Influence of Age, Sex and Breed Lines of Zebu Nellore Cattle on the Erythrocytes Osmotic Fragility, raised in São Paulo, Brazil

Maria Consuêlo Caribé Ayres (AYRES, M. C. C.)
Professor
School of Veterinary Medicine and Animal Science (EMVZ/UFBA)
Department of Anatomy, Pathology and Clinics
Salvador, Bahia Brazil

Eduardo Harry Birgel (BIRGEL, E. H.)
Retired Professor
Faculty of Veterinary Medicine and Animal Science (FMVZ/USP)
São Paulo, Brazil

Eduardo Harry Birgel Júnior (BIRGEL JR. E.H.)
Professor
Faculty of Animal Science and Food Engineering (FZEA/USP)
Department of Veterinary Medicine
São Paulo, Brazil

Laura Emilia Panelli Martins (MARTINS, L.E.P.)
Doctoral Graduate Program in Animal Sciences in the Tropics
School of Veterinary Medicine and Animal Science (EMVZ/UFBA)
Salvador, Bahia Brazil

Abstract

The average standard values of erythrocytes osmotic fragility (EOF) were established to zebu cattle of Nellore, raised in State of São Paulo , by analyzing the influence of age, sex and breed lines (Standard Nelore, Lemgruber Nellore, Mocho Nellore and Kuleia Nellore). For this purpose, 230 samples of blood from healthy animals free from Leucosis, Brucellosis, and blood parasites, were collected and submitted to standard hematological techniques and the EOF test. Blood samples obtained from calves up to three months showed the lesser fragility to hypotonic saline (50% of hemolysis= 0,43±0,03g/dL NaCl). Groups older than three months to over 72 months (50%= 0,58±0,03 to 0,48±0,02 g/dL NaCl) showed EOF decrease. The EOF was statistically different between the varieties Mocho (50%=0,54±0,05 g/dL NaCl) and Kuleia (50%=0,48±0,06g/dL NaCl). Sex had no influence. The average standard values of the EOF in 50% of hemolysis to Zebu Nellore cattle were 0,51±0,04g/dL NaCl.

Keywords: hematology, variability factors of erythrocytes, osmotic fragility, bovine

1. Introduction

The most of the Zebu breed cattle that there are in Brazil having been of breeds that originated in India. Three of these breeds are important representatives in Brazil: Guzera, Nellore and Gir. In 1930 the Nellore breed entered in the State of São Paulo, and at present there are four distinctly different types of Nellore cattle: Standard Nellore, strain Lemgruber Nellore, variety *Mocho Nellore* (resulted from recessive gene for the absence of horn) and the variety *Kuleia Nellore* (resulted from recessive gene for pink skin). Classes for the various Zebu breed were established by the Brazilian Association of Zebu cattle Breeder, and they were registered by breed characteristics and *Kuleia* variety animals were not registered by this Association because of their pink skin, despite being pure (Santiago, 1987).

The erythrocyte osmotic fragility (EOF) has been of interest since 1883, when Hamburger developed a method for determining the susceptibility of erythrocytes to hemolysis in hypotonic salt solution (Perk et al., 1964). The degree of EOF has been used as a measure of the red cells viability and also clinically, as a diagnosis characteristic (Coldman, 1969; Figueiredo et al., 2012). The fragility of the erythrocytes to hemolysis may be increased or decreased in disease (Perk et al., 1964; Binder & Mathois, 1986) and it is influenced by several factors: pH, temperature, oxygenation, size cell, membrane cell, age of animal, species, breed, lipemia, age of erythrocytes and the values obtained vary according to the laboratories (Jain, 1993).

Marked differences in osmotic behavior of erythrocytes between adult and young animals have been reported for cattle, sheep (Soliman & Amrousi, 1996; Ayres et al, 2005), and goat (Perk et al., 1964). An increase in the EOF of new born calves has been described, as the calves mature, and finally they acquire the typical osmotic fragility of the adult (Holman, 1956).

The value of NaCl concentrations which hemolyses 50% of the erythrocytes presents in the sample is more used to evaluate the erythrocytes resistance then the minimal hemolysis (H5%) and maximal hemolysis (H95%) resistance, used by the most of the authors (Coldaman, 1969; Pillai et al., 1976; Olusanya & Adepoju, 1979; Soliman & Amrousi, 1996; Sant'Ana et al., 2001).

In Brazil, the influence of the variety Zebu Nellore was observed on the hematologic values (Ayres et al., 1996) , and also the influence of cattle breed on the EOF, between Holstein and Nellore (Ayres et al., 2005)..

Zebu is very important in the production of beef cattle, and then the purpose of this research was to study the EOF in Nellore cattle, evaluating the influence of age, sex and breed lines, in animals bred in State of São Paulo, Brazil.

2. Material and Methods

For this purpose 230 sample of blood from Zebu Nellore cattle of different varieties and strain (Nellore Standard, Lemgruber, *Mocho* and *Kuleia*), clinically healthy, free from Leucosis, Brucellosis and blood parasites were drawn from the jugular vein by vacutainer system with EDTA served as an anticoagulant. To evaluate the influence of age 70 female of Nellore Standard were organized into seven different groups according to age: up to three months, 3 - 6; 6 -12; 12 - 24; 24 - 48; 48 - 72 and older than 72 months. Each group was formed by 10 animals. The influence of sex was evaluated in 80 blood sample from Nellore Standard adult, older than 24 months of age, organized into two groups: 40 females and 40 males. Breed lines influence was evaluated using 120 animals of varieties and strain: 30 Nellore Standard, 30 Nellore Lemgruber, 30 *Nellore Mocho* and 30 *Nellore Kuleia*.

All blood samples were submitted to hematological procedures that have been described and standard methods were used to calculate mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (Birgel, 1982).

Erythrocyte osmotic fragility was performed essentially as described by Jain (1986) for which briefly 20μL of blood was aspirated into 16 tubes containing 5,0mL of buffered concentration of NaCl solution according to Table 1. The suspensions were mixed by inversion and incubated at room temperature for 30 minutes and then all tubes were centrifuged at 750G for 10 minutes. A spectrophotometer was used to measure the absorbance of supernatant at 540 nm and distilled water was used as blank. The hemolysis in tube 16 was regarded as being 100% of hemolysis.

The median corpuscular fragility defined as the saline concentration causing 50% of hemolysis and the concentration tor minimal (5%) and maximal (95%) of hemolysis were calculated by Probit analysis (Parpart et al., 1947) and the comparison between the groups was analyzed by comparison of means by "t" test, using a program for statistical analysis (Graph Pad Instat, v 2.01 - 2010).

3. Results and Discussion

The mean values for the degree hemolysis of erythrocytes of different age at three NaCl concentrations are shown in Table 2 and Figure 1. The table showed that maximal hemolysis (H95%) occurs within a range of 0,28±0,06 to 0,34±0,06g/dL NaCl. Calves up to three months showed the most resistant erythrocytes and adult animal between 12 and 48 months the most fragile erythrocytes.

Perk et al. (1964) compared the fragiligrams curve obtained from adult cattle and calves showing that the blood of calves contains more resistant erythrocytes. It was also described that young calves are two populations of erythrocytes, one corresponding to adult blood and a second, more resistant to osmotic pressure (Holman, 1956). The osmotic more resistant population of erythrocytes decreases with time and completely disappears with 9 weeks after birth (Perk et al., 1964). In this present research the most of the calves included in the group up to three months were between one and 65 days of life, and this time corresponds to the lifespan of bovine erythrocytes (Römes, 1981). It has been described that in the third month the fetal hemoglobin decreases in the blood of calves (Grimes et al., 1957; Ayres et al., 2003). This study agrees with the others research that showed the influence of age in the EOF in bovine, sheep (Perk et al., 1964; Guglielmino et al., 1985), and goat (Fairley et al., 1988; Jain, 1993).

The table 3 showed that there were not statistically difference ($p > 0,05$) between the average of male and female animals. This observation agree with finding in the White Fulani cattle (Olayemi, 2004) and Kuri cattle (Olayemi et al., 2006) in which there no sex influence in the erythrocytes values. As this evaluation was performed in the Nellore Standard there was not influence of the properties of the cell membrane (Fairley et al., 1988).

In table 4 the degree of hemolysis of erythrocytes of different breed lines and variety are shown. There were statistically differences ($p > 0,05$) between the *Nellore Mocho* and the *Nellore Kuleia* variety. The lines Nellore Standard and Nellore Lemgruber have demonstrated less resistant erythrocyte when compared with the groups of *Mocho* and *Kuleia* in the degree of 5% and 50% of hemolyses. The group of the *Kuleia* variety showed the more resistant erythrocytes in the degree of medium and maximal hemolysis than the groups of the Standar, Lemgruber and *Mocho* variety. In the Figure 2 represents the accumulative curve obtained from blood of healthy adult breed lines of Nellore cattle. The difference between the curves of the *Mocho* variety and *Kuleia* variety it is evident.
The difference observed in this report between *Nellore Kuleia* and *Nellore Mocho* varieties may be explained by the effect of mutation occurred during the selection of this breed (Santiago, 1987). Difference in the behavior of erythrocyte among breeds has been associated with difference in the properties of the cell membrane (Fairley et al., 1988). Others differences between *Kuleia* and *Mocho* varieties were observed on the hematologic values of the Nellore cattle (Ayres et al., 1996).

When considering that similar research was conducted in the same laboratory using the same technique it´s possible to affirm the effect of racial patterns on the osmotic fragility in the erythrocytes of cattle (Sant'Ana et al., 2001). On the other hand and by date obtained from literature breeds of *Bos taurus* showed more fragility erythrocytes than the breeds of *Bos indicus* (Perk et al., 1964; Pillai et al., 1976; Olusanya & Adepoju, 1979; Binder & Mathois, 1986; Sant'Ana et al., 2001; Ayres et al.,2005; Olayemi et al., 2006). Mutations that occurred and gave the distinct racial types included in this research may have affected the blood characteristics of the racial types of Nellore cattle.

In table 5 a general average of erythrocyte osmotic fragility of zebu Nellore cattle, bred in São Paulo were shown and the Figure 3 represents the accumulative curve obtained from all blood sample of cattle included in this research.

The minimal and maximal degree of hemolysis were considered and evaluated, and the degree of medium hemolysis (50%) also defined as mean corpuscular fragility (Guglielmino et al., 1985). The difference between salt concentrations for which completely hemolysis occurs in the blood of adult bovine as obtained by other authors (Coldman, 1969; Soliman & Amrousi, 1996; Olayemi et al., 2006) could be explained by the difference of the method used or for the time that the blood samples were used after collected. It has been found that when washed the erythrocytes hemolysed the haemoglobin will be released at the lower ionic strength when the decrease of salt concentration of the surrounding medium is gradual (Perk et al., 1964, Jain, 1993).

4. Conclusion

Based on the results obtained in the establishing the standard values of EOF in minimal, medium and maximal degree of hemolysis in NaCl concentration (g/dL) for zebu Nellore cattle, the influence of the age and breed lines factor were observed. There is no influence of the sex factor between the animals of this breed

References

Ayres, M.C.C.; Birgel, E. H.; D'Angelino, J.L.; Benesi, F.J.; Costa, J.N. (1996). Influência de fatores varidade e linhgem sobre o eritrograma de zebuínos da raça nelore. Arquivos da Escola de Medicina Veterinária da UFBA, 18: 89-101.

Ayres, M.C.C. ; Birgel, E.H.; Birgel Junior, E.H.; Soares, P.; Rosenfeld, A.M.F. (2003) Dinâmica da hemoglobina fetal e adulta de bezerros sadios, criados no Estado de São Paulo. In: XI Congresso Latino Americano de Buiatria, 2003, Salvador Bahia. XI Congresso Latino Americano de Buiatria. Salvador, pp. 30.

Ayres, M.C.C.; Birgel, E.H.; Birgel Júnior, E.H.; Costa, J.N.; Feitosa, T.; Mirandola, R.M.S.; Barros Filho, I. R.; Hagiwara, M. K. (2005) . Determinação da Fragilidade osmótica de eritrócitos de bovinos das raças Holandesa e Nelore, criados no Estado de São Paulo. Revista Brasileira de Medicina Veterinária, 27: 118-124. 2005.

Binder, J.P.; Mayhois, H. (1986). Die osmotishe resistenz der erythrozyten von kühen. Journal Veterinary Medicine A, 33: 89-92, 1986.

Birgel, E.H. (1982). **Patologia clínica veterinária**, Sociedade Paulista de Medicina. Veterinária, São Paulo, Brasil. pp 240.

Coldman, M.F.; Gent, M.; Good, D., W. (1969) The osmotic fragility of mammalian rythrocytes in hypotonic solutions of sodium chloride. Comparative Biochemistry and Physiology, 31: 605-609.

Fairley, N.M.; Prince, G.S.; Meuten, D.J. (1988) Evaluation of red blood cell fragility in pygmy goats. American Journal Veterinary Research, 49: 1598-600.

Figueiredo, A.P.M.; Medeiros, R.M.T.; Dantas, F.P.M.; Leite, A.L.D.; Fighera, R.A.; Riet-Correa, F. (2012). Intoxicação experimental por Indigofera suffruticosa em caprinos e ovinos. Pesq. Vet. Bras. 32: 126-130.

Gugliemino, R.;Abate, O.; Cagnasso, A.; Chiaretta, G. (1985). Variazioni posnatali del tipo di emoglobina della fragilitá osmotic eritrocitário del valore hematocrito, del numero de eritrócito e del contenuto del emoglobin in vitelli di razza frisona. Annalo della Facultá de Medicine Veterinaria di Torino, 30: 344-359.

Holman, H.H. Changes associated with age in the blood picture of calves and heifers. (1956). British Veterinary Journal, 112: 91-104.

Jain, N.C. (1986). Shalm's veterinary hematology. Philadelphia : Lea & Febiger, 1986. Hematology techniques, pp.64-71.

Jain, N.C. (1993). Essentials of veterinary hematology. Philadelphia : Lea & Febiger. Comparative hematologic features of some avian and mammalian species: pp.19-53.

Olusanya, S.K.; Adepoju, F.O. (1979). Osmotic fragility of erythrocytes of some Nigerian species of domestic ruminants. Bulletin of Animal Health Production Africa, 27: 237-243.

Olayemi, F.O. (2004). Erythrocyte osotic fragility, haematological and plasma biochemical parameters of the Nigerian White Fukani cattle, 52: 208-211.

Olayemi, F.O.; Akinsiky, D.O.; Ojo, E.O.; Azeez, I.O. (2006). Haematology of Kuri breed cattle. Folia Veterinary, 50:62-65.

Parpart, A.K., Lorenz, P.B., Parpart, E.R. (1947) The osmotic resistence (fragility) of human red cells. Journal Clinical Investigation, 26: 636-640.

Perk, K.; Frei, Y.F.; Herz. (1964) A. Osmotic fragility of red blood cells of young and mature domestic and laboratory animals. American Journal Veterinary Research, 25: 1241-1248.

Pillai, M.G.R.; Nirmalan, G.; Nair, S.G. (1976). Osmotic fragility of erythrocytes of certain species of animals. Kerala Journal of Veterinary Science, 7: 139-144.

Römes, D. (1981) Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts *in vitro* and erythrocytes *in vivo.* Proceedings of the National Academy of Science of the United States of American, 78:5009-5013.

Sant'Ana, V.A.C.; Birgel, E.H.; Mourão, G. B.; Mirandola, R.M.S. (2001). Erythrocyte osmotic fragility of bovine of Holstein, Gir/Holstein, crossbreed and Gir breeds, raised in the State of São Paulo. Ciência Rural, Santa Maria, 31: 609-614.

Santiago, A.A., (1987). O gado Nelore no Brasil: 100 anos de Seleção, Editora dos Criadores: São Paulo, Brasil, pp 591.

Soliman, M.K.; Amrousi, S.E. (1996). Erythrocyte fragility of health fowl, dog, sheep, cattle, buffalo, horse and camel blood. Veterinary Record,78: 429-430.

Table 1: Concentrations of NaCl solution used to perform EOF, according Jain (1986)

Tuber number	mL of 1% NaCl	mL of destiled water	%NaCl solution
1	4,25	0,75	0,85
2	4,00	1,00	0,80
3	3,75	1,25	0,75
4	3,50	1,50	0,70
5	3,25	1,75	0,65
6	3,00	2,00	0,60
7	2,75	2,25	0,55
8	2,50	2,50	0,50
9	2,25	2,75	0,45
10	2,00	3,00	0,40
11	1,75	3,25	0,35
12	1,50	3,50	0,30
13	1,25	3,75	0,25
14	1,00	4,00	0,20
15	0,50	4,50	0,10
16	0,00	5,00	0,00

Table 2: Erythrocyte Osmotic Fragility of Zebu Nellore cattle, according age, in NaCl concentration g/dL (Mean ± SD)

Age/months	Number of animals	Degree of hemolysis g/dL		
		Minimum (5%)	Medium (50%)	Maximum (95%)
< 3	10	0,65±0,06 ^{ab}	0,43±0,04 ^{a}	0,28±0,06 ^{a}
3 – 6	10	0,69±0,04 ^{b}	0,57±0,03 ^{b}	0,32±0,04 ^{ab}
6 – 12	10	0,67±0,03 ^{b}	0,58±0,05 ^{b}	0,32±0,03 ^{ab}
12 – 24	10	0,66±0,05 ^{ab}	0,54±0,06 ^{bc}	0,34±0,06 ^{b}
24 – 48	10	0,66±0,05 ^{ab}	0,52±0,05 ^{bc}	0,34±0,04 ^{b}
48 – 72	10	0,62±0,04 ^{a}	0,50±0,04 ^{bc}	0,33±0,05 ^{b}
>72	10	0,62±0,03 ^{a}	0,48±0,05 ^{c}	0,32±0,03 ^{ab}
Total	70	0,65±0,05	0,52±0,05	0,32±0,05

Average follow of the identical letter in column are significantly different (p > 0,05)

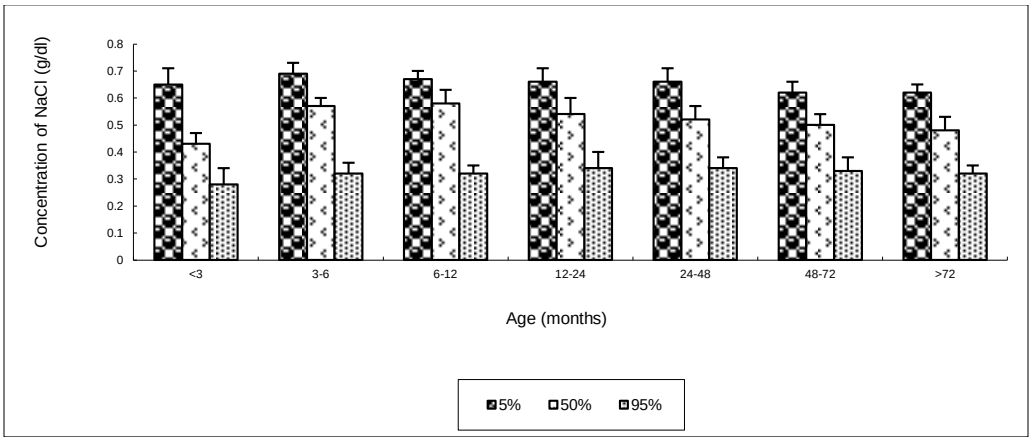

Figure 1: Erythrocytes Osmotic Fragility of Zebu Nelore cattle, according age, in Na Cl concentration (g/dL)

Table 3: Erythrocyte Osmotic Fragility of Zebu Nelore Cattle, according sex in NaCl concentration g/dL (Mean ± SD)

Sex	Number of animals	Degree of hemolysis g/dL		
		Minimum (5%)	Medium (50%)	Maximum (95%)
Female	40	0,64±0,05 [a]	0,51±0,05 [a]	0,33±0,05 [a]
Male	40	0,64±0,04 [a]	0,50±0,05 [a]	0,32±0,04 [a]
Total	80	0,64±0,05	0,51±0,05	0,32±0,05

Average follow of the identical letter in column are significantly different (p>0,05)

Table 4: Erythrocyte Osmotic Fragility of Zebu Nelore Cattle According Breed Lines, in NaCl concentration g/dL (Mean ± SD)

Breed lines of Nellore	Number of animals	Degree of hemolysis g/dL		
		Minimum (5%)	Medium (50%)	Maximum (95%)
Standard	30	0,71±0,04 [a]	0,51±0,03 [ab]	0,33±0,05 [a]
Lemgruber	30	0,69±0,03 [ab]	0,52±0,03 [ab]	0,34±0,04 [a]
Mocho	30	0,67±0,03 [b]	0,54±0,05 [b]	0,35±0,05 [a]
Kuleia	30	0,64±0,04 [c]	0,48±0,06 [c]	0,29±0,05 [b]
Total	120	0,68±0,04	0,52±0,05	0,32±0,05

Average follow of the identical letter in column are significantly different (p>0,05)

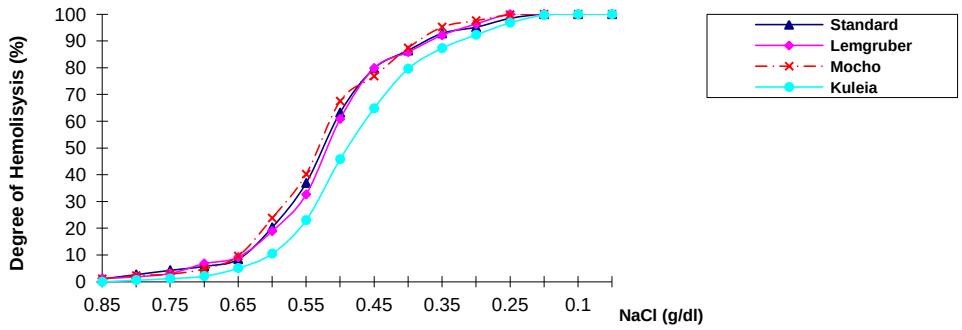

Figure 2: Accumulative curve of Erythrocyte Osmotic Fragility of Zebu Nellore Cattle, According Breed Lines

Table 5: Erythrocyte Osmotic Fragility of Zebu Nellore cattle (N=230) bred in São Paulo State, Brazil, in NaCl concentration g/dL (mean ± SD)

	Degree of hemolysis g/dL	
Minimum (5%)	Medium (50%)	Maximum (95%)
0,66±0,05	0,52±0,05	0,32±0,05

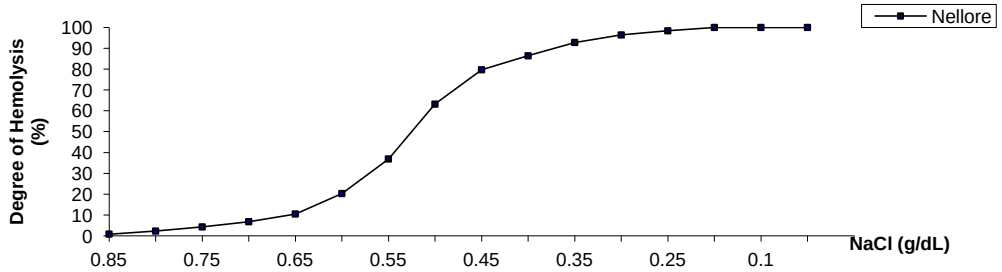

Figure 3: Accumulative curve of Zebu Nellore cattle bred in São Paulo State, Brazil