this study.

Table 2 presents the MCV, MCHC, and leukocyte count in buffalo total blood throughout the experiment. The MCV noticeable increased over time in the two conservation bags. With regards to the OD, the CPDA-1 bags showed marked changes from D35 onwards ($P<0.05$), whereas, in the CPD/SAG-M bags, such changes were seen only at D42. When the two bags were compared in this regard, significant differences were observed at all experimental times. The MCV is a marker of erythrocyte size, and its increase is directly proportional to the growth of these cells (Harvey, 2012). Therefore, an increase in MCV indicates engorgement of the red blood cells, which occurs due to faults in the sodium and potassium pumps that maintain hypertonicity in the intracellular medium, with a consequent inflow of water into the cell (Tuo *et al*., 2014). The percentage MCV found in this study indicated that a 16% increase occurred in this variable throughout the study period; this is a smaller increased than that reported by Barros (2011), who found, in a study involving donkeys, that the MCV increased by 21% in CPDA-1 bags and by 27% in CPD/SAG-M bags. Similarly, Sousa *et al*. (2012) reported a 36% MCV increase in sheep. Such precedents suggest that buffalo erythrocytes are less sensitive to storage changes than the erythrocytes of other species.

In both bag types, there was a difference in MCHC between D0 and D7 ($P<0.05$), as measured using OD. However, none of the other time

Table 1. Mean and standard deviation of red blood cell count, globular volume, and total hemoglobin in buffalo whole blood stored in CPDA-1 or CPD/SAG-M bags.

Day	Red blood cells (x 10^6)		Globular volume (%)		Total hemoglobin (g/dL)	
	CPDA-1	CPD/SAG-M	CPDA-1	CPD/SAG-M	CPDA-1	CPD/SAG-M
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
D0	7.6^{aA}±0.4	7.2^{aA}±0.3	31.8^{aA}±2.5	25.9^{aB}±2.1	9.6^{aA}±0.6	8.2^{aB}±0.6
D7	7.4^{bA}±0.4	7.0^{bA}±0.3	31.6^{aA}±3.0	25.2^{aB}±2.6	9.4^{aA}±1.2	8.3^{aB}±0.5
D14	7.1^{bA}±0.3	6.7^{bA}±0.3	29.8^{aA}±2.8	23.9^{aB}±3.6	10.3^{aA}±1.2	8.6^{aB}±0.6
D21	6.9^{bA}±0.3	6.5^{bA}±0.3	30.5^{aA}±2.0	24.5^{aB}±2.2	10.2^{aA}±0.6	8.4^{aB}±0.6
D28	6.7^{bA}±0.3	6.3^{bA}±0.3	29.1^{aA}±2.9	24.1^{aB}±2.7	9.6^{aA}±0.8	7.6^{aB}±0.8
D35	6.4^{bA}±0.2	5.9^{bA}±0.3	29.8^{aA}±2.2	23.2^{aB}±3.2	10.0^{aA}±0.5	7.5^{aB}±0.9
D42	6.0^{bA}±0.3	5.7^{bA}±0.4	29.6^{aA}±2.4	24.6^{aB}±2.9	9.7^{aA}±0.7	7.8^{aB}±0.7

Different lower case letters in the same column indicate a significant difference between baseline (D0) and the experimental time points, determined by Dunnett's multiple comparison test ($P<0.05$). Different upper case letters in the same line indicate a significant difference between the types of blood bags, determined using Sidak's multiple comparison test ($P<0.05$).

Table 2. Mean and standard deviation of mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC) of whole buffalo blood stored in CPDA-1 or CPD/SAG-M bags.

Day	MCV (fL)		MCHC (g/dL)		WBC (x 10^3)	
	CPDA-1	CPD/SAG-M	CPDA-1	CPD/SAG-M	CPDA-1	CPD/SAG-M
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
D0	40.8^{aA}±4.6	35.8^{aB}±3.3	30.2^{aA}±2.0	32.3^{aA}±2.3	12.1^{aA}±1.7	11.8^{aA}±1.8
D7	42.4^{aA}±4.1	35.9^{aB}±4.5	29.9^{bA}±3.5	33.3^{bA}±2.9	10.3^{bA}±1.5	9.3^{bA}±0.9
D14	41.9^{aA}±3.7	35.3^{aB}±6.0	35.1^{aA}±4.0	36.6^{aA}±5.0	9.5^{bA}±1.3	9.1^{bA}±1.0
D21	43.5^{aA}±2.7	37.7^{aB}±4.4	33.4^{aA}±0.9	33.5^{aA}±3.2	7.6^{bA}±1.1	7.7^{bA}±1.2
D28	43.1^{aA}±3.8	40.2^{aB}±6.3	33.2^{aA}±4.4	30.7^{aA}±3.6	7.3^{bA}±1.1	6.5^{bA}±1.3
D35	46.2^{bA}±2.3	38.8^{aB}±5.4	33.8^{aA}±2.1	32.6^{aA}±2.1	6.6^{bA}±0.8	5.8^{bA}±1.0
D42	48.6^{bA}±3.4	42.8^{bB}±5.1	32.7^{aA}±2.3	31.9^{aA}±2.4	6.1^{bA}±0.6	5.3^{bA}±0.9

Different lower case letters in the same column indicate a significant difference between baseline (D0) and the experimental time points, determined by Dunnett's multiple comparison test ($P<0.05$). Different upper case letters in the same line indicate a significant difference between the types of blood bags, determined using Sidak's multiple comparison test ($P<0.05$).

points differed from D0 in this regard (P>0.05). Furthermore, the bags did not differ from each other in terms of MCHC (P>0.05). The MCHC represents a percentage derived from the ratio of hemoglobin concentration to globular volume. The MCHC found in the present experiment stands to reason, because both the hemoglobin concentration and globular volume were constant throughout the experimental period. Clinically, this hematometric index is important in the investigation and etiological classification of anemias. Nonetheless, data about these indices are rare in blood storage studies; therefore, it is difficult to compare this parameter between species.

The leukocyte count underwent gradual decline throughout the present experiment. The reductions were significant (P<0.05) between D0 and D7. However, no differences (P>0.05) were observed between the bag types in terms of the amount of leukocytes. Such leukoreduction is commonly found during blood storage (Sousa, 2009; Hu *et al*., 2014; Hess, 2014). Razouk (2004) stated that, at the end of their storage time, blood bags should contain a maximum of 5 x 10^3 leukocytes /µl. At the end of this experiment, averages of 5.3 and 6 x 10^3 leukocytes /µl were found in the CPD/SAG-M and CPDA-1 bags, respectively. The percentages of leukocyte lysis in the present study were 50% in the CPDA-1 bag and 55% in the CPD/SAG-M bag. These values were higher than those found in goats (Tavares, 2013), where only a 5% leukocyte lysis rate occurred in the CPDA-1 bags, and a 42% rate occurred in the CPD/SAG-M bags. These findings suggest that buffalo leukocytes are more sensitive to storage, especially in the CPDA-1 pockets. Leukocytes are involved in several undesirable effects of blood transfusion, such as non-hemolytic febrile reactions, HLA antibody formation, platelet refractoriness, etc. Indeed, researchers agree that it is important to reduce

Table 3. Mean and standard deviation of plasma hemoglobin concentration and degree of hemolysis in buffalo whole blood packed in CPDA-1 or CPD/SAG-M bags.

Day	Plasma hemoglobin (g/dL)		Hemolysis degree (%)	
	CPDA-1	CPD/SAG-M	CPDA-1	CPD/SAG-M
	Mean± SD	Mean	Mean	Mean
D0	0.06^{Aa}±0.04	0.18^{Aa}±0.17	0.45^{Aa}±0.32	1.70^{Aa}±1.5
D7	0.06^{Aa}±0.03	0.45^{Aa}±0.18	0.38^{Aa}±0.23	3.41^{Aa}±1.51
D14	0.59^{Aa}±0.25	0.53^{Aa}±0.31	3.01^{Aa}±1.32	3.64^{Aa}±1.90
D21	1.16^{Ab}±0.32	1.49^{Ab}±0.72	6.46^{Ab}±1.68	11.0^{Bb}±4.44
D28	1.00^{Ab}±0.33	1.54^{Ab}±0.92	5.45^{Ab}±1.80	10.94^{Bb}±5.57
D35	1.65^{Ab}±0.59	1.46^{Ab}±0.80	8.74^{Ab}±3.59	10.2^{Ab}±4.91
D42	1.95^{Ab}±0.73	1.31^{Ab}±0.55	10.4^{Ab}±3.80	9.01^{Ab}±4.16

Different lower case letters in the same column indicate a significant difference between the experimental time points, determined using Dunnett's multiple comparison test (P<0.05). Different upper case letters in the same line indicate a significant difference between the types of blood bags, determined using the Sidak multiple comparison test (P<0.05) for each variable.

leukocytes in blood components before transfusion, and several investigators have proposed efficient means to withdraw them or even render them inactive (Serinolli *et al*., 2013).

Table 3 presents the plasma hemoglobin concentrations and degree of hemolysis. There was a significant increase in plasma hemoglobin concentration between D0 and D21 in both the CPDA-1 and CPD/SAG-M bags ($P<0.05$ in both cases).

No differences in plasma hemoglobin were observed between the bags. This increase in plasma hemoglobin was predictable; it results from red blood cell lysis, which is in turn caused by the osmotic fragility of red blood cells that develops during storage (Hess, 2014). Thus, this increase in plasma hemoglobin concentration is a normal finding in conservation studies, and it occurs in donkeys (Barros, 2011), dogs (Costa Jr., 2008), goats (Tavares, 2013) and sheep (Sousa *et al*., 2012).

CONCLUSION

Whole buffalo blood stored in CPDA-1 or CPD/SAG-M bags undergoes hematological changes during storage, but remains viable for blood transfusion when stored refrigerated for up to 42 days. In general, there are no clinically important differences in the conservation of buffalo whole blood between the two types of bags evaluated, and both are indicated for use in this species.

ACKNOWLEDGMENTS

The authors are grateful to the Coordination for the Improvement of higher education personnel, CAPES for financial assistance (AUXPE - PRO-AMAZONIA - 3288/2013). A.H.H.M is grateful to CNPq for a research productivity grant awarded.

REFERENCES

Aubron, C., A. Nichol, D.J. Cooper and R. Bellomo. 2013. Age of red blood cells and transfusion in critically ill patients. *Ann. Intensive Care*, **3**: 2.

Barros, I.O. 2011. *Evaluation of the conservation of donkey (Equus asinus) whole blood stored in CPDA-1 and CPD/SAG-M bags*. M.Sc. Thesis, Federal Rural University of Semiarid Region, Mossoró, Brazil.

Bhaduri, B., M. Kandel, C. Brugnara, K. Tangella and G. Popescu. 2014. Optical assay of erythrocyte function in banked blood. *Sci. Rep-UK*., **4**: 6211.

Cohen, B. and I. Matot. 2013. Aged erythrocytes: A fine wine or sour grapes? *Br. J. Anaesth*., **111**: i62-i70.

Filho, J.D.R., C.T. Almeida and R.C. Gonçalves. 1994. Hemogasometric changes of bovine blood during storage in glass bottles with ACD and plastic bags with CPDA-1 for 35 days. *Vet. Zoot.* **6**: 77-84.

Júnior, J.C., J.A. Viana, J.D.R. Filho, E.S. Favarato, L.C. Mata and N.A. Neto. 2008. Parameter biochemical and hemogasometric of the total blood stored of dogs in plastic stock markets I contend CPDA-1 and CPD/SAG-M. *Ciênc. Rural*, **38**(2): 378-383.

Hess, J.R. and T.J. Greenwalt. 2002. Storage of blood cells: New approaches. *Transfus. Med. Rev*., **16**: 283-295.

Latimer, K.S., R.J. Duncan and K.W. Prasse. 2011. *Veterinary Laboratory Medicine Clinical*

Pathology, 5th ed. Iowa State University Press, Ames, Iowa, USA. 524p.

Martins, S.B. 2011. *Transfusion medicine in dogs and cats: Harvesting, processing and storage of whole blood and hemocomponents*. M.Sc. Thesis, Federal University of Goiás, Goiânia, Brazil. 40p.

Nelson, D.L. and M.M. Cox. 2011. *Lehninger Principles of Biochemistry*, 5th ed. Artmed, Porto Alegre, Brazil. 1328p.

Obrador, R., S. Musulin and B. Hansen. 2015. Red blood cell storage lesion. *J. Vet. Emerg. Crit. Car.*, **25**: 187-199.

Paglia, G., B.O. Palsson and O.E. Sigurjonsson. 2012. Systems biology of stored blood cells: Can it help to extend the expiration date? *J. Proteomics*, **76**: 163-167.

Purdy, F.R., M.G. Tweeddale and P.M. Merrick. 2014. From the journal archives: The red blood cell storage lesion: past, present, and future. *Can. J. Anaesth*., **61**(6): 583-586.

Radwanski, K., M. Thill, K. Min, F. Kabi and L. Zurich. 2014. Red cell storage in E-Sol 5 and Adsol additive solutions: paired comparison using mixed and nonmixed study designs. *Vox Sang*., **106**: 322-329.

Reichmann, P. and A.C.O. Dearo. 2001. Blood and blood component transfusion in large animals. *Journal Semina Ciencias Agrarias*, **22**: 223-228.

Roback, J.D., C.D. Josephson, E.K. Waller, J.L. Newman, S. Karatela, K. Uppal, D.P. Jones, J.C. Zimring and L.J. Dumont. 2014. Metabolomics of ADSOL (AS-1) red blood cell storage. *Transfus. Med. Rev*., **28**: 41-55.

Salaria, O.N., V.M. Barodka, C.W. Hogu, D.E. Berkowitz, P.M. Ness, J.O. Wasey and S.M. Frank. 2014. Impaired red blood cell deformability after transfusion of stored allogeneic blood but not autologous salvaged blood in cardiac surgery patients. *Anesth. Analg*., **118**(6): 1179-1187.

Silva, J.B., C.T.A. Lopes, M.G.S. Souza, A.F.B. Gibson, W.M.S. Vinhote, A.H. Fonseca, F.R. Araújo and J.D. Barbosa-Neto. 2014. Serological and molecular detection of Anaplasma marginale in water buffaloes on Marajó Island, State of Pará, Brazil. *Pesqui. Vet. Brasil*., **34**: 11-14.

Sousa, R.S., I.O. Barros, M.D. Tavares, I.K.F. Sousa, G.B. Oliveira, A.H.H. Minervino and R.A. Barrêto-Junior. 2012. Storage lesions caused during blood conservation in the different species: Review. *Revista Acta Veterinaria Brasilica*, **6**: 68-79.

Sousa, R.S., R.A. Barrêto-Júnior, I.K. Sousa, D.F. Chaves, H.S. Soares, I.O. Barros, A.H.H. Minervin and E.L. Ortolani. 2013. Evaluation of hematologic, blood gas and select biochemistry parameters in whole sheep blood stored in CPDA-1 bags. *Vet. Clin. Path.*, **42**: 27-30.

Tavares, M.D. 2013. *Hematological, biochemical and hemogasometric evaluation of goat blood stored in CPDA-1 and CPD/SAG-M plastic bags*. M.Sc. Thesis, Federal Rural University of Semiarid Region, Mossoró, Brazil, 73p.

Tomczak, A.C.T.Q. 2008. *Studies on the Quality Control of Erythrocyte Transfusion Units*. Ph.D. Thesis, Federal University of Paraná, Curitiba, Brazil, 95p.

Vale, W.G., A.H.H. Minervin, K.A.L. Neve, A.C. Morini and J.A.P. Coêlho. 2013. Buffalo under threat in Amazon Valley, Brazil. *Buffalo Bull.*, **32**: 121-131.