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Influence of lactation stages on erythrocyte osmotic fragility of indigenous Badri cattle of Uttarakhand

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The indigenous cattle of Uttarakhand, Badri, is the first registered cattle breed of the state (ICAR-NBAGR 2016). These are mainly reared in the hilly areas of Kumaon and Garhwal regions of Uttarakhand. Badri is a small sized breed having 200-250 kg body weight, with bright and alert eyes, erect ears, small wide neck and a prominent hump. The coat colour is varied as black, brown, red, white or grey, and hooves and muzzle are black or brown in colour. The legs are long and straight with hard foot pads for hilly terrains. The udder is small sized and tucked up within the body, milk yield is 1.5 (0.5-2.0) kg per day. The average age at first calving is 3-5 years, lactation length is 208 days (6-8 months) and long dry period of 138 days (4-6 months) (Pundir *et al*. 2014). Badri is well adapted to the hills under the prevailing climatic conditions and resistant to many diseases (Pundir *et al*. 2014).

Pregnancy and lactation are physiological states that cause considerable alteration in individual's hematological parameters (Maria and Monika 2010). Erythrocyte osmotic fragility is defined as degree or proportion of hemolysis which occurs when a blood sample is subjected to osmotic stress by placing in a hypotonic solution. Osmotic fragility is affected by various factors like membrane composition and integrity, cell's size and/or surface area to volume ratio (Fischbach and Dunning 2008). Increased osmotic fragility is due to the damage of erythrocyte membrane by oxygen radicals in the form of lipid peroxidation and protein degradation with decreased antioxidant levels caused by imbalance in redox signalling and generation of free radicals at rates that cannot be matched by endogenous antioxidant (glutathione and superoxide dismutase) (Oyagbemi *et al.* 2009), which has influence on RBC's membrane integrity and makes cells more fragile and labile to damage. Intrinsic factors including age, species, breed, genes, phenotype, sex, pregnancy and lactation, size and difference in erythrocyte membrane composition can also affect osmotic fragility of erythrocytes (Igbokwe 2018). Lactation being one of the important physiological stage imposes stress on dairy cattle which may be reflected in terms of alteration in osmotic fragility of erythrocytes. The present study was undertaken to study the effect of various stages of lactation on erythrocyte osmotic fragility.

This study was carried out using 40 healthy cows divided into five groups with eight animals in each group during different stages of lactation, viz. group 1 (0 to 60 days), group 2 (61 to 120 days), group 3 (121 to 180 days), group 4 (181 to 240 days) and group 5 (non-lactating animals) as control group. The osmotic fragility was determined by the method described by Novozhilov *et al*. (2013). Sodium chloride solution (*p*H 7.4) was prepared at varying concentrations (0.0%, 0.1%, 0.3%, 0.5%, 0.7% and 0.9%) from 1% phosphate buffer. A volume of 5 ml of different NaCl concentrations was placed in labelled test tubes serially and 0.02 ml (20 µl) of the blood sample was pipetted into each test tube. The contents of the tubes were gently mixed by inverting the tubes and allowing them to stand at room temperature (24-26°C) for 30 min, thereafter, the tubes were centrifuged at 1500 rpm for 15 min using a centrifuge. The supernatant obtained from each tube was transferred to a clean glass cuvette and the absorbance of the supernatant was measured spectrophotometrically at a wavelength of 540 nm. The per cent haemolysis for each sample was calculated using the following Faulkner and King (1970) formula. Osmotic fragility curve of erythrocytes was determined by plotting the hemolysis percentage against various saline concentrations.

$$\% \text{ Haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 100$$

The observed values were recorded against 100±0.00 per cent haemolysis at 0% saline concentration (Table 1 and Fig. 1). The per cent haemolysis among the lactation stages and non-lactation group was found significant ($p<0.05$) at 0.9% and 0.5% saline concentrations. The maximum per cent haemolysis was observed in group 2

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Table 1. Mean±SE values of percent hemolysis during different stages of lactation and at different concentration of saline (NaCl %) solution of Badri cattle (n=8 in each group)

Saline concentration	Per cent haemolysis				
	1 (0 to 60 days)	2 (61 to 120 days)	3 (121 to 180 days)	4 (181 to 240 days)	5 (Non-lactating animals)
0.9%	2.79±0.45^{ab}	4.65±0.75^{b}	2.07±0.45^{a}	2.80±0.62^{ab}	2.64±0.55^{ab}
0.7%	4.44 ±0.66	6.09±0.81	5.24±0.74	4.83±0.72	3.78±0.66
0.5%	34.13±2.59^{b}	36.59±2.53^{b}	34.86±3.04^{b}	33.22±3.25^{b}	24.51±1.59^{a}
0.3%	83.52±4.02	88.17±6.90	85.40±2.64	80.78±4.08	75.69±2.12
0.1%	91.05±3.81	91.82±1.60	91.45±2.04	90.71±2.93	87.85±3.31
0%	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Mean values with ^{a,b} in superscript along the row differ significantly ($p<0.05$).

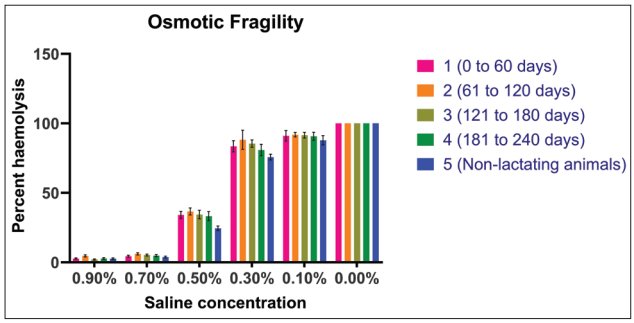

Fig. 1. Per cent haemolysis of erythrocytes at various saline concentrations.

at all saline concentrations. At 0.9% saline concentration, per cent haemolysis was significantly higher in group 2 and lower in group 3 than other stages of lactation and non-lactating animals. Whereas, at 0.5% saline concentration, per cent haemolysis tended to increase in group 2 while decreasing at later stages of lactation, whereas significantly lower levels of per cent haemolysis was observed in non-lactating animals. In present study, as saline concentration is decreased, per cent haemolysis of erythrocytes increased in all the groups. This might be due to the cell lysis caused by influx of solvent which leads to swelling and disruption of integrity of RBC's membrane when kept in hypotonic solution.

Several studies reported increase in osmotic fragility of erythrocytes during early lactation stage. The erythrocytes of lactating cows were more susceptible to osmotic fragility than erythrocytes of pregnant cows (Kadah *et al*. 2014). This could be attributed due to hemodilution as a results of increased immature RBCs (reticulocytes) production which can resist osmotic fragility more than mature RBC. Lower resistance to osmotic fragility of erythrocytes in lactating cows might be due to imbalance of oxidative stress and lipid peroxidation (Ronchi *et al*. 2000).

Habibu *et al*. (2014) reported no significant difference in erythrocyte osmotic fragility between lactating and non-lactating animals. Erythrocyte osmotic fragility decreased in late pregnancy due to improved membrane stabilization through progesterone and increased during early lactation because of low cholesterol and triglycerides concentration in the membrane (Igbokwe *et al.* 2015). Stress induces generation of free radicals which overwhelm the antioxidant defense mechanisms of the body (Nazifi *et al*. 2009). Increased free radical generation in the body has been shown to cause lipid peroxidation of cytomembranes, resulting in cell injury and death (William *et al.* 2008) of erythrocytes (Adenkola and Ayo 2009). Although the proximate mechanism of haemolysis was not investigated in the present study, it has been proved that free radicals are generated in animals subjected to stress (Tauler *et al*. 2003) which apparently occurs in cows during lactation. Droge (2002) stated that stress factors may cause damage of erythrocyte membrane by inducing increased generation of oxygen radicals which cause lipid peroxidation and protein degradation.

SUMMARY

The aim of this study was to evaluate the influence of lactation stages on erythrocyte osmotic fragility. The study was carried out using forty healthy Badri cows divided into five groups with eight animals in each group during different stages of lactation, viz. 1 (0 to 60 days), 2 (61 to 120 days), 3 (121 to 180 days), 4 (181 to 240 days) and 5 (non-lactating animals) as control animals. The per cent haemolysis of erythrocytes among different lactation groups was found significant at 0.9% during mid and early late lactation i.e. 2 and 3 group and 0.5% saline concentration during all stages of lactation as well as non-lactating animals.

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