SINGLE NUCLEOTIDE POLYMORPHISM OF THE INSULIN-LIKE GROWTH FACTOR - 1 GENE AND ITS EFFECT ON GROWTH TRAITS IN YANKASA SHEEP

BY

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DECLARATION

I declare that the work in this Dissertation entitled “**SINGLE NUCLEOTIDE POLYMORPHISM IN THE INSULIN-LIKE GROWTH FACTOR - 1 GENE AND ITS EFFECTS ON GROWTH TRAITS IN YANKASA SHEEP ”** was carried out by me in the Department of Animal Science, Ahmadu Bello University, Zaria, under the supervision of Dr. M. Kabir. The information derived from the literature has 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 this or any other Institution.

Chima Martin UMEGO **Signature:____________** **Date:_____________________**

CERTIFICATION

This Dissertation entitled **SINGLE NUCLEOTIDE POLYMORPHISM IN THE INSULIN-LIKE GROWTH FACTOR - 1 GENE AND ITS EFFECTS ON GROWTH TRAITS IN YANKASA SHEEP** by **Chima Martin UMEGO** meets the regulations governing the award of the degree of Masters of Science (Animal Science) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Dissertation is dedicated first to the ALMIGHTY GOD, Who has made everything possible, then to my father Prof. Marius Nnanna Umego and my mother, Mrs Caroline Chimezie Umego, for their tremendous contributions to my success in life. Also to all my brothers, sisters, relatives and friends all over, I cannot forget their labour of love,

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ABSTRACT

This study was conducted to determine single nucleotide polymorphism of the IGF-1 gene and its effect on some growth traits in Yankasa breed of Sheep. A random sample of 100 sheep (50 males and 50 females) were selected for the molecular study and the phenotypic evaluation. Animals were measured for growth traits namely: birth weight, average daily gain, weaning weight, weights at 6, 8 and 12 months, chest girth and height at withers. Blood samples were collected through the jugular veins into 5ml EDTA vacutainer tubes and transferred to the laboratory for DNA extraction, PCR-RFLP analysis. Total DNA extraction and PCR-RFLP was made with ZR-96 Genomic DNA miniprep. Frequency of alleles were calculated according to Hardy-Weinberg's equation and also subjected to Chi-Square analysis to test for Mendelian inheritance ratio for band. Association between genotypes and growth traits, sex and growth traits and their interaction along with correlation of measured traits were determined through statistical analysis. Both genotypic and allelic calculated χ^2 values of 9.07 and 16.94 respectively were greater than the tabulated values of 5.99 and 3.84 at 5% level of significance. All the growth traits (birth weight, weaning weight, average daily gain, weight at 6 months, weight at 12 months, height at withers and chest girth) with the exception of body weight at 8 months showed significant ($P<0.05$) variations among different genotypes. With the exception of average daily gain (g/day), which was non-significant ($P>0.05$) across the sexes, all other growth characteristics differed significantly ($P<0.05$) across sexes with the higher values recorded for males. Observed trend showed significant ($P<0.05$) genotype x sex interaction effect among the measured traits with the exception of average daily gain. Male sheep with AA and AB genotype were generally similar and more superior to males with BB genotype across all traits (birth weight, weaning weight, weights at 6 months, weights at 8 months, weights at 12 months and height at withers) with the exception of average daily gain. Significant ($P<0.05$, 0.01) correlation existed with coefficients ranging from -0.08 to 0.96. It was therefore concluded that there was a significant association of Polymorphisms in IGF-1 gene with growth traits (birth weight, weaning weight, average daily gain, weights at 6 and 12 months, height at withers and chest girth) in Yankasa sheep with AA and AB genotypes having similar but significantly higher propensity for growth than BB genotype. It is recommended that polymorphism of the IGF-I gene may be a potential molecular marker for selection for growth traits in Yankasa sheep.

CHAPTER ONE

1.0 INTRODUCTION

The economic importance of sheep in developing nations cannot be over-emphasized. Sheep with their small body size, high productive capacity and rapid growth rates are ideally suited to production by resource-poor smallholders. The population of sheep in Nigeria is currently estimated at 33.9 million making up 3.1% of the world's total (FAOSTAT, 2011). Sheep is an important livestock species in the socio-economic lives of people around the world including Nigerians (Yakubu and Ibrahim, 2011). They thrive in a wide variety of environments in the tropics and sub-tropics. Raising sheep requires less capital as they can be completely maintained on pasture, browse and agricultural waste products.

The Yankasa is a meat breed found in north and north central Nigeria. The Yankasa is a medium-sized breed of Sheep. The tail is long and thin, the ears moderately long and somewhat droopy. Rams have curved horns and a hairy white mane and ewes are polled (Mason, 1996). They have white coat colour with black patches around the eyes, ears and muzzle. Yankasa rams stand 70 to 80 cm at the withers and weigh 55 to 60kg at maturity. Mature females weigh 25 to 40kg. The milk yield (kg) per lactation is between 30 and 56kg with a lactation length of 91days. The peak milk yield per day is 0.96kg. The Yankasa Sheep are bred and selected for fecundity, high birth weight, rapid growth rate and good performance of offsprings.

The use of polymorphic genes as a detectable molecular marker is a promising alternative to the conventional methods of traits selection once these genes are proven to be associated with traits of interest in animals (Karim *et al.,* 2009). The main objective of the application of molecular biology techniques to animal genetic improvement programs currently is to identify, map and analyze polymorphisms of the genes involved in the main metabolic pathways that are related to animal growth and distribution of nutrients to the different tissues (Peter *et al.*, 2007). Recently, investigators and breeders focus on Marker-Assisted Selection (MAS) and genome analysis. MAS may increase annual rate of genetic gain in livestock by 15 to 30% without increasing the risk involved in breeding schemes (Ge *et al.,* 1997). In the livestock industry, growth traits that determine economic value of livestock are always of primary concern during breeding (Hua *et al.,* 2009). In farm animals, promising candidate genes for many traits are in the growth hormone (GH) axis.

The GH gene pathway contains various interdependent genes, such as GH, insulin-like growth factor1 (IGF1), pituitary specific transcription factor1 (PIT1), growth hormone releasing hormone (GHRH), somatostatin growth hormone releasing hormone receptor (GHRHR), growth hormone receptor (GHR), and others (Cogan and Phillips 1998). For growth traits, GH, GHR, insulin-like growth factor I (IGF-I), leptin (LEP), caprine-pituitary-specific transcription factor-1 (POU1F1), caprine myostatin (MSTN), and bone morphogenetic protein (BMP) genes are necessary for bone formation, birth weight, weaning weight, body condition, and muscle growth (Supakorn, 2009). IGF1 is a mediator of many biological effects: it increases absorption of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, stimulates the production of progesterone in granular cells, and intervenes in the synthesis of DNA, protein, RNA, and functions in cell proliferation (De la Rosa Reyna *et al.,* 2010). The IGF-1 was discovered as a skeletal growth factor produced in the liver that appeared to mediate the effects of the pituitary on whole-body somatic growth (Daughaday and Salmon, 1999). It had been shown that in addition to its production in the liver, IGF-1 is also produced in most, if not all, tissues. The liver has however been confirmed as by far the main source of the large amount of IGF found in circulation and it became increasingly apparent that pituitary Growth Hormone (GH) was not the only regulator but that they were also very strongly nutritionally dependent. Nutrition has many interacting effects upon the IGF system, including direct effects of certain nutrients upon hepatic expression and indirect effects via insulin and via changes in hepatic GH receptors (Ketelslegers *et al*., 1995). The IGFs play an important role in regulating somatic growth according to nutritional conditions. It has also become apparent that this forms part of a very fundamental control, ensuring that the development of the organism commensurate to the nutritional supply. This control system has been conserved throughout evolution from yeast to higher mammals. Insulin-like Growth Factor (IGF) plays an important role in lactation and is involved in a variety of physiological processes including reproduction, fetal development and growth (Adam *et al*., 2000; Shen *et al*., 2003). Insulin-like Growth Factor-1 (IGF-1) gene is also considered to be a factor that regulates growth, differentiation and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane-associated glycoprotein receptors (Werner *et al*., 1994).

The predicted sequence of amino acids of IGF-I peptide differs from the human, bovine, and porcine IGF-Is at a single amino acid (at position 66, alanine is substituted for proline) and differs from rat and mouse IGF-Is at positions 4 and 5, respectively. Ovine IGF-I amino-terminal peptides are 1 amino acid longer than other mammalian IGFs due to the presence of an extra amino acid (glutamine) that is present at the proposed boundary of exon1 and exon2 (Wong *et al.*, 1989). IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death. Such findings suggest that variations in IGF-1 gene in domestic animals may be important contributors to differences in their growth rate. However, studies on associations of IGF-1 polymorphism with growth traits are mainly carried out in cattle but examination of associations between SNPs in these genes and growth traits has not been reported in Sheep, in spite of the functional importance of IGF-I in the regulation of GH.

Through long evolutionary accumulation, many different instances of each type of mutation should exist in any given species, and the number and degree of the various types of mutations define the genetic variation within a species. DNA marker technology can be applied to reveal these mutations. All organisms are subject to mutations as a result of normal cellular operations or interactions with the environment, leading to genetic variation (polymorphism). In conjunction with selection and genetic drift, there arises genetic variation within and among individuals, species, and higher order taxonomic groups. For this variation to be useful to geneticists, it must be heritable and discernable to the researcher, whether as a recognizable phenotypic variation or as a genetic mutation distinguishable through molecular techniques.

At the DNA level, genetic variations also occur as a result of base substitutions, commonly referred to as single nucleotide polymorphisms (SNPs), insertions or deletions of nucleotide sequences (indels) within a locus, inversion of a segment of DNA within a locus, and rearrangement of DNA segments around a locus of interest. In fact, the SNP concept has basically arisen from dire need for very high densities of genetic markers for the studies of multi-factorial diseases, and the progress in polymorphism detection and genotyping techniques (Vignal *et al.,* 2002). Large deletions and insertions (indels) cause shifts in the sizes of DNA fragments produced upon digestion by restriction enzymes, and are among the easiest type of mutations to detect, mainly by electrophoresis of the fragments on an agarose gel; smaller indels require DNA sequencing or more elaborate electrophoretic techniques to determine smaller changes in size. Inversions and rearrangements that involve restriction sites can be easy to detect because they disrupt the ability of a restriction enzyme to cut DNA at a given site and thus can produce relatively large changes in DNA fragment sizes. Point mutations are more difficult to detect because they do not cause changes in fragment sizes. In the past, allozyme and mtDNA markers have been popular in genetics research, more recent marker types that are finding service in this field include restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite, single nucleotide polymorphism (SNP) and expressed sequence tag (EST) markers (Liu and Cordes, 2004).

1.1 Justification

DNA marker technologies have revolutionized the way genetics research is conducted. The dramatic development of molecular genetics since the first widespread use of allozymes in the 1970s, and currently exemplified by the Human Genome Project and other equally ambitious undertakings, has laid the groundwork for genomics. Broadly defined as the study of genes and their functions, genomics is rapidly impacting many facets of life, from health care and food safety to reproduction and law enforcement. Due to poor genetic potential of Nigerian livestock species ang long generation interval of ruminant animals, polymorphic traits can be used as a selection criterion for traits of great economic importance (Kubek and Bardun, 1990). A marker with high polymorphism is easier to identify which also means higher accuracy of selection and higher genetic gain (Leenstra *et al.*, 1994). With markers, the generation interval could be reduced or halved while doubling the genetic gain and maintaining the same increase in accuracy of selection (Georges and Massey, 1991). Therefore, breeding for optimal growth traits and higher gains are the main considerations in goat and Sheep breeding programs. Most genetic variation is represented by single nucleotide polymorphisms and many of them are believed to cause phenotypic differences between individuals. Identification of causative mutations that affect growth traits will greatly enhance progress towards this goal.

1.2 Objectives

The overview of this study was to investigate SNP in the IGF-I gene in Yankasa Sheep and to determine the associations between these polymorphisms and growth traits of Yankasa Sheep. This is intended to be the first step of a more in-depth study of the IGF-1 gene of the Yankasa Sheep breed in order to establish a breeding program based on marker assisted selection.

Therefore, the objectives of this study are:

- To identify single nucleotide polymorphisms in the IGF-1 gene of Yankasa Sheep.
- To determine the existing association between SNP in the IGF-1 gene and growth traits in Yankasa Sheep.
- To determine if the identified polymorphism can be used as a selection criterium for growth traits in Yankasa Sheep.

1.3 Hypotheses

The present work therefore, was designed to evaluate the following hypotheses:s

H_o: SNP in the IGF-1 gene of the Yankasa breed of Sheep has no effect on growth traits.

H_a: SNP in the IGF-1 gene of the Yankasa breed of Sheep has effect on growth traits

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and domestication of sheep

Sheep was first domesticated in the new Stone Age Evidence for the domestication of sheep dates to 9000 BC in Iraq. DNA analysis has shown that domestic sheep descended from two ancestor species, one of which is the mouflon. The mouflons comprise *O. musion* and *O.orientalis*. Although the second ancestor has not been identified, both the urial and argali have been ruled out. The urial (*O. vignei*) is found from northeastern Iran to northwestern India. It has a higher number of chromosomes (58) than domestic sheep (54) which makes it an unlikely ancestor of the latter *(http://www.unaab.edu.ng/*index.html*).*

Sheep (*Ovis aries*) are believed to have been among the first animals to be domesticated, preceded by the dog and goat. The domestication of both sheep and goats probably dates back to the pre-settled agricultural period. It is also believed that most domestication took place in western Asia where the majority of the present day small ruminant breeds likely originated. Existence of some ancestral stock of wild sheep extends from Western Europe to China (Kassahun and Solomon, 2008).

Ethiopia has long been recognized as a gateway of genetic material from Asia to Africa, and its diverse ecology served to further diversify and develop the genotypes it received (IBC, 2004) and Ethiopia is home for an estimated 28.89 million sheep (CSA, 2016) and about 14 traditional sheep populations (Solomon *et al.,* 2007). Ethiopia is one of the countries with the largest and most diverse sheep population in Africa (Getinet *et al.,* 2005). Sheep maintained virtually under the traditional subsistence oriented management systems, constitute an important livestock component in all ecological zones and agricultural systems in the country (Berhanu and Azage 2006).

2.2 Molecular Genetics in Animal Genetic Improvement

Body size, as measured by height in humans or weight in domestic species, is an archetypical quantitative or complex trait that shows continuous variation. It has been extensively recorded and studied for over a century because of its importance to ecology, its relevance in farming, and because it is an important indicator of growth and health (Kemper *et al*., 2012). Genetic progress for quantitative traits in livestock production, especially for goats and Sheep have been made by selection based on phenotypes and estimated breeding value derived from phenotypes. These strategies do not require any knowledge of genes that influence the traits or the effect of each gene because utilization of advances in molecular genetics in the past decade for selection processes is expensive. Therefore, the substantial rates of genetic improvement have been achieved and continue to be achieved on this quantitative genetic approach (Tambasco *et al*., 2003).

The success of this approach depends on accurate information concerning data or data structure and genetic evaluation methods. This selection is without any knowledge of the number of genes that affect the trait or the effects of each gene. For the last decade, molecular genetics has led to the discovery of individual genes or candidate genes with substantial effects on the traits of economic importance.

Candidate gene strategy has been proposed by direct search for quantitative trait loci (QTL) (Tambasco *et al*., 2003). In other words, the genetic variation in a gene affects the physiological pathways and phenotype. Moreover, the proportion of genetic and phenotypic variation would be likely to affect the breeding strategy for improvement of important traits in the future. Genetic markers associated with traits of interest can be searched directly by applying molecular biology techniques. These techniques can identify genetic variation at specific loci and analyze the relationship between genetic variation at QTL and production traits (Van Arendonk *et al*., 1997). Application of molecular genetics for genetic improvement relies on the ability to genotype individuals for specific genetic loci. The information utility from candidate genes in breeding programs has potential to substantially enhance the accuracy of selection (Missohou *et al*., 2006). Many studies have reported that the candidate genes influence growth, reproductive, milk, wool and disease resistance traits in goats and Sheep. In addition, some genes control more than one trait. For instance, the growth hormone (*GH*) gene influences expression of growth and milk traits. The important candidate genes for goats play a key role in productivity, metabolism, sex determination, reproduction and disease resistance (Li *et al*., 2006; Gupta *et al*., 2007; Lan *et al*., 2007).

2.3 Insulin-like growth factor 1 (IGF 1)

The insulin-like growth factor 1 (IGF1) signaling pathway arose early in evolution, possibly as a regulator of cellular proliferation in relation to nutrient availability (Longo and Finch, 2003). This function is conserved in mammals. Insulin and IGF1 have key roles in regulating cellular proliferation and apoptosis in relation to diet but additional regulatory roles related to energy metabolism, body size, longevity and various organ-specific functions 2–5 have been acquired.

Protein *IGF-1* is one of the key components of the pathway of growth hormone (Franco *et al*., 2005). The Insulin-like Growth Factor-1 (IGF-1) was discovered as skeletal growth factors produced in the liver that appeared to mediate the effects of the pituitary on whole-body somatic growth (Kazemi *et al*., 2011). Many variations in the genome affected gene expression at the transcription and translation levels. Variations in the genes of somatotropic axis could function as candidates for the evaluation of their effects on animal growth and development traits. In humans, mutations at important regulatory sites of the IGF1R gene were associated with growth. Such mutations resulted in the failure of processing of proIGF1R to mature IGF1R and caused dysfunction and short stature of IGFR. These variations affected partly the expression and physiological functions of the IGF1R gene, and subsequently affected growth (Mehdi *et al*., 2014).

It had been shown that in addition to production in the liver, IGF-1 was produced in most, if not all, tissues. The liver has however been confirmed as by far the main source of the large amount of IGF found in the circulation and it became increasingly apparent that pituitary Growth Hormone (GH) was not the only regulator but that they were also very strongly nutritionally dependent. Nutrition has many interacting effects upon the IGF system, including direct effects of certain nutrients upon hepatic expression and indirect effects via insulin and via changes in hepatic GH receptors (Ketelslegers *et al*., 1995; Kazemi *et al*., 2011). The IGFs play an important role in regulating somatic growth according to nutritional conditions. It has also become apparent that this forms part of a very fundamental control, ensuring that the development of the organism proceeds appropriately to the nutritional supply. This control system has been conserved throughout evolution from yeast to higher mammals. Also, Insulin-like Growth Factor (IGF) plays an important role in lactation and is involved in a variety of physiological processes including reproduction, fetal development and growth (Adam *et al*., 2000 and Shen *et al*., 2003). IGF1 gene is also considered to be a factor that regulates growth, differentiation and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane-associated glycoprotein receptors (Kazemi *et al*., 2011).

Understanding insulin-like growth factor-1 (IGF1) biology is of particular importance because, apart from its role in mediating growth, it plays key roles in cellular transformation, organ regeneration, immune function, development of the musculoskeletal system and aging. IGF1 bioactivity is modulated by its binding to IGF-binding proteins (IGFBPs) and the acid labile subunit (ALS), which is present in serum and tissues and has thus become a major candidate gene in livestock breeding and selection endeavors.

Insulin-like growth factor (IGF) consists of a family of polypeptide hormones structurally associated with insulin with multiple metabolic functions (Li *et al.*, 2003). The IGF are important regulators of growth, protein synthesis and cell proliferation and differentiation in a variety of cell types (Scanes *et al.*, 1999). The IGFs have been shown to regulate body and muscle growth in farm animals (Duclos *et al*., 1999). The *IGF-I* gene may play important roles in growth of multiple tissues, including muscle cells, cartilage and bone. Several studies (Amills *et al.*, 2003; Zhou *et al.*, 2005) have shown that IGF-I affects growth rate in livestock. The IGF-1 is pleiotropic in action considering its numerous functions and thus useful in selection and breeding of farm animals.

2.4 Structure of IGF-1 peptide and gene transcripts

The structure of the IGF-1 gene is well documented in several mammalian species. In both rat and human, the gene is constituted of six exons spanning over a large chromosomic region of 73 to 85 kilobases (LeRoith and Robert, 2003). A complex pattern of alternative splicing leads to numerous transcripts (Musaro *et al.*, 2001). All transcripts share the exons 3 and 4, which give rise to the mature IGF-1 peptide. Variations occur at the 5' and the 3' end of the transcripts. Alternative transcripts, encoding for different signal peptides occur from alternative use of the exon 1 or exon 2, while alternative transcripts encoding for different E peptides result from alternative use of exon 5 or exon 6 sequences.

Hepatic transcripts use the exon 2, while extra hepatic transcripts that utilize exon 1 result from the use of alternative transcription start sites. The heterogeneity of the 3' end leads to Ea (exon 5), Eb (exon 6) and Ec (exon 6) transcripts, showing different E peptide sequences. This part which is cleaved during the maturation of the preproIGF-1 peptide may influence the efficiency of the secretion of the peptide. The Ec transcripts may code for a muscle specific IGF-1 isoforms, Mechano Growth Factor (MGF), which would be specifically induced following mechanical induction or during regeneration (McKoy *et al.*, 1999). The chicken IGF-1 gene is more compact, spanning over about 48 kilobases of chicken chromosome 1. It appears to comprise only 4 exons (Kajimoto and Rotwein, 1991) related to rat exons 1, 3, 4 and 6 respectively. Further comparison of the sequences show some stretches which are conserved between chicken intron 2 and rat intron 3 (the longest intron) and between the 3' untranslated regions. This is consistent with a simpler pattern of IGF-1 transcripts (Kajimoto and Rotwein, 1989). In this context, it is not known whether chicken would express a MGF related muscle IGF-1 isoform.

2.5 IGF-1 as a local regulator of muscle growth.

After years of debate, it is now widely accepted that a significant part of IGF effect is exerted in an autocrine or paracrine mode of action. Indeed overexpression of the IGF-1 gene in the muscle tissue leads to enhanced muscle growth. This has been achieved in germ-line transgenic mice or using different viral mediated gene transfer in other model species such as the chicken. Depending on the timing of the overexpression, the muscle hypertrophy results from muscle fibre hyperplasia or hypertrophy. In the chicken, retroviral infection at embryonic day 4 (Mitchell *et al*., 2002) results in marked leg muscle hypertrophy (+80%) as a consequence of a commensurate increase in fibre number (+100%). By contrast, in germ line transgenic mice, overexpression under the control of a promoter with a later activity like the creatine kinase promoter (Coleman *et al*., 1995) or the Myosin Light Chain promoter (Musaro *et al*., 2001) leads to a muscle hypertrophy phenotype which is essentially the result of muscle fibre hypertrophy. In this last model (Musaro *et al*., 2001), the overexpression of a muscle specific isoform leads to a specific hypertrophy of the fast fibres which express the transcript.

The hypertrophy is accompanied by an increase in muscle strength, a protection against age related muscle atrophy and an improved regenerative capacity of the muscle satellite cells. It is noteworthy that a mutation in a regulatory region of the pig IGF-2 gene, which leads to overexpression of this gene in the muscle tissue specifically during post natal development, is responsible for a muscle fibre hypertrophy phenotype (Van Laere *et al*., 2003). In this case, one can assume that overexpression of IGF-2 is equivalent to overexpression of IGF-1 since both peptides act through the same receptor.

2.6 Normal IGF physiology

The IGF system involves complex regulatory networks that operate at the whole organism, cellular and subcellular levels. Key molecules involved are the ligands IGF1 and IGF2, the type 1 and type 2 IGF receptors (IGF1R and IGF2R, respectively), the IGF-binding proteins (IGFBPs), and the proteins involved in intracellular signalling distal to IGF1R, which include members of the insulin-receptor substrate (IRS) family, AKT, target of rapamycin (TOR) and S6 kinase. IGF1 has characteristics of both a circulating hormone and a tissue growth factor. Most IGF1 found in the circulation is produced by the liver. Regulation of hepatic IGF1 production is complex. Growth Hormone (GH) has a dominant role in regulating *IGF1* gene expression, but its stimulatory influence is markedly reduced by malnutrition (Thissen *et al*., 1994). GH, in turn, is produced by the pituitary gland under the regulation of the hypothalamic factors somatostatin and growth hormone- releasing hormone (GHRH).

Both IGF1 and IGF2 are ligands for IGF1R, which is a cell-surface tyrosine kinase signalling molecule. Following ligand binding, intracellular signaling pathways that favour proliferation as well as cell survival are activated. Initial phosphorylation targets for IGF1R include IRS proteins, and downstream signaling molecules include phosphatidylinositol 3-kinase, AKT, TOR, S6 kinase and mitogen-activated protein kinase. IGF2R preferentially binds IGF2, but has no intracellular kinase domain, and might not act as a signaling molecule. Bioavailability of IGFs is influenced by concentrations of specific IGFBPs (Firth and Baxter, 2002). At least six of these have been characterized, and their affinity for IGF1 and IGF2 is in the same order of magnitude as that of IGF1R. These proteins are present in the circulation as well as extravascular fluids. IGFBP3 provides most of the IGF binding capacity of serum and greatly prolongs the circulating half-life of the IGFs. IGFBPs in extracellular tissue fluid modulate interactions between IGF ligands and cell-surface IGF receptors. In different physiological contexts, the IGFBPs can either increase or decrease IGF signaling. This complexity is poorly understood; it probably arises because on the one hand IGFBPs prolong the half-lives of IGFs, but on the other they compete with receptors for free IGF1 and IGF2. Finally, there is increasing evidence that the IGFBPs have growth regulatory actions that are independent of their capacity to bind IGFs. The physiological importance of these actions is an active area of research at present.

2.7 IGF-1 in animals

Several growth factors have been identified as candidate to modulate muscle growth at each stage of development. Insulin-like Growth Factors (IGF-1 and IGF-2) exert a general effect on overall body growth (2) and both genes are expressed in the muscle tissue together with specific receptors, suggesting a paracrine mode of action. Indeed, overexpression of the IGF-1 gene in the muscle tissue of transgenic mice leads to selective muscle hypertrophy. The IGFs have been shown to stimulate the proliferation, the differentiation and the metabolism of a number of myogenic cell lines from different species as well as the anabolism of differentiated myotubes or muscle fibers. IGF-1 is expressed biallelically, whereas IGF2 is maternally imprinted in the offspring only paternal allele is expressed (DeChiara *et al*., 1991). Both of these genes, IGF1 and IGF2, are alternatively spliced in mammals. Recently, two different IGF-1 transcripts (class 1 and class 2) have been cloned and characterized in Songliao black pig (Xiao *et al*., 2009). Alternative splicing of IGF2 has been described in humans (Monk *et al*., 2006), mice (Moore *et al*., 1997) and cattle (Curchoe *et al*., 2005; Goodall and Schmutz, 2007). Recently an bovine IGF-1 intron-2 polymorphism was found to correlate positively with the twinning rate (Kim *et al*. 2009).

Alakilli *et al*. (2012) reported banding patterns (genotypes AA, AB, CC and CD) in their study on IGF-1 in goats. The frequencies of the alleles in the samples from the goat breeds varied from 0.410 to 0.620. While IGF-1 gene revealed three fragments after digestion with Haelll with genotypes AA, AB and BB and the frequencies of alleles varied from 0.432 to 0.731 in different goat breeds. Zhang *et al*. (2008) have reported A new single nucleotide polymorphism (G to C transversion) was identified at intron 4 of the *IGF-I* gene in Nanjiang Huang goats. Two alleles and three genotypes were observed. The frequencies of G and C alleles were 54.6 and 45.4% respectively. The statistical analysis showed that polymorphism of the *IGF-I* gene had a significant ($p<0.05$) association with birth weight (BW), body weight at 6 months (W6) and at 12 months (W12), heart girth at 2 months (G2), body length at 6 months (L6), wither height at 6 months (H6) and at 12 months (H12) and heart girth at 12 months (G12). The goats with genotype CC had significantly higher BW, W6, W12, G2, L6, H6, H12 and G12 than those with genotype GC which equally had significantly higher W12, H6, H12 and G12 than those with genotype GG. Also Naicy *et al*. (2017) reported significant association of IGF-1 polymorphs with growth traits in two breeds of India goats.

A number of genetic polymorphisms of the IGF1 gene were found to relate to growth parameters in chickens (Zhou *et al*., 2005), pigs (Casas-Carrillo *et al*., 1997) and goats (Zhang *et al*., 2008). Single nucleotide polymorphisms (SNPs) describe the impact in the IGF-1 gene on a number of productive performances of sheep (Pariset *et al*., 2006; Tahmoorespur *et al*., 2009; Scata *et al*., 2010, He *et al*., 2012 and Gholibeikifard *et al*., 2013). Thus, a wide range of physiological functions of IGF-1 can be attributed to polymorphism of the candidate genes in farm animals (Andrade *et al*., 2008; De la Rosa Reyna *et al*., 2010; Bahrami *et al*., 2013).

Ge *et al*. (2001) reported an effect of the *IGF-I* polymorphism (located in the regulatory region of the IGF-1 gene) on growth traits in Angus cattle and also suggested a direct impact of this polymorphism on gene transcription and consequently on phenotypic traits. Moody *et al*. (1996), Curi *et al*. (2005), Andrade *et al*. (2008) and Casas-Carrillo *et al*. (1997) reported that a polymorphic microsatellite (CA)n located in the 5´-flanking region of the IGF-1 gene has been associated with birth weight, weaning weight and yearling weight in different cattle breeds and with subcutaneous fat thickness in some swine families. Eulalia *et al*. (2006) reported a correlation between the polymorphism in the 5'-non coding region of the IGF-1 and meat and milk production traits in Polish Holstein-Friesian cattle.

Indeed, studies have described a relationship between systemic IGF-1 and carcass fat. Davis and Simmen (1997) reported that Angus bulls with lower plasma IGF-1 concentrations had higher marbling scores and dorsal fat thickness. Similarly, circulating IGF-1 was found to be negatively correlated with carcass fat percentage; fat accretion rate and fat thickness in Simmental crossbred bulls (Anderson *et al*., 1988). Furthermore, studies by Rajkumar *et al*. (1999) in transgenic mice have shown that the differentiation stage of precursor cells into mature fat cells is accompanied by enhanced expression of IGF-1 indicating a role of IGF-1 in fat cell developmental processes.

It was proved that the number of CA repeats at intron 1 of IGF-1 gene is positively correlated with plasma IGF-1 concentration and growth and fatness of pigs by Estany *et al*., (2007). On the other hand, in a noncoding fragment of porcine IGF2 gene a causative mutation (IGF2 SNP G3072A), effect on gene expression, weight of muscle and fat deposition, had been identified (Van Laere *et al*., 2003).

Insulin factor gene is exchanged among the conditions which were useful to the identification of races, as demonstrated in the Mediterranean countries (Pariset *et al*. 2006); Niżnikowski *et al*., 2014) presents a different view in relation to the research carried out on domestic Sheep breeds compared to the ancestor of the Sheep – European mouflon (*Ovis aries musimon*).

After screening a total of 4555 bp of genomic sequences in four exons and partial introns for the discovery of single nucleotide polymorphism (SNP) in common carp (*Cyprinus carpio*). Xiu *et al*. (2014) surmised that three SNPs (g.3759T>G, g.7627T>A and g.7722T>C) in intron 2 and a non-synonymous SNP (g.7892C>T) in exon 3 were identified in a pilot population including random parents and their progenies. The results of 289 progenies were further genotyped for possible associations between genotypes or combined genotypes and growth traits showed that the locus g.7627T>A was significantly associated with body weight and body length. Fish with genotype AA were averagely 5.9% heavier than those with genotype TT. No significant associations were observed between genotypes of other loci and growth traits. However, when both g.7627T>A and g.7722T>C were considered, the combined genotype TT/TT was extremely associated with the lowest values of body length and body weight and the highest K value in comparison with other diplotypes ($p < 0.01$). They concluded that these results suggested that genotype AA at g.7627T>A and its combined genotypes with alleles from another locus have positive effects on growth traits, which would be a candidate molecular marker for further studies in marker-assisted selection in common carp.

The insulin-like growth factor gene (IGF1) as a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou *et al*., 2005). Duclos (2005) have reported that in Chicken, data obtained by comparing genetic models with large variations of overall body growth show a positive relation between endocrine IGF-1 and growth rate. Data obtained using both genetic and nutritional models show a positive relation between muscle IGF-1 mRNA levels, which determine paracrine IGF-1 levels, and post hatch muscle growth. IGF-1 administration for 14 days was shown to increase weight gain (Duclos, 2005) and induce a moderate but significant effect on body composition, with decreased abdominal fat pad weight and increased nitrogen retention. Conversely, IGF-2 induced no effect on weight gain and did not increase fat deposition significantly.

Shah *et al*. (2012) studied IGF-I gene polymorphism using 90 broilers in India and they reported genotypic frequencies of 0.066, 0.166 and 0.766 for genotypes AA, AB and BB and allelic frequencies of 0.15 and 0.85 for alleles A and B, respectively. The highly significant χ^2 value of 10.80 showed that the population was not in Hardy Weinberg's equilibrium. Similar observation was made by Amills *et al*. (2003) in Black Penedesenca chicken strain who revealed the existence of three SNP on the sequence alignment of chicken IGF. One SNP (IGF-I-SNP1) was located in the 5′UTR of the IGF-I gene and consisted of one A→C substitution. This mutation was associated with a *Hinf*I Restriction Fragment Length Polymorphism (RFLP).

Abassi and Kazemi (2011) also studied IGF-I gene polymorphism using 100 Madanzaran native chickens in North of Iran. They reported that there were polymorphisms in IGF-I gene with A and B allelic frequencies of 0.51and 0.49, respectively. The genotypic frequencies were 25.88 (AA), 23.89 (BB) and 50.23 (AB). The χ^2 test revealed that the population was in Hardy-Weinberg equilibrium. Another study carried out by Li *et al*. (2010) to evaluate effect of IGF-I gene polymorphism on the egg quality of Wenchang chickens revealed three genotypes and two alleles were seen. The allelic frequencies recorded were 0.53(A) and 0.47(B) while genotypic frequencies were 0.32, 0.41 and 0.27 for genotypes AA, AB and BB, respectively.

The genotypic frequencies reported by Promwatee *et al*. (2013) in different lines of Thai synthetic chickens indicated that the AA genotype was of significantly lower frequency than the AC and CC genotypes in all the lines. Allele frequencies showed that allele A was as frequent as allele C in all lines except for Soi Pet (SP). Allele A (0.25) was of lower frequency than allele C (0.75) in the SP population with genotypic frequencies of 0.05(AA), 0.39(AB) and 0.56(BB).

Promwatee *et al*. (2013) reported association of IGF-I gene polymorphism with some growth traits in four (Kaen Thong, Khai Mook Esarn, Soi Nin and Soi Pet) synthetic chicken lines. The authors reported that day old body weight was significantly higher only among chickens with the AA genotype as compared with those of AC and CC genotypes in the Kaen Thong population. In the Khai Mook Esarn and Soi Pet populations, body weights at 4, 8, 12, and 14 weeks of age were significantly higher in the AA genotype than in the CC genotype. In the Soi Nin population, day old body weight was higher in the AA genotype than in the AC and CC genotypes; while body weight at 12 and 14 weeks of age were higher in the AA and AC genotypes than in the CC genotype.

Similarly, Amills *et al*. (2003) reported the same mutation of the IGF-I gene in two chicken strains of the Black Penedesenca breed and significant association of IGF-I SNP1 was found for body weight up to 107 days of age in one of the strains. According to the authors, IGF-I-SNP1 marker was located in the promoter region of the IGF-I gene, so the existence of suggestive associations among IGF-I-SNP1 and growth might be interpreted in the light of differences in the transcriptional rate of both alleles. In fact, the analysis of the promoter sequence revealed that the substitution A→C involved the suppression of one potential CdxA transcription factor binding site. Furthermore, Zhou *et al*. (2005) reported association of IGF-I gene to body weight in a broiler line experiment with the AA genotype having a significantly greater body weight than the AB genotype, and the AB genotype were significantly greater than the BB genotype at all ages.

Using Polish Holstein-Friesian heifers to study the association of insulin-like growth factor I gene polymorphisms (IGF1/*Tas*I and IGF1/*Sna*BI) with the growth and subsequent milk yield, Szewczuk *et al*., (2012) reported that the *CC* genotype was favourable and associated with higher body weight at two months (WT60) and daily body weight gains from the 1st to the 2nd month. For the IGF1/*Tas*I polymorphism, *AA* genotype (0.69) had the higher frequency among the other genotypes discovered. Allele frequencies were 0.75 and 0.25 for the *A* and *C* alleles, respectively. In the association study, the *AC* genotype was significantly associated with higher WT60 and average daily gains in the periods from 2nd to the 3rd month of age and for the whole rearing period.

2.8 Allelic and genotypic frequencies of IGF-I in sheep population

In the study on IGF-1 gene polymorphism in Zel sheep population, Kazemi *et al*. (2011) reported A and B alleles with frequencies of 0.71 and 0.29 respectively. The resulting genotypes AA, AB and BB also had frequencies of 0.47, 0.47 and 0.06 respectively and the population was in Hardy-Weinberg equilibrium. Polymorphism of this gene in Polish lowland Sheep, alleles C and T were te-1 gene, thus limiting its scope to determine the allele C, respectively genotype CC (Niznikowski *et al*., 2014). In the works of Behzadi *et al*. (2015) using Iranian Mehraban Sheep, they reported that two single nucleotide polymorphisms (C114G and G116A) and two amino acid exchange (S5T and G6S) with three patterns were found in IGF-1 gene fragment. Estimated frequencies were 0.24 and 0.76 for the A and B alleles, respectively.

Gholibeikifard *et al*. (2013) in their study with Iranian Baluchi Sheep detected two banding patterns in the IGF-1 locus with frequencies of 0.89 and 0.11 for AA and AB, while Hajihosseinlo *et al*. (2013) reported that in the Makoei Sheep breed three banding patterns (genotypes) named as A/A, A/G and G/G were detected in exon 1 of the IGF-1 gene with frequencies of 0.52, 0.42, and 0.06, respectively. Allelic frequencies were 0.73(A) and 0.27(G). This is an indication that the most frequent allele in the 'Makoei' Sheep breed were 0.73 and 0.52 for allele A and genotype AA, respectively. Observed heterozygosity (Hobs) value was 0.3942. The observed distribution of genotypes was not different than the distribution expected under the assumption of Hardy-Weinberg equilibrium.

2.9 Associations between polymorphisms of the sheep IGF-1 gene and growth traits

Insulin-like growth factor-1 (IGF-1) is an important regulator of cell proliferation, differentiation, and apoptosis, has acute insulin-like metabolic effects, and is important for growth and development throughout the body. The level of IGF-1 peaks during puberty and after which it declines with age. Although the IGF-1 serum level is influenced by many factors, such as nutritional status, liver function, and serum levels of sex steroids and insulin, the secretion of this peptide is mainly regulated by growth hormone (GH) (Froesch and Zapf, 1985 cited in Licht *et al*., 2014). It has been estimated that up to 60% of the variance in IGF-1 serum level has a genetic basis (Harrela *et al.*, 1996 and Hong *et al*., 1996 cited in Licht *et al*., 2014).

Several polymorphisms in the promoter region of the IGF-1-gene have been identified, comprising a variable length cytosine-adenine (CA) repeat sequence (Kato *et al*., 2003). These polymorphisms are thought to influence the transcription rate of IGF-1, which in turn affects serum IGF-1 levels (Fletcher *et al*., 2005). The 192 bp allele is the most common allele and therefore is called the wild type (Fletcher *et al*., 2005; van Turenhout *et al*., 2011). Results of studies that evaluated the relationship between the IGF-1 promoter polymorphisms and IGF-1 levels are contradictory; the homozygote 192 bp genotype has been associated with both higher and lower IGF-1 levels compared to the heterozygote and non-carrier genotypes (Fletcher *et al*., 2005; Euser *et al*., 2011).

The greatest numbers of known genes that affect Sheep meat production code for various regulatory peptides such as myostatin (Clop *et al*., 2006), calpastatin (Cockett *et al*., 2004) and others. Another important regulatory protein, which controls growth and development in mammalian muscle structures, is insulin-like growth factor 1 (*IGF-1*). Along with IGF-2, growth hormone (GH) and growth hormone releasing hormone (GHRH), it is a member of the so-called somatotropic axis (GH / IGF-1 axis), which plays a key role in the growth of vertebrates (Curi *et al*., 2005 and Zhang *et al*., 2008). IGF-1 mediates the stimulatory effect of growth hormone and testosterone on the growth and development of muscle fibers (Oksbjerg *et al*., 2004; Mateescu and Thonney, 2005).

Association of polymorphism in the 5´ flanking region of IGF-1 gene of goat has been studied and the results have shown that this polymorphism is significantly associated with twinning rate (Wang *et al*., 2011), but the polymorphism of IGF-1 gene in Sheep did not have significant association with twinning rate (He *et al*., 2012). Also polymorphism in 5´ flanking region had a significant effect on growth traits, live weight and carcass weight in Zel Sheep (Kazemi *et al*., 2011). No association was found between the polymorphism in the 5´ flanking region and body size, milk, yield and birth weight in Chinese dairy goats (Deng *et al*., 2010; Wang *et al*., 2011). In Kurdish goat, the polymorphism of IGF-1 gene was associated with growth traits and yearling fleece weight (Kurdistani *et al*., 2013). Also association of the IGFBP-*3* genotypes with production traits has been reported in Sheep (Kumar *et al*., 2006).

Gholibeikifard *et al*. (2013) have reported no significant association detected between the polymorphism of *IGF-I* and body weights in Baluchi Sheep at birth, weaning, 6 months and 12 months respectively. Behzadi *et al*. (2015) reported significant ($p<0.05$) association between IGF-I patterns and blood cholesterol and blood triglycerides; dorsal fat thickness and carcass weight which is a function of liveweight.

The association of IGF-1 gene polymorphism (short random repeat in the 5'-flanking region) with body weight, both at birth and weaning, has also been described (Li *et al*. 2004; Moody *et al*. 1996). However such an association was not found by Curi *et al*. (2005). Recently an IGF-I*1* intron-2 polymorphism was found to correlate positively with the twinning rate (Kim *et al*., 2009). A positive correlation was found between the level of IGF-1 in the plasma, birth weight and muscle eye depth (Afolayan and Fogarty, 2008) of lambs at the age of 100 days. Because of their function in muscle development, genes for IGFs became candidate genes for meatiness of farm animals. Proskura and Szewczuk (2014) have reported significant ($P<0.001$) association between the T allele of the IGF1R gene with body weight and average daily gain and no significant ($P>0.05$) association between these traits and the T allele of IGF1 in Pomeranian Coarse wool Sheep.

Trukhachev *et al*. (2016) in studying the polymorphisms of the IGF-1 gene in Russian Sheep breeds and their influence on some meat production parameters stated that 18 single nucleotide polymorphisms (SNPs) were found in this breed. Only one SNP – c.-81T>C – was found in the coding region. All other SNPs were located in introns 5'UTR and 3'UTR. The c.-5363C>T, c.-

5188G>C, c.-5186G>A and c.-4088G>A polymorphisms, presented together in two alleles of the gene, correlate with a high live weight in a heterozygous state. The synonymous substitution of c.81T>C in the exon was not found to have any influence on the analyzed meat production parameters. One of the detected SNPs – c.-91A>C – had a positive correlation with weight, height, croup parameters and other attributes in rams.

2.10 Insulin-like Growth Factors and Neoplasia.

The insulin-like growth factor 1 (IGF1) signalling pathway has important roles in regulating cellular proliferation and apoptosis. Converging results from epidemiological research and *in vivo* carcinogenesis models indicate that high levels of circulating IGF1 are associated with increased risk of several common cancers in humans (Jul *et al*., 2002 and Vasan, 2003). This potential for accelerating neoplasia should be kept in mind in breeding and selection to avoid carcinogenic complications in farm animals.

2.10.1 *Effect of breed at different ages on bodyweight*

Sheep are the second most numerous pastoral species in Nigeria after cattle, and small flocks usually accompany many cattle herds in the North and in the Middle Belt of the country (Bourn *et al*., 1994). The productivity of WAD sheep is substantially lower than that of other breeds. The skin of all Nigerian sheep can be used to process leather. The species are rarely milked in Nigeria (RIM, 1992). Sheep however have the potential to supply (yield) significant portion of the milk deficit in the country because sheep far exceed cattle in number in both rural and urban communities in Nigeria (Adewunmi, 2005).

The Balami is the fastest growing, attaining a weaning weight of 18kg in 12 weeks (Oni, 2002). The breed has good potential as a meat producer and its ability to survive under arid conditions is a characteristic that can be exploited in the utilization of non-arable land (Akinyemi, 2010). The Yankasa sheep has been reported to be superior to the WAD sheep interms of yearling weight, liveweight, productivity per animal and annual rate of return (Adu and Onwuka, 1991). Sheep of Northern Nigeria are good producers of meat for human consumption (Ozung *et al.*, 2011). According to Acharya (1998), liveweight constitutes an important economic trait in meat animals.

The estimates of average body weight reported by various workers at different stages have been presented in Table 2.1. Birth weight ranged from 1.89 ± 0.76 kg in Sonadi breed (Tailor and Yadav, 2010) to 3.75 ± 0.08 kg in Munjal breed (Poonia, 2004), 3 months body weight ranged from 7.89 ± 0.11 kg in Mecheri sheep (Shivkumar, 2003) to 17.70 ± 0.05 kg in Pugal sheep (Dass, 2007), 6 months body weight ranged from 12.92 ± 0.08 kg in Ganjam sheep (Patnayak *et al.,* 2003) to 27.14 ± 0.57 kg in Munjal breed (Poonia, 2004), 9 months body weight ranged from 16.49 ± 0.41 kg in Malpura sheep (Singh, 1980) to 32.01± 0.23 kg in Malpura sheep (Arora et al., 1999) and 12 months body weight ranged from 15.91 ± 0.25 kg in Mecheri breed (Shivkumar, 2003) to 30.44 ± 0.12 kg in Marwari sheep (Narula *et al.,* 2010).

2.10.2 Effect of sire

The body weight at birth, 3 months, 6 months and 12 months of age were significantly affected by sire groups as reported by Malik *et al.* (1980) in crosses of Chokla, Malpura and Jaisalmeri with Rambouillet sheep, Singh and Dhillon (1992) in Avikalin, Singh and Kushwaha (1995) and Tomar *et al.* (2000a) in Bharat Merino, Arora *et al.* (1999) in Avikalin and Malpura sheep and Ahmad *et al.* (2004) in Avikalin sheep. Weaning weight was significantly affected by sire as reported by Nawaj *et al.,* (1998) in Kajli and Lohi sheep. The 3 months and 12 months body weights of Madras Red sheep was significantly affected by sire groups as reported by Devendran et al. (2007). Body weights at all ages up to yearling significantly affected by sire as reported by Gohil (2010) and Singh (2012) in Marwari sheep and Parihar (2012) in Magra sheep.

Table 2.1: Least-square means(kg) of body weights in different breeds of sheep at different ages

Breed/genetic group	Birth	3 months	6 months	9 months	12 months	Authors
Polled Dorset	3.35±0.09					Khan *et al*.(2011)
Marwari	3.14±0.01	15.13±0.13	21.14±0.18	25.85±0.16	28.87±0.21	Singh (2012)
Magra	3.04±0.01	14.20±0.10	21.86±0.22	25.67±0.26	27.89±0.33	Parihar (2012)
Deccani	2.87±0.01	13.86±0.10	18.02±0.12	21.17±0.15	23.94±0.18	Chikurdekar *et al*. (2012)
Madras Red	2.76	9.90	14.53	18.16	21.05	Balasubramanyam *et al*. (2012)

2.10.3 *Average daily gain in body weight from birth to yearling age*

The average daily gain (absolute growth rate) estimated by various workers in different breeds are shown below: Mandal *et al.* (2003) recorded pre weaning and post weaning average daily gain as 127.8 ± 3.3 and 49.4 ± 1.6 g, respectively in Muzaffarnagari sheep. Patnayak *et al.* (2003) observed average daily gain as 72.6 and 37.0 g/day in Ganjam sheep during 0-3 and 9-12 months of ages, respectively. Shivkumar (2003) observed daily live weight gain as 0.063 ± 0.001 kg during 0-3 months in Mecheri sheep. Dey and Poonia (2005) observed pre and post-weaning daily weight gain in Nali sheep as 84.72 ± 1.06 and 37.36 ± 1.09 g, respectively. Body weight gain during 0-3, 3-6 and 6-12 months of age groups were recorded as 91 ± 1.0, 75 ± 1.9 and 32 ± 0.6 g in Chokla sheep by Prince *et al.* (2008). Mishra *et al.* (2009a) observed average daily gain (g) in Garole × Malpura half bred as 104.91 ± 1.27 and 63.12 ± 1.07 g in 0-3 and 3-6 months, respectively. Narula *et al.* (2009) observed average daily weight gain as 137.41 ± 0.83, 84.09 ± 0.75 and 34.89 ± 0.41 g during 0-3, 3-6 and 6-12 months, respectively in Magra sheep. Narula *et al.* (2010) observed daily weight gain as 143, 75.14, and 40.77 g/day during 0-3, 3-6 and 6-12 months, respectively in Marwari sheep.

Tailor and Yadav (2010) observed absolute growth rate during 0-3, 3- 6, 6-9 and 9-12 months of age as 92, 60, 33 and 41 g/day, respectively in Sonadi sheep. Gohil (2010) recorded the average daily weight gain as 134.80 ± 2.14, 66.47 ± 1.73, 48.96 ± 2.02, 31.23 ± 2.25 and 68.28 ± 1.00 g/day during 0-3, 3-6, 6-9, 9-12 and 0-12 months of age in Marwari sheep, respectively. Singh (2012) recorded the average daily weight gain as 133.59 ± 1.430, 66.75 ± 1.031, 38.67 ± 1.170 and 31.23 g/day during 0-3, 3-6, and 6- 12 months of age in Marwari sheep, respectively. Parihar (2012) recorded the average daily weight gain as 124.20 ± 1.135, 78.88 ± 1.792, 32.99 ± 0.895 and 68.52 ± 0.745 g/day during 0-3, 3-6, 6-12 and 0-12 months of age in Magra sheep, respectively.

2.11 Association between ovine ADRB3 intron, 3’UTR genotypes and key growth traits in the sheep breed

In sheep, variation in both coding and non-coding regions of ADRB3 has been reported in breeds that include traditional European/English breeds and crossbred sheep (Byun *et al.,* 2008; Forrest *et al.,* 2003) and some Chinese sheep breeds (Yang *et al.,* 2009). Among all of the ovine ADRB3 variants identified to date, the putative polypeptide encoded by variant D shows two amino acids substitutions (Val52Ala and Leu322Val, Forrest *et al.,* 2003), which occur in positions that are normally conserved in all ADRBs and that are thought to be involved in ligand-binding (reviewed in Strosberg, 2000). It is notable that most of the variation detected in the ADRB3 are in the non-coding region. In the ADRB family, an intron only exists in ADRB3 and not in ADRB1 or ADRB2 (reviewed in Strosberg, 2000). This unique existence of intron in ADRB3 suggests that this region may play important role in regulation of ADRB3 expression and be important for the function of ADRB3. Forrest *et al.* (2007) found an association between variation in the ovine ADRB3 intron and lamb cold-survival by genotyping 13427 lambs from 13 different breeds born on 22 different farms throughout the South Island of NZ. These results underpin the use of ovine ADRB3 as a gene-marker for breeding more cold-tolerant lambs, and a gene-marker 26 test for lamb cold-tolerance is now commercially available from the Gene-Marker Laboratory, Lincoln University, NZ.

Forrest *et al*. (2003) also reported the association between variation in ovine ADRB3 intron and variation in birth weight, pre-weaning growth-rate and carcass traits. In the NZ Romney sheep, an association between variation in the ovine ADRB3 intron and variation in post-weaning growth-rate has been reported (Horrell *et al.,* 2009), but no association between intron variants and birth weight were detected. In Horrell's study (2009), the presence of intron variant A was found to be associated with pre-weaning growth-rate. It is noteworthy that ninety-four percent of those Romney lambs studied possessed intron variant A. In addition, in previous studies (Byun *et al.,* 2008; Forrest and Hickford, 2000; Forrest *et al.,* 2007; Forrest *et al.,* 2003; Forrest *et al.,* 2006; Horrell *et al.,* 2009), intron A was found to be a common variant in some sheep breeds. However, because of the ambiguities in the sequence data derived from the Polymerase Chain Reaction-Single Strand Conformational Polymorphism (PCR-SSCP) banding pattern of variant A, greater sequence variation within the extended ADRB3 A sequence has been suggested

(Forrest *et al.,* 2003). Therefore, it would be helpful for using ovine ADRB3 as a gene-marker if extended variation and haplotypes in the gene could be identified. Research reveals that the 3'UTR of ADRB2 mRNA may have roles in post-transcriptional regulatory mechanisms that are involved in the stability and degradation of various cellular RNAs, sub-cellular targeting and translation of many transcripts (Tholanikunnel and Malbon, 1997; Yang *et al.*, 1997).

Deletion of the 3'UTR sequences resulted in 2-2.5 fold increases in ADRB2 expression (Subramaniam *et al.,* 2011). The similarities of the ADRB2 3'UTRs from humans and rodents were calculated to be 73% and 79%, respectively (Nakada *et al.,* 1989), thus, it is presumed that this regions contain genetic elements that are required to regulate receptor expression and therefore have been subject to selective pressure to preserve their sequence (Nakada *et al.,* 1989). The ADRB1 mRNA has also been reported to be post-transcriptionally regulated at the level of mRNA stability and undergo accelerated agonist-mediated degradation via interaction of its 3'UTR with RNA binding proteins (Nakada *et al.,* 1989).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The research was carried out at the Small Ruminant Research Programme of National Animal Production Research Institutte (NAPRI) Shika, Zaria, Kaduna State, Nigeria. Shika lies between latitude 11^0 12'N, longitude 7^0 33'E and at an altitude of 640m above sea level. The area falls within the Northern Guinea Savannah having an average annual rainfall of 1100mm which starts from late April or early May and ends in mid-October and followed by a dry period (which is divided into early and late dry periods). The early dry period is characterized by cold period and lasts from November to February. The mean temperature is about 24.4^{o}C (14.5-39.5^{o}C) with the lowest and highest humidity of 21% and 72% occurring during the early dry and wet seasons respectively (Institute for Agricultural Research Meteorological unit, 2016).

3.2 Experimental Animals and their Management

The experimental animals were isolated and reared under semi-intensive system. The sheep were housed during night in sheds covered with asbestos sheets with open sides during winter and in open corrals made by chain link fencing during summer months. Space of about 1.5 sq. meters per sheep was provided. The sheep were fed concentrate supplements (3% of body weight) in the morning at 8:00 am. The concentrates were compounded using cotton seed cake, ground maize grain or maize offals, bone meal, vitamin and mineral premix and salt to make a diet of 18% crude protein (CP) for weaners and 15% CP for yearlings and other adult sheep. Gamba grass (*Andropogon gayanus*) hay was also provided in addition to the concentrates. The animals were watered twice, once in the morning and again in the evening. Nursing ewes and their lambs were kept intensively up to weaning at 90 days while the other sheep in the flock were daily released to graze on improved pastures of *Digitaria smutsii; Bracharia decumbens;* Gamba grass; *Cynodon dactylon and Hyperenia rufa*, between 8:00 am and 4:00 pm. Routine medication of application of anti-helminthic and drenching (deworming) was carried out every month for the suckling lambs and every 45 to 60 days for the weaned and adult sheep. Ectoparasite bath (dipping) was carried out every 2 weeks; as well as vaccination against endemic diseases.

3.3 Collection and Analysis of Blood Sample

3.3.1 Blood Collection

Blood samples (2ml) were collected through jugular venipuncture in the morning (7.00am) from 140 randomly selected ewes (n=70) and rams (n=70) of Yankasa sheep within the age range of 2-3 years. The blood samples were placed in ethylenediamine tetra-acetic acid (EDTA) tubes to prevent coagulation and were transported in ice-pack to the African Biosciences Laboratory, Ibadan, Nigeria for analysis.

3.3.2 Genomic DNA extraction

The blood samples were transferred to eppendorf tubes and frozen at -40˚C for storage. The DNA extraction procedure was performed at room temperature (15-30˚C).

a) Lysis step

50uL of whole blood sample was placed in the eppendorf tube. Add 200uL of genomic lysis buffer (mercaptoethanol added) to make a 4:1 volume of genomic lysis buffer to the sample. Samples were vortexed for 4-6 seconds, then kept at room temperature for 5-10 minutes. The mixture was transferred to a spin column in a collection tube and centrifuged at 10,000g for one minute, then the collection tube was discarded with the flow.

b) Wash step

The spin column was transferred to a new collection tube. 200uL of DNA was added to pre-wash buffer to the spin column and centrifuged at 10,000g for one minute. 500uL of g-DNA wash was added to the buffer in the spin column and was centrifuge at 10,000g for one minute**.**

c) Elution step

The spin column was transferred to a clean micro centrifuge tube. 50uL DNA Elution buffer was added to the spin column. The samples were incubated at room temperature for 2-5minutes and centrifuge at top speed (16,000g) for 30seconds to elute the DNA and stored at -200^{o}C for future use.

3.3.3 Gel check

One percent (1%) agarose gel was prepared by placing 1g of agarose powder in a conical flask, make up to 100ml with Tris buffer and allowed to dissolve using a microwave for 2-3minutes. The gel was allowed to cool by running water at the base of the flask for a few minutes until it is no longer hot to touch (ensure the gel does not solidify while cooling). 5uL of ethidium bromide was added to the flask. Gel was poured into the tray and insert combs and the gel was allowed to solidify for approximately 20minutes. Samples were prepared by mixing 5UL of the extracted DNA sample with 1uL of the loading dye. 6uL of sample/loading dye was loaded into a well (one sample per well). The gel was run at 100volts for 50minutes and the gel was placed in the UV transilluminator to visualize.

3.4 Primer sequencing results

Primers were designed according to the mRNA sequence of *Ovis aries* IGF1 gene (GenBank No. NM_001009774) and two EST sequences of *Ovis aries* IGF1 gene (GenBank No. X69472 and X17229). Primer sequence, amplified region, annealing temperature and product size are as listed in Table 3.1. Three genotypes with two alleles were detected by the primer used.

Table 3.1 :The sequence, amplified region, annealing temperature and product size of the primer for Sheep IGF-1 gene analysis

Primer	Primer Sequence(5’ - 3’)	Amplified Region	Annealing Temp (^{0}C)	Product Size (bp)

P1	R: CATATTTTTCTGCATAACTTGAACCT F: TGAGGGGAGCCAATTACAAAGC	5’ regulatory region	55	294

PCR-RFLP Analysis

Reaction setup

PCR amplicon - 5uL

MluCl (10,000 units/ml) - 0.5uL

10x Enzyme buffer - 0.95uL

Water - 8.55uL

Total - 15uL

1ml of stock MluCl = 10,000units

1uL of stock MluCl = 10units

Therefore, 5 units = 0.5uL of stock MluCl

To reduce the number of pipetting and hence errors, a reaction cocktail can be prepared, which will then be aliquot in 10uL per sample

For 50 samples in this experiment, a cocktail was prepared for 55 to make allowance for

MluCl (10,000 units/ml)	-	0.5uL X 55 =	27.5uL
10x Enzyme buffer	-	0.95uL X 55 =	52.25uL
Water	-	8.55uL x 55 =	470.25uL
Total	-	**10uL**	**550uL**

The allele and genotype frequency was estimated by direct gene counting method as described by Christensen (2003) and was used to score IGF-1 bands based on the separation of electrophoretic migration as follows;

- A high molecular weight band was designated as AA homozygote.
- The presence of a single lower molecular weight band was designated as BB homozygote.
- Any of a mixture midway between low and high bands was designated as AB heterozygotes.

This analysis was carried out at African Biosciences Laboratory, Ibadan.

3.5 Bodyweights and Body Measurements

The body weight of lambs at birth was recorded within 24 hrs of lambing. The lambs were subsequently weighed at three months of age(weaning weight) and thereafter six, eight and twelve months of age in the morning before having access to water.

Weaning weight: Measured as the weight of the offspring when it was separated from the dam.

Average daily gain

$$\textbf{ADG} = \frac{W_2 - W_1}{t_2 - t_1}$$

Where: W_2= Body weight (kg) at age 2.

W_1= Body weight (kg) at age 1.

t_2 = Age 2 (days)

t_1 = Age 1(days)

The linear body measurements taken include:

Height at withers: Measured as the distance from the surface of the platform to the withers of the animal.

Chest girth: Measured by taking the circumference of the chest behind the forelegs with a measuring tape.

3.6 Data Collection

As stated earlier, the Yankasa Sheep was measured for growth traits (birth weight, weight at 3, 6 and 9 months, average daily gain, weaning weight, chest girth and height at withers) each of the experimental Sheep was scored according to its band for the SNPs either as high (AA), midway (AB) or low (BB) molecular weights.

3.7 Statistical analysis

On the basis of identified genotypes of Yankasa Sheep, the frequency of alleles was calculated according to Hardy-Weinberg’s equation (Kubek and Bardun, 1990). This equation is based on the binomial expansion of $(p+q)^2=1$ which gives $p^2+2pq+q^2=1$

Where; p = Dominant allele

q = Recessive allele

To calculate frequency of alleles:

$p = f_A = N_A/N_A+N_B$ $q = f_B = N_B/N_B+N_A$

To determine associations, the traits of interest was analyzed using the General Linear Model (GLM) procedure of the SAS program (Statistical Analysis System, 2000), the following statistical model was used for association of SNP with measured variable

$Y_{ij} = \mu + G_i + S_j + (G \times S) + e_{ij}$

Where:

Y_{ij}= growth traits,

μ = Overall mean,

G_i= Fixed effect of the i^{th} genotype for IGF-1,

S_j= the fixed effect of the j^{th} sex for IGF-1

(G x S) = interaction effect

e_{ij}=Random residual error.

Mendelian inheritance ratio for band (genotype) inheritance was tested using chi-square test. The observed Chi-square value was calculated as:

$$\chi^2_{calc} = \sum \frac{(O-E)^2}{E}$$

Where, O= Observed number of alleles

E= Expected number of alleles

$\chi^2_{calc.}$: Calculated Chi-square

CHAPTER FOUR

4.0 RESULTS

4.1 Banding Pattern of DNA and Images of Detected Polymorphs of IGF-1 gene in Yankasa Sheep.

Plate 4.1 shows the images of the presence of DNA in blood samples after extraction.

Plate 4.2 shows the images of the banding pattern of the AA, BB and AB bands discovered from the analyzed blood samples.

4.2 Frequency distribution of genotype and alleles observed for IGF-1 polymorphism in Yankasa sheep.

Table 4.1 shows the frequency of genotypes and alleles detected in Yankasa Sheep. The AA genotype had the highest frequency with a value of 0.58 followed by AB (0.28) and BB (0.14). Allelic frequency observed were 0.72 for the faster polymorph (A) and 0.28 for the heavier but slower polymorph (B).

4.3 Chi Square distribution of IGF-1 gene polymorphism in the sheep population

The Chi Square distribution table of IGF-1 gene polymorphism in the Sheep population is illustrated in Table 4.2. Both genotypic and allelic calculated χ^2 values (9.07 and 16.94) for Mendelian inherited ratio were greater than the tabulated values of 5.99 and 3.84 at 5% level of significance.

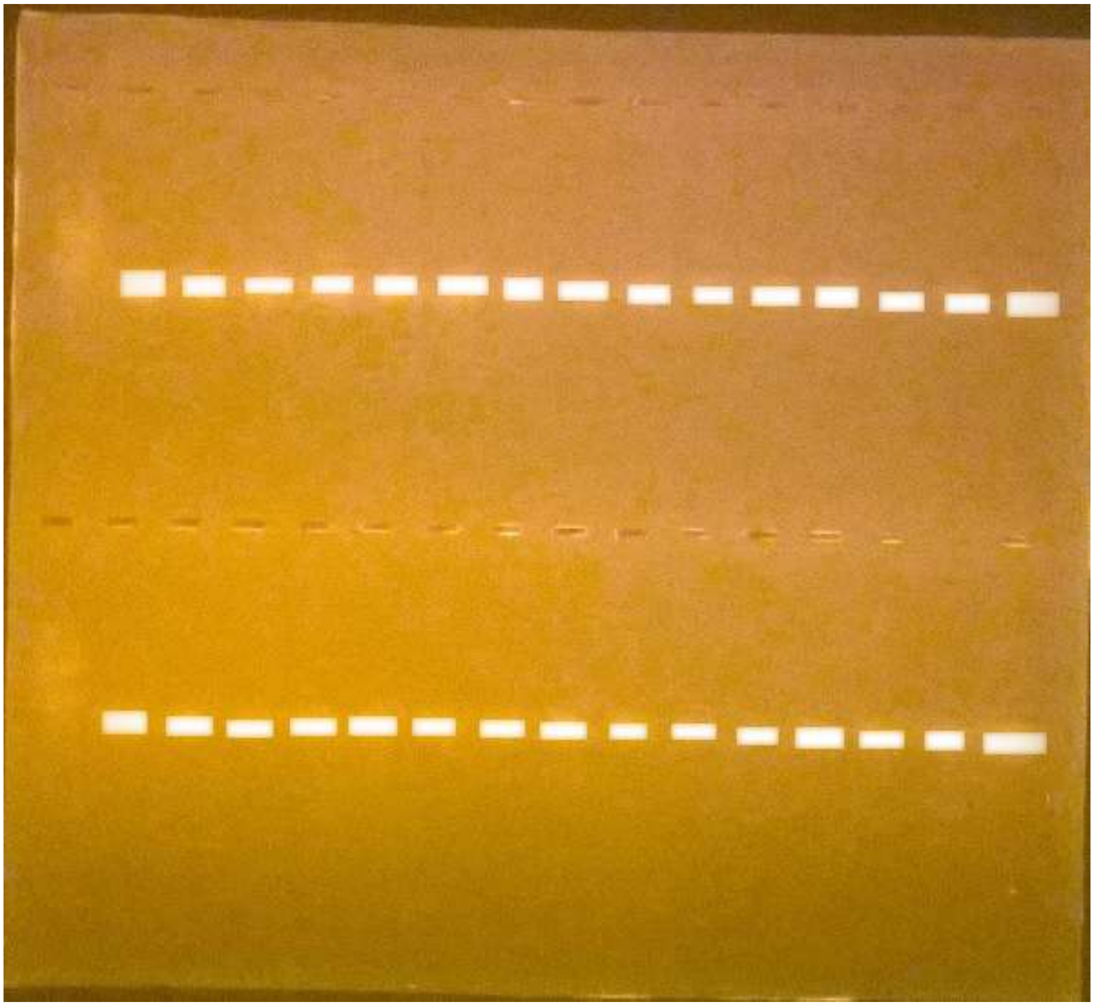

Plate 4.1: Image of the presence of bands representing DNA in blood samples after extraction

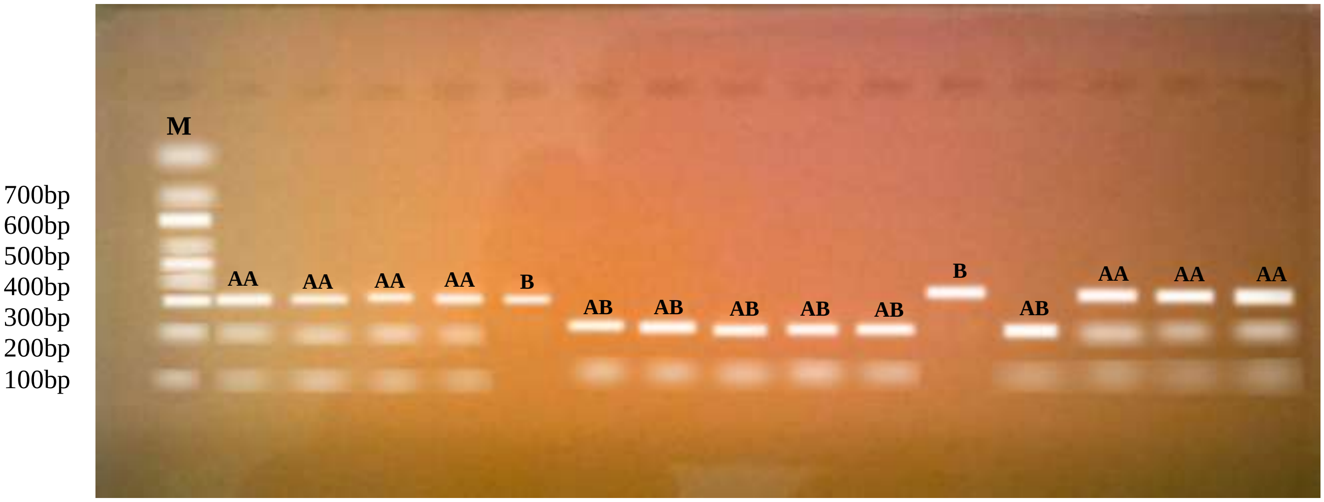

Plate 4.2: Image of the banding pattern of the AA, BB and AB bands discovered from the analyzed blood samples

Table 4.1: Frequency distribution of genotype and alleles observed for IGF-1 polymorphism in Yankasa sheep

	Genotypes			Alleles	
	AA	AB	BB	A	B
Frequency	0.58	0.28	0.14	0.72	0.28

Table 4.2: Chi Square distribution table of IGF-1 gene polymorphism in the sheep population

Genotypes	No of Animals	Expected	Observed	***Calc.*** χ^2	Tab. χ^2	P <0.05
AA	140	70	81.2	9.07	5.99	*
AB		35	39.2			
BB		35	19.6			
Alleles						
A		75	100.8	16.94	3.84	*
B		25	39.2			

Calc. = Calculated value. Tab. = Tabulated value

4.4 Association between Igf-1 genotypes and growth traits in sheep

The association between the polymorphic forms of IGF-1 genotypes and growth traits in the Yankasa sheep breed is outlined in Table 4.3. All growth traits (birth weight, weaning weight, average daily gain, weight at 6 months, weight at 12 months, height at withers and chest girth) with the exception of Body weight (Kg) at 8 months showed significant ($P<0.05$) association with IGF-1 genotypes. The AA and AB genotypes were mostly similar for birth weight, weaning weight, weight at 6 months, weight at 12 months, height at withers and chest girth and differed significantly ($P<0.05$) from the BB genotypes. Animals with the AA and AB genotypes were similar and had the highest birth weight, weaning weight, average daily gain, weights at 6 and 12 months, height at withers and chest girth, BB individuals had the lowest growth characteristics of the studied population.

4.5 Sexual differences in growth traits of Yankasa sheep

Table 4.4 shows the differences in growth traits among the sexes. With the exception of average daily gain (g/day), which was not significantly ($P>0.05$) affected by sex, all other growth characteristics (birth weight, weaning weight, weight at 6 months, weight at 8 months, weight at 12 months, height at withers and chest girth differed significantly ($P<0.05$) across sexes with the higher values recorded for males.

Table 4.3: Association between IGF-1 genotypes and growth traits in sheep

Traits	AA	AB	BB	SEM	LOS
Birth weight (Kg)	2.35^{a}	2.33^{a}	1.80^{b}	0.25	*
Weaning weight (Kg)	12.42^{a}	12.29^{a}	11.16^{b}	0.50	*
Average Daily Gain (g/day)	0.14^{a}	0.11^{b}	0.11^{b}	0.01	*
Weight at 6 months (Kg)	23.58^{a}	22.01^{ab}	20.12^{b}	1.05	*
Weight at 8 months (Kg)	28.12	28.66	29.09	1.31	ns
Weight at 12 months (Kg)	33.19^{a}	32.57^{a}	29.01^{b}	1.82	*
Height at withers (cm)	58.07^{a}	57.51^{a}	51.45^{b}	2.90	*
Chest Girth (cm)	69.34^{a}	68.79^{a}	67.25^{b}	0.60	*

^{ab}means across columns differ significantly ($P<0.05$); ns: non-significant

Table 4.4: Sexual dimorphism in growth traits of studied sheep

	Male	Female	SEM	LOS
Birth weight (Kg)	2.80^{a}	2.08^{b}	0.31	*
Weaning weight (Kg)	13.29^{a}	10.19^{b}	1.47	*
Average Daily Gain (g/day)	0.11	0.11	0.01	ns
Weight at 6 months (Kg)	23.29^{a}	18.73^{b}	2.04	*
Weight at 8 months (Kg)	30.78^{a}	27.22^{b}	1.21	*
Weight at 12 months (Kg)	33.99^{a}	28.77^{b}	2.23	*
Height at withers (cm)	59.4^{a}	52.82^{b}	3.86	*
Chest Girth (cm)	69.46^{a}	64.06^{b}	2.01	*

^{ab} means across columns differ significantly ($P<0.05$); ns: non-significant

4.6 Interaction between polymorphic forms of IGF-1 and sexual differences in growth Traits of Yankasa sheep

Table 4.5 shows the interaction between polymorphic forms of IGF-1 and Sex on growth traits in Yankasa Sheep. Observed trend showed significant ($P<0.05$) interactions among the measured traits with the exception of average daily gain. Male Sheep with AA and AB genotype were similar in performance across all traits (birth weight, average daily gain, weaning weight, weight at 6 months, weight at 8 months, weight at 12 months and height at withers) with the exception of chest girth (cm) where AA was significantly ($P<0.05$) higher than AB. Both AA and AB males had higher growth characteristics than BB males with the exception of weight at 8 months where all genotypes were similar for the males. Body weight at 12 months showed the AA female having higher value than BB males.

Table 4.5: Interaction between polymorphic forms of IGF-1 and sex on growth traits in Yankasa sheep

	AA		AB		BB		SEM	LOS
Traits	Male	Female	Male	Female	Male	Female		
Birth weight (Kg)	2.75^{a}	2.14^{b}	2.70^{a}	2.08^{b}	2.32^{b}	2.01^{b}	0.18	*
Weaning weight (Kg)	13.63^{a}	11.25^{b}	13.98^{a}	11.81^{b}	12.15^{b}	12.11^{b}	0.65	*
Average DailyGain (g/day)	0.10	0.10	0.11	0.11	0.11	0.11	0.01	ns
Weight at 6 months (Kg)	23.96^{a}	21.26^{b}	22.91^{a}	20.35^{b}	22.75^{a}	19.27^{b}	1.23	*
Weight at 8 months (Kg)	29.77^{a}	27.42^{b}	28.83^{a}	26.54^{bc}	28.84^{a}	25.35^{c}	0.93	*
Weight at 12 months (Kg)	32.05^{a}	28.78^{a}	31.18^{a}	27.16^{b}	29.39^{b}	26.90^{c}	1.29	*
Height at withers (cm)	62.98^{a}	58.05^{b}	63.27^{a}	53.55^{c}	55.31^{bc}	53.71^{c}	2.23	*
Chest Girth (cm)	71.63^{a}	67.46^{bc}	69.08^{b}	65.60^{c}	65.71^{c}	62.59^{c}	1.16	*

^{abc} means across columns differ significantly ($P<0.05$); ns: non-significant

4.7 Pearson correlation coefficients of growth traits in Yankasa sheep.

Table 4.6 outlines the correlation coefficients amongst measured traits. Significant ($P<0.05$, 0.01) correlation existed with coefficients ranging from -0.08 to 0.96. Birth weight was positively and significantly correlated with weaning weight ($r=0.26$), average daily gain ($r=0.31$) and height at withers ($r=0.26$). Weaning weight correlated significantly and positively with all traits with the exception of chest girth ($r=0.13$) and height at withers ($r=0.17$). Average daily gain was moderate and significantly ($P<0.05$) correlated with weight at 6 months, weight at 8 months and chest girth. Average daily gain had strong and significant ($P<0.05$) association with height at withers ($r=0.43$). Weight at 6 months had moderate and significant ($P<0.05$) correlation with weight at 8 months, weight at 12 months and chest girth. Weight at 8 months was moderate and significantly ($P<0.05$) correlated with weight at 12 months and chest girth with the exception of height at withers ($r=0.16$). Weight at 12 months was moderate and significantly ($P<0.05$) correlated with of height at withers ($r=0.38$) and chest girth ($r=0.23$). Low correlation existed between height at withers and chest girth ($r=0.18$).

Table 4.6: Pearson Correlation Coefficients for growth traits in Yankasa Sheep

Traits	Birth weight (Kg)	Weaning weight (Kg)	Average Daily Gain (g/day)	Weight at 6 months (Kg)	Weight at 8 months (Kg)	Weight at 12 months (Kg)	Height at withers (cm)	Chest Girth (cm)
Birth weight (Kg)								
Weaning weight (Kg)	0.26*							
Average Daily Gain (g/day)	0.31*	0.96**						
Weight at 6 months (Kg)	0.08^{ns}	0.21*	0.17					
Weight at 8 months (Kg)	-0.08^{ns}	0.22*	0.24*	0.26*				
Weight at 12 months (Kg)	0.12^{ns}	0.33*	0.21*	0.25*	0.35*			
Height at withers (cm)	0.26*	0.17	0.43**	0.17	0.16	0.38*		
Chest Girth (cm)	0.11^{ns}	0.13^{ns}	0.33*	0.24*	0.35*	0.23*	0.18*	

*; **: (P<0.05; 0.01); ns: non-significant

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Distribution of observed polymorphism of IGF-1 gene in Yankasa sheep.

Observed allelic frequencies of 0.72 and 0.28 for the A and B polymorphs of IGF-1 genes respectively, were similar to the corresponding values (0.71 and 0.29) reported in Zel sheep population and (0.76 and 0.24) in Mehraben sheep (Kazemi *et al*., 2011 and Behzadi *et al*., 2015). They however differed from the values of (0.89 and 0.11) reported in Iranian Baluchi sheep (Gholibeikifard *et al*., 2013). This difference in the frequency of the polymorphs of IGF-1 gene variants may due to different genetic selection programs being used in different countries, or because some of the IGF-1 gene variants are breed-specific, or simply because of small population size. These genetic differences need further clarification by investigating larger population of different breeds, from different areas or countries. There existed conspicuous absence of other polymorphic variants such as the C and G alleles reported in literature (Hajihosseinlo *et al*., 2013 and Niznikowski *et al*., 2014). The frequency of Variant B polymorphs of IGF-1 gene was low in Yankasa sheep (28.0%). This might suggest that variant B may postively associate with a trait or traits that might have been selected against historically.

The frequency of A variant in this study was low compared to the frequency of 0.89 for A variant reported by Gholibeikifard *et al*., (2013) in Iranian Baluchi sheep. Therefore, it is conceivable that the growth traits association previously seen with the Merino and Romney sheep is the result of a different intron A- 3’UTR haplotype than that present in the Suffolk population and/or the effect is moderated by some other genetic influence or environment effects.

Genotypic frequencies obtained were consistent with those observed and reported in different sheep (Kazemi *et al*., 2011; Behzadi *et al*., 2015 and Gholibeikifard *et al*., 2013), while reports of non-deviation from Hardy-Weinberg equilibrium reported by these authors were at variance with what was obtained in this study. The principle of HWE states that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. This could be due to the sheep having been "selected" and being derived from related rams and thus the frequency of the IGF-1 gene variants may be different from the unselected or original population. It may also be the result of some other factors including breed and genotype and environmental interactions. However the proclivity of the A allele and its hybrid genotype in this study have shown that this fast moving band of IGF-1 gene may be superior as a target candidate gene for selection and breeding of sheep in Nigeria since it has been estimated that up to 60% of the variation in IGF-1 serum level has a genetic basis (Licht *et al*., 2014).

5.2 Association between IGF-1 genotypes and growth traits in Yankasa sheep.

Observed differences in growth traits due to the various genotypes observed in this study agree with the findings of Kazemi *et al*. (2011) that polymorphism in 5´ flanking region had a significant effect on growth traits, live weight and carcass weight in Zel sheep and also that of Kurdistani *et al.* (2013) in Kurdish goat, where the polymorphism of *IGF-I* gene was associated with growth traits and yearling fleece weight (Deng *et al*., 2010) and Gholibeikifard *et al*. (2013) in Baluchi sheep.

This significant impact of the various genotypes on birth weight, weaning weight, average daily gain, weight at 6, 8 and 12 months, height at withers and chest girth in this study supports the assertion that Insulin-like growth factor-1 (IGF-1) is an important regulator of cell proliferation, differentiation, and apoptosis, as well as its acute insulin-like metabolic effects, and importance for growth and development. The level of IGF-1 peaks puberty and declines thereafter (Licht *et al*., 2014). According to Fletcher *et al.* (2005), the polymorphisms are thought to influence the transcription rate of IGF-1, which in turn affects serum IGF-1 levels which influences growth trait. It is however cautionary to note that results of studies that evaluated the relationship between the IGF-1 promoter polymorphisms and IGF-1 levels are contradictory; the homozygote 192 bp genotype has been associated with both higher and lower IGF-1 levels compared to the heterozygote and non-carrier genotypes (Fletcher *et al*., 2005 and Euser *et al*., 2011).

Literature reviews have shown that results vary in association between *IGF-I* gene polymorphism and growth traits among different breeds. Linkage disequilibrium of the *IGF-I* gene with QTLs could be the main reason of the inconsistent results. Therefore, quantitative traits are regulated by the large number of genes and also are affected by the interaction of these genes, so it is usual to observe the different effect of a candidate gene associated with a particular trait in a population. More studies are required in larger populations of Yankasa sheep and in other native Sheep breeds in order to verify the relevant effects of this SNP marker.

5.2.1 Birthweight

Birthweight of Yankasa sheep in this study ranged from 1.80 to 2.35 kg is lower than 3.55 kg reported in Kheri sheep (Gowane and Arora, 2009) and the values reported for Nilagiri Synthetic sheep (Sandyno) 2.56 kg (Rajendran, 2005) and 2.81 kg (Iyue, 1993).

5.2.2 Weaning weight (WW)

The weaning weights of Yankasa sheep in this study which ranged from 11.16 to 12.42 kg are higher than the lowest weaning weight of 7.11 kg in Gaddi sheep (Arora and Batta, 1983) but lower than 18.16 kg reported in Madgyal breed of sheep (Waghmode *et al*., 2008). Among the synthetics, Kashmir Merino had the maximum weaning weight of 21.80 (Khan and Singh, 1974). The values reported for Nilagiri Synthetic sheep were 10.00 (Iyue, 1993) and 10.66 kg (Rajendran, 2005).

5.2.3 Average daily gain

The average daily gains ranging from 0.11-0.14g/day which are higher than the 0.063 ± 0.001 kg reported by Shivkumar (2003) in Mecheri sheep. The variations could be linked genotype x environment interactions.

5.2.4 Weight at 6 months

The weights at 6 months which ranged from 22.01-23.58kg are lower than the 28.56 kg recorded in Keezhakkaraisal ewe lambs (Ganesakale and Rathnasabapathy, 1973) and the higher value of 26.46 kg was reported for Muzaffarnagri sheep.

5.2.5 Weight at 8 months

The weights at 8 months which ranged from 28.12 to 29.09Kg are higher than 12.09 and 27.55 kg recorded for Coimbatore (Singh and Singh, 1984) and Madgya breeds of sheep respectively (Waghmode *et al.*, 2008). The mean 8 months weight of 17.85kg for Nilagiri (Panneerselvam, 1993) and 18.57kg for Nilagiri Synthetic (Iyue, 1993) sheep are lower to the values obtained in this study.

5.2.6 Weight at 12 months

The weights at 12 months which ranged from 29.01 to 33.19 Kg in Yankasa sheep compared with the yearling weight of 26.63 and 32.85 kg recorded in Garole (Karim *et al.*, 2006) and

Muzaffarnagri (Bhat *et al.*, 1978) breeds of sheep respectively. The mean YW for Nilagiri and Nilagiri Synthetic sheep were reported as 20.26 and 21.05kg respectively (Panneerselvam, 1993; Iyue, 1993).

5.3 Sexual differences in growth traits of Yankasa sheep

Sex as a highly significant source of variation observed in the present investigation was supported by various authors in both mutton and wool breeds of sheep (Nehra and Singh, 2006; Waghmode *et al.*, 2008). The observed differences in studied traits between the male and female of Yankasa sheep were consistent with the reports of Afolayan *et al.* (2006) in Yankasa sheep using animals from the same stock. Birth weight ranges of 2.08-2.80 Kg obtained were within the range of 2.64-3.02Kg obtained by these authors. Similarly, males were heavier, taller and broader than the females according to Afolayan *et al*. (2006) which is similar to what was obtained in this study. This might partly be due to the pre-natal (Williams, 1968 cited in Afolayan *et al*., 2006) and pre-weaning advantage in male as compared to female lambs. These differences between male and female lambs were similar to those reported in beef cattle (Afolayan *et al.*, 2002ab, Afolayan *et al*., 2006 and Otoikhian *et al*., 2008). In those reports, steers were heavier, taller and bigger in girth; more muscled but with less fat compared to heifers both at weaning and post-weaning ages.

However, sex of lambs did not affect average daily gain in this study contrary to the results from other investigations (Mokhtari *et al*., 2008; Mohammadi *et al*., 2010; Thiruvenkadan *et al*., 2011). Klindt (2005) had explained that endocrine functions are often sexually dimorphic, different in males and females. He explained further that programming of sexual dimorphism begins with embryonic expression of the sex-determining gene (SRY) in males and secretion of Mullerian-inhibiting hormone (anti-Mu¨llerian hormone, MIH), which prevents development of internal reproductive tracts of females. The expression of sexual dimorphism in terms of overall body weight gain (considering the entire population) was possibly masked by genetic potentials for fast growth expressed by males.

5.4 Interaction effect of polymorphic forms of IGF-1 and sex on growth traits in Yankasa sheep

It has been reported that IGF-1 serum level is influenced by many factors, such as nutritional status, liver function, and serum levels of sex steroids and insulin, the secretion of this peptide is mainly regulated by growth hormone (GH) (Licht *et al*., 2014). This pre-supposes that there exist interaction between sex (through sex steroids) and IGF-1 gene and its circulating genotypes. Findings in this study has confirmed this assertion due to the significant ($P<0.05$) differences obtained for the interaction between genotype and sex on growth traits measured with the exception of average daily gain.

5.5 Pearson correlation coefficients for growth traits in Yankasa sheep

In this study, the mostly positive and significant correlation between growth and linear traits were consistent with the reports of Afolayan *et al*., (2006) and Otoikhian *et al*., (2008) in Nigerian Sheep breed though there existed variations in coefficient values. This may be due to sample size, differences in environmental and experimental set up and stock differences. Similarly, Afolayan (2003) obtained a higher genetic correlation between weight and other growth measures across weaning and post-weaning ages of some *Bos taurus* cattle breeds. This indicates that using IGF-1 gene as a candidate gene for selection and improvement of sheep will not only influence live weight but other conformational traits since they are positively associated. The phenotypic correlations among body weight traits were positive and showed a tendency to decline as the age interval between weights increased. Correlations between successive weights especially for later ages were high in most instances. The stronger positive correlations between early body weight traits and late body weights could be used for indirect selection on these early traits to get higher body weights at later ages which are economically important. In Nilagiri sheep, Panneerselvam (1993) found similar results for birth weight and weight at 8 months. Birth weight had low and negative phenotypic correlation with body weight at 8 months.

The negative estimates could be due to the seasonal fluctuations in availability of nutrition for the animals. The lambs up to one year age were continuously provided with concentrate feed of high protein content, while in the adults only pregnant and lactating animals received the concentrate feed. The negative phenotypic correlation could also be due to possible genotype x environment interaction, where in the animals with superior body weights reared under favourable nutrition could become inferior during harsh conditions. Negative phenotypic correlations observed could be due to the distinct seasonal fluctuations with respect to climate and nutrition prevailing in the Zaria, Kaduna State. The estimates of phenotypic correlation of 6, 8 and 9 months were generally positive and medium. This is in agreement with the earlier reports on different Indian breeds of sheep (Dixit *et al.*, 2001). Phenotypic correlations among successive weights were higher than those with a longer intervening period of time. The high estimates between successive weights may be artificial, due to autocorrelations of the error terms. In general, the estimates obtained at various age intervals were similar to earlier estimates reported for different breeds in India (Nehra and Singh, 2006).

Body weight and most of body linear measurements in this study showed significant variability in a polynomial trend as animal age advances. This implies that growth patterns of the animal might not be explained well by body measurements. This result is in line with (Alayu Kidane *et al.*, 2014) who found similar result on indigenous goat types in north Gondar zone. In addition the variations show that those measurements might be too dependent on age rather than environmental perturbations (Hamayun *et al*., 2006). Height at wither and chest girth significantly showed a polynomial trend as the age advances implies that animals were increased in their body size or skeletal size and also increased in their body condition as reported by (Fajemilehin *et al.*, 2008) that height at withers at any given time reflects the animal's skeletal size and that heart girth reflects body condition.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

In other to determine single nucleotide polymorphism of the IGF-1 gene and its effect on some growth traits in Yankasa breed of Sheep, a random sample of 50 sheep (25 males and 25 females) were selected. Animals were measured for growth traits namely: birth weight, average daily gain, weaning weight, weight at 6, 8 and 12 months, chest girth and height at withers. Average daily gain (ADG) was calculated as the difference between the initial and final body weights divided by the number of days in between the two measurements. Blood samples was collected from the animals neck region through the jugular veins into 5ml EDTA vacutainer tubes and transferred to the laboratory for DNA extraction. Total DNA extraction was made with ZR-96 Genomic DNA miniprep.

Obtained band of the polymorphic bands were designated as

- A high molecular weight band as AA homozygote.
- The presence of a single lower molecular weight band as BB homozygote.
- Any of a mixture midway between high and low molecular weight bands as AB heterozygotes.

Frequency of alleles were calculated according to Hardy-Weinberg’s equation and also subjected to Chi-Square analysis to test for Mendelian inheritance ratio for band. Effects of sex and genotype and their interaction on growth traits along with correlation between measured traits were investigated.

Allelic frequencies were 0.72 and 0.28 for the A and B alleles respectively while genotypic frequencies of 0.58, 0.28 and 0.14 were discovered for the AA, AB and BB bands respectively. Mendelian inheritance test showed that the population was not in Hardy-Weinberg equilibrium. Association between genotype and growth traits showed that the AA and AB genotype were superior to the BB for most measured traits, and there was genotype and sex interaction effect. Correlated analysis also showed high, positive and significant associations between most of the traits measured in the study. This further solidifies the position of IGF-1 gene as a candidate gene for selection and improvement of Yankasa sheep breed in Nigeria.

6.2 Conclusions

1. There was significant Polymorphism in the IGF-1 gene of Yankasa sheep.
2. These polymorphisms were significantly associated with growth traits (birth weight, weaning weight, average daily gain, weights at 6 and 12 months, height at withers and chest girth).
3. The Yankasa sheep with AA and AB genotypes had similar but significant higher propensity for growth than BB genotype.

6.3 Recommendations

1. My current results indicate that polymorphism of the IGF-I gene can be a potential molecular marker for growth traits in Yankasa sheep.
2. AA and AB genotypes could be used for genetic improvement of growth traits in Yankasa sheep in Nigeria.
3. Further studies on non-selected population and larger population of other breeds of sheep can beused to get a better picture on IGF-1 gene polymorphism among Nigerian Sheep.

6.4 Contribution to Science

1. Polymorphism of the IGF-1 gene for AA and AB genotypes are strongly associated with the male animals with respect to growth traits.

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