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Full length Research Article

Haematological and Serum Biochemical Reference Intervals for Nigerian White Fulani Neonatal Calves

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Summary: Reference ranges for blood and serum parameters aid in diagnosing diseases, monitoring health, and distinguishing between normal and abnormal values. There is a lack of available information and research data establishing reference ranges for haematological and serum biochemical parameters in newborn White Fulani calves. We aim to establish reference intervals for haematology and serum biochemistry analytes in apparently healthy White Fulani neonatal calves. A cross-sectional study was conducted on 30 White Fulani neonate calves under 28 days old from different farms in Ibadan, Nigeria. Blood samples were collected for haematology and serum chemistry. The haematological analysis involved packed cell volume, haemoglobin, red and white blood cell counts, differential leukocyte counts, and platelet counts using standard methods. Serum was analysed for proteins, enzymes, metabolites, electrolytes and lipid profiles using spectrophotometric techniques. Normally distributed data was analysed using 2.5th-97.5th percentiles as 95% reference intervals, with 90% confidence intervals per IFCC recommendations, using SPSS software. Haematological intervals included packed cell volume (30.11-32.29%), haemoglobin (9.26-10.04 g/dL), and white blood cell count (4.61-5.18 x 109/L) among others. Key serum biochemistry intervals were total protein (5.61-6.50 g/dL), glucose (67.12-76.78 mg/dL), cholesterol (49.98-60.52 mg/dL), creatinine (0.52-0.61 mg/dL), and electrolytes like sodium (122.25-143.95 mmol/L). The study establishes haematological and serum biochemical reference intervals for White Fulani neonate calves, suggesting their use for future research and comparisons.

Keywords*: Newborn calves, haematology, serum biochemistry, White Fulani, reference interval*

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INTRODUCTION

Establishing reference intervals for haematological and serum biochemical parameters in healthy newborn calves is important for clinicians to accurately diagnose and monitor disease conditions, and to determine whether any alterations are due to physiological changes or pathological processes and in the determination of animal welfare. Laboratory diagnoses are often made by comparing an animal's values to reference intervals from clinically healthy animals (Mohri *et al*., 2007; Marcato *et al*., 2020).

If performed properly, laboratory testing and interpretation of laboratory data have the potential to offer valuable insights into diseases and their treatment (Thrall, 2004). Specific reference intervals are required for haematological and serum biochemical test results to be accurately interpreted for each animal species. In some cases, a distinct reference value may be needed for an analyte from a particular age or breed within a species. Many analyte values fluctuate with the age of the animal, with significant changes often occurring before puberty. Therefore, some analytes necessitate separate reference intervals for different age groups (Meyer and Harvey, 2004) to account for developmental variations. Establishing species-specific and age-adjusted reference ranges allows for meaningful comparison of individual animal results in the context of normal physiological ranges for that parameter.

Alterations in haematological and serum biochemical parameters have been associated with various neonatal diseases in calves. Common disorders presenting with haematological changes include neonatal isoerythrolysis, diarrhoea, pneumonia and sepsis. Elevations in liver enzymes indicate hepatic disorders while abnormal kidney function biomarkers point to renal issues. Metabolic abnormalities are reflected by perturbations in serum electrolyte, protein and enzyme levels. Serial monitoring aids in disease diagnosis, progression and response to treatment.

These parameters are important for clinical evaluation and in identifying animal diseases. Diseases of the newborn and neonatal mortality are major causes of economic loss in livestock production, as newborn calves are highly susceptible to infectious diseases and metabolic disorders in their first few weeks of life (Windeyer *et al*., 2014) due to an immature immune system. Thus, the knowledge of

specific haematological and serum biochemical reference ranges can underscore the need to improve and promote the ability of clinicians to accurately interpret clinical pathology data and diagnose neonatal diseases.

Factors such as species, breed, age, rearing systems, feeding, and number of parturitions influence serum biochemical values; thus, the identification of these factors and their interactions is crucial for the correct interpretation of the blood parameters (Klinkon and Jezek, 2012). This study focuses on the White Fulani breeds of cattle in their first three weeks of life. White Fulani is the most numerous and widely accepted cattle breed in Nigeria (Mbap and Bawa, 2001). It is an indigenous breed derived from Bos indicus, sourced through the derived Guinea savannah, Sahel and subarid climatic zones of northern Nigeria and border countries such as Niger, Chad and Mali (Jeremiah and Banwo, 2019). The breed is highly prized for its adaptability to tropical environments, endurance, and milk production, making it a crucial component of the livestock sector and agriculture in the West African region. There is scarce breed-specific data on White Fulani calves from the first three weeks of life, a period greatly associated with birth-related changes and colostrum intake (Pérez-Santos *et al*., 2015) in normal birth with access to colostrum in the first week of life. This helped control for potential confounding effects during the critical neonatal stage when reliable reference values were being established.

There is a dearth of data on reference intervals for haematology and serum biochemistry analytes in White Fulani neonatal calves. This study aims to establish reference intervals for haematology and serum biochemistry analytes in apparently healthy White Fulani neonatal calves. Understanding reference values is crucial for veterinarians to utilize clinical pathology effectively as a diagnostic and monitoring tool.

MATERIALS AND METHODS

Study design: A cross-sectional study, which considered data at a single point in time, was carried out.
Study animals: A total of 30 White Fulani neonate calves (6 males and 24 females) under 28 days old were sampled in the present study. The neonate calves were owned by different individuals and farms in Ibadan, Oyo State, Nigeria. Only healthy neonate calves were included in the present study while calves with known health issues or under any medical treatment were excluded.

Sample collection: A 6 ml jugular venous blood sample was obtained early in the morning before feeding using a 10 ml syringe and 21G needle by slow suction. The sample was divided into EDTA bottles (3 ml) for haematological analysis and plain bottles (3 ml) for serum chemistry analysis. Within 30 minutes, the samples were transported in an ice chest to the Clinical Pathology Laboratory, Department of Veterinary Pathology, University of Ibadan.

Laboratory analysis: The haematological indices of the samples were analysed using standard methods. The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002). The haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008). The red blood cell (RBC) and white blood cell counts were determined manually using a Neubaeur haematocytometer. Differentiation of white cells into segmented neutrophils, lymphocytes, eosinophils, and monocytes was carried out by microscopic examination of Giesma-stained thin blood smear. The platelet count (PC) was done following the Rees and Ecker direct counting method as adopted by Ihedioha and Agina (2014).

Blood samples were subjected to centrifugation at 1008 g (3,000 revolutions per minute) for 10 minutes using a table centrifuge (TDL4®, B. Bran Scientific and Instruments Co., England) to isolate the serum. The blood serum was then analysed for various parameters, including total protein and its fractions (albumin and globulin), urea, creatinine, glucose, sodium, potassium, chloride, phosphorus, calcium, and the activity of creatine kinase, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), as well as triglycerides, total cholesterol, and high-density lipoproteins (HDL). These analyses were conducted through spectrophotometry using RANDOX® laboratory reagent kits sourced from RANDOX Laboratories Ltd., Ardmore, United Kingdom, following the manufacturer's instructions. The content of low-density lipoproteins (LDL) in the blood serum was calculated based on the Friedewald equation (Friedewald *et al*., 1972).

Data analysis: Exploratory analysis revealed a normal distribution of the parameters. Mean, standard deviation (SD), and 95% reference intervals were calculated by excluding the upper and lower 2.5% of the range for each haematological and serum biochemical parameter, resulting in the 2.5th and 97.5th percentiles. Additionally, 90% confidence intervals (CI) were calculated for each reference limit to assess their precision for clinical use, following the International Federation of Clinical Chemistry's (IFCC) Approved Recommendations on the Theory of Reference Values (Solberg, 1987). The Statistical Package for Social Sciences (SPSS®, version 26) was used for the analysis.

RESULTS

Haematological reference intervals established for White Fulani neonate calves: The haematological reference interval for White Fuani neonate calves in the present study include the following: packed cell volume (30.11-32.29 %), haemoglobin (9.26-10.04 g/dL), red blood cell count (4.58-4.95 x 106/µL), mean corpuscular volume (63.80-66.72 fL), mean corpuscular haemoglobin concentration (32.32-33.03 g/dL), mean corpuscular haemoglobin (21.18-22.05 pg), white blood cell count (4.61-5.18 x 109/L), platelets (105.38-113.91 x 109/µL), lymphocyte count (2.77-3.20 x 109/L), neutrophil (1.32-1.56 x 109/L), monocyte (0.12-0.15 x 109/L), and eosinophil (0.18-0.22 x 109/L) (Table 1).

Serum biochemistry reference intervals established for White Fulani neonate calves: The serum biochemical reference interval for White Fuani neonate calves in the present study include the following: total protein (5.61-6.50 g/dL), albumin (2.51-2.94 g/dL), globulin (3.11-3.62 g/dL), aspartate transferase (168.72-186.28 IU/L), alanine

transferase (10.79-13.01 IU/L), alkaline phosphatase (170.66-199.04 IU/L), blood urea nitrogen (11.30-12.48 mg/dL), creatinine (0.52-0.61 mg/dL), total bilirubin (0.1-0.14 mg/dL), glucose (67.12-76.78 mg/dL), cholesterol (49.98-60.52 mg/dL), triglyceride (24.29-29.29 mg/dL), high-density lipoprotein (23.95-30.14 mg/dL), low-density lipoprotein (144.18-188.62 U/L), creatine kinase (151.64-195.26 U/L), sodium (122.25-143.95 mmol/L), potassium (2.44-2.79 mmol/L), chloride (93.10-96.90 mmol/L), phosphorus (2.19-2.68 mmol/L), and calcium (9.07-11.09 mmol/L) (Table 2).

DISCUSSION

The key finding of the results is the establishment of haematological and serum biochemical reference intervals for White Fulani neonate calves. The results suggest a comprehensive set of reference intervals for various haematological and serum biochemical parameters in White Fulani neonate calves.

The haemoglobin (9.26-10.04 g/dL) and red blood cell count (4.58-4.95 x 106/µL) of the neonatal calves in the present study are lower than those of the White Fulani adults reported in several studies (Ihedioha *et al*., 2017; Ewuola *et al*., 2014; Unigwe *et al*., 2022). This could be because neonatal calves are born with a lower number of red blood cells, a condition known as physiological anaemia of the newborn. This is a normal, transient phenomenon that occurs due to the rapid breakdown of foetal red blood cells after birth and the slower rate of red blood cell production by the calf's bone marrow initially (Mohri *et al*., 2007).

Table 1:
Haematological reference intervals established for White Fulani neonate calves

Parameter	Mean reference value	SD	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit
PCV (%)	31.20	2.33	30.11-32.29	29.23-30.99	31.41-33.17
HB (g/dL)	9.26	0.84	9.26-10.04	8.94-9.58	9.73-10.36
RBC (x 10^6/µL)	4.76	0.39	4.58-4.95	4.43-4.73	4.80-5.09
MCV (fL)	65.80	3.12	63.80-66.72	62.62-64.98	65.54-67.91
MCHC (g/dL)	32.67	0.77	32.32-33.03	32.03-32.61	32.74-33.32
MCH (pg)	21.62	0.93	21.18-22.05	20.83-21.53	21.70-22.40
WBC (x 10^9/L)	4.90	0.62	4.61-5.18	4.37-4.84	4.95-5.42
Platelets (x 10^9/ µL)	109.65	91.20	105.38-113.91	101.92-108.83	110.46-113.91
Lymph (x 10^9/L)	2.99	0.45	2.77-3.20	2.60-2.94	3.03-3.37
Neut (x 10^9/L)	1.44	0.26	1.32-1.56	1.22-1.42	1.46-1.66
Mono (x 10^9/L)	0.13	0.04	0.12-0.15	0.10-0.13	0.14-0.16
Eos (x 10^9/L)	0.20	0.04	0.18-0.22	0.16-0.19	0.20-0.24

Reference interval= 2.5 and 97.5 percentiles, SD= standard deviation, CI= confidence interval, PCV= packed cell volume, HB= haemoglobin, RBC= red blood cell, MCV= mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH= Mean corpuscular haemoglobin, WBC= white blood cell, lymph= lymphocyte, neut= neutrophil, mono= monocyte, and eos= eosinophil

Table 2:
Serum biochemical reference intervals established for White Fulani neonate calves

Parameter	Mean reference value	SD	Reference interval	90% CI for lower reference limit	90% CI for upper reference limit
Total Protein (g/dL)	6.05	0.95	5.61-6.50	5.24-5.97	6.20-6.80
Albumin (g/dL)	2.73	0.46	2.51-2.94	2.34-2.68	2.77-3.22
Globulin (g/dL)	3.37	0.55	3.11-3.62	2.90-3.32	3.41-3.83
AST (IU/L)	177.50	18.77	168.72-186.28	161.60-175.83	179.17-193.40
ALT (IU/L)	11.90	2.38	10.79-13.01	9.88-11.69	12.11-13.92
ALP (IU/L)	184.85	30.31	170.66-199.04	159.17-182.15	187.44-210.53
BUN (mg/dL)	11.89	1.25	11.30-12.48	10.83-11.78	12.00-12.95
Creatinine (mg/dL)	0.57	0.09	0.52-0.61	0.49-0.56	0.57-0.64
Total bilirubin (mg/dL)	0.12	0.06	0.1-0.14	0.0746-0.1166	0.13-0.17
Glucose (mg/dL)	71.95	10.32	67.12-76.78	63.21-71.03	72.87-80.69
Cholesterol (mg/dL)	55.25	11.27	49.98-60.52	45.71-54.25	56.25-64.79
Triglyceride (mg/dL)	26.70	5.14	24.29-29.29	22.34-26.24	27.16-31.05
HDL (mg/dL)	27.05	6.61	23.95-30.14	21.45-26.16	27.64-32.65
LDH (u/L)	166.40	47.48	144.18-188.62	126.18-162.17	170.63-206.62
LDL (mg/dL)	45.07	12.49	39.22-50.92	34.49-35.95	46.19-55.65
Creatine kinase (u/L)	173.45	46.60	151.64-195.26	133.98-169.30	177.60-204.92
Sodium (mmol/L)	133.10	23.19	122.25-143.95	113.46-131.04	135.16-152.74
Potassium (mmol/L)	2.61	0.37	2.44-2.79	2.29-2.58	2.64-2.93
Chloride (mmol/L)	95.00	4.05	93.10-96.90	91.10-94.64	95.36-98.44
Phosphorus (mmol/L)	2.44	0.53	2.19-2.68	2.14-2.23	2.64-2.73
Calcium (mmol/L)	10.08	2.16	9.07-11.09	8.25-9.89	10.27-11.91

Reference interval= 2.5 and 97.5 percentiles, SD= standard deviation, CI= confidence interval, AST= aspartate transaminase, ALT= alanine transaminase, ALP= alkaline phosphatase, BUN= blood urea nitrogen, HDL= high density lipoprotein, LDH= lactate dehydrogenase, LDL= low density lipoprotein

The mean corpuscular volume (MCV) (63.80-66.72 fL) of the neonatal calves in the present study is higher than the MCV of White Fulani adults reported in several studies (Ihedioha *et al*., 2017; Ewuola *et al*., 2014; Unigwe *et al*., 2022). Since neonatal calves are born with a lower number of red blood cells compared to adult cattle (Mohri *et al*., 2007), the body compensates for this physiological anaemia by producing larger red blood cells, leading to an increased MCV (Mohri *et al*., 2007). However, the mean corpuscular haemoglobin concentration (MCHC) (32.32-33.03 g/dL) of the neonatal calves is lower than the MCHC of White Fulani adults reported in several studies (Ihedioha *et al*., 2017; Ewuola *et al*., 2014; Unigwe *et al*., 2022). This can be attributed to the fact that neonatal calves are born with lower haemoglobin levels due to physiological anaemia, which can lead to a lower MCHC value (Brun-Hansen *et al*., 2006). The white blood cell count (4.61-5.18 x 109/L), lymphocyte count (2.77-3.20 x 109/L), and neutrophil count (1.32-1.56 x 109/L) in the present study are lower than those of adult White Fulani cattle reported by Ihedioha *et al*., (2017). This could be because neonatal calves are born with an immature immune system, which results in lower production of white blood cells, including lymphocytes and neutrophils. The immune system develops gradually during the first few weeks and months of life (Barrington and Parish, 2001; Chase *et al*., 2008).

Our study shows that the total protein level in the White Fulani neonate calves is in the same range as adult White Fulani cattle in studies carried out by Ihedioha *et al*., (2017) and Ewuola *et al*., (2014). However, studies (Knowles *et al*., 2000; Brun-Hansen *et al*., 2006; Mohri *et al*., 2007) have shown that neonatal calves have lower total protein than adult cattle. Our result, being different from other studies, might be due to the absorption of colostral immunoglobulins, which contribute significantly to the total protein content, and can result in neonatal calves having similar or even higher total protein levels compared to adult cattle. This mechanism of immunoglobulin transfer from the mother to the calf through colostrum can help explain why some cattle breeds like White Fulani calves may exhibit comparable total protein levels in both neonatal calves and adult cattle, despite their different physiological stages and protein requirements (Quigley and Drewry, 1998).

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the White Fulani neonates in this study are higher than AST and ALT levels in adult White Fulani cattle reported by Ewuola *et al*., (2014). This is in agreement with several studies where they compared the levels of these liver enzymes between neonatal calves and adult cattle. The general consensus is that neonatal calves tend to have higher levels of AST and ALT compared to adult cattle (Knowles *et al*., 2000; Mohri *et al*., 2007).

The blood urea nitrogen and creatinine levels in the White Fulani neonates in this study are lower than the blood urea nitrogen and creatinine values in adult White Fulani cattle reported by Ewuola *et al*. (2014). The lower blood urea nitrogen and creatinine levels in neonatal calves are attributed to several factors: (1) immature renal function and lower glomerular filtration rate in neonatal calves; (2) lower muscle mass and lower protein turnover in neonatal calves, resulting in lower creatinine production; and (3) lower dietary protein intake and lower urea production from protein metabolism in neonatal calves. Also, several studies (Knowles *et al*., 2000; Brun-Hansen *et al*., 2006; Mohri *et al*., 2007) have shown that blood urea nitrogen and creatinine levels are lower in neonatal calves compared to adult cattle.

The cholesterol level in the neonate calves in the present study is lower when compared with a study on adult White Fulani cattle. In agreement with this, several other studies have shown lower cholesterol levels in neonate calves when compared to adult cattle (Knowles *et al*., 2000; Brun-Hansen *et al*., 2006; Mohri *et al*., 2007). The lower cholesterol levels in neonatal calves are thought to be due to several factors, including immature lipid metabolism and cholesterol synthesis pathways in newborn calves, lower dietary intake and absorption of cholesterol in neonatal calves as they are primarily consuming milk which is low in cholesterol, and lower cholesterol requirements for growth and development in neonatal calves compared to adult cattle (Rauprich *et al*., 2000).

Abnormal values outside these reference intervals in White Fulani neonate calves may signal potential health issues, and continuous monitoring of these parameters can aid in the early detection of diseases or nutritional imbalances. Veterinarians can utilize these reference intervals as a diagnostic tool to assess the health status of individual calves and the overall herd, while researchers can use the data as a foundational basis for further studies on the health and physiology of White Fulani neonate calves.

A potential limitation of this study is the modest sample size of 30 White Fulani neonate calves included in the cross-sectional study. This limited sample size may not fully represent the diversity within the population, and the findings might not be entirely reflective of the broader range of health conditions or variations in this specific calf population. The sample size could potentially limit the external validity and applicability of the established reference intervals to a larger population of White Fulani neonate calves. Further research with an expanded sample size could help validate and refine the reference intervals established here, strengthening their application and relevance to the broader population.

In conclusion, the study has successfully established comprehensive haematological and serum biochemical reference intervals for White Fulani neonate calves. We recommend that researchers use these reference intervals as a foundation for further studies, comparisons with other breeds, and investigations into factors influencing haematological and serum biochemical parameters in neonate calves.

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