COMPARATIVE GENOMICS ANALYSIS OF GROWTH HORMONE (GH), INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) AND MYOSTATIN (MSTN) GENE SEQUENCES IN CHICKEN, RABBIT AND SHEEP

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JUNE, 2018

DECLARATION

I hereby declare that the work in this dissertation entitled ‘’Comparative Genomics Analysis of Growth hormone (GH), Insulin-like Growth Factor 1 (IGF-1) and Myostatin (MSTN) Gene sequences in Chicken, Rabbit and Sheep’’ was carried out by me at Animal Science Department, Faculty of Agriculture, Ahmadu Bello University Zaria, under the supervision of Dr. M. Kabir and late Prof. G. N. Akpa. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented the degree or diploma at any other university.

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled **''COMPARATIVE GENOMICS ANALYSIS OF GROWTH HORMONE (GH), INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) AND MYOSTATIN (MSTN) GENE SEQUENCES IN CHICKEN, RABBIT AND SHEEP''** by MUSA Abdulrahman, meets the regulations governing the award of the degree of Masters of Science (M.Sc.) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicated this research work to my beloved parents - Alhaji Abdulrahman Ahmed and Hajia Asabe Abdulrahman Suleiman – for their understanding, believing in my ability and always give me all their necessary support in all ways.

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Alhamdulillah; my greatest gratitude to Allah (SWT) for honoring me among many of his creations and guiding me always to what I am today and to the future, being under His cover all my life.

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ABSTRACT

The gene sequences of Growth hormone (GH), Insulin-like Growth Factor 1 (IGF-1) and Myostatin (MSTN) were downloaded from National Center for Biotechnology Information (NCBI) database, through Entrez of the database as non-redundant reference sequence in FASTA format, using respective accession numbers of the various genes in the GenBank to access the necessary gene information. They were subjected to different computational tools, on-line softwares and programs; for Multiple sequence alignment, phylogenetic tree, BLAST-like alignment tool (BLAT), Basic Like Alignment Search Tool (BLAST)were used to analyzed for gene number in a genome, exon type and number per gene, number of codons per gene; gaps within the alignment of the three species for each gene; single nucleotide polymorphism between the alignments of chicken by rabbit, chicken by sheep, and rabbit by sheep; the conserved regions between the alignments of chicken by rabbit, chicken by sheep, rabbit by sheep, and chicken by rabbit by sheep and other parameters by submitting the genes respective sequences in FASTA format to the tool. Results indicated that higher body weight and size of sheep might have been due to two categories of GH, high gene number of GH, high exon number of GH, long sequence length of GH which resulted to higher predicted coding sequence and higher predicted peptide size. Rabbit and chicken never shared common ancestor in GH, the number of chromosomes GHgene distributed in sheep genome was higher than that in rabbit and chicken, and all are found on the opposite strand (negative). Rabbit and sheep once shared common ancestor in IGF-1 and MSTN gene. Rabbit and sheep IGF-1 has longer gene length, higher number, exon number, predicted coding sequence, predicted peptide size which might have been the reason for higher body weight at maturity compare to chicken. It is concluded that; the number of GH gene of sheep is higher than that of GH of chicken, than GH of rabbit but the

number IGF-1 gene of rabbit and sheep are higher than that of chicken IGF-1 gene, while the number of rabbit myostatin is higher than that of chicken and sheep Myostatin. The number of genes in chicken, rabbit and sheep genomes plays a key role in establishing effective gene function. The genes shares some conserved regions but the length/size, gene number, exon number, exon type, number of gene/genome, gene DNA strand, codons/gene, gaps, SNP varied greatly among chicken, rabbit and sheep species. The gene conserved regions similarities are for conserved functions while, the differences are for different expression pathways among the three species. Myostatin gene depresses animal growth process through the action of IGF-1 gene. Frame shift mutations in the upstream or downstream regions of the genes, lead to differential gene regulation without actually changing the structure and functions of the protein. It is recommended that; Chicken and sheep growth could be improved through increasing the length/size and number of their IGF-1 gene by gene modification techniques. Rabbit growth could be improved through increasing the length/size and number of the GH gene by gene modification techniques. Some missing nucleotides in rabbit and chicken GH and IGF-1 gene sequences could be modified by gene modification technology and use as a useful marker for economic traits. It is recommended that more experiments could be conducted to knock-off or knock-out some of these extra length sequence regions of sheep GH and IGF-1 gene not found in rabbit and chicken GH and IGF-1 gene, when aligned.

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CHAPTER ONE

1.0 INTRODUCTION

Bioinformatics is the science of storing, extracting, organizing, analyzing, interpreting and utilizing information from biological sequences and molecules (Khalid, 2010). Bioinformatics is often defined as the application of computational techniques to understand and organize the information associated with biological macro-molecules (Luscombe *et al.*, 2001). It has been mainly fueled by advances in DNA sequencing and mapping techniques (Khalid, 2010). Over the past few decades, rapid developments in genomic, other molecular research technologies and information technologies have combined to produce a tremendous amount of information related to molecular biology. The primary goal of bioinformatics is to increase the understanding of biological processes (Khalid, 2010).As biology is increasingly becoming a technology-driven science, databases have become indispensable to store not only data, but also the results of experiments generated by different research projects around the world (Hey *et al*., 2009). A biological database is a collection of information, or data from a biological system, stored in a computer readable format. Some databases are also called data repositories if they function as a place where large biological datasets can be stored and retrieved by users. Sharing of data between scientists accelerates the speed of discoveries and has the potential to greatly advance a scientific field as a whole (this is known as the Fourth Paradigm of Data-Driven Scientific Discovery (Hey *et al*., 2009). There are two types of biological databases: public databases that are freely accessible on-line, and private databases that require payment before you can access them (Dutilh and Keşmir, 2016).

The genome of a species encodes genes and other functional elements, interspersed with non-functional nucleotides in a single uninterrupted string of DNA (IHGSC, 2001).

Recognizing protein-coding genes typically relies on finding stretches of nucleotides free of stop codons called Open Reading Frames (ORFs) that are too long to have likely occurred by chance. Since stop codons occur at a frequency of roughly 1 in 20 random sequence, ORFs of at least 60 amino acids will occur frequently by chance (5% under a simple Poisson model), and even ORFs of 150 amino acids will appear by chance in a large genome (0.05%). This poses a huge challenge for higher eukaryotes in which genes are typically broken into many, small exons (on average 125 nucleotides long for internal exons in mammals (IHGSC, 2001).

Some regions within a protein sequence are more conserved than others during evolution (Dutilh and Keşmir, 2016). These regions are generally important for the function of a protein and/or the maintenance of its three dimensional structure, or other features related to its localization or modification. By analyzing constant and variable properties of such groups of similar sequences, it is possible to derive a signature for a protein family or domain, which distinguishes its members from other unrelated proteins by sequence alignment, which allows us to discover these signatures (Dutilh and Keşmir, 2016). Sequence alignment is defined as the bioinformatics task of locating equivalent regions of two or more sequences, and aligning their nucleotide or amino acid residues side by side, to maximize their similarity (Dutilh and Keşmir, 2016). Multiple sequence alignments allow for identification of conserved sequence regions. This is very useful in designing experiments to test and modify the function of specific proteins, in predicting the function and structure of proteins, and in identifying new members of protein families (Dutilh and Keşmir, 2016).

DNA Sequencing is a technique/method by which the exact order of nucleotides within a DNA molecule is determined (Mayor *et al.*, 2000). Comparative data analysis provides the

opportunity to determine what is shared and what is unique to each species (Mayor *et al*., 2000).

Growth in animals is controlled by a complex system, in which the somatotropic axis plays a key role. The genes that operate in the somatotropic axis are responsible for the postnatal growth, mainly GH that acts on the growth of bones and muscles mediated by IGF-1 (Sellier, 2000). The growth hormone (GH) and insulin-like growth factor 1 (IGF-1) genes are candidates for growth in bovine, since they play a key role in growth regulation and development (Hossner *et al*., 1997; Tuggle and Trenkle, 1996). Effects of GH on growth are observed in several tissues, including bone, muscle and adipose tissue. These effects result from both direct action of GH on the partition of nutrients and cellular multiplication and IGF-1-mediated action stimulating cell proliferation and metabolic processes associated to protein deposition (Boyd and Bauman, 1989). IGF-1 stimulates protein metabolism and is important for the function of some organs, being considered a factor of cellular proliferation and differentiation (Andrea*et al*., 2005). Polymorphisms in GH gene have been used as a genetic marker associated with different performances and productions traits such as body weight, birth weight and weaning weight in goat (Wickramaratne *et al*., 2010), The rabbit GH gene has already been sequenced by Wallis and Wallis (1995) and has been investigated as a gene associated with market weight of commercial rabbit (Fontanesi *et al*., 2012). Mutations of this GH gene have been described in goats (Malveiro *et al*., 2001), and poultry (Feng *et al*., 1997) to affect important production traits.

In chickens divergently selected for high or low growth rates, there were significantly higher IGF-1 mRNA levels in the high growth rate line than in the low growth rate line (Beccavin, *et al*., 2001). The growth hormone receptor (GHR), insulin-like growth factor-1 (GH-IGF-1) system controls the number of follicles in animals that are recruited to the

rapid growth phase (Roberts *et al*., 1994; Monget, *et al*., 2002). It is also known that the GH-IGF-1 system has been modified as a result of selection for enhanced growth rate (Ballard *et al*., 1990; Ge *et al*., 2001). The insulin-like growth factor gene (IGF1) is 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). Earlier research on GHR, IGF-1 and IGFBP-3 in cattle, goats and chickens showed genetic polymorphisms and their association with production traits (Liu *et al*., 2010). The IGF1 gene is essential for normal embryonic and postnatal growth in mammals (Bian *et al*., 2008).

Myostatin (MSTN), previously called Growth differentiation factor 8 (GDF8), is a member of transforming growth factor-β (TGF-β) superfamily. It is a negative regulator for both embryonic development and adult homeostasis of skeletal muscle (Tu *et al*., 2014). Myostatin (MSTN) is a negative regulator of the muscle growth factor, which belongs to the transforming growth factor beta superfamily (McPherron *et al*., 1997). It is able to negatively control the growth of muscle cells by inhibiting the transcriptional activity of MyoD family members. Its expression is negatively correlated with muscle weight (Weber *et al*., 2005). Mutations in the myostatin gene have also been shown to cause double muscling in humans and other species (Clop *et al*., 2006). These findings suggest that strategies for inhibiting myostatin function may be applied to improve animal growth. Homozygote and heterozygote cattle with mutations of the MSTN gene-conserved Ribbon bases exhibit the advantage of strong muscle in increase birth weight, and obvious double-hip muscle characteristics (Casas *et al*., 1999). As the candidate gene in pig double-hip muscle, the MSTN gene has an important impact on the amount of lean meat and fat deposition (Sonstegard *et al*., 1998). The rabbit is a high quality and efficient meat producing livestock as well as a common experimental animal. Therefore, providing

information on its genetic basis and regulation mechanism of skeletal muscle growth and development has an important theoretical and practical significance (Qiao, 2014). The effects of the SNPs of myostatin gene on chicken growth in a F_2 resource population are associated with increase in abdominal fat weight, abdominal fat percentage, birth weight and breast muscle percentage (Zhiliang *et al*., 2004). Notably, these data suggest that myostatin could be an ideal molecular marker for marker-assisted selection for skeletal muscle and adipose growth in chicken breeding program. It was reported that TTTTA deletion phenomenon occurred in MSTN gene was unique for goats when compared with sheep, cattle, water buffalo, domestic yak, pigs, and humans (Grisolia *et al*., 2009; Zhang *et al*., 2013) Khichar *et al*. (2016) found an important effect of a 5-base pair (bp) deletion on early body weight and size of a goat.

1.1 Justification

Identification of a candidate gene is a powerful method for understanding the direct genetic basis involved in the expression of quantitative traits and their differences between individuals (Rothschild and Soller, 1997; Nagaraja *et al*., 2000). Mutations of the MSTN gene-conserved region bases in chicken, rabbit and goat will lead to the activation or inhibition of the gene expression product and the loss or increase in function or inhibiting muscle growth, which will result in excessive muscle development and expression (Lee and McPherron, 1999). Indeed, there have been several recent examples in which comparative sequence data have led to the discovery and understanding of function of previously undefined genes. The complete human/mouse orthologous-sequence dataset proved particularly valuable in the characterization of gene families in humans and mice (Dehal *et al*., 2001). For instance, by comparing olfactory receptor gene families on human

chromosome 19, computational analysis indicated that humans have approximately 49 olfactory receptor genes, but only 22 had maintained an open reading frame and appeared functional. This contrasts with the vast majority of the homologous mouse genes that have retained an open reading frame. This finding of reduced olfactory receptor diversity in humans is consistent with the reduced olfactory needs and capabilities of humans relative to rodents (Pennacchio and Rubin, 2003).

Growth hormone gene (GH) a single polypeptide produced in the anterior pituitary gland is a promising candidate gene marker for improving milk and meat production in goats and other farm animals (Min *et al.,* 2005). IGF1 is a mediator of many biological effects; it increases the absorption of glucose, stimulates myogenesis and production of progesterone, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, and intervenes in the synthesis of DNA, protein, RNA , and in cell proliferation (Mohammadi *et al.,* 2011)

The increasing availability of genomic sequence from multiple organisms has provided biomedical scientists with a large dataset for orthologous-sequence comparisons. The rationale for using cross-species sequence comparisons to identify biologically active regions of a genome is based on the observation that sequences that perform important functions are frequently conserved between evolutionarily distant species, distinguishing them from nonfunctional surrounding sequences. (Pennacchio and Rubin, 2003). Sequence alignment is a good way of predicting the function of a gene or protein. Moreover, sequences contain a lot more information, such as from which organism the gene or protein is derived, and what are the evolutionary relationships of the gene or species with other genes or species. Much of this information can only be discovered by finding homologs of the gene or protein in other species (Dutilh and Keşmir, 2016).

To justify this study, a comparative genomics analysis to access the similarities and differences between these three growth genes; Growth hormone (GH), Myostatin (MSTN) and Insulin-like growth factor-1 (IGF-1) gene among chicken, rabbit, and sheep will identify the similarities or differences in the rate of increase in growth and body size to maturity, final body size at maturity, and body conformation at maturity. The analysis of sequences conserved between these three species will further enrich available information of biologically active sequences in these species.

1.2 Objectives of the Study

The main objective of this study was to determine gene sequence conservations and variations of GH, IGF-1 and MSTN gene in chicken, rabbit and sheep that can be utilize for animal production improvement. The specific objectives are;

1. To determine the distribution of GH, IGF-1 and MSTN genes and their chromosomal and DNA strand contained in the genome of chicken, rabbit and sheep.
2. To determine the similarities and differences in nucleotide sequences of GH, IGF-1 and MSTN genes in chicken, rabbit, and sheep.
3. To establish the kind of conserved nucleotides regions, gaps, single nucleotide polymorphism between GH, IGF-1 and MSTN genes that affect the codons of chicken, rabbit and sheep.
4. To determine the evolutionary nucleotide substitution and relationship of GH, IGF-1 and MSTN genes in chicken, rabbit and sheep.
5. To establish the nucleotide sequence variation in GH, IGF-1 and MSTN genes that result in weight differences between chicken, rabbit and sheep at maturity.

1.3 Hypotheses

H_0 = Variation in nucleotide sequences and conservation of these genes does not have specific effect on the growth, development and size at maturity of Chicken, Rabbit and Sheep.

H_a = Variation in nucleotide sequences and conservation of these genes have specific effect on the growth, development and size at maturity of Chicken, Rabbit and Sheep.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Genes and Mutations

A candidate gene approach has been successfully applied to identify several DNA markers associated with production traits in livestock (Rothschild and Soller, 1997). The principle is based on the fact that variability within genes coding for protein products involved in key physiological mechanisms and metabolic pathways directly or indirectly are involved in determining an economic trait (e.g. feed efficiency, muscle mass accretion, reproduction efficiency, disease resistance, etc.) might probably explain a fraction of the genetic variability for the production trait itself (Fontanesi *et al.,* 2008). The first step is the identification of mutations in candidate genes that can be analyzed in association studies in specific designed experiments (Yao *et al.,* 1996).

Genetic marker is a commonly occurring genetic variation that can be easily tracked in genetic studies and can be used on entire alleles, repetitive stretches of DNA or single nucleotide polymorphisms (SNPs) (Gu *et al.,* 2004). SNP is the most frequent form of genetic variation and is a resource for mapping complex genetic traits. A major goal in animal agricultural genetics is to understand the role of common genetic variants in controlling quantitative traits. The development of high quality SNP maps for agriculturally important species can be viewed as a fundamental tool for animal breeding program. This will involve investigating the nature of gene variation in animal populations, assembling a large database of SNPs in candidate genes and studying association genetics of SNPs with particular quantitative trait and genome-wide association scans (Gu *et al.,* 2004). Marker Assisted Selection (MAS) allows for the accurate selection of specific DNA variants that

have been associated with variation in growth and meat production traits for breeding of superior individuals for genetic improvement. The body weights (at birth, at weaning, 6-month and twelve-month weights) are the indicators of individual performance on growth rate and they have direct effect on the health and productivity of sheep (Zaffer *et al.,* 2015). The study of genes underlying phenotypic variation can be performed in two different ways, first, from phenotype to genome, which is performed by linkage disequilibrium (LD) based association mapping or by targeting particular candidate genes identified based on homology to known genes, and second, from genome to phenotype, which involves the statistical evaluation of genomic data to identify likely targets of past selection using selective sweep analysis (Asadi, 2012; Azizi, 2012). In contrast to natural populations, domesticated species provide an exciting opportunity to understand how artificial selection promotes rapid phenotypic evolution (Moore *et al.,* 2003). With the hypothesis that different selection pressures operated in thin and fat tail breeds over the history of time, and somehow the selection acts on a variant that is advantageous only in one breed, it is expected that the frequency of that variant may differ across populations to a greater extent than predicted for variants evolving neutrally in all populations (Azizi, 2012). Identifying these genome regions, which have been subject to such selective sweeps, could reveal the mutations which are responsible for the expression of the variant in these breeds. The examination and identification of variation in SNP allele frequencies between populations, can be quantified by the statistic fixation index (FST), is a promising strategy for detecting signatures for selection (Azizi, 2012). Measures of body size and form are desired in many experiments with sheep, including studies of growth, inheritance and nutrition (Nejati-Javarem *et al.,* 2007).In meat-producing species, body conformation and growth rate of animals are important selection criteria (Duguma *et al.,* 2002).

A gene mutation is a permanent alteration in the DNA sequence that makes up the gene, such that the sequence differs from the normal sequence found in most animals (Genetics Home Reference, 2017). Mutations range in size; they can affect anywhere from a single DNA building block (base pair) to a large segment of a DNA double helix that includes multiple genes. Gene mutations can be classified in two major ways: Hereditary mutations that are inherited from a parent and are present throughout animal's life in virtually every cell in the body. These mutations are also called germ-line mutations because they are present in the parent's egg or sperm cells, which are also called germ cells. When an egg and a sperm cell unite at fertilization, the resulting fertilized egg cell receives DNA from both parents. If this DNA has a mutation, the offspring that grows from the fertilized egg will have the mutation in each of his or her cells requiring the regulation of the mutagens (Genetics Home Reference, 2017).Mutational fitness effects are generally divided into three categories: deleterious mutations reduce the fitness of the organism, neutral mutations have very small effects on fitness and advantageous mutations increase the fitness (Kimura, 1983).The majority of the advantageous mutations will cause a very small increase in fitness, but due to clonal interference these will rarely be the winners in laboratory or natural populations (Rozen, *et al*., 2002).

Genetic alterations that occur in less than 1 percent of the population are called polymorphisms. They are common enough to be considered a normal variation in the DNA. Polymorphisms are responsible for many of the normal differences between animals such as eye color, hair color, and blood type. Although many polymorphisms have no negative effects on animal's health, some of these variations may influence the risk of developing certain disorders (Genetics Home Reference, 2017).

In a study of Fay *et al*. (2001), to attempt to partition human amino acid substitutions with respect to their phenotypic consequences, which was based on common polymorphism and sequence divergence data from human genes. Estimated that 60% of missense mutations were deleterious, 20% were slightly deleterious, and 20% were neutral. The vast majority of mutations listed in Human Gene Mutation Database reside within the coding region (86%), the remainder being located in either intronic (11%) or regulatory (3%, promoter, untranslated or flanking regions) sequences (David *et al*., 2010).Frame shift mutation occurs when the addition or loss of DNA bases changes a gene's reading frame. A reading frame consists of groups of 3 bases that code for one amino acid. A frame shift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions, and duplications can all be frame shift mutations (Genetics Home Reference, 2017).

2.2 Phylogenic Analysis

Reconstructing the evolutionary history of genes is also of great importance for the bioinformatics prediction of the functions of genes that have not been experimentally determined. Both the prediction of gene function and the discovery of the evolutionary relationships between species rely on the reconstruction of phylogenetic trees of genes (Dutilh and Keşmir, 2016). Phylogenetic trees reveal evolutionary relationships between organisms or sequences (e.g. genes or proteins) (Dutilh and Keşmir, 2016). Phylogeny study a set of sequences that are likely to have evolved from a common ancestor to find out how the species or sequences diverged from each other, in which order and at what time. This procedure that allow information of the evolutionary relationship between organisms,

individuals, genes, prediction functions and structures, among others (Junqueira *et al*., 2014).

Often it is not possible to reliably estimate divergence times from molecular data. If the data set does not allow for such an estimate, the final tree branches do no longer represent Million years (Ma). If it is known that, the sequences under study evolve with similar rates, the branch lengths can again be drawn to scale, to represent evolutionary distance (e.g. the number of nucleotides that have been substituted) instead of Ma (Dutilh and Keşmir, 2016). Alignment techniques are necessary to whole genome analysis, in which the comparison between different genomes or from the same species allows us to identify variations in the sequences and associate them with specific phenotypes (Junqueira *et al*., 2014). Discrepancies between gene trees and species trees can arise because genes have their own dynamics within populations and genomes. For example, divergence within genes typically occurs prior to the splitting of populations during speciation. This occurs particularly frequently in genes, for which (allelic) diversity in population is advantageous. Phylogenetic trees of individual genes are inconsistent, the whole-genome analysis, e.g., the gene content (the presence/absence of gene families over genomes) is becoming an attractive approach to extracting the bulk phylogenetic signals (Xun and Hongmei, 2004).

Gene duplication within the genome is another reason for differences between gene trees and species trees. Gene duplication means that a copy of a gene is inserted somewhere else on the chromosome. This process affects trees over much larger time-scales than the allelic divergence. The genes are still similar in the sense that one species can harbor multiple variants of a homologous gene, and that a tree of genes from these species can display multiple genes per species (Dutilh and Keşmir, 2016). The field of molecular phylogenetic

can be defined as the study of evolutionary relationships of genes and other biological macromolecules by analyzing mutations at various positions in their sequences and developing hypotheses about the evolutionary relatedness of the biomolecules. Based on the sequence similarity of the molecules, evolutionary relationships between the organisms can often be inferred (Jin, 2006). Mutations in the coding sequence of the gene may lead to changes in the protein structure and consequently its enzymatic function, or also by mutations in the upstream or downstream regions of the gene, leading to differential gene regulation without actually changing the structure and function of the protein (Dutilh and Keşmir, 2016). In a gene tree, the internal node indicates a gene duplication event (Jin, 2006).

2.3 Growth Hormone Gene (GH)

Growth, is a complex process that involves both increase in mass and differentiation, and maturation of many tissues especially skeletal and muscles, therefore a number of complications (such as reduced reproductive performance, increased carcass fat, skeletal abnormalities, and ascites) have arisen with intense mass selection for high growth rate (Gu *et al.,* 2004). This disturbance of physiological homeostasis has become a selection barrier to continued selection for genetic improvements in growth performance. However, Growth rate is the most important area for the poultry breeding industry due to its economic value and has been improved dramatically in the past, through mass selection due to it is moderate to high heritability values (Gu *et al.,* 2004). As it has been demonstrated that genetic polymorphisms are associated with quantitative traits like growth rate (Dunnington *et al.,* 1990), therefore it may be possible to use SNPs as genetic markers for direct

genome-wide assessments in order to eliminate undesirable alleles and maximize the degree of homozygosity or heterozygosity for desirable alleles (Gu *et al.,* 2004).

Growth hormone (GH) treatment increased the growth rate in achondroplasia (ACH) in parallel with the increment of serum levels of insulin-like growth factor IGF-1, suggesting an important role of IGF-1 in skeletal development. GH administration increased height velocity in a larger number of ACH patients with no adverse effects (Tanaka *et al.,* 1998; Seino *et al.,* 1999). It is well known that IGF-1 mediates the action of GH on cartilage and bone (Daughaday *et al.,* 1972; Yakar *et al.,* 1999). It is, therefore, possible that GH improves the disturbed bone growth in patients with ACH, at least in part, through production of IGF-1. IGF-1 mediates its biological effects through a specific receptor, IGF-1 receptor (IGF-1R), which contains tyrosine kinase in the intracellular domain (Daughaday and Rotwein, 1989). IGF-1 is known as a mediator of GH action on cartilage and bone (Tanaka *et al.,* 1998; Seino *et al.,* 2000).

Growth hormone gene (somatotropin; GH) is a protein isolated from the pituitary, which regulates somatic growth in most vertebrates, and has effects on various metabolic activities. Like other mammalian GH genes for which sequences are available, the rabbit growth hormone gene consists of five exons split by four introns (Wallis and Wallis, 1995). Southern blotting analysis revealed that unlike other species, this gene is present as a single copy gene without GH-like genes in the rabbit genome (Fontanesi *et al.,* 2008). Fontanesi *et al.*, (2012) have re-sequenced a fragment of 1337 bp of the growth hormone gene in rabbits (EMBL accession numbers: HE646284 and HE646285) from different breeds and identified two single nucleotide polymorphisms (SNPs) in the 5′-flanking region: c.-78C>T andc.-33A>G.

In animal industry, growth traits of animal are always of primary concern during breeding for its determinant economic value (Zhang *et al.,* 2008). Main application and potential for use of markers to enhance genetic improvement in livestock is through within-breed selection (Dekker, 2004). The genes of the growth hormone axis affect a wide variety of physiological parameters such as appetite control, growth, body composition, ageing and reproduction (Byatt *et al.,* 1993) as well as immune responsiveness (Kelley and Felton, 1995). The structure of the ovine growth hormone gene is similar to that found for other growth hormone genes and, as expected, is very homologous to the bovine gene (Gordon *et al.,* 1983).

Growth hormone gene is encoded by 1800 base pairs (bp), consisting of five exons, separated by four intervening sequences (Gordon *et al.,* 1983). The complete ovine GH DNA sequence and its predicted amino acid sequence have been established by (Orian *et al.,* 1998) from an ovine pituitary genomic library. In the ovine, two alleles of the GH gene have been described. The Gh1 allele contains a single gene copy (GH1), whereas in the Gh2 allele the gene is duplicated (copies GH2-N and GH2-Z) with the two copies being located 3.5 kb apart (Valinsky *et al.,* 1990). Sequence differences between the GH2-N and GH2-Z copies have been demonstrated and polymorphisms have been found in ovine GH coding and non-coding regions (Ofir and Gootwine, 1997). Use of genetic markers in selection can greatly accelerate the breeding process.Growth traits are complex quantitative traits involving multiple genes, loci and interactions.

It is well documented that growth hormone (GH) influences animal processes such as growth (Breier, 1999), lactation (Baldi, 1999), and reproduction (Scaramuzzi *et al.,* 1999), since its discoveries in the 1920 s. In most mammals, GH is a product of a single gene and is normally secreted in a pulsatile manner by the pituitary gland (Veldhuis *et al.,* 2001). GH

affects cell growth and proliferation either directly or indirectly through stimulation of the insulin-like growth factor (IGF) system. GH activity is first detected in the fetal pituitary and in circulation of fetal lambs around days 50-60 of pregnancy (Gluckman *et al.,* 1979). The components that constitute the growth hormone (GH) axis have a major influence on a diverse array of biological processes, ranging from growth and differentiation to reproduction (Chase *et al*., 1998, Feng *et al*., 1997). The growth hormone receptor (GHR), insulin-like growth factor-1 (GH-IGF-1) system controls the number of follicles in animals that are recruited to the rapid growth phase (Roberts *et al.,* 1994, Monget *et al.,* 2002). It is also known that the GH-IGF-1 system has been modified as a result of selection for enhanced growth rate (Ballard *et al*., 1990, Ge *et al*., 2001). In chickens divergently selected for high or low growth rates, there were significantly higher IGF-1 mRNA levels in the high growth rate line than in the low growth rate line (Beccavin *et al.,* 2001). There are obvious physiological connections between body weight homeostasis and the reproductive axis in both sexes. The rate of sexual maturation is much more closely associated with body growth than with chronological age (King, 2000). Researchers have demonstrated that the identified markers in the IGF-1 and GH-receptor genes, which are still segregating in many non-inbred strains of White Leghorn chickens, were associated with changes in body weight (Feng *et al*., 1998). The latter studies suggest that IGF is a local mediator of GH or gonadotropin action in the ovary (Hui-fang *et al.,* 2008). The growth hormone is the main constituent of the somato-tropic axis and plays crucial role in the postnatal growth and metabolism regulation. Additionally, GH affects indirectly, by controlling the secretion of other hormones including IGF1, which interacts with insulin-like growth factor 1 receptors (IGF1R) in target tissues (Proskura and Szewczuk, 2014).

The chicken growth hormone (cGH) gene is considered one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism (Byatt *et al.,* 1993; Copras *et al.,* 1993; Vasilatos-Younken *et al.,* 2000). The gene encodes a 191– amino acid mature growth hormone protein and a 25– amino acid signal peptide. The cGH gene has 4,101 base pairs and consists of five exons and four introns, differing in this regard from its mammalian counterpart (Mou *et al.,* 1995; Tanaka *et al.,* 1992). A 50 bp deletion in intron 4 of the cGH gene was found in Chinese native Taihe Silkies chickens (Nie *et al.,* 2002). The cGH gene in another native breed, Yellow Wai Chow, was found to have one silent substitution, 31 insertions, and other substitutions spread among the introns (Ip *et al.,* 2001). Considerable diversity in the cGH gene existed between Chinese native breeds and commercial breeds such as Avian Parental, Arbor Acre broilers, and Hy-Line layers (Ip *et al.,* 2001; Nie *et al.,* 2002). Chinese native chickens are genetically diverse (Zhang *et al.,* 2002) and have distinctive characteristics, including differences in feather color, growth rate, meat characteristics, and reproductive performance. Most of the variations in a gene are single nucleotide polymorphisms (SNPs) arising from substitution, deletion, or insertion of a single nucleotide. A single SNP can greatly affect performance traits of an animal.

Most of these SNPs (36 of 46) were located in introns, with four in the 5' un-translated region, one in the 3' un-translated region, and five in coding exons. Two of the five coding SNPs led to amino acid changes. All 46 SNPs were nucleotide substitutions, and transitions (38) occurred more frequently than transversions (8). One of two non-synonymous coding SNPs (Gþ951A) altered an amino acid in the cGH precursor (A13T), and the other (Gþ1532A) changed an amino acid in the mature cGH (R59H). The nucleotide diversity of the cGH gene was somewhat higher, even within similar base populations (Nie *et al.,*

2005). Polymorphisms in introns of the cGH gene indicated associations with chicken growth, fat deposition, and egg production (Feng *et al.,* 1997; Fotouhi *et al.,* 1993; Kuhnlein *et al.,* 1997).

GH plays important functions in animal growth and development and several studies have analysed the GH gene as a candidate for economic traits in livestock species. Mutations of this gene have been described in dairy cattle (Lagziel *et al.,* 1996; Yao *et al.,* 1996), beef cattle (Taylor *et al.,* 1998; Barendse *et al.,* 2006), sheep (Marques Mdo *et al.,* 2006), goats (Malveiro *et al.,* 2001), pigs (Knorr *et al.,* 1997) and poultry (Feng *et al.,* 1997; Kuhnlein *et al.,* 1997) to affect important production traits. The rabbit GH gene was isolated and sequenced by Wallis and Wallis (1995). It comprises four introns and five exons that code for a deduced protein of 216 amino acids with 26 amino acids signal peptides and 190 amino acid mature peptides. Southern blotting analysis revealed that the GH gene is present in the rabbit genome as a single copy gene without GH-like genes that, instead, are reported for other species (Wallis and Wallis, 1995).

2.4 Myostatin Gene (MSTN)

Myostatin, or Growth Differentiation Factor 8 (GDF8) is a member of Transforming Growth Factor beta (TGF-β) superfamily of secreted growth and differentiation factors. This is one among the important candidate genes for growth and development and thus has potential applications in animal husbandry. GDF8 is a negative regulator of muscle cell development (Lee and Mc Pherron, 1999). It has been demonstrated that Myostatin-null mice exhibit a 2-3 fold increase in the skeletal muscle mass due to hyperplasia and hypertrophy. Mutations in Myostatin gene were also associated with double muscling phenotype in cattle (Mc Pherron *et al.,* 1997; Grobet *et al.,* 1997). In chicken, Zhiliang *et*

al. (2004) observed significant association of different genotypes with skeletal muscle growth (fat, body weight and muscle parameters). Zhiliang *et al.* (2002) studied Myostatin gene in different chicken populations and identified seven SNPs in promoter and untranslated regions. Association of myostatin gene and production trait has been reported in other livestock such as cattle (Sellick *et al*., 2007; Gill *et al*., 2009; Wiener *et al*., 2009), sheep (Tellam *et al*., 2012), pig (Stinckens *et al*., 2008), chicken (Zhang *et al*., 2011), horse (Dall'Olio *et al*., 2014), and rabbit (Bindu *et al*., 2012).

Myostatin is expressed specifically in developing an adult skeletal muscle and functions as a negative regulator of skeletal muscle mass in mice (McPherron *et al.,* 1997). Myostatin null mice generated by gene targeting shows a dramatic and widespread increase in skeletal muscle mass. Individual muscles in myostatin null mice weigh 2-to3-fold more than those of wild-type mice, primarily due to an increased number of muscle fibers without a corresponding increase in the amount of fat (McPherron and Lee, 1997). Myostatin gene is highly conserved among vertebrate species, two breeds of cattle that are characterized by increased muscle mass (double muscling), Belgian Blue (Hanset, 1982) and Piedmontese (Masoero and Poujardieu, 1982), have mutations in the myostatin coding sequence. These results demonstrate that the function of myostatin has been highly conserved among vertebrates. The high degree of sequence conservation of myostatin across species suggests that the function of myostatin has also been conserved.

Double muscled breed of cattle, Belgian Blue, the double muscling phenotype segregates as a single genetic locus designated muscular hypertrophy (Hanset and Michaux, 1985). The muscular hypertrophy mutation, which is partially recessive, causes an average increase in muscle mass of 20–25%, a decrease in mass of most other organs (Ansay and Hanset, 1979;

Hanset, 1991), and a decrease in intramuscular fat and connective tissue (Hanset *et al.*, 1982). The muscular hypertrophy locus is tightly linked to markers on a region of bovine chromosome 2 (12) that is synthetic to a region of human chromosome 2 (2q32) (13) to which the human myostatin gene had been mapped the human myostatin gene by fluorescence in situ hybridization. The similarities in phenotype between the myostatin null mice and the Belgian Blue cattle breed and the similar map positions of the myostatin gene and the muscular hypertrophy locus suggested that, the bovine homolog of myostatin could be a candidate gene for the muscular hypertrophy locus (McPherron and Lee, 1997). The Belgian Blue myostatin coding sequence was identical to the Holstein sequence except for a deletion of nucleotides 937–947 in the third exon. This 11-nucleotide deletion causes a frame-shift which is predicted to result in a truncated protein that terminates 14 codons downstream of the site of the mutation (McPherron and Lee, 1997). The deletion is expected to be a null mutation because it occurs after only the first 7 amino acids of the C-terminal region, resulting in a loss of 102 amino acids (amino acids 274–375). This mutation was similar to the targeted mutation in myostatin null mice in which the entire region encoding the mature protein was deleted (McPherron *et al.*, 1997).

2.4.1. Mutation of myostatin gene

This mutation is likely to result in a complete or almost complete loss of function, as the cysteine residue is invariant, not only among all myostatin sequences but also among all known members of the transforming growth factor β superfamily (McPherron and Lee, 1996). This cysteine residue is known to be one of the amino acids involved in forming the intra-molecular cystine knot structure in members of this superfamily for which the three-dimensional structure is known (Schlunegger and Gru¨tter, 1992; Mittl *et al.*, 1996). When

the corresponding cysteine in activin A (cysteine-44) was mutated to alanine, the mutant protein had only 2% of wild-type receptor binding and biological activity (Mason, 1994). The similar map positions of the myostatin gene and the muscular hypertrophy locus and the identification of relatively severe mutations in the myostatin gene of two different double-muscled cattle breeds suggest that these mutations are responsible for the double muscling phenotype (McPherron and Lee, 1997).

Unlike in mice, a myostatin null mutation in cattle causes a reduction in sizes of internal organs and only a modest increase in muscle mass (20–25% in the Belgian Blue breed as compared with 200–300% in myostatin-deficient mice). It is possible that cattle may be nearer to a maximal limit of muscle size after generations of selective breeding for large muscle mass, unlike mice, which have not been similarly selected (McPherron and Lee, 1997). There are some disadvantages to double-muscled cattle, namely the reduction in female fertility, lower viability of offspring, and delay in sexual maturation (Me´nissier, 1982). The increased muscle mass and increased feed efficiency in the Belgian Blue breed largely offset these drawbacks (Hanset *et al.*, 1987). The fact that a null mutation in the myostatin gene in cattle results in animals that are still viable and fertile and produce high-quality meat demonstrates the potential value of producing an increase in muscle mass in other meat animals such as sheep, pig, chicken, turkey, and fish by disrupting myostatin function (McPherron and Lee, 1997). The high degree of sequence conservation in animals ranging from mammals to birds to fish suggests that the biological function of myostatin has been conserved widely throughout the animal kingdom (McPherron and Lee, 1997).

Myostatin gene expression in mice has indicated that the myostatin gene is first expressed in myogenic precursor cell of the myotome compartment of developing somites

(McPherron *et al.,* 1997), and later, in adult axial and paraxial muscles. The level of myostatin expression, however, varies in different axial and paraxial muscles. In practice where a gene has been implicated in controlling quantitative traits (by chromosomal position relative to linkage peaks, known biological function, or expression pattern), it is desirable to exhaustively survey allelic variation for any association to that quantitative trait (Gu *et al.,* 2004). Most sequence variations are attributable to SNPs, with the rest due to insertion or deletion of one or more bases, repeat length polymorphisms, and rearrangements (Gu *et al.,* 2004).

Myostatin circulates in the blood of adult mice, and systemic overexpression of myostatin in adult mice is found to induce profound muscle and fat loss (Zimmers *et al.,* 2002). The myostatin-null mice revealed a significant reduction in fat accumulation with increasing age compared with wild-type litter mates (McPherron and Lee, 2002). The mice lacking myostatin gene might cause a switch between myo- genesis and adipogenesis (Lin *et al.,* 2002).

The myostatin gene is expressed almost exclusively in cells of skeletal-muscle lineage throughout embryonic development as well as in adult animals and functions as a negative regulator of muscle growth (McPherron *et al.,* 1997; Amthor *et al.,* 2002). Targeted disruption of the myostatin gene in mice resulted in doubles skeletal-muscle mass (McPherron *et al.,* 1997). Conversely, systemic overexpression of the myostatin gene leads to a wasting syndrome characterized by extensive muscle loss (Zimmers *et al.,* 2002). In adult animals, myostatin appears to inhibit the activation of satellite cells, which are stem cells resident in skeletal muscle (McCroskery *et al.,* 2003; Mauro, 1961). The potential relevance of myostatin to the treatment of disease in humans has been suggested by studies involving muscular dystrophy mutation (mdx) mice, which carry a mutation in the

dystrophin gene and therefore serve as a genetic model of Duchenne's and Becker's muscular dystrophy (Sicinski *et al.,* 1989). The mdx mice that lacked myostatin were found not only to be stronger and more muscular than their mdx counterparts with normal myostatin, but also to have reduced fibrosis and fatty remodeling, suggesting improved regeneration of muscle (Wagner *et al.,* 2002). The injection of neutralizing monoclonal antibodies directed against myostatin into either wild-type or mdx mice increases muscle mass and specific force, suggesting that myostatin plays an important role in regulating muscle growth in adult animals (Whittemore *et al.,* 2003; Bogdanovich *et al.,* 2002). The phenotypes of mice and cattle lacking myostatin and the high degree of sequence conservation of the predicted myostatin protein in many mammalian species have raised the possibility that myostatin may help regulate muscle growth in humans. The identification of a myostatin mutation in a child with muscle hypertrophy, provide strong evidence that myostatin does play an important role in regulating muscle mass in humans (Markus *et al.,* 2004).

Myostatin expression is detected at an earlier stage of myogenesis when quiescent stem cells rapidly expand in number to generate the myoblasts needed to repair tissue damage (Glass, 2010). Several studies have demonstrated that MyoD is sufficient and necessary for the formation or survival of skeletal myoblasts (Emerson, 1993; Buckingham, 2006). Myostatin is a secreted growth and differentiation factor belonging to the transforming growth factor (TGF)-beta superfamily. It exert an important modulator role for body composition in animals by controlling the proliferation of myoblasts, and by inhibiting the differentiation of this cells (Rodgers and Garikipati, 2008; Glass, 2010).

Myostatin knock out rabbits had shown phenotype of double muscle with hyperplasia or hypertrophy of muscle fiber. Similar phenotype was found in the F1 generation, suggesting

that the mutation of myostatin could be stably inherited in the myostatin knock out rabbits (Qingyan *et al.,* 2016). It has been reported that spontaneous mutations of myostatin in cattle (Fries *et al.,* 1997) and sheep (Clop *et al.,* 2006) causes muscle hypertrophy. A double-muscled phenotype with the characteristics of increased muscle mass was also obtained in myostatin knock out sheep (Crispo *et al.,* 2015), pigs (Qian *et al.,* 2015) and dogs (Zou *et al.,* 2015), which encourages the use of Myostatin knock out rabbits for the study of muscle development and improvement of animal traits for agriculture in the future. The Myostatin null rabbits were obviously heavier than wild-type rabbits after 6 weeks of age. In addition, the typical double-muscled phenotype was also found in myostatin null rabbits at 2 months of age. The enlarged tongue in the myostatin knock out rabbits suggested that, is due to fiber hypertrophy. The increased muscle mass of gluteus maximus in the myostatin knock out rabbits was due to both fiber hyperplasia and hypertrophy (Qingyan *et al.,* 2016).

The increased muscle mass makes it attractive to create myostatin knock out livestock, it use is limited by the calving difficulty induced by enlarged body size of fetus in the myostatin -mutant animals (Arthur, 1995). It has been reported that the myostatin -deficient animals exhibited disorders similar to large offspring syndrome (LOS) (Lee, 2004; Mosher *et al.,* 2007). The myostatin knock out rabbits showed a typical double-muscled phenotype and a dramatically increased body weight, there was no significantly difference in body size and weight at birth compared to the wild-type controls. Animals with myostatin mutations are characterized by increased muscle mass, which results from a combination of increased muscle fiber size and number (Lee, 2004). The myostatin -regulated muscle proliferation and differentiation are also affected by expression levels of myostatin mRNA, the health state and the individual developmental stage of the organism (Qingyan *et al.,* 2016).

The myostatin gene is specifically expressed during embryonic development, expressed at high level in adult skeletal muscle and controls skeletal muscle growth (Joulia *et al.,* 2003). Molecular analysis of the myostatin gene in different species has showed that it consists of three exons and two introns and were found to affect both the amount and composition of muscle fibers. The muscle mass of Myostatin knockout mice was two to three times greater than that of wild-type mice (McPherron *et al.,* 1997), which is primarily due to an increased number of muscle fibers, followed by muscle cell hypertrophy and suppression of body fat accumulation (McPherron and Lee, 2002). Myostatin gene is one of the most conserved genes among vertebrate species (Karim *et al.,* 2000). However, there are many reports of mutations, disrupting the myostatin function, which cause double-muscle phenotypes in cattle and increase in body mass in mice (Grobet *et al.,* 1998). About 30% more muscle mass with less bone and fat is found in double-muscled cattle per animal on the same food intake as normal cattle (Kambadur *et al.,* 1997).

The PCR amplification of Myostatin gene with the designed primer resulted in amplified product of size (797 bp) which includes part of 5'UTR, exon 1 and part of intron 1 in Madras Red, Mecheri and Nilagiri breeds of sheep. The restriction endonuclease, Msp I had cleavage site (C/CGG) at nucleotide position 571 of the amplified region which yielded two fragments viz. 226 bp and 571 bp in all the three sheep breeds studied. All the parts of the gene studied did not show any polymorphism (Sahu *et al.,* 2016). The restriction endonuclease Hae III with recognition sequence GG/CC on digestion of amplicon yielded two fragments 366 bp and 431 bp suggested monomorphism, which indicates that Myostatin gene is highly conserved among all the three breeds studied (Sahu *et al.,* 2016).

The transforming growth factor b superfamily encompasses a large group of secreted growth and differentiation factors that play important roles in regulating development and

tissue homeostasis (McPherron and Lee, 1996). The muscular hypertrophy mutation, which is partially recessive, causes an average increase in muscle mass of 20–25%, a decrease in mass of most other organs (Ansay and Hanset, 1979; Hanset, 1991), and a decrease in intramuscular fat and connective tissue (Hanset *et al.*, 1982). Trukhachev *et al.* (2015) investigated the polymorphism of the Myostatin gene and its influence on body parameters in Russian sheep breed Dzhalginsky Merino and detected 20 single nucleotide polymorphism (SNP), that is SNP in promoter, 5'UTR, exon I, intron 1-2, intron 2-3, 3'UTR. Three of the detected SNP have a negative effect on the body parameters; decrease weight, height and others (Trukhachev *et al.*, 2015). The three SNP, who influence the size of the animal, were located in the 5' regulatory region of the myostatin gene. Thus, at least two of three substitutions can affect the functional properties of the gene promoter Myostatin (Trukhachev *et al.*, 2015).

Myostatin gene coding regions are highly conserved. It is known about two single nucleotide substitutions in exons. Missense mutation of c.101 G>A in the first exon leads to the substitution of glutamic acid for glycine. Substitution of c.384G>A in the second exon synonymous and does not change the encoded amino acid leucine (Zhou *et al.*, 2008). Most mutations account for introns, 5'UTR and 3'UTR. Substitution of c.1232 G>A in the 3'UTR of Myostatin gene of sheep was offered for use as a marker for genomic selection (Kijas *et al.*, 2007; *Han et al.*, 2013). Genetic changes in the border regions of introns may affect mRNA splicing, changing the amino acid sequence and, accordingly, meat quality (Sjakste et al., 2011). Mutation of c.101A>G in the first exon alters the structure of the myostatin pro peptide (Dunner *et al.*, 2003). The whole coding region (CDS) of the myostatin gene in Texel double-muscling sheep was sequenced, but no sequence differences were found in the coding region (Marcq *et al.*, 1998).

Mutation(s) in the myostatin 3'UTR have been found at the molecular or cellular level in a recent study by Clop *et al.* (2006). The mutations create an illegitimate miRNA binding site which might affect the double muscling trait of the Texel sheep. In addition, mutations in the promoter regions of the swine myostatin were reported to affect the stability of myostatin mRNA, and therefore affect animal phenotype (Cieślak *et al.*, 2003). The fact that no variation in the coding region was found in double muscling sheep myostatin has confused many researchers (Marcq *et al.*, 1998), but it does not exclude the functional involvement of myostatin in double-muscling sheep, because polymorphisms in other regions of myostatin could be equally important (Gan *et al.*, 2008). Mutations in regions upstream of initial codon ATG may control the transcript level of mRNA by increasing or destroying the number of transcription factor binding sites; and mutations in 3'UTR may create microRNA targets, whose binding to some well-paired miRNAs might suppress or block myostatin mRNA translation and lead to differences in sheep phenotypes (Gan *et al.*, 2008). In a study, based on sequence analysis of the noncoded region of the sheep myostatin gene, four SNPs were identified in the 5' promoter region, 5'-UTR and 3'-UTR, and dozens of SNPs were identified in the introns. The main emphasis was on the regulatory region (putative promoter and UTRs) because they were most likely to influence the meat traits by modulating the level of Myostatin expression.

SNP(6223 (G→A)), which was identified as a QTN (quantitative trait nucleotide) located in myostatin 3'UTR and reported in Texel sheep by Clop *et al.* (2006), was also found in Beltex and B×H sheep flocks with a high percentage but was not found in the other breeds. Beltex and Texel sheep are both renowned for their extraordinary ability for meat production (Banks, 1997; Busboom *et al.*, 1999). G to A transition found in the 3'UTR of myostatin was verified to create a target site for miR-1 and miR-206 which are highly

expressed in skeletal muscle (Clop *et al.,* 2006). The miRNA binding results in translational inhibition of myostatin mRNA and thus in the reduction of myostatin protein level, which weakens the inhibition of myostatin protein in skeletal muscle development and growth (Clop *et al.,* 2006). This may provide the biological mechanism for double muscling sheep. Double-muscled cattle also deposit less fat than other breeds (Potts *et al.,* 2003). These animals have less bone, less fat, and 20% more muscle on an average (Shahin and Berg, 1985; Hanset, 1991). Muscular hypertrophy allele (mh allele) in the double muscle breeds involved mutation within the myostatin gene (Kambadur *et al.,* 1997). Such a major effect of a single gene on processing yields opened a potential channel for improving processing yields of animals using knockout technology (Arif *et al.,* 2002). Sequencing of the myostatin locus from farm animals is important to produce genomic resources for development of knockout technology as well as for understanding the structure, function, and evolution of the gene (Shah *et al.,* 2006). To date, all vertebrate myostatin genes, whose genomic sequences are publicly available, had three exons and two introns: exon 1 (379 bp), exon 2 (371 bp) and exon 3 (381 bp); intron 1 (363 bp) and intron 2 (811 bp) (Jeanplong *et al.,* 2001; Ko *et al.,* 2006; Liangyi *et al.,* 2006).

2.5 Insulin-like Growth Factor-1 Gene (IGF-1)

Insulin-like Growth Factor-1 is a key regulator of muscle development and metabolism in birds and other vertebrate species (Duclos, 2005). 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 (Jones and Clemmons, 1995) and both genes are expressed in the muscle tissue together with specific receptors, suggesting a paracrine mode of action. Indeed, over expression 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 (Duclos, 2005). (Musaro *et al.*, 2001).The over expression 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 over expression 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). It is worthy to assume that over expression of IGF-2 is equivalent to over expression of IGF-1 since both peptides act through the same receptor.

Unlike mammalian cells, chicken cells do not express a second IGF receptor type (type 2 IGF receptor) with a selective specificity for IGF-2 (Duclos *et al*., 1991). In fact, the chicken cation independent mannose 6-phosphate receptor gene does not include the sequences, which allow IGF-2 binding in mammals (Zhou *et al.,* 1995). IGF-1 stimulates glucose uptake, amino acid uptake and protein synthesis, and inhibits protein degradation by satellite cell derived myotubes (Duclos *et al.,* 1993). IGF-1 is much more potent than insulin for all these effects, showing that they must be mediated by the IGF receptor rather than the insulin receptor which appears to be present at very low level in these cells. Mature chicken muscle has been shown to possess specific insulin receptors and type 1 IGF receptors (Oudin *et al.,* 1998). Therefore, chicken muscle could be susceptible to direct

modulation of its metabolism by IGF's and insulin through their specific receptors. Interestingly, several reports suggest that muscle IGF-1 expression in the chicken could be largely independent of growth hormone unlike in mammals (Rosselot *et al.,* 1995; Goddard *et al.,* 1996).

IGF-1 administration for 14 days was shown to increase weight gain (Tomas *et al.,* 1998) 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 (Tomas *et al.,* 1998, Spencer, *et al.,* 1996). 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 Roberts, 1991).

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 un-translated regions. It is now widely reported that a significant part of IGF effects is exerted in an autocrine or paracrine mode of action. Indeed over expression of the IGF-1 gene in the muscle tissue leads to enhanced muscle growth. This has been achieved in germline transgenic mice or using different viral mediated gene transfer in other model species such as the chicken. Depending on the timing of the over expression, the muscle hypertrophy results from muscle fibre hyperplasia or hypertrophy. 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.

Comparing broiler and layer strains, in one study, where the two genotypes were bred under the same conditions, no significant differences in circulating IGF-1 concentrations were observed between 1 and 10 weeks of age (Goddard *et al.,* 1988). But, in another study were layers and broilers were obviously not reared under the same conditions, higher IGF-1 concentrations were observed in layers compared to broilers at 6 weeks of age and later on (Lee *et al.,* 1989). In a broiler strains model divergently selected for high or low growth potential, IGF-1 levels were significantly higher in the high growth line compared to the low growth line at seven weeks of age (Scanes *et al.,* 1989). This difference was no longer significant when measured in much older chickens (46 weeks of age and above). The IGF-2 levels were also measured in this study and did not differ between the two genotypes. But, introduction of the dwarf gene further lowered circulating IGF-1 and IGF-2 concentrations in both the high and the low growth genotype (Scanes *et al.,* 1989). The circulating levels of IGF-1 have been shown to have low heritability in the chicken (Pym *et al.,* 1991). In turkeys, the comparison of a medium weight and a heavy weight line was performed between 1 and 28 weeks of age and a positive phenotypic correlation was observed between plasma IGF-1 levels and growth rate until 7 weeks of age and higher circulating concentrations were indeed observed in the selected line (Bacon *et al.,* 1993). This relationship was not conserved in older birds (Bacon *et al.,* 1993). If differences in the IGF system are involved in differences in chicken growth rates, they can be located either at the level of the peptides and/or at level of target tissues (for the responsiveness to IGF) (Duclos, 2005). The two hypothesis that explored the divergently selected lines of broilers with high (HG) or low (LG) growth rate, differ for this character. These lines were reported

to hatch at comparable body weights but already exhibited a 20% difference in the number of muscle fibers at this stage, which resulted from different rates of embryonic muscle growth (Remignon *et al.,* 1994). Most of the differences occurred at post hatch and, at 11 weeks of age, the two lines of chickens exhibited a large difference (about 2 fold) in body weight and muscle weight (Remignon *et al.,* 1994). This was associated with larger muscle fibers and a higher number of myonuclei, which suggests that the proliferative activity of satellite cells could be higher in the line selected for higher growth (Duclos, 2005).

2.5.1. Expressions of insulin-like growth factor-1 gene (IGF-1) and potential as a marker assisted selection

The possible differences at receptor level has been investigated, whole muscle has been solubilised and receptors partially purified from leg and breast muscle of 1 and 7 weeks-old High growing (HG) and low growing (LG) chickens (Oudin *et al.,* 1998). IGF-1 binding decreases with age, but does not differ between genotypes. The IGF-1 stimulated tyrosine kinase activity of receptors was similar between genotypes at one week of age. Total IGF-1 and IGF-2 were measured by radio-immunoassay in serum from 1 to 12 week-old HG and LG chickens, showing an increase with age and higher circulating levels in HG compared to LG chickens (Beccavin *et al.,* 2001).

Therefore, higher circulating concentrations of IGF-1 and -2 in HG compared to LG chickens, together with comparable levels of IGF receptors in the target tissues are consistent with the difference in growth rate between the two genotypes (Duclos, 2005). The contribution of IGFBP is uncertain as only a small difference is observed for IGFBP-(34 kDa). Recently, chickens selected for high breast meat yields and their controls have been compared (Le Bihan-Duval *et al.,* 2001). The selected chickens exhibited slightly but significantly higher circulating IGF-1 concentrations, but similar IGF-2 concentrations

(Tesseraud *et al.,* 2003). In vivo between 14 days and 6 weeks post-hatch, the selected chickens exhibited higher breast but similar leg yields. They showed larger muscle fibers in their Pectoralis Major and Sartorius muscles from 2 weeks post-hatch. In the Pectoralis Major, IGF-1 mRNA levels were significantly higher in the selected chickens at 4 and 6 weeks of age, when breast meat yields were most different (Guernec *et al*., 2003).

The insulin-like growth factors (IGFs), both IGF-1 and IGF-2, are peptide hormones that have important roles in mammalian growth and development. IGF-1 is structurally similar to insulin, but has a much higher growth-promoting activity. IGF-1 binds to the IGF-1 receptor (IGF-1R) homodimer or the IGF-1R/insulin receptor heterodimer and provokes intracellular signaling cascades. (Fagan and Yee, 2008; Pollak, 2008). Similar to insulin, IGF-1 not only acts as a growth factor at the cellular level but also functions as a hormone regulating growth and energy metabolism at the whole-organism level (Pollak, 2008). IGF-1R is activated by extracellular IGF-1 and is auto phosphorylated at multiple tyrosine residues in its kinase domain (Riedemann and Macaulay, 2006). Activation of IGF-1R induces the diverse signaling pathways such as the phosphoinositide 3-kinase (PI3K)/AKT and the mitogen-activated protein kinase (MAPK) pathways that are important to cell proliferation, transformation and survival (Pollak, 2008; Law *et al.,* 2008). Van Laere *et al.* (2003) showed that a quantitative trait loci for muscle growth in pigs was caused by a nucleotide substitution in intron 3 of the insulin-like growth factor 2 gene (IGF2). Amills *et al.* (2003) identified three SNPs in chicken IGF1 and IGF2 that were associated with growth and feeding traits.

The insulin-like growth factor gene (IGF1) is 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). The insulin-like growth factor 1 receptor

(IGFIR) is a membrane glycoprotein mediating most biological actions of IGF-1 and IGF-2, which have an important effect on chicken growth, carcass, and meat quality traits. Two receptors (IGF1R and IGF2R) were found in the mammals but only one (IGF1R) was found in the birds. IGF1R not only regulated the half-life time and activity of IGFs, but also played important roles on the key developmental stage and adult stage such as the cell life cycle, transplantation, metabolism, subsistence, proliferation and differentiation (Lei *et al.,* 2008). Many variations in the genome affected gene expression at the transcription and translation levels. Variations in the genes of somato-tropic axis could function as candidates for the evaluation of their effects on animal growth and development traits. These variations affected partly the expression and physiological functions of the IGF1R gene, and subsequently affected growth. However, few studies on associations of the IGF1R gene with growth and carcass traits were reported in chickens (Lei *et al.,* 2008). However, the genetic potential in almost all chicken breeds has not yet been much revealed. Based on the literatures, the chicken IGFs are considered to be the most important candidate genes that can influence chicken performance traits like growth, body dimensions, carcass and reproduction.

In a prior study, Gouda and Essawy, (2010) analyzed the polymorphism of IGF-I gene among Egyptian chicken breeds, and reported that, their effect on the growth traits of chicken was significant. IGFBP2 and STAT5b act as modulators for the biological action of IGF gene in various signaling pathways, so their expression levels as well as SNPs play important role in the action of IGF protein. To improve production traits and health simultaneously it is appropriate to use molecular markers associated with one or two characteristics. The IGF1 gene is, therefore, a potential marker for use in marker-assisted selection programmers. The Insulin-like Growth Factors (IGF-1 and 2) were discovered as

skeletal growth factors 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 production in the liver, both IGF-1 and IGF-2 were 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 of growth, but that they were also very strongly nutritionally dependent (Seyed *et al.,* 2011). 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 been reported 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 (Seyed *et al.,* 2011). 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). 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 (Werner *et al.,* 1994).

Insulin-like Growth Factor I (IGF-I) is a hormone like polypeptide related to several economically important traits including growth and reproduction in cattle (Spicer and Chamberlain, 1998). Some experiments have shown, that normal growth can be maintained even if circulating IGF-I levels are less than normal, these experiments do not rule out the

importance of circulating IGF-1 as a regulator of growth hormone concentration and its correlation with local IGF-1 production in several tissues (Yakar *et al.,* 1999). Locally produced IGF-1 is more important than circulating IGF-1 in maintaining tissue growth and development (Yakar *et al.,* 1999). However, IGF-I receptors have been detected throughout the body and IGF-I deficiency is associated with growth abnormalities in cattle (Kitagawa *et al.,* 2001). Several studies have shown a close association between male reproductive traits and IGF-I measurements (Glander *et al.,* 1996; Vickers *et al.,* 1999). Tahmoorespur *et al.* (2009) found the positive influence of IGF-1 exon1 in average daily Gain from Birth to Weaning. Ge *et al.* (1997) has reported the cytosine/thymine (C/T) transition at position -472 in the 5'-noncoding region of the IGF-1gene in Angus cattle. Similar results were reported by Mehmannavaz *et al.* (2010) in cattle using RFLP/SnaBI. Yilmaz *et al.* (2005) confirmed it using single strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP) and Tahmoorespur *et al.* (2009) using SSCP in sheep when detecting the polymorphisms purposed. Yilmaz *et al.* (2005) has identified two single nucleotide polymorphisms; A and T to C transition and a G to C transversion at positions 179 and 181, respectively, of the amplified PCR product that were digested separately and resulting in recognition sites for Bsp143II and HaeI enzymes.

The mature IGF-I is a relatively small peptide, its gene is surprisingly large in mammals, comprising 80 to 100 kb of genomic DNA (Rotwein *et al.,* 1986; Shimatsu and Rotwein, 1987). In humans, pigs, goats, rats, and chickens, the IGF1 nucleotide sequence is about 70-90 kb (Shimatsu and Rotwein, 1987; Kajimoto and Rotwein, 1991). Exon numbers differ between species; for example, goats, pigs and sheep have 1-6 exons (Mikawa *et al.,* 1995) and humans and rats 1-5 exons (Rotwein *et al.,* 1986; Shimatsu and Rotwein, 1987). The IGF-1 gene that is located on chromosome 3 in sheep is a marker for growth rate and meat

production and has an important role in mammary gland cell differentiation and prolife ratio (Imam-Ghali *et al.,* 1991).

2.5.2 IGF Family and other expressions roles

The IGF family is made up of the following three related ligands: insulin, IGF-I, and IGF-II. Both IGFI and II are found in circulation and extracellular fluids conjugated to any one of six different IGF binding proteins (IGFBP) (Clemmons, 1999). Insulin-like growth factors one and two (somatomedins- IGF-I and IGF-2) are structurally related proteins that have a key role in cell differentiation, embryogenesis, growth and regulation of metabolism (Siadkowska *et al.,* 2006). IGF-I has been reported to be necessary for progression of cells through both the G1 and the G2/M phases of the cell cycle (Adesanya *et al.,* 1999).

In earlier studies of a dinucleotide repeat polymorphism in the 5 flanking region of the IGF-I gene in cattle and swine, a possible role of this somato-mediater in production traits was evident (Kirkpatrick, 1992). There have been studies of the allelic frequency of IGF-I gene in different cattle breeds, and beef cattle is considered to be a model for this system (Ge *et al.,* 1997; Spicer *et al.,* 2002; Curi *et al.,* 2005). Most studies of beef cattle have revealed an association between gene polymorphism and body condition scoring and weight, particularly in early life stages. The role of IGF-I and its binding proteins has been reviewed (Sara and Hall, 1990; Jones and Clemmons, 1995). IGF1 is a mediator of many biological effects; for example, it increases the 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 in cell proliferation (Mohammadi *et al.,* 2011).

Also the AA, AG conformational patterns of IGF1 gene individuals have a significant effect on fat thickness (the thick rump). And these patterns of IGF1 gene (AA, AG) had the highest Fat thickness (the thick rump). It has been reported that no significant associations of the SNPs in the 5' flanking region of the ovine IGF-I Gene with carcass traits in Zel (tailed) and Lori-Bakhtiari (fat-tailed) sheep breed (Honarvar *et al.*, 2012).There was significant association between genotypes of exon 17 region of the DGAT1 gene with carcass weight and dressing percentage in two Lori-Bakhtiari and Zel breed (Dekker, 2004). It has been reported that the relationship seen between band patterns with tail length and tail down circumference was close to the significant level in fourth exon of growth hormone gene in Kermanian sheep (Asadi, 2012). Also a significant relationship between the band patterns (exon 3 Ovine Leptin gene) with the tail, chest, abdomen and neck circumference and body length had been reported in the Zel breed. In the Bakhtiari breed, the patterns were associated with Stature, gap tail length, middle and down tail width (Azizi, 2012). A significant association was reported between band patterns (part of exon and intron 2 Leptin gene in fat-tailed Lori-Bakhtiari and tailed Zel breed) and hip circumference, tail length and blood triglyceride in the Zel breed. There were no significant association between observed bands and measured traits in the Bakhtiari breed (Azizi, 2012). Significant association between band patterns 16 – 17 exon of DGAT1 gene in Lori-Bakhtiari sheep (LB) and Zel sheep (Z) breeds. At the DGAT1 locus, CC sheep showed the significantly greater fat-tail weight and back fat thickness. The results of these studies demonstrated novel associations in which the C allele had a positive effect on fat-tail weight and back fat thickness in fat-tailed sheep (Mohammadi *et al.*, 2011).

2.6 On-line Tools/Programs

The development of Bioinformatics computational tools and databases and the application of these tools and databases in generating biological knowledge to better understand living systems, Jin, X. (2006). The main challenge is to make sense of the enormous amount of structural data and sequences that have been generated at multiple levels of biological systems (Pevsner, 2015). Statistical or computational tools to help synthesize recorded data and integrate various types of information in the process of answering a particular biological question (Jin, 2006). In bioinformatics, development of tools is necessary (statistical and computational) capable of assisting in understanding the mechanisms underlying biological questions in the study (Pevsner, 2015).

These tools are used in three areas of genomic and molecular biological research: molecular sequence analysis, molecular structural analysis, and molecular functional analysis. The analyses of biological data often generate new problems and challenges that in turn spur the development of new and better computational tools. The tool development includes writing software for sequence, structural, and functional analysis, as well as the construction and curating of biological databases (Jin, 2006). Data mining research has led to the developments of numerous efficient and scalable methods for mining interesting patterns and knowledge in large databases, ranging from efficient classification methods to clustering, outlier analysis, frequent, sequential and structured pattern analysis methods, and visualization and spatial/temporal data analysis tools (Dasu *et al*., 2002).

To evaluate the evolutionary relationships of camel myostatin with the myostatin of other farm animals from the GenBank, a phylogenetic tree was constructed by using CLUSTAL W (1.8) computer software. All analyzed farm animals were divided into three subgroups at

this gene locus (Shah *et al.*, 2006). Trukhachev *et al.*(2015) Sequencing was performed using a genomic sequencer (GS) Junior (Roche, USA). The resulting sequence fragments mapped to the reference genome assembly Ovis aries oviAri3 (The National Center for Biotechnology Information, Genome (2012). Ovis aries (sheep), (2015) by software GS Reference Mapper v2.9 (Roche, USA). Phylogenetic analysis was performed using the software Unipro UGENE 1.15.1 (Unipro, Russia) (Trukhachev *et al.*, 2015).

Sequence analysis was further validated by multiple sequences alignment of myostatin gene from different species in FASTA format using Clustal W (http://www.ebi.ac.uk/ Tools/clustalw/) available at European Molecular Biology Laboratory (EMBL) website. Mutation detection was done by multiple sequence alignment of consensus sequences of rabbit in Bioedit (v 7.0.7.1) and phylogenetic tree was constructed using Neighborhood joining method of bootstrap test of phylogeny in MEGA4 (Kurkute *et al.*, 2011). By using SeqScape Software (Applied Biosystems), forward and reverse sequences of representative sample of each gene fragment were assembled against most closely related reference sequence of respective gene to obtain total sequence length. Similarity was looked in to the non-redundant database of GenBank with BLAST algorithms (http://www.ncbi.nlm.nih.gov/BLAST/) (Kurkute *et al.*, 2011).

Clustal Omega is currently one of the most popular multiple sequence alignment programs (Sievers *et al.*, 2011). Clustal W, the predecessor of Clustal Omega, uses the principle of progressive sequence alignment that consists of three main steps (Thompson *et al.*, 1994). With a sequence-to-sequence alignment, a similarity matrix such as BLOSUM62 is used to obtain a score for a particular substitution between the pairs of aligned residues. In aligning alignments, however, each of the two input alignments are treated as a single sequence, and

can calculate the score at aligned positions as the average substitution matrix score of all the residues in one alignment versus all those in the other (Dutilh and Keşmir, 2016).

VISTA combines a global-alignment program with a running-plot graphical tool to display the alignment (Mayor *et al*., 2000) (http://www-gsd.lbl.gov/vista/). Global alignments are produced when two DNA sequences are compared and an optimal similarity score is determined over the entire length of the two sequences. In contrast, PipMaker uses BLASTZ, a modified local-alignment program, and displays plots with solid horizontal lines to indicate un-gapped regions of conserved sequence (i.e., blocks of alignments that lack insertions or deletions) (http://bio.cse.psu.edu/ pipmaker/) (Schwartz, *et al.*, 2000). Local alignments are generated when two DNA sequences are compared and optimal similarity scores are determined over numerous sub-regions along the length of the two sequences (Pennacchio and Rubin, 2003).

Custom comparison to whole genomes, In addition to preprocessed whole-genome comparative data, several additional tools allow for any sequence from any organism to be compared with previously assembled and annotated genomes. They include Genome Vista and a server available through UCSC Genome Browser. Genome Vista uses the same data sources and algorithmic methods as are used to generate the alignments for the VISTA Genome Browser, but it allows users to input their own sequence of interest for direct comparison with the human, mouse, or rat genome. One can acquire these sequence files from in-house sequencing projects, or automatically retrieve them from sequence databases such as GenBank by simply inputting the accession number for the desired sequence at the Genome Vista website. The Genome Vista data output is similar to that of the VISTA Genome Browser but allows species other than those available in the current alignment to

be examined in the context of the annotated human or mouse genome (Pennacchio and Rubin, 2003).

The two most commonly used comparative genomic tools are Visualization Tool for Alignment (VISTA) and Percent Identity Plot Maker (PipMaker) (Mayor *et al*., 2000). The primary goal of both programs is to turn raw orthologous-sequence data from multiple species into visually interpretable plots to drive biological experimentation. Some of their common features include the ability to compare multiple mega-bases of sequence simultaneously from two or more species, web accessibility, and the option to customize numerous features by the user. While each program uses different overall strategies, they both allow for the identification of conserved coding as well as noncoding sequences between species (Pennacchio and Rubin, 2003).

Most biological research involves application of some type of mathematical, statistical, or computational tools to help synthesize recorded data and integrate various types of information in the process of answering a particular biological question (Jin, 2006).According to Luscombe *et al*. (2001),the goals of bioinformatics are to organize the data so that researchers can access the information and create new entries, to develop tools and resources that help in the data analysis, and also to use these tools to analyze the data and interpret them significantly.

Bioinformatics depends on experimental science to produce raw data for analysis. It, in turn, provides useful interpretation of experimental data and important leads for further experimental research (Jin, 2006).

Bioinformatics and their tools have a major impact on many areas of biotechnology and biomedical sciences. It has applications; in knowledge-based drug design, forensic DNA analysis, and agricultural biotechnology. Computational studies of protein–ligand interactions provide a rational basis for the rapid identification of novel leads for synthetic drugs. It is worth mentioning that genomics and bioinformatics are now poised to revolutionizeourhealthcaresystembydevelopingpersonalizedandcustomizedmedicine.Bioinformatics tools are being used in agriculture as well. Plant genome databases and gene expression profile analyses have played an important role in the development of new crop varieties that have higher productivity and more resistance to disease (Jin, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Research Centre/Location

This Comparative genomic research study was conducted at A.B.U. ICT/Digital Centre, Animal Science Department, Faculty of Agriculture, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

3.2 Genes Sequence Download and Alignment MEGA7 Computational Tool

The GH, IGF-1 and MSTN genes sequence were downloaded from National Center for Biotechnology Information (NCBI) database through Entrez of the database as non-redundant reference sequence in FASTA format (http://www.ncbi.nlm.nih.gov/) using respective accession numbers of the various genes in the GenBank. To access the necessary gene information (Table 3.1). Multiple sequence alignment was done using a built-in CLUSTAL W implementation and MUSCLE program (for the complete sequence or data in any rectangular region) of MEGA7.0.26, software. The number of codons for each gene were detected; gaps within the alignment of the three species for each gene; single nucleotide polymorphism between the alignments of chicken by rabbit, chicken by sheep, and rabbit by sheep; the conserved regions between the alignments of chicken by rabbit, chicken by sheep, rabbit by sheep, and chicken by rabbit by sheep by submitting the genes respective sequences in FASTA format to the tool.

Table 3.1: Accession Numbers of GH, IGF-1 and Myostatin Genes of Chicken, Rabbit and Sheep.

Specie	GH gene Accession	IGF-1 gene Accession	Myostatin gene Accession
Chicken	NC_006114.4	NC_006088.4	NC_006094.4
Rabbit	NC_030827.1	NC_013672.1	NC_013675.1
Sheep	NC_019468.2	NC_019460.2	NC_019459.2

Source: GenBank, National Center for Biotechnology Information (NCBI) database

3.3 Evolutionary Phylogenetic Tree Using MEGA7 Computational Tool

Evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). This method finds a tree based on probability calculations that best accounts for the large amount of variations of the data (sequences) set. It performs its analysis on each position of the multiple alignments. The phylogenetic trees were drawn to reveal evolutionary relationships between the genes of the three species by submitting the genes respective sequences in FASTA format to MEGA7 tool. The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then the topology with superior log likelihood value were selected. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). All positions containing gaps and missing data were eliminated from MEGA7.0.26. (Kumar *et al*., 2016).

3.4 Vista Computational Tool

Chicken/rabbit, Chicken/sheep and rabbit/sheep of GH, IGF-1 and MSTN genomic-sequence comparisons was conducted using VISTA online software by submitting the genes respective sequences in FASTA format to the tool. (a) VISTA analysis with chicken sequence shown on the x axis and percentage similarity to rabbit on alignment 1 axis. The graphical plot was based on sliding-window analysis of the underlying genomic alignment. A 100-bp window was used that slides at different-bp nucleotide increments. Blue and pink shading indicate conserved coding and noncoding DNA, respectively. Green and yellow bars immediately above the VISTA plot correspond to various repetitive DNA elements for

GH, IGF-1 and MSTN genomic-sequence. (b) VISTA analysis with chicken sequence shown on the x axis and percentage similarity to sheep on alignment 1 axis for GH, IGF-1 and MSTN genomic-sequence. (c) VISTA analysis with rabbit sequence shown on the x axis and percentage similarity to sheep on alignment 1 axis for GH, IGF-1 and MSTN genomic-sequence. (d) VISTA analysis with chicken sequence shown on the x axis and percentage similarity to rabbit and sheep on alignment 1 axis for GH, IGF-1 and MSTN genomic-sequence, according to the procedure of Grehan *et al.*, 2001 (http://genome.lbl.gov/vista/index.shtml).

3.5 BLAT Analysis of UCSC Genome Browser Computational Tool

For Custom comparison to whole genomes; University of California at Santa Cruz (UCSC) Genome Browser output was analyzed using Blast like alignment tools (BLAT) for Chicken, rabbit and sheep whole genome comparison of the GH, IGF-1 and MSTN gene sequence according to the procedure of Kent *et al.*(2002). The different scoring system for percent identity range within 50 – 100% level of conservation. The number of GH, IGF-1 and MSTN genes all over the genome, number of chromosomes in which the genes are distributed, and the type and number of DNA strand (input or opposite strand), in which the genes are found in each of whole chicken, rabbit and sheep genome by submitting the genes respective sequences in FASTA format to the tool (http://genome.ucsc.edu/).

3.6 Genscan Computational Tool

To identify the number of a particular category of gene in a genome; number of exons in each gene; type of exons either initial, intermediate, terminal exon, poly-A signal or promoter; the DNA strand on which each type of exons is found; number of open reading frame; predicted peptide size (amino acid) per gene and predicted coding sequence size (bp)

per gene by submitting the genes respective sequences in FASTA format to the tool, Using genome comparison Genscan (http://genes.mit.edu/GENSCAN.html).

3.7 The BLAST Analysis of Ensembl Computational Tool and Vista Genome

Sequence analysis was further validated by multiple sequences alignment of GH, IGF-1 and MSTN gene of chicken, rabbit and sheep in FASTA format by submitting the genes respective sequences in FASTA format to the tool, as query gene, against the genome of each other species. To detected number of significant alignment in the genome, percent identity range (http://www.ensembl.org/index.html) and conserved regions in base pair size and percentage was carried out using Vista genome tool (http://genome.lbl.gov/vista/index.shtml).

CHAPTER FOUR

4.0 RESULTS

4.1: GENSCAN Output Result of GH Gene

GENSCAN showing the similarities and differences in GH gene of chicken, rabbit and sheep. The chicken GH gene has one gene number; three internal exons, one terminal exon and one poly-A signal, all within the input strand but no initial exon and promoter. Rabbit GH gene has one gene number; one initial exon, two internal exons and one terminal exon but no poly-A signal or promoter, all within input strand, while Sheep GH gene has two gene number; one initial exon, seven internal exons, one terminal exon and one promoter, all within first GH gene but no poly-A signal while the second GH gene has one initial exon, four internal exons, one terminal and one poly-A signal but no promoter, all within opposite strand.

The chicken GH gene has five exons, gene length of 3525 bp and three open reading frames in the three internal exons and two open reading frames in one terminal exon and no open reading frame in the poly-A signal. Rabbit GH gene has four exons, gene length of 1288 bp and no open reading frame in the initial exon, four open reading frames in the two internal exons and two open reading frames in one terminal exon. The Sheep GH gene has ten exons in the first gene and seven exons in the second gene, gene length of 31240 bp and one open reading frame in one initial exon, eight open reading frames in seven internal exons, two open reading frames in one terminal exon and no open reading frame in the promoter of the first gene, while the second gene has no open reading frame in initial exon, three open reading frames in four internal exons, two open reading frames in one terminal exon and no open reading frame in the poly-A signal.

The chicken GH gene has a predicted peptide size of 304 (Amino acid) and a predicted coding sequence size of 915 bp. Rabbit GH gene has a predicted peptide size of 187 (Amino acid) and a predicted coding sequence size of 564 bp while the sheep GH gene has a predicted peptide size of 759 (Amino acid) and a predicted coding sequence size of 2280 bp in the first gene and the second gene has a predicted peptide size of 525 (Amino acid) and a predicted coding sequence size of 1578 bp.

4.2: GENSCAN Output Result of Myostatin Gene

GENSCAN showing the similarities and differences in Myostatin gene of chicken, rabbit and sheep. The chicken Myostatin gene has one gene, one initial exon, one internal exon and one terminal exon, all within an input strand. Rabbit Myostatin gene has one gene, one initial exon, one internal exon and one terminal exon, all within an input strand, while Sheep Myostatin gene has one gene, one initial exon, one internal exon and one terminal exon, all within an input strand.

The chicken Myostatin gene has three exons, gene length of 5514 bp but no open reading frame. Rabbit Myostatin gene has three exons, gene length of 4941 bp and two open reading frames in the initial exon, one open reading in the internal exon but no open reading frame in the terminal exon, while Sheep Myostatin gene has three exons, gene length of 5018 bp and no open reading frame in the initial exon, and internal exon but with two open reading frames in the terminal exon.

The chicken Myostatin gene has a predicted peptide size of 362 (Amino acid) and a predicted coding sequence size of 1089 bp. Rabbit Myostatin gene has a predicted peptide size of 375 (Amino acid) and a predicted coding sequence size of 1128 bp, while Sheep

Myostatin gene has a predicted peptide size of 375 (Amino acid) and a predicted coding sequence size of 1128 bp.

Table 4.1: GENSCAN Output Result of GH Gene

Type	DNA strand	Gene number	Exon number	Query Gene length (bp)	No. of reading frame	Predicted peptide size (aa)	Predicted coding sequence size (bp)
Chicken							
3 intr	+	1	5	3525	3	304	915
1 term	+				2		
1 phyA	+				0		
Rabbit							
1 init	+	1	4	1288	0	187	564
2 intr	+				4		
1 term	+				2		
Sheep							
1 init	-	1	10	31240	1	759	2280
7 intr	-				8		
1 term	-				2		
1 prom	-						
1 init	-	2	7		0	525	1578
4 intr	-				3		
1 term	-				2		
1 plyA	-						

bp: base pair, aa: amino acid, Init : initial exon, Intr : internal exon, term : terminal exon, prom : promoter, PlyA : poly-A signal, + : input strand; - : opposite strand

Table 4.2: GENSCAN Output Result of Myostatin Gene

Type	DNA strand	Gene number	Exon number	Query Gene length (bp)	No. of reading frame	Predicted peptide size (aa)	Predicted coding sequence size (bp)
Chicken							
1 init	+	1	3	5514	0	362	1089
1 intr	+				0		
1 term	+				0		
Rabbit							
1 init	+	1	3	4941	2	375	1128
1 intr	+				1		
1 term	+				0		
Sheep							
1 init	+	1	3	5018	0	375	1128
1 intr	+				0		
1 term	+				2		

bp: base pair, aa: amino acid, Init : initial exon, Intr : internal exon, term : terminal exon, prom : promoter, PlyA : poly-A signal, + : input strand; - : opposite strand

4.3: GENSCAN Output Result of IGF-1 Gene

GENSCAN showing the similarities and differences in IGF-1 gene of chicken, rabbit and sheep. The chicken IGF-1 gene has two gene numbers; four internal exons, one terminal exon, and one poly-A signal, all within the first chicken IGF-1 gene in the input strand, while the second chicken IGF-1 gene has one internal exon, one terminal exon and one poly-A signal, but no promoter in the opposite strand. Rabbit IGF-1 gene has three gene numbers; four internal exons, one terminal exon, and one poly-A signal, all within the first rabbit IGF-1 gene in the input strand. The second rabbit IGF-1 gene has one initial exon, six internal exons, one terminal exon, one poly-A signal and one promoter, in input strand, while the third rabbit IGF-1 gene has one initial exon, three internal exons, one terminal exon and one poly-A signal, all within the opposite strand. While the Sheep IGF-1 gene has three gene numbers; one initial exon, eight internal exons, one terminal exon and one poly-A signal in the first sheep IGF-1 gene, all within input strand. The second sheep IGF-1 gene has one initial exon, two internal exons, one terminal exon, one poly-A signal and one promoter, found in the input strand. While the third Sheep IGF-1 gene has one initial exon, one terminal exon and one poly-A signal in the opposite strand.

The chicken IGF-1 gene has gene length of 48447 bp, six exons and six open reading frames in the four internal exons and no open reading frame in the terminal exon and poly-A signal in the first chicken IGF-1 gene, the second IGF-1 gene has three exons, two open reading frames in one internal exon and one open reading frame in the terminal exon but no open reading frame in the poly-A signal. Rabbit IGF-1 gene has gene length of 76818 bp, six exons, and two open reading frames in the four internal exons and no open reading frames in the terminal exon and poly-A signal in the first rabbit IGF-1 gene, the second

rabbit IGF-1 gene has ten exons, one open reading frame in the initial exon, seven open reading frames in the six internal exons and no open reading frames in the terminal exon, poly-A signal and the promoter, while the third IGF-1 gene has six exons, two open reading frames in the initial exon, two open reading frames in the three internal exons, one open reading frame in the terminal exon and no open reading frame in the poly-A signal. While Sheep IGF-1 gene has gene length of 75362 bp, eleven exons, no open reading frame in the initial exon, ten open reading frames in the eight internal exons, two open reading frames in the terminal exon and no open reading frame in the poly-A signal in the first gene, the second sheep IGF-1 gene has six exons, one open reading frame in one initial exon, three open reading frames in two internal exons, one open reading frames in the terminal exon and no open reading frame in the poly-A signal and promoter, while third sheep IGF-1 gene has three exons, one open reading frame in initial exon, one open reading frames in the terminal exon and no open reading frame in the poly-A signal.

The chicken IGF-1 gene has a predicted peptide size of 238 (Amino acid) and a predicted coding sequence size of 717 bp in the first gene and predicted peptide size of 132 (Amino acid) and a predicted coding sequence size of 399 bp in the second gene. Rabbit IGF-1 gene has a predicted peptide size of 293 (Amino acid) and a predicted coding sequence size of 882 bp, predicted peptide size of 340 (Amino acid) and a predicted coding sequence size of 1023 bp in the second gene and predicted peptide size of 219 (Amino acid) and a predicted coding sequence size of 660 bp in the third gene, While Sheep IGF-1 gene has a predicted peptide size of 502 (Amino acid) and a predicted coding sequence size of 1509 bp, predicted peptide size of 206 (Amino acid) and a predicted coding sequence size of 621 bp in the second gene and predicted peptide size of 109 (Amino acid) and a predicted coding sequence size of 330 bp in the third gene.

Table 4.3: GENSCAN Output Result of IGF-1 Gene

Type	DNA strand	Gene number	Exon number	Query Gene length (bp)	No. of reading frame	Predicted peptide size (aa)	Predicted coding sequence size (bp)
Chicken							
4 intr	+	1	6	48447	6	238	717
1 term	+				0		
1 phyA	+						
1 intr	-	2	3		2	132	399
1 term	-				1		
1 phyA	-						
Rabbit							
4 intr	+	1	6	76818	2	293	882
1 term	+				0		
1 phyA	+						
1 init	+	2	10		1	340	1023
6 Intr	+				7		
1 term	+				0		
1 phyA	+						
1 prom	+						
1 init	-	3	6		2	219	660
3 intr	-				2		
1 term	-				1		
1 phyA	-						
Sheep							
1 init	+	1	11	75362	0	502	1509
8 intr	+				10		
1 term	+				2		
1 phyA	+						
1 init	+	2	6		1	206	621
2 intr	+				3		
1 term	+				1		
1 plyA	+						
1 prom	+						
1 init	-	3	3		1	109	330
1 term	-				1		
1 phyA	-						

bp: base pair, aa: amino acid, Init : initial exon, Intr : internal exon, term : terminal exon, prom : promoter, PlyA : poly-A signal, + : input strand; - : opposite strand

4.4: MEGA7 Alignment Result

MEGA7 Alignment result. The chicken GH gene has 1169 codons, IGF-1 gene codons of 15250 and Myostatin gene codons of 1815. The rabbit GH gene has 420 codons, IGF-1 gene codons of 24778 and Myostatin gene codons of 1538, while Sheep GH gene has 10338 codons, IGF-1 gene codons of 24566 and Myostatin gene codons of 1578.

The Alignment results of chicken, rabbit and sheep GH gene revealed gaps/deletions of 27723, 29968 and 210, respectively. Chicken, Rabbit and Sheep IGF-1 gene revealed gaps/deletions of 36075, 7712 and 9157, respectively. Chicken, rabbit and sheep Myostatin gene revealed gaps/deletions of 51, 632 and 553, respectively.

The GH gene single nucleotide polymorphism of chicken by rabbit, chicken by sheep and rabbit by sheep are 560/31235, 2272/31235 and 656/31235, respectively. The IGF-1 gene single nucleotide polymorphism of chicken by rabbit, chicken by sheep and rabbit by sheep are 25675/84503, 26210/84503 and 19514/84503, respectively. The Myostatin gene single nucleotide polymorphism of chicken by rabbit, chicken by sheep and rabbit by sheep are 2229/5544, 2261/5544 and 1042/5544, respectively.

The GH gene conserved regions of chicken by rabbit, chicken by sheep, rabbit by sheep and chicken by rabbit by sheep are 687/31235, 1121/31235, 496/31235 and 1045/31235, respectively. The IGF-1 gene conserved regions of chicken by rabbit, chicken by sheep, rabbit by sheep and chicken by rabbit by sheep are 20677/84503, 21135/84503, 48598/84503 and 32721/84503, respectively. The Myostatin gene conserved regions of chicken by rabbit, chicken by sheep, rabbit by sheep and chicken by rabbit by sheep are 2647/5544, 2690/5544, 3744/5544 and 2382/5544, respectively.

Table 4.4: MEGA7 Alignment Result

Gene	Codons/gene	Gaps	Chicken Xrabbit SNP	Chicken X sheep SNP	Rabbit Xsheep SNP	ChickeX rabbit conserved	ChickeX sheep conserved	Rabbit X sheep conserved	ChickenX rabbitX sheep conserved
				Chicken					
GH	1169	27723/ 31230	560/ 31235	2272/ 31235	656/ 31235	687/ 31235	1121/ 31235	496/ 31235	1045/ 31235
IGF-1	15250	36075/ 84503							
Myostatin	1815	51/ 5544							
				Rabbit					
GH	420	29968/ 31230							
IGF-1	24778	7712/ 84503	25675/ 84503	26210 /84503	19514/ 84503	20677/ 84503	21135/ 84503	48598/ 84503	32721/ 84503
Myostatin	1538	632/ 5544							
				Sheep					
GH	10338	210/ 31230							
IGF-1	24566	9157/ 84503							
Myostatin	1578	553/ 5544	2229/ 5544	2261/ 5544	1042/ 5544	2647/ 5544	2690/ 5544	3744/ 5544	2382/ 5544

4.5: BLAT Analysis Result Using Genome Browser

BLAT analysis using genome browser. The numbers/copies of chicken GH gene predicted within chicken genome is 201, having 102 copies on the input strand and 99 copies on the opposite strand with identity copies ranging 58 – 100 percent found in 29 different chromosomes. The copies of chicken IGF-1 gene predicted in chicken genome is 209, with 105 copies on input strand and 104 copies on opposite strand with identity copies ranging 85 – 100 percent found among 22 different chromosomes. The copies of chicken Myostatin gene predicted in chicken genome is 3, one copy on input strand and two copies on opposite strand with identity copies ranging 81 – 100 percent found in 3 different chromosomes.

The numbers/copies of rabbit GH gene predicted in rabbit genome is 11, with 7 copies on input strand and 4 copies opposite strand with identity copies ranging 94 – 100 percent found in 9 different chromosomes. The rabbit IGF-1 gene information could not be display due to large size of data available on the website for the gene. The copies of rabbit myostatin gene predicted in rabbit genome is 5, with 2 copies on input strand and 3 copies on opposite strand with identity copies ranging 78 – 100 percent found in 5 different chromosomes.

The numbers/copies of sheep GH gene predicted in sheep genome is 232, with 112 copies on input strand and 120 copies on opposite strand, having identity copies ranging 71 – 100 percent found in 46 different chromosomes. The sheep IGF-1 gene information could not be display due to large size of data available on the website for the gene. The copies of sheep myostatin gene predicted in sheep genome is 3, with 2 copies on input strand and 1 copy on opposite strand, having identity range of 80 – 100 percent found in 2 different chromosomes.

Table 4.5: BLAT Analysis Result Using Genome Browser

Gene	No. of Gene in Genome	DNA strand	Identity % range	No. of Chromosomes
Chicken				
GH	201	102+, 99-	58 – 100	29
IGF-1	209	105+,104-	85 - 100	22
Myostatin	3	1+,2-	81 – 100	3
Rabbit				
GH	11	7+,4-	94 – 100	9
IGF-1	Not available on this website due to large size			
Myostatin	5	2+,3-	78 – 100	5
Sheep				
GH	232	112+,120-	71 – 100	46
IGF-1	Not available on this website due to large size			
Myostatin	3	2+,1-	80 – 100	2

+ : input strand; - : opposite strand

4.6: BLAST Analysis Result Using Ensembl Browser and Vista Genome

BLAST analysis using Ensembl browser and Vista genome. The rabbit GH gene has 22 significant alignments in chicken genome, with an identity range of 81 – 100 percent with a conserved region of 658 bp at 78.0 percent. The sheep GH gene has 19 significant alignments in chicken genome, with an identity range of 93 – 100 percent with a conserved region of 2306 bp at 76.5 percent. The rabbit IGF-1 gene has no significant alignments in chicken genome using Ensembl browser, with a conserved region of 2338 bp at 75.9 percent. The sheep IGF-1 gene has 65 significant alignments in chicken genome, with an identity range of 82 – 100 percent with a conserved region of 1172 bp at 84.3 percent. The rabbit Myostatin gene has 18 significant alignments in chicken genome, with an identity range of 83 – 100 percent with a conserved region of 1214 bp at 81.1 percent. The sheep myostatin gene has 23 significant alignments in chicken genome, with an identity range of 84 – 100 percent with no conserved region found using VISTA tool.

The chicken GH gene has 40 significant alignments in rabbit genome, with an identity range of 81 – 100 percent with a conserved region of 673 bp at 76.5 percent. The sheep GH gene has no significant alignments in rabbit genome, no identity range using Ensembl browser or conserved region information using VISTA tool. The chicken IGF-1 gene has 87 significant alignments in rabbit genome, with an identity range of 82 – 100 percent with a conserved region of 40396 bp at 75.9 percent. The sheep IGF-1 gene has 152 significant alignments in rabbit genome, with an identity range of 82 – 100 percent with a conserved region of 7866 bp at 77.3 percent. The chicken Myostatin gene has no significant alignments in rabbit genome, with no identity range using Ensembl browser but with a conserved region of 1160 bp at 84.4 percent. The sheep myostatin gene has 25 significant

alignments in rabbit genome, with an identity range of 82 – 100 percent and a conserved region of 4046 bp at 79.3.

The chicken GH gene has 22 significant alignments in sheep genome, with an identity range of 82 – 100 percent but no conserved region information was found using VISTA tool. The rabbit GH gene has 14 significant alignments in sheep genome, with an identity range of 86 – 100 percent and a conserved region of 2328 bp at 75.9. The chicken IGF-1 gene has 53 significant alignments in sheep genome, with an identity range of 82 – 100 percent with a conserved region of 40513 bp at 75.8 percent. The rabbit IGF-1 gene has 94 significant alignments in sheep genome, with an identity range of 82 – 100 percent with a conserved region of 1226 bp at 80.0 percent. The chicken Myostatin gene has 31 significant alignments in sheep genome, with an identity range of 80 – 100 percent with a conserved region of 4018 bp at 79.4 percent. The rabbit myostatin gene has 21 significant alignments in sheep genome, with an identity range of 83 – 100 percent with no conserved region found using VISTA tool.

Table 4.6: BLAST Analysis Result Using Ensembl Browser and Vista Genome

Query Gene	No. of sig. Alignment in Genome	Identity % range	Conserved region/gene (bp) (VISTA)	Conserved region/gene % (VISTA)
		Chicken Database		
Rabbit GH	22	81 – 100	658	78.0
Sheep GH	19	93 - 100	2306	76.5
Rabbit IGF-1	-	-	2338	75.9
Sheep IGF-1	65	82 -100	1172	84.3
Rabbit Myostatin	18	83 - 100	1214	81.1
Sheep Myostatin	23	84 – 100	-	-
		Rabbit Database		
Chicken GH	40	81 – 100	673	76.5
Sheep GH	-	-	-	-
Chicken IGF-1	87	82 – 100	40396	75.9
Sheep IGF-1	152	82 - 100	7866	77.3
Chicken Myostatin	-	-	1160	84.4
Sheep Myostatin	25	82 – 100	4046	79.3
		Sheep Database		
Chicken GH	22	82 – 100	-	-
Rabbit GH	14	86 - 100	2328	75.9
Chicken IGF-1	53	82 - 100	40513	75.8
Rabbit IGF-1	94	82 - 100	1226	80.0
Chicken myostatin	31	80 - 100	4018	79.4
Rabbit Myostatin	21	83 – 100	-	-

4.7: Phylogenetic Analysis Result of Growth Hormone Gene of Chicken, Rabbit and Sheep

At nucleotide substitution at 0.08, there was divergence from the common ancestor of chicken and rabbit. Chicken had nucleotides substitution of 0.15 in the gene sequence, and rabbit had nucleotide substitution of 0.17 in the gene sequence. While, the sheep GH gene had nucleotide substitution of 0.25 in the gene sequence, but have not shared a common ancestor with chicken and rabbit GH gene. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). All positions containing gaps and missing data were eliminated. There were a total of 1262 positions in the final dataset.

4.8: Phylogenetic Analysis Result of Myostatin Gene of Chicken, Rabbit and Sheep

At nucleotide substitution at 0.24, there was divergence from the common ancestor of rabbit and sheep. Rabbit had nucleotides substitution of 0.14 in the gene sequence, and sheep had nucleotide substitution of 0.12 in the gene sequence. While, the chicken myostatin gene had nucleotide substitution of 0.38 in the gene sequence, but have not shared a common ancestor with rabbit and sheep myostatin gene. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). All positions containing gaps and missing data were eliminated. There were a total of 4761 positions in the final dataset.

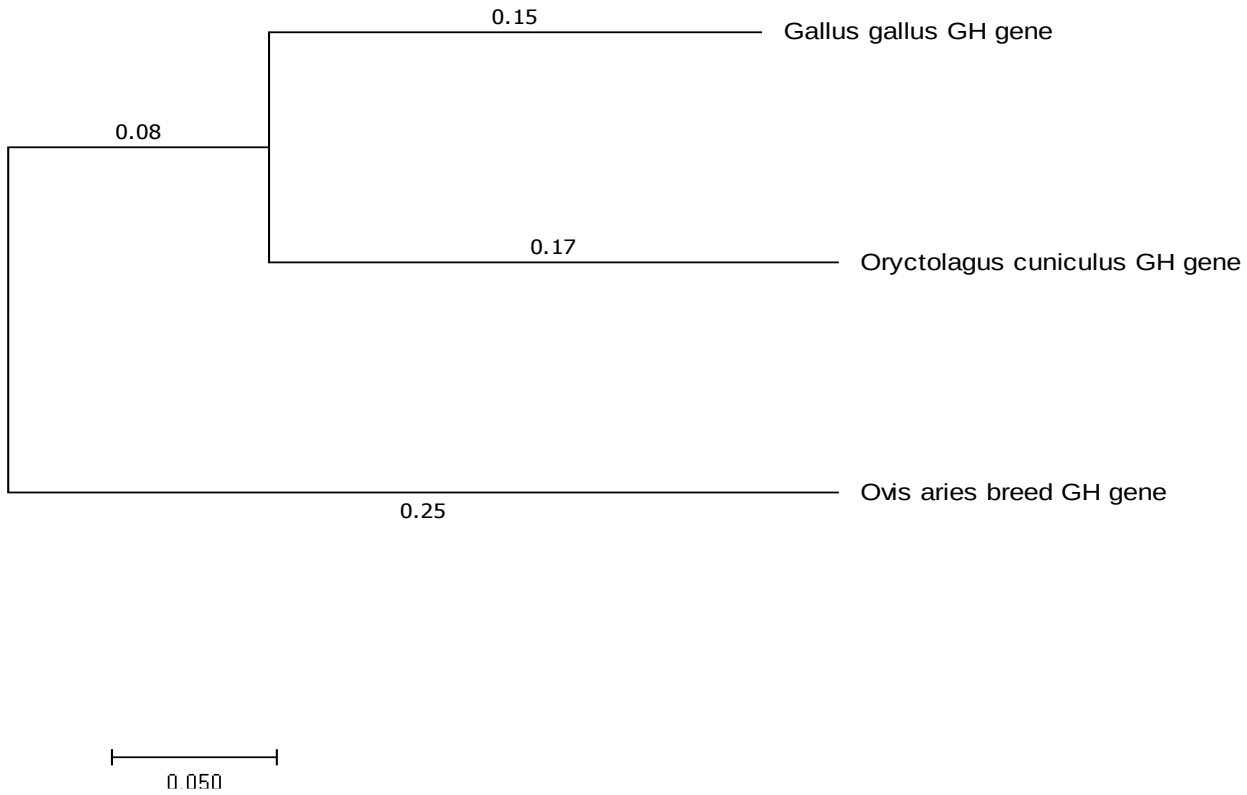


Figure 4.7: Phylogenetic Analysis Result of Growth Hormone Gene of Chicken, Rabbit and Sheep

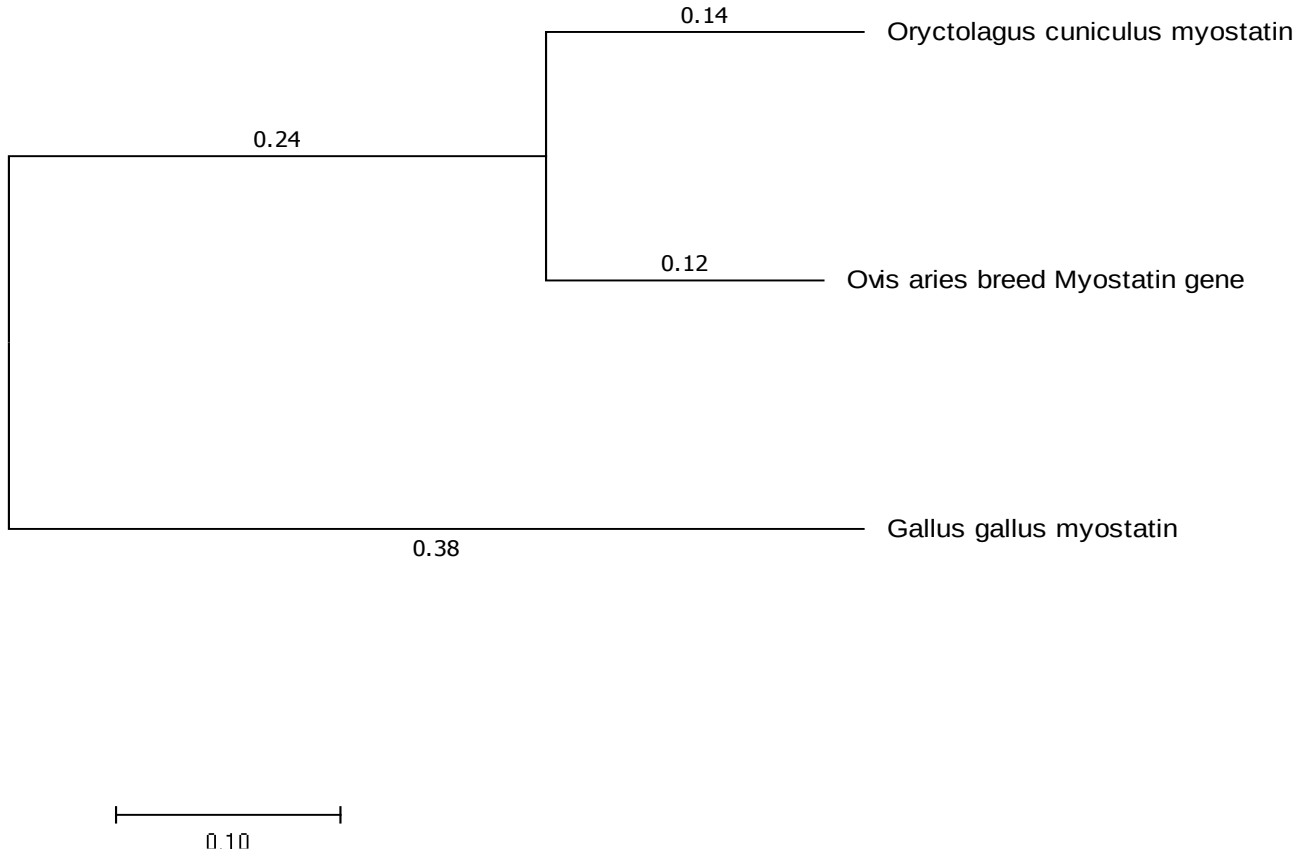


Figure 4.8: Phylogenetic Analysis Result of Myostatin Gene of Chicken, Rabbit And Sheep

4.9: Phylogenetic analysis result of Insulin-like Growth Factor-1 Gene of Chicken, Rabbit and Sheep.

At nucleotide substitution at 0.38, there was divergence from the common ancestor of rabbit and sheep. Rabbit had nucleotides substitution of 0.18 in the gene sequence, and sheep had nucleotide substitution of 0.17 in the gene sequence. While, the chicken IGF-1 gene had nucleotide substitution of 0.57 in the gene sequence, but have not shared a common ancestor with rabbit and sheep IGF-1 gene. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). All positions containing gaps and missing data were eliminated. There were a total of 45747 positions in the final dataset.

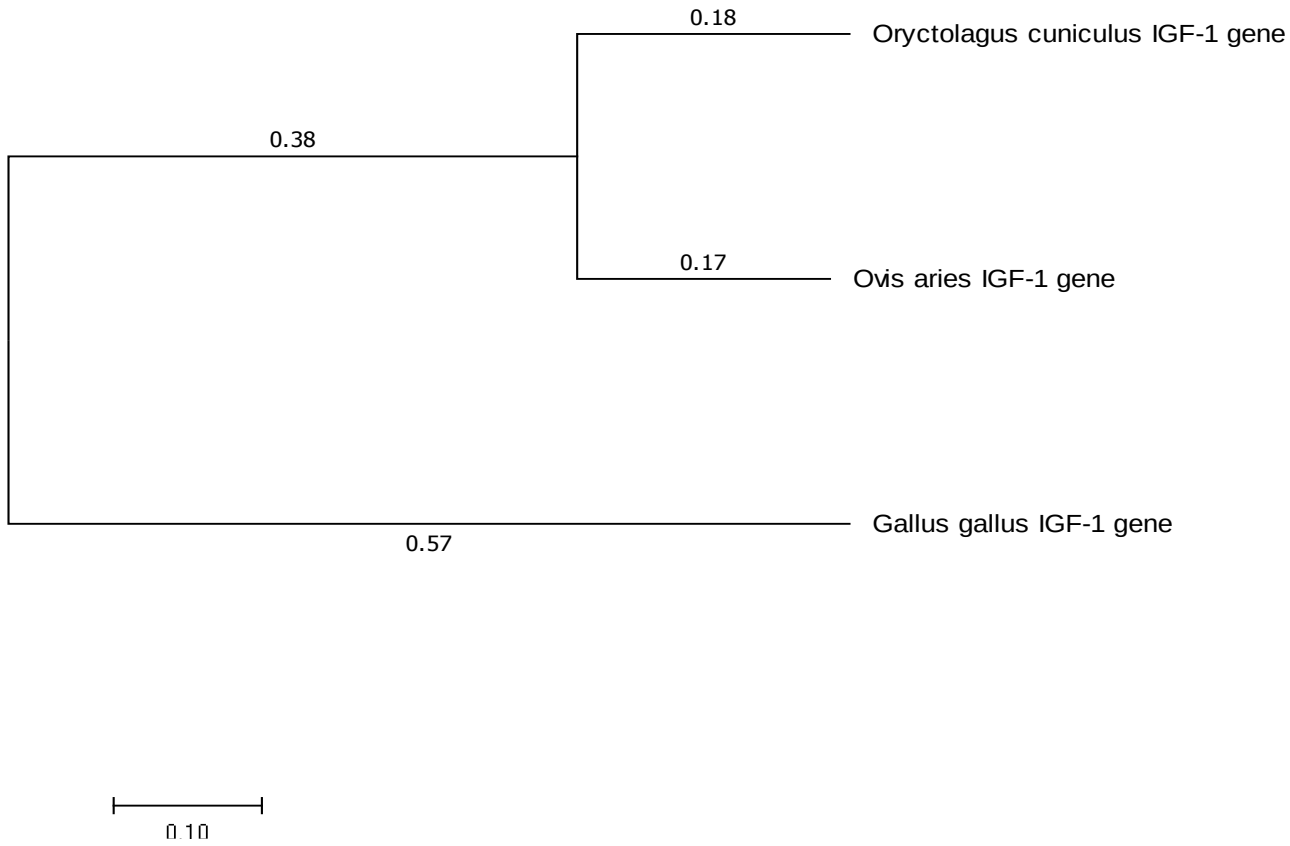


Figure 4.9: Phylogenetic analysis result of Insulin-like Growth Factor-1 Gene of Chicken, Rabbit and Sheep.

4.10: Alignment Result of Rabbit GH X Chicken GH:1-3507bp

The VISTA analysis of chicken GH sequence shown on the x axis and percentage similarity to rabbit GH on the y axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on sliding-window analysis of the underlying genomic alignment. In this illustration, a 100-bp window is used for this slide at different bp nucleotide increments. The necessary keys for the regions, alignment name and axis are position at left.

4.11: Alignment Result of Rabbit Myostatin X Sheep Myostatin X Chicken Myostatin:1-5493bp

The VISTA analysis of chicken myostatin sequence shown on the x axis and percentage similarity to rabbit myostatin and sheep myostatin on the y axis in alignment 1and 2 respectively, with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on sliding-window analysis of the underlying genomic alignment. In this illustration, a 100-bp window is used for this slide at different bp nucleotide increments. The necessary keys for the regions, alignment name and axis are position at left.

4.12: Alignment Result of Rabbit IGF-1 X Chicken IGF-1 Gene:1-48428bp

The VISTA analysis of chickenIGF-1 sequence shown on the x axis and percentage similarity to rabbitIGF-1 on the y axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on sliding-window analysis of the underlying genomic alignment. In this illustration, a 100-bp window is used for this slide at different bp nucleotide increments. The necessary keys for the regions, alignment name and axis are position at left.

Figure 4.10: Alignment Result of Rabbit GH X Chicken GH:1-3507bp

Figure 4.11: Alignment Result of Rabbit Myostatin X Sheep Myostatin X Chicken Myostatin:1-5493bp

Figure 4.12: Alignment Result of Rabbit IGF-1 X Chicken IGF-1 Gene:1-48428bp

Figure 4.12: Alignment Result of Rabbit IGF-1 X Chicken IGF-1 Gene:1-48428bp

4.13: Alignment Result of Sheep IGF-1 X Chicken IGF-1 Gene:1-48428bp

The VISTA analysis of chickenIGF-1 sequence shown on the x axis and percentage similarity to sheepIGF-1 on the y axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on sliding-window analysis of the underlying genomic alignment. In this illustration, a 100-bp window is used for this slide at different bp nucleotide increments. The necessary keys for the regions, alignment name and axis are position at left.

4.14: Alignment Result of Sheep IGF-1 X Rabbit IGF-1 Gene: 1-76791bp

The VISTA analysis of rabbit IGF-1 sequence shown on the x axis and percentage similarity to sheep IGF-1 on the y axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on sliding-window analysis of the underlying genomic alignment. In this illustration, a 100-bp window is used for this slide at different bp nucleotide increments. The necessary keys for the regions, alignment name and axis are position at left.

Figure 4.13: Alignment Result of Sheep IGF-1 X Chicken IGF-1 Gene:1-48428bp

Figure 4.13: Alignment Result of Sheep IGF-1 X Chicken IGF-1 Gene:1-48428bp

Figure 4.14: Alignment result of Sheep IGF-1 X Rabbit IGF-1 Gene: 1-76791bp

Figure 4.14: Alignment result of Sheep IGF-1 X Rabbit IGF-1 Gene: 1-76791bp

CHAPTER FIVE

5.0 DISCUSSION

5.1 GENSCAN Output Result of GH Gene

The chicken GH gene has one gene number; three internal exons, one terminal exon and one poly-A signal, all within the input strand but no initial exon and promoter. Rabbit GH gene has one gene number; one initial exon, two internal exons and one terminal exon but no poly-A signal or promoter, all within the input strand. The two genes have each single category of specific GH gene, there was variation in exon number, coding-sequence size, and length of the gene sequence. There might have been as a result of insertion or deletion found in the gene sequences which are both classified as a frame shift mutation (Genetics Home Reference, 2017). This might have affected some of the important regulatory regions of the genes, or even exons and rendered them inactive in the gene. This was possible because of the gaps during alignment were found at the extreme ends of the sequences where most of the regulatory codons are found, while some within the sequences (intronic regions) might have been as a result of evolution, speciation or mutations. Transcriptional regulation is mediated by proteins binding to regulatory elements on the DNA in a specific combinatorial manner, where particular combinations of transcription factor binding sites establish specific regulatory codes (Leelavati and Ivan, 2009).

The exons of rabbit and chicken GH gene were found within the input strand, which may be to direct the effect of the gene through a specific regulatory pathways and mode of function. According to Leelavati and Ivan (2009), Regulatory motifs are short DNA sequences that are used to control the expression of genes, dictating the conditions under which a gene will be turned on or off. Each motif is typically recognized by a specific DNA-binding protein

called a transcription factor (TF). A transcription factor binds precise sites in the promoter region of target genes in a sequence-specific way, but this contact has the potential to tolerate some degree of sequence variation (Leelavati and Ivan, 2009). The effect of these variations in the gene sequences can be easily depicted by the resulting predicted coding sequence size variation. The predicted coding sequence variation are different predicted peptide sizes (amino subgroup) in chicken, rabbit and sheep, which may translate into more complex enhancing protein fast growth rate of chicken and rabbit.

Sheep GH gene has two gene number categories (which might be paralog genes), first sheep GH gene has; one initial exon, seven internal exons, one terminal exon and one promoter, but no poly-A signal while the second GH gene has one initial exon, four internal exons, one terminal and one poly-A signal but no promoter, all within the opposite strand. These two genes makes up the sequence length of the sheep GH gene, but the effect of these variations in two sheep GH gene category predict the coding sequence size variation sinomous to the two different predicted peptide sizes (amino sub group) of the sheep GH gene. The two different GH gene in sheep means that, each has it is own route of expression and function to perform as the number of exons vary between the two sheep GH gene, this might explain the variations in higher mature body weight and size of sheep compared to chicken and rabbit. The sheep GH gene exons been on opposite strand might be to take different expression pattern and pathways compared to rabbit and chicken.

5.1.1 Regulatory elements and expressions of GH, IGF-1 and MSTN genes

Regulatory elements play a major role in controlling temporal and spatial expression of genes in the cellular environment. The genomic code of gene regulatory elements is encrypted by combinatorial patterns of TF binding sites (Leelavati and Ivan, 2009). By their

nature, they are very short (6 to 15 bp), frequently degenerate and can appear at varying distances and orientations upstream of target genes. Unlike genes that contain clear start and stop codons, as well as well-defined splicing signals, motifs have no detectable sequence features and they are indistinguishable from random sequences of the same length (Leelavati and Ivan, 2009).

Open reading frames are found in chicken, rabbit and sheep GH gene, to give an identification of sequence coding region of the genes. According to IHGSC (2001), the genome of a species encodes genes and other functional elements, interspersed with non-functional nucleotides in a single uninterrupted string of DNA. Recognizing protein-coding genes typically relies on finding stretches of nucleotides free of stop codons (called Open Reading Frames, or ORFs) that are too long to have likely occurred by chance. Since stop codons occur at a frequency of roughly 1 in 20 in random sequence, ORFs of at least 60 amino acids will occur frequently by chance (5% under a simple Poisson model), and even ORFs of 150 amino acids will appear by chance in a large genome (0.05%). This poses a huge challenge for higher eukaryotes in which genes are typically broken into many, small exons (on average 125 nucleotides long for internal exons in mammals (IHGSC, 2001).

5.1.2 Growth hormone genes, regulatory elements and transcription

In some other transgenic animal trials, growth hormone expression was effectively increased in domestic animals such as pig. This modification improved body weight and growth rate by increasing the meat quality (Prather *et al.*, 2008). The transcript was not efficiently transcribe and regulated in these transgenic pigs ‘as a result’ the high level of growth hormone expression produced side effects such as lameness, reduction in fertility, and vulnerability to stress (Pursel and Rexroad, 1993). These defects in the transgenic

animals might have been as a result of differences in the sequences of the gene of one specie to specie, which led to transcription of some regulatory regions correctly while some sequence regions were missing or changed. The polymorphisms at the 3'UTR may be within or near the miRNA-binding site; thus these polymorphisms may affect the miRNA function and lead to differential expression of genes thereby affecting the phenotype (Trott *et al.,* 2014). Transgenic pigs were also developed with ovine growth hormone (oGH), clearly the pigs gained more muscle content compared to wild or non-transgenic pigs. Unfortunately, the pigs suffered from arthritis and bone thickness (Pursel *et al.,* 1997).

When GH gene from the same species is used to improve the transgenic animal, the necessary regulatory elements may be present in the non-coding regions to transcribe the genes effectively, but if there is missing sequences or increased sequences that would be left un-transcribed or transgenes may not function at all, the transgenic animals are without morphological defects. A number of transgenic pigs and sheep were produced with human, bovine, rat, porcine, or ovine GH under the control of several gene promoters, one pig expressing porcine GH (pGH) grew at an exceptionally rapid rate compared to littermates (1273 vs. 781 g/day) (Peter *et al.,* 1988).

In contrast to the GH transgenic pigs, transgenic lambs expressing high levels of ovine GH (oGH) or bGH did not grow faster or utilize feed more efficiently than control lambs, but they were much leaner (Pursel *et al.,* 1989). Severely bowed front legs were a frequent anatomical abnormality observed in the transgenic lambs. A research with modified oGH transgenic that provided only a slight increase in circulating level of oGH resulted in lambs that grew faster and were leaner, but had significantly lower depth of eye muscle than their siblings (Peter *et al.,* 1988). These might explain one of the functions of the two sheep GH genes and the reason why sheep is mostly leaner than rabbit and chicken when compared to

the body size. It was recognized from the outset that regulation of transgene expression would be required to circumvent deleterious effects from continuous exposure of animals to elevated GH (Peter *et al*., 1988).

Transgenic pigs have been produced with a fusion gene composed of avian skeletal z-actin promoter, first intron and 3'-noncoding flanking regions and human insulin-like growth factor-1 (hIGF-1) coding region. Coleman *et al.*(1995) reported that this fusion gene directs high levels of expression specifically in striated muscle in transgenic mice, and elicit myofiber hypertrophy. To this end, this indicate that for any gene to be transcribed efficiently the regulatory gene sequences have to be in place like in sheep GH gene compared to rabbit and chicken GH gene sequences with a lot of gaps/deletions mostly at the extreme ends. According to Karin and Richards, (1982), plasmid contains the hMT-IIA promoter fused to a hybrid pGH gene consisting of pGH cDNA sequences encoding amino acid residues 1-158 of pre-pGH, and pGH genomic sequences (Vize and Wells, 1987) encoding pre-pGH residues 158-216. The genomic sequence also contains approximately 700 bases of pGH gene 3' non-coding sequences. The 3' non-coding sequences were included to ensure the efficient processing and polyadenylation of the hybrid messenger RNA (Peter *et al*., 1988).

5.2 GENSCAN Output Result of Myostatin Gene

The high conservation of myostatin gene in DNA strand, gene number and exon number among rabbit, chicken, sheep and most different species, also indicate ortholog features, which seems to function by specific target regulating sequence regions in genes common to all the species that are responsible for growth in animals. The other few variations observed between the alignment length of myostatin gene of rabbit, chicken and sheep might be due

to the regions where the specie different activation sites are residing. According to McPherron *et al.* (1997), deletion is expected to be a null mutation because it occurs after only the first 7 amino acids of the C-terminal region, resulting in a loss of 102 amino acids (amino acids 274–375). This mutation is similar to the targeted mutation in myostatin null mice in which the entire region encoding the mature protein was deleted. This means any gene modification in these regulatory sequences of myostatin or the binding site of the target genes can also render the activity of myostatin suppression inactive. This might however not bring about the defect of complete knock-out myostatin gene, since other regulatory elements might still be active. Thus, the regulatory regions of the myostatin or target genes seems to developed by increasing in length mostly with the age of the animal as it approaches maturity for the myostatin gene to effectively function.

This myostatin gene's most notable effect is on the hypertrophy and hyperplasia of muscle fibers for both pre- and post-natal growth. Myostatin expression in livestock species can be modulated in a way that increases meat production without the negative attributes associated with natural-occurring myostatin mutations. Animals with reduced, but not eliminated, myostatin will display an increase in muscle mass and potentially avoid the detrimental effects of double muscling, which decrease fertility and encourage dystocia. The chicken myostatin sequence has no open reading frame, while the rabbit myostatin sequences has three open reading frames. The sheep myostatin sequence has two open reading frames, which might be the reason why chicken can grow more muscle in proposition to it is body size compared to rabbit and sheep.

Sheep and rabbit MSTN gene are closer than that of chicken MSTN gene, when translated sequences were used for preparation of phylogeny. It was observed that rabbit MSTN gene

was nearest to M. *musculus* MSTN gene and S. *scrofa* farthest from G. *gallus* MSTN gene. Apart from these, other variations were also observed in the intronic regions of myostatin gene. However, the nucleotide variations and the amino acid changes seen in some animals showing mutations did not affect the myostatin protein structure and conformation. Thus from this, it can be concluded that Myostatin gene is highly conserved in and between different species of other animals. The multiple sequence alignment of MSTN amino acid sequences of various species also showed many other variations even though they were highly similar (Schuelke *et al.*, 2004). The predicted coding sequence of rabbit and sheep were the same in size, but the chicken MSTN gene differed little in size, which also transcribe to a very little difference in size of predicted peptide size (amino acid subgroup) of rabbit (375), chicken (362) and sheep (375). The Belgian Blue myostatin coding sequence was identical to the Holstein sequence except for a deletion of nucleotides 937–947 in the third exon. This 11-nucleotide deletion causes a frame-shift which is predicted to result in a truncated protein that terminates 14 codons downstream of the site of the mutation (McPherron *et al.*, 1997).

5.3 GENSCAN Output Result of IGF-1 Gene

5.3.1.IGF-1 gene of chicken

This research indicated two categories of the chicken IGF-1gene; the first IGF-1 gene having the largest predicted coding sequence which translate to larger predicted peptide size (amino acid subgroup) with six open reading frames, where all the six exons were found within the input strand. While the second chicken IGF-1 gene may be a paralog to the first chicken IGF-1 but may function through a different pathway, as all the three exons are found within opposite strand and a smaller predicted coding sequence which also translated to smaller predicted peptide size (amino acid subgroup) compared to the first chicken IGF-1

gene. Both chicken IGF-1 genes are smaller in length compared to rabbit and sheep IGF-1 genes. As a mediator or mechanism through which GH genes exerts their effects in animals, this might explain why chicken specie final matured body weight and size is smaller compared to that of rabbit and sheep species, as some of the effects of the chicken GH gene are limited by the gene length, number and the DNA strand of the IGF-1 gene in chicken. Even though the chicken GH gene is larger than rabbit GH gene, its effect might be limited by its mediator. According to Lupu *et al.(*2001), the insulin-like growth factor (IGF) signaling system is the major determinant of growth in mammalian organism. It provides the main common downstream conduct for the action of most growth-promoting gene products controlling body size. The mechanisms controlling mammalian IGF-1 gene transcription are complex, they may involve two promoters and also dispersed regulatory elements that serve GH signals acting through the Stat5b pathway (Eleswarapu *et al*., 2008).

5.3.2.IGF-1 gene of rabbit

The rabbit IGF-1 gene is of three categories, the first IGF-1 gene has two open reading frames, with six exons found within the input strand. The second rabbit IGF-1 gene may be a paralog to the first rabbit IGF-1 but has the largest predicted coding sequence which translate to larger predicted peptide size (amino acid subgroup) may function through a different pathway, because all the ten exons and eight open reading frames, are found within the input strand. This might be the reason why rabbits species final body weight is higher than that of chicken as it also compensate for the small size of rabbit GH genes and manifest/expressed its effect through this second rabbit IGF-1 gene. The first rabbit and chicken IGF-1 gene have the same number of exons (6) and all are within the input strand,

there are small variation in the predicted coding sequences and predicted peptide sizes, these might explain their fast growth within a short period. The third rabbit IGF-1 gene may also be a paralog to the first and second rabbit IGF-1 gene, it has a smaller predicted coding sequence which also translated to smaller predicted peptide size (amino acid subgroup) but larger than sheep third IGF-1 gene, even though both third IGF-1 gene are within the opposite DNA strand, as might be unique to non-avian species. As reported by Davis and Simmen, (2006),Insulin-like growth factor 1 (IGF1), is an important component of somatotropic axis, it plays an important role in growth, reproduction, foetal development and cell proliferation. In the development and growth of muscles, IGF1 acts as a mitogen and a differentiating factor by controlling the mitogenic and myogenic processes during muscle development (Ewton *et al.*, 1994; Florini *et al.*, 1996).

5.3.3 IGF-1 gene of sheep

The sheep IGF-1 gene also consist of three categories; the first sheep IGF-1 gene has twelve open reading frames, with eleven exons found within the input strand that have the largest predicted coding sequence which translate larger predicted peptide size (amino acid subgroups) may function through a different pathway, while the second sheep IGF-1 gene may be a paralog to the first sheep IGF-1, with six exons and five open reading frames, found within the input strand, which might be the reason why sheep species final body weight is higher than that of rabbits and chicken species. The sheep GH gene is higher in size and with higher size of the first IGF-1 gene to manifest its effect, while the second sheep IGF-1 gene is not as high as the second rabbit IGF-1 gene, to compensate for the fast growth compared to rabbit second IGF-1 gene, this might be the effect of this small variation in the predicted coding sequences and predicted peptide sizes. The third sheep

IGF-1 gene may also be a paralog to the first and second sheep IGF-1 genes, it has a smaller predicted coding sequence which also translated to smaller predicted peptide size (amino acid units),it is found within the opposite DNA strand, and might function in different pathways.

IGF-1 gene transcription is GH-dependent in skeletal muscles, adipose tissue, ovary, and kidney (Venken *et al.,* 2007; Liu *et al.,* 2000). The large size of rabbit IGF-1 gene may also be another why rabbit IGF-1 gene controls growth by expressing the effect of GH gene effectively. Even though the chicken GH gene is almost two to three times higher in size and which supposed to have more effect in final body weight of chicken species than rabbit species, it is not so because of the compensatory effect of the rabbit IGF-1 size. The sheep IGF-1 gene is also large in size but it compensate to the larger size of sheep GH gene, which may also explain the final large body weight and size of sheep species. According to Coleman *et al*.(1995) and Musaro *et al.*(2001), reports describing muscle-specific transgenic overexpression of IGF-1 gene, they found no change in serum IGF-1, despite a 47-fold higher than normal level of the peptide in muscle extracts in one of the study. Between breed comparison indicates a significantly higher IGF-1 expression in muscles of Malabari than Attappady Black breed of sheep. During postnatal skeletal muscle growth in vivo or in fully differentiated muscle cells, it was reported that IGFs stimulated the rate of protein synthesis and inhibits the rate of protein degradation, thereby enhancing myofibre hypertrophy and increase in myofibre diameter, which may affect muscle tenderness (Koohmaraie *et al.,* 1995; Florini *et al.,* 1996).

5.4 MEGA7 Alignment Result

5.4.1. Growth hormone gene (GH) deletions and codons

The condition in eukaryotes is that regulatory sites often act in both directions, binding sites are usually distant from regulons because of large intergenic regions, and transcription regulation is usually a result of combined action by multiple transcription factors in a combinatorial manner (Luscombe *et al.*, 2001). The Alignment result of chicken, rabbit and sheep GH gene revealed gaps/deletions. This is an indication that a lot of deletion (29968 gaps) and translated to 420 codons of the available sequence, might have occurred more in rabbit GH gene in the course of evolutionary change, which might have rendered some regulatory elements in-active in rabbit species, than in chicken GH gene with (27723 gaps) and translated to 1169 codons of the available sequence, which might have additional function in chicken species than in rabbit species due to increase in size of the sequences that will translate to more amino acids to produce more functional proteins. The sheep GH gene had only few deletions (210 gaps) and translated to 10338 codons of the available sequence, along the gene sequences, which might result to possessing a lot of regulatory elements, coding and non-coding regions and also functions not normally found in rabbit and chicken species. These might also translate to large body weight and size of sheep species at early stage and at maturity than rabbit and chicken species. In ensuring that the regions compared are orthologous, assumptions can be about the rate of change of different regions, and apply statistical models to interpret the significance of strong or weak conservation in discovering biological signals (IHGSC, 2001).

5.4.2. Insulin-like growth factor-1 gene (IGF-1) deletions and codons

Chicken IGF-1 gene had deletions of (36,075 gaps), and translated to IGF-1 gene codons of 15,250 of the available sequence, which might rendered a lot of regulatory element non-functional as large deletions in gene sequences mostly lead to mutation or change the reading frame of the gene as a result of frame shift mutation and might not function properly in expressing the effect of the GH gene effectively as somatomedin. Sheep IGF-1 gene had a deletions of (9,157 gaps) and translated to IGF-1 gene codons of 24,566 of the available sequence, which are comparatively few to chicken IGF-1 deletions but might compensate to expressing a lot of regulatory regions of the large sheep GH gene. The Rabbit IGF-1 gene revealed fewer deletions of (7,712 gaps) and translated to IGF-1 gene codons of 24,778 of the available sequence, which is resulted to large sequence of IGF-1 gene and the ability to manifold the function of few rabbit GH gene, which seems to have compensated with fast growth and large body weight of rabbit species compared to chicken species. Laron (2001), and Estany *et al.* (2007), report suggested that IGF-1 deficiencies causes pronounced growth retardation and delayed onset of puberty.

5.4.3. Myostatin (MSTN) deletions and codons

McPherron and Lee, (1997) reported that sequencing GDF-8 gene in Belgian Blue breeds of cattle showed mutation which resulted in 11-bp deletion in coding region of myostatin cause stop decoding after amino acid 287, it also causes forming an inactive and incomplete protein so it stops the controlling role of myostatin protein on muscles growth then it appears double muscling phenotype. Mutation exon 3 in piedmontes breed of cattle changes nucleotide G into A and cysteine amino acid codon changes into tyrosine and probably inhibited myostatin gene function.

Chicken myostatin gene, had only (51 gaps), resulted to Myostatin gene codons of 1815 translation which might indicate presence of more functional regulatory elements as more sequence regions were maintained and as a negative regulator of growth, this extra sequences length might be the effective region which restrict the matured chicken species body weight less than rabbit and sheep species. Rabbit myostatin gene, had deletions of (632 gaps), resulted to Myostatin gene codons of 1538 translation which might be the reason why rabbit species have higher matured body weight than chicken species, as some of the functions of the rabbit myostatin gene might be limited by these deletions as negative regulator of growth and might have been during process of speciation. While, sheep Myostatin gene revealed deletions of (553 gaps), translated to Myostatin gene codons of 1578 translation which might have also lost some of it is functions as a negative regulator of growth, as a result of these large deletion and lost some regulatory elements in the sheep myostatin gene sequence. The six mutations, located in exons 2 and 3, result in premature stop codons, each of these mutations causes the negative effect of myostatin to stop the muscles growth and create double muscling phonotype (Bellinge*et al.,* 2005).

5.4.4. Growth hormone gene (GH) SNP and conservations

The GH gene single nucleotide polymorphism of chicken by rabbit (560/31235), chicken by sheep (2272/31235) and rabbit by sheep (656/31235). These variations in genes sequence might have been due to mutation along speciation process, and which may vary direction of function base on species to a different pathway of expression and bring variation in specie morphology. Even though the genes may be ortholog or paralog. The large single nucleotide variation in chicken by sheep GH gene might be an indication of how wide they vary in gene expression pathway. While chicken by rabbit is an indication of how close

they are in gene expression pathway. The GH gene conserved regions of chicken by rabbit (687/31235), chicken by sheep (1121/31235), rabbit by sheep (496/31235) and chicken by rabbit by sheep (1045/31235). These conservations might be the indication of conserved functions, even though they are found in different species. The large conserved region in chicken by sheep GH gene might be an indication of how close they are in gene expression function. While rabbit by sheep GH gene might be an indication of how wide they are in gene expression function. According to Dutilh and Keşmir (2016), genes or proteins that belong to the same family, may have been derived from a common ancestor. The proteins within one family often share some level of functional similarity, although their precise function may be different.

5.4.5. Insulin-like growth factor-1 gene (IGF-1) SNP and conservations

The IGF-1 gene single nucleotide polymorphism of chicken by rabbit (25675/84503), chicken by sheep (26210/84503) and rabbit by sheep (19514/84503). The large single nucleotide variation in chicken by sheep IGF-1 gene might be an indication of how wide they vary in gene expression pathways. While rabbit by sheep IGF-1 gene might be an indication of how close they are in gene expression pathway. The IGF-1 gene conserved regions of chicken by rabbit (20677/84503), chicken by sheep (21135/84503), rabbit by sheep (48598/84503) and chicken by rabbit by sheep (32721/84503). The large conserved region in rabbit by sheep IGF-1 gene might be an indication of how close they are in gene expression function. While chicken by rabbit IGF-1 gene might be an indication of how wide they are in gene expression function. But, chicken by rabbit by sheep IGF-1 gene conservation might be an indication of how similar they all function, a feature that is common between the species. Gene sequences are both subject to different random genetic

events like mutations, insertions, duplications and deletions, the sequences of the two duplicated genes will diverge. This means that their sequences will increasingly lose similarity to one another over time (Dutilh and Keşmir, 2016).

5.4.6. Myostatin gene (MSTN) SNP and conservations

The Myostatin gene single nucleotide polymorphism of chicken by rabbit (2229/5544), chicken by sheep (2261/5544) and rabbit by sheep (1042/5544). The large single nucleotide variation in chicken by sheep myostatin gene might be an indication of how wide they vary in gene expression pathways. While rabbit by sheep myostatin gene might be an indication of how close they are in gene expression pathways. The Myostatin gene conserved regions of chicken by rabbit (2647/5544), chicken by sheep (2690/5544), rabbit by sheep (3744/5544) and chicken by rabbit by sheep (2382/5544). The large conserved region in rabbit by sheep myostatin gene might be an indication of how close they are in gene expression function. While chicken by rabbit myostatin might be an indication of how wide they are in gene expression function. But, chicken by rabbit by sheep myostation gene conservation might be an indication of how similar they all function, a feature that is common between the species. Similarly Tay *et al.*(2004) reported 38 nucleotide differences between the myostatin sequences in cattle and that of the goat. The authors found that there were 25 non-synonymous changes and 13 synonymous changes and identified three SNPs, two in exon II and one in exon III. Grisolia *et al.* (2009) found 37 polymorphisms in the un-translated region segment, one SNP in intron I and three SNPs in intron II of Nellore cattle breed. They concluded that this high degree of allelic heterogeneity in the myostatin gene could be related to its high mutation rate, and it also could be the result of a long history of

artificial selection for meat production, which has probably favored such modifications and maintained them in cattle populations.

In sheep, the myostatin gene is located on chromosome 2. Twenty single-nucleotide polymorphisms (SNPs) were identified in the region of GDF-8 in Texel sheep, among there were, SNP g+6223G>A in the 3' untranslated region (3'UTR) that cause the body muscular hypertrophy (Clop *et al*., 2006).

5.5 BLAT Analysis Result Using Genome Browser

5.5.1. Growth hormone (GH) gene

The copies of chicken GH gene predicted within chicken genome was 201, distributed over the chicken specie, which might have been as a result of paralog process that might have been through mutation, deletions or insertions in some of the regulatory elements and regions. These mutations might have led to significant variation in the gene sequences and also vary a little in function and might have also led to the different identity percentage (58 – 100), distributed over 29 different chromosomes at different locations but on the two different DNA strand, having 102 copies on the input strand and 99 copies on the opposite strand, which might be to function through different pathways as the orientations of the genes on different strands might change. The input strand seems to per-take most in establishing some of the functions, as the percentage of the chicken GH genes are higher in this strand. The chicken GH gene large number compare to the body size might be one of the reasons behind IGF-1 gene small number since the chicken GH gene abundance in the genome, the effect is almost in every cell.

Rabbit GH gene predicted in rabbit genome was 11 copies only, which might also be as a result of paralog process that was also distributed only within 9 different chromosomes in the rabbit specie, which the gene effect might cover few locations and with 7 copies on

input strand and 4 copies opposite strand though with higher identity percentage of the copies (94 – 100), which is an indication of few single nucleotide polymorphisms or mutations between the rabbit GH genes found in the rabbit genome, this was also an indication of how close they are in function. The input strand also seems to per-take most in establishing some of the functions, as the percentage of the rabbit GH genes are higher in this strand.

The sheep GH gene numbers predicted in sheep genome was 232 copies, which is expected given the large body size of sheep species at birth and at maturity, but in comparison with chicken GH gene of 102 number and body size, it is an indication that the GH gene is not the sole determinant of the large body size of sheep species, this might explain the role of large size of IGF-1 gene in sheep and the role of the opposite DNA strand in which most of the sheep GH genes are found might explain the large body size pathways of sheep species at birth and at maturity (112 copies on input strand and 120 copies on opposite strand), all with high identity percentage of (71 – 100) distributed all over a wide range of 46 different chromosomes in the sheep genome. Duplications of pieces of a genomic sequence can occur during replication, sometimes leading to the copying of an entire gene. In principle these errors are a random process, and whether the result is fixed in a descendent lineage depends on the frequency of the mutation and natural selection (Dutilh and Keşmir, 2016).

5.5.2. Insulin-like growth factor-1 (IGF-1) gene

The chicken IGF-1 gene predicted in chicken genome was 209 copies, which vary little in size with chicken GH gene with only (8 copies), almost a 1:1 mapping ratio between the genes and the DNA strands (105 copies on input strand and 104 copies on opposite strand) with high identity percentage (85 – 100), found among 22 different chromosomes in the

chicken genome. This might explain the reason for the final small body size of chicken because chicken GH gene is not complemented by the chicken IGF-1 genes compared to rabbit and sheep. So the chicken GH gene effect is not multiple in folds by the chicken IGF-1 gene. The chicken IGF-1 gene is also distributed over small range of different chromosomes compared to the chicken GH gene, which might also limit the spread of the effect of the chicken GH gene. Transgenic mice had been used to study the expressed increased insulin-like growth factor-I (IGF-1),it was observed that they too grew larger than normal (Mathews *et al.*, 1988). When the expressed transgenes encodes a growth promoting hormone(growth hormone or growth hormone releasing factor), transgenic mice were reported to grow up to twice their normal size (Palmiter *et al.*, 1983; Hammer *et al.*, 1985a). The rabbit IGF-1 gene information could not be display due to large size of the rabbit IGF-1 gene and large size of the gene in the genome data available on the website for the gene. The sheep IGF-1 gene information could not be retrieved due to large size of the sheep IGF-1 gene and large size of the gene in the genome data available on the website for the gene.

5.5.3. Myostatin (MSTN) gene

The chicken Myostatin gene predicted in chicken genome was only 3 copies, which might explain more on the negative effect of myostatin gene on fast growth and body weight, which might have retarded the growth to the normal chicken specie at high level, if the chicken GH gene regulate fast growth through the input strand, might also explain the one copy of the chicken myostatin gene on input strand to reduce the effect of myostatin gene and the two copies of chicken myostatin gene on the opposite strand to reduce the matured body size and weight, with identity percentage of (81 – 100) found only in 3 different

chromosomes may be to also further reduce the effect of the myostatin gene since the chicken IGF-1 gene is also small in size.

The rabbit myostatin gene predicted in rabbit genome is 5 copies, higher than that of chicken and sheep species, which might be as a result of large size of rabbit IGF-1 gene that multiply the effect of small size of rabbit GH gene. The 2 copies of rabbit myostatin gene on the input DNA strand might be a pathway to reduce excessive fast growth from birth to maturity of rabbit species while the 3 rabbit myostatin gene copies on opposite strand might be the pathway to reduce the final body weight at maturity of rabbit species. The identity percentage of the rabbit myostatin gene copies (78 – 100) found in 5 different chromosome may be to further increase the effect of the rabbit myostatin gene compared to chicken and sheep myostatin genes.

The copies of sheep myostatin gene predicted in sheep genome was 3, with 2 copies on input strand might explain the slow growth rate from birth to maturity of myostatin gene of sheep species compared to rabbit and chicken species and 1 copy on opposite strand might also explain the less effect of myostatin gene on the large body weight and size of sheep species compared to rabbit and chicken species, at identity percentage of (80 – 100) found in 2 different chromosomes. This might also indicate the route of action of myostatin gene on growth might be through suppressing IGF-1 gene effect. In the muscle tissue of high growth line broilers, it has been reported that a positive relationship between muscle IGF-1, mRNA levels, which determine paracrine IGF-1 levels, and post hatch muscle growth (Duclos, 2005).

5.6 BLAST Analysis Result Using Ensembl Browser and Vista Genome

5.6.1. Chicken genome

The rabbit GH gene has 22 significant alignments in chicken genome, which indicate some level of conservation and maintenance of some level of function between rabbit and chicken species GH gene, with high identity percentage of (81 – 100) and a conserved region of 658 bp at 78.0 percent per rabbit GH gene. The sheep GH gene has 19 significant alignments in chicken genome, with higher identity percentage of (93 – 100) and a conserved region of 2306 bp at 76.5 percent per sheep GH gene. The significant alignment is an indication of how similar chicken GH gene might function in 22 different genome locations like rabbit GH more than that of other 19 different locations like sheep GH gene.

The rabbit IGF-1 gene has no significant alignments in chicken genome, but a conserved region of 2338 bp at 75.9 percent per gene. The sheep IGF-1 gene has 65 significant alignments in chicken genome, with high identity percentage of (82 – 100) and a conserved region of 1172 bp at 84.3 percent per gene. This means chicken IGF-1 gene might function similarly in 65 different locations like sheep IGF-1 gene, as a result of the similar conserved regions but even though there is conserved regions with rabbit IGF-1 gene but no significant alignment between the genes.

The chicken genome has 18 significant alignments to function similarly like rabbit Myostatin gene, with an identity percentage of (83 – 100) as a result of conserved region of 1214 bp at 81.1 percent per gene and 23 more significant alignments like sheep myostatin gene, with an identity percentage of (84 – 100).The relationship between pairs of proteins or the genes from which they are derived: analogous proteins have related folds, but unrelated sequences, while homologous proteins are both sequentially and structurally similar (Luscombe *et al.*, 2001).

5.6.2. Rabbit genome

The rabbit genome has 40 significant alignments to function similar to chicken GH gene, with high identity percentage of (81 – 100) as a result of conserved region of 673 bp at 76.5 percent per gene. But, has no significant alignments with sheep GH gene.

The rabbit genome has 87 significant alignments to function similar to chicken IGF-1 gene, with high identity percentage of (82 – 100) as a result of conserved region of 40396 bp at 75.9 percent per gene and with 152 significant alignments like sheep IGF-1 gene, with high identity percentage of (82 – 100) as a result of conserved region of 7866 bp at 77.3 percent per gene. This might also be reason for the fast growth from birth to maturity like chickens and the heavy body weight at maturity more than chickens.

The rabbit genome has no significant alignments with chicken Myostatin gene, even though there is a conserved region of 1160 bp at 84.4 percent. But, has 25 significant alignments to function like sheep myostatin gene, with a high identity percentage of (82 – 100) as a result of conserved region of 4046 bp at 79.3 per gene. Gene expression level differs under different environmental conditions, different stages of the cell cycle and different cell types in multicellular organisms (Holstege *et al.,* 1998).

5.6.3. Sheep genome

The sheep genome has 22 significant alignments to function similar to chicken GH gene, with high identity percentage of (82 – 100) and has 14 significant alignments to function like rabbit GH gene, with high identity percentage of (86 – 100) as a result of conserved region of 2328 bp at 75.9 per gene.

The sheep genome has 53 significant alignments to function like chicken IGF-1 gene, with an identity percentage of (82 – 100) as a result of conserved region of 40513 bp at 75.8

percent per gene and has 94 significant alignments to function like rabbit IGF-1 gene, with an identity percentage of (82 – 100) as a result of conserved region of 1226 bp at 80.0 percent per gene. This might explain the reason for large body size of sheep species compared to rabbit and chicken.

The sheep genome has 31 significant alignments to function similar to chicken Myostatin gene, with an identity percentage of (80 – 100) as a result of conserved region of 4018 bp at 79.4 percent per gene and has 21 significant alignments to function like rabbit myostatin gene, with an identity percentage of (83 – 100). This might also explain the limited fast growth from birth to maturity and limited very large body weight, despite very large size of sheep GH and IGF-1 gene in sheep specie.

Genes can be clustered into those with particular functions (eg enzymatic actions) or according to the metabolic pathway to which they belong (Kanehisa and Goto, 2000). Normally, orthologues retain the same function while paralogues evolve distinct, but related functions (Tatusov *et al.*, 1997).

5.7 Phylogenetic Analysis Resultof Growth Hormone Gene of Chicken, Rabbit and Sheep

The tree is drawn to scale in 100, with branch lengths measured in the number of substitutions per site (next to the branches). The rooted tree with branch lengths scaled according to the amount of evolutionary change thought to might have happened on each branch. The branch leading to rabbit GH gene is longer than to chicken GH gene, meaning that the rate of evolution has been faster in rabbit GH gene than to chicken GH gene because, more nucleotides have changed in rabbit GH gene than in chicken GH gene since their last common ancestor. The sheep GH gene has never share a common ancestor, even though the rate of evolution has been faster than that of chicken GH gene but at the same

rate with rabbit GH gene, but does not tell us the precise time of speciation. Sequences always diverge due to the high dimensionality of sequence space: each nucleotide or amino acid in a sequence is a separate "dimension" that can occur in 4 or 20 states for nucleotides or amino acids, respectively (Dutilh, 2014).

5.8 Phylogenetic AnalysisResult of Myostatin Gene of Chicken, Rabbit And Sheep

The tree is drawn to scale in 100, with branch lengths measured in the number of substitutions per site (next to the branches). The rooted tree with branch lengths scaled according to the amount of evolutionary change thought to might have happened on each branch. The branch leading to rabbit Myostatin gene is longer than to sheep Myostatin gene, meaning that the rate of evolution has been faster in rabbit Myostatin gene than to sheep Myostatin gene because, more nucleotides have changed in rabbit Myostatin gene than in sheep Myostatin gene since their last common ancestor. The chicken Myostatin gene has never share a common ancestor, even though the rate of evolution has been faster than that of sheep Myostatin gene and rabbit Myostatin gene, but does not tell us the precise time of speciation.

Among homologues, it is useful to distinguish between orthologues, proteins in different species that have evolved from a common ancestral gene, and paralogues, proteins that are related by gene duplication within a genome (Fitch, 1970).

5.9 Phylogenetic Analysis Result of Insulin-like Growth Factor-1 Gene of Chicken, Rabbit and Sheep.

The tree is drawn to scale in 100, with branch lengths measured in the number of substitutions per site (next to the branches). The rooted tree with branch lengths scaled according to the amount of evolutionary change thought to might have happened on each

branch. The branch leading to rabbit IGF-1 gene is longer than to sheep IGF-1 gene, meaning that the rate of evolution has been faster in rabbit IGF-1 gene than to sheep IGF-1 gene because, more nucleotides have changed in rabbit IGF-1 gene than in sheep IGF-1 gene since their last common ancestor. The chicken IGF-1 gene has never share a common ancestor, even though the rate of evolution has been faster than that of sheep IGF-1 gene and a little fast than that of rabbit IGF-1 gene, but does not tell us the precise time of speciation. Distinct proteins frequently have comparable sequences, organisms often have multiple copies of a particular gene through duplication while different species have equivalent or similar proteins that were inherited when they diverged from each other in evolution (Luscombe *et al.,* 2001).

5.10 Alignment of Rabbit GH X Chicken GH:1-3507bp

The VISTA analysis of chicken GH sequence shown on the x axis and percentage similarity to rabbit GH on alignment axis with the percentage similarity scaling between 50 – 100% only on the right of the plot. The graphical plot is based on the regions where chicken GH gene and rabbit GH gene have a common conservation, in which the least conserved region between chicken and rabbit GH gene is 109bp at 78.9 percent and the highest conserved region between is 223bp at 78.5 percent with a total conserved regions of 673bp at different regions, which are found in the non-coding regions which might also explain the differences in expression of the chicken and rabbit GH as the conserved regions are not found in the coding regions, which means the translation into protein will also be different. But, the high similarities percentage in the conserved non-coding regions, might explain the similarities in mode of function of the GH gene in chicken and rabbit species in growth

process. Different species have equivalent or similar proteins that were inherited when they diverged from each other in evolution (Luscombe *et al.,* 2001).

5.11Alignment of Rabbit Myostatin X Sheep Myostatin X Chicken Myostatin:1-5493bp

The VISTA analysis of chicken myostatin sequence shown on the x axis and percentage similarity to rabbit myostatin and sheep myostatin on alignment axis in alignment 1and 2 respectively, with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on the regions where chicken, rabbit and sheep myostatin gene have a common conservation, almost at the same size (bp) and distance at different regions as they are highly similar in conservation, but still are found in the non-coding regions which might also explain the differences in expression as negative regulator of muscles as the conserved regions are not found in the coding regions, which means the translation into protein will also be different. But, the high similarities percentage in the conserved non-coding regions, might explain the similarities in mode of function of the myostatin gene in chicken, rabbit and sheep species in growth process. Polymorphisms that are associated with the function of these genes have been observed in different species (Seyed, 2012).

5.12 Alignmentof Rabbit IGF-1 X Chicken IGF-1 Gene:1-48428bp

The VISTA analysis of chicken IGF-1 sequence shown on the x axis and percentage similarity to rabbit IGF-1 on alignment axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on the regions where chicken IGF-1 gene and rabbit IGF-1 gene have a common conservation, in which the least conserved region between chicken and rabbit IGF-1 gene is 117bp at 74.4 percent and the

highest conserved region between is 387bp at 86.8 percent with a total conserved regions of 2275bp at different regions with a wide distance between the conserved regions over the large size of the IGF-1 gene, which are also found in the non-coding regions which might also explain the differences in expression of the chicken and rabbit IGF-1 as the conserved regions are not found in the coding regions, which means the translation into protein will also be different. But, the high similarities percentage in the conserved non-coding regions, might explain the similarities in mode of function of the IGF-1 gene in chicken and rabbit species in growth process. The IGFs play an important role in regulating somatic growth, according to nutritional conditions, 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 (Shahabodin and Hamid, 2015).

5.13Alignment of Sheep IGF-1 X Chicken IGF-1 Gene:1-48428bp

The VISTA analysis of chicken IGF-1 sequence shown on the x axis and percentage similarity to sheep IGF-1 on alignment axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on the regions where chicken IGF-1 gene and sheep IGF-1 gene have a common conservation, in which the least conserved region between chicken and sheep IGF-1 gene is 138bp at 71.7 percent and the highest conserved region between is 547bp at 83.7 percent with a total conserved regions of 2328bp at different regions with a wide distance between the conserved regions over the large size of the IGF-1 gene, which are also found in the non-coding regions which might also explain the differences in expression of the chicken and sheep IGF-1 as the conserved regions are not found in the coding regions, which means the translation into protein will

also be different. But, the high similarities percentage in the conserved non-coding regions, might explain the similarities in mode of function of the IGF-1 gene in chicken and sheep species in growth process. Locally produced IGF-I is more important than circulating IGF-1 in maintaining tissue growth and development (Yakar *et al.*, 1999).

5.14 Alignment of Sheep IGF-1 X Rabbit IGF-1 Gene:1-76791bp

The VISTA analysis of rabbit IGF-1 sequence shown on the x axis and percentage similarity to sheep IGF-1 on alignment axis with the percentage similarity scaling between 50 – 100% on the right of the plot. The graphical plot is based on the regions where rabbit IGF-1 gene and sheep IGF-1 gene have a common conservation, in which the least conserved region between rabbit and sheep IGF-1 gene is 86bp at 81.4 percent and the highest conserved region between is 1673bp at 84.8 percent with a total conserved regions of 40396bp at different regions with very little distance between all the conserved regions over the large size of the IGF-1 gene, which are also found in the non-coding regions which might also explain the differences in expression of the rabbit and sheep IGF-1 as the conserved regions are not found in the coding regions, which means the translations into protein will also be different. The very high similarities percentage in the conserved non-coding regions, might also explain the very high similarities in mode of function of the IGF-1 gene in rabbit and sheep species in growth process.

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 (Werner *et al.*, 1994).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Chicken, rabbit and sheep genes (GH, IGF-1 and Myostatin) sequences where downloaded from NCBI database, and were subjected to computational analysis using computational and on-line tools/programs to access similarities and differences between the three orthologs growth genes, that results to differences in growth rate to maturity and final body weight and size between the three species. The results shows that the main differences were in the sequence length of the GH and IGF-1 genes, as some of the regulatory elements sequences might have been deleted in chicken IGF-1 and rabbit GH genes which shorten the length of the genes and causes loss of some functional regions, which might be as a result of selection, mutation or evolution.

Even though, chicken GH gene is higher in size and resulted in faster growth rate in chicken specie than rabbit species, though both chicken and rabbit GH genes are found in the same input strand, which might explain their general faster growth rate than sheep species but rabbit has higher IGF-1 gene size that manifold the effect of rabbit GH and resulted in large final body weight of rabbit species. Sheep had higher size (length) of GH and IGF-1 gene than chicken and rabbit species which resulted to the large body size of sheep at birth and maturity but might have grown bigger with larger IGF-1 gene to manifold the effect of sheep GH.

The myostatin gene negative regulatory effect on growth seems to suppress growth through IGF-1 gene, as they share same pattern of conservation in sequences in rabbit and sheep species, while chicken has its own pattern of conservation of myostatin gene. With gene

editing technology and construction, some of the deleted sequences might be restored, to restore some short hormonal sequences and regulatory element and improve on the growth rate, final body weight and size of animals.

6.2 Conclusion

1. The number of GH gene of sheep (232) is higher in size than that of GH gene of chicken (201) and GH of rabbit (11) but the number of IGF-1 gene of rabbit and sheep are higher in size than that of chicken IGF-1 gene, while the number of rabbit myostatin (5) is higher than that of chicken (3) and sheep (3) Myostatin, and the number of genes (GH, IGF-1, MSTN) in a chicken, rabbit and sheep genomes plays a key role in establishing effective gene function.
2. The genes (GH, IGF-1, MSTN) shares some conserved regions but the length/size, gene number, exon number, exon type, number of gene/genome, gene DNA strand, codons/gene, gaps, number of chromosomes distributed and SNP varied greatly among chicken, rabbit and sheep species.
3. The gene similarities in conserved regions are for conserved functions while, the differences are for different expression pathways among the three species.
4. Variations in the genes (GH, IGF-1, MSTN) length/size and numbers mainly affect the growth and development of chicken, rabbit and sheep While, Myostatin gene depresses animal growth process through the action of IGF-1 gene.
5. Most of the mutations, deletions and insertions are in the upstream or downstream regions of the genes (GH, IGF-1, MSTN) occurred mostly in chicken and rabbit compared to sheep, which leads to differential gene regulation without actually changing the structure and functions of the protein.

6.3 Recommendations

1. Chicken and sheep growth could be improved through increasing the length/size and number of their IGF-1 gene by gene modification techniques. While, Rabbit growth could be improved through increasing the length/size and number of the GH gene by gene modification techniques.
2. Some missing nucleotides in rabbit and chicken GH and IGF-1 gene sequences could be modified by gene modification technology and use as a useful marker for economic traits.
3. It is recommended that use of the short sequences/regulatory regions of genes on body weight and size at maturity for Agricultural improvement could be more effective than whole transgene.
4. It is recommended that more studies could be conducted to knock-down or knock-out some of these extra length sequence regions of sheep GH and IGF-1 genes not found in rabbit and chicken of GH and IGF-1 gene, when aligned.
5. Studies could be done on the association between IGF-1 and Myostatin gene on growth process, and on the gene DNA strand.

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APPENDIX I

Rabbit BLAT Results

BLAT Search Results

Go back to chr9:11026346-11032288 on the Genome Browser.

Custom track name: blat Oryctolagus Custom track description:

blat on Oryctolagus

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browserdetails	Oryctolagus	4912	1	4912	4912	100.0%	7	-	130429151	130434062	4912
browserdetails	Oryctolagus	89	4595	4749	4912	78.8%	4	+	39334805	39334959	155
browserdetails	Oryctolagus	34	1788	1899	4912	91.9%	1	-	30348331	30348441	111
browserdetails	Oryctolagus	29	3082	3117	4912	91.2%	11	+	18924710	18924748	39
browserdetails	Oryctolagus	22	1489	1513	4912	95.9%	13	-	85405440	85405466	27

Missing a match?

APPENDIX II

BLAT Search Results

Chicken BLAT Results

Go back to chr7:195700-201192 on the Genome Browser.

Custom track name: blat Gallus (1) Custom track description:

blat on Gallus

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browserdetails	Gallus	40236	1	40248	48428	100.0%	1	+	55432530	55472776	40247
browserdetails	Gallus	627	8608	25633	48428	90.9%	1	-	55441136	55458161	17026
browserdetails	Gallus	346	8591	8997	48428	93.3%	4	+	45850431	45850838	408
browserdetails	Gallus	345	8591	8998	48428	92.6%	1	-	167329623	167330030	408
browserdetails	Gallus	345	8591	9000	48428	92.6%	1	-	57276329	57496113	219785
browserdetails	Gallus	344	8591	9000	48428	92.7%	1	-	2891990	2892406	417
browserdetails	Gallus	343	8591	9001	48428	92.6%	Z	-	23841505	23841916	412
browserdetails	Gallus	343	8591	9000	48428	92.2%	1	-	184738674	184843449	104776
browserdetails	Gallus	343	8591	9007	48428	90.8%	3	+	74910369	74910783	415
browserdetails	Gallus	343	8591	9000	48428	92.2%	1	+	55085626	55086035	410
browserdetails	Gallus	341	8591	9000	48428	91.7%	4	-	85582652	85583060	409
browserdetails	Gallus	341	8591	9000	48428	92.0%	2	-	48775006	48775415	410
browserdetails	Gallus	341	8595	9000	48428	92.4%	1	-	173604471	173604876	406
browserdetails	Gallus	341	8591	9009	48428	90.8%	1	-	81631149	81631564	416
browserdetails	Gallus	341	8591	9009	48428	90.5%	Un_AADN03011932	+	4377	4791	415
browserdetails	Gallus	340	8591	9000	48428	92.2%	1	+	176452080	176452489	410
browserdetails	Gallus	339	8591	9000	48428	91.1%	6	+	1091856	1092263	408
browserdetails	Gallus	339	8591	9000	48428	91.7%	2	+	144331574	144331983	410
browserdetails	Gallus	339	8591	9000	48428	91.9%	1	+	171778184	171778596	413
browserdetails	Gallus	338	8591	9027	48428	90.2%	4	+	12519115	12519762	648
browserdetails	Gallus	338	8591	8999	48428	91.7%	1	+	184820552	184820960	409
browserdetails	Gallus	337	8591	9000	48428	91.5%	4	-	50597685	50598094	410
browserdetails	Gallus	337	8591	9000	48428	91.5%	3	-	91559341	91559750	410
browserdetails	Gallus	337	8591	8999	48428	91.8%	3	-	86223830	86224237	408
browserdetails	Gallus	337	8591	9000	48428	91.5%	3	-	78572219	78572628	410
browserdetails	Gallus	337	8591	9001	48428	92.3%	4	+	28664415	28664826	412
browserdetails	Gallus	337	8591	9000	48428	92.1%	3	+	76004256	76004665	410
browserdetails	Gallus	337	8591	9000	48428	91.2%	2	+	147988519	147988927	409
browserdetails	Gallus	337	8591	9001	48428	91.7%	1	+	130658116	130658526	411
browserdetails	Gallus	337	8591	9000	48428	91.7%	1	+	13152545	13152955	411
browserdetails	Gallus	336	8591	9001	48428	92.2%	3	+	65731181	65731590	410
browserdetails	Gallus	336	8591	9010	48428	90.5%	2	+	99110607	99111023	417
browserdetails	Gallus	335	8591	9000	48428	91.3%	4	-	88895316	88895724	409
browserdetails	Gallus	335	8591	9000	48428	91.6%	1	-	170046819	170047228	410
browserdetails	Gallus	335	8591	9000	48428	91.2%	1	-	152297493	152297902	410
browserdetails	Gallus	335	8591	9000	48428	91.8%	1	-	129680837	129681247	411
browserdetails	Gallus	335	8591	9000	48428	91.2%	1	-	122277262	122277671	410
browserdetails	Gallus	335	8591	9000	48428	91.2%	1	-	111404669	111405078	410
browserdetails	Gallus	335	8591	9000	48428	91.5%	4	+	51655439	51655847	409
browserdetails	Gallus	335	8591	9000	48428	91.2%	2	+	138202847	138203256	410
browserdetails	Gallus	335	8591	9001	48428	91.8%	1	+	18827540	18827951	412
browserdetails	Gallus	334	8591	9000	48428	91.4%	3	-	43622372	43622781	410
browserdetails	Gallus	334	8591	8997	48428	91.8%	1	-	135024781	135404082	379302
browserdetails	Gallus	334	8591	9000	48428	91.4%	3	+	75762261	75762670	410
browserdetails	Gallus	334	8591	8996	48428	91.5%	1	+	169628110	169628514	405
browserdetails	Gallus	334	8596	9000	48428	91.6%	1	+	167223654	167224058	405
browserdetails	Gallus	334	8591	9000	48428	91.4%	1	+	24601425	24601834	410
browserdetails	Gallus	333	8591	9000	48428	90.7%	7	-	15702936	15703344	409
browserdetails	Gallus	333	8591	9006	48428	91.2%	3	-	75625040	75625457	418
browserdetails	Gallus	333	8591	9000	48428	91.4%	1	-	105294479	105294896	418
browserdetails	Gallus	333	8591	9000	48428	90.8%	1	-	54811884	54812292	409
browserdetails	Gallus	333	8591	9000	48428	90.7%	4	+	85727739	85728147	409
browserdetails	Gallus	333	8591	9001	48428	90.7%	4	+	44642587	44642996	410
browserdetails	Gallus	333	8595	9000	48428	91.1%	1	+	154530891	154531295	405
browserdetails	Gallus	333	8591	8997	48428	91.8%	1	+	65302288	65302694	407
browserdetails	Gallus	332	8591	9001	48428	93.1%	2	-	96196482	96196895	414
browserdetails	Gallus	332	8591	9000	48428	90.8%	1	-	121199800	121200208	409

APPENDIX III

GENSCAN Output Chicken GH

View gene model output: PS

<http://genes.mit.edu/07_20_17-10:09:47.ps> | PDF

<http://genes.mit.edu/07_20_17-10:09:47.pdf>

GENSCAN 1.0 Date run: 20-Jul-117 Time: 10:09:47

Sequence /tmp/07_20_17-10:09:47.fasta : 3525 bp : 54.20% C+G : Isochore 3 (51 - 57 C+G%)

Parameter matrix: HumanIso.smat

Predicted genes/exons:

Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..

----- ---- - ------ ------ ---- -- ----- ---- ----- ----- ------

1.01 Intr + 977 1137 161 0 2 89 88 116 0.736 11.92

1.02 Intr + 1580 1696 117 1 0 76 89 85 0.785 8.57

1.03 Intr + 1998 2159 162 2 0 93 73 165 0.989 16.19

1.04 Term + 2955 3428 474 2 0 50 54 588 0.255 46.87

1.05 PlyA + 3507 3512 6 1.05

Suboptimal exons with probability > 1.000

Exnum Type S .Begin ...End .Len Fr Ph B/Ac Do/T CodRg P.... Tscr..

----- ---- - ------ ------ ---- -- -- ---- ---- ----- ----- ------

NO EXONS FOUND AT GIVEN PROBABILITY CUTOFF

Predicted peptide sequence(s):

Predicted coding sequence(s):

>/tmp/07_20_17-10:09:47.fasta|GENSCAN_predicted_peptide_1|304_aa

XSWFSPLLIAVVTLGLPQEAAATFPAMPLSNLFANAVLRAQHLHLLAAETYKEFERTYIP

EDQRYTNKNSQAAFCYSETIPAPTGKDDAQQKSDMELLRFSLVLIQSWLTPVQYLSKVFT

NNLVFGTSDRVFEKLKDLEEGIQALMRLQLHVQHHFQILLSTDTSAGADNRYADHLHPFH

CCLLTPNTSEPRGVSSGCSSQLHDGQGWVRALPTRKLEQRQHHFASSPSLSRSPLVPQEL

EDRSPRGPQLLRPTYDKFDIHLRNEDALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGES

NCTI

APPENDIX IV

Chicken X Rabbit IGF-1 gene Alignment

chicken Gallus:1-48428 (+)

rabbit Oryctolagus:44-70732 (+)

Conservation Visible

000000001 GCTGTTTCCTGTCTACAGTGTCTGTGTAATGTAGATAAATGTGAGGATTTTCTCTAAATC 000000060

>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||||||| | |||||||||||||||| | ||||||<<<<<<<<<

000000044 GCTGTTTCCTGTCTACAGTGTCTGTGTTTTGTAGATAAATGTGAGGATTTTCTCTAAATC 000000103

000000061 CCTCTTCTGTTTGCTAAATCTCACTGTCACTGCTAAAATCAGAGCAGATAGAGCCTGCGC 000000120

>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||||||| | |||||||||||||||||||| | ||||||<<<<<<<<<

000000104 CCTCTTCTGTTTGCTAAATCTCACTGTCACTGCTAAATTCAGAGCAGATAGAGCCTGCGC 000000163

000000121 AATGGAATAAAGTCCTCAATATTGAAATGTGACATTGCTCTCAACATCTCACATCTCTCT 000000180

>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||||||| | |||||||||||||||| | | | | | ||||||<<<<<<<<<

000000164 AATGGAATAAAGTCCTCAAAATTGAAATGTGACATTGCTCTCAACATCTCCCATCTCTCT 000000223

000000181 GGATTTCTTTTT-TCTCATCATTACTGCTAACAAATTCATTTCCAGACTTTGCACTTTTA 000000239

>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||||||| | |||||||||||||||| | |||||<<<<<<<<<

000000224 GGATCTCTTTTTGCCTCATTATTCCTGCTAACCAATTCATTTCCAGACTGTGTACTTC-- 000000281

000000240 AGAAGCAATGGAAAAAATCAACAGTCTTTCAACACAATTAGTTAAGTGCTGCTTTTGTGA 000000299

>>>>>>>>> |||||||||||||||||||||||||||||||| | |||||||||||||||||||| | ||||||||||||| | ||||||<<<<<<<<<

000000282 AGAAGCAATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGCTGCTTTTGTGA 000000341

000000300 TTTCTTGAAGGTAAAT---CTTTATTACTATTTCAAATAATT-------TTTTAGTTTTA 000000349

>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||||||| | ||||||||||||||||<<<<<<<<<

000000342 TTTCTTGAAGGTAAATATTTTTTTTTTCTCTTTGAAGTCATTGGGGGAATTTTACTTAAA 000000401

000000350 TTTTACTGTGTGTTGTCTG-CTTCTGCTTTGAAGAG-----TATTGTGCACTTTTCAATA 000000403

>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||| | ||||||||||||||||||<<<<<<<<<

000000402 TT-----GTGTACTGTTTTTCTTCTGCCTAGAACTGATATTCACTTTACATTTTTCA--- 000000453

000000404 GCATTATTTTAGTATTGAGAATTATTTCTAAATTGCTGAACATGCAATTTTATGTGATCT
000000463

>>>>>>>>>|||||||||||||||||||||||||||||||||||||||||| | |||||||||||||||| | |||<<<<<<<<<

000000454 ---TTGTTTCGGAACTGGGAGTTATTTATAAATTGCTGAATATGCAGTTCTGTGGGATCT
000000510

APPENDIX V

BLASTN 2.2.30+

Reference: Zheng Zhang, Scott Schwartz, Lukas Wagner, and Webb

Miller (2000), "A greedy algorithm for aligning DNA sequences", J

Comput Biol 2000; 7(1-2):203-14.

Database: Gallus_gallus.Gallus_gallus-5.0.dna.toplevel.fa

23,475 sequences; 1,230,258,557 total letters

Query= Ovis aries breed Myostatin gene

Length=4991

Sequences producing significant alignments:	Score (Bits)	E Value
7 dna:chromosome chromosome:Gallus_gallus-5.0:7:1:36946936:1 REF	357	2e-95
3 dna:chromosome chromosome:Gallus_gallus-5.0:3:1:111302122:1 REF	46.1	0.082
1 dna:chromosome chromosome:Gallus_gallus-5.0:1:1:196202544:1 REF	46.1	0.082
10 dna:chromosome chromosome:Gallus_gallus-5.0:10:1:20435342:1 REF	44.1	0.32
6 dna:chromosome chromosome:Gallus_gallus-5.0:6:1:35467016:1 REF	44.1	0.32
5 dna:chromosome chromosome:Gallus_gallus-5.0:5:1:59825302:1 REF	44.1	0.32
AADN04017456.1 dna:scaffold scaffold:Gallus_gallus-5.0:AADN0401...	42.1	1.3
AADN04003070.1 dna:scaffold scaffold:Gallus_gallus-5.0:AADN0400...	42.1	1.3
AADN04007000.1 dna:scaffold scaffold:Gallus_gallus-5.0:AADN0400...	42.1	1.3
AADN04004803.1 dna:scaffold scaffold:Gallus_gallus-5.0:AADN0400...	42.1	1.3
Z dna:chromosome chromosome:Gallus_gallus-5.0:Z:1:82310166:1 REF	42.1	1.3
22 dna:chromosome chromosome:Gallus_gallus-5.0:22:1:4729743:1 REF	42.1	1.3
14 dna:chromosome chromosome:Gallus_gallus-5.0:14:1:15595052:1 REF	42.1	1.3
8 dna:chromosome chromosome:Gallus_gallus-5.0:8:1:29963013:1 REF	42.1	1.3
4 dna:chromosome chromosome:Gallus_gallus-5.0:4:1:91282656:1 REF	42.1	1.3
AADN04004771.1 dna:scaffold scaffold:Gallus_gallus-5.0:AADN0400...	40.1	5.0
AADN04002137.1 dna:scaffold scaffold:Gallus_gallus-5.0:AADN0400...	40.1	5.0
KQ759484.1 dna:scaffold scaffold:Gallus_gallus-5.0:KQ759484.1:1...	40.1	5.0
24 dna:chromosome chromosome:Gallus_gallus-5.0:24:1:6280547:1 REF	40.1	5.0

18 dna:chromosome chromosome:Gallus_gallus-5.0:18:1:11053727:1 REF 40.1 5.0

13 dna:chromosome chromosome:Gallus_gallus-5.0:13:1:18407460:1 REF 40.1 5.0

9 dna:chromosome chromosome:Gallus_gallus-5.0:9:1:24091566:1 REF 40.1 5.0

2 dna:chromosome chromosome:Gallus_gallus-5.0:2:1:149560735:1 REF 40.1 5.0

> 7 dna:chromosome chromosome:Gallus_gallus-5.0:7:1:36946936:1

REF

Length=36946936

Score = 357 bits (180), Expect = 2e-95

Identities = 339/392 (86%), Gaps = 0/392 (0%)

Strand=Plus/Plus

Query 2195 ATTCATTTATAGCTGATCTTCTAGCAGAAGTGCAAGAAAAACCCAAATGTTGCTTCTTTA 2254

Sbjct 220535 ATTCATTTATAGCTGATTTTCTTGTACAAATGGAGGGAAAACCAAAATGTTGCTTCTTTA 220594

Query 2255 AATTTAGCTCTAAGATACAACACAATAAAGTAGTAAAGGCCCAACTGTGGATATATCTGA 2314

Sbjct 220595 AGTTTAGCTCTAAAATACAATATAACAAAGTAGTAAAGGCACAATTATGGATATACTTGA 220654

Query 2315 GACCTGTCAAGACTCCTACAACAGTGTTTGTGCAAATCCTGAGACTCATCAAACCCATGA 2374

Sbjct 220655 GGCAAGTCCAAAAACCTACAACGGTGTTTGTGCAGATCCTGAGACTCATTAAGCCCATGA 220714