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WBC differential in Gir and Jaffarabadi animals at different physiological stages

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Abstract
A study was conducted to establish the leucogram in Gir cattle and Jaffarabadi buffaloes at different age's *viz*.1 wk, 1m, 3m, 6m, 12m, 24m and 36m, in lactating (1m, 2m, 3m) and non-lactating (pregnant and non-pregnant) and in castrated males and bulls. Thin blood smears were made and stained with Eosin and Methylene Blue (Field Stain) and the different types of leucocytes (DLC) were enumerated. The percentage of neutrophils in the blood smear is more than the percentage of lymphocytes at 1 wk age, indicating the pre-ruminant status of the animal. However, from one month of age onwards it was observed that the percentage of lymphocytes was more than the percentage of neutrophils as seen in ruminant animal.

Keywords: Gir cattle, Jaffarabadi buffalo, lactation, pregnant, WBC

1. Introduction
Haematological values help to assess the general health status of the individual animal and also the herd. The large number of species and breeds of domestic animals all over the country makes it is necessary to establish the normal baseline values for these breeds. Haematological values vary according to the species, breed, age, nutrition, physiological status, stress and climate. Leucocytes play an important role in the immune defense of the animal and make the infectious agent inactive by firstly destroying the bacteria or virus by phagocytosis and secondly by producing sensitised antibodies. As they can be transported quickly to the area of infection, they provide a very fast and strong defense against infectious agents.

2. Materials and Methods
The study was carried out on male and female Gir cattle and Jaffarabadi buffaloes of various ages and physiological stages maintained under standard feeding and management conditions at the Cattle Breeding Farm, Junagadh Agricultural University, Junagadh, Gujarat and the haematological evaluation was carried out at the Department of Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, Gujarat. The project was approved by the Institutional Animal Ethics Committee (IAEC). Fresh whole blood (1ml) collected aseptically from the jugular vein of Gir cattle and Jaffarabadi buffaloes at different age's (1 wk, 1, 3, 6, 12, 24 and 36 months) and physiological stages (lactating (1,2 and 3 months) and non-lactating (pregnant and non-pregnant), castrated males and bulls) were immediately subjected to haematological analyses. Thin blood smears were made and stained with Eosin and Methylene Blue (Field Stain) and the different types of leucocytes (DLC) were enumerated [1]. The data obtained were subjected to statistical analyses by using the Completely Randomised Design (CRD) [2].

3. Results and Discussion
The Mean ± S.E. differential leucocyte count (DLC) at different physiological stages for male and female Gir cattle and Jaffarabadi buffaloes are given in Table -1 and 2.

3.1 Lymphocytes (%): The lymphocyte count was significantly (P<0.05) lower at 1 wk age in both the sexes of both the species studied. The values increased significantly (P<0.05) at 1 m age and increased as age advanced. The counts at 36 m in Gir and Jaffarabadi females and that at 12 m age in Gir and Jaffarabadi males was significantly (P<0.05) higher than the values at 1 wk age.

The lymphocyte count increased significantly (P<0.05) in lactating Gir cows and increased non-significantly in lactating Jaffarabadi buffaloes as the lactation progressed. Significant (P<0.05) differences were noted in among non-lactating Gir cows whereas non-significant effect was seen in non-lactating Jaffarabadi buffaloes. Non-significant difference was noted between bulls and castrated males of Gir breed. Significant (P<0.05) difference was observed between Jaffarabadi male and female at 12 m age. Significantly (P<0.05) higher values were observed at 36 m age and in non-lactating pregnant females of Jaffarabadi breed as compared to Gir breed.

3.2 Monocytes (%): No significant trend was observed for monocytes count (%) in the females of Gir and Jaffarabadi buffaloes. However, in males of both the species a non-significant increase in count was observed at 12 m age from that at 1 m age. In lactating Gir cows, the count at 2 m lactation was significantly (P<0.05) higher than that in Jaffarabadi buffaloes. Non-significant differences for monocyte counts were noted for all other comparisons between the two species.

3.3 Neutrophils (%): Neutrophils count (%) was significantly (*P<0.05*) higher at 1 wk of age than that observed at the rest of the ages studied in both the sexes of Gir cattle and Jaffarabadi buffaloes. The values at 36 m in Gir and Jaffarabadi females and at 12 m age in Gir and Jaffarabadi males was significantly (P<0.05) lower than the values at 1 wk age. In lactating females, the values from 1 m lactation to that at 3 m lactation decreased significantly (P<0.05) in Gir cows and non-significantly in Jaffarabadi buffaloes. Non-significant differences were noted among non-lactating stages of both the species. The variation in the values between bulls and castrated males of Gir breed and between bulls of Gir and Jaffarabadi breed was non-significant.

3.4 Eosinophils (%): Lower eosinophil count (%) was noted at 1 wk age in both Gir and Jaffarabadi females and males. The values increased as age advanced with significant (P<0.05) differences observed in both Jaffarabadi females and males. The count at 2 m lactation in Gir females was significantly (P<0.05) higher than in Jaffarabadi buffalo at the same stage. No significant differences were detected among the non-lactating stages of both Gir and Jaffarabadi females. The difference between the count in Gir bulls and castrated males and between Gir and Jaffarabadi bulls was non-significant.

3.5 Basophils (%): The basophil count (%) differed non-significantly between all the ages and physiological stages studied in Gir cattle and Jaffarabadi buffaloes and also between the two species studied.

Studies conducted by [3] in Gir Cattle and [4] in Jaffarabadi buffaloes, belonging to the same age groups and physiological stages as reported here, found that the TLC (10^3/µl) values ranged from 6.85 to 10.07 in Gir cattle and from 6.74 to 9.26 in Jaffarabadi buffaloes. Significant (P<0.05) effect of age was seen in Gir females and males and the values at 1 wk of age were found to differ significantly (P<0.05) between male and female Gir cattle. Higher count (non-significant) of leucocytes was observed at pregnancy as compared to lactating stages. Non-significantly higher values were obtained in lactating buffaloes over that of pregnant buffaloes. The detailed values at each age and physiological stage along with the discussion on the same are present in the above mentioned articles cited at [3] and [4].

The percentage of different leucocytes in Gir cattle was in the range observed by [5, 6]. The observation in our study of significant (P<0.05) increase in the lymphocyte values and decrease in the neutrophil values as age advanced in Gir cows was similar to the findings of [5] in crossbred cows and [7] in different breeds of cattle. Slightly higher values of neutrophils observed at 1m and 2m lactation over that of adult cows (36 m) and dry cows was in line with the finding of [6] in Frieswal cattle. Non-significant effect of sex in the leucocyte values of Gir cattle was in accordance to the findings of [8]. The count of different leucocytes in Jaffarabadi buffaloes was in the range reported by [9, 10, 11, 12]. A reference range of 6.0 – 12.8 x 10^3/µl for leucocytes in cyclic and acyclic Nili-Ravi buffaloes was reported by [13] with neutrophil, lymphocyte, monocyte and eosinophil reference values being between 24 – 61.4, 31.7 – 70.0, < 8.0, < 10.0 percent, respectively. In a study on pre-parturient Murrah buffaloes [14] the TLC was reported to be around 8743 with neutrophil, lymphocyte, eosinophil, monocyte, and basophil levels being 31.2, 55.6, 7.83, 4.4 and 0.47 percent, respectively. The values in lactating, pregnant, non-pregnant and heifers were in the range recorded by [15] in Murrah buffaloes and [16] in Nili-Ravi buffaloes. The DLC values obtained in the current studies at ages above 1 month and all physiological stages studied were in the range reported by [17] in buffaloes during transition period. In their studies on haematological differences between the genders in buffaloes in Egypt [18] found no significant effect between the TLC and DLC of male and female buffaloes. Comparison of differential leucocyte counts between Gir cattle and Jaffarabadi buffaloes obtained in the current study with the results of other scientists was not possible due to paucity of literature. The higher levels of neutrophil and lower levels of lymphocytes at 1 wk age in both Gir cattle and Jaffarabadi buffaloes is a pointer towards their pre-ruminant status (which is equivalent to simple stomach) at this age as in simple stomach animals the percentage of neutrophils is higher than the other leucocytes. In both the species the shift in neutrophils to lymphocytes by 1 m age indicates the development of rumen i.e. shifting from simple stomach to ruminant stage. Reece and Swenson [19] found that lymphocytes are the most numerous in cattle from 6 weeks age onward. The rise in neutrophil numbers at lactation might be due to lactational stress and increase in numbers may be due to release of endogenous corticosteroids [6]. The increase in eosinophils as age advanced in both the species could be a response of the body to various internal and external parasites and allergens to which it is exposed over a period of time. Further, it is absolutely essential to read the DLC values with the total WBC count to understand the changes in the Leucocyte picture of the body.

Table 1: Differential Leucocyte Count (DLC -%) at different Physiological stages in Gir and Jaffarabadi females (Mean ± S.E.)

Age	Wk	Months						Lactation month			Non Lactating	
Animal	1	1	3	6	12	24	36	1	2	3	P	NP
L FG	42.25^{a}±1.27	54.25^{b}±1.36	65.00^{c}±0.36	59.38^{bc}±2.27	60.63^{bc}±2.52	64.63^{c}±1.60	58.87^{bt}±2.31	53.13^{h}±3.64	54.50^{h}±1.91	63.75^{i}±0.99	53.13^{ft}±1.49	60.63^{g}±2.32
L FJ	44.75^{a}±0.88	54.00^{b}±1.25	61.13^{c}±0.26	66.63^{d}±2.61	61.63^{cdx}±1.64	63.88^{cd}±1.33	66.63^{du}±2.25	56.88±0.83	57.75±2.34	60.13±1.83	61.38^{u}±2.74	59.00±2.51
N FG	52.25^{c}±1.32	39.13^{b}±1.48	28.88^{a}±0.26	33.88^{a}±2.23	33.88^{a}±1.42	29.13^{a}±1.61	32.75^{at}±2.07	39.38^{i}±3.14	38.13^{i}±2.25	29.63^{h}±1.22	37.25±1.44	32.00±2.23
N FJ	49.75^{d}±0.56	37.00^{c}±1.90	31.13^{ab}±0.28	28.50^{ab}±2.18	32.50^{b}±1.98	29.83^{ab}±1.91	26.63^{au}±1.95	36.63±0.96	36.83±2.35	33.13±1.47	31.63±2.74	32.83±2.88
E FG	3.13±0.29	4.38±0.76	3.75± 0.07	4.38±0.38	4.38±0.49	4.38±0.68	5.63±0.46	5.50±0.78	6.25^{m}±0.41	4.63±0.56	5.50±0.37	4.00±0.59
E FJ	2.88^{a}±0.35	6.00^{c}±0.65	5.00^{bc}±0.05	3.38^{ab}±0.59	3.88^{ab}±0.35	4.25^{b}±0.45	4.38^{b}±0.39	4.880±.55	3.88^{n}±0.44	4.63±0.26	4.50±0.54	5.38±0.49
M FG	2.13± 0.22	1.75±0.16	2.25±0.04	1.88±0.22	2.13±0.23	1.750.31	2.50±0.33	1.88±0.12	2.38^{m}±0.26	1.75±0.25	3.63±0.59	3.13±0.35
M FJ	2.13±0.48	2.75±0.62	2.63±0.03	1.50±0.27	2.00±0.33	2.00±0.33	2.130.29	1.62±0.26	1.50^{n}±0.18	2.00±0.26	2.50±0.27	2.75±0.37
B FG	0.25±0.16	0.50±0.18	0.13±0.01	0.50±0.19	0.25±0.16	0.13±0.12	0.25±0.16	0.13±0.12	0.0	0.25±0.16	0.50±0.19	0.25±0.16
B FJ	0.50±0.18	0.25±0.16	0.50±0.02	0.0	0.0	0.0	0.25±0.16	0.0	0.0	0.13±0.12	0.13±0.12	0.0

Note: Means having the same superscript do not differ significantly from each other (P<0.05).F: n = 8 at each stage; M: n = 6 at each stage.
Key: L – Lymphocyte; N – Neutrophil; E – Eosinophil; M – Monocyte; B – Basophil.
FG – Female Gir FJ – Female Jaffarabadi MG – Male Gir MJ – Male Jaffarabadi P – Pregnant NP – Non Pregnant

Table 2: Differential Leucocyte Count (DLC -%) at different Physiological stages in Gir and Jaffarabadi males (Mean ± S.E.)

Age Animal	Wk	Months				Bulls	Castrated
	1	1	3	6	12		
L MGM	43.33^{a}±1.56	52.17^{b}±1.11	62.00^{d}±2.68	58.33^{cd}±1.82	59.83^{cdv}±1.08	54.67^{bc}±2.11	58.50^{cd}±2.33
L MJ	43.66^{a}±0.49	51.66^{b}±0.84	58.17^{bc}±1.44	60.17^{c}±3.00	70.17^{dwy}±3.72	57.83^{bc}±2.36	-
N MGM	51.50^{c}±1.36	41.33^{b}±1.44	30.33^{a}±2.64	34.00^{ab}±1.81	35.00^{abv}±1.93	37.17^{b}±2.41	34.00^{ab}±2.31
N MJ	50.00^{d}±0.93	40.67^{c}±1.02	32.33^{b}±1.31	34.50^{bc}±2.49	23.83^{aw}±3.89	35.00^{bc}±1.78	-
E MGM	3.50±0.34	4.50±0.22	5.17±0.70	5.17^{v}±0.61	4.00±0.57	6.17±0.47	5.17±0.98
E MJ	3.67^{ab}±0.21	5.17^{bc}±0.60	6.50^{c}±0.56	3.17^{aw}±0.31	3.67^{ab}±0.21	4.83^{b}±0.87	-
M MGM	1.67±0.21	1.83±0.48	2.33±0.42	2.00±0.11	2.17±0.31	2.00±0.25	2.33±0.33
M MJ	2.17±0.40	2.17±0.60	2.67±0.33	2.17±0.48	2.33±0.42	2.33±0.33	-
B MGM	0.0	0.16±0.16	0.16±0.16	0.50±0.22	0.67±0.33	0.0	0.0
B MJ	0.50±0.22	0.33±0.21	0.33±0.21	0.33±0.21	0.0	0.0	---

Superscripts
a – e: between females and between male's
f – g: between non-lactating pregnant and non-lactating non pregnant
h – j: within lactating Gir and lactating Jaffarabadi
m – n: between lactating Gir and lactating Jaffarabadi
t – u: between Gir females and Jaffarabadi females
v – w: between Gir males and Jaffarabadi males
x – y: between male and female

4. Summary

The leucogram of female and male Gir cattle and Jaffarabadi buffaloes at different ages and physiological stages revealed that the percentage of neutrophils in the blood smear are more than the percentage of lymphocytes at 1 wk age, indicating the pre-ruminant stage of the animal. However, from one month of age onwards it was observed that the percentage of lymphocytes was more than the percentage of neutrophils as seen in ruminant animal.

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