MYOSTATIN GENE SEQUENCE AND ITS MORPHOMETRIC IMPLICATIONS IN HELMETED GUINEA FOWL BREEDS

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DECLARATION

I, Damilare Olaniyi ARIJE, an **M.Sc**. student in the **Department of Animal Production** (**Breeding and Genetics**), Landmark University, Omu-Aran, hereby declare that this dissertation entitled "**Myostatin Gene Sequences and its morphometric implications in Helmeted Guinea Fowls Breeds**", submitted by me is based on my original work. Any material(s) obtained from other sources or work done by any other persons or institutions have been duly acknowledged.

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Student's Full Name and Matriculation Number

Signature and Date

CERTIFICATION

This is to certify that this dissertation has been read and approved as meeting the requirements of the Department of Animal Science, Landmark University, Omu-Aran, Nigeria, for the Award of Master in Science (M.Sc.) Animal Production (Animal Breeding and Genetics).

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ABSTRACT

Various methods and tools have been utilized to establish genetic variability in poultry production which remains a platform for genetic improvement of poultry species. Although, myostatin gene sequences of some poultry species have been fully sequenced on various exons, that of guinea fowl is yet to gain the needed attention making the guinea fowl database incomplete. The genetic enhancement of guinea fowls would increase the production potentials of the species as well as providing data on their genetic differences, which is critical for strategic breeding plans and genetic improvement. The study aimed to explore the polymorphisms of the gene encoding for myostatin in helmeted guinea fowl using Single Nucleotide Polymorphisms.

Ten matured guinea fowls were randomly selected for DNA assay. A 640bp fragment of myostatin gene was amplified using genomic DNA extracted from the blood. Production traits of sixty birds of three plumages were measured using standard procedures. Blood samples were taken by wing venepuncture and the samples were subjected to DNA amplification and electrophoresis to determine genetic polymorphisms of the myostatin gene on several sites. Morphometric data were analyzed using descriptive statistics and one-way ANOVA.

Body weight ranged from 1.35 to 2.67kg while head lengths were 4.00cm, 4.80cm, and 5.00cm for white, pearl, and exotic guinea fowls, respectively. Morphologically, exotic guinea fowl was superior in all production traits than their counterparts except for neck length. Averagely, pearl guinea fowls showed better performance than white guinea fowls. Nucleotide diversity among the guinea fowl sequence was 0.02887, which is quite low. This suggests that guinea fowl sequences are quite closely related, due to indiscriminate interbreeding among domestic guinea fowl that reduced nucleotide diversity.

The study, therefore, recommends sequencing of the whole genome of helmeted guinea fowl, which is yet to be established. This will allow the improvement of the birds and the genetic database.

Keywords: Myostatin, Polymorphism, Sequence, Morphometric, Helmeted Guinea Fowl.

Word Count: 303

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Farming families in numerous countries heavily depend on poultry, especially chicken, for food and money (MOLD 2010.; Aswani *et al*., 2017). There are several different ways in which chickens (poultry) have helped humanity, from producing food and many other materials because it serves as a regenerative resource of which food by far provides the most significant input. The poultry meat production includes pheasant, pigeons, guinea fowl, chickens, ducks, ostrich, turkeys, emu, geese, etc., some also give eggs, such as ducks, guinea fowl and chickens, and others provide manure (chickens, ducks, guinea fowl, pigeons, ostrich, emu, pheasant, etc. Poultry is a superior supplier of protein than calories when it comes to meat, eggs, and feathers.

It is also helpful for young people, the aged, and pregnant women to get protein from meat and eggs, as they are economical sources of nutrition, which also combats malnutrition in Africa countries like Kenya and Nigeria (Martin *et al.,* 2012). A large number of people desired to consume native poultry, quail, and guinea fowl meat, due to their exceptional taste and excellent environmental benefits. They're also chosen because they have desirable characteristics including heat tolerance, disease resistance, and low input needs (Ogada *et al.,* 2016). In arid and semi-arid climates, Moraa *et al.,* (2015) discovered that indigenous chickens have phenotypic adaptations for coping with heat stress, while Panyako *et al.,* (2016) hypothesized that guinea fowl have adapted to their local climatic conditions due to their wide distribution. According to Ommeh *et al.,* (2010), an *in vitro* avian influenza susceptibility might be created through a Single Nucleotide Polymorphism (SNP) in the myostatin (MSTN) gene in indigenous chickens, resulting in these poultry-tolerant strains for rural farmers.

Chickens are ubiquitous proteinaceous food, commonly eaten in huge quantities. Since poultry products can increase the levels of ω−3 fatty acids in the diet, they can serve as a transporter for delivering those critical nutrients to the human system (Laudadio and Tufarelli, 2011). An increase in the amount of genetically modified poultry meat, diet and breeding methods have been previously reported to alter the flavour (integrity) of poultry

products (Aletor *et al*., 2003; *Rymer et al*., 2010; Nasr and Kheiri, 2011). The additions of anticarcinogenic chemicals to poultry products are expected to yield additional health benefits, including lower cholesterol and decreased overweight (obesity) (Crespo and Esteve-Garcia, 2001; Aletor *et al*., 2003).

The poultry industry has a big impact on Nigeria's economy, as well as the international market. Initially, it started as a small-scale agricultural industry, it has transformed into a highly capitalistic, capital-intensive commercial enterprise, contributing significantly to Nation's economy's three main sectors (petroleum, mining, and agriculture). Nigeria chicken population, which was reported to be 114.3 million, and other poultry, including ducks, guinea fowls, turkeys, and pigeons, totalled about 31.9 million. While commercial chickens constituted about 11% of this total, the rest were not commercially raised (RIM, 1992). When poultry is compared to farming other domestic animals, it is found that they provide cheap methods of production and this could be due to an increase in their productivity, good feed change proficiency, increased prolificacy, deducibility, development rate, egg production, and quality of meat through hereditary qualities and improved within an ample period and with the low capital venture. Meat and egg-producing poultry are vital sources of nutritious amino acids. A little over 30% of the meat consumed in Nigeria is provided by poultry (guineas, chickens, etc.) which is more than four times higher than it was just a few decades ago (FAO, 2009). For instance, this is due to a recent increase in public knowledge of the benefits of dietary protein consumption for urban households (Nigerian average) as well as the fact that hereditary factors, such as egg production, hen-day egg output, and egg weight quality features of indigenous and exotic guinea fowl, thereby improving meat and egg quality traits while taking into account the economic need to increase edible guinea fowl production. The ease of finding and maintaining adequate animal food supply depends upon consumers' tolerance of and preferences for meat and eggs (traits). Schedule hereditary and breeding experimentation ought to be carried out ceaselessly via hereditary measures for some guinea fowl characteristics, especially to make strides of commercial breeds, to choose the most excellent performance with regards to significant characteristics by centralizing and improving traits in the birds. The upgraded stock would be saved and expanded for use in production. Improvements in Nigerian poultry and animal production can be made

with the addition of commercial guinea fowl, which can be used as a hedge against poultry commodity shortages.

As a result, the overall aim of this project is to genetically sequence the muscle fibre of the Nigerian guinea fowl to increase its economic potential.

Indigenous guinea fowl is Nigeria's second-largest and most numerous sources of dietary protein following domestic fowl, as reported by Ayorinde, (2004). A large number of undomesticated guinea fowl are domicile in Nigeria's wood-grassland where they are well adapted. Despite these noticeable populations of guineas, key numbers of poultry farmers concentrated on exotic chickens that have been bred with time, forgetting our native poultry species, which has received minimal or no investigation (experimentation) and has remained largely unenhanced. Ikani and Dafwang, (2004), ascertain more than 50 million estimated semi-domesticated (docile) guineas (N. Meleagris) in the country, which amount to close to 25 per cent of the total domesticated poultry species in head count.

The farming of guinea fowl is rampant and domicile in the northern part of the country (Nigeria) as a backyard domestic production unit, where they are adapted to a low practice (production) and small output production which is a similar approach used in the production of northern Vietnam's Hmong chicken (Vo Van Su *et al.,* 2001). Guineas grow meat and eggs, and their meat is said to be tastier than that of other birds. Guinea fowl (Numida meleagris) have been of interest to geneticists since the early 1980s in New Bussa, Kanji Lake Research Institute, in Niger State, as documented in the literature. The bird got quite a lot of attention, in particular on diet and performance variables, but was ultimately abandoned. An interesting finding to notice is that while chickens and pigeons have attracted considerable attention, the hardy bird has been neglected by the African Network for Rural Poultry Development (ANRDP). Out of the resources ANRDP produces, including newsletters, journals, and books, only a handful discuss the hardy bird. For the animal to be of practical use in large-scale development, its performance attributes must be upgraded. Access to genetic variations and successful methods of exploiting these variations are needed for improvement programs. These differences can be measured on three levels: morphological, protein, and nucleotide, all of which are useful for detecting genetic relationships between breeds of domestic animals (Salako

and Ngere 2002; Zaitoun *et al*., 2005 and Luc *et a*l., 2007). While it may be possible to measure certain morphological, protein, and nucleotide differences among various breeds of domestic animals, the first two levels do not distinguish one species from another (Salako and Ngere 2002; Zaitoun *et al*., 2005 and Luc *et al*., 2007). As to the variations in colour seen in the varieties of indigenous guinea fowl, the black, lavender, pearl, and white feathered groups have a richly varied colour palette, Ayorinde, (1987). Despite this, the various four-colour varieties of guinea fowl appear to represent different genetic subpopulations. It is essential to first identify the genetic diversity before pursuing genetic enhancement. There is little doubt that it is viable to run a Guinea fowl meat processing plant in various locations (Nahashon *et al*., 2006). After the discovery that guinea fowl meat could replace chicken in many European countries, meat from guinea fowl became a successful operation in Canada, the United States, and other European markets such as France, Belgium, and Italy (Nahashon *et al*., 2005; Tufarelli *et al*., 2007). For countries to make informed decisions about the potential management of their animal genetic capital, they need comprehensive information. This needs to be done because local breeds specific to certain geographic regions are getting lost in the mix in favour of exotic breeds, which tend to have higher output. The urgent need for quick financial gain has taken precedence over preserving the greater long-term wealth of genetic resources. National Action Plans (NAPs) have already been formed in some nations, such as Ghana, whereas others have allocated additional funds for managing genetic resources. It is best to approach the issue of animal genetic resources depletion from the perspective of an organized and cohesive effort rather than individually trying to reverse the problem to enable researchers and farmers to get used to production changes.

Guinea fowls are significant in the Nigerian animal genetic resources because of their abundance in the wild which provides access to a researcher to investigate its genetic makeup. Guinea fowls are an important source of animal genetic resources in Nigeria. Guinea fowls are undomesticated animal species that are found in dryland (arid rural areas) of some countries in Africa, and their numbers have increased dramatically in recent years as organic agriculture has improved (Yildirim, 2012). As guinea fowl rearing has been more widely established, the total amount of poultry has also expanded since these birds may be successfully raised in semi-intensive circumstances, such as farms or

villages, with less effort. A physiological and sexual mature guinea can reproduce, can survive in a range of situations, and has impressive resistance to mostly other poultry diseases (Boko, 2004). Embury (2001) and Champagne (2003) ascertain that some countries in Europe: Belgium, Italy, and France have genetically improved strains of guineas that may lay a maximumly of 190 eggs each year (Mulal, 2001).

Chicken growth is known to be regulated by several genes. Some genes enhance muscle growth, while others, including myostatin (MSTN), are inhibitory to muscle growth, especially before and after hatching (Sato *et al.,* 2006). Myostatin is part of the transforming growth factor (TGF) family and is expressed mainly in muscle tissues, where it contributes to the development and tissue homeostasis in both adults and embryos (Lee and McPherron, 2001; Hickford *et al*., 2010). Practically, deficiency of myostatin consequently leads to muscle fibre development, demonstrating the presence of an effective instrument to control muscle fibre in healthy organisms (McPherron *et al.*, 1997). MSTN's arrangement (sequence) and result (function) appear to be profoundly moderate across animals (vertebrate species), and its part amidst chicken formation of muscles tissues during myogenesis was anticipated to be comparable to that researched in other warm-blooded animals (mammals) (Scheuermann et al., 2004). Myo-D gene expression is impacted by myostatin influence on Pax-3, and Pax-3 inhibition is connected to myogenic process inhibition in chickens at the developmental phases (Amthor *et al.,* 2002). McPherron *et al.,* (1997) reported that mice missing the myostatin gene had around double the bone and muscle of mice that were not undomesticated, and this was attributable to muscle fibre hyperplasia and hypertrophy.

Myostatin is a protein synthesized via skeletal fibre, which serves as a limiter to the constant growth of muscle fibres (Lee and McPherron, 2001). The role of myostatin and other muscle inhibitors may present an explanation as to how muscle sizes of different parts of the body are regulated and controlled (Lee, 2004), however, myostatin is considered to be the most powerful of muscle inhibitor proteins characterized so far (Amthor *et al.*, 2004). There is a paucity of details available on myostatin characterization in helmeted guinea fowls, and even less that focuses on Helmeted guinea fowls, hence the design of this study is a timely one.

In cattle with a mutant MSTN gene, muscle mass increased substantially, along with huge swelling (surge) in the muscles (Grobet *et al*., 1997; McPherron and Lee, 1997). The mutation led to several notable growths in the human population (Schuelke *et al.,* 2004). Further research indicates that MSTN is more frequently detected in persons with critical conditions, such as those with HIV or AIDS, as well as patients who suffer from rapid muscular weakening (dystrophy) (Ivey *et al.*, 2000; Reardon *et al.*, 2001). With these facts in mind, the study's objectives were to identify the effects of myostatin genes on the growth traits of indigenous and exotic guinea fowl.

Growth characteristics are important economic characteristics in animal production. Even though manual preference (selection) for phenotypic features of poultry species has resulted in consequential advancement in rates of growth and meat production over the last 50 years, poultry breeders and experts face new demands presently due to counterbalance relationship (unbalanced correlation) between raising the birds (production) and vigour features (traits). As a result of the advancement of genomic instruments and biotechnology, it is now possible to recognize and interpret genes of interest that is related to economic traits.

1.2 Statement of the Problem

Despite the abundance of poultry species in Nigeria, most poultry may not be able to withstand tough weather circumstances, helmeted guinea fowls are unique because they are resistant to most poultry diseases and can feed themselves by scavenging for grains and insects (Ikani and Dafwang, 2014). Because of its thick shell, its eggs have a longer shelf life. With the above positive aspects of guinea fowl, production, management, production and husbandry of the birds are still limited to rural Savannah zone rural communities where a lack of suitable housing, nutrition, management, and egg production, and strong predation rates restrict the potentials of the animals (Moreki, 2009; Kebede *et al.*, 2012). Due to the lack of attention given to the helmeted guinea fowls in Nigeria, a knowledge vacuum has emerged that needs urgent attention to bridge the gap for the better performance of the great bird. It appears that there is a lot of information that is still unknown about their adaptability, nutritional requirements, and body size and egg-producing capability. Because of this dearth of information, their conservation, improvement, and commercialization have all been significantly affected. The United Nations (UN), (2015). projected that Nigeria's population would exceed 400 million inhabitants by 2050. This is predicted to quadruple the need for animal protein in this population. In addition, climate change, soil problems, and water issues are all projected to be difficult for livestock producers (UN, 2015). Unless proper plans are put in place in terms of profiling and development of more resilient breeds the aforementioned poverty indicators will create more poverty and malnutrition. This piece of work will seek among others to provide a solution to the following questions:

1. Will there be variance in the genome of Helmeted guinea fowls due to myostatin genes?
2. Will there be any morphometric variations between the indigenous and exotic breeds of guinea fowls?

1.3 Justification of the study

Poultry by-products, such as chicken meat and eggs, are in greater demand in Nigeria because of an increase in demand for animal protein. However, the poultry industry is plagued by several other issues which include: Poor nutrition, a lack of technical competence, the complexities of weather conditions, poor growth, minimal meat production, a small number and size of eggs, and ample cost-effective, and rapid death rate are among them. Genetics research initiatives, as well as increased funding for molecular research, are critical to ensure the long-term growth and productivity of poultry. Livestock species such as guinea fowls, which are considered to be harder and more disease-tolerant, while having good meat taste, should be promoted to assist in their sustainability. It is therefore of necessity to have a complete and comprehensive understanding of guinea fowl production qualities and molecular genetic traits to successfully increase their output and productivity. There is a need to utilize bioinformatics assay as a tool to study the utilization of various growth-related genes, such as myostatin, to better enable the genetic optimization and improved output of this noble bird (guinea fowls).

1.4 Aim of the research

This research's general goal is to examine if there are differences in myostatin gene sequence in helmeted guinea fowl breeds.

Specific objectives are:

- To determine the various occurrence of polymorphisms of the myostatin gene in exon 1.
- To analyse the different morphometric traits between local and exotic breeds of guinea fowls.
- To determine the implications of sex in morphometric traits of guinea fowl’s breeds.

1.5 Significance of the study

The current research will encourage researchers to fully understand the whole genome of guinea fowl and help to know how to improve this species through genetic tools and methods. It will also encourage poultry farmers to massively go into the production of these birds to reduce over-reliance on chicken meats and products which in turn provides a source of livelihood to the farmers.

Immediate beneficiaries, however, also include the policy and decision-makers, planners and managers in the State and Federal Department of Poultry production, who are policy makers on sustainable poultry management and development, which will help project the research on a large scale.

CHAPTER TWO

2.0 Literature Review

2.1 Origin and Domesticated of Guinea Fowl

Originated in Africa, guinea fowl (*Numidia meleagris*) is rather common in the woods where they roam freely and scavenge for feed (Gracey *et al*., 1999; Saina, 2005). It was emphasized that guinea fowl emerged from an Asian francolin-like progenitor species, but only in Africa did the species evolve and radiate (Ayorinde, 2004). The domestic guinea fowl has its origin in the African helmeted guinea fowl, according to Embury, (2001). While he was making his proposal, Delacour (1977) emphasized that only the Guinea fowl (Numididae) of the world's five Galliformes is native to Africa. Crowe (1978b), proposed that helmeted guinea fowl, which he believed to be a sub-Saharan species, is found in Ethiopia, Senegal, and southern Africa. According to Church and Taylor (1992), the birds are found all over the world, although there has been a drop in populations in certain areas, such as habitat destruction and poaching for their meat. Domestication of guinea fowl was traced back to around three millennia (3000 years) ago in South European countries, as it is for ancient Romans and Greeks who preferred the flavour of its meat; domestic guinea fowl came from the wild guinea fowl (*Numida meleagris*), which notwithstanding occupies the grassland geography of Madagascar and South Africa, as reported by Świreczewska *et al.*, (1999). Sequel to all the facts and findings given above and comes from the classification of the species, which groups the birds according to their geographic locations. Given that "Guinea" is a country on the western coast of Africa, it would be impertinent to conclude that the species originated in Africa. The guineas earned their name since they were initially discovered on the West African shore, which was also known as Guinea (Roy and Wibberley, 1979; Anthony, 1990; Payne, 1990; Annor *et al*., 2012). Guinea fowls are tame and may usually live alongside other domesticated fowls like chickens and geese. The families Numididae and Galliformes contain the guinea fowl as members (Payne, 1990; Annor *et al*., 2012). The groups of guinea fowl are listed from the most ancient to the recent in taxonomic order in Table 2.1

In a deep and tapering nest, guinea could lay 25–30 eggs. The eggs laid have oval, darkened, as well have a very hard shell. The hens always demonstrate a character of masking (hiding) and sharing their nest with hens of the same order till they have a vast number of laid eggs. After 26–28 days of incubation, young hatched chicks are named "keets." Keets are prone to wetness (they are endemic to Africa's arid environments) and can die when following their mother through moist grass. They can, however, be among the most difficult domestic land fowl after the first two to six weeks of growing.

Table 2.1 Taxonomic Order of Guinea Fowl (*N. meleagris*) Species

Genus	Species	Description
Agelastes	Agelastes meleagrides Agelastes niger	White breasted guinea fowl Black breasted guinea fowl
Numida	Numida meleagris	Helmeted guinea fowl
Guttera	Guttera plumefera Guttera pucherani	Plumed guinea fowl Crested guinea fowl
Acryillium	Acryillium vulturinum	Vulturine guinea fowl

Source: Annor *et al*, (2012)

2.2 Description of Guinea Fowls Breeds in Nigeria

There are numerous poultry species present in Nigeria, which include chickens, guinea fowls, turkeys, ducks, and pigeons. Africa has been where Guinea fowl (Numida meleagris) have been found for thousands of years, and, as a result, has had a notable impact on world agriculture, which is how it made its way to such countries as France, Canada, Belgium, and Australia, where the birds are utilized for a wide range of purposes (Robinson, 2000; Embury, 2000). In nations like Nigeria, Zimbabwe, and Malawi, guinea fowl production is still in its infancy (Dondofema, 2000; Ligomela, 2000; Smith, 2000). Guinea fowl, which can be found in Nigeria, include the species Numida ptilorhycha and Numida meleagris. The Nigerian variety, N. ptilorhycha, is endemic to the southern section of the country where the deciduous (trees and shrubs) tropics are found, but the northern variety, N. meleagris, is being adopted into peasant farming communities (Ayorinde, 1987). It is known in Africa as a “poor man's pheasant,” because it is farmed by subsistence farmers (Smith, 2000.; Bonds, 1997). There are farmers that maintain guinea fowls around their farms as “security animals” since they have sharp eyesight, a

strident call, and raise a terrible howl at any stress as observed by Smith, (2000). Additionally, guinea fowl are kept to produce income and to eliminate mice, ticks, snakes, and other pests, all of which serve as biological control of such pests. Farming has spurred the rise of guinea fowl traders, many of whom deal with guinea fowl breeding and consumption throughout the year.

2.3 Morphology of Guinea Fowls

The bird has tough leg bones and a hard bony crown (Sibley and Monroe, 1990). Oval shaped and inclined relative to the ground, the trunk is found in the middle of the ovoid figure, the crowns of rectrices are lowered and almost touch the ground (hump-backed posture). An adult bird's head is around 9 cm long and its hairless. The bird's skin is blue and its helmet is brown: its structure varies depending on the sex of the bird. While in other species, such as certain types of birds, sex-specific changes in feather shape, colour, and size are due to gonadal hormones, the guinea fowl does not exhibit sexual dimorphism in plumage (Ayorinde, 2004). It is quite little and has a crimson wattle. The bird has short, dark brown or greyish legs, and does not have spurs. The tibia, which is about 12 cm long, and the shank, which is 8 cm long, make up the lower leg. The average body length is approximately 55–61 cm, and the wingspan is 74–76 cm (Ayorinde, 2004). The plumage of Guinea fowl is dense and thick due to the numerous colours that it has. The bird's rounded wings make long flights uncomfortable for it. When threatened, the helmeted Guinea fowl (*Numida meleagris*) will sometimes fly into trees. In addition to their naked heads, the helmeted Guinea fowl is distinguished by its distinctive "helmet" which is built above the noggin. Males and females differ only a little in their helmet size; otherwise, there is no sexual dimorphism (Crowe, 1978a).

2.4 Breeding Season

Quite a number of wild guinea fowl birds reproduced over a certain period of the season, regardless of the altitude of location as reported by Sharp, (1998). The cyclical or repeated accessibility of the suitable feed resources needed for nourishment and sustaining the keets usually cause bottlenecks thereby, imposing the restriction of its production (Murton and Westwood, 1977). The guinea hen (female) of subtropical helmeted guinea fowls is monogamous and breeds seasonally (Oke *et al.,* 2003.;

Maganga and Haule, 1994; Nwagu and Alawa, 1995). Due to the seasonality in reproduction, guinea fowl farming is not likely to be a big economic activity. Although the reasons for seasonality remain unknown, it appears that it is the result of several different variables. (Oke *et al.,* 2003).

2.5 Economic Importance of Guinea Fowls

Guinea meat is inexpensive and nutritious meat that low-income individuals can include in their meals because guinea meat is often considered a cheaper alternative to other meats. The main attraction of this notable bird is that its flavour which is of the quality as other poultry meats, as well as its moderate saturated fat content, and can be complemented with some vital nutrients, which make its meat consumed globally. Guinea fowls live for an average of 15 years. Guinea fowls are sturdy and cheaper to raise than chickens, which is why they are less vulnerable to poultry diseases (salmonellosis, Newcastle), Ayorinde, et al., (2004). Guineas make a lot of noise, which most farmers can employ to keep the farm safe. They are also used to get rid of fleas, ticks, snakes, termites, and rodents on the farm because they serve as biological control of the above organisms. As a result of their ability to withstand high temperatures, they are appropriate for transportation at any time of the day, unlike chicken that requires early or late transportation. Their sociable nature and preference for flocking means that if one goes missing, the flock will come together to find them. The fact that guinea fowls are resistant to the preponderance of poultry diseases makes them an excellent choice for resource-constrained farmers with access to veterinary services. House building is few, and health-care procedures heavily rely on ethno-veterinarian medicine. There has been minimal genetic advancement in the subsector throughout key markets in Africa, where birds are still predominantly raised as free-roaming scavengers.

Some of the economic advantages of guinea fowl are reported as follows:

2.5.1 Meat Production

The meat is low in calories, high in vital fatty acids, and rich in vitamins, with a breast meat yield of roughly 25 per cent of live weight and an outstanding flesh-to-bone ratio. The meat is exceptionally lean. Its meat is, on the other hand, a deeper red than that of a

chicken. Younger guinea fowl have soft and luscious meat, similar to other meat types, guinea fowl meat also gets tougher as it gets older.

2.5.2 Egg Production

It has been observed that it is possible for guinea fowl to produce as many as 100 eggs each year if they are carefully cared for and maintained in semi-intensive management. Their strong shells mean that the eggs are less likely to shatter when being handled than chicken egg shells are. The eggs are brown in colour and speckled, and they have a deep flavour, as well as their delicacy taste. In accordance with http://www.fourseasonsfam.com, Guinea eggs had a number of important amino acids, particularly high in fat and dietary cholesterol, and contained Omega-3 fatty acids as well as B vitamins, cholin, and minerals.

2.5.3 Farming System

The type of agricultural system to choose will be largely determined by what you plan to produce (eggs or meat). Most farmers practise extensive farming or free-ranging, in which the guineas are left to their own devices and are raised primarily for meat production. However, intensive farming is preferable for egg production since it is more secure. You may regulate the guineas' mobility and gather their eggs while keeping them in cages. Guineas have a reputation for being fiercely protective of their eggs, and they'll hide in the woods, tall grass, or anywhere else they can get away from predators.

2.6 Phenotypic Variation

The phenotypic characteristics of organisms are the features that can be either be quantitative or non-quantitative. Subjective qualities often referred to as non-metric traits, are measured in a visual assessment while objective traits are measured using measurable metrics (such as height, length, and width). The results of the genetics, environment and other elements interacting together to express the phenotype is known as phenotypic expression. In an experiment, carried out by Ken Meghen *et al.*, (1994), it was found that many phenotypic characteristics are commonly used to identify animals, including coat colour, horns (form and size), hair, weight, and other body parameters.

Breed characterization consists of certain factors that are seen more important, including:

- Constituent identification: Country, species, variety, variety group, and geographic place where the variety is found.
- number of residents, herd size, and the average age of animals being used for breeding by sex for the year being reported
- The observable physical qualities, such as coat colour, horns, and hair.
- All adult size and weight measurements, as well as various body measurements
- Practical applications.
- The dominating system has a quantitative description of itself (nomadic, transhumance housing etc.).
- Production characteristics

Although a significant number of farmers in the sector are illiterate, an extensive investigation into the measurements of domestic animals in the tropics is vitally important, as a large number of farmers depend on these animals for their livelihood. Weighting scales and other equipment are not available; thus, they do not perform the necessary exercise on these tools. Even when scales and other equipment are present, they lack the propensity to use them (Gerald, 1994; Sharples and Dumelow, 1988). Rather than assess several factors at different periods in time, they rely on visual assessments of the variables to predict various phenomena such as sales, medication, feeding, and breeding.

The collection of data regarding the phenotypes of breeds, as well as knowledge about how these phenotypes change during development, aids in steering a breeding effort. Animals that display high diversity in phenotypic features are utilized to distinguish between species. Even within the same species, though, genetic variation may be limited. Specific breeds have been developed because animals have been domesticated, which has led to increase within-species diversity. Animals are housed in a range of environments from temperate to tropical, where they will express a wide range of features for various purposes.

2.7 Myostatin (MSTN)

Myostatin (referred to be Growth Differentiation Factor 8, or called GDF-8) is a proteoglycan peptide (myokine), a protein secreted and produced by myocytes to hinder

myogenesis (cell growth muscle and disparity) via autocrine signalling. MSTN gene-encoded structure is also found in humans as it was reported by Gonzalez-Cadavid *et al.*, (1998); Carnac G *et al.,* (2006); Joulia-Ekaza D *et al.,* (2007). Myostatin, as a factor that facilitates growth is from the transforming growth factor (TGF) superfamily, is a specific inhibitor of muscle tissue growth. In mice, for example, knocking out the MSTN gene resulted in several folds that increase the fibre mass (McPherron *et al.,* 1997). A nonproductive alteration in the growth differentiation factor (myostatin) gene was reported in double-muscled cattle, which have more muscle mass than cattle with normal genes (Grobet *et al.*,1997; McPherron and Lee, 1997; Kambadur *et al.,* 1997). In addition to inhibiting muscle cell hyperplasia, myostatin seems to be responsible for the inhibition of both hypertrophy and hyperplasia. The introduction of either recombinant or overexpressed myostatin is available in skeletal muscle cell cultures interfered with the growth of myoblast (Thomas *et al.,* 2000; Taylor *et al.,* 2001). Because inactivated myostatin that inhibits the growth of myoblasts in cattle and small vertebrate (mice) show hyperplasia of muscle fibres. The protein myostatin appears to stop myoblasts from moving through the cellular cycle, from G1 to the S phase, which is regulated by Rb protein phosphorylation (Thomas *et al.,* 2000). In the same cell culture, p21 expression was increased whilst cyclin-dependent kinase (Cdk2) expression was suppressed (Thomas *et al.,* 2000). C2C12 cultured myostatin-overexpressing muscle cells also increased p21 (Rios *et al.*, 2001).

Myostatin-or myostatin-inhibiting substance-deprived animals display much higher muscle hypertrophy. The myostatin gene has been found to be altered on both copies result in individuals having a much greater amount of muscle mass and strength. Muscular dystrophy, which now has no cure, may one day be treated using research on myostatin, which may result in an effective medication, Tsuchida K, (2008).

It is commonly thought that alteration in MSTN is linked to the double-muscled phenotype found in so many different species, including the Belgian Blue and Piedmontese cattle, sheep and dogs, breeds. McPherron, and Lee, (1997); Clop, and Marcq *et al,* (2006).; Mosher, *et al.* (2007).; and Shelton, (2007), indicating that MSTN function is evolutionarily conserved among above-listed animals. Schuelke *et al*., (2004)

ascertain the discovery of a human kid with a mutation in the MSTN gene and enhanced musculature, which has fueled interest in the gene's research in recent years.

The tissue's ability to maintain homeostasis, which includes and consists of skeletal muscle development and growth, is regulated after birth by MSTN, as well as throughout skeletal muscle creation. Indeed, people suffering from muscle wasting processes including cachexia, muscular dystrophies, and other muscle illnesses may have increased levels of MSTN protein in their blood or muscle tissue (Jespersen *et al.*,2006; Joulia-Ekaza *et al*.,2007; Wójcik *et al.,* 2005). Importantly, certain antibodies that inhibit MSTN promote muscle hypertrophy in dystrophic animals, making it a key target for the treatment of muscle wasting diseases (Bogdanovich *et al.,* 2002). Additional methods to stop MSTN protein activity in vivo were tested, and they all failed. To stop myostatin protein function in vivo, researchers used a variety of strategies. The body mass and skeletal muscle mass has both been exhibited to ameliorate in response to a DNA vaccination, with no observable differences in blood biochemistry or side effects (Tang, 2007). MSTN protein function was also inhibited using an antibody that neutralizes and have a dominant-negative receptor (Takata H., 2007). As a result, many technological tools exist to control MSTN activity, both for the treatment of human health and to advance livestock development.

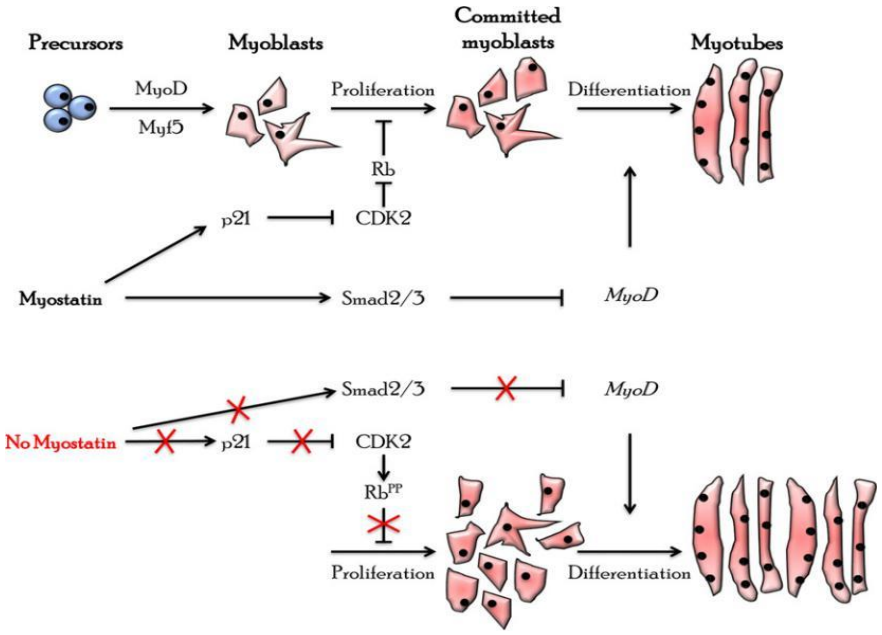


Figure 1: Action of myostatin during myoblast proliferation and differentiation (modified from Langley et al,.2002). Cell division is inhibited by retinoblastoma protein (Rb) in a low phosphorylated state. Rb activity is reduced as a result of hyper-phosphorylation caused by CKD2 kinase activation. CDK2 activity, on the other hand, is blocked by p21, which is activated by MSTN. MSTN also promotes Smad2/3 effectors, which suppresses MyoD expression, which is required for normal myoblast development. In the absence of MSTN, CDK2 activity is not inhibited, allowing it to inactivate Rb, resulting in enhanced myoblast proliferation. Simultaneously, MyoD expression is no longer regulated by Smad2/3 signaling pathways, allowing it to promote differentiation of extra numerary myoblasts development.

2.8 Effects of Myostatin in Different Species of Animals

2. 8.1 Human Myostatin (MSTN) Gene Promoter

A 3.3-kb portion of the 5′ regulatory site was the initial step in investigating how to modulate MSTN promoter function in humans (Ma *et al.,* 2001). In this region, there are numerous places where both overall and muscle-specific signalling pathways could bind. TATA and CAAT boxes are among the most relevant, and the 12 E-boxes are all part of a group of regulatory factors that are comprised of, MRF4, MYF5, MYOD and MYOGENIN proteins, all of which play a role in skeletal myogenesis (Zammit, 2017). To add to this, MYOD, which binds to the promoter region, was demonstrated to become an upstream regulatory of the myostatin gene (Spiller *et al.*,2002). Additionally, both

MEF2 sites are extremely important for myogenic differentiation (Taylor and Hughes, 2017).

Essential regulatory elements were reported in the 5′ regulatory region of the human, which includes a 327 bp proximal segment of sequence that features characters for basal promoter activity and responsiveness to glucocorticoids, and this sequence results in a measurable increase in MSTN, which was shown to have a dose-dependent effect on promoter action (Ma *et al.,* 2001). Affecting the control of myostatin gene expression in diverse settings, this promoter upstream of this proximal promoter has the following possible elements: probable bases 3322–2062 and 1447–1187) contain suppressive factors as well as suspected promoters (between 1187 and 529 and 529–327). The research has already revealed that the 5′ regulatory region of myoblasts has higher activity than that of myotubes (Ma *et al.,* 2001) in the process of differentiation, as demonstrated by MSTN mRNA and endogenous MSTN (Rios, *et al.,* 2001, Artaza *et al.,* 2002). This additionally supports the theory that MSTN promoter is linked to the expression of genes in a myogenesis context, which may help medical personnel with their work. Despite that, most studies regarding the processes regulating MSTN promoter activity have been done *in vitro* or in animal models, primarily the mouse as discussed below.

2.8.2 Double Muscled Cattle

The discovery of the nucleotide sequence of a myostatin gene, and the resulting attempts to clone and confirm the sequence, were first made in Belgian Blue and Piedmontese cow breeds. Researchers reported mutations mostly in the myostatin gene (different variations in each breed), and these mutations altered the myostatin gene in some way, as a result of which functional myostatin is absent in some animals (McPherron *et al.,* 1997; Kambadur, *et al.,* 1997 and Grobet, *et al.,* 1997). These cattle breeds' muscle cells grow, rather than multiply when myostatin is absent. Some breeders refer to this breed of cattle as "doubled muscled," however there is only a 40% increase in all muscles. (Kambadur, *et al.,* 1997; Builtreport.com, 2019, and McPherron, *et al.,* 1997). Myostatin is essential for muscle growth, yet treatment with constituents like follistatin blocks the myostatin receptor and causes considerable muscular growth. Thus, a 20% decrease change in

myostatin levels has a considerable impact on muscle growth in animals as reported by Kota, (2009).

Despite having offspring that are larger and huge than other animals, some animal breeds became homozygous for myostatin deficiency, resulting in a reproduction issue and an increased cost of feeding. This decreases the value of myostatin-deficient breeds and so makes them irrelevant in the marketplace. While myostatin-deficient beef meat, such as beef from the Piedmontese, which has a niche in the specialised market because of its distinctive qualities, myostatin-deficient cattle, on the other hand, have a drawback in the market due to the need for veterinary care, De Smet (2004).

There was an investigation to discover if the upstream region of the bovine animal was significantly similar to the human 5' regulatory region. Using a sequence identity percentage of 79%, this 1.6 kb upstream region exhibited several prospective binding sites, along with a CAAT site, three TATA boxes, except a MEF2, which had shown to increase the effectiveness of the promoter in vitro (Spiller *et al.*, 2002; Crisà *et al.*, 2003). Bovine promoter (Fig. 2) has ten putative E-boxes grouped into three clumps, each having six E-boxes. These numbers are equal to the degree of the regulatory sequence found in all the ten E-boxes. It appears that the 6th E-box is critical for regulation, and is the preferred site for MRF MYOD and MYF5. It is capable of replacing the other E-boxes if needed. According to the findings from this research, it was shown that an E-box is required for muscle-specific gene production through MRFs. As a result, the muscle-specific promoter has been demonstrated, indicating limited activity during translation into fibroblasts (Spiller *et al.*, 2002).

Several polymorphic sites with their mutations were shown in table 2.2 below:

Table 2.2 Myostatin Gene Polymorphisms in Cattle

	Polymorphisms		
Specie	Location	Mutation	References
Australia de los Valles	c.821	del 11	Grobet *et al*. (1997)
Belgian Blue	c.821	del 11	McPherron and Lee (1997)
Blonde	c.821	del 11	Kambadur *et al*. (1997)
d'Aquitaine	g.3811	T>G	Bouyer *et al*. (2014)
Charolaise	c.610	C>T	Kambadur *et al*. (1997)
Gasconne	c.938	G>A	Kambadur *et al*. (1997)
			Dunner *et al*. (2003)
Limousine	c.821	del 11	Kambadur *et al*. (1997)
	c.610	C>T	Cappuccio *et al*. (1998)
	g.433	C>A	Sellick *et al*. (2007)
Maine-Anjou	c.419	del-7-ins 10	McPherron and Lee (1997)
	c.676	G>T	Grobet *et al*. (1997)
Marchigiana	g.874	G>T	Cappuccio *et al*. (1998)
Nellore	g.76	A>T	Grissolia *et al*. (2009)
	g.111	G>T	
	g.267	A>G	
	g.374	del 16	
	g.414	C>T	
	g.420	T>G	
	g.433	A>T	
	g.445	A>T	
	g.527	T>A	
	g.641	G>A	
	g.694	G>A	
	g.840	A>G	
	g.951	T>G	
	g.1083	C>T	
Parthenoise	c.821	del 11	Kambardur *et al*. (1997)
Piedmontese	c.938	G>A	Kambardur *et al*. (1997)
Rubia Gallega	c.821	del 11	Kambardur *et al*. (1997)

The wild-type myostatin booster upstream of the luciferase reporter gene was cloned and then was transfected into C2C12 cells. Concentrations of wild-type MSTN were tested and shown to have an inhibitory effect on luciferase activity Myostatin protein. As a result, there was a significant reduction in the promoter activity, suggesting that MSTN protein maintains a negative control on the MSTN promoter (Forbes *et al.,* 2006). The Piedmontese cattle transition from cysteine to tyrosine, which is shown at the C-terminal region of the MSTN protein, did not significantly alter promoter activity. Moreover, it was found that MSTN promoter negatively regulates MSTN protein via ActRIIB, ALK5 receptors, and SMAD7 protein. Wildtype MSTN protein as well as the mutant Piedmontese MSTN were shown to activate the MSTN gene booster, while the MSTN enhancer activity was decreased in response to SMAD7 transcription. The findings show that the myostatin enhancer is negatively restricted by myostatin proteins, which occurs in double-muscled cow breeds that carry the mutant MSTN gene. This allows for an increase in MSTN mRNA, which is thought to occur in those breeds (Forbes *et al.,* 2006).

While the 5' regulatory region of the Limousine, Belgian Blue, Marchigiana, Piedmontese, Black and White, Hanwoo, Jeju Black, Holstein Cattle, and Qinchuan cattle has single nucleotide polymorphisms (SNPs) other breeds have SNPs elsewhere in their genomes, such as the Belgian Blue, Piedmontese, Limousine, Marchigiana, Black-and-White, Holstein, Hanwoo, Jeju Black Cattle, and Qinchuan cattle (Crisà *et al.,* 2003; Han *et al.,* 2012; He *et al.,* 2013; Santi *et al.,* 2014; Jank *et al.,* 2006). To provide the most relevant information, the performance of the research is organised into Table 2.2. While there are connections between specific promoter polymorphisms and phenotypes in some of these models, in many cases the specific promoter polymorphisms were correlated with the phenotype. In these cases, the MSTN promoter could be a genomic marker and a candidate region for improving economic parameters in breeds of cattle, as more thoroughly discussed in the Prospective Applications section. Because other SNPs were also seen to influence fat deposition (Han *et al.,* 2012), this SNP also has a role in regulating myoblast and preadipocyte differentiation, with an impact on fat accumulation.

Plate 1: Belgian Blue cattle: Builtreport.com. Retrieved 2019-06-03.

2.8.3 Sheep myostatin gene

This fragment of the sheep's 5' regulatory region is well conserved, with the corresponding values in the goat, cattle, pig, human, and mouse being 98.1%, 95.8%, 86.9%, 80.2%, and 67.7% respectively (Du R, *et al.,* 2005). Three separate TATA boxes were detected at − 139, − 163, and − 523 bp while one CAAT box was found at − 206 bp upstream of the ATG. BOTH sequence AND position of the TATA 1,2 boxes are retained in all ruminants, with TATA box 3 being exempt in the swine MSTN promoter (Du *et al.,* 2005). Song *et al*., (2016) succeeded in locating the TATA box (contained about 156–165 bp) and thus the CAAT box (situated at 202–207 from the beginning of the codon) within 150 to 220 bp of the region in using three different software.

Some genes, for instance, PRE, were silent in sheep as well as in goats, while others, such as MEF2, were conserved among sheep, goats, and cattle as well as GRE which is stored in sheep, porcine and goats. The promoter activity and GRE can both be regulated by MEF2 and MTBF binding sites, which were demonstrated to be significant in promotion as well as GRE activation, which appears to occur through a glucocorticoid receptor-mediated route (Du, *et al.,* 2007).

An analysis conducted using the genomic sequences of sheep, goat, and porcine found that there are eight E-boxes located within approximately 1.2 kilobases of the promoter, of which seven (with the exception of E5) remain in region and sequence, are equivalent to the corresponding porcine E-boxes. The positions of six E-boxes are close to those of E1, E2, E3, E4, E5, and E7 in the bovine enhancer, excluding E4 and E5 (Du, *et al.*, 2005). A deletion experiment found that the activity of the promoters was significantly altered by the modifications in the E-boxes 3, 4, 5, and 7, with E-box 7 being the most essential of all. For more stable DNA-protein binding, E-boxes 3, 4, and 5 are arranged in a cluster. In some e-boxes 3, 5, and 7, we can deduce that myoblasts differentiate into myotubes because cells with alterations in these three binding areas had reduced transcriptional activity, whereas the rest of the 1.2 kb 5′ regulatory region increased transcription after the cells underwent differentiation.

In the enhanced green fluorescent protein (eGFP) insertion 5′ regulatory region (which encodes an eGFP protein) upstream of the reporter gene (which encodes an eGFP protein) from the sheep MSTN gene, 1.2 kb of genomic DNA from sheep was cloned and transfected into mouse C2C12 myoblasts or sheep fibroblasts (Du, *et al.*, 2007). In keeping with previous studies using bovine (Spiller *et al.*, 2002) and porcine (Li *et al.*, 2012) cells, the results reveal that the MSTN promoter is muscle-specific. In deletion tests, it was found that the presence of the first 272 base pairs of the promoter is sufficient to drive reporter performance, but the level of action is the greatest when the entire 1.2 kilobase is present. Even if these regulators are not the direct negative influences, they are presumed to exist within 0.7 and 0.9 kb or between 0.3 and 0.4 kb. To sum up, while MSTN is known to have an inhibitory effect on cell proliferation, increasing C2C12 cell density led to MSTN inhibiting promoter activity (Du *et al.*, 2007).

Table 2.3 Myostatin Gene Polymorphisms in Sheep

	Polymorphisms		
Specie	Location	Mutation	References
Texel	g.6723	G>A	Kijas *et al*. (2007)
	g.391	G>T	
	g.2449	C>G	
	g.2379	C>T	
	g.1405	A>T	
	g.1402	G>A	
	g.1214	C>T	
	g.1129	C>T	
	g.41	A>C	
	g.39	T>C	
	g.474	C>T	
	g.613	T>C	
	g.616	G>A	
	g.619	T>C	
	g.622	T>C	
	g.632	G>T	
	g.696	C>T	
	g.3135	C>T	
	g.4036	A>C	
	g.4044	C>T	
Norwegian	c.960	del 1	Wang *et al*.
(2016)			
White Sheep	c.2360	G>A	
New Zealand	c.101	G>A	Kijas *et al*. (2007)
Romney	c.-959	C>T	Wang *et al*.
(2016)			
	c.-784	A>G	
	c.373+18	A>G	
	c.373+563	A>G	

	c.373+607	G>A	
	c.676-654	T>C	
	c.374-54	T>C	
	c.*83	A>G	
	c.*455	C>A	
	c.*709	insA	
	c.*123A	T>G	
	c.-2449	G>C	
	c.-2379	T>C	
Charollais	c.*123A		Kijas *et al*. (2007)
White Suffolk	c.*123A		Kijas *et al*. (2007)
Poll Dorset	c.*123A		Kijas *et al*. (2007)
Lincoln	c.*123A		Kijas *et al*. (2007)
Indian sheep	c.*539	T>G	Pothuraju *et al*. (2015)
	c.821	T>A	
Stavropol	c.373+396	T>C	Trukhachev *et al*. (2018)
Merino	c.374-362	A>T	
	c.374-16	delT	
	c.747+185	C>A	
	c.748-194	C>T	
	c.782-783	insT	
	c.940	G>T	
	c.*310	G>T	

2.8.4 Myostatin role in muscle atrophy

A glucocorticoid is a well-known inducer of muscle atrophy, and the mouse promoter only specifically responded to glucocorticoids in vitro (Allen *et al.*, 2010). It appears that the induction process here is oblique and interdependent on the overexpression of C/EBP, which led to the identification of two potential binding sites within 100 bp of the myostatin enhancer.

In a curious way, these binding sites are complementary because the first mutation nearly completely abolished the ability of the basal promoter to regulate C/EBP or GR (glucocorticoid receptor) activity, but had no effect on the promoter's sensitivity to

C/EBP or GR (glucocorticoid receptor. The other (second) mutation, on the other hand, reduced the promoter's sensitivity to C/EBP, but this did not have any effect on the basal promoter (Allen *et al.,* 2010). In yet another situation, C/EBP signalling was found to reduce myostatin activator function in pluripotent C3H10T1/2 cells stimulated to differentiate into adipose tissues. This suggested that, while they may have different biological functions in distinct cellular contexts, these molecules have the same regulatory mechanism when functioning in each context. MSTN transcription, however, is influenced by glucocorticoids *in vivo* studies. MSTN activity increased by 34% when plasmids in form of vectors were implanted in the thigh muscles of rodents given high doses of dexamethasone. There is a mutant version of the GRE motif present, and its mutation decreases the promoter activity by 17 per cent (Qin *et al.,* 2013). In conjunction, these findings show that glucocorticoids impact on MSTN promoters in multiple ways, both indirectly and directly, depending on the species, cellular environment, and through distinct mechanisms.

It was shown that the murine MSTN promoter is likewise activated by FOXO1, a gene that is known to affect muscle atrophy and is elevated with caloric restriction (Allen *et al.,* 2007). Researchers were able to identify five putative FOXO sites across a total of 1177 bp in the 5′ regulatory location, and the closest one is conserved throughout several species such as humans, pig, goat and cattle. Five Sites deletion causes the expression of FOXO-inducible genes to decrease dramatically, but not completely, showing that this process also occurs indirectly. Additional binding sites for the SMAD proteins are also conserved and are found near to proximal FOXO site, and SMAD proteins attach to the promoter and activate it without any involvement from the FOXO site (Allen *et al.*, 2007). Also, in hypothyroid rats, MSTN level was significantly higher (Carneiro, 2004) It was postulated that the variation in the hypothyroid animal's MSTN promoter activity could be related to the muscle loss found in these animals because the MSTN promoter contains many areas responsive to hormones.

2.8.5 Equine myostatin gene

A 670-bp fragment was analysed and shown to display a similarity of 90% and 77% with the promoter of the pig and mouse, respectively (Dall'Ollio *et al.,* 2010). Three

consensus binding sites - including a TATA box which the second is derived (TATA-2) was preserved across all species, and TATA-1 and TATA-3 were not maintained in mice and pigs, respectively, while TATA-2 was preserved several species but well-aligned in the equine sequence with other species (the others included the pig, mouse, and sheep sequences). Lastly, four E-boxes (the only ones not conserved in humans and mice) were found, with E-4 being the only one that is not present in both species (Dal'Olio *et al.*, 2010). Two promoter alterations (T26, -T156; C26, -T156) were found in many horse breeds. The findings suggest that there are four potential haplotypes: 1 (T26, -T156), 2 (T26, -C156), 3 (C26, -T156), and 4 (C26, -C156). According to repeated studies, haplotype 1 may be the most ancestral, as it was found in every investigation. (Dal'Olio *et al.,* 2010). A 227-bp insertion was found at position 146 in the myostatin enhancer domain of thoroughbred horses (Hill EW *et al.,* 2010). Further study has identified this region as a horse-specific repetitive DNA sequence element (SINE) named equine repetitive element 1 (ERE-1), positioned 373/147 bp downstream of the ATG site, and revealed that it may impair or displace numerous key binding sites, including FOXO, CCAAT, E-boxes and SMAD binding sites (Hill *et al*, 2010; Petersen *et al,* 2013). Additional possible binding sites for the Upstream Stimulator Factor (USF), a regulator of cell growth and proliferation, include RAS-Responsive element-binding protein (RREB-1) and members of the NKX-homeodomain factor family (Van den Hoven *et al,* 2015). Additionally, a possible extra E-box and a motif like the TATA box were found (Dall'Olio *et al.,* 2014). Additionally, it was demonstrated that the presence of the SINE causes the transcription beginning point to be displaced into the SINE insertion sequence, resulting in the formation of an mRNA roughly 200 nucleotides longer (Rooney MF *et al.*, 2018). Additional data showed that the insertion caused the formation of a new CpG island, which includes a whole downstream section that is possible to epigenetically modify (Van den Hoven *et al.*, 2015). To verify if the SINE affected the strength of the MSTN promoter, two versions were constructed and cloned in a vector that contained the GFP reporter gene upstream. These constructs were then transfected into human HeLa cells and horse fibroblast cells. Both SINEs induce a significant drop in the levels of the reporter gene. This indicates that this inclusion interferes with the MSTN promoter activity (Santagostino M *et al.,* 2015).

Table 2.4 Myostatin Gene Polymorphisms in Horse

	Polymorphisms		
Specie	Location	Mutation	References
American Quarter Horse	g.66495326_66495327	INS227	Petersen *et al*. (2013)
Andalusian	g.26	T>C	Dall'Olio *et al*. (2010)
	g.156	T>C	
	g.1634	T>G	
	g.2024	G>A	
	g.2115	A>G	
	g.2327	A>C	
	g.4230	T>A	
Arabian horses	g.2279	A>C	Baron *et al.* (2012), Stefanik *et al*. (2016)
	g.66495696	T>C	
	g.66495254	C>T	
Bardigiano	g.156	T>C	Dall'Olio *et al.* (2010)
Haflinger	g.156	T>C	Dall'Olio *et al*. (2010)
Hucul	g.26	T>C	Stefanik *et al*. (2014,2016)
	g.66495696	T>C	
	g.66493737	T>C	
	g.66490010	T>C	
Italian Saddle	g.26	T>C	Dall'Olio *et al*. (2010)
	g.156	T>C	
Italian trotter	g.26	T>C	Dall'Olio *et al*. (2010)
Polish Konik	g.66495254	C>T	Stefanik *et al*. (2014,2016)
	g.66495696	T>C	
	g.66493737	T>C	
	g.66490010	T>C	
Lipizzan	g.26	T>C	Dall'Olio *et al*. (2010)
Maremmano	g.156	T>C	Dall'Olio *et al*. (2010)
Murgese	g.156	T>C	Dall'Olio *et al*. (2010)
Noric	g.26	T>C	Dall'Olio *et al*. (2010)

	g.156	T>C	
Polish Heavy Draft	g.26	T>C	Stefanik *et al.* (2014,2016)
	g.66495254	C>T	
	g.66495696	T>C	
	g.66493737	T>C	
	g.66490010	T>C	
Rapid Heavy Draft	g.26	T>C	Dall'Olio *et al*. (2010)
	g.156	T>C	
Salernitano	g.156	T>C	Dall'Olio *et al*. (2010)
Soraia	g.2478	G>C	Baron *et al*. (2012)
Thoroughbred horse	g.156	T>C	Dall'Olio *et al*. (2010)
	g.1634	T>G	Petersen *et al*. (2013)
	g.2115	A>G	Petersen *et al*. (2013)
	g.2327	A>C	Petersen *et al*. (2013)
Tolfetano	g.156	T>C	Dall'Olio *et al*. (2010)
Uruguayan Creole	g.156	T>C	Dall'Olio *et al*. (2010)
Ventasso	g.26	T>C	Dall'Olio *et al.* (2010)

2.8.6 Whippets

Whippets have a rare variant of the myostatin gene, known as a whippet myostatin mutation, that has a deletion of two base pairs that result in a shortened and almost certainly myostatin protein that is inactive. Affected organisms have a unique body shape: with a big and prominent head, short legs, and a thicker tail. These animals are referred to as "bully whippets" in the dog-breeding community. Despite being substantially more muscular, they are less competent runners than some other whippets. Individual whippets that had a double mutant had a large increase in representation in elite racing classes, according to Mosher *et al*. (2007).

Plate 2: A whippet with a myostatin homozygous mutation, Mosher, *et al.,* (2007)

2.8.7 Mouse mammary tumour (MSTN) gene promoter

Following an examination of the 2.5-kb portion of the murine 5′ regulatory region, a few target genes, mostly for MEF2, C/EBP, and CCAAT, were discovered. According to Salerno *et al.,* (2004), seven E-boxes were arranged into four categories (Fig. 2), pointing that these components are necessary for myostatin expression in the cell, and moreover, these researchers found that MSTN expression increased in vitro after adding more than one copy of each element. In the proximal 1kb, the first five E-boxes, which were found to have the highest promoter activity in vitro, were shown to be sufficient for obtaining it, and E-box number 5 seems to be essential and proficient in increasing the promoter activity six-fold and by itself since it was preferentially bound by MYOD and MYF5, which increased the promoter activity by 2-fold and 6-fold, respectively (Salemo *et al.,* 2004). However, the study of the 3' untranslated region (3'UTR) revealed that 260-bp, which includes all of the orthologous elements (E-boxes 1–4), could activate genes, but at a lower level and to a reduced extent, and this demonstrated sequence identity with the human ortholog to be 92% similar (Grade *et al.,* 2009). To discover the MSTN promoter's human relevance, research has focused on it and its relationship to the human MSTN gene, as shown below.

Several activators of MSTN gene enhancers had already been specified in the myogenic setting. Additionally, since insulin-like growth factor (IGF) is secreted within the cells, IGF would boost promoter activity by increasing cytosolic calcium (Valdés *et al.,* 2013), which results in the activation of NFAT and CREB binding protein (CREB) (Zuloga *et*

al., 2013). The calcium levels increase, leading to the dephosphorylation of NFAT, which gets accumulated in the nucleus, where it then interacts and activates gene promoter activity. Although several NFAT binding regions were discovered in the mouse myostatin genome (Valdés *et al.,* 2013), additional ones were found. No studies have confirmed that the binding sites for CREB are also necessary for the CREB/NF-Y/MEIS1 basal regulation of MSTN transcription during early myogenesis (Grade *et al.,* 2017). A putative link between IGF-I signalling and CREB activation was shown, with transfection of CREB siRNA vectors suppressing IGF-I-mediated promoter activation (Zuloga *et al.,* 2013). However, no direct DNA-protein binding tests were undertaken, making it unclear whether these reported increases in activity directly affect the gene promoter.

Despite being opposed to the MSTN promoter, some MSTN antagonists are also known. The nuclear factor I/X (NFIX) binds to particular locations on the MSTN gene promoter and represses the promoter's expression in murine myoblasts and in vivo (Rossi *et al.,* 2016). That is done to ensure that satellite cell differentiation and muscle development occur at the right time.

In addition, during muscle cell differentiation, neuronal orphan receptor 1 (NOR-1) inhibits myostatin enhancer activity, and hence, possibly influences fatty acid use during skeletal muscle (Pearen *et al.,* 2006). This work may provide insights for the development of MSTN transcription inhibition techniques, focusing on the promoter.

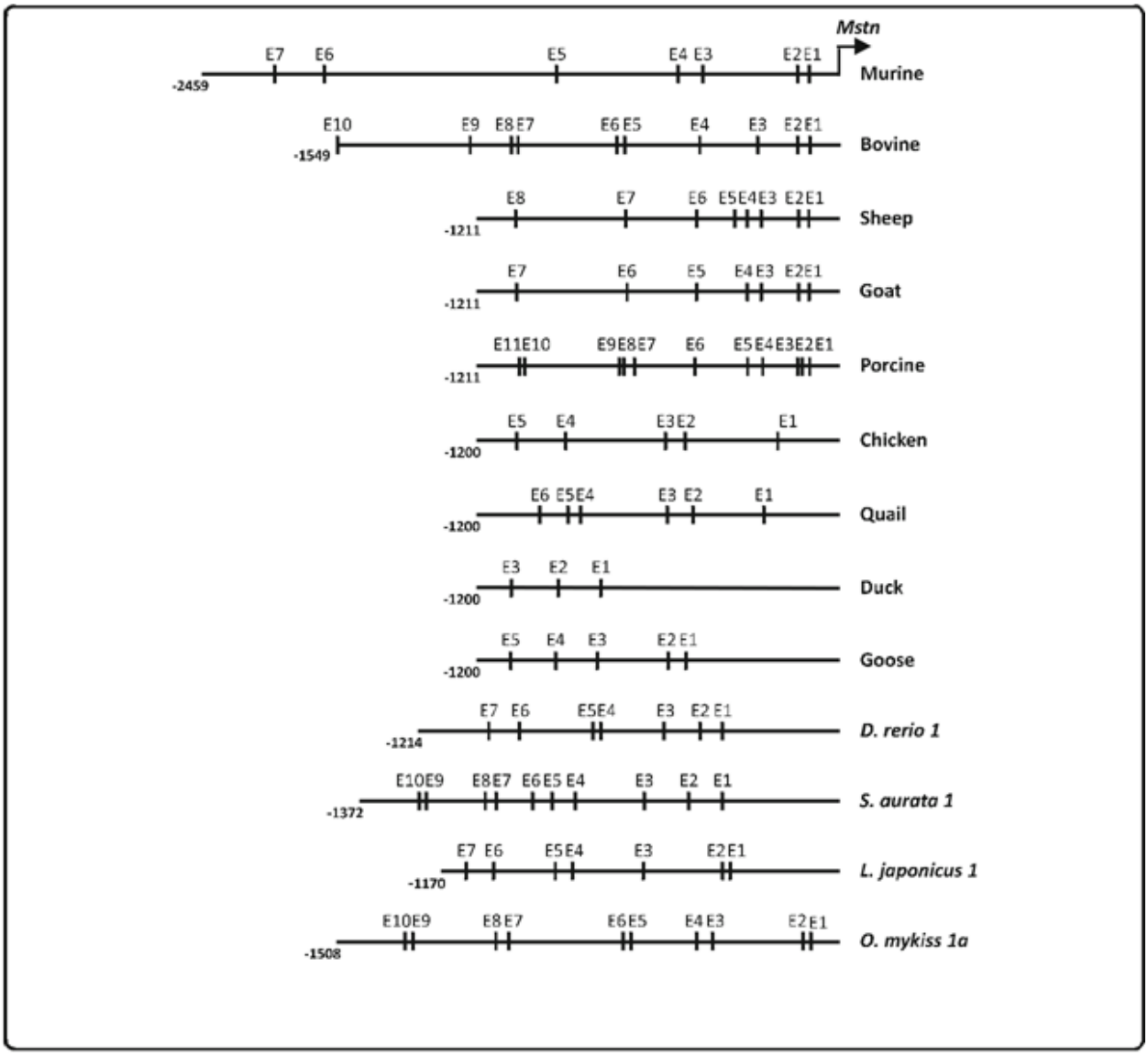


Fig. 2 E-boxes in the MSTN/MSTN-1 promoter region of various animal species is represented schematically. E-boxes are sequentially numbered, as modified from (Salemo *et al.*, 2004; Du *et al.*, 2005; Gu *et al*., 2004; Funkenstein *et al.*, 2009.

2.8.8 Rabbits

In the year 2016, Guo, *et al.,* (2016) genetically edit goats and rabbits without functioning copies of the myostatin gene through the use of the CRISPR/Cas9 technology. The animals from this genetic modification were found to be substantially thicker in both cases. While low lifespans and stillbirths were seen in rabbits lacking myostatin, this particular group of rabbits had an expanded tongue and greater rates of early and late stillbirths.

Plate 3: Rabbit Lacking MSTN https://www.scoop.it/topic/transgenesis-by-geg-tech, (2021).

Table 2.5 shows various breeds of rabbits and their mutation of different sites:

Table 2.5 Myostatin Gene Polymorphisms in Rabbit

	Polymorphisms		
Specie	Location	Mutation	References
Belgian hare	c.108	C>T	Fontanesi *et al*. (2011)
	c.713	T>A	
	c.*194	A>G	
	c.747+34	C>T	
Burgundy fawn	c.108	C>T	Fontanesi *et al*. (2011)
	c.713	T>A	
	c.*194	A>G	
	c.747+34	C>T	
Checkered giant	c.108	C>T	Fontanesi *et al*. (2011)
	c.713	T>A	
	c.*194	A>G	
	c.747+34	C>T	
Commercial breeds	g.478	T>C	Qiao *et al*. (2014)
(Not specified)			
Giant grey	c.108	C>T	Fontanesi *et al*. (2011)
	c.713	T>A	
	c.*194	A>G	
	c.747+34	C>T	
Giant grey	c.-125	T>C	Sternstein *et al*. (2014)
	c.373+234	T>C	
New Zealand White	c.-125	T>C	Sternstein *et al.* (2014)
	c.373+234	T>C	

2.8.9 Goat myostatin gene

The same approximately 1 kb sequence from numerous Indian goat varieties had 96.8% homology with sheep, 94.8% with cattle, 75.6% with pigs, and > 60.3% with cats. A set of three distinct TATA boxes, along with six E-boxes (see Fig. 2) as well as other binding regions were discovered (Singh *et al.*, 2014). This observation agrees with the results of a 5-bp TTTTA deletion (position − 10 to − 6) in numerous goat breeds but does not exist in other species (Singh *et al.*, 2014). A considerable change in body mass and size is also

reported (Li *et al.*, 2008). 16 SNPs were detected in many Indian breeds of goat (Singh *et al.*, 2014), but the significance of these genes on attributes of interest to the Indian economy has yet to be examined. An overview of the genetic variations discovered in goats is provided in Table 2.6.

Table 2.6 Myostatin Gene Polymorphisms in Goat

	Polymorphisms		
Specie	Location	Mutation	References
Anhui white	g.197	G>A	Zhang *et al*. (2013)
	g.345	A>T	Nguluma *et al*. (2018)
Boer	g.1256	TTTA/-	
	g.197	G>A	
	g.1388	T>A	
	nt345	A>T	
	nt298	T>C	
Haimen	nt1256	TTTA/-	
Motou	nt1256	TTTA/-	
Nubi	nt1256	TTTA/-	

2.8.10 Porcine Myostatin gene

Using 1.2 kb of the 5′ promoter regions of the pig myostatin gene, the research found that the copy number is 84.4 % similar in humans, 71.6 % in mice, 83.7 % in cattle, 82.2% in sheep, and 82.5% goats (46,47). This demonstrates that the activity of the porcine promoter was stronger in myoblasts than in myotubes, despite the similar structural attributes of these two cell types. This is different from the findings of the human (Ma *et al.*, 2001), implying the fact that control of this gene may be distinct to all these organisms (Deng *et al.*, 2012; Liss *et al.*, 2012). Myoblasts (i.e., immature muscle cells) had positive reactions to the porcine promoter, but fibroblasts (i.e., mature muscle cells) did not. This supports the idea that there is indeed a degree of tissue-specificity control in every species except for mice, where two TATA boxes are present, which also co-occurred in the alignment of these regions. It was concluded that primarily on mRNA sequences, it was observed there are differences in transcription-associated structures among mammals, the most distant TATA box found within nucleotides − 71 and − 67 in

pigs and humans is associated with transcription in both species, leading to a relatively small 5′ UTR in the rodent (Yu *et al.*, 2005). Numerous conserved transcription factor binding sites were found while harmonizing the pig and human 5' regulatory regions (Deng *et al.*, 2012; Stinckens *et al.*, 2008; Yu *et al.*, 2005). It has been established that MEF2 is capable of binding and activating the porcine MSTN promoter, as reported in mice and cattle (Li *et al.*, 2012). Eleven E-boxes were clustered in clusters and displayed very high levels of conservation between the porcine regulatory regions as well as bovine MSTN 5′ sequence (Stinckens *et al.*, 2008) (see Fig. 2). There was also an increase in the activity of the promoter, which is what MYOD was shown to be the cause (Deng *et al.*, 2012). A 386-number insertion of base pairs with four additional E-boxes had already been documented in several breeds of pigs, in which length polymorphism was detected (Yu *et al.*, 2007).

An overview of the discovered genetic variations in porcine is provided in Table 2.7

Table 2.7 Myostatin Gene Polymorphisms in Pig

	Polymorphisms		
Specie	Location	Mutation	References
Belgian Pietrain	g.435	G>A	Stinckens *et al*. (2008)
	g.447	A>G	
	g.879	T>A	
Chinese	g.879	T>A	Qiqan *et al*. (2015)
Yorkshire pig	g.383	T>A	Jiang *et al*. (2002)

Meishan pigs, a sluggish Chinese endemic variety, were the subject of research, have indicated that maternal diet affects MSTN protein expression (Liu *et al.*, 2011). The C/EBPβ binding to the myostatin regulator was identified at 35 days of age in the weaning piglets, a low protein diet which may have contributed to the reduced MSTN expression found at this age. It was reported, however, that in the late-stage (8 months age) of the piglet, a C/EBPβ-binding to the myostatin regulator region is increased, and MSTN expression is also increased, which indicates that both present and future effects of protein intake on offspring myostatin transcription are controlled by C/EBPβ (Liu *et al,* 2011). Furthermore, at 28 days of age, commercial pigs fed a low protein diet

demonstrated a significant increase in the expression of FOXO3 and GRE in their putative binding sites in the MSTN promoter (—3708, —3535), which may play a role in MSTN expression activation in these animals when protein intake is restricted in pregnant sows (Jia *et al*, 2016). As previously mentioned by other authors (Stinckens *et al*, 2008; Yu *et al.*, 2007; Tu *et al*, 2014; Guimeraes *et al*, 2007), multiple MSTN SNP markers have already been discovered, and the pertinent ones are included in Fig 3. These little changes may have adverse consequences, such as those that may prevent the transcription factors MEF3 (Stinckens *et al*, 2008; Tu *et al*, 2014) and NFAT (Yu *et al.*, 2007) from binding, as well as impact key binding sites like CREB and ATF2. Muscle deposition is elevated when the MSTN promoter is activated, resulting in enhanced MSTN expression. What's noteworthy is that the three SNPs A (A435 -G447 -T879), B (G435 -A447 -T879), C (A435 -A447 -A879) and D (A435 -A447 -T879), may be arranged into four potential haplotypes (B, C, D, and A), in which D is the most progenitors (ancestral), B and C came from D by artificial selection, and A originated from B (Liu *et al.*, 2011). Haplotype A is expressed more actively in MSTN compared to other variants and has been shown to be positively linked with weight gain and the proportion of meat in breeds like Laiwu, Landrace, Yorkshire, and Dapulian (Tu *et al.*, 2014). In contrast, haplotype D was detected solely in wild boars, and this haplotype exhibited the lowest activity (Liu *et al.*, 2011). The second weakest allele, the dominant haplotype in Duroc pigs, had already been associated with improved body weight, as well as daily gain and increased backfat thickness in Yorkshire and Duroc pigs (Yu *et al.*, 2007; Tu *et al.*, 2012). This research suggests that multiple variants (haplotypes) A and C also converse with transcription factors (TFs) that are not muscle-specific, lending further evidence to the hypothesis that the variants can influence the transcription of TFs that are not muscle-specific (Bongiomi *et al.*, 2014).

2.8.11 Poultry

Many genes have been mapped and sequenced in poultry that is consistent with their role in growth (Duclos, 2005). These candidates' genes serve as an ideal subject for genetic observations on how dietary change influences gene expression. The somatotropic pathway governs chicken growth and development in the same way that it regulates

mammalian maturation. (Zhao *et al,* 2004). The somatotropic pathway, which includes various important growth factors like insulin-like growth factors (IGF-1 and IGF-2), somatostatin (SS), growth hormone-releasing hormone (GHRH), growth hormone (GH), with their linked cofactor proteins and receptors, and many other hormones such as leptin, insulin, and thyroid hormones or glucocorticoids, is formed from multiple hormones, such as GH, GHRH, IGF-1, and IGF-2, and the linked carrier proteins and receptors, and other hormones like leptin, insulin, and glucocorticoids or thyroid hormones (Renaville *et al*, 2002; Nie *et al,* 2005). The binding of GH to the GHR on the outer layer of hepatocytes triggers a GH-stimulated increase in hepatic IGF-1 levels, which encourages bone and muscle cell differentiation as well as proliferation (McMurtry *et al.,* 1997; Kuhn *et al.,* 2002). It is a peptide hormone that acts as a mediator for GH's development factors in poultry (Kita *et al.,* 2002; Anh *et al.,* 2015). IGF-1 and IGF-2 are components of the protein kinase B (PKB)/AKT/mTOR (mammalian target of rapamycin) route and play a role in the regulation of muscle fibre improvement (Clemmons, 2009). The mTOR pathway regulates protein synthesis in hypertrophy and results in the production of IGF-1 and IGF-2 (Kimball and Jefferson, 2006). A growth factor in TGF-superfamily members also serves as a cytokine by decreasing the proliferation of myogenic precursor cells and myoblast differentiation and hindering muscle growth. It is one of the primary inhibitory regulators of muscle mass (Acosta *et al.,* 2005). Other, notable examples of the IGF system component IGFBP family include influencing the ability of IGFs to maintain a constant level in the body, reducing the levels of IGFs in the circulation, having an association with IGFs as a carrier, and redistributing IGFs to different tissues and interstitial fluid (Kim, 2010). As part of the maintenance of this dynamic equilibrium, this route constantly produces both positive and negative impulses, which affects muscle development in birds.

Table 2.8 Myostatin Gene Polymorphisms in Poultry

Specie	Polymorphisms		References
	Location	Mutation	
Arbor Acre	g.167	G>A	Gu *et al*. (200)
	g.177	T>C	Zhang *et al*. (2012a, b)
	g.304	G>A	
	g.322	A>G	
	g.326	A>G	
	g.344	C>T	
	g.344	C>G	
	g.1346	C>T	
	g.1375	G>A	
	g.6935	A>G	
	g.7263	A>T	
Bian chicken	g.234	G>A	Zhang *et al*. (2021a, b)
Gaoyou ducks	g.2701	G>A	Liu *et al.* (2012)
Jinghai	g.326	A>G	Zhang *et al.* (2012a, b)
	g.334	C>G	
	g.1346	C>T	
	g.1375	G>A	
Pekin duck	g.129	T>C	Xu *et al*. (2013)
	g.708	T>C	
	g.952	T>C	
Sansui duck	g.106	G>A	Zhao *et al*. (2016)
	g.120	A>G	
	g.159	G>A	
	g.5368	G>A	
	g.5389	A>C	
	g.5410	G>A	
Youxi	g.326	A>G	Zhang *et al.* (2012a, b)
	g.334	C>G	
	g.1346	C>T	
	g.1375	G>A	

2.8.12 Fish myostatin gene

Teleost fishes had an extra cycle to complete genome replication, as opposed to Vertebrates with jaws, and so the research of myostatin genes is quite challenging in this class of species (Meyer A *et al.,* 1999). Due to this extra duplication, the genetic codes of teleost fish have double myostatin genes, which are: MSTN-1 (MSTNb, GDF8) and MSTN-2 (MSTNa, GDF8I), and also four copies in salmonids, as in many other species (Osibye T *et al,* 2007).

Seven putative E-boxes are found in 1.2 kb of the MSTN-1 5′ regulatory domain the zebrafish when observed as a model organism (Danio rerio) by Xu *et al.* 2003, several of which are in positions comparable to those in cattle, supporting the likelihood that they play a conserved role in regulating promoter activity. The GFP construct was injected into early-stage embryos and muscles were stained with reporter antibodies, revealing that the 5′ regulatory region of the analyzed area contained muscle-specific regulatory components (Xu *et al*., 2003). We did, however, find reporter gene expression in the brain, which suggests that MSTN-1 is participating in some processes outside than myogenesis. (Xu *et al.,* 2003; Kerr T *et al.,* 2005). MSTN-1 and MSTN-2 activators, including TATA boxes, E-boxes, and MEF2 and MEF3 locations, were identified as TFBSs by many research groups.

Complementary DNA (cDNA) existence was discovered using sequence analysis comprised of 10 E-boxes grouped in three clusters (see Fig. 2), plus several possible promoter factor binding locations, including CCAAT and TATA boxes (Funkenstein *et al.,* 2009). When fish orders, like all other phyla, arrange themselves around a TATA box, a CAAT box, a GRE, and a CRE, the locations of a GRE, a CAAT box, and a POU1F1a (PIT1a) site relative to the TATA box, a POU1F1a (PIT1a) site, and a GH-CSE are observed to be silent. Using an in vitro technique, the MSTN-1 promoter deletion constructs were able to promote luciferase actions with the 1113 bp length segment resulting in the maximum activity. This may be indicative of regulatory elements present upstream of this region (Funkenstein *et al.,* 2009). Three alleles promoter (MSTN-2a, MSTN-2b, and MSTN-2c), as well as various SNPs (Nadjar-Boger et al., 2011), were found in reference to Sparus aurata MSTN-2 (Nadjar-Boger E *et al.,* 2011).

2.8.13 Myostatin impacts in sarcopenia

The study also implicated MSTN as being a possible source of sarcopenia, a muscular wasting condition associated with liver cirrhosis (Qiu *et al,* 2013). Hyperammonemia was observed to be related to a rise in myostatin levels and a decrease in muscle mass, suggesting that reduced hepatic function leads to hyperammonemia. These experiments demonstrated that ammonium acetate treatment generated an upsurge in the attachment of the NF-κB p65 subunit to the MSTN transcription, thereby resulting in gene expression activation. When the promoter was cleaved away from NF-κB-p65 locations as well NF-κB gene expression was reduced, the stimulatory signal was dampened (Qiu *et al.,* 2013).

2.8.14 Myostatin impacts on other tissues

Myostatin is also expressed in cardiac, adipose, liver and other tissues (McPherron *et al.,* 1997; Sundaresan *et al.,* 2006) and the promoter of MSTN was found to have a crucial role in these different tissues. Because 3T3-L1 preadipocytes had lower MSTN promoter activity after 4 days of differentiation, the MSTN gene is upregulated when cotransfected with a construct for C/EBPα, PPARγ, and SREBP-1c, demonstrating an increase in MSTN gene expression during adipose tissue growth. MSTN promoter appears to be involved in variations in lean and fat tissue development and metabolism during obesity (Allen, *et al.,* 2008).

Angiotensin II (ANGII), is a crucial nucleotide in cardiovascular remodelling along with hypertrophy, is triggered by the MSTN promoter in rat cardiac myocytes (Wang B.W *et al.,* 2008). However, when MSTN promoter DNA was mutagenized, the additional activity observed with ANGII was eliminated. The compound ANGII was found to stimulate the activity of the MSTN promoter in MEF2 siRNA-treated cells through p38 MAP kinase inhibition, resulting in negative feedback that counters the pathological hypertrophy effects of ANGII (Wang *et al.,* 2008).

2.8.15 Myostatin Gene on Bone Formation

Myostatin indirectly reduces the amount of pressure on the bone by blocking muscle growth (Hamrick, 2003; Tarantino *et al.,* 2015). This has an immediate signalling response in bone formation (Oestreich *et al.,* 2016) as well as degradation (Dankbar B, *et al.,* 2016; Tarantino U *et al.,* 2015). The production of multinucleated cells responsible

for bone tissue disintegration known as osteoclasts has been found to be reduced in animal models of rheumatoid arthritis (mice) due to myostatin inhibition (Dankbar, *et al*., 2016). In addition to bone degeneration, there are numerous other features of rheumatoid arthritis that might damage joint integrity. It has been found that myostatin, on the other hand, is not required for the creation of mature osteoclasts from macrophages, but it does appear to have a co-activator role.

Myostatin manifestation is more common in places where a fracture has occurred. Myostatin suppression on the fracture site causes more callus growth and bone size, which buttress the fact that myostatin inhibits bone production. Dankbar *et al.,* (2015) discovered that a considerable reduction in inflammation occurred at the fracture site due to a lack of myostatin (Dankbar *et al.,*2016). Myostatin has receptors on osteoclastic macrophages and is involved in osteoclastogenesis by attaching to the receptors, activating a signalling pathway, and inducing osteoclast differentiation. Osteoporosis, a disease defined by bone tissue deterioration, likewise sarcopenia, the age causes of deterioration in muscle quantity as well as quality, have been discovered to be linked by Tarantino *et al.* (2015). Although it's unknown whether this interaction is the direct cause of regulation or a subsequent impact, one study claims it's the product of direct regulation. Oestreich *et al.,* 2016 have discovered that offspring with low levels of myostatin in the prenatal environment had better bones than those with osteogenesis imperfecta, thus partially countering the consequences of this condition (brittle bone disease). The mutation that results in the synthesis of defective Type I collagen produces the disorder known as osteogenesis imperfecta. Mice with dysfunctional myostatin were generated by substituting the sequence coding for myostatin's C-terminal domain containing a neomycin cassette. Interbreeding mice with deficient Type I collagen and mice with myostatin deletion resulted in a torsional maximum toughness increased by 15%, tensile strength increased by 29%, and energy to failure increased by 24% in their femurs. when compared to other osteogenesis imperfecta mice, demonstrating the beneficial influences of greatly reduced myostatin on bone development and strength, Kawao, (2015).

2.8.16 Myostatin gene of Invertebrates

Because the MSTN gene promoter had already been investigated in certain invertebrate species, the conservation of its structure across evolutionary time was discovered. Additionally, 1.4-kb sequence analysis of the MSTN gene's 5′ region was used to look for and identify numerous TFBSs, including as a TATA box, MTBF, MEF2, and COMP, alongside three E-boxes (Hu *et al.*, 2010).

In addition to TATA and CAAT boxes, E-boxes, and MEF2 activating regions, a 2-kb fragment from the *Fenneropenaeus merguiensis* (Banana Shrimp) myostatin enhancer has several additional elements, such as TATA and CAAT boxes, as well as E-boxes and MEF2 binding sites (Zhuo *et al.,* 2017). Several binding sites were identified in the 1.3 kb MSTN 5′ region of *Apostichopus japonicus* (sea cucumber), including a TATA box (Li *et al.*, 2016).

Fig 3: Multiple alignments of Myostatin (MSTN)

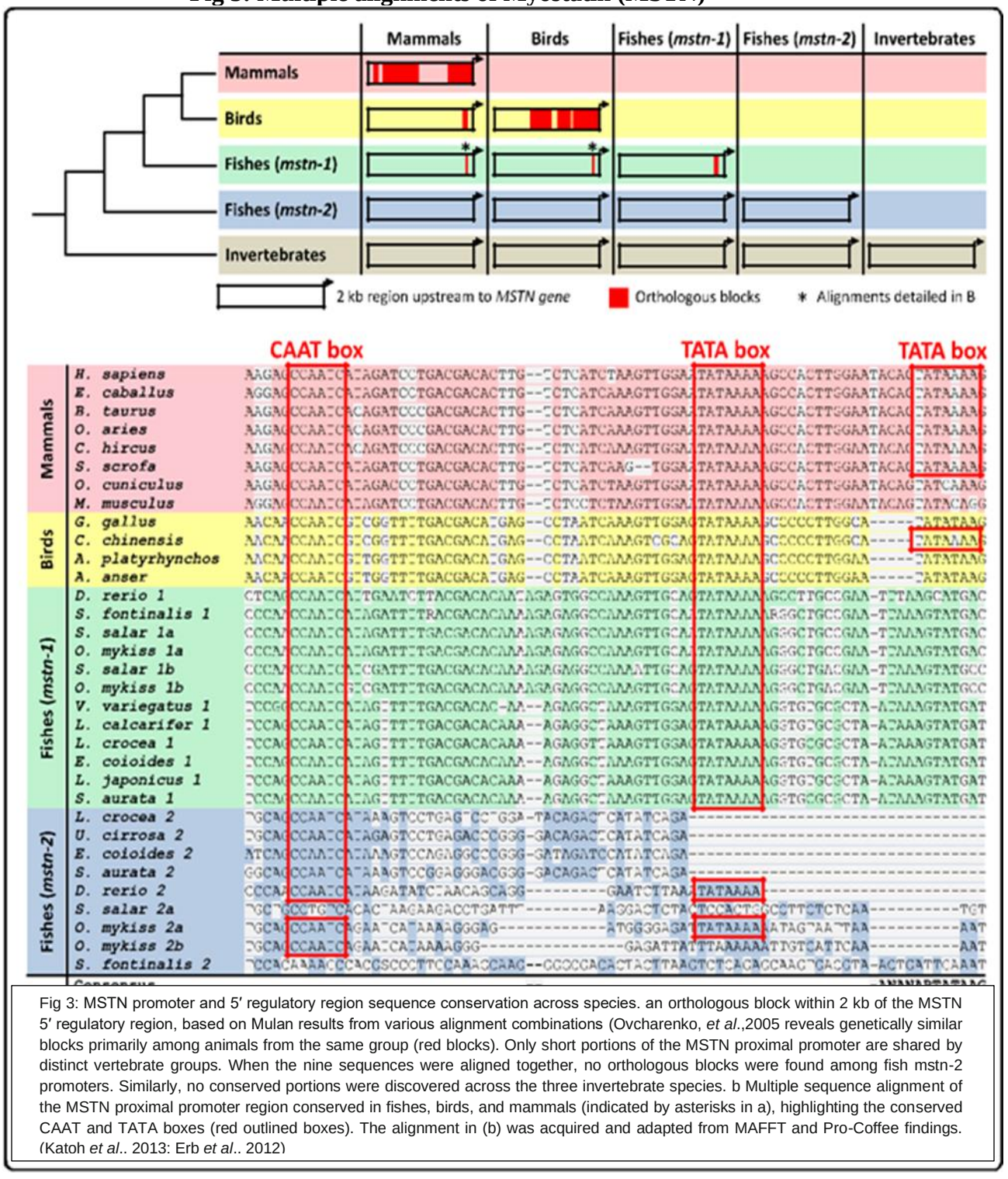


Fig 3: MSTN promoter and 5′ regulatory region sequence conservation across species. an orthologous block within 2 kb of the MSTN 5′ regulatory region, based on Mulan results from various alignment combinations (Ovcharenko, *et al*.,2005 reveals genetically similar blocks primarily among animals from the same group (red blocks). Only short portions of the MSTN proximal promoter are shared by distinct vertebrate groups. When the nine sequences were aligned together, no orthologous blocks were found among fish mstn-2 promoters. Similarly, no conserved portions were discovered across the three invertebrate species. b Multiple sequence alignment of the MSTN proximal promoter region conserved in fishes, birds, and mammals (indicated by asterisks in a), highlighting the conserved CAAT and TATA boxes (red outlined boxes). The alignment in (b) was acquired and adapted from MAFFT and Pro-Coffee findings. *(*Katoh *et al*.. 2013: Erb *et al*.. 2012)

2.9 Molecular Determinants for Guinea Fowl Genetic Biodiversity

A molecular (genetic) marker is a consistent and hereditary variation that may be discovered using a suitable technology to determine the existence of one or more other genotypes or phenotypes besides itself (Fulton, 2009). The introduction of these novel genetic markers has opened up a plethora of fresh options for selection and genetic improvement opportunities for animals. These variations in DNA are employed in many applications such as selecting progeny based on inherited characteristics, testing lineage relations, distinguishing species, and studying population genetics (Naqvi, 2007). Variation of this kind also lays the groundwork for functional genomic maps, which are employed to find variations that drive profit and to speed up productivity traits (Zhang et al., 2015). This led to an evaluation that was done to see whether there was a better way to create and manage animal breeding and management programmes based on using molecular and biochemical markers (Toro *et al.,* 2009). Weigend and Romanov, (2002).; Bruford *et al.,* (2003); Gibson *et al., (*2006); and Teneva, (2009) stated that molecular markers are being used to quantify animal variety, uniqueness, and population structure, (Gholizadeh and Mianji, 2007), as well as for genetic conservation of confined populations in order to avoid extensive inbreeding (Zanetti, 2009). In evaluating guinea fowl improvement initiatives, some of the most notable DNA-based molecular approaches employed are explained below:

2.9.1 Restricted Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is reported to be the most extensively employed genetic assay created through hybridization as reported by Tazeb, (2018). Inherited RFLPs are Mendelian traits with point mutations in the 40 restriction endonucleases target sequence site, causing mutations in the fragments, and uneven crossing over as a result of evolutionary processes like gene mutation in the restriction enzyme recognition sites. The benefits of RFLPs are their co-dominant nature, their high level of reliability in linkage analyses, and their use inbreeding. Restriction digest and Southern blotting both require substantial amounts of DNA, which is a shortcoming of the RFLP marker. The RFLP is a substantially costly procedure and risky proposition because a radioactive isotope is essential. Only one out of every few markers may be

polymorphic, making the test method time-consuming and labour-intensive and causing it to be slow to implement in the avian breeding sector (Fulton, 2012). Single base alterations are beyond its capability; thus, polymorphism-detecting areas can only identify point mutations.

2.9.2 Random Amplification of Polymorphic DNA

Another way to identify mutation indicators is with the random amplified polymorphic DNA (RAPD) assay. These oligonucleotide primers are of variable sequence and used to amplify various segments of the genome using the polymerase chain reaction (PCR) as reported by Tingey and de, (1993) and Kumar and Gurusubramanian, (2011). A single template with 10 sequencing bases (oligo-nucleotide PCR) along with a minimum GC content of 50 per cent was used in the development of this PCR-based DNA marker in 1990 by William and his colleagues (Smiths *et al.*, 1996). The RAPD method is quite a simple, swift, and low-cost procedure for investigating both population and genetic variations in DNA using random amplified polymorphic DNA (RAPDs). It is extensively utilised in poultry research, especially when using dominant markers (Shivashankar, 2014). RAPD genetic markers were adopted to identify mutations (polymorphism) in five chicken breeds: White Leghorn (chosen for part-litter output and egg density) and Kadaknath (a native breed) and Red Cornish (selected for early body weights), as well as White Plymouth Rock (a long-term egg producer) (Sharma *et al.*, 2001). Heritability (how much of a genetic difference comes from genetics and how much from non-genetic influences) was also measured in the study, in which scientists also tested genetic diversity and heritability in and between four chicken breeds and two breeds of turkey flocks observed by Smiths *et al.*, (1996). RAPDs are the most cost-effective, versatile, and simple to perform PCR-based DNA markers. Because the method involves no previous knowledge of DNA sequences and uses small amounts of genetic material, it can even be practised on unusual plant populations. Banding patterns and dominant inheritance are two main drawbacks to RAPD markers. Due to the imprecise patterns produced from fingerprints, as well as fact about homozygotes and heterozygotes neither being discriminated as a function of predominant inheritance, the probability of incorrect conclusions about an individual's identity is significantly increased. RAPD has a couple

of limitations. First, the resulting fingerprint patterns are ambiguous, and homozygous and heterozygous variants cannot be discriminated since RAPD is a dominant trait (Rege and Okeyo, 2006). Furthermore, the mechanism by which the observed genetic variation is produced is not fully understood, trying to make reconfiguration of genetic evolution histories from RAPD data prove difficult.

2.9.3 Microsatellite

Sporadic simple sequence length polymorphisms (SSLP) sometimes called microsatellites, simple tandem repeats (STRs), or single nucleotide sequence repeats (SNPs) (Bruford *et al.,* 2003). Simple repetitive DNA sequences, or SSLPs, are among the tiniest types of DNA sequences. These are co-dominant, multi-allelic, and greatly polymorphic. It is commonly accepted that prokaryotic and eukaryotic genomes have introns and these elements are seen both in coding and non-coding locations. Tandem repeat loci can provide reliable and accurate measures of genetic diversity for use in researching genetic connections between chicken breeds (Weigend and Romanov, 2002). It is possible to detect and calculate these small genetic segments easily (Zhang *et al.,* 2002). It is quite possible that a lot of microsatellites have already achieved widespread popularity in chickens, and they have been mapped in a number of relevant databases (Gholizadeh and Mianji, 2007). Both within- and between-breed genetic diversity is an advantage when the sites where these sequences are found repeat the same short sequence several times. Additionally, the sites where these sequences are found exhibit rapid mutation frequency and a multilocus tendency, which makes them excellent platforms for study (Toro *et al.,* 2009).

2.9.4 Amplified Fragment Length Polymorphism (AFLP)

AFLP uses PCR amplification to identify restriction fragments. After performing two separate restriction endonuclease restrictions, a fraction of the resulting genomic DNA is amplified using a modified PCR and sequenced using computerized sequencing (Rege and Okeyo, 2006). In the case of the AFLP, there is no need for prior genome sequence information, thereby providing an advantage. A huge number of polymorphic bands are produced, with a high degree of repeatability, and standard kits are available (Duim *et al.,* 2000). Toro *et al.,* (2009) found that AFLPs are bi-allelic dominant indicators with an

incredibly simple genetic sequence variance testing method. The high resolution makes it possible to delineate complicated genomic architecture with the AFLPs (Rege and Okeyo, 2006). The challenge of evaluating related breed variance as a result of a strong mode of inheritance, as well as their potential usefulness in evaluating among breed variance, presents itself when using the theory of quantitative genetics.

2.9.5 Single Nucleotide Polymorphism (SNP)

A novel and extremely exciting molecular tool that provides a variety of assessments of genetic diversity by examining how changes occur in a specific area of the genome is known as a single nucleotide polymorphism (SNP) (Weigend and Romanov, 2002). In particular, genetic variety is defined as any differences between two genomes, including the interchange of strands of nucleotides (Fulton, 2008). The most prevalent kinds of genetic variants may be identified in DNA, and these discoveries, in addition to insertions/deletions, have served as the basis for most of the different allele phenotypes (Gu, 2004; Ye *et al.,* 2007). SNPs are most frequently found in the coding region, while they may also be found in the non-coding region (mostly in the mentioned region) as well as the intergenic regions. SNPs are present in the genome in large numbers; therefore, they are a solid source of genetic information and can be analysed by high-throughput automated methods, making them one of the more interesting methods in the identification of species (Teneva and Petrovic, 2010). SNPs appear around once every 1,000 to 2,000 base pairs, and as such could be utilised as a genetic marker to detect and examine SNPs regarding quantitative characters (Gu, 2004; Ye *et al.,* 2007). The Galliformes genome has shown to have one SNP per every 225 base pairs, that's 5 times more than in person (Jalving *et al.,* 2004; Orsini *et al.,* 2011). In their paper "Toward More Accurate Identification of Neutral and Efficient Genetic Variation", Toro *et al.*, (2009) showed that while most genetic variation is present in non-coding areas, some correlate to mutations that alter gene expression.

2.9.6 Mitochondria DNA

The mitochondria are tiny organelles, and each one contains its own separate, non-nuclear genome, called an extrachromosomal genome. Because it is inherited through the maternal line, mitochondrial DNA does not recombine, therefore allowing for the

investigation of numerous populations' phylogenetic relationships and subspecies, as well as the species (Shen *et al.,* 2002). The team of researchers at Indiana University are the first to attempt an mtDNA study in the Chunky broiler, and it is their goal to use the information to make comparisons amongst chicken breeds for egg production. The new mitochondrial cytochrome gene evidence they discovered was the result of studies conducted on the mitochondrial cytochrome genes of their control chicken (Chunky) and the other three replicates chicken breeds, Shen *et al.,* (2002).

2.10 Polymorphisms

A polymorphism (mutation) is a genetic variation that is found in nothing less than one-tenth of a population's genetic makeup. By establishing a cut-off of one per cent, it is possible to rule out random alterations that may have originated within and spread through the generations of a single-family. Polymorphism occurs commonly in enzymes. A population of enzymes encoded by a gene nucleotide may have two or more variants of the enzyme expressed by that locus. The amino acid sequences of the variants differ slightly, and this frequently results in the variants migrating in different directions when electrophoresed. It is possible to detect the presence of the enzyme by loading the gel with the enzyme's substrate. Alleles can be identified from one another based on the speed at which their genetic code moves in the organism. Allozymes are enzyme variants that can be detected by electrophoresis and are found in a population. Rognon, et al., (1998)

2.11 Distance Measures and Genetic distance

The combination of clustering techniques employs the differences (similarities) or distances amongst objects when the groups are formed. The similarity is a set of guidelines that act as requirements to group or separate objects. This distance may be as a result of a single or several observations with a set of procedures and conditions to group organisms in each dimension. The easiest technique to calculate distances (length) among components in multiple dimensional spaces is to calculate Euclidean length (space). It is determined as follows: distance $(x,y) = (\sum_i (x_i - y_i)^2)^{½}$

Note that the distances of Euclidean (and Euclidean squared) is commonly calculated using raw data and not standard data. This approach provides a number of benefits (For example, the addition of new items to the study, which may be outliers, does not affect the distance between any two objects). However, discrepancies of scale between the parameters from which the distances are calculated can be substantially altered. Genetic distance refers to the genetic difference between species or across populations in an organism that can be linked by several measures. Smaller genetic distances show a tight genetic link, whereas wider genetic distances show a more distanced genetic relation. Genetic proximity can be used to examine the relatedness in human and chimpanzee species. Within such a mammal genetic distance, the divergence between the various sub-species can be measured. The genetic difference between two populations in its simplest form is the difference in the frequencies of a feature. The genetic distance with several attributes (traits) can then be averaged nearly for computing the genetic distance overall (Cavalli-Sforza, 2001).

2.12 Measure of Genetic Distance

Theoretically, several actions show genetic distances, including the Fixation Index, a popular measurement of genetic distance ranging from 0 to 1. A 0 value suggests that two genetically identical populations, while the 1 value shows that two species are unique breeds; Genetic differences originate as a result of mutations, genetic drift, developed by Nei (1973) and Cavalli Sforza and Edwards (1967) as well as Reynolds *et al.,* (1983) assume that gene variation only results via genetic drive.

Genotype or haplotype drift is a shift in genetic variability (allele) frequency in a group of organisms because of generic samples (Masel, 2011). The alleles in the progeny are a sampling of the ancestors, and chance helps to determine that a certain person lives and replicates. A population alone frequency is the proportion of a gene that shares a specific shape (Futuyma, 1998). Genetic drift can cause gene variations to totally vanish and hence limit genetic variety. If there exist fewer duplicates of an alternative gene, haplotype drift is stronger and the effect is reduced when there are several copies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Overview of the research area

Nigeria is positioned between Global Positioning System (GPS) coordinates (latitudes) 0f 4^0 - 14^0N and 2^0 - 14^0E longitudes respectively. It has a landmass of 923,768km^2 (FOS, 2012). It is surrounded northward by Niger and the Republic of Chad, westward by the Republic of Benin, eastward by Cameroon, and southward by the Atlantic Ocean. The vegetation of Nigeria is separated into two large belts which are rain forest and grassland. The Grasslands ecosystem of Nigeria, like that of the rest of West Africa, is segregated into three primary zones that extend from south to north: Guinea Grasslands, Sudan Grasslands, and Sahel Grasslands. The majority of the birds live in Sudan Savannah, a vegetation belt in the northwest that stretches from the plain of Sokoto at the west region to the northern parts of the intermediate plateau. This encompasses nearly all northern communities that shared a border with the Republic of Niger which accounts for more than a quarter of Nigeria's overall landmass. Sokoto State is located in Nigeria's extreme northwest, near the confluence of the Sokoto and rivers Rima.

Landmark University Teaching and Research Farm is located in Omu'Aran, Kwara State, Nigeria. The farm shares a borderline with Ifelodun Local Government Area northward, Osun State southward, Ekiti and Offa Local Government Areas to the eastward and westward, respectively, and has residents of 148,610 people as reported in the 2006 population census and have a land cover area of 737 square kilometres (https://en.wikipedia.org/wiki/Irepodun, 2021). Omu'Aran can be found at Latitude 8.9^{0}N and Longitude 5.61^{0}E. It is situated on a section of Elliu Hill and is the highest point in Kwara State, standing at 1,824 feet above sea level (weatherspark.com, 2021).

3.2 Climatic condition

The climatic condition in the research area is humid tropical, with clear rainy and dry seasons. They are distinguished by high temperatures and relative humidity. The average yearly rainfall is around 1367mm, with a lower quantity difference of close to 10 per cent. The rainfall is highly seasonal, with distinct wet and dry seasons and double

maxima as a result of the August "little dry season." The changing prevalence of moist maritime southwest downpour of winds from the Atlantic Ocean and dry continental northeasterly harmattan from the Sahara Desert is associated with these distinct rainy and dry seasons. In an average year, the rainy season lasts from April to November, with a break in August when rainfall is relatively low due to the prevalence of stratiform clouds, which are not thick enough to produce large amounts of rain. From November to early April, the dry season lasts. The average number of rainy days per year is 112. The temperature of the atmosphere is nearly the same all through the year, with noticeable variation from the annual mean of 27 degrees Celsius. The hottest months are February and March, with temperatures of 28°C and 27°C, respectively, while June is the coolest, with a temperature of 25°C. During the peak of the harmattan in January, the lowest mean temperature (20°C) is recorded. The lowest mean maximum temperature is recorded in August when the value of solar radiation incidents on the ground is at its lowest due to the thick cloud cover.

3.3 The Experimental Design

All morphometric data collected from experimental birds were subjected to a completely randomized design to examine any differences among parameters across the three breeds of birds used in this study. The design used was completely randomized in a factorial experiment.

3.4 The Experimental Birds

A total of sixty (60) birds within 3 plumages was used for morphometric analysis, of which 40 are local and 20 are exotic adult guinea fowls. The mixed sexes of these birds were utilized. The birds were wing tagged for identification purposes and raised on deep litter. They were individually wing-tagged and quarantined for on-station blood collection for DNA extraction, Molecular and Biochemical analyses.

3.5 Identification

The grey helmeted guineas were reported to have five varieties in Nigeria: Pearl, White-Breasted, Lavender, Black and White (Ogundipe, 1983). However, Ayorinde (2004) only

recognised four varieties from the bird breeding behaviour: Black, white, Lavender and Pearl. The plumage colours of the variants are:

- The pearl is frequently marked with pure grey plumage with little white circular patches of white feathers on its breast.
- The Lavender is filled with white ash or light grey feathers
- The black is wholly black in plumage, although circular white spots are particularly noticeable on the flying plumes.
- The white variety is pure white

The photographs catalog of different varieties of guineas are presented in plates 4 - 7.

Plate 4: Guinea bird (Pearl)

Plate 5: Guinea bird (Lavender)

Plate 6: Guinea bird (Black)

Plate 7: Guinea bird (White)

3.6 Measurement of Quantitative Traits

Individual bird weight was collected with a 5-kg sensitivity weight scale of 0.01 kg. The measurements of length (distance) and girth (circumference) were performed with a centimetre measuring line (cm). Morphometric parameters were measured in the morning by skilled personnel (to prevent mistakes and biases caused by personal idiosyncrasies). Each measurement is taken at least twice as well as the average variable value of the measurements is reported.

3.7 Quantitative Traits

On each of the animals, body weight (BWT) and 13 major biometric characteristics were obtained. The biometrical variables include head length (HDL), head thickness (HDT), helmet length (HL), helmet width (HW), wattle length (WL), wattle width (WW), neck length (NL), neck circumference (NC), wing length (WGL), truck length (TL), the keel length (KL), the chest circumference (CC), thigh length (THL), thigh circumference (THC), shank length (SL), shank thickness (ST), wing length (WGL) and wingspan (WGS). Data were grouped by variety.

3.8 Description of Reference Points of Measurements

As illustrated in plate 5, the anatomical reference points adopted were based on the standard descriptor (FAO, 2016).

Body Weight (BW): It is the measurement of the body mass of an organism (guinea), determine in kilogram (kg) with a sensitive scale.

Breast Girth (BG): This is the radius of the girth at the deepest location of the breast. A tape rule was used in taking the measurement.

Body Length (BDL): A tape rule was used to measure this. It measured length between the cervical base and pygostyle. The distance from the first cervical vertebra (Atlas) to the posterior end of the ischium.

Head length (HDL): Taken between the most protruding point of the occipital and the frontal (lacrimal) bone.

Head thickness (HDT): Head thickness is the distance of curvature around the middle of the head.

Helmet length (HL): This is the evaluation of the length of the helmet to the head base, usually in centimetres (cm).

Helmet width (HW): Measured as the distance between the broadest part of the helmet

Wattle length (WL): It is taken as the length between the broadest portion of the wattle to beak.

Wattle width (WW): It is the evaluation of distance between the broadest part of wattles.

Neck length (NL): Distance of the cranial dermal bone and the choroid plexus margins.

Neck circumference (NC): Taken with measuring tape, the widest point of the neck.

Wing Length (WL): The length between the tip of the phalanges and coracoid-humerus joint. A tape rule was used in taking this measurement.

Wing Span (WS): Distance between the two wings at a stretch, from tip to tip.

Trunk Length (cm): The distance of ischium from the posterior end to shoulder attachment.

Keel length (cm): Keel length (Sternum or breast bone) to be evaluated from the keel's anterior tip to its posterior end.

Chest circumference (cm): This is the circumference of the body around the breast region.

Thigh-length (TL): This is an evaluation in between the knee and the hip. Measurement between the hock to femoral joints, it's measured in centimetres.

Thigh circumference (TC): Evaluated as the diameter from the widest point of the thigh;

Shank length (SL): This is evaluated as the size in between the gambrel joint to the tarsometatarsus. The measuring rule was used to measure most especially, the right leg of the bird.

Shank thickness (ST): Measurement used to find the overall thickness of the shank was done by measuring the midpoint of the shank.

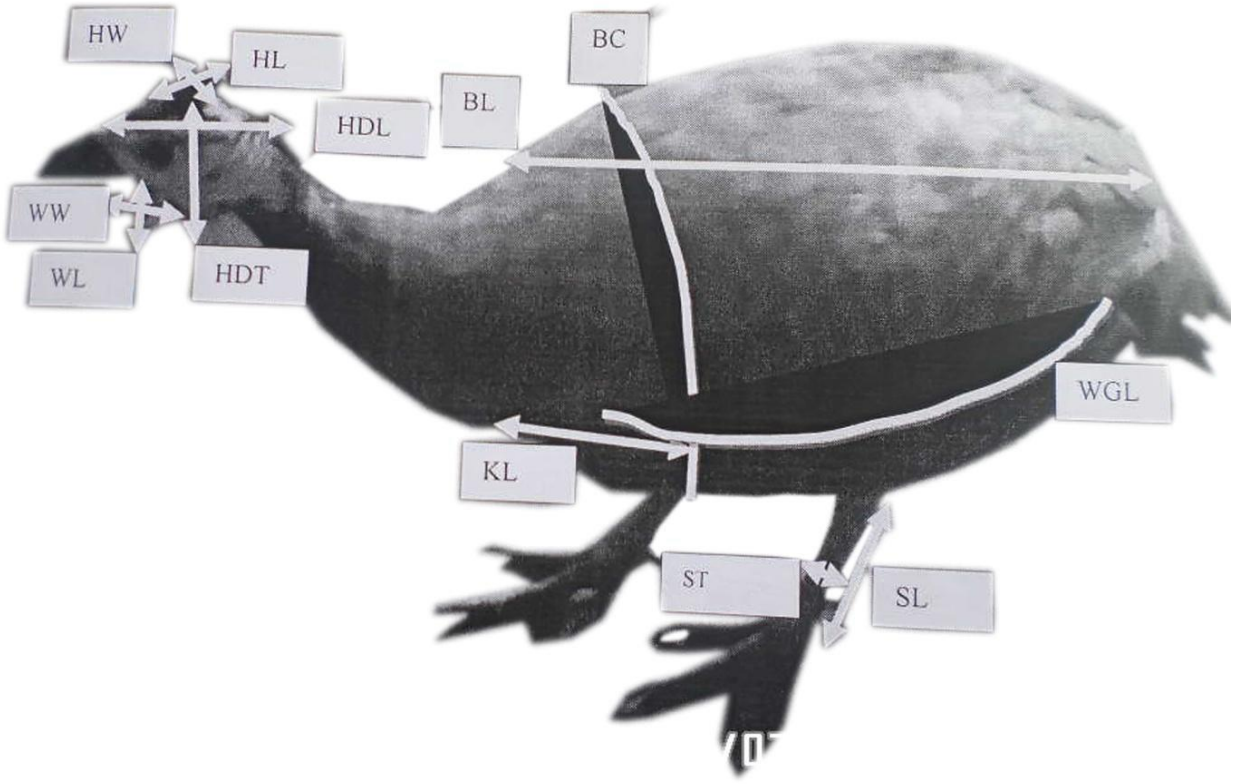


Plate 8: Some reference points for body measurements of Guinea fowl (Fajemilehin, 2014)

HW-Helmet width, HL-helmet length, WW-Wattle width, WL-Wattle length, HDT–head thickness, HDL –head length, BL - Body length, BC- Body circumference, KL- Keel length, SL –Shank length, ST-shank thickness, and WGL-Wing length

3.9 DNA Extraction

The FTA paper in which the blood was collected was punched five times with a micro punch and released into the 200µl Eppendorf (centrifuge) tube that was labelled 1 – 10. 150µl of TrisSDS was added to the sample collected in the Eppendorf tube to open the nuclear and cell membrane of the blood. The mixture (Tris and SDS) formed a soap-like liquid to emulsify the fat and proteins that surround the cell. The Eppendorf tube was closed and manually shaken gently (to avoid foaming reaction) for 30 minutes. After shaking; a micropipette was used to remove (170 - 190µl) liquid (desiccants) from each sample. Also, 150µl of distilled water was added to each sample to completely remove TrisSDS buffer with a gently shake for 10 minutes.

3.10 Electrophoresis

Electrophoresis is a scientific method used to determine Genetic code (DNA), RNA, or polypeptides (Proteins) based on size and electrical potential (charge). An electric current is applied to transport molecules to be differentiated across a gel. The settings involved throughout electrophoresis can be modified to separate molecules in a specific size range. The process of electrophoresis employed for this research include:

- Pouring the Gel: Agarose powder was dissolved in boiling buffer TBE which was a mixture of Tris, Boric Acid and EDTA and allowed to cool at 55^{o}C before casting in the electrophoresis chamber.
- Mixing the DNA: 4µl to 6µl of SYBR Green dye was used to stain DNA samples to provide colour and allow the samples to sink in the gel.
- Loading the DNA: Each DNA sample was placed in the well of the electrophoresis chamber.
- Running the Gel: The chamber was connected to a power source to initiate electrophoresis.
- Visualizing the Gel: The gel running was snapped with a camera for viewing.

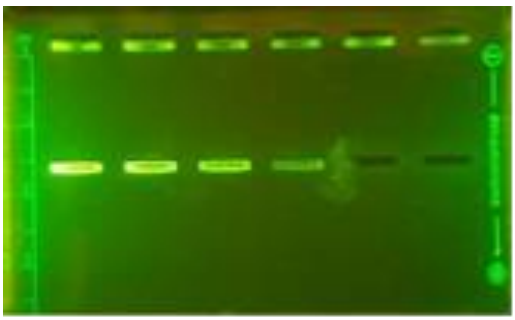


Plate 9a: Electrophoresis No run, no marker

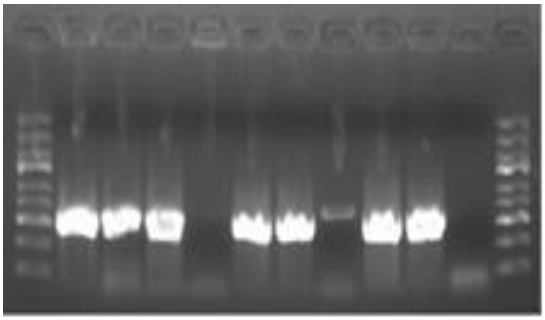


Plate 9b: Electrophoresis, No DNA visible on samples 4, 7 and 10

96^0C	15min	
95^0C	30 sec	
58^0C	30 sec	
70^0C	1:40 s	
70^0C	5 min	
12^0C		

	1x	11
H20	12.8	140.8
PCR Buffer	2.5	27.5
$MgCl_2$	1.5	16.5
dNTP's	1	11
FWD	1	11
REV	1	11
Hot Taq	0.5	2.2
gDNA	5	

Table 3.1: Electrophoresis Primer

3.11 Primer Design and Sequencing

AAAAGCTGCAGTGACTGTAAGATCATGCAAAAGCTAGCAGTCTATGTTTATA
TATACCTGTTCATGCAGATTTCTGTTGATCCGGTGGCTCTTGATGGCAGTAGT
CAGCCCACAGAAAACGCTGAAAAAGACGGACTGTGCAATGCTTGTACGTGGA
GACAGAATACAAAATCCTCCAGAATAGAAGCCATAAAAATTCAAATCCTCAG
CAAACTGCGCCTGGAGCAAGCACCTAACATTAGCAGGGATGTTATTAAACAA
CTTTTACCCAAAGCTCCTCCACTGCAGGAACTGATTGATCAGTATGACGTCCA
GAGAGACGACAGTAGCGATGGCTCTTTGGAAGACGATGACTATCATGCCACA
ACCGAAACGATTATCACAATGCCTACGGAGTGTAAGTAATAACCCTGCTGCT
TTCG

“The bases in green are the end of the untranslated 5 prime regions just before exon 1, while the red bases are the beginning of intron 1-2. The blue bases are the region of interest (Guinea fowl Myostatin Exon 1).

Primer:

Fowl-Myo-ex1-FWD – 5’ AGTTCACCCTTGGCTGTAAC ‘3

Fowl-Myo-ex1-REV – 3’ ACACTCCGTAGGCATTGTG ‘5 Tm 59ºC

The primer design and sequencing of the DNA in this research were carried out at Stabrigar Biotechnology Laboratory, Portugal.

3.12 Data Analysis

- Descriptive statistics was adopted to describe the mean values of morphometric parameters for significant differences, at a 95% confidence interval ($p \leq 0.05$)
- The least-square means were used to evaluate the substantial difference between body weight and other morphometric traits as well as sexual dimorphism.
- Myostatin gene isolated from the blood was compared with the already available Myostatin sequence in the repository of the National Center for Biotechnology Information (NCBI) with (accession number XM-021400400.1) using DNASTAR software (Lasergene Inc., Madison, 2015).
- Myostatin gene sequence chromatogram was analysed with DNASp software version 6

CHAPTER FOUR

4.0 RESULTS

4.1 Pairwise

The sequences of the seven Nigerian indigenous helmeted guinea fowl samples displayed 43 mutations on 41 polymorphic sites, on a 640-bp sized segment. Of these polymorphic sites, 15 are singleton variable sites, while the remaining 26 are parsimony informative sites. Each of the singleton variable polymorphisms had 2 variants, while 24 of the parsimony-informative polymorphisms had 2 variants, and the remaining 2 had 3 variants. Nucleotide diversity was estimated at 0.02887, The D test statistic proposed by Fu and Li was 0.36503 (not significant; $p > 0.1$), Fu and Li's F test statistic was 0.36528 (not significant; $p > 0.1$), and Tajima's D test was 0.20995 (not significant; $p > 0.1$). A pairwise distance table was also generated to observe the genetic distances among the sequences used in this study.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 5**	**Sample 6**	**Sample 8**	**Sample 9**
Sample 1							
Sample 2	0.0331						
Sample 3	0.0252	0.0346					
Sample 5	0.0395	0.0394	0.0378				
Sample 6	0.0314	0.0330	0.0377	0.0283			
Sample 8	0.0236	0.0346	0.0299	0.0300	0.0172		
Sample 9	0.0220	0.0204	0.0283	0.0300	0.0220	0.0220	

Table 4.1: Pairwise distances between the Helmeted guinea fowl sequences used in this study.

A neighbour-joining phylogenetic tree was drawn using 32 sequences (seven sequences acquired from Helmeted guinea fowl specimens, along with twenty-five avian myostatin sequences selected by BLAST and downloaded from NCBI (details in Table 4.4 and figure 4, page 65 and 66 respectively), and tested with 1000 bootstrap replications. It showed all the helmeted guinea fowl sequences clustering into a large clade, though individually they did not all group together into smaller common clades. The Helmeted guinea fowl sequences shared a larger common clade with the guinea fowl sequence downloaded from NCBI, and this common guinea fowl clade was more closely related to

the clade which contained other gallinaceous birds (chicken, turkey, ring-necked pheasant, and Japanese quail) than any of the other avian clades.

Among the Helmeted guinea fowl sequences alone, haplotype diversity was 1, with each guinea fowl sequence having its haplotype.

Table 4.2: Singleton Variable Polymorphism Information of Helmeted Guinea fowl MSTN Exon 1

Site	Polymorphisms
4	A / G
269	G / C
322	T / G
362	G / A
368	A / G
396	A / G
422	A / G
425	C / G
456	G / C
480	G / C
553	A / G
593	G / A
598	G / T
628	C / A
634	G / A

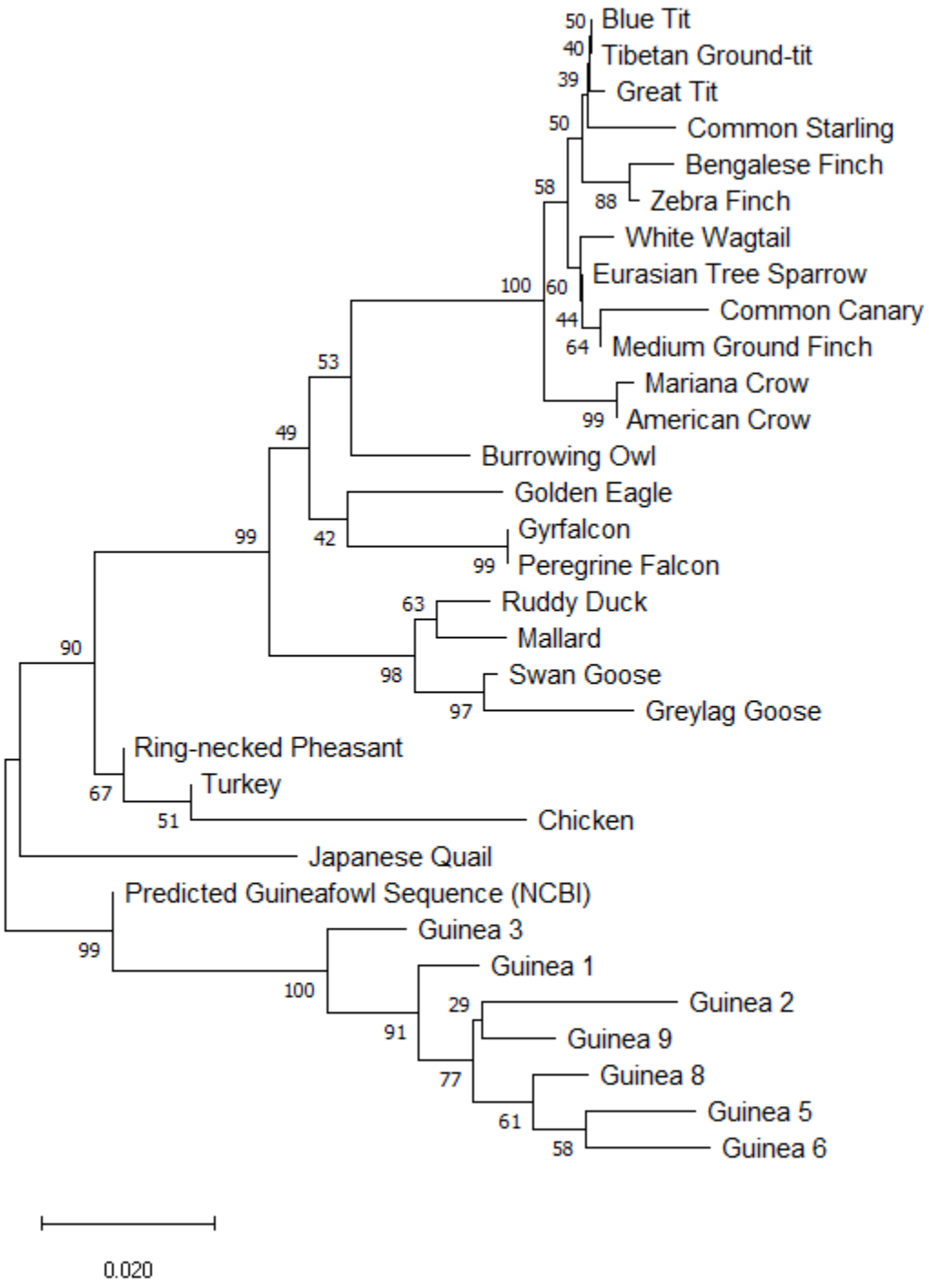
Table 4.3: Parsimony Informative Polymorphism Information of Helmeted Guinea fowl MSTN Exon 1

Site	Polymorphisms
2	T / C
3	C / A / T
7	A / C
222	A / G
273	G / C
276	G / T
311	T / A
312	G / T
330	T / G
342	G / T
346	G / C
359	C / G
403	T / A
447	G / C
459	T / G
469	A / C
555	A / G
563	A / G
590	G / A
608	G / A
613	G / A
616	A / T
627	C / A / T
629	A / C
631	C / G
635	A / G

Table 4.4: Information of the twenty-five avian sequences downloaded from Genbank

Accession Number	NCBI Information	Common Name
XM 042040706.1	PREDICTED: Corvus kubaryi myostatin (MSTN) mRNA	Mariana Crow
XM 039728754.1	PREDICTED: Passer montanus myostatin (MSTN) mRNA	Eurasian Tree Sparrow
XM 038143386.1	PREDICTED: Motacilla alba alba myostatin (MSTN) mRNA	White Wagtail
XM 037396897.1	PREDICTED: Falco rusticolus myostatin (MSTN) mRNA	Gyrfalcon
XM 035331618.1	PREDICTED: Oxyura jamaicensis myostatin (MSTN) mRNA	Ruddy Duck
XM 031604466.1	PREDICTED: Phasianus colchicus myostatin (MSTN) mRNA	Ring-necked Pheasant
XM 030016954.1	PREDICTED: Aquila chrysaetos chrysaetos myostatin (MSTN) transcript variant X2 mRNA	Golden Eagle
XM 026852905.1	PREDICTED: Athene cunicularia myostatin (MSTN) mRNA	Burrowing Owl
XM 023930719.1	PREDICTED: Cyanistes caeruleus myostatin (MSTN) mRNA	Blue Tit
XM 021531392.2	PREDICTED: Lonchura striata domestica myostatin (MSTN) mRNA	Bengalese Finch
XM 021400400.1	PREDICTED: Numida meleagris myostatin (MSTN) mRNA	Helmeted Guineafowl
XM 015867858.2	PREDICTED: Coturnix japonica myostatin (MSTN) mRNA	Japanese Quail
XM 015634184.3	PREDICTED: Parus major myostatin (MSTN) mRNA	Great Tit
XM 014880960.1	PREDICTED: Sturnus vulgaris myostatin (MSTN) transcript variant X1 mRNA	Common Starling
XM 013178647.1	PREDICTED: Anser cygnoides domesticus myostatin (MSTN) mRNA	Swan Goose
XM 009088136.3	PREDICTED: Serinus canaria myostatin (MSTN) mRNA	Common Canary
XM 008630155.2	PREDICTED: Corvus brachyrhynchos myostatin (MSTN) mRNA	American Crow
XM 005530338.1	PREDICTED: Pseudopodoces humilis myostatin (MSTN) mRNA	Tibetan Ground-tit
XM 005415977.2	PREDICTED: Geospiza fortis myostatin (MSTN) mRNA	Medium Ground Finch
XM 005241307.3	PREDICTED: Falco peregrinus myostatin (MSTN) mRNA	Peregrine Falcon
XM 005011412.5	PREDICTED: Anas platyrhynchos myostatin (MSTN) mRNA	Mallard
XM 002195107.6	PREDICTED: Taeniopygia guttata myostatin (MSTN) mRNA	Zebra Finch
NM 001303161.1	Meleagris gallopavo myostatin (MSTN) mRNA	Turkey
DQ355160.1	Exons 1, 2 with partial cds of the Anser Anser myostatin gene	Greylag Goose
AY670651.1	Gene for myostatin (MSTN) from Gallus gallus exon 1 and partial cds	Chicken

Figure 4: Neighbour-joining Phylogenetic Tree showing seven Helmeted guinea fowl myostatin exon 1 sequence, and 25 myostatin sequences of other avian species



4.5 Morphometric Traits

Table 4.5 shows the effect of helmeted guinea fowl plumage on selected morphometric parameters, which are bodyweight, head length (HDL), head thickness (HDT), helmet length (HL), helmet width (HW), wattle length (WL), wattle width (WW), neck length (NL), neck circumference (NC), wing length (WGL), wingspan (WGS), body length (BL), trunk length (TRL), keel length (KL), chest circumference (CC), thigh length (TL), thigh circumference (TC), shank length (SL), and shank thickness (ST). In almost all parameters studied significant ($p<0.05$) differences were obtained due to plumage colour, exotic helmeted guinea fowls had higher values than the pearl and white, and the white plumage showed the least values in other parameters with the exception of HL, WL, WW, NL, WGL, KL, CC, TL and ST where it either performed nominally or significantly better than the pearl variety.

Table 4.5: Effect of plumage type on bodyweight and morphometric traits

Traits	Exotic	Pearl	White	SEM	P-value
Bodyweight (g)	2.67[a]	1.74[b]	1.35[c]	0.11	0.0003*
HDL	5.00[a]	4.80[b]	4.00[c]	0.08	0.0138*
HDT	12.75[a]	11.75[b]	11.50[b]	0.40	0.017*
HL	3.20[a]	1.40[c]	2.25[b]	0.15	0.0002*
HW	2.63[a]	1.63[b]	1.65[b]	0.18	0.010*
WL	3.95[a]	2.05[c]	2.60[b]	0.28	0.005*
WW	2.85	2.10	2.85	0.29	0.200
NL	10.88	11.30	12.00	0.95	0.796
NC	8.00	7.50	7.50	0.23	0.308
WGL	31.50[a]	16.00[b]	16.25[b]	1.09	0.001*
WGS	71.63[a]	38.13[b]	37.25[b]	1.88	0.001*
BL	37.75	35.93	29.00	2.31	0.155
TRL	31.25	26.77	26.25	1.36	0.09
KL	23.50[a]	10.95[b]	12.00[c]	0.92	0.001*
CC	38.13	32.00	35.50	1.48	0.061*
TL	13.42[a]	11.38[c]	12.00[b]	0.45	0.04*
TC	12.25	11.00	10.65	0.96	0.121
SL	14.00[a]	7.25[b]	7.00[b]	0.42	0.001*
ST	4.83	4.22	4.89	0.39	0.597

*abc superscript highlights significant ($p<0.05$) variations in mean scores across rows

4.6 Sexual Dimorphism

The impacts of sexual dimorphism on the above-mentioned morphometric parameters in helmeted guinea fowls are displayed in table 4.6. Females had higher body weights than males on average.

Table 4.6: Effect of sexual dimorphism on bodyweight and morphometric traits

Traits	Male	Female	SEM	P-value
Bodyweight (g)	1.97	2.10	0.28	0.75
HDL	4.74	4.86	0.13	0.53
HDT	12.30	11.90	0.42	0.52
HL	2.48	2.10	0.41	0.53
HW	1.92	2.14	0.28	0.59
WL	3.28	2.56	0.46	0.29
WW	2.50	2.60	0.30	0.82
NL	11.40	11.40	0.81	0.83
NC	7.80	7.60	0.23	0.54
WGL	22.20	22.30	3.88	0.98
WGS	52.84	49.86	8.39	0.81
BL	35.54	35.00	2.52	0.88
TRL	28.42	28.50	1.61	0.97
KL	16.66	15.70	3.08	0.83
CC	34.00	36.30	1.76	0.38
TL	12.30	12.34	0.60	0.96
TC	11.46	11.40	0.51	0.93
SL	9.70	10.10	1.70	0.87
ST	4.68	4.50	0.35	0.73

4.7 Phenotypic characterization of Exotic guinea fowl

Table 4.7 describes the phenotypic correlation concerning differences in body weight as well as certain selected morphometric qualities in the exotic guinea fowl. Guinea body mass was shown to be significantly ($p<0.05$) and showed a positive correlation with the selected parameters except for NL and SL where it was negatively and significantly correlated. It is also noteworthy to observe that both NL and SL were mostly negatively associated with other selected morphometric variables except where SL is positively correlated with BL and WL respectively. Coefficients ranged from 0.09 (WGL and HL) to 0.99 (BW and HL) in the positive direction and -0.23 (NL and HL) to -0.68 (NL and WGL).

Table 4.7: Phenotypic characterization of Exotic guinea fowl

	BW	**HL**	**WL**	**NL**	**WGL**	**BL**	**SL**
BW	1.0000	0.9949	0.5854	-0.2548	0.1741	0.6714	-0.3169
HL	0.9949	1.0000	0.6537	-0.2390	0.0907	0.7387	-0.2478
WL	0.5854	0.6537	1.0000	-0.4219	-0.2669	0.9836	0.5539
NL	-0.2548	-0.2390	-0.4219	1.0000	-0.6831	-0.2928	-0.4620
WGL	0.1741	0.0907	-0.2669	-0.6831	1.0000	-0.3333	-0.2428
BL	0.6714	0.7387	0.9836	-0.2928	-0.3333	1.0000	0.4162
SL	-0.3169	-0.2478	0.5539	-0.4620	-0.2428	0.4162	1.0000

4.8 Phenotypic characterization of Pearl guinea fowl

Table 4.8 displays similar information for the Pearl guinea fowl variety of Nigeria, however, bodyweight was revealed as being negatively and significantly ($p<0.05$) associated with the selected morphometric parameters except for BL and SL that shows positive association existed. HL, WL, NL and WGL had significant association phenotypically and were positive in direction, their interaction with BL and SL was however mainly negative and significant except for the interaction between NL and BL being positive in this regard. Positive coefficient values obtained ranged from 0.24 (NL

and BL) to 0.91 (BW with SL, HL with WGL) in the positive direction and -0.40 (HL and BL) to -0.89 (WL and SL) in this indigenous variety.

Table 4.8: Phenotypic characterization of Pearl guinea fowl

	BW	HL	WL	NL	WGL	BL	SL
BW	1.0000	-0.5541	-0.8261	-0.4135	-0.4655	0.1801	0.9137
HL	-0.5541	1.0000	0.7792	0.7861	0.9102	-0.4005	-0.8437
WL	-0.8261	0.7792	1.0000	0.3465	0.8561	-0.6639	-0.8987
NL	-0.4135	0.7861	0.3465	1.0000	0.4626	0.2488	-0.6679
WGL	-0.4655	0.9102	0.8561	0.4626	1.0000	-0.7435	-0.7303
BL	0.1801	-0.4005	-0.6639	0.2488	-0.7435	1.0000	0.2830
SL	0.9137	-0.8437	-0.8987	-0.6679	-0.7303	0.2830	1.0000

4.9 Phenotypic characterization of White guinea fowl

Table 4.9 indicates obtained results in the white guinea fowl variety on the phenotypic correlation of body measures. With the exception of SL that indicated non-significant (trivial) ($p>0.05$) and non-existing relationship with all measured traits, all other traits showed significantly perfect coefficients of relationship in either direction for this variety of guinea fowl.

Table 4.9: Phenotypic characterization of white guinea fowl

	BW	HL	WL	NL	WGL	BL	SL
BW	1.0000	1.0000	1.0000	-1.0000	-1.0000	-1.0000	0.0000
HL	1.0000	1.0000	1.0000	-1.0000	-1.0000	-1.0000	0.0000
WL	1.0000	1.0000	1.0000	-1.0000	-1.0000	-1.0000	0.0000
NL	-1.0000	-1.0000	-1.0000	1.0000	1.0000	1.0000	0.0000
WGL	-1.0000	-1.0000	-1.0000	1.0000	1.0000	1.0000	0.0000
BL	-1.0000	-1.0000	-1.0000	1.0000	1.0000	1.0000	0.0000
SL	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phylogenetic Tree

Phylogenetic characterization information presented by Figure 4 shows that all the myostatin guinea fowl sequences are closely related to the predicted myostatin sequence downloaded from Genbank, and less closely related to those of other gallinaceous birds (chicken, turkey, Japanese quail and ring-necked pheasant). This is expected, as sequences of taxonomically related organisms should be more similar, and is in line with the taxonomical classification of the helmeted guinea fowl as a gallinaceous bird by Crowe *et al.,* (2006). Nucleotide diversity among the guinea fowl sequences was 0.02887, which is quite low, and might suggest that the guinea fowl sequences are quite closely related, due to a bottleneck in recent history, or some selective sweep that reduced nucleotide diversity (VanBuren *et al.*, 2016). It could also be due to indiscriminate interbreeding among domestic guinea fowl flocks (Walker *et al.*, 2004) or as the result of the low number of samples used in this study. Subsequent studies with larger sample numbers may be needed to verify this result.

5.2 Single Nucleotide Polymorphism

The myostatin exon 1 has 43 polymorphisms. These are possible potential regions for polymorphisms that may affect production traits in helmeted guinea fowls. Zhang *et al.* (2012a) suggested that a G > A mutation in myostatin's exon 1 of Bian Chinese chickens caused chickens with haplotypes AA and GA to have greater muscle mass than chickens with GG genotypes, making this polymorphism a possible good candidate for a genetic marker. With the number of polymorphisms on exon 1 of guinea fowls alone, such an orthologous polymorphism variant may exist in guinea fowls as well, though more research needs to be carried out to identify whether this ortholog exists. There is also a strong possibility that other polymorphisms present in the helmeted guinea fowls might have positive or negative genetic effects on the body parameters of these birds, especially two polymorphisms (on sites 3 and 627) which have two different nucleotide variants, as opposed to the others with just one. It may be expedient to focus subsequent research on the examination of these polymorphisms.

Fijabi *et al.,* (2020), in their research on the impact of myostatin polymorphism on the growth of indigenous Nigerian turkey, reported that there exists a general absence of distinctive growth differences due to this gene's polymorphism in Nigerian Turkey breed. "Preliminary research with avian, likewise with the vast majority of non-mammal vertebrates, attempted to link variations in myostatin expression to critical skeletal muscle growth periods or even to physiological responsiveness to a catabolic stimulus. The research was the first to show that myostatin's growth inhibitory properties may be conserved by avian and fish skeletal muscle, even if the results were purely descriptive". Recent research, on the other hand, is beginning to analyse not only the effects of myostatin in various vertebrates but also the LAP attenuating actions of myostatin. In chickens, polymorphisms in the myostatin gene were linked to variations in body weight. (Ye *et al*., 2007). Consequently, it is imperative to further explore the impact of myostatin gene study in the genetics and improvement of helmeted guinea fowl variety.

5.3 Morphometric of Guinea Fowl

Regarding qualitative parameters measured in this research, exotic guinea fowls were able to perform better than pearl and white guineas (counterparts) in all components, except neck length. This agrees with findings by Ayorinde (1991), who reported that unimproved local guinea fowls are considerably smaller than their exotic counterparts. This is because these exotic birds have most likely been selected extensively for commercial raising, with a selection focusing on larger body size and more meat production than the local birds, which have been mostly subjected to natural selection and are thus selected more in the direction of survival than commercial production. Between the pearl and white local guinea fowls, pearl showed higher components than the white on average. This agrees with a research result by Bernacki *et al.* (2012), in a study carried out to compare breast muscle structure and carcass composition in pearl and white plumaged guinea fowls. They reported that pearl plumaged guinea fowls showed higher body weights and muscle proportions than white guinea fowls. Although concordant findings by Fajemilehin (2010) report the superiority of pearl plumage over ash and black plumages in terms of morphometric body parameters, other works (Premavalli, 2013; Brown *et al.*, 2017) have reported that white guinea fowls are superior to pearl plumaged

birds in terms of body morphometric parameters. Premavalli (2013), mentioned though that pearl guinea fowls have a faster growth rate initially than white guinea fowls, though the white eventually overtake them in growth rate and body size after about 8 to 10 weeks of age. The observation in the result of this study showing that pearl was larger than white may be as a result of peculiar Helmeted guinea fowl genetics, and more studies on the comparison of white and pearl plumaged Helmeted guinea fowls might shine a more explanatory light on the subject.

5.4 Sex Dimorphism

With respect to sex, guinea fowls show very little sexual dimorphism especially as keets, as far as visual observation is concerned, although sex can be done by cloacal observation, or listening to the guinea fowl's cry; the male has a monosyllabic sound, while the female's sound has two syllables. The wattles and helmets of the males also seem to be larger on average, but females tend to have higher body weights, although these differences are not easily discernable by an observer, and cannot be reliably used to distinguish between males and females (Moreki, 2007). The study's findings are in agreement with the above citation, as females had higher body weights on average, although the males had higher values in wattle size, body length, keel length, thigh circumference, shank thickness and wingspan. Baeza *et al.*, (2001) presented similar findings, with female guinea fowls being heavier than males, although the male carcass of a guinea fowl yield was heavier than that of females. Also, values recorded in this study of shank thickness and thigh circumference are in accord with Kokoszynski *et al.* (2011), who noted, in findings similar to this study and Moreki (2009), that male guinea fowls had lower body weights, carcass weights, dressing percentage and abdominal fat accumulation, but had higher proportions of leg muscles, than female guinea fowls. Kokoszynski *et al.* (2011) also noted that female guinea fowls had higher breast muscle content, but this observation is at variance with the outcomes of this research work, which noted that male guinea fowls had higher keel length measurements than females. This may be a peculiarity of the differences between guinea fowls of different climes, although there's still work to be done in this area, to establish any suspected reason.

5.5 Phenotypic Correlation:

According to the conducted study, the strong connection reported between weight and most quantitative measures indicated that birds of the exotic and white plumage variety could be selected for using any of its associated parameters since similar trends were not obtained with the pearl variety, it goes to show the lower potential of such selection method in the bid to improve the variety, the observed results on the exotic and white variety agreed while that of the pearl variety was discordant with the findings of Subir *et al.* (2019) with significant results have already been observed by Daikwo *et al*. (2011). Differences in the correlation between body weight and other morphometric measures based on variety may point to deeper underlying genetic mechanism between varieties, hence the need to further investigate the impact of a gene such as myostatin on this indigenous variety as exemplified by the report that "significant association among body weight and body linear parameters indicated by the prediction equations revealed that regression equations could be useful in predicting body weight. While also indicating differences based on variety for the predictive potential of body measures as concluded by (Dudusola *et al.* 2021) that the highest coefficient of determination (R2) is in the wattle length which had the highest accuracy to predict body weight compared to the other body linear measurements in pearl, black and lavender varieties while the wingspan had the highest coefficient of determination for the white variety. The substantial and positive relationship between body weight and other morphological traits (such as body length, keel length, and chest circumference) shows that the traits are under the same gene action (pleiotropism), and that selection for improvement in one variable leads to improvement in the other because of the associated response. A similar observation was reported in the work of Ogah (2013) on guinea fowl.

CONCLUSION

This study presents information on exon 1 sequences of the myostatin gene in helmeted guinea fowls. There is relatively low nucleotide diversity, suggesting that these birds have a poor phylogenetic structure, due to possible bottleneck events or breeding within populations. Exotic birds had the highest morphometric parameters, and females on average weighed heavier than males. Differences in the correlation between body weight and other morphometric measures based on variety may point to deeper underlying genetic mechanisms between varieties, hence the need to further investigate the impact of a gene such as myostatin on this indigenous variety.

RECOMMENDATIONS

In respect to several mutations on different sites of polymorphisms in exon 1 of this myostatin gene which could have positive or negative on the performance of this bird; this research thereby recommends the following:

1. The whole-genome sequence of our local guinea fowl should be done to further look into the genome for genetic manipulation of these noble birds.
2. Gene mapping of the myostatin gene can be done on the loci to encourage gene insertion and trigger the inserted genes in the animals.

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