

**STUDIES ON PRODUCTIVE TRAITS AND RELATIONSHIP AMONG
THREE GENETIC GROUPS OF CATTLE USING BLOOD BIOCHEMICAL
POLYMORPHISM**

BY

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**DEPARTMENT OF ANIMAL SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA**

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**DEPARTMENT OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA**

FEBRUARY, 2016

DECLARATION

I declare that the work in the dissertation entitled “**STUDIES ON THE PRODUCTIVE TRAITS AND RELATIONSHIP AMONG THREE GENETIC GROUPS OF CATTLE USING BLOOD BIOCHEMICAL POLYMORPHISM** has been carried out` by me in the Department of Animal Science under the Supervision of Prof. I.A. Adeyinka, Prof.B.I, Nwagu and Dr. (Mrs.). M. Orunmuyi.

All the pieces of information derived from literature have been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any University.

Bunjah, Umar Danladi Shuaib

NAME OF STUDENT

SIGNATURE

DATE

CERTIFICATION

This dissertation entitled **Studies on productive traits and relationship among three genetic groups of cattle using blood biochemical polymorphism** by **BUNJAH, UMAR. DANLADI SHUAIB**, meets the regulation governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, Nigeria and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty Allah, the maker of Heaven and Earth, My Wife Rahinat and our children Aminat, Halimat, Asma'u, Fatima, Shuaib and Umar Umar whose help on this work I gratefully acknowledged. Also to my late daughter Safiya Umar Bunjah

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Abstract

This study was undertaken to evaluate some selected productive traits and relationship between three genetic groups of cattle (Bunaji, Friesian X Bunaji cross and Sokoto Gudali) using blood biochemical polymorphism. A total of 150 cows consisting of 50 per genetic group was randomly sampled from the (NAPRI) cattle herd and used for the study. The selected productive traits evaluated include body weight (BW) in Kg, body length in cm (BL) height at withers (HW) in cm, chest width (cw), heart girth (HG), rump width ((Rumw) teat length in cm (TL), rear udder height (RUH) udder circumference (UC), total milk yield (TY), average daily milk yield (ADY) and lactation length in days (LL). Blood biochemical parameters, namely Haemoglobin, Transferrin and carbonic anhydrase were evaluated for genotypic and allelic frequencies of these blood protein polymorphism and their impacts on productive traits, correlated analysis of the measured trait, principal component analysis of the variables, stepwise linear regression and multivariate analysis involving discriminant genetic distance and classification components between breeds were all computed. Results showed that BW and other measured traits differed significantly ($P < 0.005$) among the genetic groups BW and BL were higher in the Friesian X Bunaji than the Bunaji and Sokoto Gudali which showed no significant ($P > 0.05$) difference for these traits. The haemoglobin locus revealed overall allelic frequencies of 0.44 and 0.13 for HbAA and HbBB respectively. Low frequencies of alleles (0.20, 0.20 and 0.40) were observed in the Bunaji, Friesian X Bunaji and the Sokoto Gudali respectively. In the transferrin locus, only the A and B alleles were observed with overall frequencies of 0.16 and 0.09 respectively. Low genotypic frequency of 0.04 for AA was obtained in the Sokoto Gudali while the Bunaji and the Friesian X Bunaji recorded frequencies of 0.30 and 0.12 respectively. In the carbonic anhydrase locus, only the F and S alleles were observed with the overall frequencies of 0.17 and 0.23 respectively. Both the Transferrin and Carbonic Anhydrase locus were not in Hardy-Weinberg equilibrium for the studied population. Significant ($p < 0.05$) differences were obtained in all body and milk production traits measurements of the three studied population indicating clear genetic group distinction. It was noted that the Sokoto Gudali was superior to the Bunaji in most of these traits though the Sokoto Gudali is a relatively poor milker than the Bunaji. Study of blood protein polymorphism and productivity indicated significant ($p < 0.05$) influence of the haemoglobin, transferrin and carbonic anhydrase on both body and milk

production traits. Correlated studies were observed to be very significant between variables in the pooled analysis by majorly insignificant for individual genetic groups, also estimates were generally low though ranging from 0.87 – 0.84. Principal component analysis observed to show factors ranging from 3 in the pooled data to 5 in the Bunaji and 6 in the Friesian X Bunaji and Sokoto Gudali genetic groups. Generally communalities ranged from 0.31 – 0.99 while proportion of variance accounted for by factors were 47% in the pooled and Bunaji and 58% in the cross breed and Sokoto Gudali. Multivariate analysis indicated TY, CW, ADY, RUH, LL, Rumwi, UC, TL and HG as the most discriminating variable among the genetic groups. Their respective partial R^2 and F values were (0.84, 0.58, 0.22, 0.15, 0.13, 0.09, 0.08, 0.15 and 0.04) and (2278.64, 613.22, 123.85, 81.12, 65.12, 43.02, 37.10, 79.68 and 16.52) with high significant value of ($p < 0.0001$). Genetic distance among the genetic groups revealed high Mahalanobis value (3.94 – 4.95) between the Bunaji and Sokoto Gudali and Friesian X Bunaji and Sokoto Gudali. The Highest proper classification (84%) was in the Sokoto Gudali and it indicated greater genetic group homogeneity. However, multivariate studies clearly indicated manifestation of gene introgression across genetic groups through indiscriminate cross breeding. Perhaps as evidenced by the high levels of misclassification in the Bunaji, Friesian X Bunaji and Sokoto Gudali. There is a need for generic study using protein and DNA microsatellite markers to compliment the results arisen from morphometric differentiation of the two most populous Nigerian breeds of cattle in the NAPRI herd.

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CHAPTER ONE

1.0 INTRODUCTION

Genetic characterization of indigenous breeds and their biochemical traits form one important component in a strategy to expand food production. Several studies have been published on various livestock species in Nigeria (Das and Deb, 2008). The indigenous breeds have received less attention due to low performance in productivity which has shifted the interest of the breeders towards temperate cattle breeds to upgrade their local genetic resources. It is generally accepted that the highest amount of genetic diversity in the populations of livestock is found in the developing world where record keeping is poor, and the risk of extinction is high and on the increase. Recently, loss of genetic diversity within indigenous livestock breeds has been a major concern (Kastelic *et al.*, 2005).

Most genetic research is now directed towards the investigation of the relationship between physiological, biochemical and metabolic products/markers to the productive efficiency of farm animals. Biochemical traits, including blood groups, blood proteins and enzymes have been studied with a view to explaining the physiological basis of performance traits. The classical approach to the breeding of superior animals is based on phenotypic variations observed among and between related groups of individuals, but these are only partly due to genetic variations and partly due to environmental influences. There may be however a great variety of genetic variations of a completely different nature that may reflect more accurately the genetic differences and productive efficiency between individuals (Desmarais and Pare, 1974). Polymorphism of blood protein first offered the possibility to study genetic differentiation before the advent of molecular markers. Consequently several livestock breeds including the domestic cattle, sheep and goat have been characterized for variations in major blood proteins (Di Stasio,

1997). In addition to several important functions of blood proteins, several studies in cattle and sheep have already linked these markers to production traits and environmental adaptation (Vicovan and Rascu, 1989; Charon *et al.*, 1996, Akpa *et al.*, 2010).

Blood polymorphism studies have been conducted extensively to identify biodiversity among livestock. Biochemical particles of blood can be determined easily at the post-natal period of young animals, and these components are merely or not affected by the environmental factors. Many research works have been conducted to detect the different types of blood components such as haemoglobin, albumin, glutathione, transferrin and potassium (Kuwar *et al.*, 2000).

Rako *et al.* (1964) observed that higher productivity may be as result of special traits arising from certain biochemical processes in the organism and suggested the introduction of selection tests based on these biochemical traits. Existence of any significant relationship between activities of enzymes and other biochemical features with performance traits may help to identify a selection criterion which can be used in early life and minimize later recording of performance traits (Singh *et al.*, 1983).

The existence of blood potassium and glutathione polymorphisms in cattle (Evans and Phillipson, 1957; Gonzales *et al.*, 1984), sheep (Gurcan *et al.*, 2010) and goats (Soysal and Ulku, 1998) have been established in some studies. Also several studies were carried out to establish the association between potassium and glutathione polymorphism of blood and various traits (Alpan and Ertugrul, 1991). If an association exists, then the erythrocyte potassium and glutathione types in blood can be utilized as a polymorphic marker among domestic animals (Lush, 1977). Even if some studies did not find any significant relationship with production traits (Soysal, 1983), a few studies displayed an important association between glutathione types and milk production traits in Finn sheep (Atroshi and Soudholm, 1982) and between the potassium

and sodium types and some economical yield traits in livestock (Antunovic *et al.*, 2004; Milewski and Szczepanski, 2006).

Haemoglobin variants have been extensively studied in Zebu cattle and at least eight variants have been identified. Four migration bands were found, Hba, Hbl, Hbc and HbB, but the last band (HbB) may be possibly broken into two, named; HbB1 and HbB2. The respective gene frequencies were 0.563 ± 0.012 , 0.007 ± 0.01 , 0.021 ± 0.002 , 0.188 ± 0.007 and 0.221 ± 0.007 . The genetic frequencies were in equilibrium (Mario, *et al.*, 1982). The existence of two types of haemoglobin; Hb and HB has been established (Huisman, *et al.*, 1959). They are expressed as homozygous Hb^{AA} and Hb^{BB} and phenotypes with Hb^{AC} being a pre-adult form of Hb (Johnson *et al.*, 2002). Discrete differences in oxygen affinity of haemoglobin type A and B could also be established in cattle (Huisman, 1959).

1.1 Justification of the Study

Knowledge of the type of biochemical polymorphism and their association with productive and reproductive performances in animals have been successfully utilized in selecting superior performers in recent time (Guney *et al.*, 2003 and Das *et al.*, 2004). However few experiments linked various body features or physical characteristics, biochemical polymorphism and production traits. Some studies are available on some breeds of cattle from East Africa and Brazil on blood biochemical polymorphism, but not much study has been carried out on the breeds of cattle in Nigeria. This therefore necessitated this work.

1.2 Objectives of the Study

- i. To determine blood biochemical activities in relation to morphology and milk traits among three genetic groups of cattle.
- ii. To determine the blood biochemical polymorphism and their gene and genotypic frequencies among three genetic groups of cattle.
- iii. To establish the existing relationship among three genetic groups of cattle using Multivariate analysis of Morphometric and milk trait measures.

1.3 Hypotheses of the Study

- i. Ho: There is no association in blood biochemical activities in relation to morphology and milk traits among three genetic groups of cattle
- ii. Ho: There is no association among the blood biochemical polymorphism and their gene and genotypic frequencies among three genetic groups of cattle
- iii. Ho: There is no relationship between analysis of Morphometric and milk trait measures among three genetic groups of cattle

CHAPTER TWO

2.0 Literature Review

2.1 Biotechnology options for Improving Livestock Production

Biotechnology is defined as any technique that uses living organisms or substance and from such organism make or modify a product to improve plants or animals or to develop microorganisms for specific purposes (McLaren *et al.*, 2001). Biotechnology is not new. Man has used it for thousands of years to manufacture products such as beer, wine and bread. Conventional plant and animal breeding which involves selection and mating of phenotypically preferred individuals is a good example of age-old application of biotechnology. What is new about biotechnology comes from more recent breakthroughs such as recombinant DNA technology and associated techniques, monoclonal antibody techniques and embryo manipulation technology. These have enhanced possibilities for manipulating biological systems for the benefit of mankind. Techniques of modern biology such as molecular cloning of genes, gene transfer, genetic manipulation of animal embryo transfer, genetic manipulation of rumen microbes, chemical and biological treatments of low quality animal feeds for improved nutritive value, genetically engineered immunodiagnostic and immunoprophylactic agents as well as vaccines are a reality today and are finding their ways into research and development programmes of developing countries (Olayemi *et al.*, 2002). Genetic improvement of livestock depends on access to genetic variation and effective methods for exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production in a range of environments (McLaren *et al.*, 2001).

In developed countries, breeding programmes are based upon performance recording and this has led to substantial improvements in animal production. Developing countries have distinct disadvantages for setting up successful breeding programmes; because infrastructure needed for performance testing is normally lacking; herd size are normally small and variability between farms, farming systems and seasons of the year are wide while reproductive efficiency is low, due mainly to poor nutrition.

2.1.1 Biochemical polymorphism

Biochemical diversity popularly called biochemical polymorphism is the occurrence of the varieties attributed to biochemical difference, which are under genetic control. A population is said to exhibit genetic polymorphism when two or more distinct inherited variables co-existed in the same population. A genetic character is known to be polymorphic when the rarest phenotype has a frequency greater than one percent.

2.1.2 Genetic control on biochemical polymorphism

Authors such as Das and Deb (2008) reported that single or more allelic pair of genes controls all types of biochemical polymorphism. It is also proved that several types of protein variations are due to the different number of amino acids present in the protein molecules and their effects in different manner. A triplet code of nucleotide of DNA and RNA is responsible for coding of a particular amino acid to participate in the formation of a protein. In this way the different triplet codes present in a particular DNA or RNA sends different codes of words to different amino acids to come in a particular arrangement to form a particular protein which is permanently or temporarily required for a particular function of the body. If the allelic pair of genes has the same nucleotide sequences in both the genes, they can send the same types of codes to the amino acids to come in contact to form the similar type of protein.

2.2 Electrophoresis

The term electrophoresis generally defines movement of charged particles through an electrolyte (buffer) when subjected to an electric current. In the case of protein the overall charge originates from the carboxyl (COOH) and amino (NH₂) groups, which can assume an electric charge according to the pH of the medium (Di Stasio, 1997). The technique has been used over the years as a tool for characterizing macromolecules and for assaying their purity. Electrophoresis methods can be used to detect polymorphisms of major animals' blood proteins and protein variants determined by co-dominant alleles. Dally *et al.* (1980), Harris and Warren (1955) demonstrated the existence of variable haemoglobin (Hb) types in small ruminant (sheep). Evans *et al.* (1956) designated the two Hb alleles as A and B; thus the three electrophoretic genotypes are AA, AB, and BB. Akinyemi and Salako (2010) observed that Hb AA had higher oxygen affinity than Hb BB. Evans *et al.* (1956) identified the HbAA and HbBB as single bands with HbAA being the fastest single band towards the anode during electrophoresis while HbBB was the slowest. The HbAB, however, was identified as a double band having both the fast moving AA and slow moving BB. They also discovered a fourth, rare haemoglobin type; HbAC which is mostly found in young (pre-adult) animals before maturity. This HbAC was referred to as the 'foetal haemoglobin'. Das *et al.* (2004) and Salako *et al.* (2007) also reported similar haemoglobin types, but occurrences for each haemoglobin type vary from location to location. Johnson *et al.* (2002) recorded frequencies for HbAA and HbAB but had no records of the homozygous HbBB or the pre-adult.

2.2.1 Protein electrophoresis

Protein polymorphisms, although still widely used in population studies, are of limited value in the assessment of genetic variation at the level of cattle breeds. This is largely because of the

relatively low levels of polymorphism found in protein loci, resulting in a lower taxonomic limit to the resolving power of protein electrophoresis.

2.2.2 Cellulose acetate electrophoresis

In 1950 Joachim Kohn took a position in the pathology service at Queen Mary Hospital in Roehampton London, in that same year. Durrum introduced filter paper as a solid support for electrophoresis of serum proteins (Durrum, 1950). At a Central Institute for Brackish Aquaculture (CIBA) foundation symposium on paper electrophoresis in July 1955, Durrum reviewed the limitation of filter paper and said that “in the future, probably we will have a better supporting medium (Durrum, 1955). Seventeen months later, November 1956, at a Biochemical Society meeting, Kohn presented a new support medium that solved many of the problems of filter paper. He described for the first time the use of a cellulose acetate (CA) microbiology filter as a solid support for electrophoresis (Kohn, 1957).

Unlike filter paper, protein bands on Cellulose Acetate Membranes were sharply separated, and the membranes could be made transparent with a simple clearing agent. Years later, he wrote that the idea for the use of CA membranes came to him from a remark made by a laboratory sales person that standard CA microbiology filters could be made transparent for use with light microscopy (Kohn, 1982). Kohn presented his second paper on CA electrophoresis at a protein conference in Bruges Belgium in May 1957 (Kohn, 1957). The stained and dried CA membranes were made transparent with immersion oil, sandwiched between two glass slides and scanned on a densitometer. After the Bruges conference Kohn submitted his first full report on CA electrophoresis to the *Journal Clinica Chimica Acta*. This land mark paper was published in August 1957 issue (Kohn, 1957) and described a method in which CA strips 20cm long by 5cm wide were run in a standard paper electrophoresis chamber. Five protein fractions plus a pre

albumin (Transthyretin) band were separated in half the time required for paper media. The strips were stained with Nigrosin (acid black2) dried and made transparent with microscope immersion oil that had a refractive index of 1.474. Protein bands were measured by either elution or optical densitometry of the cleared membrane. Another advantage of cellulose acetate membranes over paper was that for elution, cellulose acetate (CA) membranes could be completely dissolved in a mixture of ethanol and chloroform.

In 1958, Kohn published a series of 3 papers in which he reported redesigning the paper electrophoresis chamber and reducing the size of the cellulose acetate membranes. Conventional horizontal paper electrophoresis chamber contained 2 running buffer tanks separated by an air gap over which the paper strip was mounted. In Kohn's new design, the 2 buffer chambers were brought together in the middle with no air gap, allowing shorter strips to be used and reducing convection currents by eliminating the air chamber under the mounted solid support media, cellulose acetate strips 12cm long and 2.5cm wide were used with the new chamber. Separation time for serum protein was reduced from 14hr on paper to 2hr with cellulose acetate membranes in the new chamber.

Serum samples of 1.0NL stained with poncean stain or Nigrosin were adequate for qualitative densitometry or elution. The shadon scientific company later commercialized his new chamber for use with cellulose acetate membrane. Enthusiastic and positive reports on Kohn's new electrophoresis technique began to appear in the literature in 1959. In July, Smith and muchison reported their results with cellulose acetate membranes for the electrophoresis of 400 serum samples over 11 month period. Albert – Rech stained proteins with lisamine green and made a careful study of quantitative densitometry, compared protein recovery between paper and cellulose acetate membranes. Kohn expanded the capability of cellulose acetate electrophoresis

with the development of a concentration procedure for body fluids that contained low protein concentrations.

In 1960, Kohn wrote a 34 page chapter in the book *Chromatographic and Electrophoretic technique: vol II, Zone electrophoresis*, his chapter was the first procedure manual on the technique of cellulose acetate electrophoresis for serum proteins. Kohn described the low protein binding nature of cellulose acetate membranes and their ability to be made transparent with immersion oils, with a refractive index of 1.474. Cleared cellulose acetate membranes were suitable for optical densitometry between 250 and 1000nm. Typical results were achieved with as little as 0.1ul of serum with a separation time of 1.5hr. Protein bands were well separated with little to no tailing. The X, Y band was well separated from the much larger albumin band. Ponceau stain and Nigrosin protein staining techniques were presented.

He also designed a multi-position stamp applicator that dispersed 8 different serum samples onto a single cellulose acetate membrane in 1968, Kohn expanded his chapter on cellulose acetate electrophoresis for Ivor Smith's book (Kohn, 1968). Kohn described procedures for the separation of serum and spinal fluid proteins, haemoglobin, haptoglobin and methods of the isozymes of lactic dehydrogenase and leucine aminopeptidase.

2.2.3 Sodium dodecyl sulphate poly acrylamide gel electrophoresis

The 1960's was a revolutionary time for electrophoresis and protein analysis. During the Sixties, Samuel Raymond helped the scientific community by introducing poly acrylamide gel electrophoresis (PAGE). He had engineered a vertical gel slab which when submerged in a buffer solution could be hooked up to current. The model integrated the concepts of molecular sieves and molecular filtration described by Oliver Smithes. Raymod's model served as a prototype for successive protein gel apparatus (Righetti, 2004).

In 1967, Shapiro and his colleagues came up with the idea to let proteins swim in an anionic surfactant using sodium dodecyl sulphate (SDS). Later, a technique was developed to reduce disulfide linkages which help to separate both tertiary and quaternary structures (Righetti, 2004). This technique is called sodium Dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE). SDS – PAGE is run on the basis of gel filtration as the proteins are separated according only to their molecular mass. First, the great negative charge of the SDS overwhelms the charge of the protein, so that charge is not a factor. Second, SDS, as an amphiphilic molecule, disturbs the hydrophobic interactions of the proteins, forcing them to assume a rod – like shape. This eliminates shape or configuration of the protein as a factor when migrating through the gel. Today, SDS – PAGE is one of the most common electrophoretic techniques used in biochemical and molecular biological processes (Righetti, 2004).

By adding an ionic detergent to the proteins and heating the solution, SDS molecule bind to the protein and allow them to unfold. Approximately one SDS molecule bind to two amino acid residues making the whole protein extremely negative, thus charge become insignificant (Nelson and Cox, 2005). SDS – PAGE not only unfolds the proteins but the proteins subunits are unfolded as well. This gives them a nearly uniform mass to charge ratio thereby allowing proteins and subunits to be separated solely on the basis of their size. Without SDS, different proteins with similar molecular weights would travel through the gel differently due to differences in folding (Righetti, 2004). The advantage of the molecule being unfolded is that they become nearly linear and molecules of similar size travel more consistently. The polyacrylamide gel is a cross linked matrix that acts like a filter by providing resistance to the proteins trying to pass through. Smaller proteins travel faster because they can fit through the pores of the gel more

easily while larger proteins have difficulty travelling through the pores. Therefore, larger proteins travel more slowly.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis is very commonly used in biochemistry and molecular biology procedures. By studying gels produced by SDS – PAGE and comparing proteins to a molecular weight marker, researchers can determine the size of proteins. Many other characteristics can be determined by using SDS – PAGE such as identification of proteins, determination of sample purity, identification of disulfide bonds, and application in blotting procedures (Righetti, 2004). Commonly, SDS – PAGE is the first technique used to test protein purity due to its reliability and ease (Nelson and Cox, 2005). A gel that shows a single band indicates that the protein being tested is pure, since there is only one protein (band) visible, if there were other proteins. While agarose gels are superior to running larger molecules, like DNA, SDS PAGE gels are more geared towards running smaller molecules, like proteins (Righetti, 2004). By using SDS – PAGE in a 2 D – PAGE technique, valuable information can be attained for use in proteomic analysis.

During the 1990's, with the help of 2D – PAGE and the development of mass spectrometry (MS), large numbers of proteins/gene data bases were created. This allowed researchers to track and identify the genes and the proteins that they encode. The combination of using iso electric focusing and SDS – PAGE in a 2 D – page combined with MS has been incredibly advantageous for fields such as biology, medicine and bio analysis (Righetti, 2004).

. The separation gel is composed of water, acrylamide, SDS, TEMED, and Tris Hcl of pH 8.8, one important thing to note is that SDS (Sodium Dodecyl Sulphate) has highly negatively charged groups. When SDS is added to a protein sample, the sample takes on a large negative charge.

This overwhelming charge due to the SDS negates any effects of charge on the separation of the proteins in the gel. After this gel is poured and solidified, the stacking gel is added. The stacking gel consists of the same ingredients; however, the Tris HCl is at pH of 8.8. Once this gel is poured, the combs are inserted into the gel to create wells after solidification. Prior to adding the protein samples to each well, a loading dye consisting of beta – mercapto-ethanol must be added to each sample and boiled. This betamercaptoethanol reduces the disulfide bonds and allows proteins to be separated by size. The smaller sized molecules will travel further down the agarose gel than the larger molecules. Also, the molecules will be separated by their surface area. This means that uncut plasmids will travel further than cut plasmids. Gels which are composed of both separating and stacking gels are considered to be discontinuous.

An electric current applied to the gel causes the negatively charged proteins to migrate across the gel. The gel is run at two different voltages. Initially, a lower voltage is applied to the gel while the proteins are still in the stacking gel. In the stacking gel, the proteins essentially line up and form a single sharp band. When the proteins reach the border between the stacking and separating gel, the voltage is increased and the proteins begin to separate as they move through the separating gel. The smaller proteins move more quickly through the gel. After the proteins have reached the end of the gel or run for a set amount of time, the voltage is turned off. The gel is then stained with Coomassie Blue, and the unique protein bands become visible. After staining the gel with Coomassie Blue for approximately 15 minutes, the staining solution is removed. Next, a destaining solution consisting of methanol and acetic acid at a ratio of 1:3 is added to cover the gel and remove the stain. The resulting gel displays the various bands at different locations. Each band corresponds to a protein. A molecular weight marker allows the size of the bands to be established and aids in identifying the protein bands.

2.3 Blood Protein and Enzyme Types

2.3.1 The haemoglobins

Haemoglobin is the iron – protein compound in red blood cells that gives blood its red colour and transports oxygen, carbon dioxide and nitric oxide. It is present in all but the least complex of animals. Haemoglobin is contained entirely in the red blood cells, amounting to perhaps 35% of their weight.

To combine properly with oxygen, red blood cells must contain adequate haemoglobin. Haemoglobin, in turn, is dependent on iron for its formation. A deficiency of haemoglobin caused by lack of iron in the body leads to anaemia.

Haemoglobin was once described by the American Physiologist (L.J. Henderson), as the second most interesting substance in the world. It is probable that he thought of chlorophyll as occupying the premier position. However, haemoglobin is not a single substance but a group of related globins to which the same prosthetic group, heme, is attached (Lehmann and Huntsman, 1974).

Those haemoglobins that exist in nature today must represent the end result of a very long period of evolution and are superbly adapted to the specialized and varied functions that they undertake in the cells of even the lowest animal (Lehmann and Huntsman, 1974). On the evolution of haemoglobin functions, Wells (1999) writes:

(i) At an early stage in evolution, worm – like animals had large, polymeric aggregations of the subunits circulating through primitive circulatory system and some possessed monomeric Hb in blood cells functioning as an oxygen source.

(ii) The circulating vertebrate red cell provides an environment allowing heme units to interact among themselves and with various organic phosphates to allow a responsive and highly regulated system of gas transport.

During metazoan evolution the burden of physiological regulation has shifted from the cells to organic systems as endothermy and aerial breathing permit a relatively constant environment.

An understanding of the adaptive possibilities of Hb has helped us to understand the ontogeny of oxygen transport and interpret recently described functional properties of human embryonic haemoglobins. Just as it would be erroneous to regard the red cells as a fixed biconcave disc, so it would also be wrong to regard the haemoglobin molecule as an “inert table tennis ball”. The red cell expands and contracts at each respiratory cycle, because of the passage of the charged ions through the membranes, and is also to change its shape when it is squeezed on its way through the capillary bed. At the same time, the haemoglobin molecule is also changing its shape, expanding when the oxygen molecules are released and contracting when they are inserted onto the heme groups (Lehman and Huntsman, 1974).

The marvel of mammalian haemoglobin is not that it can combine with oxygen but that it can free it by donating it when physiologically convenient. The evolution of haemoglobin demonstrates the development of a potential to part with acquired oxygen; the mere combination of a chemical compound with molecular oxygen is by comparison an easy trick (Lehman and Huntsman, 1974). This chemically functions as a carrier of oxygen and to a lesser extent as a carrier of carbon – dioxide. The characteristics of haemoglobin also assist in the maintenance of acid – base homeostasis within the body fluid (Breazile, 1971).

In addition to oxygen and carbon dioxide, haemoglobin takes up and releases a third gas, nitric oxide. Nitric oxide plays an important role in regulating blood pressure by relaxing the blood vessel walls, thus increasing the blood flow. Haemoglobin controls the expansion and contraction of blood vessels, and thus blood pressure by regulating the amount of nitric oxide to which the vessels are exposed.

Haemoglobin carries more than 20 times its volume of oxygen. Some chemicals such as carbon monoxide combine so firmly with haemoglobin that it can no longer combine with oxygen and asphyxiation results. After a life of 120 days, red blood cells are destroyed in the spleen, or in course of circulation, their haemoglobin is broken into its constituents, including iron, which enters new blood cells formed in the bone marrow (Ganong, 1995).

When blood vessels rupture, as in injury, the red cells are released and escape into tissue, where they are broken down. The haemoglobin is converted into pigments, the colour of which is responsible for the appearance of bruises. Alteration in the structure of haemoglobin can lead to life threatening illnesses. The most important of these conditions is sickle – cell anaemia, which involves a hereditary change in one of the amino acids that make up haemoglobin. The thalassemys are a group of hereditary diseases of similar origin (Uthman, 2007).

Haemoglobin is a complex protein with a molecular weight of approximately 66,000 which has four polypeptide chains, to each of which a heme group is attached. The heme groups, being the functional position of the haemoglobin molecule consists of the tetrapyrrol protoporphyrin IX within an iron atom in coordinate linkage with the central nitrogen of the pyrrol ring (Breazile, 1971). Blunt and Huisman (1971) reported that haemoglobins with similar designations or even similar electrophoretic properties, do not necessarily have the same structure.

Most haemoglobins consists of 2 α and 2 non α (P and Y) globulin polypeptide chains and neither the α - chains nor the non - α chains need be identical (Lehmann and Huisman, 1974). There are at least four physiological haemoglobins in humans. Haemoglobin A comprises about 98% of adult haemoglobin.

Haemoglobin A2 is a small fraction, which is present in all individuals, but may be raised or lowered above or below the usual proportion of 2% in certain haemoglobinopathies.

Thirdly, there is Haemoglobin F which forms more than half of the haemoglobin of the new born but disappears almost completely during infancy. There is yet physiological haemoglobin which is present in very young embryos, but which disappears by the third month of gestation (Lehmann and Huntsman, 1974).

Animal haemoglobin, like that of humans have been classified based on electrophoretic mobilities as haemoglobins AA, AB, AC, AH, BB, BC, CC, CH, FA, FAB, FF, HH, in cattle (Senepati *et al.*, 1997) Haemoglobins AA, AB, DAB, DBB, N(c), F, BD, DD, Gower 1 and Gower 2 in sheep (Ndamukong, 1995); Haemoglobin AA, AB, F, BB, S, in cattle and goats (Baruah and Bhat, 1980; Sartore *et al.*, 1981., Bhat, 1986; Ndamukong, 1995).

Haemoglobin AA, AB, A/BII, BI/BII, ABI, BIBII, AIA, A₁A₂, A⁵₁ A₂, A₁ A₂^m + M -, A₁A₂^{m+m+} and A₁-----1, 1/0 and 0 in horses (Singhvi and Khanna, 1987; Silva *et al.*, 1996) and lastly evidence of haemoglobin polymorphism in the beagle dog is absent (LeCrone, 1970), but was found in erythrocytes of dogs of seven Japanese nature breeds (Tanabe *et al.*, 1978).

2.3.1.1 Types and haemoglobin factors.

In the past, some authors have discovered that HbB cattle were associated with higher erythrocyte values than HbA cattle, while leukocyte values were highest with Hb AB and

lowest with HbB (Anosa and Obi, 1980). Zhang *et al.* (1984) put forth the idea that in Tibetan sheep, Hb type BB, had significantly higher packed cell volume than that of Hb AA sheep.

Pieragostini *et al.* (1994) confirmed the differences in hematocrit and haemoglobin concentration between HbA and HbB in Leccese sheep, with HbA sheep exhibiting a higher PCV and Hb concentration followed by Hb AB, Hb BB, Hb DAB, and Hb DBB. Hb DAB and Hb DBB exhibited the highest leukocyte value, followed by Hb BB and Hb AB sheep.

2.3.1.2 Haemoglobin types and anaemia.

Braend (1964) discovered an animal having atypical haemoglobin (HbN) when investigating the Hb phenotypes of experimental sheep with the technique of starch gel electrophoresis. It has been reported that an association exist between haemoglobin switching and anaemia in sheep (Ndamukong, 1995). The Haemoglobin variant C (N) has been produced experimentally by heavy bleeding in sheep of haemoglobin Type A and AB, but not in B individuals (Tucker *et al.*, 1983). However, Ndamukong (1995) have reported Hb CC in sheep with phenotype BB. This type of haemoglobin is distinguishable from haemoglobin A and B by the lower electrophoretic mobility at alkaline pH and by characteristic motilities in cation and anion exchange chromatography (Moore *et al.*, 1966).

The appearance of haemoglobin C in sheep having previous phenotypes AA or AB haemoglobin types occurred 5 – 7 days after severe haemolysis and a relationship exist between the severity of anaemia and the amount of Hb C produced (kitchen *et al.*, 1968). Thurmon *et al.* (1970) compared the degree of A - C haemoglobin switching following the administration of a human urine erythroprotein preparation to a non – anaemic sheep, in the same animal, with the switching obtained with anemia sheep plasma containing known amounts of erythroprotein. A

strong correlation emerged between the dosage of erythroprotein given and the degree of A - C switching observed (Thurmon *et al.*, 1970)

Bell and Huisman (1968) observed in an anaemic cow that a new haemoglobin variant, replacing either HbA or HbB was not produced during severe blood loss anaemia; and that any new variant produced during severe anaemia in the cow must have the same chromatographic and electrophoretic motilities as the haemoglobin which is replaced. It was also concluded that significant variation did not occur in the relative production of HbA and HbB during anaemia.

2.3.1.3 Haemoglobin types and disease resistance.

Hodges *et al.* (1976) put forth the idea that sheep possessing type AB haemoglobin exhibited more marked effects following inoculation with *Leptospira* Serotype *Pomona* than those with haemoglobin types A or B. Jilek and Bradley (1969) stated that ewes with HbA may be more resistant to infection with *Haemonchus contortus* than ewes with HbB in Florida Native and Rambouillet breeds of sheep. Allonby and Urquhart (1976) mentioned that in merino sheep, those with HBA show 'self-cure' more frequently and effectively than those with HbB and HbAB types in haemonchosis. Anosa (1977) reported signs of severe parasitism including marked stunting and a normocytic normochronic anaemia characterized by low packed cell volumes, low red cell counts and depressed haemoglobin concentrations in two groups (B and C) of Nigerian dwarf sheep exposed to natural outbreak of helminthosis predominantly caused by *Haemonchus contortus*. However, sheep belonging to group A showed negative fecal egg counts and had normal red cell values and live weights.

Preston and Allonby (1979) also reported that in Merino sheep in addition to a more frequent and effective self-cure in AA type sheep, there are fewer deaths and consistently lower egg counts which they attributed to differences in the establishment of the adult *Haemonchus contortus*

rather than a suppression of egg laying capacity or an alteration of male to female adult worm ratio. Hooda *et al.* (1999) reported the mean values of haemoglobin, packed cell volume, total serum protein and peripheral eosinophil counts to be significantly higher in responders than non-responders. However, serum pepsinogen concentration was significantly less in responders than in non-responders, which demonstrate a distinct resistance in responders to haemonchosis.

Bachman *et al.* (1997) found no conclusive relationship between frequencies of the HbA and HbB genes and the severity of parasitemia in experimentally – induced bovine babesiosis in Drought master cattle. The presence of HbF was not associated with any limitation on the severity of infection in calves aged 1½ months (Bachman *et al.*, 1997). Anosa and Obi (1980) put forth the evidence that in cattle with heterogenous haemoglobin pattern the fast HbB band, and to a lesser extent, HbAB, were associated with increased resistance to infection as measured by percentage of parasite-free animals, incidence of blood parasite and fecal helminth eggs.

2.4 Haemoglobin Types and Economic Traits:

2.4.1 Haemoglobin types and production traits.

Samarineanu *et al.* (1982) reported that Romanian Brown cows in Moldavia with haemoglobins type AB were superior to other types (AA, BB) in milk yield (2271 – 2900 vs 1900 – 2471 litres) and milk fat yield (84.6 – 105.0 vs 67.5 – 103.7 Kg).

Cows of the BB type were found to be inferior to the other 2 types (AB, AA) in milk yield and with fat percentage. Petre *et al.* (1982) reported that Romanian Brown Cows of Hb types A, AB and B showed an average first calving age of 45.14, 40.52, and 36.66 months respectively, average milk yield of 2478, 2578 and 2277kg, and average fat yield of 95.78, 97.62 and 85.56kg with differences in all three characters being significant. Senapati *et al.* (1997) observed that in crossbreds (Holstein X Haryana, Brown Swiss X Haryana, and Jersey X Haryana). Hb types

significantly influenced age at first calving in all groups except the Holstein crossbreds, service period in Brown Swiss crossbreds and milk yield in brown Swiss crossbreds and Haryana purebreds.

Kumaran *et al.* (1984) reported that in crossbred cattle (Holstein-Friesian X Haryanas, Brown Swiss X Haryanas, Jersey X Haryanas and back crosses to the exotic breed) there were no significant relationships of Hb type with body weight at birth, 12 or 14 weeks, age at first calving, first or second lactation milk yields, first or second dry periods or the calving interval. However, in Jersey crossbred, first-lactation length was significantly shorter for Hb type BB animals (226.22 days) than for all types (310.62 – 338.00 days) Barowics and Pacek (1984) observed that in Polish long wool sheep, ewes of Hb type B had a higher lambing rate, litter size, lamb birth weight and fleece weight 87.5%, 1.36, 4.3kg and 3.91kg respectively, than ewes of Hb types A and AB; the lowest values for the first 3 of these traits were for type A ewes (71.0%, 1.31 and 3.87kg). Lambs of type B were superior to lambs of the other types (A, AB) for body weight, wool length, and fleece weight (Barowics and Pacek, 1984).

Weimer *et al.* (1984) reported no significant relationships of Hb and Tf types with litter sizes at birth and weaning, percentage of multiple births, incidence of reproductive failure and wool production in Corriedale and Romney – Marsh sheep in Brazil. Sadykulov and Kim (1985) observed that in degeres sheep, slaughter weight, dressing percentage and meat yield were greater for animals with phenotype TfAB, HbBB or TfCC, HbAB than for TfCC, HbBB and TfAC, HbAB. They recommended the use of male lambs with Tf type AA, CC, AB, AD, CE, or BC for breedings and that replacement females should be chosen from those with Tf type AA, BC, AD, or CE. Dalal *et al.* (1985) reported that in Patanwadi, sheep birth weight did not differ significantly between haemoglobin types.

Yaman *et al.* (1986) observed that the average weaning weight in Ramlic lambs of Hb types AA, AB, and BB (41.55, 41.54, and 41.76kg respectively) were not significant when compared with the body weight (55.35, 55.54, and 53.96kg, respectively) at the end of 3 – months fattening period. Fleece weight averaged 3.63, 3.80 and 3.62kg, respectively, staple length 8.72, 8.56 and 8.36cm and fibre diameter 20.87, 20.17 and 20.10mpm in Ramlic yearling lambs of Hb types AA, AB and BB, respectively.

2.4.2 Haemoglobin types and adaptation traits:

Vicovan and Rascu (1989) observed that in local Romanian breeds (Palas Merino, Transsylvanian Merino, Spanca, Tsigai, Turcana and Karakul), imported breeds (Australian Merino, Romney Marsh, Drysdale Corriedale, Polwarth and Finish Landrace) and two synthetic lines (a meat-finewool line and a high-prolificacy line), the frequency of A allele was higher in the imported breeds than the local breed (28.8 – 100 vs 5.4-19.3%) expression of the gene frequencies in different age groups showed that there was a tendency for the frequency of the A allele to be lower in older than in younger animals (Vicovan and Rascu, 1989). Mortality to weaning also tended to be higher in AA lambs than in lambs of other phenotypes and the number of lambs weaned per ewe lambing was higher for AB and BB than for AA phenotypes (Vicovan and Rascu, 1989). Al-Timemi and Al-Murrani (1990) reported that the Hb genotype significantly affected body temperature and heat tolerance in Iraq local sharabi cows.

2.4.3 Haemoglobin physiological function

Haemoglobin carries oxygen from the lungs or gills, where blood is oxygenated, to body cells. When saturated with oxygen, haemoglobin is called oxyhaemoglobin. After releasing oxygen to the body tissues, haemoglobin reverses its function and picks up carbon dioxide, the waste product of cellular respiration, for transport to the lungs, where it is expired. When saturated

with Carbon dioxide haemoglobin is known as carboxy haemoglobin. Haemoglobin controls the expansion and contraction of blood vessels, and thus blood pressure, by regulating the amount of nitric oxide to which the vessels are exposed (Ganong, 1995).

2.5 The Transferrins

Transferrins are iron-binding blood plasma glycoproteins that control the level of free iron in biological fluids (Crichton *et al.*, 1987). Transferrin is encoded by the Tf gene.

Transferrin glycoproteins bind iron very tightly, but reversibly. Although iron bound to transferrin is less than 0.1% (4mg) of the total body iron, it is the most important iron pool with the highest rate of turnover (25mg/24h) (Yang *et al.*, 1984). Transferrin has a molecular weight of around 80 moles and contains two specific high-affinity Fe (iii) binding sites. The affinity of transferrin for Fe(iii) is extremely high (10^{23} M^{-1} at pH7.4) but decreases progressively with decreasing pH below neutrality, when non-bound to iron it is known as apotransferrin (Aisen *et al.*, 1978).

2.5.1 Transferrin transport mechanism

When a transferrin protein loaded with iron encounters a transferrin receptor on the surface of a cell (e.g. to erythroid precursors in the bone marrow), it binds to it and as a consequence, is transported into the cell in a vesicle by receptor-mediated endocytosis. The pH of the vesicle is reduced by hydrogen ion pumps (H^+ ATPases) to about 5.5 causing transferrin to release its iron ions. The receptor (with its ligand, transferrin bound) is then transported through the endocytic cycle back to the cell surface, ready for another round of iron uptake. Each transferrin molecule has the ability to carry two iron ions in the ferric form (Fe^{3+}).

2.5.2 Structure of transferrin

Transferrin consists of a polypeptide chain containing 679 amino acids. The protein is composed of alpha helices and beta sheets to form two domains. The N- and C- terminal sequences are represented by globular lobes and between the two lobes is an iron binding site (Dassauer *et al.*, 1981). The amino acids which bind the iron ion to the transferrin are identical for both lobes, two tyrosines, one histidine and one aspartic acid for the iron ion to bind an anion is required preferably carbonate (CO₂⁻).

Transferrin also has a transferrin iron-bound receptor; it is a disulfide-linked homodimer (Macedo *et al.*, 2008). In humans, each monomer of transferrin consists of 760 amino acids. It enables ligand bonding to the transferrin, as each monomer can bind to one or two molecules of iron. Each monomer consist of three domains; the protease, the helical, and the apical domains, the shape of transferrin receptor resembles a butterfly-like complex, due to the three clearly shaped domains (Dassauer *et al.*, 1981).

Macedo *et al.* (2008) reported that the liver is the main source of transferrin synthesis, but other tissues and organs, such as the brain, also produce it. The main role of transferrin is to deliver iron from absorption centres in the duodenum and white blood cell macrophages to all tissues transferrin plays a key role where erythropoiesis and active cell division occur, the receptor helps maintain iron homeostasis in the cells by controlling iron concentrations (Macedo *et al.*, 2008).

2.5.3 Transferrin in immune system:

Ritchie *et al.* (1999) reported that transferrin is associated with the innate immune system, it is found in the mucosa and binds iron. This creates an environment low in free iron that impedes bacterial survival in a process called iron withholding, hence the level of transferrin decreases in inflammation.

2.5.4 Role of transferrin in disease control

Transferrin imbalance can have serious health effects for those with low or high serum transferrin levels. Macedo *et al.* (2008) reported that a patient with an increased serum transferrin level often suffers from iron deficiency anaemia, and a patient with decreased plasma transferrin can suffer from iron overload disease and protein malnutrition. An absence of transferrin results from a rare genetic disorder known as a transferrineamia a condition characterized by anaemia and hemosiderosis in the heart and liver that leads to many complications, including heart failure. Most recently transferrin and its receptor have been shown to diminish tumour cells by using the receptor to attract antibodies (Macedo *et al.*, 2008).

2.5.5 Transferrin types and economic traits:

2.5.5.1 Transferrin types with prolificacy traits:

Taraphder *et al.* (2011) reported that ewes with T^{fAD} phenovariant showed best performance with respect to lamb production (2.71 ± 0.28 lambs per lambing) in the analysed flock, in the first lambing, the highest (2.50 ± 0.52 lamb per lambing) and lowest ($.25 \pm 0.25$ lambs per lambing) number of lambs were born by ewes with genotype T^{fBC} and T^{fAA} respectively. He further stated that in the second lambing the highest number of lambs (3.00 ± 0.49) was born by ewes with both genotype T^{fAD} and T^{fBC}, and the lowest number (1.50 ± 0.49) by ewes with genotype T^{fDE}. In the third lambing the highest number of lambs produced was observed for ewes with genotype both T^{fAD} and T^{fBD} which produced 3.00 ± 0.40 and 3.00 ± 0.33 lambs per lambing while lowest number by ewes with T^{fBC} genotype viz. 1.50 ± 0.49 lambs per lambing; it was then observed that ewes with T^{fAD} genotype showed best performance with respect to prolificacy; and type T^{fDE} is least productive in the analysed flock.

Osman (1961) studied serum transferrin polymorphism in desert sheep of Sudan and also suggested that some phenotypic combination may have some selective advantage for production traits in certain environments. Vanti and Bas (1994) observed that transferrin polymorphisms affected the number of lambs born in Awassis sheep.

Rasmusen *et al.* (2009) reported that Finish landrace ewes of transferrin type BD had smaller litter than ewes of other types. Taraphder *et al.* (2011) observed that the heterozygous ewes at the Tf locus were more prolific than the homozygous ones at the same locus. He also observed that most prolific homozygous ewes were those with T^{fCC} phenotype, He however concluded that transferrin heterozygous ewes have an obvious superior genotype comparatively with that of the Tf homozygous ewes genotype.

2.5.6 Transferrin types with age at first lambing

Ghatak (2001) reported that age at first lambing was higher for T^{fAA}, T^{fBD}, T^{fDD} types transferrin in Muzaffarnagri sheep T^{fAC}, T^{fAD}, T^{fCD}, and T^{fDD} transferrin types in shahabadi sheep and T^{fBC}, T^{fBD}, T^{fCD} and T^{fDD} transferrin types in Garole sheep.

Taraphder *et al.* (2011) reported that transferrin genotype had non-significant effect in case of age at first lambing, in the Garole sheep. Gopinathan and Nair (1976) did not find any significant relationship of transferrin with age at first kidding. It has however been observed that no significant association of age at first lambing with transferring phenotypes (Erokhin 1978; Ghatak, 2001).

2.5.7 Transferrin types with lambing interval

Paul (2002) observed that there were no significant differences in kidding interval due to transferrin types in Bengal goats. Erokhin and Bash keeva (1978) found no clear relationship between transferrin alleles and reproduction traits of kuibysher sheep. However, Shamsuddin *et*

al. (1988) recorded significant association of kidding interval with TfAA (homozygous) in Saanen X Malabari crossbreds which may be due to the effect of exotic breed or crossbreeding. Taraphder (2011) then concluded that the heterozygous ewes tend to have a significantly better prolificacy than the homozygous ewes.

2.5.8 Physiological function of transferrin

The function of transferrin is to transport iron from the intestine, reticulo-endothelial system, and liver parenchymal cells to all proliferating cells in the body. In addition to its function in iron transport, this protein may also have a physiological role as a granulocyte/pollen binding protein (GPBP) involved in removal of certain organic matter/allergens from serum. (Macedo and de Sousa, 2008) iron/ transferrin is essential in haemoglobin synthesis and for certain type of cell division since 70% of iron in the body is in haemoglobin and most iron transported are bound to transferrin. Thus in iron deficiency, the amount of transferrin in the plasma is increased and its percent saturation with iron is decreased (Ganong, 1995).

2.6 Carbonic Anhydrase.

The carbonic anhydrases (or carbonate dehydrates) form a family of enzymes that catalyze the rapid interconversion of carbon dioxide and water to bicarbonate and protons (or vice-versa), a reversible reaction that occurs rather slowly in the absence of a catalyst (Badger and Price, 1994). The active site of most carbonic anhydrases contains a zinc ion; they are therefore classified as metalloenzymes (Badger and Price, 1994).

One of the functions of the enzyme in animals is to interconvert carbon dioxide and bicarbonate to maintain acid-base balance in blood and other tissues, and to help transport carbon dioxide out of tissues.

This enzyme was first identified in 1933, in red blood cells of cows. Since then, it has been found to be abundant in all mammalian tissues, plants, algae and bacteria. Carbonic anhydrase has three distinct classes – alpha, beta and gamma carbonic anhydrase; carbonic anhydrase from animals belong to the alpha class.

2.6.1 Carbonic anhydrase in health and disease:

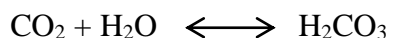
Shuahismita *et al.* (2004) reported the role of carbonic anhydrase in the production and usage of protons and bicarbonate ions in the body. He reported that carbonic anhydrase play a key role in the regulation of pH and fluid balance in different parts of the body. In the stomach lining it plays a role in secreting acid, while the same enzyme helps to make pancreatic juices alkaline and saliva neutral. The transport of the protons and bicarbonate ions produced in the kidney and eyes influence the water content of the cells at these locations (Shuahismita *et al.*, 2004).

2.6.2 Physiological function of carbonic anhydrase:

Parietal cells in the stomach secrete massive amounts of acid (i.e. hydrogen ions or protons) unto the lumen and a corresponding amount of bicarbonate ion into the blood.

Pancreatic duct cells do essentially the opposite, with bicarbonate as their main secretory product. Secretion of hydrogen ions by the renal tubules is a critical mechanism for maintaining acid base fluid balance. Carbon dioxide generated by metabolism in all cells is removed from the body by red blood cells that convert most of it to bicarbonate for transport, and then back to carbon dioxide to be exhaled from the lungs.

The relevance of the points stated above is observed in this reaction:



This important reaction proceeds slowly in either direction unless the enzyme carbonic anhydrase is present (Ganong, 1995). The transport of CO₂, the control of pH of body fluids and selection for the production of carbonate ions are facilitated by carbonic anhydrase.

2.7 Genetic Types of Carbonic Anhydrase.

Ca^s was observed to be fixed in Yankasa, Uda, Mbororo and West African Dwarf sheep breeds and in Merinoland, East Friesian Milk and German Grey Health Sheep breeds (Ibeagha – Awemu and Eerhadt, 2004). The fixation of Ca^s is contradicted by the report of polymorphism Ca^m and Ca^s on similar breeds (Missohou 1999). This allele was also fixed in three German breeds and is generally the most occurring allele in most breeds (Bius and Tucker, 1983; Zanotti *et al.*, 1990). Ca^m which was specific to the Black Faced Mutton sheep is rare but also of wider distribution as it has also been reported to occur in some breeds of sheep (Ordas, 2004) Italy (Zanotti Casati *et al.*, 1988) and Africa (Missohou *et al.*, 1999).

In a study on the genetic variability in nineteen south African breeds Sageant *et al.* (1999) reported that six of the breeds were polymorphic at the locus, C allele was found in Russian Red woolled Persian and Border Leicester breeds at low frequencies, A allele was found in the remaining four i.e. Landrace, Afrino, Namaqua and vendor also at low frequency. In a study by Ordas and Primitivo (1986) on Churra sheep breeds a new allele M was found at the CA locus, CA^s was also found in all the breeds studied (Sageant *et al.*, 1999), the allele was reported fixed in most of the breeds.

2.8 Performance Traits

2.8.1 Morphometric measures in cattle

Characterization of livestock breeds is the first approach to a sustainable use of its animal genetic resources (Lanari *et al.*, 2003). The first step in the characterization of local genetic resources is

based on the knowledge of variations in the morphological traits (Delgado *et al.*, 2001). Yakubu *et al.* (2010) have reported that generally, the linear body measurements of Sokoto Gudali were significantly ($P < 0.05$) higher than those of the Bunaji cattle with the exception of body length and face length respectively. They stated that comparative measurements of morphometric traits can provide evidence of breed relationships and size and the considerable variation in body dimensions of the two cattle breeds might not be unconnected with individual breed's potential and peculiarities. While the Bunaji cattle is noted for milk production, their Sokoto Gudali counterparts which rank second in milk production produce more meat and appear to have more draught power than the former.

Several authors have reported height at withers of cattle breeds such as Nandi (110–122 cm), Mongalla (100–110cm) (Rege, 1999), Mexican Criollo Chinampo (101–117cm) (Espinoza *et al.*, 2009) and Sudan Baggara (115.9–148.80cm) (Alsiddig *et al.*, 2010). The chest circumference were reported as 122-127cm for North Bengal Grey cattle in Bangladesh (Al-Amin *et al.*, 2007) among other morphometric measures, these goes to show that significant breed variation exist in morphometric measures and could be exploited both for classification, conservation and production purposes. For the purpose of characterization, Yakubu *et al.* (2010) have reported that rump width, withers height and face length were found to be the most discriminating variables to assign Bunaji and Sokoto Gudali cattle into distinct genetic groups.

2.8.2 Udder characteristic and milk production

Morphologically udder traits are very important for dairy animals. These traits are of interest to the breeder because of their influence on applicability to mechanical milking, udder health and milk yield (Dag and Zulkadir, 2004). In goats, udder characteristics, milk production, milking time and rate are traits with adequate genetic variation to allow selection response (Akpa, 1999;

Bhuiyan *et al.*, 2004). The knowledge of the relationships between individual characteristics of udder morphology is important for their inclusion into total selection indexes or construction of partial selection indexes for udder morphology and it will enable farmer to predict future correlated responses in milk-oriented selection programmes (Dag and Zulkadir, 2004). There are few studies on the udder and teat dimensions in goats (Prasad *et al.*, 2010; Akporharho *et al.*, 2011). Factors that affect udder traits in goats include age, parity and season of kidding (Akpa, 1999; Akporharho *et al.*, 2011).

The most important factors contributing significantly to the variability of the depth, width and circumference which define udder size are lactation month, flock and yield (Milerski *et al.*, 2006). Other traits cistern height, teat position, length and width, showed high repeatability and were less dependent on yield and lactation month, which made them potential candidates for genetic improvement (Akpa, 1999). Moreover, cistern height, teat position and teat angle traits are the most important for udder adaptation to mechanical milking (Mahthar and Vrla-Anesti, 1994). Cistern height, vertical teat position and minimal teat angle favour letdown and drainage of the milk, which eliminate lengthy stripping which wide teat angle are responsible for teat cup slipping and inhibition of the ejection reflex (Labussiere, 1988). Stage of lactation has been reported to significantly influence mammary traits (Bhuiyan *et al.*, 2004; Akpa *et al.*, 1999). Udder measurement increases with parity (Akpa *et al.*, 2003). As lactation progresses, these traits diminished with milk yield. Teat size (length and width), decreases with stage of lactation; while udder width and cistern height increased with parities (Akpa *et al.* 2003). Milk yield has been reported to greatly influence udder size (Akpa, 1999; Prasad *et al.*, 2010). The traits (depth, width and circumference) defining udder size, were highly dependent on total milk yield per lactation and test day milk yield (Labussiere, 1988).

Prasad *et al.* (2010) reported a positive correlation between all udder measurements (udder width, udder length and udder depth) and daily yield. Although the correlation was significant only with udder width, Czarnik (1994) had earlier reported similar results. Mavrogenis *et al.* (1988) observed that mammary characteristics (except udder size) can be selected for without modifying milk yield because genetic correlations with milk yield were low. Udder size was observed to be more of a limiting factor to yield in first lactation than in later ones (Akpa *et al.*, 2003). The correlation between udder circumference, udder height, and distance between teat had been reported to be high and positive (Fernandez *et al.*, 1997). It has been observed that cows with pointed teat ends had the lowest milk flow rate (Seykora and McDaniel 1985); and bowl shaped udder in dairy cows yielded the maximum test yield (3.4kg), medium in goaty shaped udder (3.1kg) and less in round shaped udder (2.8kg) (Bhuiyan *et al.*, 2004). Contrary to this, other authors observed that milk yield did not differ significantly by classification of teat end shape (Milerski *et al.*, 2006). Appleman (1970) found that teat ends that were disk or inverted had wider streak canals than those with pointed or round ends. Teat with large diameter has slow milking than a small one. However, selection of a more pointed teat could increase resistance to mastitis, but milk yield may not increase because of a tendency to milk incompletely animals with slower milk flow (Seykora and McDaniel 1985). Increased residual milk would tend to lower total yield; hence, faster milk flow in the flatter teat ends may compensate for increased infection rate (Seykora and McDaniel 1985; Dag and Zulkadir, 2004).

2.10 Genetic Introgression and Breeding Herds Morphometric Attributes

The wide range of breeds and species that have evolved in various environments represent unique sets of genetic diversity. Genetic diversity has been defined as the variety of alleles and genotypes present in a population, and this is reflected in morphological, physiological and

behavioural differences between individuals and populations (Frankham *et al.*, 2002). Morphometric measurements have been used to evaluate the characteristics of various breeds of animals, and could provide useful information on the suitability of animals for selection (Martins *et al.*, 2009 and Yakubu, 2010). The outcome of genetic improvement programmes could also be evaluated on morphological basis (Riva *et al.*, 2004). Although recent analyses have focused on molecular techniques, most mammalian species and subspecies originally were described on the basis of morphological characteristics (Feldhamer *et al.*, 2004). Previous efforts on the phenotypic characterization of breeds of livestock have been restricted to the use of analysis of variance, whereas the current trend in livestock classification involves the use of multivariate statistical tools (Yakubu and Akinyemi, 2010). This is because univariate statistical analysis, according to Dossa *et al.* (2007), analyze each variable separately and do not explain how the populations under investigations differ when all measured morphological variables are considered jointly. Multi-factorial discriminant analyses have been found to be more suitable in assessing variation within a population and can discriminate different population types when all measured morphological variables are considered jointly.

Since the general aim of Breeding and genetic conservation is to maintain within and across breed diversity, where within breed diversity refers to the genetic management of one population and the across breed diversity implies the genetic management of many populations. Within breed diversity it is needed for the breed to genetically adapt to changes in the production and economic environment, and to avoid inbreeding problems. The herds of cattle being employed for research in NAPRI needs to be evaluated for gene introgression which contradicts these goals. Population studies which elucidate the relationship existing between the different breeds of a given species may offer useful information for the conservation and management of

animal genetic resources (AnGR) such as the evolution of the breeds, the development of gene pools and the magnitude of genetic differentiation. According to Mariante *et al.* (2008), national AnGR conservation programmes should use the association of phenotypic data, molecular polymorphisms and adequate statistical methods which reflect the real condition of a population. This was buttressed by Berthouly *et al.* (2010) who studied genetic diversity of Vietnamese H'mong cattle using multivariate analysis on morphometric and genetic data. In multivariate analysis and classification of studied herds into genetic groups, Traoré *et al.* (2008) have attributed the cause of large misclassifications to the manifestation of introgressions across breeds resulting from the actions of most stock breeders who intend to obtain products with bigger conformation. Such misclassification example includes the (30.2-31.7%) in classifying the Sahel as the Djallonke sheep (Birteeb *et al.*, 2012) and implies gene introgression within the breeding stock probably due to attempt at cross breeding. Yakubu *et al.* (2010) reported a lower error rate (3%) of misclassification for the Sokoto Gudali and a higher error rate (15%) for the Bunaji in Nasarawa state. The larger restriction of the Sokoto Gudali to its ecological zone, and the wide dispersal of the Bunaji may account in part for these observations. It therefore follows that, the gene pool of the Bunaji is being eroded at a faster rate than the Sokoto Gudali counterpart.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the Experiment

The research was conducted at the Dairy Breeding Unit of National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State. NAPRI is geographically located between latitude 11⁰ and 12⁰N and longitude 7⁰ and 8⁰E at an altitude of 640m above sea level (Ovimaps, 2014).

The mean annual rainfall in this zone is 1,100mm which commence from May and last till October, of which 90% fall during the wet raining season (June - September). Following the wet season is a period of dry, cool weather called hammer tan which marks the onset of the dry season, this extend from mid-October - January. The dry season (February - May) is characterized by very hot weather conditions. At this period daily temperature range from 21°C-36°C, the mean relative humidity is 21% and 72% during “hammer tan” and the raining season respectively.

3.2 Animals and Management

A total of 150 equal number of Bunaji (50), Friesian X Bunaji (50) and Sokoto Gudali (50) cows were used for this research. The Bunaji (50) and Friesian X Bunaji (50) were source from National Animal Production Research Institute (NAPRI) Shika Zaria, while Sokoto Gudali was source from the NAPRI out station in Talata Mafara, Zamfara State. They were raised under semi intensive system of management. The animal were raised during raining season on paddock-sown pasture comparison of elephant grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*), Giant star grass (*Cynodon plectostacynum*) Gamba grass (*Andropogon gayanus*) and the legumes Muccuna spp, Alfafa (*Medicago sativa*), Lablab (*Lablab prurpureum*)

Desmodium canum (luicaena leucocephala) as a browse plant, while hay or silage with cotton seed cake were offered during the dry season. They had access to water and salt lick and water ad-libitum, unrestricted grazing was allowed under the supervision of the herd men for 7-9 hours per day. Routine spray against ticks and other ectoparasites was observed.

3.3 Bodyweights

The bodyweight (BW) was measured once a month, in the morning prior to any feed intake, because in grazing animals the digesta present in the gastro intestinal tract is likely to be minimal in quantity. The weight was taken in kilogram (kg) using weigh bridge

3.4 Body linear Measurement

Body Length (BL): Body length was measured using a flexible tape, as the distance from the occipital protuberance to the base of the tail.

Height at Withers (HW): The height was measured as the distance from the ground to the withers using a measuring tape.

Heart girth (HG): The heart girth was measured by taking the measurement of the circumference of the chest with measuring tape.

Chest Width (CW): This is measured from the inside surface between the top of the front legs.

3.5 Udder Measurements

The Udder and teat measurements were done using flexible tape (cm) as follows:

Udder Circumference (cm): Measured at the widest point of the Udder round it.

Udder height (cm): Measured from the rear attachment of the Udder to the front of it where it blends with the body.

Teat length (cm): measured as the distance from the upper part of the teat, where it hangs perpendicularly from the Udder to the tip of the teat.

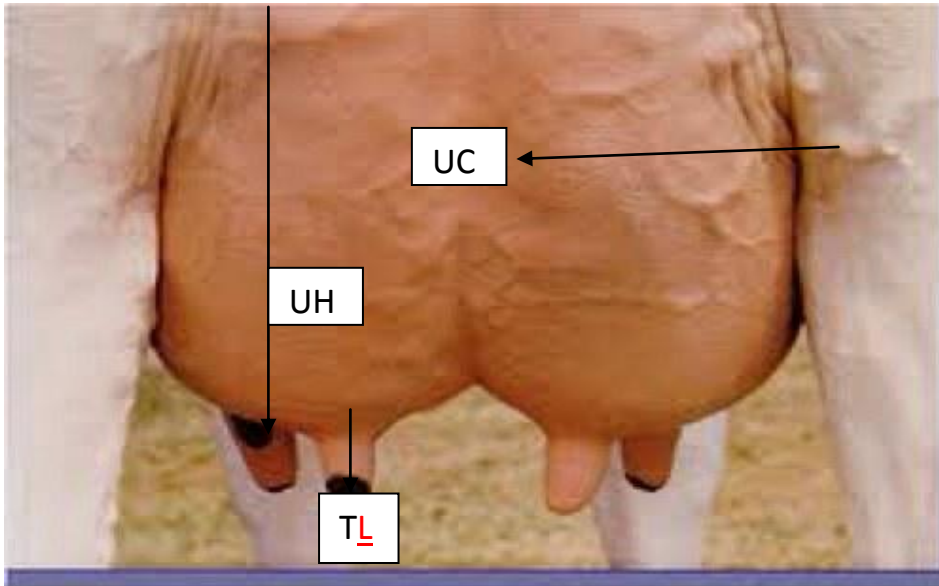


Plate I Showing Udder Measurement

Key: UC: Udder Circumference, UH: Udder Height, TL: Teat Length

3.6 Milk yield characteristics

Milk yield characteristics were measured as follows:

Average Daily Yield (ADY): - Average daily milk yield was measured as the average of all test day yields within the milking period.

Total Yield (TY): - Total yield was measured as milk production during the lactation period up to the point where the production of the cow dropped below 100ml.

Lactation Length (LL): - This was calculated as the period from calving to the point when the milk yield of the cow falls below 100ml

3.7 Cellulose Acetate Electrophoresis

3.7.1 Blood Collection and sample preparation

Five milliliter (5ml) of blood was collected from each of the sampled animal by jugular venepuncture using needle and syringe into test tube containing Ethylene Diamine Tetra Acetic

Acid (ADTA) as anticoagulant and sample were properly labelled. It was then refrigerated at 8⁰C for two hours and thereafter carried into the laboratory of the department of animal science university of Ibadan Nigeria.

3.8 Sample Preparation

3.8.1 Blood haemolysates and plasma collection

Red blood cell (RBC) was prepared from the erythrocyte fraction of heparinised blood by centrifuging at 2500 – 3000rpm for 10mins at 4⁰C. The RBC were washed in saline (0.155M NaCl) three times and centrifuged at 2500 – 3000 rpm for 5mins at 4⁰C. The RBCs were lysed with a fourfold volume of distilled H₂O to release the haemoglobin. The plasma fraction was separated from the erythrocyte fraction of heparinized blood by centrifuging at 2500 -3000 rpm. The supernatant was used to analyse for transferrin and carbonic anhydrase.

3.9 Experimental Procedure:

3.9.1 Gel soaking

Cellulose acetate plates were soaked in the same buffer as the electrode buffer (Continuous buffer system). Multiple gels were simultaneously soaked in an 800ml beaker with individual gel plates separated by glass rods to ensure complete soaking of every plate. Care was taken, however, to prevent the formation of bubbles on the gel plate as it was immersed. This was accomplished by submerging the plates at a slow, constant rate into the gel buffer. Plates were soaked for 20 minutes.

3.9.2 Sample loading

Blood samples were prepared and added to the wells of the sample plate. The cellulose acetate paper was blotted dry between sheets of filter paper to remove excess moisture. Care was taken to ensure that the cellulose acetate paper did not shift when the samples were loaded. To prevent movement, the aligning plate was moistened with a drop of gel buffer before the cellulose acetate sheet was set on it. The plate was also centered on the aligning base to ensure that all samples were applied. Using the applicator, samples were applied twice to the same position on the plate. Once loaded, plates were rested on the wicks in the tank without current being applied while subsequent plates were loaded. The teeth of the applicator were blotted each time before other applications were

3.9.3 Gel running

The side bearing the acetate was placed on the wick. Care was taken to ensure that the load zone located near the end of the gel did not come into contact with the wicks. Since the current runs from the cathode to the anode electrode (negative to positive), the loaded zones on the plate was positioned at the cathodal end of the tank for the majority of the enzyme systems which migrate anodally e.g. carbonic anhydrase, the loading zone was placed on the anodal end.

3.9.4 Gel staining

When the gel run was completed, the plates were removed from the tank and placed in an empty petri dish. Again, care was taken to ensure that the cellulose acetate plate lied horizontally. Once plates were removed from the tank, they were stained with Ponceau stain before they dry out.

3.9.5 Gel and bands scoring

Bands were scored visually as described by RIKEN (2006) according to the migration of the bands. Direct counting was used for calculating gene frequencies. Frequencies generated were used to compute genotypic frequencies

3.9.6 Estimation of the gene and genotypic frequency

Gene and genotypic frequencies were estimated using the Hardy–Weinberg Model which consists of two equations: One that calculates the allelic frequencies and one that calculate genotype frequencies. Since these involve frequencies, the rule of thumb is that both equations must add up to 1. Thus the equation used were:

$$P + q = 1, \text{ where } A = p \text{ and } q = B$$

1. Gene (Allele) frequency

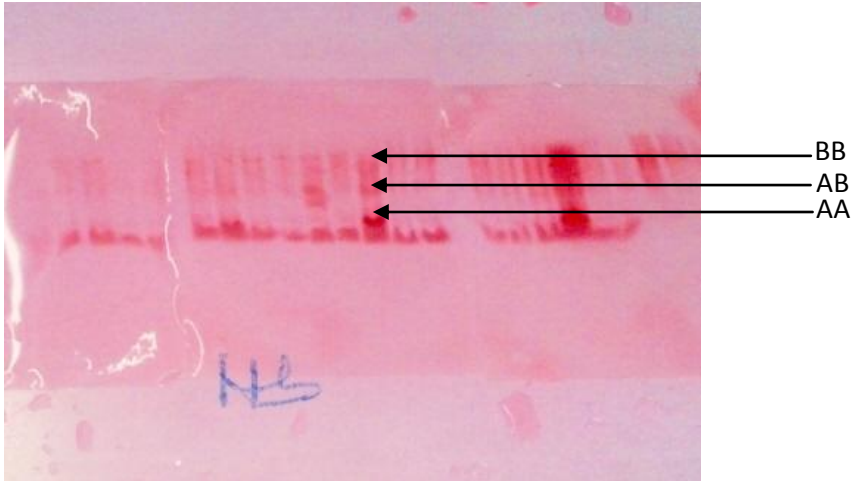
$$A = \text{Ratio AA} + \frac{1}{2} (\text{Ratio AB})$$

$$B = \text{Ratio BB} + \frac{1}{2} (\text{Ratio AB})$$

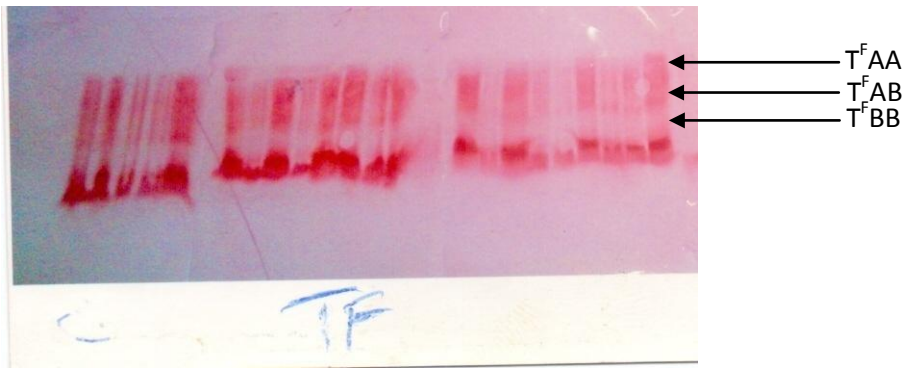
2. Genotype Frequency

$$P^2 + 2pq + q^2, \text{ where } P^2 = A^2 \text{ (or AA), } 2pq = 2(\text{AB}) \text{ and } q^2 = B^2 \text{ (or BB)}$$

$$A^2 + 2AB + B^2$$



PlateII: Haemoglobin bands



PlateIII: Transferrin bands

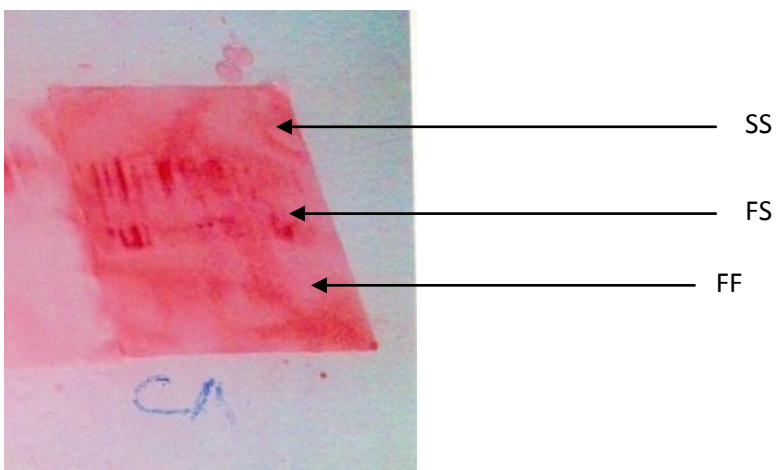


Plate IV: Carbonic anhydrase bands

3.10 Electrophoretic Conditions

The method used was described by RIKEN (2006) as shown in Table 3.1.

Table 3.1 Electrophoretic Condition

MARKERS	BUFFER	TIME (MINS)	P ^H	STAIN
Haemoglobin	Tris EDTA borate	40	8.4	Ponceau
Carbonic Anhydrase	EDTA Sodium	45	5.6	Ponceau
Transferrin	Tris glycine	15	8.5	Ponceau

3.10.1 Measurement of genetic distance.

Gene frequency, genotypic frequency and Genetic distances among the genetic groups (estimated from gene frequencies) were calculated using S.A.S, 2003

3.11 Statistical Analysis

The effect of breed and blood enzyme polymorphism on measured traits and their interaction were determined using the PROC Generalized Linear Model (GLM) of Statistical Analysis System (SAS) 9.2 (2003). Significant ($p < 0.05$) differences in means were separated using Duncan Multiple Range Test (DMRT) Duncan (1955)

3.11.1 Model for the analysis was a factorial anova design as illustrated below:

$$Y_{ijkl} = \mu + B_i + P_j + BP_{ij} + E_{ijk}$$

Where Y_{ijk} is the record observed

μ = population mean

B_i = Fixed effect of the i^{th} breed of cattle

P_j = Fixed effect of the j^{th} polymorphic types of blood protein

BP_{ij} = interaction between breed and blood protein polymorphic class

E_{ijk} = random error particular to the ijk^{th} observation assumed to be independently randomly distributed with mean zero and variance NIID ($0, \sigma^2_e$).

3.11.2 Correlation Analysis

The degree of relationship between all pairs of metric variables was computed for all the animals within each breed using Correlation procedure of the SAS 9.2 (2003) package.

Phenotypic correlation $r = \frac{\text{COV}_{xy}}{\sqrt{\sigma^2_x \sigma^2_y}}$

Where:

σ^2_x = variance of x

σ^2_y = variance of y

σ^2_{xy} = covariance between x and y

Variables x and y are assumed to be random normal variables jointly distributed with a bivariate normal distribution.

3.11.3 Principal component analysis procedures

The correlation coefficients of body weight and the linear body measurements were also determined. From the correlation matrix, data for the principal component factor analysis were generated. According to Everitt *et al.* (2001), principal component analysis is a method for transforming the variables in a multivariate data set x_1, x_2, \dots, x_p , into new variables, y_1, y_2, \dots, y_p which are uncorrelated with each other and account for decreasing proportions of the total variance of the original variables defined as:

$$y_1 = a_{11} x_1 + a_{12} x_2 + \dots + a_{1p} x_p$$

$$y_2 = a_{21} x_1 + a_{22} x_2 + \dots + a_{2p} x_p$$

$$y_p = a_{p1} x_1 + a_{p2} x_2 + \dots + a_{pp} x_p$$

with the coefficients being chosen so that y_1, y_2, \dots, y_p account for decreasing proportions of the total variance of the original variables, x_1, x_2, \dots, x_p .

Or in matrix form:

$$Y_1 = a'x$$

The a_{ji} are scaled such that $a_1'a_1=1$. Y_1 accounts for the maximum variability of the p variables of any linear combinations. The variance of Y_1 is λ_1 . The next, principal component Y_2 is

formed such that its variance, λ_2 is the maximum amount of the remaining variance and that it is orthogonal to the first principal component. That is,

$$a_1' a_2 = 0$$

Components are extracted until some stopping criteria is encountered or until p components are formed. The weights used to create the principal components are the eigenvectors of the characteristics equation:

$$(R - \lambda I) a = 0$$

Where R is the correlation matrix. The λ are the eigenvalues, the variances of the components.

The eigenvalues are obtained by solving $(R - \lambda I) a = 0$ for λ .

During the evaluation, factors were rotated with varimax rotation of Kaiser. The aim of the Varimax rotation is to maximize the sum of variances of a_{ij}^2 quadratic weight. Anti-image correlations, Kaiser – Meyer Olkin measures of sampling adequacy and Barlett's Test of Sphericity were computed to test the validity of the factor analysis of the data sets. The appropriateness of the factor analysis was further tested using communalities and ratio of cases to variables.

The stepwise variable selection multiple regression procedure was used to obtain models for predicting body weight from body measurements (a) and from established principal components (b).

$$BW = a + B_1 x_1 + \dots + B_k x_k \dots \dots \dots (a)$$

$$BW = a + B_1 PC_1 + \dots + B_k PC_k \dots \dots \dots (b)$$

where; BW is the body weight, a is the regression intercept, B_1 is the i -th partial regression

coefficient of the i th linear body measurement, X_i represents independent variables (i.e body measurements) or PC_i represents the i -th principal component and k represents infinity.

3.10.2.3 Discriminant analysis

Stepwise discriminant procedure (SAS, 2003) was applied using PROC STEPDISC to determine which morphological traits have more discriminant power than others. The relative importance of the morphometric variables in discriminating between the three cattle populations were assessed using the level of significance, partial R^2 and F-statistic. The CANDISC procedure was employed to perform univariate and multivariate one-way analysis that were used to calculate the Mahalanobis distance between the breeds. The ability of these canonical functions to assign each individual animal to its breed was judged as the percentage of correct assignment to each genetic group using the DISCRIM procedure (Nearest Neighbour Discriminant Analysis). A discriminant function was computed to classify the breeds into respective genetic groups based on the measurements. Two thirds of the data were randomly selected across the genetic groups and used as training data for the discrimination, while the remaining one-third was used as test data for the discriminant analysis.

CHAPTER FOUR

4.0. RESULTS

4.1 Genotype and Gene Frequencies of Blood Proteins among Three Genetic Groups

4.1.1 Genotype and gene frequencies of haemoglobin among three genetic groups

Table 4.1 presents the gene and genotypic frequencies of haemoglobin variants. The observed and expected numbers were also indicated. The HbAA overall count was 40 with a frequency of 0.44, HbAB was 91 and 0.61 and HbBB was 19 and 0.13. Allelic count and frequency were (85 and 0.62) and (65 and 0.38) for the A and B variants. In the Bunaji the count and frequency were HbAA (10.00 and 0.20), HbAB (33.00 and 0.66), HbBB (7.00 and 0.14) while allelic frequency indicated similar count and frequency of HbA (30.00 and 0.60) and HbB (20.00 and 0.40). The Friesian X Bunaji cross indicated (3.00 and 0.25), (7.00 and 0.58) and (2.00 and 0.17) respectively for genotypes observed; (6.50 and 0.54) and (5.50 and 0.46) for allelic counts and frequencies. In the Sokoto Gudali the following were observed for the genotype; (20.00 and 0.40), (28.00 and 0.56) and (2.00 and 0.04) while the allelic counts and frequencies were (34.00 and 0.68) and (16.00 and 0.32) respectively. The χ^2 value 1.27 was not significant ($p>0.05$) indicating conformity to Hardy-Weinberg equilibrium for the haemoglobin loci across all the genetic groups.

4.1.2 Genotype and gene frequencies of transferrin among three genetic groups

Observed and expected counts of the gene and genotypic frequencies of the Transferrin locus is presented in Table 4.2. χ^2 value of 15.48 showed high level of significance ($p<0.01$) and non-conformity to Hardy-Weinberg equilibrium. For the T^{fAA} , T^{fAB} and T^{fBB} genotypes and the alleles T^{fA} and T^{fB} , the overall counts and frequencies showed the following (24.00 and 0.16), (112.00 and 0.75) and (14.00 and 0.09) genotypes and (83.50 and 0.56), (66.50 and 0.44) alleles.

In the Bunaji, these were indicated as (15.00 and 0.30), (35.00 and 0.70) and (2.00 and 0.04) genotypes and (33.00 and 0.66) with (17.00 and 0.34) alleles. The Friesian X Bunaji cross were outlined for genotypes as (6.00 and 0.12), (38.00 and 0.76) and (6.00 and 0.12) and for alleles as (30.00 and 0.60) and (20.00 and 0.40) respectively. The Gudali genotypes were (2.00 and 0.04), (41.00 and 0.82) and (7.00 and 0.14) respectively and the alleles were (22.50 and 0.45) and (27.50 and 0.55) respectively.

Table 4.1: Genotype and gene frequencies of haemoglobi among three genetic groups

Variable	Genotype			Gene		χ^2 df=2	LOS
	AA	AB	BB	A	B		
Overall						11.12	***
Observed	40.00	91.00	19.00	85.00	65.00		
Expected	50.00	50.00	50.00	50.00	50.00		
Frequencies	0.44	0.61	0.13	0.62	0.38		
Bunaji							
Observed	10.00	33.00	7.00	30.00	20.00		
Expected	16.70	16.70	16.70	16.70	16.70		
Frequencies	0.20	0.66	0.14	0.60	0.40		
Friesian X Bunaji							
Observed	10.00	30.00	10.00	30.00	20.00		
Expected	16.70	16.70	16.70	16.70	16.70		
Frequencies	0.20	0.60	0.20	0.60	0.40		
Gudali							
Observed	20.00	28.00	2.00	34.00	16.00		
Expected	16.70	16.70	16.70	25.00	25.00		
Frequencies	0.40	0.56	0.04	0.68	0.32		

LOS-Level of significance; *p<0.05-significance, p>0.05-not significance

Table 4.2: Genotype and gene frequencies of transferrin among three genetic groups

Variable	Genotype			Gene		χ^2 df=2	LOS
	T ^{fAA}	T ^{fAB}	T ^{fBb}	T ^{fA}	T ^{fB}		
						15.48	**
Overall							
Observed	24.00	112.00	14.00	83.50	66.50		
Expected	50.00	50.00	50.00	50.00	50.00		
Frequencies	0.16	0.75	0.09	0.56	0.44		
Bunaji							
Observed	15.00	33.00	2.00	33.00	17.00		
Expected	16.70	16.70	16.70	25.00	25.00		
Frequencies	0.30	0.70	0.04	0.66	0.34		
Friesian X Bunaji							
Observed	6.00	38.00	6.00	30.00	20.00		
Expected	16.70	16.70	16.70	25.00	25.00		
Frequencies	0.12	0.76	0.12	0.60	0.40		
Gudali							
Observed	2.00	41.00	7.00	22.50	27.50		
Expected	16.70	16.70	16.70	25.00	25.00		
Frequencies	0.04	0.82	0.14	0.45	0.55		

LOS-Level of significance; *p<0.05-significance, p>0.05-not significance

Table 4.3: Genotype and Gene Frequencies of Carbonic Anhydrase among three genetic groups

Variable	Genotype			Gene		χ^2 df=2	LOS
	FF	FS	SS	F	S		
Overall							
Observed	25	88	37	40	110	76.25	***
Expected	50	50	50	75	75		
Frequencies	0.17	0.59	0.25	0.27	0.73		
Bunaji							
Observed	11	27	12	15	35		
Expected	16.7	16.7	16.7	25	25		
Frequencies	0.22	0.54	0.24	0.30	0.70		
Friesian X Bunaji							
Observed	23	8	19	10	40		
Expected	16.7	16.7	16.7	25	25		
Frequencies	0.46	0.16	0.38	0.20	0.80		
Gudali							
Observed	6	4	40	8	42		
Expected	16.7	16.7	16.7	25	25		
Frequencies	0.12	0.08	0.8	0.16	0.84		

LOS-Level of significance; *p<0.05-significance, p>0.05-not significance

4.2 Performance of Three Genetic Groups in Morphometric and Selected Milk Production

Traits

The mean of morphometric and selected milk production traits of the three breeds is presented in Table 4.4. Body Weight (BW) and all other measured traits differed significantly ($p < 0.05$) between the breeds; the highest BW and BL were obtained in the Friesian–Bunaji and this differed from the Sokoto Gudali and the sokoto Gudali differed from the Bunaji which had the least BW and BL. The Sokoto Gudali had the highest HW, CW, HG, Rumwi and TY (178.42cm, 31.69cm, 127.78cm, 50.18cm and 1388.52litres), followed by the Friesian–Bunaji (174.99cm, 25.13cm, 124.09cm, 43.53cm and 1097.59litres). TL was significantly higher in the hybrid (5.10cm) and differed from the Bunaji (4.67cm) which also differed from the Sokoto Gudali (4.47cm). The same trend was observed with RUH and UC except that UC was similar in the Bunaji and Gudali. TY in this study was significantly higher in the Sokoto Gudali (1203.52litres), this is due to the fact that age and parity were not considered during the sampling. While the Bunaji and its hybrid were statistically ($p > 0.05$) similar; ADY indicated a reversed trend with the Friesian–Bunaji having the highest production while the Bunaji and Sokoto Gudali were similar. LL was higher and similar between the Bunaji and Friesian–Bunaji compared to the Sokoto Gudali which had the least LL value.

4.3 Effect of Haemoglobin Types on Morphology and Milk Production

The effect of the haemoglobin polymorphism on growth and milk traits of the breeds of cattle is presented in Table 4.5: Polymorphic forms of haemoglobin only significantly ($p < 0.05$) influenced Bw, Bl and cw. While the other traits indicated non-significant variations due to haemoglobin types. The highest Bw and Bl was observed with the AB genotype (391.15kg and 178.95cm) while the BB and AA homozygote individuals exhibited statistically similar ($p > 0.05$)

values for these traits. cw was also larger with the homozygote individual and differed from the BB genotype, while the BB genotype differed from the AA genotype. It was also noted that for most traits studied which did not show significant variation, the heterozygote had higher mean values for most of these.

4.4 Influence of Transferrin types on Morphology and Milk Production

Table 4.6 presents the influence of polymorphic forms of transferrin protein on studied traits. BW showed significant ($p < 0.05$) variation, the highest mean value was noted with the AB and BB individuals and they were statistically similar but differed from the AA genotype with the least Bw. Bl, Hg, Ty and Le showed variations of means with the highest value established in the BB genotype, while the AB was similar to the BB and AA genotype that exhibited the least mean value for all these traits. Hw and Cw displayed the highest mean with the BB individual and this differed significantly from the AB genotype and the AB genotype values differed from the AA which had the least values for these traits.

Table 4.4: Performance of Genetic Groups in morphometric and selected milk Production traits

Genetic Groups	Bunaji	Friesian X Bunaji	Gudali	SEM
BW	379.95 ^c	395.40 ^a	388.42 ^b	3.35
BL	175.48 ^c	180.63 ^a	178.35 ^b	1.01
HW	170.02 ^c	174.99 ^b	178.42 ^a	0.78
CW	22.22 ^c	25.13 ^b	31.69 ^a	1.13
HG	124.94 ^b	124.09 ^b	127.78 ^a	1.20
Rumwi	44.00 ^b	43.53 ^b	50.18 ^a	3.08
TL	4.67 ^b	5.10 ^a	4.47 ^c	0.36
RUH	19.69 ^b	24.43 ^a	17.45 ^c	1.95
UC	41.35 ^b	44.08 ^a	40.08 ^b	1.56
TY	1042.87 ^b	1097.59 ^b	1203.52 ^a	47.71
ADY	4.37 ^b	7.40 ^a	5.43 ^b	0.76
LL	245.33 ^a	255.68 ^a	218.99 ^b	9.61

^{abc}Means with different superscript across rows differ significantly (p<0.05)

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

Table 4.5: Effect of Haemoglobin Types on Morphology and Milk Production

Haemoglobin	AA	AB	BB	SEM
BW	383.83 ^b	394.15 ^a	387.92 ^b	3.35
BL	176.99 ^b	180.95 ^a	178.59 ^b	1.01
HW	174.17	174.87	173.88	0.78
CW	24.98 ^c	28.58 ^a	25.75 ^b	1.13
HG	126.5	125.63	125.26	1.20
Rumwidth	45.29	46.43	44.80	3.08
TL	4.66	4.83	4.72	0.36
RUH	20.42	20.59	20.72	1.95
UC	41.52	42.04	41.99	1.56
TY	1187.81	1187.81	1172.24	47.71
ADY	4.49	5.45	5.37	0.76
LL	233.34	244.82	241.64	9.61

^{bc}: Means with different superscript across rows differ significantly ($p < 0.05$)

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

Table 4.6: Influence of Transferrin types on Morphology and Milk Production

TF	AA	AB	BB	SEM
BW	385.17 ^b	388.31 ^a	392.75 ^a	3.35
BL	177.73 ^b	178.15 ^{ab}	179.76 ^a	1.01
HW	172.62 ^c	174.81 ^b	176.7 ^a	0.78
CW	23.66 ^c	26.92 ^b	28.58 ^a	1.13
HG	124.23 ^b	125.87 ^{ab}	127.05 ^a	1.20
Rumwi	44.02	46.29	47.63	3.08
TL	4.70	4.76	4.71	0.36
RUH	21.66	20.24	20.17	1.95
UC	42.49	41.7	41.32	1.56
TY	1170.04 ^b	1211.21 ^{ab}	1481.54 ^a	47.71
ADY	4.97	5.04	5.82	0.76
LL	235.42 ^b	240.32 ^{ab}	254.56 ^a	9.61

^{abc}: Means with different superscript across rows differ significantly ($p < 0.05$).

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

Table 4.7: Influence of Carbonic anhydrase on Morphology and Milk Production

CA	FF	FS	SS	SEM
BW	387.91	385.07	391.49	3.35
BL	178.21	177.54	178.88	1.01
HW	176.48 ^a	174.80 ^b	173.27 ^b	0.78
CW	25.9 ^b	28.16 ^a	24.26 ^b	1.13
HG	125.40	126.47	124.6	1.20
Rumwi	45.8 ^b	47.65 ^a	43.77 ^{ab}	3.08
TL	4.56	4.59	5.02	0.36
RUH	21.09 ^{ab}	18.72 ^b	22.78 ^a	1.95
UC	42.47 ^{ab}	40.63 ^b	44.67 ^a	1.56
TY	1238.43 ^b	1002.21 ^c	1482.61 ^a	47.71
ADY	5.14 ^{ab}	4.23 ^b	6.09 ^a	0.76
LL	240.94	236.93	243.45	9.61

^{abc}: Means with different superscript across rows differ significantly ($p < 0.05$).

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

4.5 Influence of Carbonic Anhydrase on Morphology and Milk Production

Effect of Carbonic Anhydrase on growth and milk production traits is shown in Table 4.7, significant ($p < 0.05$) differences existed with HW, CW, Rumwi, RUH, UC, TY and ADY among the forms of Carbonic Anhydrase forms. HW was highest (176.48) with the FF genotype and differed from the FS and SS which were similar. CW showed the FF and SS individuals having statistically similar means that differed from the FS which had the highest mean, similar trend was obtained with Rumwi. RUH, UC and ADY indicated similar variation with the homogeneous forms being higher and similar in means, while differing from the heterozygote form. Distinct variation in TY existed among the various forms of Carbonic Anhydrase; the SS form had the highest TY and differed from the FF which was next in rank and these all differed from the heterozygote form with the least TY.

Table 4.8: Effect of Breed and Haemoglobin forms on Morphology and Milk traits

Genetic Group	BUNAJI			FRIESIAN X BUNAJI			SOKOTO GUDALI			SEM
	AA	AB	BB	AA	AB	BB	AA	AB	BB	
HB	AA	AB	BB	AA	AB	BB	AA	AB	BB	SEM
BW	379.75 ^c	379.67 ^c	381.48 ^c	389.89 ^b	398.81 ^a	392.32 ^b	387.68 ^b	388.63 ^b	388.97 ^b	2.82
BL	175.51 ^c	175.12 ^c	175.99 ^c	180.70 ^a	180.66 ^a	180.40 ^a	176.83 ^c	178.86 ^b	178.95 ^b	0.99
HW	170.22 ^c	170.06 ^c	168.92 ^d	174.72 ^b	174.77 ^b	176.22 ^{ab}	178.27 ^a	177.23 ^a	170.32 ^c	0.94
CW	22.12 ^c	22.42 ^c	22.38 ^c	25.03 ^b	25.16 ^b	25.19 ^b	31.98 ^a	31.63 ^a	31.30 ^a	0.37
HG	174.35 ^c	175.53 ^b	176.76 ^b	174.53 ^c	173.88 ^c	174.11 ^c	183.3 ^{4a}	176.74 ^b	176.54 ^b	1.44
Rumwi	43.82 ^d	43.95 ^d	45.03 ^c	43.62 ^d	43.62 ^d	43.07 ^d	50.66 ^b	49.82 ^b	51.38 ^a	0.59
TL	4.63 ^b	4.82 ^{ab}	4.59 ^b	4.83 ^{ab}	5.23 ^a	5.04 ^a	4.53 ^b	4.48 ^b	4.35 ^c	0.11
RUH	19.90 ^c	19.29 ^c	19.39 ^c	25.38 ^a	24.23 ^b	23.58 ^b	17.36 ^d	17.48 ^d	17.51 ^d	0.38
UC	41.68 ^{ab}	40.89 ^b	40.57 ^b	43.50 ^a	44.37 ^a	44.00 ^a	39.12 ^b	40.41 ^b	40.40 ^b	1.26
TY	742.50 ^c	732.24 ^c	731.14 ^c	1855.00 ^a	1927.00 ^a	1837.00 ^a	1071.51 ^b	1074.10 ^b	1234.00 ^b	9.18
ADY	3.38 ^d	3.36 ^d	3.36 ^d	7.30 ^a	7.52 ^a	7.17 ^{ab}	4.36 ^c	4.39 ^c	4.96 ^b	0.15
LL	219.67 ^d	217.93 ^d	217.60 ^d	254.11 ^a	256.25 ^a	256.2 ^{0a}	245.76 ^b	244.66 ^b	248.79 ^b	1.85

abcd: Means with different superscript across rows differ significantly (p<0.05).

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

4.6 Effect of Genetic Groups and Haemoglobin forms on Morphology and Milk Traits

Table 4.8 shows the result of the interaction between breed and haemoglobin forms. All measured characteristics varied significantly ($p < 0.05$) within and between breed due to the effect of haemoglobin polymorphs. The highest BW (398.81 Kg) was observed in the AB phenotype of Friesian -Bunaji, followed by the BB (392.32 Kg) which was similar to the AA in this breed and all the other polymorphs in the Sokoto Gudali but differed from the Bunaji were all the Haemoglobin polymorphs were similar and had the least BW. Body Lengths were highest but similar among forms in the Friesian X Bunaji (180.40 – 180.70 cm) and differed from the Sokoto Gudali where the AB and BB ranked next (178.86 and 178.95cm) but differed from the AA with the least length in this breed (176.83) that was statistically similar to the records obtained among the polymorphs in the Bunaji breed (175.12 – 175.99 cm). Generally for HW, CW, HG and Rumwi, the interaction between polymorphic forms of Haemoglobin and the different breeds indicates lower measures in the Bunaji irrespective of forms and medium to higher values for the Bunaji-Friesian and the best performance for these traits in the Sokoto Gudali. TL, RUH, UC, TY, ADY and LL means indicated a reversal of these patterns with the best mean profile existing with the Friesian X Bunaji followed by the Bunaji and finally the Sokoto Gudali. Also it was observed that for most studied characteristics, the highest mean were noted for the AB phenotype among haemoglobin forms with the exception of HW, CW and HG.

Table 4.9: Effect of three genetic groups and transferrin forms on morphology and milk traits

Breed	BUNAJI			FRIESIAN X BUNAJI			SOKOTO GUDALI		
TF	AA	AB	BB	AB	BB	AA	AB	BB	SEM
BW	380.39 ^c	379.74 ^c	390.45 ^b	396.94 ^a	397.47 ^a	391.13 ^b	388.23 ^b	388.84 ^b	2.82
BL	175.78 ^b	175.33 ^b	180.64 ^a	180.66 ^a	180.31 ^a	176.30 ^b	178.39 ^a	178.95 ^a	0.99
HW	168.77 ^d	170.62 ^d	174.83 ^c	174.94 ^c	175.90 ^b	177.39 ^b	177.59 ^b	179.66 ^a	0.94
CW	22.01 ^c	22.32 ^c	24.98 ^b	25.15 ^b	25.41 ^b	28.39 ^{ab}	31.92 ^a	31.20 ^a	0.37
HG	173.42 ^c	175.67 ^{bc}	174.72 ^c	173.88 ^c	173.91 ^c	188.0 ^{9a}	178.01 ^b	177.36 ^b	1.44
Rumwi	43.76 ^d	44.12 ^d	43.66 ^d	43.55 ^d	42.97 ^e	47.71 ^c	50.16 ^b	51.49 ^a	0.59
TL	4.61 ^c	4.70 ^b	4.87 ^b	5.17 ^a	5.12 ^a	4.60 ^c	4.49 ^c	4.35 ^{cd}	0.11
RUH	19.44 ^d	19.81 ^d	25.49 ^a	24.17 ^b	23.38 ^c	17.21 ^e	17.46 ^e	17.51 ^e	0.38
UC	42.27 ^a	40.90 ^{ab}	43.38 ^a	44.50 ^a	42.73 ^a	39.34 ^b	40.11 ^{ab}	40.15 ^{ab}	1.26
TY	732.23 ^c	740.92 ^c	1851.74 ^a	1918.11 ^a	1801.44 ^a	1091.60 ^b	1075.11 ^b	1197.72 ^b	9.18
ADY	3.33 ^d	3.39 ^d	7.28 ^a	7.49 ^a	7.03 ^{ab}	4.45 ^c	4.39 ^c	4.82 ^b	0.15
LL	219.89 ^d	218.56 ^d	254.36 ^a	256.09 ^a	256.25 ^a	245.30 ^c	244.9 ^c	248.49 ^b	1.85

abcde: Means with different superscript across rows differ significantly ($p < 0.05$).

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

4.7 Effect of Three Genetic Groups and Transferrin Forms on Morphology and Milk Traits

Table 4.9 displays the output of the interaction among three genetic groups and transferrin polymorphism on growth and milk Production parameters. As with the interaction between Hb and genetic groups, significant ($p < 0.05$) variations were observed among all traits. BW showed high mean values in the Friesian X Bunaji for AA and AB (396.94 and 397.47kg respectively) forms of transferrin, which were equal but significantly different ($P > 0.05$) from the BB variant. BW in the BB of Bunaji was similar to those of AB and BB in the Sokoto Gudali (the only variants in the Sokoto Gudali) and the AA of Friesian X Bunaji. BW in the AA and AB of Bunaji were least in value and were statistically similar ($P > 0.05$). BL indicated similarity of higher means among the BB of Bunaji, AA and AB of Friesian X Bunaji and the AB and BB of Sokoto Gudali that differed from the AA and AB of the Bunaji and BB of the Friesian X Bunaji with lower but equal means. HW, CW and Rumwi revealed trend of superiority among forms of transferrin in the Sokoto Gudali compared to the two breeds, with higher means noted for the BB form compared to the heterozygote AB. While the Friesian X Bunaji existed as intermediate among the breeds, the Bunaji ranked lowest. HG only varied slightly from this trend with the highest mean obtained with the BB phenotype of the Friesian X Bunaji. TL, RUH, UC, TY, ADY and LL showed patterns of superior mean values consistently among forms in the Friesian -Bunaji compared to the other breeds followed by the Bunaji and then the Sokoto Gudali, RUH indicated inferior means for the BB of Friesian X Bunaji and all the other forms in the Sokoto Gudali breeds.

Table 4.10: Effect of three genetic groups and carbonic anhydrase forms on morphology and milk traits

GENETIC GROUPS	BUNAJI			FRIESIAN X BUNAJI			SOKOTO GUDALI		SEM
	FF	FS	SS	FF	FS	SS	FF	FS	
BW	381.30 ^b	379.39 ^b	380.11 ^b	393.25 ^a	383.78 ^b	397.08 ^a	388.86 ^a	388.35 ^a	2.82
BL	175.71 ^c	175.51 ^c	175.35 ^c	180.39 ^a	181.14 ^a	180.62 ^a	178.39 ^b	178.34 ^b	0.99
HW	168.23 ^{cd}	170.88 ^c	169.63 ^c	174.69 ^b	175.00 ^b	175.05 ^b	178.48 ^a	177.67 ^a	0.94
CW	22.20 ^d	22.08 ^d	22.41 ^d	24.87 ^c	25.24 ^c	25.17 ^c	30.91 ^b	31.82 ^a	0.37
HG	173.16 ^{bc}	174.65 ^b	176.02 ^b	174.73 ^b	174.57 ^b	173.90 ^{bc}	187.11 ^a	176.96 ^b	1.44
Rumwi	44.48 ^b	43.60 ^{bc}	44.32 ^b	43.71 ^{bc}	43.51 ^{bc}	43.49 ^{bc}	49.51 ^a	50.29 ^a	0.59
TL	4.49 ^b	4.68 ^b	4.74 ^b	4.68 ^b	5.27 ^a	5.17 ^a	4.51 ^b	4.47 ^b	0.11
RUH	19.38 ^c	20.05 ^c	19.36 ^c	26.05 ^a	24.07 ^b	24.12 ^b	17.34 ^d	17.47 ^d	0.38
UC	44.3 ^{2a}	40.85 ^b	40.79 ^b	44.24 ^a	42.38 ^a	44.23 ^a	38.54 ^b	40.33 ^b	1.26
TY	744.26 ^c	738.36 ^c	734.26	1898.90 ^a	1782.40 ^a	1902.15 ^a	1081.11 ^b	1089.40 ^b	9.18
ADY	3.33 ^d	3.38 ^d	3.37 ^d	7.49 ^a	6.94 ^b	7.43 ^a	4.41 ^c	4.44 ^c	0.15
LL	223.50 ^c	218.45 ^d	217.88 ^d	253.52 ^a	256.83 ^a	256.01 ^a	245.15 ^b	245.36 ^b	1.85

abcd: Means with different superscript across rows differ significantly ($p < 0.05$).

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

4.8 Effect of Three Genetic Groups and Carbonic Anhydrase Forms on Morphology and Milk Traits

Table 4.10 shows the result of the effect of carbonic anhydrase on body weight, morphometric and milk Production characteristics of the different genetic groups studied. Significant ($p < 0.05$) variations were observed among carbonic anhydrase variants within breeds and between breeds for all studied traits. The highest BW was observed with the homozygote forms in the Friesian-Bunaji and this was similar to the means obtained in the Sokoto Gudali for the FF and FS type but differed from the Bunaji breeds where lower means were observed for all the variants. BL showed higher and similar means among variants in the Friesian X Bunaji and differed from the Sokoto Gudali which ranked next with equality of means among variants while differing from the Bunaji with lesser but similar means among the variants. Similar trends as observed in the interaction between breeds and Hb types for HW, CW, HG and Rumwi where higher means were observed among the variants in the Sokoto Gudali for these traits were observed here also. For TL, RUH, UC, TY, ADY and LL, similar pattern with HB and breed interaction were also observed. However no clear cut pattern of superiority of homozygous and heterozygous phenotypes among the variants could be established for Carbonic anhydrase.

4.9 Correlated Studies

4.9.1 Correlation of growth and milk traits for all genetic groups

Table 4.11 reveals the Pearson correlation coefficient among growth and milk traits pooled across all genetic groups. BW was significantly ($p < 0.05$, 0.0001) and positively correlated with BL, RUH, TY, ADY and LL. Only with TY, ADY and LL was it highly correlated. BL also was found to be positively and significantly related to RUH, TY, ADY and LL; HW had significant and negative association with CW, HG and Rumwi while been positively correlated with TL,

RUH, UC, TY and ADY and LL. CW had positive and significant correlation with HG, Rumwi, TY and LL but negatively with TL, RUH and UC. HG was only positively correlated with Rumwi and LL but negatively with TL, RUH, UC, TY and ADY. Significant and negative correlation existed between Rumwi and TL, RUH, TY, ADY and LL; TL showed highly positive and significant association with RUH, TY, ADY and LL, same trend was observed among udder conformation traits and milk Production parameters, with the exception of UC which had non-significant ($p>0.05$) positive relationship with UC.

4.9.2 Correlation of growth and milk production traits in the Bunaji

Phenotypic correlation for growth and milk Production traits in the Bunaji breed is described in Table 4.12. Observed correlations in the Bunaji breed were low and mostly non-significant ($p>0.01$) among measured characteristics. BW was negatively and significantly ($p<0.05$) correlated with HG. While BL was so with TL and TY; HW was positively correlated with HG and TL; HG with Rumwi and negatively correlated with TY. Negative correlation existed between Rumwi and TY, TL and UC; while UC was positively correlated with ADY.

4.9.3 Correlation of growth and milk production traits in the Friesian X Bunaji

Table 4.13 shows the correlation between studied traits in the Friesian X Bunaji cross: positive and significant ($p<0.05$) relationship existed between BL, TL and RUH; HW, UC and TY; Rumwi and ADY; TY and ADY. Significant and negative relationship was observed between BW and TL; CW and RUH; HG and TY; TL and TY and between RUH and ADY.

4.9.4 Correlation of growth and milk production traits in the Sokoto Gudali

Phenotypic correlation for growth and milk Production traits in the Sokoto Gudali breed is described in Table 4.14. Significantly ($p<0.05$) positive relationship in these breed among

measured characteristics existed between BW and BL; HG and RUH; TL and RUH; RUH, UC and TY; and UC with ADY. Significant and negative relationship existed between BW and HG; CW with HG and TL. All other relations were low and non-significant.

4.10 Multivariate Principal Component Analysis

4.10.1 Principal component analysis of morphometric traits for all genetic groups

Table 4.15 presents the share of total variance, factor loading and Eigen value of principal components of morphometric and milk traits pooled for all the genetic groups of cattle. The factor pattern coefficients were used to assess the relative contributions of the various body measurements in determining the numerical value of the corresponding factor (principal component). Three components were extracted from the original 12; PC1 with Eigenvalue of 2.73 accounted for 23% of total variation observed with loadings for Total Yield (TY) and Average Daily Yield (ADY). PC2 (1.78) and PC3 (1.07) accounted for 15 and 9 % respectively and loaded for Chest Width (CW) and Rump Width (Rumwi); and Body weight (BW) and Teat Length (TL) respectively. Community estimates ranged from 0.06 to 0.99.

4.10.2 Principal component analysis of morphometric traits in the bunaji

Table 4.16 presents the PC for the Bunaji indicating share of total variation, Eigenvalues and factor loadings. Five components were extracted from the initial 12. PC1 showed Eigenvalue of 1.36 and 11% of total variation, it loaded for Heart Girth (HG), Rumwi, and negatively for TY, PC2 had 10% of the total variation and 1.22 Eigenvalue and loaded for TL negatively and positively for Udder circumference (UC). PC3, PC4 and PC5 accounted for 9% of total variation each with Eigenvalues of 1.13, 1.12 and 1.03 respectively. PC3 loaded for Body length negatively, PC4 loaded negatively for CW and positively for ADY and PC5 loaded for CW and negatively for Lactation Length (LL). Community estimates ranged from 0.32 to 0.89.

Table 4.11: Correlation of growth and milk traits for all genetic groups

	BW	BL	HW	CW	HG	Rumwi	TL	RUH	UC	TYA	ADY	LL
BW												
BL	0.08*											
HW	0.00	0.01										
CW	0.06	0.05	-0.67***									
HG	0.01	0.03	-0.81***	0.65***								
Rumwi	-0.01	0.00	-0.47***	0.37***	0.46***							
TL	-0.05	0.04	0.21***	-0.12**	-0.17***	-0.10**						
RUH	0.08	0.11**	0.49***	-0.26***	-0.43***	-0.25***	0.18***					
UC	0.06	0.02	0.10**	-0.09*	-0.10**	-0.04	0.02	0.11**				
TY	0.22***	0.21***	0.23***	0.10**	-0.17***	-0.11**	0.18***	0.47***	0.11**			
ADY	0.19***	0.18***	0.26***	0.03	-0.19***	-0.14***	0.18***	0.40***	0.13**	0.72***		
LL	0.14***	0.12**	-0.10**	0.28***	0.12**	0.08*	0.11**	0.20***	0.05	0.58***	0.46***	

*: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.0001$.

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

Table 4.12: Correlation of growth and milk production traits in the Bunaji

	BW	BL	HW	CW	HG	Rumwi	TL	RUH	UC	TYA	ADY	LL
BW												
BL	-0.001											
HW	-0.02	0.03										
CW	0.00	-0.02	-0.04									
HG	-0.09*	0.03	0.11**	0.03								
Rumwi	-0.07	0.00	0.04	0.06	0.14***							
TL	0.04	-0.08*	0.08*	-0.04	0.04	-0.01						
RUH	-0.01	0.03	0.01	-0.03	0.07	0.02	-0.05					
UC	-0.04	0.06	-0.03	-0.02	0.00	0.06	-0.08*	0.07				
TY	0.03	-0.11**	0.00	0.00	-0.12**	-0.12**	0.00	-0.01	0.01			
ADY	-0.06	0.02	-0.02	0.03	0.01	-0.02	0.05	0.02	0.11**	0.06		
LL	-0.06	-0.01	-0.01	0.02	-0.04	0.06	0.01	-0.02	0.04	0.02	-0.02	

*: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.0001$.

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

Table 4.13: Correlation of growth and milk production traits in the Friesian X Bunaji

	BW	BL	HW	CW	HG	Rumwi	TL	RUH	UC	TYA	ADY	LL
BW												
BL	-0.01											
HW	0.01	-0.01										
CW	-0.03	-0.03	0.01									
HG	0.01	0.02	0.01	-0.07								
Rumwi	0.02	-0.07	0.04	-0.05	-0.02							
TL	-0.12**	0.14***	0.03	0.03	0.01	0.03						
RUH	-0.02	0.14***	-0.06	-0.12**	-0.02	0.00	0.04					
UC	0.07	0.01	0.09*	0.00	0.01	-0.02	-0.02	0.01				
TY	0.03	0.06	0.09*	0.00	-0.08*	0.03	-0.08*	-0.07	0.01			
ADY	0.03	-0.03	-0.06	-0.05	0.01	0.08*	0.01	-0.13**	0.03	0.08*		
LL	-0.06	0.01	-0.07	-0.01	0.03	-0.02	0.10	0.02	-0.04	-0.06	-0.01	

*: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.0001$

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

Table 4.14: Correlation of growth and milk production traits in the Sokoto Gudali

	BW	BL	HW	CW	HG	Rumwi	TL	RUH	UC	TY	ADY	LL
BW												
BL	0.09*											
HW	-0.01	0.04										
CW	-0.03	-0.03	0.02									
HG	-0.13***	-0.01	0.05	-0.10**								
Rumwi	-0.03	0.00	0.03	0.02	-0.07							
TL	0.02	-0.04	-0.02	-0.13***	0.02	-0.03						
RUH	0.01	0.07	-0.05	0.02	0.09*	0.06	0.12**					
UC	-0.04	-0.02	-0.01	-0.07	-0.06	0.04	0.02	0.08*				
TY	0.06	0.02	-0.09*	0.07	-0.02	0.01	0.01	0.12**			0.01	
ADY	0.02	0.02	0.00	0.03	0.01	-0.05	0.04	0.00		-0.03		
LL	-0.04	-0.10	-0.09*	0.03	-0.02	0.03	0.05	0.00	0.01	0.03	-0.03	

*: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.0001$.

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

4.10.3 Principal component analysis of morphometric traits in the Friesian X Bunaji

Principal component analysis of morphometric and milk traits in the Friesian X Bunaji cross is presented in Table 4.17. Six factors were extracted, share of total variance and Eigenvalue were (11%, 10%, 10%, 9%, 9% and 9%) and (1.34, 1.18, 1.14, 1.13, 1.07 and 1.03) respectively. PC1 loaded for TL and Rear Udder height (RUH), PC2 loaded for BL and RUH, PC3 loaded for Height at withers (HW) and CW, PC4 loaded for Rumwi, TL and ADY, PC5 loaded for HW, HG and UC and PC6 loaded negatively for Rumwi. Community ranged from 0.30 to 0.97.

4.10.4 Principal component analysis of morphometric traits in the Sokoto Gudali

Principal component analysis of morphometric and milk traits in the Sokoto Gudali is presented in Table 4.18. Six factors were extracted similar to that of the Friesian X Bunaji cross, share of total variance and Eigenvalue were (11%, 10%, 10%, 9%, 9% and 9%) and (1.27, 1.24, 1.20, 1.10, 1.09 and 1.04) respectively. PC1 loaded for TL, Rear Udder height (RUH) and TY PC2 loaded CW and negatively for HG, PC3 loaded BW, BL and negatively for LL, PC4 loaded for HG, PC5 loaded for Rumwi and UC and PC6 loaded negatively for CW and ADY. Community ranged from 0.31 to 0.99.

4.11 Stepwise Linear Regression Predictor for Total Milk Yield

Table 4.19 presents the stepwise linear regression for Total milk yield equation pooled for all breeds and within breeds. Prediction equation of TY showed R^2 values ranging from moderate (23.70) in the Bunaji to high (64.54) in the pooled data. BW, BL, CW, HG, Rumwi, RUH, ADY and LL were traits that featured in the overall prediction equation. In the Bunaji with R^2 value of 23.70 consisted of BL, HG and Rumwi as predictors for TY while HW and ADY were observed

in the Friesian X Bunaji cross with R^2 value of 51.55. The Sokoto Gudali only had one predictor component for predicting TY and this was RUH and it showed a R^2 value of 43.32.

4.12 Discriminant Analysis of Selection Traits

Table 4.20 shows the summary of stepwise selection of discriminating traits, TY, CW, ADY, RUH, LL, Rumwi, UC, TL and HG were shown to be the most discriminating variables among the three breeds. Their respective partial R^2 and F values were (0.84, 0.58, 0.22, 0.15, 0.13, 0.09, 0.08, 0.15 and 0.04) and (2278.64, 613.22, 123.85, 81.12, 65.12, 43.02, 37.10, 79.68 and 16.52) with high significant values of ($p < 0.0001$).

4.13 Genetic Distance and Classification into Genetic Groups

Table 4.21 presents the Mahalanobis genetic distance between the three genetic groups. The squared distance to breed indicated that the pairwise distance (0.495) between the Bunaji and Friesian X Bunaji cross was not significant ($p > 0.05$) and there exist a closer link between the two, while the significant ($p < 0.0001$) 3.938 distance between the Bunaji and Sokoto Gudali and 4.952 between the Friesian X Bunaji and Sokoto Gudali shows greater diversity away from the Sokoto Gudali, this was substantiated by the classification result presented in Table 4.22.

Table 4.15: Principal component analysis of studied traits for all genetic groups

Traits	PC1	PC2	PC3	Communality
BW	0.19	0.11	-0.54*	0.53
BL	0.19	0.09	0.04	0.34
HW	0.06	0.32	0.27	0.73
CW	0.02	0.60*	-0.04	0.53
HG	-0.09	0.21	0.41*	0.36
Rumwi	-0.12	0.49*	-0.04	0.49
TL	0.18	-0.17	0.59*	0.99
RUH	0.38	-0.30	0.07	0.99
UC	0.12	-0.11	-0.34	0.29
TY	0.54*	0.08	0.00	0.06
ADY	0.51*	0.02	0.01	0.47
LL	0.40*	0.30	0.03	0.71
Eigenvalue	2.73	1.78	1.07	
% Variance	23	15	9	

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days). *-loadings

Table 4.16: Principal component analysis of studied traits for the Bunaji

Traits	PC1	PC2	PC3	PC4	PC5	Communality
BW	-0.32	-0.17	-0.34	0.02	0.13	0.48
BL	0.25	0.19	-0.48*	0.13	-0.11	0.56
HW	0.21	-0.37	0.08	0.37	-0.27	0.74
CW	0.08	0.07	0.24	-0.42*	0.59*	0.32
HG	0.53*	-0.25	0.08	0.14	0.20	0.44
Rumwi	0.49*	-0.05	0.19	-0.31	-0.04	0.58
TL	-0.06	-0.45*	0.36	0.30	-0.03	0.91
RUH	0.20	0.23	-0.18	0.30	0.02	0.88
UC	0.19	0.56*	0.07	0.19	-0.12	0.52
TY	-0.42*	0.17	0.34	0.19	-0.05	0.64
ADY	0.04	0.33	0.37	0.45	0.34	0.89
LL	0.05	0.16	0.35	-0.33	-0.62*	0.59
Eigenvalue	1.36	1.22	1.13	1.12	1.03	
% Variance	11	10	9	9	9	

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).*-loadings

Table 4.17: Principal component analysis of studied traits for the Friesian X Bunaji

Traits	PC1	PC2	PC3	PC4	PC5	PC6	Communality
BW	-0.32	0.30	-0.30	-0.13	0.04	0.22	0.6
BL	0.34	0.42*	0.19	0.30	-0.14	0.39	0.37
HW	-0.23	0.27	0.41*	0.14	0.41*	-0.35	0.77
CW	-0.08	-0.29	0.56*	-0.30	-0.03	0.15	0.47
HG	0.11	0.01	-0.30	0.01	0.63*	0.22	0.41
Rumwi	-0.16	-0.13	-0.20	0.46*	-0.01	-0.58*	0.47
TL	0.41*	-0.13	0.31	0.43*	0.23	-0.03	0.97
RUH	0.42*	0.45*	-0.19	-0.02	-0.23	-0.23	0.97
UC	-0.18	0.35	0.10	0.03	0.42*	0.14	0.38
TYA	-0.35	0.19	0.25	0.34	-0.36	0.18	0.32
ADY	-0.26	-0.27	-0.22	0.51*	-0.03	0.38	0.30
LL	0.34	-0.32	-0.05	0.09	0.10	0.15	0.63
Eigenvalue	1.36	1.18	1.14	1.13	1.07	1.03	
Proportion	11	10	10	9	9	9	

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days). *-loadings

Table 4.18: Principal component analysis of studied traits for the Sokoto Gudali

Traits	PC1	PC2	PC3	PC4	PC5	PC6	Communality
BW	0.07	0.27	0.50*	-0.33	-0.24	-0.20	0.54
BL	-0.01	0.03	0.61*	0.18	0.00	-0.02	0.37
HW	-0.37	-0.19	0.21	0.24	0.30	-0.06	0.65
CW	-0.16	0.52*	-0.13	0.22	0.07	0.47*	0.49
HG	0.04	-0.55*	-0.06	0.47*	-0.20	0.23	0.31
Rumwi	0.08	0.23	-0.06	0.30	0.51	-0.43*	0.55
TL	0.45*	-0.30	0.02	-0.20	-0.10	-0.18	0.99
RUH	0.57*	-0.03	0.18	0.39	0.10	0.12	0.98
UC	0.28	-0.09	0.01	-0.25	0.64*	0.06	0.35
TY	0.48*	0.37	0.08	0.22	-0.20	0.19	0.52
ADY	0.04	-0.12	0.16	-0.39	0.26	0.63*	0.54
LL	0.24	0.14	-0.50*	-0.13	-0.08	-0.08	0.89
Eigenvalue	1.27	1.24	1.20	1.10	1.09	1.04	
Proportion	11	10	10	9	9	9	

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days). *-loadings

Genetic group	Dependent Variable	Model	R ² %
Overall	TY	-222.97+0.52BW+1.15BL+3.29CW-1.27HG-1.27Rumwi+7.22TL+9.75RUH+212143.74ADY+2.43LL	64.54
Bunaji	TY	1741.79-3.82BL-0.98HG-4.33Rumwi	23.70
Friesian X Bunaji	TY	1281.30+0.29HW+0.60ADY	51.55
Sokoto Gudali	TY	508.77+21.65RUH	43.32

Table 4.20: Summary of Stepwise Selection of Traits

Step	Entered	Partial R ²	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average Correlation	Squared Canonical	Pr > ASCC
1	TY	0.84	2278.64	<.0001	0.16	<.0001	0.42		<.0001
2	CW	0.58	613.22	<.0001	0.07	<.0001	0.71		<.0001
3	ADY	0.22	123.85	<.0001	0.05	<.0001	0.73		<.0001
4	RUH	0.15	81.12	<.0001	0.05	<.0001	0.75		<.0001
5	LL	0.13	65.12	<.0001	0.04	<.0001	0.76		<.0001
6	Rumwi	0.09	43.02	<.0001	0.04	<.0001	0.78		<.0001
7	UC	0.08	37.10	<.0001	0.03	<.0001	0.79		<.0001
8	TL	0.15	79.68	<.0001	0.03	<.0001	0.81		<.0001
9	HG	0.04	16.52	<.0001	0.03	<.0001	0.82		<.0001
10	HW	0.02	9.07	0.0001	0.03	<.0001	0.82		<.0001
11	BL	0.01	3.76	0.0236	0.03	<.0001	0.82		<.0001
12	BW	0.01	3.18	0.0423	0.03	<.0001	0.82		<.0001

Keys: BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days).

4.14 Percentage of Individual Cattle Classified into Groups

The posterior probability of membership of individual cattle in each population is presented in Table 4.22. The proportion of Bunaji classified as Bunaji was 63.33%, while 36.67% were classified as Friesian X Bunaji cross and 0% as Sokoto Gudali, 26% Friesian X Bunaji cross were classified as Bunaji, 66% as Friesian X Bunaji and 8% as Sokoto Gudali. 16% Sokoto Gudali were classified as Bunaji, 0% as Friesian X Bunaji and 84% were correctly assigned as Sokoto Gudali. Error level of classification was lowest for the Sokoto Gudali (0.16), while it was similar and higher for the Bunaji (0.37) and Friesian X Bunaji (0.34).

Table 4.21: Genetic Distance Among Three Genetic Groups

Mahalanobis Squared Distance to group			
Genetic groups	Bunaji	FRxBunaji	Sokoto Gudali
Bunaji	0.000	0.495	3.938
FrXBu	0.495	0.000	4.952
Sokoto Gudali	3.938	4.952	0.000

Table 4.22: Percent of individual Cattle classified into groups

Genetic group	Bunaji	Friesian X Bunaji	Sokoto Gudali
Bunaji	63.33	36.67	0
Friesian X Bunaji	26	66	8
Sokoto Gudali	16	0	84
Error Level	0.3667	0.34	0.16
Priors	0.3333	0.3333	0.3333

CHAPTER FIVE

5.0 DISCUSSION

5.1 Genotype and Gene Frequencies of Blood Proteins among three Genetic Groups

5.1.1 Genotype and gene frequencies of haemoglobin among three genetic groups

The frequencies of 0.44 (HbA) and 0.13 (HbB) obtained for the pooled Bunaji, Friesian X Bunaji and Sokoto Gudali cattle populations were lower to the report of 0.64 and 0.36, respectively reported by Abdussamad *et al.* (2004) for Zebu cattle and their crosses in Zaria. The frequencies of HbA (0.20, 0.20 and 0.40) obtained respectively for the Bunaji, Friesian X Bunaji and Sokoto Gudali cattle were contrary with the observation of Essien *et al.* (2011) that most of the animals studied revealed a high genotypic frequency of 0.518 for HbAA in haemoglobin types in Bunaji cattle and their Friesian crosses in Shika, Zaria-Nigeria. The preponderance of the HbAB over the HbAA and HbBB in all the genetic groups and in the pooled information were consistent with the report that Zebu cattle and their crosses with dominant HbAB genotype show a high degree of dominance over HbAA and HbBB and this was a result of natural selection (Abdussamad *et al.*, 2004). Also the lack of significant ($p>0.05$) variation among the frequencies observed also agreed with the findings of Essien *et al.* (2011) and may perhaps, apart from pointing to the stability of the population with regards to these genes, indicate that the Zaria environment may be favouring the HBAA genotype by natural selection, this observation confirms the conclusion of Esseine *et al.* (2011) that Hb type AA in the Nigerian Friesian-Bunaji crosses of cattle of Zaria environment was favoured by natural selection, possibly for adaptation against the hot tropical weather and tolerance to tick and helminthic infestation which was not achievable with exotic cattle breed. Also Akpa *et al.* (2013) in reporting the effect of age, sex and haemoglobin type on adaptive and blood biochemical characteristics of Red Sokoto goats have stated that the variation of Hb type with adaptive and blood biochemical characteristics was significant ($P<0.05$) and that the

relationship between Hb types and heart rate can be linked to the different oxygen carrying capacity of the Hb types. In their study, higher Heart Rate was observed in goats with HbAA and HbAB, and HbA is known to be the haemoglobin allele with highest affinity for oxygen. This is in line with the earlier report of Huisman *et al.* (1969) who related the preponderance of HbA to greater affinity for oxygen, thus explaining the high adaptive coefficient they observed on goats with Hb types AA and AB since adaptive coefficient is a function of Heart Rate and Rectal Temperature.

5.1.2 Genotype and gene frequencies of transferrin among genetic groups

The overall allelic frequency of 0.16 and 0.09 for T^{f-A} and T^{f-B} observed respectively were lower than the range (0.17 – 0.24) reported for T^{f-A} in the Gudali (Queval, 1982 and Tawah and Rege, 1994). The deviation from Hardy-Weinberg equilibrium at the Tf locus significantly ($p < 0.01$) ($\chi^2 = 15.48$) were consistent with the findings of Ibeagha-Awenu (2004) on Polymorphisms in Blood Proteins of *Bos indicus* and *Bos taurus*. Bouquet and Osterhoff (1969) have reported that the nature and function of transferrin polymorphism has been subjected to speculations, theories and investigations. Few detailed researches on transferrin polymorphism have been carried out in recent times, however, though fraught with contradictions and inadequate explanations, the transferrin locus has been associated with fertility. The overall frequency of A (0.16) and B (0.09) allele were lower to the findings of Lukac *et al.*, (2013) in Holstein cattle, however it differed in the respect of conformity to Hardy-Weinberg equilibrium. The absence of the BB genotype in the Bunaji and the low frequency (0.08) of BB recorded in the Friesian X Bunaji in this work confirmed the findings of Ashton (1968) that the B and F allele were only present in the *Bos indicus* and not in *Bos taurus*. Since the Bunaji and its cross with Friesian are taurine breeds, the observation is credible. Also the lower frequency (0.04) of the AA and (0.45) of the A allele in the Sokoto Gudali compared to the Bunaji and Friesian X Bunaji (0.30 and 0.04) and (0.12 and 0.12) respectively observed supports the findings of Mukherjee and Yap (1979) that temperate

breeds have higher frequency of the A allele compared to the Zebus. Observed frequencies for the taurine cattle in this study were comparable to the observation of 0.22 – 0.53 reported for the Muturu (Abogaye *et al.*, 1998)

5.1.3 Genotype and gene frequencies of carbonic Anhydrase among genetic groups

The observed overall frequencies of 0.25 (S) observed in this study were lower to the range 0.79 - 0.91 (S) observed and reported for different genetic groups (Panepucci, 1989) and well within the range 0.59 – 1.00 required as stated that the S allele is the predominant allele in all breeds (Sartore *et al.*, 1969; Shanker *et al.*, 1983) with these range. There was a marked absence of the Z allele in this study, the value obtained for the Bunaji concerning F: S (0.22: 0.24) was similar from the findings in Pitangueiras cattle with the ratio were 0.13:0.82 (Lemos *et al.*, 1990). However the ratio observed in the hybrid (Friesian X Bunaji) and Gudali (0.16:0.46 and 0.16:0.84) were comparable. Penedo (1981) showed a 1.00 for 5/8 Holstein Friesian, this may undoubtedly have modified the ratio observed in the hybrid towards something slightly closer to what is predominantly obtained in this locus for cattle. Similar ratio (0.99:0.01) was reported by Petit and Queval (1973) as cited in Abogaye *et al.* (1998).

5.2 Performance of Genetic Groups in Morphometric and Selected Milk Production

Traits

The observed significant ($p < 0.05$) differences in all body and milk production traits measurements of the three studied genetic groups indicates clear group distinction. Observed value for the Bunaji BW (379.95kg) was higher than the value of 249kg reported for the NAPRI cow herd (Kanai *et al.*, 2013) but fell within the range 250 – 380kg as reported by Tawah and Rege (1994), while the value 395.40 obtained was lower than the value 491kg recorded by these authors for the Friesian X Bunaji. This may be attributed to differences in age of sampled herds, season of study amongst other things. The value of 388.42 kg observed

in the Sokoto Gudali was higher than the range of 241-353kg, 335-336kg and 360 – 363kg reported for the Sokoto Gudali, Adamawa Banyo and Yola Gudali but within the range 330-408kg for the Nguadere Gudali of Adamawa (Tawah and Rege, 1994). Since it has been noted that the Gudali is more of a beef cattle compared to the Bunaji, the observed superiority of the Gudali to the Bunaji in BW appears tenable. The Superiority of the Gudali over the Bunaji in HW, CW, HG and Rumwi were consistent with the findings of Yakubu *et al.* (2010). They stated that generally, the linear body measurements of Sokoto Gudali were significantly ($P<0.05$) higher than those of the Bunaji cattle with the exception of body length and face length respectively, this study however showed differences in BL between the two breeds.

Comparative measurements of morphometric traits can provide evidence of genetic groups relationships and size. The considerable variation in body dimensions of the two cattle breeds might not be unconnected with individual breed's potential and peculiarities. While the Bunaji cattle is noted for milk production, their Sokoto Gudali counterparts which is often ranked second in milk production produce more meat and appear to have more draught power than the former Yakubu *et al.*, (2010). The superiority of cross bred animals to local breeds in this study needs not be emphasised as it is a generally accepted trend in animal improvement work. The estimates for BL (175.48 – 180.63) were comparable to 175.29 – 179.02 reported by Yakubu *et al.* (2010). HW estimates of 170.02 -178.42 were higher than 110-148.40 reported by various authors for different cattle breed (Rege, 1999; Espinoza *et al.*, 2009 and Alsiddiq *et al.*, 2010). Observed measures of HG range of 124.09-127.78 cm obtained were lower than the values 141-151cm reported for Bunaji (Kanai *et al.*, 2013). Average daily yield of 3.37 to 4.43 l observed were comparable to 4.8l reported for Bunaji and Friesian X Bunaji (Kanai *et al.*, 2013). But the value 7.40 observed for the hybrid was higher than Bunaji and Sokoto Gudali estimate. LL (days) of 245.33 was within the range of 173 – 249.5

reported by Tawah and Rege, 1994. While the LL of 218.99 days obtained for the Sokoto Gudali were comparable to the values of 216 – 225 for Yola Gudali in Kafare station (Tawah and Rege, 1994). The significant superiority of the Sokoto Gudali in milk production compared to the Bunaji was contrary to the claim by Tawah and Rege (1994) that generally the Sokoto Gudali is a relatively poor milker compared to the White Fulani and the other important zebu breeds in this region.

5.3 Blood Polymorphism and Productivity

5.3.1 Effect of haemoglobin types on morphology and milk production

It was observed that BW, BL and CW were significantly ($p < 0.05$) influenced by Hb types. There exists a deficit of literature reports on the impact of Hb types on growth and production traits. However, Lemos *et al.* (1990) reported that haemoglobin types had no significant influence on LL. No direct evidence exists of differences among the three Hb genotypes (AA, AB and BB) for fitness in cattle (De Vito *et al.*, 2002). Bangham and Blumberg (1958) reported that Hb type did not significantly affect milk yield and butterfat percentage in dairy cattle. There exist variations in literature reports on the effect of polymorphic forms of Hb on body traits; Guney and Darcan (2000), Darcan and Guney (2001) had reported that haemoglobin type had influence on performance of sheep and goats, while Das *et al.* (2004) found no significant relationship between Hb type and body weight, body length, heart girth and height at withers in Garole sheep. It should be noted that goats exhibit a very complex Hb polymorphism due to the presence of a number of allelic and non-allelic chains both in the alpha and beta globin systems (Pieragostini *et al.*, 2005). This may be responsible for the lack of clear pattern and accord in obtained results and literature reports on their impact on morphological traits. However, the significant effect of the interaction between breed and Hb types on growth and milk production traits in this study may point to the impact of Hb on fitness which may in turn influence growth and productivity. However, the lack of preceding

literatures makes it difficult to compare and contrast. It may be posited that breed and Hb interaction may be a good source of variation in adaptability and productivity.

5.3.2 Effect of transferrin types on morphology and milk production.

As observed in the Hb locus, where no definite literature exists as to the impact of these blood proteins on growth and productive traits, the transferrin locus presents the same dilemma. However the superiority of the AB and BB genotype to the AA was observed for all traits. Mukherjee and Yap (1979) have previously shown that no significant variation ($p > 0.05$) between the effects of the different transferrin genotype (AA, AD, AE, DD and DE) on body weight and body conformation traits existed while Ashton and Hewetson (1968) reported that the allele D has a significant positive influence on milk yield; however other authors reported weak to negative influence (Jamieson and Robertson, 1967 as cited in Bouquet and Osterhoff, 1969). Bouquet and Osterhoff (1969) reported that in Afrikaner cattle breeds, animals that were homozygous for E allele were better able to withstand stress than the A and D allele bearing animals. Our observation on the superiority of the B allele over the A allele were similar to the report of Stratil (1968) who observed that chickens with type 'T^{fB}' have the advantageous egg production over the chicken with T^{fA}. While according to Lush (1966) as cited in Das and Deb (2008) the effect of heterozygous transferrin (T^{fBC}) appears to be significant including variability in the fertility, hatchability and egg production (at least 90 days' production). Chicken with T^{fA} appears to have delayed sexual maturity while the chicken with the T^{fB} has the earlier age of sexual maturity. In the interaction between breed and transferrin variants, the heterozygote AB and homozygote BB were observed to be better for most growth and milk related traits, this trend were comparable and similar to the findings of Shoyombo (2014) in goats but differed from the report of Guney *et al.* (2003) who found no significant effect of transferrin genotype on performance of Damascus goats. It can thus

be stated that selection of heterozygote animals for transferrin protein will yield animals with moderate to high growth performance. The advantage of this study lies in the fact that selection can be carried out at earlier ages than most morphometric and allometric traits of interest.

5.3.3 Effect of carbonic anhydrase types on morphology and milk production.

Reports of the impact of Carbonic anhydrase on morphometric traits are few; also morphological characteristics do not necessarily correspond to the genetic characteristics of blood protein and non-protein polymorphisms (Tsunoda *et al.*, 2010). This enzyme performs purely a buffering role in carbon, carbonate and pH regulation in the living cells. Findings obtained in this study however, revealed that highest BW was observed with the homozygote forms in the Friesian X Bunaji and this was similar to the means obtained in the Sokoto Gudali for the FF and FS type but differed from the Bunaji breeds where lower means were observed for all the variants. The FF form of it was superior for most indices and could serve as a potential tool for selection in growth and adaptation studies especially in environments where acid base balance is significantly influenced such as when animals travel long distance or during pre-slaughter stress which serves to disrupt body pH balance.

5.4 Correlated Relationship Studies

In this study, BW and BL were only significantly ($p < 0.05$) and positively correlated with body length when pooled for all genetic groups and in the SokotoGudali, the estimate was low, this observation may not be unconnected with the report that the Bunaji is generally taller and narrow-bodied than most European cattle breeds (Hall, 1991 as cited in Tawah and Rege, 1994). They are fairly medium to large size, with a well-balanced body of good depth and width, this general shallowness of the body and lack of width give the animal a "leggy" appearance. This characteristic of the breed has been described as an adaptation to long distance trekking (Oyenuga, 1967; Capitaine, 1972). While the Sokoto Gudali is generally

long with well-balanced and relatively compactly built animal possessing deeper and wider body than the Bunaji, so that every increase in length will lead to increase in body weight in the Sokoto Gudali. The pooled correlation revealed a strong positive and negative correlation between body morphometrics and milk productivity traits, however this trend was not observed to be so within breeds and this may have been due to the relatively low sample size employed per breed. The varying positive estimates of inter-correlatedness among traits for pooled data could be attributed to the fact that postnatal growth does not take place proportionally in all tissue categories and body regions. Instead, it gives preference in the different growth phases to particular tissue types or body regions within those tissue categories (Kallweit, 1993) since variations in age of sampled animal was not taken into cognizance to ensure uniformity of sample. Observed negative correlation between certain morphometric traits both in the pooled and individual genetic groups correlations were at variance with the observations of Okpeku *et al.* (2011) in goat breeds, Yakubu *et al.* (2009) in the white Fulani cattle. This trend indicates that selection on the basis of any of these traits will lead to a decrease in its associated trait. The non-uniformity of animals used with respect to age, differences in source of animal amongst others may give plausible explanation for this trend. However, this trend was comparable to the recurring negative associations (weak and strong) between BW, HG and BL at the different ages for the different breeds of goats by Shoyombo (2014). Yakubu *et al.*, (2011) showed that as age advanced coefficients of determination decreased while residual mean square increased. Also Thiruvankadan (2005) reported this trend in Kanni Adu kids under farmers management in Southern part of India. These observations may however be connected with the body condition score of the animals employed in this study which was not studied as animals could be tall or long but weigh less than stockier animals. Relationship of linear conformation traits with body weight body, condition score and milk yield in Friesian X Bunaji cows were positive indicating that taller, wider, deeper and fatter cows tended to be heavier (Alphonsus *et al.*, 2010). It has been

stated that the magnitude of the coefficient reflects active or passive growth at different age group in the species (Oke and Ogbonnaya, 2011).

5.5 Principal Component Analysis of Morphometric Traits

Obtained total sum of variance (47%) accounted for by the three PC in the pooled analysis and the five PC in the Bunaji were lower than the percentage of variance reported by Okpeku *et al.* (2011), while the six PC in the Friesian X Bunaji and Sokoto Gudali (58%) were within the range 55.3-95.2% reported by Yakubu *et al.* (2009) in the Bunaji but comparable to 52.48% in Red Sokoto and 54.49% in WAD goats (Shoyombo, 2014). The loading of PC1 for TY and ADY across all breeds supports the assertion of positive relationship between milk yield and general body condition based on morphometric composition (Alphonsus *et al.*, 2010). The variables associated with factor 2 CW and Rumwi described general body volume and broadness. Ignoring the negative loading, the PC3 loaded for TL. All this loadings goes to show that most of the animals studied are good milk producer.

Communalities, ranging from 0.31-0.99 for pooled and individual breed were wider than 0.91-0.99 reported by Okpeku *et al.* (2011) in the Maradi goats. This wideness lends moderate credence to the appropriateness of the factor analysis. It was observed that the components of the Gudali were closer to that of the pooled. However no clear pattern among breeds for corresponding principal components. This may lend credence to differences in breeds with regards to conformational and production traits and can also be attributed to low level of correlation coefficients among traits in this age, since in PCA analysis, the extent to which a set of variables decomposes into fewer factors depends on the level of redundancy (correlations) in original variables (Mavule *et al.*, 2013). Since principal components are uncorrelated by definition, the selection to improve body size which is an important target for beef production implies little or no variation in milk production across and within genetic groups.

5.6 Stepwise Linear Regression Predictor for Total Milk Yield

Observed constancy of BL, HG, Rumwi, HW, ADY and RUH as major predictors in the regression equations pooled for all genetic groups and within individual groups partly agrees with the report of Baffour-Awuah *et al.* (2000) that body lengths, width at shoulder and heart girth were significant predictors. However, the superiority of heart girth over other linear body measurements has been reported by other workers for growth targets (Baffour-Awuah *et al.* 2000; Leng *et al.*, 2010). This is not unexpected considering the high environmental sensitivity of heart girth. In this study however, BL appear to be superior to HG in influencing total milk yield. It should be noted that clear breed distinctions observed for major predictors across the breeds for total milk yield indicates differences in breed based on growth and development of body parts, so that programmes designed for selection and cross breeding must take this into recognition. The rump at udder height was the only predictor noted for influencing total yield in the Sokoto Gudali, this may be a pointer to the fact that the udder of the Sokoto Gudali is seemingly poorly attached unlike the Bunaji's that is fairly well-developed, is of good shape and is strongly attached with teats that are well positioned and are of medium to reasonably large size (Tawah and Rege, 1994).

5.7 Multivariate Analysis of Selection Traits

The Observation of TY, CW, ADY, RUH, LL, Rumwi, UC, TL and HG points to the fact that these genetic groups are different in all traits of interest most especially milk production traits as all traits pertaining to milk production were involved, only CW and HG were discriminating among growth related traits. The Presence of Rumwi as a discriminating factor in this study was in agreement with similar observations in the work of Yakubu *et al.* (2010) in the analysis of phenotypic differentiation in Bunaji and Sokoto Gudali cattle and in the report of Birteeb *et al.* (2012) in sheep. According to Herrera *et al.* (1996) Morphological variables are easy to monitor and may facilitate the use of ethnological characterization and at

the same time institute reliable racial discriminants. The three variables obtained in the present study are more important and informative and could be used to assign the cattle genetic groups into distinct populations, thereby reducing the errors of selection in future breeding and selection programmes especially in a research institute like NAPRI.

5.8 Genetic Distance Study

Genetic variation is vital for the populations to adapt to varying environments and to respond to artificial selection; therefore, any conservation and development scheme should start from assessing the state of variation in the population (Toro *et al.*, 2011). Observations of high morphological distance (3.94 and 4.95) between the Bunaji and Sokoto Gudali and Friesian X Bunaji and Sokoto Gudali were comparable to the values of 7.19 obtained between the Bunaji and Sokoto Gudali (Yakubu *et al.*, 2010) and were within the range of 5.72 – 31.88 reported between ewe and rams of Djallonke and Sahel sheep (Birteeb *et al.*, 2012).

In the classification of studied animals into genetic groups it was observed that the considerable (37% and 34%) erroneous classification of the Bunaji and Friesian X Bunaji as belonging to each other were comparable to those obtained (30.2%-31.7%) in classifying the Sahel as the Djallonke sheep (Birteeb *et al.*, 2012) and implies gene introgression within the breeding stock probably due to attempt at cross breeding. Similar reason is adduced for the lower error rate (16) encountered in classifying the Sokoto Gudali as either the Bunaji or the Friesian X Bunaji , this error rate was similar to 15% in the Bunaji (Yakubu, *et al.*, 2010). The higher proper classification (84%) of Sokoto Gudali compared to the Bunaji was comparable to the findings of Yakubu *et al.* (2010). Traoré *et al.* (2008) have attributed the cause of large misclassifications to the manifestation of introgressions across breeds resulting from the actions of most stock breeders who intend to obtain products with bigger conformation. The lower misclassification in the Sokoto Gudali breed may be an indication of more uniformity as a result of more genetic homogeneity of this breed than the other two

genetic groups. This inference is further supported by an error rate of 3% established in the Sokoto Gudali (Yakubu, *et al.*, 2010) compared to the Bunaji. The larger restriction of the Sokoto Gudali to its ecological zone, and the wide dispersal of the Bunaji may account in part for this observations and inference. It therefore follows that, the gene pool of the Bunaji is being eroded at a faster rate than the Sokoto Gudali counterpart. And efforts should be made to curtail this alarming trend.

The present information on the phenotypic differentiation of Bunaji and Sokoto Gudali could therefore be exploited in designing appropriate strategies for their management and conservation. However, there is a need for a genetic study using protein and DNA microsatellite markers to complement the results arisen from morphometric differentiation of the two most populous Nigerian breeds of cattle (Yakubu, *et al.*, 2010).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS.

6.1 Summary

1. A total of 150 animals, 50 per genetic group of the Bunaji, Friesian X Bunaji and Sokoto Gudali were used to study the relationship among genetic groups. The animals were sourced from National Animal Production Research Institute (NAPRI). They were raised under semi intensive system of management.
 2. Variables measured were BW: Body weight (Kg); BL: Body Length (Cm); HW: Height at withers (cm); CW: Chest width (cm); HG: Heart Girth (cm); Rumwi: Rump width (cm); TL: Teat Length (cm); RUH: Rear Udder Height (cm); UC: Udder Circumference (cm); TY: Total Yield (Litres); ADY: Average Daily Yield (Litres/day) and LL: Lactation Length (days). Also blood protein polymorphism of the Haemoglobin, Transferrin and Carbonic Anhydrase locus were evaluated.
 3. The Frequencies of blood protein polymorphism among genetic groups, the impact of this on measured variables, interaction among genetic groups and polymorphic protein forms and their impact on measured variables, correlated analysis of measured traits, principal component analysis of these variables, stepwise linear regression and multivariate analysis involving discriminant, genetic distance and classification components among the genetic groups were all computed.
1. Observed results showed overall frequencies of 0.44 (HbA) and 0.13 (HbB). Moderate to higher frequencies of the A allele (0.20, 0.20 and 0.40) were obtained respectively for the Bunaji, Friesian X Bunaji and Sokoto Gudali cattle in the Haemoglobin locus. Also the heterozygote genotype predominated across all breed and the population existed in Hardy-Weinberg equilibrium. In the Transferrin locus, only the A and B allele were observed. Overall allelic frequency of 0.16 and 0.09 for T^{f-A} and T^{f-B} were obtained, lower frequencies of 0.04 (AA) in the Sokoto Gudali and

higher and lower frequencies of (0.30 and 0.12) in the Bunaji and Friesian X Bunaji were also noted. For the Carbonic Anhydrase locus, only the F and S allele were observed, the overall S allele was 0.25 and the F was 0.17. The F:S in the Bunaji were (0.22: 0.24), Friesian X Bunaji and Sokoto Gudali (0.46:0.38 and 0.16:0.84). Both the Transferrin and Carbonic Anhydrase locus were not in Hardy-Weinberg equilibrium for the studied population.

2. Study of blood protein polymorphism and productivity indicated significant ($p < 0.05$) influence of the haemoglobin, transferrin and carbonic anhydrase on both body and milk production traits with no definite patterns, but rather it was more of the by product of the fitness and adaptability they confer on the animals. However, it was noted that the heterozygote genotype for most of the locus and genetic groups were superior to the homozygote.
3. Correlated studies were observed to be very significant among variables in the pooled analysis but majorly insignificant for individual genetic group, also estimates were generally low though ranging from -0.87 – 0.84 in the pooled which may be unconnected with sampling inadequacy
4. Principal component analysis observed to show factors ranging from 3 in the pooled data to 5 in the Bunaji and 6 in the Friesian X Bunaji and Sokoto Gudali.
5. Generally communalities ranged from 0.31 – 0.99 while proportion of variance accounted for by factors were 47% in the pooled and Bunaji and 58% in the cross bred and Gudali. However, no clear pattern for loaded variables could be established among the genetic groups.
6. Stepwise linear regression of variables indicated BL, HG, Rumwi, HW, ADY and RUH as major predictors in the regression equations pooled for all breed and within individual genetic group. In this study, BL appear to be superior to HG in influencing total milk yield, also clear genetic group distinctions were observed for major

predictors across the genetic groups for total milk yield indicates differences among the genetic groups and points to good selection and conservation potentials within and among genetic groups

7. Multivariate analysis indicated TY, CW, ADY, RUH, LL, Rumwi, UC, TL and HG as the most discriminating variables among the genetic groups. Their respective partial R^2 and F values were (0.84, 0.58, 0.22, 0.15, 0.13, 0.09, 0.08, 0.15 and 0.04) and (2278.64, 613.22, 123.85, 81.12, 65.12, 43.02, 37.10, 79.68 and 16.52) with high significant values of ($p < 0.0001$).

6.2 Conclusion

1. Considerable variation were obtained in all body and milk production traits measurements of the three studied population indicating clear genetic group distinction. It was noted that the Sokoto Gudali was superior to the Bunaji in most of these traits though the Sokoto Gudali is a relatively poor milker than the Bunaji
2. The overall frequencies at haemoglobin locus was 0.44 (HbA) and 0.13 (HbB), transferrin locus was 0.16 and 0.09 for T^{fA} and T^{fB} while carbonic anhydrase was 0.17 for S allele and 0.25 for F allele. This indicated that Transferrin and Carbonic Anhydrase locus were not in Hardy-Weinberg equilibrium for the studied population.
3. Genetic distance among genetic groups revealed high Mahalanobis values (3.94 and 4.95) between the Bunaji and Gudali and Friesian X Bunaji and Sokoto Gudali. Classification of studied animals into genetic groups based on discriminating variables revealed considerable (37% and 34%) erroneous classification of the Bunaji and Friesian X Bunaji as belonging to each other and 16 % Sokoto Gudali were also erroneously classified as either Bunaji or its crossbred counterpart. The Highest proper classification (84%) was in the Sokoto Gudali and it indicated greater genetic group homogeneity.

4. However, multivariate studies clearly indicated manifestation of gene introgression across genetic groups through indiscriminate cross breeding perhaps, as evidenced by the high levels of misclassification in the Bunaji, Friesian X Bunaji and Sokoto Gudali. However, this was more pronounced in the Bunaji herd. Consequently, the present information on the phenotypic differentiation of Bunaji and Sokoto Gudali could therefore be exploited in designing appropriate strategies for their management and conservation.

6.3 Recommendations

There is a need for a genetic study using protein and DNA microsatellite markers to complement the results arisen from morphometric differentiation of the two most populous Nigerian breeds of cattle in the NAPRI herd.

Effort must be made to correct the erosion of the gene pool of the Bunaji that is building up within the institute's herd in order to conserve their uniqueness. A more detailed study using animals with uniform age of the different genetic group is further recommended in carrying out relationship analysis based on protein and DNA markers.

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