



# Comparison of time frame critical for feed supplement on haematological indices of azawak cattle in semi–arid zone of Niger Republic

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**Abstract**

The haematological parameters of apparently healthy of Azawak cattle consisting of 36 animals (18 bulls and 18 heifers) at CERRA, Maradi in semi–arid zone in Niger Republic were studied. Data were analyzed for the effect of the time frame critical for utilization of feed supplement per sex. According a time frame critical for utilization of feed supplementation (bull and heifer) higher and lower values of Packed Cell Volume (PCV) was obtained from S2DM (26.32±3.78%) and NS (23.44±4.26%), there was no significantly lower ( $P>0.05$ ) for Azawak cattle breed in semi–arid zone. Haemoglobin (Hb) values was higher from S2DM (10.66±0.57 g/dl) and lower SMED (9.92±1.28 g/dl). Red blood cell count (RBC) was no significantly ( $P>0.05$ ) for Azawak cattle breed. According treatment, the Mean Corpuscular Haemoglobin (MCH) was higher from NS (18.89±2.28 pg) and lower value was from S2DE (17.53±0.53 pg). The Mean Corpuscular Haemoglobin Concentration (MCHC) was significantly higher ( $P<0.05$ ) for Azawak cattle breed after feeding. The Mean Corpuscular Volume (MCV) was observed to be higher from S2DE (44.55±1.37 fl) while the values were much higher from SMED (45.25±3.05 fl) of heifer and NS (44.59±0.87 fl) of bull. SED (10.07±2.73  $\times 10^9/L$ ) for all sex had the highest white blood cell count (WBC). White blood cell differential shows that lymphocytes was no significantly higher ( $P>0.05$ ) Azawak cattle breed. Neutrophils was significantly higher ( $P<0.05$ ) for Azawak cattle breed (male and female). Include Eosinophil, Monocyte and Basophil was observed a higher value from S2DE (12.90±1.05%) while the values were much higher from S2DE (13.70±0.50%) for heifer and SMD (13.20±1.97%) for bull. Conclusively, the haematological profile level, higher values for leucocytes and lymphocytes were noticed with increased differences between the investigated categories of animals taken into study.

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**Keywords:** Haematology; Supplement; Azawak cattle; Semi–arid; Zone



## Introduction

Blood is a special type of connective tissue composed of formed elements in a fluid matrix. Plasma is the fluid portion called serum when depleted of fibrinogen [1]. The formed elements include erythrocytes (red blood cells), leukocytes (white blood cells) and platelets [2]. Hence, the haematological values during different physiological situations should be known for the diagnosis of various pathological and metabolic disorders which can adversely affect the productive and reproductive performance of cows, leading to heavy economic losses [3]. Many of haematological parameters are influenced by many factors like breed, age, sex, seasonal variations, lactation, pregnancy, health and nutrition status [4-6]. It is acknowledged that for comparisons between individuals and with reference data in a clinical diagnostic situation, it is necessary to consider normal variations due to age, sex and breed in order to increase diagnostic precision [7]. It is recognized that normal values for the various blood cell parameters not only differ from species to species but can vary between the breeds within a species [8]. Red Blood Cells (RBCs) are small, disc-shaped, anucleate cells and the primary function of them is to transport hemoglobin which carries oxygen and carbon dioxide to and from tissues, therefore red blood cells play an important role in pH regulation [9]. White Blood Cells (WBCs) are basic cellular components of the immune system and can be divided into neutrophilic, eosinophilic and basophilic granulocytes, monocytes and lymphocytes. Only a small percentage (0.5 to 3%) of the leukocytes of domestic mammals are basophils. Hence, they are not often found in blood smears. Platelets play an important role in hemostasis [2,9]. Mean Corpuscular Volume (MCV) is more valuable than blood film examination in assessing the true size of erythrocytes. Using automated cell counting systems, a histogram or volume distribution curve of the erythrocyte population can be generated [1]. The aim of this study was comparison of time frame critical for feed supplementation on haematological indices of Azawak cattle breed in semi-arid zone of Niger Republic.

## Materials and methods

A total number of thirty six (36) Azawak cattle (18 bulls and 18 heifers) with an average initial Body Weight (BW) of  $184 \pm 40$  kg aged 3 to 4 years owned by CERRA, Maradi ranch was used for the experiment. The animals were quarantined for 3 weeks and ear tagged for identification. The trial was conducted at CERRA, Maradi. The cattle were grouped into six (6) animals per experimental diet. They were arranged in six different treatments of feed. The experimental animals were allowed to move out for normal grazing within the rangeland twice daily (8:30am – 1:30pm and 2:30pm – 4:30pm) and supplement were given daily. The houses were disinfected with IZAL® solutions and were allowed to dry for one week before the commencement of the experiment. The pens were cleaned fortnightly. The animals were housed in a cage (3 m long, 3 m wide and 2 m high). The cages were enriched with iron platforms and parallel iron bars. The house is located at the border of a gallery forest, in an area of environmental protection. Before the commencement of the experiment, animals were given prophylactic treatment of Ivermectin (0.2 mg/kg sub-cutaneous), Oxytetracycline (5 mg/kg intra-muscular) and multivitamin (10 ml/kg intra-muscular) injections were administered. The animals were supplemented to provide necessary minerals for 90 days. Water was supplied *ad-libitum* through the watering place.

The composition of experimental diet contained 35, 15, 10, 15 and 1% of millet stalk, cotton seed cake, *Faidhebia albida*

pod, wheat bran and salt. Also, added to it were cowpea haulms (13%), urea (1%), phosphorus (5%) and calcium (5%).

The proximate composition of feed supplement and experimental diet was carried out according to the method described by [10]. All the samples were properly labeled and analyzed in Animal Feed and Nutrition Laboratory of Faculty of Agriculture, Bayero University, Kano.

The animals were allocated into six treatment groups of three replicates per sex each containing 6 cattle (3 bulls and 3 heifers). There were thus 6 cattle per group in the Completely Randomized Design experiment. The treatments were evaluated by time of supplementation of the diet to the experimental animals. NS: No supplementation (control), SMD: Supplementation early (8:00 am) in the morning daily before moving out for grazing, SED: Supplementation in evening (5:00 pm) daily after the afternoon grazing, SMED: Supplementation in the morning (8:00 am) and in the evening (5:00 pm) daily, S2DM: Supplementation once every two days in the morning (8:00 am) before morning out for grazing and S2DE: Supplementation once every two days in the evening (5:00 pm).

Blood sample was collected from each animal twice; on the first day of experiment and the last day of experiment through the jugular vein, bloodletting was performed from apparently health cattle. Blood samples were collected in test tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant. These tubes were placed in an icebox and carried to the laboratory within 4 h of collection. In the laboratory, these samples were centrifuged at 3000 rpm for 10 minutes; the plasma was separated and stored at  $-4^{\circ}\text{C}$  for further analysis.

Red Blood Cell (RBC) mass, White Blood Cell (WBC) mass, Haemoglobin (Hb) concentrations, Packed Cell Volume (PCV or hematocrit), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), MCH and platelet mass were determined with methods cell counter set (Coulter T 860, England) and methods that described by Thrall (2004). Statistical analysis was performed using the SPSS version 20.

The most appropriate reference range is generated from a group of healthy animals with environmental and physiological characteristics as similar to the patient as possible. As in all species, a certain amount of physiological variability is observed in haematologic profiles of cattle. Variables that contribute to the thresholds and width of reference intervals include age, sex, stress, diet, body condition, reproductive status, recent activity, hydration, ambient temperature, and altitude.

The comparison of some normal haematological parameters according a time frame critical for utilization of supplement is shown in Table 2. The comparison of some normal haematological parameters according a time frame critical for utilization of supplement per male is shown in Table 3. The comparison of some normal haematological parameters according a time frame critical for utilization of supplement per female is shown in Table 4.

The data from this study were sorted out, stored in EXCEL sheet and later analyzed using statistical package for social science (SPSS) version 20. The data of chemical composition were analyzed by the following statistical procedures: one-way ANOVA. Significant differences were detected with  $P < 0.05$  and means were separated by Duncan's Multiple Range Test (DMRT). The data from haematological indices after feeding supplementation were analyzed by using  $2 \times 6$  factorial laid in Completely

Randomized Design (CRD). The factors evaluated were two (2) sexes (bull and heifer) by the six (6) time frame (treatments) of supplementation. Differences between sex and treatment were determined by the T-test mean $\pm$ SD separation and Least Significant Difference (LSD). For all analyses, a P value <0.05 was considered statistically significant and a P value >0.05 was considered not significant.

## Results and discussion

The nutritive characteristics of feed supplementation ingredients are shown in Table 1. This dry matter content indicates all constituents excluding water of the ingredients used in the formulation. DM was recorded in feed supplementation as (91.09%). The DM in this study is slightly above (95.4%DM) reported by [11] but similar to the range of 93.80%-98.70% DM reported by [12]. The crude protein content of the feed was 22.11% this value is slightly lower than what was reported by [13] who reported in a similar experiment 10.00% - 11.20% CP range. The crude protein content of the diet is sufficient for ruminants which will provide ammonia required by rumen microorganism to support optimum microbial activity [14]. This differences and variation in crude protein percentage among diets may be due to the type of protein source and its level of inclusions in the rations. This is completely different from what was reported by [15] and [6] who reported 10.9% to 14.8% CP and 11.0% - 13% CP respectively. The differences observed could also be associated with soil nitrogen condition, level of maturity of the crop residue and varietal differences [16]. Crude fibre was obtained at 45.64% CF which agrees reported by [13] and also in line with (11–45%) CF reported by [17]. This feed supplementation was recorded highest crude fibre level. Such high crude fibre content of the diet could be due to the quality and fibrous nature of ingredients used which reduces digestibility rate of the diet as well. Ether extract was obtained at 9.85% EE higher value to the work of Kinfermi *et al.*, (2009) with 6.13% EE. The mean ash content of feed supplementation was (9.18%) which is a little higher than the value of (6.34%) reported by [18] for semi-arid browse plants. The ME content in MJ/kg DM was 2086.57 kcal/kg DM. However, The NDF content from feed was higher than that reported by [19] and [20]. The ADF content from diet was higher than that reported by [19] and [21]. Many factors affect chemical composition such as oil extraction process [22] stage of growth [23] maturity and species or variety [24,25]. Ca was recorded 148.53 mg/kg Ca and TP was 45.86 mg/kg TP. The observed differences in mineral composition in these products may be due to genetic factor and added in different level (Ikram

*et al.*, 2010). Decrease in minerals, energy and protein contents also contribute to lower intake, reduced digestibility and consequently losses in weight by grazing animals. The report of [26] was in line with the findings of this study who reported that the crude protein content of tropical forages decreases during the advanced periods of the dry season.

The results of this study showed that application of feed supplementation contributed to an increased level of selected haematological parameters. In end of treatment, RBC, PCV, PLT, WBC, Hb, MID and MCV increased in both the control and experimental group. However, the observed increase was higher at S2DM. A reverse association was observed for the WBC, which decreased in the experimental group. Nevertheless, the haematological parameters values obtained in the study fell within the range of normal values [27] Similar changes in the levels of the aforementioned haematological indicators were observed in calves, which received the herbal mixture in the amount of 3.5% as a concentrate supplement [28]. Packed Cell Volume (PCV) in this study was higher from S2DM for all sexes (bull and heifer) and per each sex obtained. In contrast, [29], attributed increase in PCV values in cattle to increase in environmental temperature. The PCV values obtained for heifer were comparable to those obtained for bull. This observation is not in contrast with values obtained for Red Sokoto goats in Nigeria [30] in which female animals have higher values than males. PCV in Angus was significantly lower than Sharuleh [31]. Unlike this study, [32] reported significantly higher RBC count in Brown-swiss cattle than other strains.

Sex and nutritional status of animals could cause differences in values observed for MCHC was differed significantly (P<0.05) of heifer while PLT, Hb, NEUT and MCV were differed significantly (P<0.05) of bull. A significant sex effect was also observed, with males having higher values of PCV and RBC and females have shown higher value on WBC. A significant age effect was observed for MCV and MCHC. Significant sex effect was evident with heifers having highest value on MCHC while bulls had higher MCV. A significant sex effect was observed on Hb concentration. Another study conducted by [33] revealed the influence of age and sex on haematological values of goats and sheep; age and sex had remarkable influence on the RBC counts of goats, age influenced the Hb and PCV values, age and sex greatly influenced the MCV and age influenced MCHC. Age and sex influenced neutrophil and eosinophil counts in sheep. Sex influenced the RBC values of sheep. Sex significantly (P<0.05) influenced the total WBC and monocyte counts (which was higher in bulls and heifers).

Table

**Table 1:** Chemical composition of experimental diet.

Feed materials	Percentage
Millet stalk	35
Cowpea haulms	13
Faidhebia albida pods	10
Wheat bran	15
Cotton seed cake	15
Common salt	1
Urea	1
Phosphorus	5
Calcium	5
Total	100

Constituents	Mean $\pm$ SD of nutritive values
DM(%)	91.09 $\pm$ 0.59
Ash(%)	9.18 $\pm$ 0.25
CP(%)	22.11 $\pm$ 1.22
CF(%)	45.64 $\pm$ 0.54
EE(%)	9.85 $\pm$ 0.35
NFE(%)	13.24 $\pm$ 2.37
ME (kcal/kg DM)	2086.57 $\pm$ 10.16
ADF(%)	40.65 $\pm$ 0.35
NDF(%)	50.28 $\pm$ 0.55
Ca (mg/kg)	148.53 $\pm$ 5.35
TP (mg/kg)	45.86 $\pm$ 1.36

**Abbreviations:** DM: Dry matter; CP: Crude protein; CF: Crude fiber; EE: Ether extract; NFE: Nitrogen free extract; ADF: Acid detergent fiber; NDF : Nitrogen detergent fiber; ME: Metabolizable energy; Ca: Calcium; TP: Total Phosphorus; SD: Standard deviation.

**Table 2:** Effect of time frame of feed supplementation on haematological profile of Azawak cattle (bull and heifer).

Haematological Indices	Treatment						Pr>F
	NS	SMD	SED	SMED	S2DM	S2DE	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
RBC (x10 <sup>12</sup> /L)	5.37±0.79	5.78±0.30	5.59±0.67	5.59±0.66	6.00±0.52	5.72±0.57	0.422ns
PCV (%)	23.44±4.26	25.57±1.87	24.18±3.73	24.40±3.53	26.32±3.78	25.50±3.12	0.348ns
PLT (x10 <sup>9</sup> /L)	877.78±460.96	850.17±447.72	948.33±424.09	928.17±262.95	736.00±491.03	702.50±449.91	0.457ns
WBC (x10 <sup>9</sup> /L)	9.54±1.89	8.83±1.67	10.07±2.73	9.58±1.80	9.24±2.26	8.93±1.82	0.824ns
Hb (g/dl)	10.00±0.65	10.25±0.81	10.05±1.04	9.92±1.28	10.66±0.57	10.02±0.99	0.309ns
LYMP (%)	62.22±4.50	57.28±7.97	55.14±5.85	51.68±12.78	59.70±6.12	57.67±5.78	0.015*
NEUT (%)	26.42±2.63	30.20±7.24	32.12±6.26	36.42±11.13	29.48±4.27	29.43±4.82	0.004*
MID (%)	11.36±1.88	12.52±2.32	12.75±2.35	11.90±2.25	10.82±2.81	12.90±1.05	0.518ns
MCHC (g/dl)	4.37±0.75	4.02±0.29	4.19±0.30	4.07±0.18	4.09±0.35	3.94±0.15	0.050*
MCH (pg)	18.89±2.28	17.71±0.80	18.02±0.68	17.74±0.66	17.82±0.81	17.53±0.53	0.172ns
MCV (fl)	43.47±2.36	44.19±1.80	43.14±1.94	43.58±1.65	43.74±2.99	44.55±1.37	0.385ns

**Abbreviations:** NS: No Supplementation; SMD: Supplementation early in the morning (8:00am) daily before moving out for grazing; SED : Supplementation in the evening (5:00pm) daily after the grazing; SMED: Supplementation in the morning (8:00am) and in the evening (5:00pm) daily; S2DM: Supplementation once every two days in the morning (8:00am) before moving out for grazing; S2DE: Supplementation once every two days in the evening (5:00pm); PCV: Packed cell volume, RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; WBC: White Blood Cell; LYM: Lymphocyte; PLT: Platelets; NEUT: Neutrophil; MID: include–Eosinophil, Monocyte and Basophil; SD: Standard deviation

**Table 3:** Effect of time frame of feed supplementation on haematological profile of bulls.

Haematological Indices	Treatment						Pr>F
	NS	SMD	SED	SMED	S2DM	S2DE	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
RBC (10 <sup>12</sup> /L)	5.72±0.24	5.57±0.26	5.47±0.67	5.27±0.78	5.69±0.16	5.64±0.72	0.078ns
PCV (%)	24.95±0.07	24.83±1.54	23.53±3.56	22.77±3.67	23.60±0.57	24.80±3.38	0.085ns
PLT (10 <sup>9</sup> /L)	1044.00±12.73	800.33±405.08	1064.67±158.02	979.00±160.12	1071.00±29.70	872.00±558.85	0.021ns
WBC (10 <sup>9</sup> /L)	8.85±1.77	7.47±0.70	8.57±2.48	10.63±1.57	9.70±0.71	10.07±1.17	0.095ns
Hb (g/dl)	10.40±0.14	9.63±0.57	10.13±1.24	9.27±1.33	10.30±0.14	9.93±0.93	0.035*
LYMP (%)	62.45±6.86	56.33±9.00	56.87±4.91	51.43±12.50	63.45±7.85	62.53±1.74	0.057ns
NEUT (%)	26.35±3.89	30.47±9.30	30.30±7.50	35.60±10.86	27.25±6.43	25.37±1.31	0.047*
MID (%)	11.20±2.97	13.20±1.97	12.93±2.77	12.97±1.86	9.30±1.41	12.10±0.78	0.390ns
MCHC (g/dl)	4.17±0.04	3.88±0.16	4.32±0.24	4.08±0.07	4.37±0.05	4.02±0.17	0.075ns
MCH (pg)	18.21±1.01	17.31±0.84	18.53±0.08	17.61±0.43	18.11±0.25	17.67±0.56	0.085ns
MCV (fl)	43.66±1.96	44.59±0.87	42.98±2.31	43.12±1.21	41.48±0.14	43.97±0.48	0.024*

**Abbreviations:** NS: No Supplementation; SMD: Supplementation early in the morning (8:00am) daily before moving out for grazing; SED : Supplementation in the evening (5:00pm) daily after the grazing; SMED: Supplementation in the morning (8:00am) and in the evening (5:00pm) daily; S2DM: Supplementation once every two days in the morning (8:00am) before moving out for grazing; S2DE: Supplementation once every two days in the evening (5:00pm); PCV: Packed cell volume, RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; WBC: White Blood Cell; LYM: Lymphocyte; PLT: Platelets; NEUT: Neutrophil; MID: include–Eosinophil, Monocyte and Basophil; SD: Standard deviation



**Table 4:** Effect of time frame of feed supplementation on haematological profile of heifers.

Haematological Indices	Treatment						Pr>F
	NS	SMD	SED	SMED	S2DM	S2DE	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
RBC (10 <sup>12</sup> /L)	5.13±1.00	6.00±0.14	5.71±0.81	5.91±0.42	6.20±0.62	5.79±0.53	0.148ns
PCV (%)	22.43±5.70	26.30±2.18	24.83±4.56	26.03±3.10	28.13±4.01	26.20±3.39	0.440ns
PLT (10 <sup>9</sup> /L)	766.97±615.50	900.00±574.09	832.00±619.72	877.33±373.45	512.67±542.90	533.00±327.93	0.544ns
WBC (10 <sup>9</sup> /L)	10.00±2.19	10.20±0.95	11.57±2.40	8.53±1.54	8.93±3.10	7.80±1.73	0.655ns
Hb (g/dl)	9.73±0.75	10.87±0.42	9.97±1.07	10.57±1.03	10.90±0.66	10.10±1.25	0.623ns
LYMP (%)	62.07±4.11	58.23±8.67	53.41±7.25	51.93±15.88	57.20±4.54	52.80±3.06	0.069ns
NEUT (%)	26.47±2.51	29.93±6.66	33.93±5.64	37.23±13.77	30.97±2.73	33.50±2.61	0.048*
MID (%)	11.47±1.63	11.83±2.85	12.57±2.45	10.83±2.40	11.83±3.30	13.70±0.50	0.291ns
MCHC (g/dl)	4.51±1.03	4.15±0.35	4.05±0.33	4.07±0.27	3.91±0.36	3.86±0.11	0.026ns
MCH (pg)	19.35±3.01	18.12±0.65	17.51±0.62	17.88±0.92	17.63±1.07	17.40±0.58	0.075ns
MCV (fl)	43.34±3.02	43.80±2.62	43.31±2.00	43.97±2.21	45.25±3.05	45.13±1.85	0.750ns

**Abbreviations:** NS: No Supplementation; SMD: Supplementation early in the morning (8:00am) daily before moving out for grazing; SED : Supplementation in the evening (5:00pm) daily after the grazing; SMED: Supplementation in the morning (8:00am) and in the evening (5:00pm) daily; S2DM: Supplementation once every two days in the morning (8:00am) before moving out for grazing; S2DE: Supplementation once every two days in the evening (5:00pm); PCV: Packed cell volume, RBC: Red blood cell; Hb: Hemoglobin; MCV: Mean cell volume; MCH: Mean cell hemoglobin; MCHC: Mean cell hemoglobin concentration; WBC: White Blood Cell; LYM: Lymphocyte; PLT: Platelets; NEUT: Neutrophil; MID: include—Eosinophil, Monocyte and Basophil; SD: Standard deviation

## Conclusion

In haematological profile level, higher values for leucocytes (white blood cells) and lymphocytes were noticed with increased differences between the investigated categories of animals taken into study. The best time frame critical for utilization of supplement of Azawak cattle in Semi-Arid zone was obtained per treatment with increased difference between the investigated categories of sex taken into study. In hematologic profile examination, comparing obtained results and appreciation must be carried out taking reference in individuals belonging not only to the same sex but to the same line or breed.

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