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COLLEGE OF BASIC AND APPLIED SCIENCES

ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS IN INSULIN-LIKE GROWTH FACTOR 1 AND INSULIN-LIKE GROWTH FACTOR 2 GENES AND GROWTH TRAITS, AND SURVIVABILITY IN THREE LOCAL CHICKEN ECOTYPES OF GHANA

BY

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DECLARATION

I, Roland Atabe Kanlisi, declare that the content of this thesis is result of my own research, field, and laboratory work. This thesis has not been presented for the award of any degree at any institution anywhere in the world.

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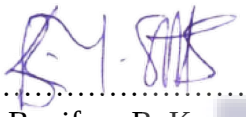

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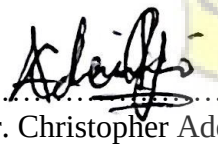

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ABSTRACT

Local chickens have comparatively smaller body sizes and slower growth rates. As a result, many smallholder farmers often tend to cross them with heavier breeds of chicken with the aim of improving their growth traits, a process that can lead to the dilution of their genetic composition and subsequent loss of important adaptive traits. Insulin-like growth factor 1 (cIGF1) and Insulin-like growth factor 2 (cIGF2*)* hormones have been widely reported to be associated with embryonic and post-hatch growth and development of chicken and the genes that code them have also been reported as candidate genes for growth and carcass traits of chicken.

This research was conducted with the aim of assessing the growth, carcass, and survivability traits of three chicken ecotypes of Ghana, to analyze the diversity of the *cIGF1* and *cIGF2* genes in these chicken ecotypes and assess the extent to which polymorphisms in these genes influence the growth and carcass traits of the three chicken ecotypes of Ghana.

Eggs of the Forest (FO), Interior Savannah (IS) and Coastal Savannah (CS) chicken ecotypes of Ghana were obtained from breeder stocks at the Livestock and Poultry Research Center (LIPREC) of the University of Ghana. These eggs were hatched and reared by ecotype up till 22 weeks of age. During this period, the body weights were taken fortnightly up till the end of the experiment after which the chickens were all euthanized, and some carcass traits measured. The quantity of feed consumed per bird during this period and the number of deaths were recorded daily. From these measurements, growth rates, survivability, Feed Conversion Ratios, and carcass traits were computed. DNA was also extracted from the chicken, and Polymerase Chain Reaction used to amplify targeted regulatory and exonic regions of the two genes. Thereafter, Restriction Fragment Length Polymorphism was used to detect single nucleotide

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polymorphisms (SNPs) in the PCR amplicons. These polymorphisms were confirmed with the aid of sanger sequencing and sequence alignments.

The results of this research showed that the IS and FO chicken ecotypes were heavier and grew at a faster rate than the CS ecotype. However, the FO ecotype had significantly ($p<0.05$) higher breast weight and breast yield than the IS and CS chicken ecotypes. The results also showed that the CS ecotype had the highest survival rate under intensive management conditions while the IS ecotype had the lowest survival rate. The three chicken ecotypes could therefore be used together in a breed improvement programmes that seek to enhance the growth and carcass traits of local chickens of Ghana by leveraging the higher growth rates of the Interior savannah chicken ecotype, the higher breast yield of the Forest chicken ecotype and the higher survival rate of the Coastal savannah chicken ecotype.

The RFLP digestion of *cIGF1* and subsequent sequencing revealed a *HinfI* C>A single nucleotide polymorphism (SNP) in the 5' UTR, a *PstI* T>C SNP in the promoter of the 5' flanking region, and a *BstBI* T>C SNP in the 3'UTR, while a *NlaIII* C>T SNP was identified in exon 3 of *cIGF2*.

At the *HinfI* C>A locus, the AC heterozygote was the predominant genotype amongst the FO and CS ecotypes, while the CC genotype was predominant in the IS ecotype. The C allele had the highest frequency amongst all the three chicken ecotypes. The observed heterozygosity values were higher than the expected heterozygosity values in the FO and CS ecotype populations. There was no significant association of the *HinfI* C>A SNP with body weights, weight gain and the carcass traits. This SNP locus may therefore not be an ideal molecular marker for selection to improve body weight and carcass traits in Ghanaian local chickens.

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At the *PstI* T>C locus, the CT heterozygote was the most predominant genotype among the three Ghanaian chicken ecotypes, with the T allele having the highest frequency. The observed heterozygosity amongst the FO and CS chicken ecotypes was higher than the expected heterozygosity. *PstI* T>C was associated with body weights at hatch and at 16 and 22 weeks of age ($p<0.05$) but it did not influence the carcass traits that were investigated. *PstI* T>C could therefore be a potential molecular marker for use in marker-assisted selection for the improvement of body weight in the local chicken ecotypes of Ghana.

At the *BstBI* T>C locus, CT heterozygote was the common genotype among the three chicken ecotypes while the C allele had the highest frequency. The observed heterozygosity of this polymorphism was higher in the FO and CS ecotypes than in the IS ecotype. *BstBI* T>C was significantly ($p<0.05$), associated with body weights at hatch, 2, and 10 weeks of age, but it was not associated with any of the carcass traits studied. This SNP locus could be useful in future marker-assisted selection for the improvement of body weight traits in the local chicken ecotypes of Ghana.

An analysis of the diversity of the *NlaIII* C>T SNP showed that the AB heterozygote was the predominant genotype amongst the FO and CS ecotypes while the BB genotype was predominant in the IS ecotype. The A allele had the highest frequency in the FO ecotype, while the B allele was the predominant allele in the CS and IS ecotypes.

The FO and IS ecotypes had lower observed heterozygosity values than the expected heterozygosity values. However, in the CS ecotype, the observed heterozygosity was higher than the expected heterozygosity. *NlaIII* C>T had no significant effect on body weights and any of the carcass traits studied. *NlaIII* C>T would therefore not be a good molecular marker for use in selection to improve body weight of chicken ecotypes in Ghana.

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The results of this research suggest that *PstI* T>C and *BstBI* T>C could be useful molecular markers for the improvement of body weight in future marker-assisted breeding programmes of local chicken ecotypes in Ghana. However, *HinfI* C>A, *PstI* T>C, *BstBI* T>C and *NlaIII* T>C SNPs may not be the ideal molecular makers for carcass traits improvement among the three chicken ecotypes of Ghana.

These studies conclude that genetic improvements in the economic traits of importance of the local chicken ecotypes of Ghana such as body weight could be carried out by taking advantage of the higher growth rates of the IS chicken ecotype, the higher breast yield of the FO chicken ecotype and the higher survival rate of the CS chicken ecotype. Furthermore, the *PstI* T>C and *BstBI* T>C SNPs could be considered as molecular markers for the improvement of body weight in future marker-assisted breeding programmes of local chicken ecotypes in Ghana

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DEDICATION

I dedicate this endeavour to

My parents Moses Musah Kanlisi and Kadah Kanlisi of blessed memory for instilling the spirit of tenacity in me and for all the sacrifices they made for my siblings and me.

To my brothers and sisters who encouraged me to pursue my dreams and passion.

To my wife and children for their support and tolerance.

To Prof. Boniface Kayang, Prof. Augustine Naazie, Dr. Chris Adenyo and Dr. Raphael Ayizanga for their professional guidance and mentorship.

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ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
AnGR	Animal Genetic Resources
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CS	Coastal Savannah
cIGF1	Chicken Insulin-like Growth Factor 1 hormone
cIGF1	Chicken Insulin-like Growth Factor 1 gene
cIGF2	Chicken Insulin-like Growth Factor 2 hormone
cIGF2	Chicken Insulin-like Growth Factor 2 gene
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
FCE	Feed Conversion Efficiency

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FCR	Feed Conversion Ratio
FO	Forest
GEBV	Genomic Estimated Breeding Value
GH	Growth Hormone
GHRH	Growth Hormone-releasing Hormone
GS	Genomic Selection
GWAS	Genome-Wide Association Studies
HWE	Hardy-Weinberg equilibrium
IGF	Insulin-like Growth Factor
IGF1	Insulin-like Growth Factor 1 hormone
IGF1	Insulin-like Growth Factor 1 gene
IGF2	Insulin-like Growth Factor 2 hormone
IGF2	Insulin-like Growth Factor 2 gene
IGFBP	Insulin-like Growth Factor binding protein

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IS	Interior Savannah
LD	Linkage disequilibrium
LIPREC	Livestock and Poultry Research Centre
LSM	Least Square Means
MAI	Marker Assisted Introgression
MAS	Marker Assisted Selection
ME	Metabolizable Energy
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Center for Biotechnology Information
NDPC	National Development Planning Commission
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RFI	Residual Feed Intake

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RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SAS	Statistical Analysis System
SNK	Student-Newman-Keuls
SNP	Single Nucleotide Polymorphism
TH	Thyroid hormone
TRH	Thyrotropin-releasing Hormone
T_3	Triidothryonine
UN	United Nations
UNDP	United Nations Development Programme
UNICEF	United Nations Children Fund
UTR	Untranslated Region

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CHAPTER 1

GENERAL INTRODUCTION

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1.1 Background

Ghana has made some progress in child nutrition but anaemia, stunting and underweight are still major challenges among children in the country (UNDP and NDPC, 2015). About 17.5% of children in the country under 5 years of age are moderately stunted while about 6.8% are severely stunted (Global Nutrition Report, 2021). About 35.4% of women aged 15 to 49 years are anaemic, while malnutrition is still pervasive in the northern parts of the country, with the Northern region being the most affected with about 28.8% stunting and 9.1% wasting rates. The Volta and Upper East regions are also severely impacted by malnutrition with stunting and wasting rates of 20.9% and 7.2% and 7.5% and 7.2%, respectively. In general, 19.5% of men in the country are stunted compared to 15.6% women. There are also more underweight men (14.1%) than women (11%) (Global Nutrition Report, 2021).

It has been widely reported that there is a significant association between malnutrition and dietary diversity, to the extent that the likelihood of being stunted, wasted, or underweight among children decreases as the number of food groups increase (Khamis *et al*., 2019; Modjadji *et.al*., 2020). In Ghana, the diversity of the diet of the average person is low and is composed mainly of starchy roots (cassava, yams), fruits (plantain) and cereals (maize, rice). Starchy roots and cereals provide about three quarters of the dietary energy in Ghana, while the share of protein in the dietary energy supply is only about 9% (Agble *et al.*, 2009).

Apart from low dietary diversity, another contributory factor to malnutrition is low access to high quality nutrient dense foods, especially animal source protein. Unfortunately, animal source protein is quite expensive in Ghana with many resource-constrained households unable to afford it. Paradoxically, many small holder subsistence farmers who rear livestock would rather keep them as a store of wealth and social status or as a source of funds during

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emergencies than use them to improve their nutritional status (Nyantakyi-Frimpong *et al*., 2018).

Notwithstanding the fact that chicken and eggs are important sources of high-quality of protein, vitamins, and minerals, they are still inexpensive and readily available in most places (Mingle *et al*., 2021). Furthermore, there are no major barriers or taboos to the consumption of chicken and eggs as human food (Moges *et al.*, 2010).

There is a growing demand in Ghana and other African countries for the meat of local chickens and poultry products (Atuahene *et al*., 2014; Mujyambere *et al*., 2022). This increased demand is mainly driven by increases in disposable incomes, population growth and rapid urbanization. This demand is greatest in the urban areas of Ghana (Mingle *et al*., 2021), as well as among the middle class and health-conscious Ghanaians. Several authors, including, Atuahene *et al*. (2014); N'dri *et al*. (2018); Asante-Addo and Weible (2020), and Ragasa *et al.* (2020) have observed that most Ghanaians would rather have the meat of local chickens than consume imported chicken because of quality, taste, and food safety issues. Similarly, Kyarisiima *et al*. (2011) also reported that most people in Uganda prefer local chicken to the meat of exotic chicken strains, while in Bangladesh, most rural dwellers prefer the meat and eggs of local chickens because of their taste, toughness, pigmentation, low fat content and suitability for special dishes (Islam, 2000). Despite the apparent high demand for local chicken in Ghana and many African countries, its availability, especially in the urban areas remains a major issue (Ragasa *et al*., 2020). According to MOTI, (2010), the demand for poultry products far outstrips the supply with only about 10% of the national demand being met through local production (Flake and Ashitey, 2009).

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It is estimated that Ghana had a chicken population of about 68, 511 million in 2014 out of which about 11.35% was made up of local chickens (FAO, 2014). The national production of chicken currently stands at about 58,000 metric tonnes while the national demand is about 460,000 metric tonnes (Poultry World, 2020). The gap between the demand and supply of chicken in Ghana is mainly being addressed through importations. In 2018, Ghana was reported to have imported about 204,062 tonnes of chicken worth about $130 million (UN ComTrade, 2019). Other estimates have it that the country imports over 79% of its total chicken meat supply (Zamani *et al*., 2021). However, these figures may not depict the whole picture because most of the live chickens coming into Ghana from the neighbouring countries are not properly recorded (FAO, 2019).

The gap between the supply of poultry and the national demand does not seem to be closing. There is therefore an urgent need to increase local production of chickens, especially local chickens since they are reported to be very hardy, can thrive under suboptimal conditions of feed and health care (Tadelle *et al*., 2003a; Conan *et al*., 2012) and produce meat that is in high demand in Ghana and in the subregion (Asante-Addo and Weible, 2020; Ragasa *et al*., 2020)

In Ghana, the rearing of local chickens is being carried out mainly at small-scale subsistence levels, with average household flock sizes being in the range of 10-88 chickens (Dankwa *et al*., 2000). Despite several efforts in the past to promote the commercial production of local chickens in the country through crossbreeding schemes amongst others, there has been little success in this direction. One of the main reasons why the commercial rearing of local chickens in Ghana and for that matter Africa has not caught on, in part, could be attributed to government policies that do not adequately prevent the dumping of cheap poultry products in the country. Consequently, the local markets are inundated with imported frozen chicken meat which in turn is suffocating the local poultry producers and the whole poultry value chain. The relatively

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slower growth rates of local chickens compared to exotic breeds of chickens (Minga *et al.*, 2001; Hassen *et al.*, 2006; Osei-Amponsah *et.al.*, 2014; Birteeb *et.al.*, 2016; Chen *et al.*, 2019) seems to be another major reason why many farmers hesitate to rear them on a commercial basis. To obtain birds with bigger body sizes or higher growth rates, many farmers tend to cross their local chickens with exotic cocks of unknown genetic makeup, a process that often leads to either breed replacement or to the loss of some useful traits like resistance to poultry diseases and adaptation to extreme environmental and climatic conditions.

Growth in chicken, like in many other livestock species, is a complicated process that is regulated by a wide variety of neuroendocrine pathways. Hence, it is very difficult to achieve rapid progress in growth rates or body sizes by means of the conventional within breed genetic selection methods (Zhang *et al.*, 2008). Fortunately, "the current and ongoing advances in molecular genetics technology have provided new opportunities and pathways to evaluate genetic variability at the DNA level" (Zhang *et al.*, 2008). Genomic approaches such as candidate genes or molecular markers like single nucleotide polymorphisms (SNPs), could be used alongside the traditional breeding methods to improve the growth rates and egg production of local chickens within the shortest possible time.

Growth in farm animals is influenced by many genes, among which Insulin-like growth factor 1 (*IGF1*) and Insulin-like growth factor 2 (*IGF2*) genes have been widely reported as candidate genes (Wang *et al.*, 2005; Qiao *et al.*, 2015). Insulin-like growth factor 1 (IGF1) and Insulin-like growth factor 2 (IGF2) are protein hormones that are part of the somatotropic axis and play important roles in the differentiation, proliferation and growth of tissues (Kim, 2010). These hormones are secreted by the liver under the influence growth hormone but are ubiquitously present in other organs of the body. The effects of IGF1 are more pronounced in the postnatal

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stages of growth, while IGF2 is thought to be a primary growth factor required for embryonic and early development.

The association of single nucleotide polymorphisms in the genes that encode the IGF1 and IGF2 hormones, with growth traits have been extensively studied in several livestock species. Since these hormones are known to be associated with the growth of chicken (Tang *et al.*, 2010; Anh *et al.*, 2015; Yan *et al.*, 2017), it is possible that the genes that encode them have some single nucleotide polymorphisms which may be associated with growth and carcass traits, and for that matter, could be used as molecular markers in breeding programs that seek to rapidly improve the growth and carcass traits of local chickens in Ghana.

To a large extent, the genetic improvement of livestock depends on the genetic variation that exists within populations of the species in question. Genetic diversity is a fundamental source of biodiversity, and it can generally be defined as the differences in the DNA of individuals within a species. Species diversity on the other hand is the variety of species that exist within a particular region. Livestock populations that are genetically diverse are more likely to be able to cope and thrive in changing environmental and climatic conditions while species with low genetic diversity are more likely to get extinct over time. Within a population, genetic diversity can be reduced by selection, inbreeding and genetic drift, while mutations and gene flow tend to increase the genetic diversity. On the other hand, amongst populations, gene flow tends to reduce genetic diversity while natural selection, mutations and genetic drift increase it. Globalization and the use of reproductive technologies like artificial insemination have encouraged the spread out of a few highly productive breeds of farm animals, mostly of European and American origin around the world, leading to the crowding out of local livestock breeds and a reduction in genetic diversity of farm animals. In addition, climate change,

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environmental degradation, habitat loss, and changes in consumer demand and preferences are also important factors that affect genetic diversity.

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1.2 Justification

In Ghana, local chickens have been classified into three ecotypes i.e., the Interior Savannah ecotype (IS), the Forest ecotype (FO), and the Coastal Savannah (CS) ecotype (Osei-Amponsah *et al*., 2011; Kayang *et al.*, 2015;). Local chickens are generally very hardy and cope quite well under conditions of minimum feed availability and health care (Birteeb and Essuman, 2016). They are mainly produced for subsistence purposes but are gradually finding some space in the commercial sector (Aryemo *et al*., 2016). The rearing of local chickens is potentially a powerful agent of change in smallholder farming systems in Ghana as it offers rural households` opportunities to increase their incomes and take care of their pressing needs (Mpenda *et al*., 2018). They also facilitate the diversification of smallholder farm enterprises, enhance food security, create employment, and help to improve soil nutrient status through manure use (Padhi, 2016).

Despite the above-mentioned importance, opportunities, and advantages, one of the main reasons why local chickens are not being commercially reared in Ghana is their comparatively slower growth rates, even under optimal production conditions. This seems to suggest that genetic factors may be at play. In a quest for bigger and fast-growing chickens, Ghanaian farmers often try to improve the production traits of local chickens by crossing them with exotic breeds of chicken, a process that can lead to the erosion of the genetic composition of local chickens and to the loss of important traits like disease resistance, broodiness, ability to withstand high ambient temperatures etc. Therefore, the development of fast-growing local chickens will not only contribute to meeting the current high demand and preference for local chicken by boosting the availability of meat and eggs, but it may also contribute to a reduction in the importation of frozen chicken, leading to huge foreign exchange savings. Furthermore, it could also contribute to the preservation of local animal genetic resources (AnGR) in Ghana.

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Marker assisted selection is one of the approaches that finds a lot of use in genetic improvement programs of traits of livestock. Single nucleotide polymorphisms are molecular markers that have found space in animal breeding. Several genes, including the Insulin-like Growth Factor 1 (*IGF1*) and Insulin-like Growth Factor 2 (*IGF2)* genes have been reported as candidate genes for some production and reproduction traits in livestock.

Furthermore, some SNPs in *IGF1* and *IGF2* genes have also been reported to be linked with the growth and carcass traits of several livestock species including chicken (Zhou *et al.*, 2005; Moe *et al.*, 2009; Sato *et al.*, 2012; Promwatee *et al.*, 2013; Bhattacharya *et al.*, 2015; Yan *et al.*, 2017), guinea fowls (Ahiagbe, 2018), pigs (Markljung, *et al.*, 2009; Niu *et al.*, 2013; Yue *et al.*, 2014), small ruminants (Deng *et al.*, 2010; Naicy *et al.*, 2017) and cattle (Reyna *et al.*, 2010; Abo-Al-Ela *et al.*, 2014; Gui *et al.*, 2018).

To the best of my knowledge, studies on the association of SNPs in *IGF1* and *IGF2* genes of the Ghanaian local chicken ecotypes with growth, carcass and survivability traits have not been documented. Therefore, it is very important to analyse the extent to which SNPs in the *IGF1* and *IGF2* genes are associated with the survivability, growth, and carcass traits of the local chicken ecotypes of Ghana, so that they could eventually be used as molecular makers for the improvement of the growth and carcass traits of these chickens. Currently, there is a high demand for the meat of these chickens and yet due to their slow growth rates, they are not being produced on a scale that will meet the existing demand. Apart from the high demand for the meat of local chickens in the country, the hardy nature of these birds calls for concerted efforts to be made to improve and conserve them, especially within the context of an intensification of the effects of climate change.

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This study contributes to the literature by providing empirical evidence on the diversity of *IGF1* and *IGF2* genes in the Forest, Coastal Savannah, and Interior Savannah chicken ecotypes of Ghana. In addition, the findings of the study will provide researchers and the Government of Ghana with vital insights into the extent to which polymorphisms in these genes can be used as molecular markers when making policy or operational decisions for the improvement of the growth and carcass traits of the three chicken ecotypes of Ghana.

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1.3 Objectives

1.3.1 Main Objective

This research aimed to investigate the extent to which Single Nucleotide Polymorphisms in the untranslated and coding regions of *IGF1* and *IGF2* genes influence survivability, growth, and carcass traits of local chicken ecotypes from the Forest, Coastal Savannah, and Interior Savannah agroecological zones of Ghana.

1.3.2 Specific objectives

This research was carried out with the following specific objectives.

1. To assess the growth, carcass, and survivability traits of chicken ecotypes of the Interior Savannah, Forest, and Coastal Savannah agroecological zones of Ghana.
2. To assess the genetic diversity of the *cIGF1* and *cIGF2* genes in chicken ecotypes of the Interior Savannah, Forest, and Coastal Savannah agroecological zones of Ghana.
3. To estimate the associations of SNPs within the *cIGF1* and *cIGF2* genes of chicken ecotypes of the Interior Savannah, Forest, and Coastal Savannah agroecological zones of Ghana with their growth and carcass traits.

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CHAPTER 2

LITERATURE REVIEW

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2.1 The history of the domestic chicken

The genus *Gallus* (junglefowl) is composed of four species i.e., *Gallus gallus* - red junglefowl, *Gallus varius* - green junglefowl, *Gallus lafayettei* - Ceylon junglefowl, and *Gallus sonneratii* - grey junglefowl (Lawal, 2017; Eda, 2021). *Gallus gallus* has the widest distribution and is said to be the main progenitor of the domestic chicken (Larson and Fuller, 2014). However, some hybridization experiments have also implicated the genetic contribution of the other species of *Gallus* (Eriksson et al., 2008).

Gallus gallus has five subspecies namely: Cochin-Chinese red junglefowl (G. g. *gallus)*, Burmese red junglefowl (G. g. *spadiceus)*, Tonkinese red junglefowl (G. g. *jabouillei)*, Indian red junglefowl (G. g. *murgha)*, and Javan red junglefowl (G. g. *bankiva)* (Lawal, 2020). *Gallus gallus* ranges from sub-Himalayan northern India through southern China and Southeast Asia (Fuller *et al.*, 2011). It has thin, dark legs and can fly long distances. The females do not have combs while the roosters have elaborate plumage (Lawler, 2012).

An earlier study of mitochondrial DNA by Fumihito *et al. (*1996) showed that the domestic fowl originated from *Gallus gallus* and from a single domestication event that occurred in Thailand and its adjacent regions. However, later studies showed that the domestic fowl was domesticated in three separate regions in Asia (Liu *et al.*, 2006) from where dispersals to various parts of the world took place.

There is still no conclusive evidence of when and how domestic chickens were first introduced into Africa, but terrestrial and maritime introductions may have played a key part in this process (Mwacharo *et al.*, 2013). Gifford-Gonzalez and Hanotte (2011) reported that there were two main episodes of chicken introductions into Africa. The first episode occurred during the Ptolemaic period (300 B.C.) from the Mediterranean Sea through Egypt and later spreading via the Nile valley to West Africa (Fuller *et al.* 2011). The second episode is said to have occurred

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during the beginning to middle of the 1st millennium A.D from the Indian Ocean to the east coast of Africa by traders (Fuller *et al*., 2011; Mwacharo *et al.*, 2013).

2.2 Characteristic features and roles of local chickens

According to Conan *et al.* (2012), about 80% of chickens that are raised in Africa are local chickens. Over the centuries, these chickens have been crossbred with exotic breeds or by random breeding within flocks thus making it extremely difficult to standardize their characteristics and performance (Sonaiya and Swan, 2004). Depending on the country of origin, these chickens may be called by various names including, indigenous, local, native, backyard, traditional, family, or village chickens. In this thesis, they shall henceforth be referred to as local chickens.

Local chickens are domestic fowls that have adapted very well to harsh climatic and environmental conditions, including extensive, free range, and organic production systems (Birteeb *et al*., 2016). They can thrive under conditions of feed and water scarcity, and little or no health care (Tadelle *et al.*, 2003a; Conan *et al.*, 2012). They are often unimproved, have no identified description (Horst, 1989), have a multi-purpose use, including for egg and meat production, as well as for various social and religious purposes (Guèye, 2005).

These types of chickens are reared in almost every rural setting, especially amongst smallholder farming households in the developing world (Guèye, 2005; Okeno *et al.*, 2010; Bett *et al.*, 2011; de Bruyn *et al.*, 2015). The rearing of these types of chicken has several advantages, including the fact that there are virtually no religious or social restrictions to their rearing (Moges *et al.*, 2010); they have an ability to efficiently transform waste foods into high quality protein (Smith *et al.*, 2013); they require low capital investments; they do not require large

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spaces for their rearing and hence can be reared even by landless families and resource constrained households; their eggs and meat do not require large or elaborate storage and preservation facilities (Tadelle *et al*., 2003a).

Local chickens are kept by smallholder farmers for a variety of reasons. Tadelle et *al.* (2001) observed that in the central highlands of Ethiopia, they are kept for breeding purposes and to produce eggs and meat, which may be sold for income or for household consumption. Local chickens are a major source of income for rural households and are often the first asset to be sold whenever a family is urgently in need of cash to take care of its immediate pressing needs (de Bruyn *et al.*, 2015). Furthermore, these chickens also play major roles in religious sacrifices, and can also be used as gifts (Tadelle et al., 2001; Guèye, 2005).

In Africa, local chickens are generally kept under extensive free-range farming systems with flock sizes ranging from about 5 to 25 birds (Mpenda *et al.*, 2018; Guèye, 2005). According to Hagan *et al.* (2013), the flock sizes of local chickens in the Coastal, Forest and Savannah agroecological zones of Ghana are in the range of 10-25, 12-36 and 8-15 respectively. Local chickens most often scavenge for themselves, with limited access to inputs like supplementary feed, water, housing, and health care (Ajayi, 2010). There are also virtually no planned breeding processes in the rearing of these chickens (Menghesha, 2012).

2.3 Varieties of local chickens

Based on quantitative and qualitative traits, local chickens of Africa are said to have a high genetic diversity (Mtileni *et al.*, 2011; Conan *et al.*, 2012). Local chicken ecotypes come in different plumage colours, comb types, plumage cover, body sizes and shapes, and productive performance variables (Msoffe *et al.*, 2002; Mtileni *et al.*, 2011). The pattern of variations in the phenotypic parameters of these chickens depends a lot on where they are located, the

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genotype and the ecotype (Mpenda *et al.*, 2018). Variations in the phenotypes of local chicken ecotypes have been reported in Ghana (Adomako, 2009; Osei-Amponsah, 2010a), in Tanzania (Msoffe *et al.*, 2002; Mpenda *et al.*, 2018), in South Africa (Mtileni *et al.*, 2011), in Ethiopia (Halima *et al.*, 2007; Moges *et al.*, 2010), in Kenya (Okeno *et al.*, 2010), in Tunisia (Larbi *et al.*, 2013), and in Nigeria where Ajayi (2010) observed that the composition of normal feathered local chickens was about 91.8% while those of the naked neck and frizzle feathered local chickens were 3% and 5.2 respectively.

In Ghana, different phenotypes of local chickens are reared across the three major agroecological zones i.e., the Interior Savannah (IS) which covers the northern parts of the country, the Forest zone (FO) which consists of the Rain and Deciduous forests and the Coastal Savannah (CS) which straddles the coast in the south-eastern parts of the country. Studies undertaken by Osei-Amponsah *et al.* (2010b) and Kayang *et al.* (2015) on the chicken populations in these agroecological zones concluded that they have a variety of unique alleles and a high genetic diversity which was not influenced by their agroecological zones. Kayang *et al.* (2015) also postulated that the three local chicken ecotypes are probably derived from meat and egg type breeds of chicken. Furthermore, they also concluded that the IS and FO ecotypes were genetically similar but different from the CS population and attributed this to the proximity of the Forest and Interior agroecological zones. A recent study by Walugembe *et al.* (2020) concluded that the local Ghanaian chicken ecotypes were quite different from those of Tanzania. According to Walugembe *et al.* (2020), there is some overlap between the local chicken ecotypes of Ghana with the FO and CS ecotypes being closer to each other than the IS ecotype.

According to Horst (1989) and Leroy *et al.* (2012), the genetic resource base of local chickens in the tropics is very rich. As reported by Horst (1989), the following genes are present in local

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chickens and can be used in genetic improvement programmes: Dwarf (dw); naked neck (Na); frizzle (F); silky (h); slow feathering (K); non-inhibitor (id); fibromelanosis (Fm); peacomb (P); and blue shell (O). These genes are implicated in traits like improved fitness and disease tolerance, improved ability for heat convection, protection against UV radiation and improved shell stability (FAO, 1998).

2.4. Genetic improvements of local chickens

Several ecotypes of chicken in Africa have been described (Table 1). These chickens are reported to have high genetic diversity (Khobondo *et al*., 2015), an attribute that bodes very well for their chances of surviving in the future, especially in the face of the ongoing changes in the climatic conditions. The high diversity of these chickens is said to a result of variations in agroecological, environmental and climatic conditions and as well as the purposes for which they are being reared (Padhi, 2016).

Three categories of major genes are associated with the phenotypes of local chicken i.e., body weight reducing genes, feather reducing genes and plumage colour genes. While local chickens are reported to have attributes like good mothering ability, resiliency to harsh environmental conditions and immunity to common poultry diseases (Tixier-Boichard and Rognon, 2009; Manyelo *et al*. 2020), they also tend to have comparatively low production traits. However, it has been argued by several authors that the genetics of local chicken is not the main constraint to their ability to fully express their production potentials, but rather, attention should also be paid to nutritional and environmental factors (Dessie et al., 2011; Ul Haq *et al*., 2022). Improvements in the diets of local chicken has been reported to bring about increases in some the production traits of local chickens (Abdelqader et al., 2007).

A few examples of genetic improvements in the production traits of local chicken ecotypes have been reported. This includes the Fayoumi breed of chicken whose genetic improvement

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is reported to have started around the 1940s in Egypt (Hossaryl and Galal, 1994). Since then, two lines of the Fayoumi chicken have been developed, one line for the number of eggs produced and the other for body weight at 8 weeks. The medium-high heritability rates of the Kuchi and Tanzanian *Medium* chicken ecotypes, as estimated by Lwelamira (2009), suggests that these chickens could be improved through selection. The moderate-to-high heritability estimates for body weigh among populations of the Forest and Interior Savannah chicken ecotypes of Ghana also indicates that this trait can also be improved through selective breeding (Osei-Amponsah, 2013).

On station crossbreeding of local chickens with exotic breeds of chicken i.e., Rhode Island Red, White Leghorn, and Dahlem Red, has been caried out leading to significant increases in some production traits (Fulla, 2022). For example, crossbreeding the Fulani ecotype with the exotic Dominant Black egg type significantly improved body weight at first egg, egg weight, total number of eggs produced and egg fertility in the offspring compared to the pure Fulani ecotype (Sola-Ojo, and Ayorinde, 2011). Similarly, as reported by Adedokun and Sonaiya (2002), crossing the dual purpose Dahlem Red with the Fulani chicken or with Yoruba chicken produced offspring that had higher egg and body weights than the pure Fulani and Yoruba chicken ecotypes. Unfortunately, most of the crossbreeding programs that were implemented in Africa, though successful under experimental conditions, could not be sustained for various reasons (Dana, 2011).

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Table 1. Some breeds/ecotypes of chicken in Africa

Country	Breed/ecotype
Sudan	Baladi.
Lesotho	Basotho
Niger	Kolonto, Tswana
Morocco	Beldi
Chad	Djided Baladi, Karmout
Egypt	Fayoumi, Dandarawis, Baladi Beheri, Sina
Ethiopia	Kei, Tilili, Horro, Chefe, Jarso and Tepi
Algeria	Kabyle
South Africa	Venda, Ovambo
Nigeria	Fulani, Yoruba
Mali	Kokochie, Belachie, chiedieman,Doufowchie, Dakissechie, Kolokolochie, Chiefiman, Segachie
Burkina Faso	Poulet Du Djelogodij, Peulh, Souche Konde
Ghana	Necked neck, frizzle,

Source: http://dagris.ilri.cgiar.org/regions/65/breeds?order=name_1&sort=asc&page=5

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2.5 Local chicken production practices

2.5.1 Feeding

Local chickens in Africa, and for that matter Ghana, are mainly produced under the extensive, free range scavenging systems where they must fend for themselves most of the time. However, from time to time, they may be provided with supplementary feed. The main feed resources that they scavenge include insects, worms, herbage, scattered grains, and waste food. According to Tadelle and Ogle (2001), while the amount of feed resources that are available to scavenging chickens varies from season to season, it also depends on factors like the life cycle of insects and other invertebrates and on the activities of the farmstead such as land preparation, sowing and harvesting.

The supplementary feeding of local chickens in extensive farming systems is often carried out mainly because of decreases in the scavengeable feed resource base and the absence or limited availability of natural feed resources (FAO, 2014). According to Moges *et al.* (2010), about 97.5% of people in the North-western part of Ethiopia, who kept local chickens provided them with some form of supplementary feed, with grains and household leftovers being the major feed stuffs. They also observed that 85.4% of the farmers watered their flock only during the dry season while 14.3% of them provided water throughout the year. In Tunisia, it has been reported by Larbi *et al.* (2013) that about 90% of farmers who rear local chickens practice supplementary feeding.

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2.5.2 Housing

The housing of local chickens under free-range production systems is often rudimentary and comes in various types and forms including mud and wooden structures. Where it exists, the structures are mainly for the protection of the chicken at night from predators and extreme weather conditions (Kingori *et al.*, 2010).

In a survey conducted in the Greater Accra region of Ghana, Blackie (2014) reported that different forms of housing structures made of mud or wood with thatch roof were provided for local chickens by 49.1% of farmers, whilst 17.3% of them allowed their birds to perch on trees/roofs during the night. This is however at variance with the findings of Awuni (2002) who reported that in the coastal areas of Ghana, 91.7% of households provided some shelter for their local chickens while 8.3% allowed them to perch on trees in the night. Awuni (2002) also indicated that in the forest agroecological zone of Ghana, 38.9% of farmers provided shelter for their flocks while 27.8% did not provide shelter.

2.5.3 Breeding

Most smallholder farming households in Africa that keep local chickens do not engage in selective breeding of the chicken for future progenies. In Ethiopia, smallholder farmers who practised selective breeding of local chicken selected their breeding stock with a high preference for traits like mothering ability, disease resistance, and meat and egg taste (Terfa *et al*., 2019). It has been reported by Okeno *et al.* (2010) that in Kenya about 30% of smallholder farmers often select their chickens for breeding purposes, with about 99.2% of them using egg size as a major criterion. Other preferred traits among these farmers include body size, growth rate, disease tolerance, egg yield, and fertility (Okeno *et al.*, 2010).

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Generally, the small flock sizes that are typical of smallholder farming systems in Africa do not encourage selective breeding. As a result, there is inbreeding depression amongst local chickens, which is further exacerbated by diseases and poor feeding conditions (Khobondo *et al*., 2015).

2.6 Constraints of local chicken production

Local chickens are known for their adaptation superiority in terms of their immunity to endemic diseases and harsh environmental conditions (Ajayi *et al*., 2010). This notwithstanding, Newcastle disease (ND) is the most prevalent and economically important constraint affecting local chickens in Africa (Guèye, 2005; Moges *et al*., 2010; Ochieng *et al*., 2013; FAO, 2014). In Ghana, it has been reported by Awuni (2002) and Blackie (2014) that about 80% of chickens are lost annually because of outbreaks of Newcastle disease and other diseases like diarrhoea, fowl pox, worm, and lice infestations. Predation is also another important constraint. According to Hagan *et al.* (2013), the mortality rate of local chicks in the Coastal agroecological zone of Ghana is about 49.9%, while in the Forest and Savannah agroecological zones it is about 28.7% and 31.4% respectively. In Tanzania, over 50% of the mortality of local chickens occur amongst the chicks (Minga *et al*, 2001; Mwalusanya *et al*., 2002). In contrast, Malago *et al*., (2009) observed that under intensive rearing conditions, local chickens in the Morogoro area of Tanzania had a 14.7% mortality rate during the first four weeks of life and about 6.7% during the second month of life. Olwande (2010) reported that in Kenya only about 10-13% of the chicks of local chickens survived between post-hatch and 8 weeks of age, while Egahi *et al*., (2013) observed that the mortality rates of local chickens in the Guinea Savannah of Nigeria from hatch to 20 weeks of age were 32.18%, 17.33% and 26.03% amongst the normal feathered, frizzle and naked neck genotypes. The mortality rates of local chickens in Burkina

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Faso are reported by Kondombo *et al*. (2003) to be 4.2% for hens, 7.8% for cocks, 9.5% for cockerels, 7.5% for pullets and 31.7% for chicks.

2.7 Growth and development

Growth is generally defined as an increase in body mass while development is the progression from fertilization towards sexual maturity (Baker *et al*., 1993; Cogburn *et al*., 2000). Growth is a complicated process in which the endocrine system, genetics, nutrition, and other environmental factors, play very crucial roles (Kim, 2010). Growth occurs by the processes of an increase in the number of cells of an organic tissue (hyperplasia) or by an increase in the size of cells (hypertrophy) (Christopherson, 2010). Hyperplasia can be the result of mitotic division of cells or due to cytoplasmic fragmentation, while hypertrophy is caused by an increase in organelles and structural proteins in cells (Jo *et al*., 2009). Cells are made up of water, carbohydrates, proteins, lipids, nucleic acids, and minerals, all of which are constantly being degraded, synthesized, accumulated, or eliminated (Christopherson, 2010). Therefore, for an increase in mass or size to take place in an animal, the rate of anabolism must be more than the amount catabolism in cells. Normally, growth in animals takes a steady form until it reaches a mature size. However, at puberty, there may be a deacceleration in growth. At birth and at weaning the growth rate may also temporarily slow down as the animal switches from one source of nutrients to another (Swatland, 1994).

Development in chicken encompasses five stages i.e., embryonic, post hatch, juvenile, pubertal, and adult stage with the most rapid growth occurring during the late embryonic, post-hatch and juvenile stages (Cogburn *et al.*, 2000; Ku¨hn *et al.*, 2002). The growth rate of chicken is sexually dimorphic with males achieving higher body weights at sexual maturity than females (Osei-Amponsah *et al*., 2012).

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2.7.1 Growth Curves

Growth in poultry, just like in mammals, consists of four cycles (Yang *et al*, 2006) i.e., an accelerating growth phase which occurs during the embryonic phase and immediately after birth, a point of inflection where maximum growth rate has been achieved, deaccelerating growth phase and a limiting mature weight (Lui and Baron, 2011). A reduction in growth often takes place when there is a decline in both hyperplasia (cell proliferation) and hypertrophy (cell enlargement) (Lui and Baron, 2011).

Understanding the growth trajectory of chicken over time is a very important component of poultry improvement programmes. The trajectory of growth is usually depicted by a growth curve which relates body weight to age. Growth curves can be used to elucidate the changes in the growth of poultry and predict body weights at specific ages (Norris *et al*., 2007; Osei-Amponsah, 2010a). Furthermore, the knowledge of growth curve parameters, such as body weight, can be used as a basis for the management, feeding and improvement in the traits of animals (Manjula *et al*., 2018; Do and Miar, 2019).

Several growth equations have been proposed as models of weight versus time to describe growth. These include the Gompertz, Brody, Von Bertalanffy, Logistic, and Richards non-linear mathematical models (Norris *et al*., 2007; Osei-Amponsah, 2011; Do and Miar, 2019). The Gompertz, Brody, Von Bertalanffy, and Logistic are three-parameter models while Richards is a four-parameter model. These models make it possible for us to better appreciate the patterns of growth in animals. However, a correct choice of models is very important as this could lead to inaccurate growth curves and unrealistic values of the growth parameters (Do and Miar, 2019).

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2.8 Growth and growth traits of local chicken ecotypes

The production parameters of local chickens vary widely depending on the geography in which they are reared. Generally, local chickens have been reported by several authors to have slower growth rates compared to exotic breeds of chicken (Minga *et al.*, 2001; Osei Amponsah *et.al.*, 2011; Birteeb *et.al.*, 2016; Chen *et al.*, 2019).

Several studies on the growth rates of local chicken ecotypes in Ghana, Nigeria, Tanzania and in many other countries in Africa have been carried out. Youssao *et al*., (2012) observed that in the Republic of Benin, the Savannah and Forest chicken ecotypes had similar body weights during the first 8 weeks after hatch, but beyond the 8th week the Savannah ecotype was significantly heavier than the Forest type. Similarly, Osei-Amponsah *et al*. (2014) reported that under intensive management, the Savannah chicken ecotype of Ghana had higher body weights from six weeks onwards than the Forest ecotype, with the Savannah males and females weighing about 1.66 kg and 1.38 kg, respectively, while the males and females of the Forest ecotype weighed about 1.52 kg and 1.18 kg respectively. Hagan *et al.* (2013) also reported that in Ghana, the average weight of local hens in the Coastal, Forest and Savannah regions were in the region of 1.3 kg, while the cocks weighed about 1.8 kg, 1.7 kg and 1.7 kg respectively. On the other hand, Adomako (2009) reported the average weight of local hens in the Forest zones of Ghana to be 1.13 kg while the cocks weighed about 1.55 kg.

In a comparative study on the performance of Nigerian local chickens, Adetayo and Babafunso (2001) observed that local chickens from the derived savannah ecological zone were significantly heavier than local chickens from the rain forest ecological zone. According to them, the cocks and hens of the derived savannah ecotype weighed about 1.1 kg and 0.95 kg respectively at 20 weeks while those from the rain forest weighed about 0.95 kg and 0.77 kg respectively. In another study in Nigeria, Ajayi and Agaviezor (2009) observed that on the

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average, local cocks weighed about 1.5 kg while the hens weighed about 1.29 kg. Similarly, Rotimi *et al.* (2016) reported that in the Benue State of Nigeria, the mean body weight of cocks of local chicken (1.38 kg ± 0.20) was significantly heavier than that of the hens (1.23 kg ±0.19). Olawunmi *et al.* (2008) also concluded that cocks and hens of the Fulani chicken weighed about 1.76 kg and 1.46 kg respectively, while the cocks and hens of the Yoruba ecotype weighed about 1.01 kg and 0.92 kg respectively.

In the Nyanza area of Kenya, Olwande *et al*. (2010) observed that the mature body weighs of local cocks and hens were in the range of 1.2-2.5 kg and 1.0-2.25 kg respectively. On the other hand, Okeno *et al.* (2010) reported the average mature body size of the hens and cocks in some parts of Kenya to be 2.22 kg and 1.58 kg respectively.

Osei-Amponsah *et al*. (2012) reported sexual dimorphism in the growth rates of local savannah and forest chicken ecotypes of Ghana, with the males having higher growth rates than the females from the 20th to the 28th week of age. In addition, they also reported that between hatch and 10 weeks of age, the male and female chickens of the savannah ecotype had higher growth rates (7.8 g/bird/day and 6.2 g/bird/day) than the male and female chickens of the forest ecotype (7.0 g/bird/day and 5.6 g/bird/day), respectively. These results are similar to those of Semahoro *et al.* (2018) who also reported a significant effect of sex on the body weight of chicken, with cocks of Ugandan normal feathered, frizzle feathered and Kuroiler chicken being significantly heavier than the females. The study of Semahoro *et al.* (2018) also revealed that body weight was significantly affected by both ecotype and sex. The growth rate of indigenous chickens of Kenya in the Nyanza region were reported by Olwande *et al*. (2010) to be in the range of 4.3-4.7g/day for cocks and 3.8 -4.3g/day for hens.

Under improved management conditions, Aseel chickens of India had higher body weight gains than Kadaknath chickens, except from hatch to 2 weeks (Chatterjee *et al.*, 2007). Hassen *et al.*

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(2006) reported a significant difference in the average body weight and body weight gain among five different lines of local chickens in Ethiopia under intensive management. Similarly, Cosmas *et al.* (2015) also reported significant differences in the body weight, body weight gain, feed intake and feed conversion rates among local breeds of chicken in Nigeria.

Generally, there seems to be a wide variation in the body weights and growth rates of local chickens from country to country and from one area to another. However, Hagan *et al.* (2013) observed that there were no significant variations in the body weights of local chickens from the three agroecological zones of Ghana.

2.9 Regulation of growth in chicken

The neuroendocrine system plays a very important role in the growth of animals, with the hypothalamus - pituitary - somatotropic axis playing a pivotal role in the regulation of growth (Jia *et al.*, 2018; Vaccaro *et al.*, 2022). Growth hormone plays a crucial role in the growth of chicken. The production and secretion of this hormone from the anterior pituitary gland is regulated by several hormones, including growth hormone-releasing hormone, thyrotropin-releasing hormone, and somatostatin, which are an integral part of the somatotropic axis of chicken (Porter, 2005). The somatotropic axis also has the cell membrane-bound growth hormone receptor (GHR), GH-binding protein (GHBP), Insulin-like Growth Factors (IGF1), several IGF-binding proteins (IGFBPs) and the type-I IGF receptor (Cogburn *et al.*, 2000).

GH exerts two types of actions; directly on the metabolism of target organs, and indirectly by affecting the stimulation of the differentiation and proliferation of target cells through the mediation of IGF1 (Ku¨hn *et al.*, 2002). GH stimulates many tissues, especially the liver, to synthesize and secrete IGF1, which then stimulates hypertrophy and hyperplasia of most tissues.

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The production of IGF2 does not depend so much on the actions of GH, as is the case with the production of IGF1, and IGF2 is much less important in stimulating linear growth (Rosengren, (2007). The actions of GH and IGF1 in the modulation of growth are shown in Figure 1 below.

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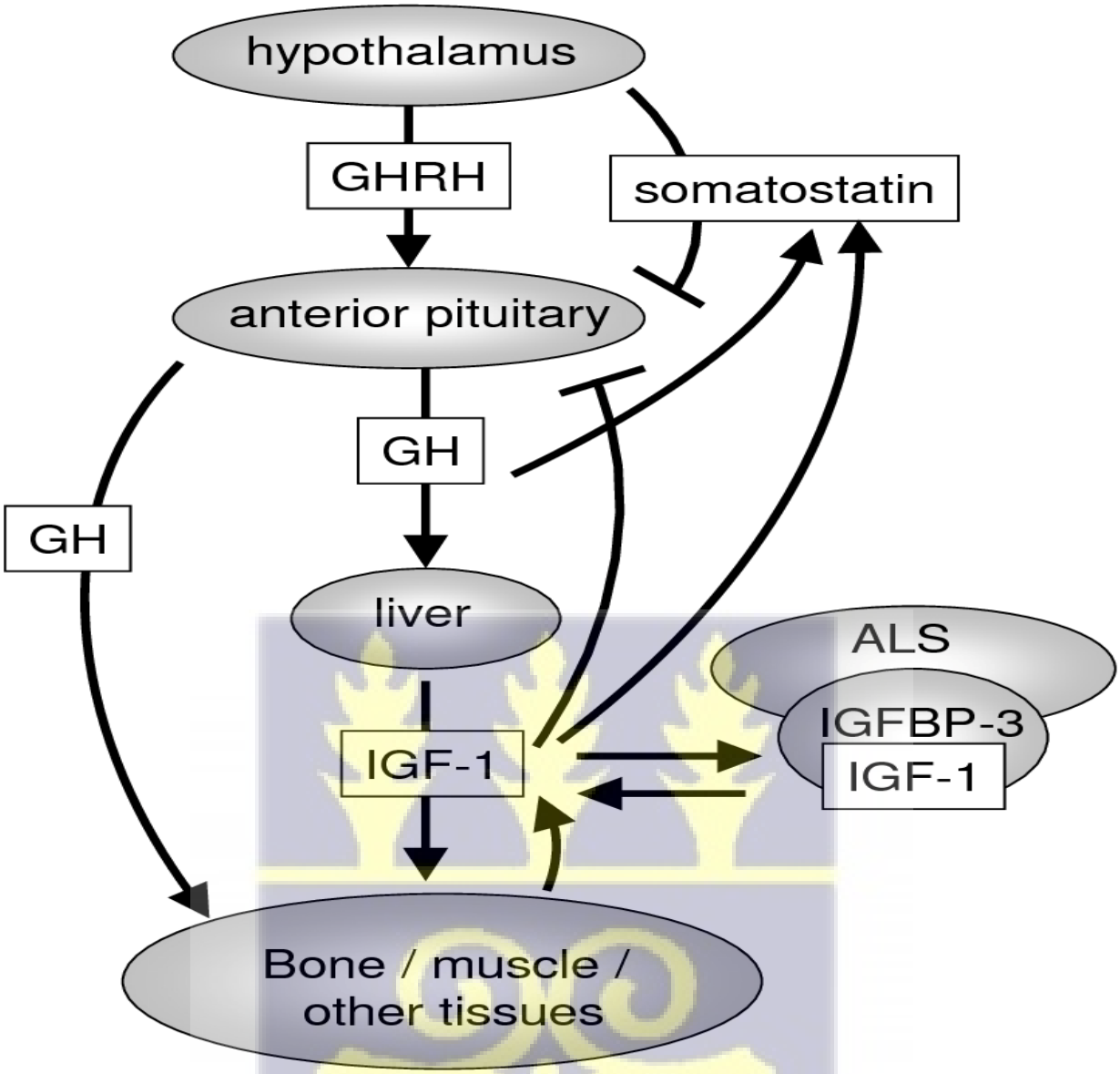

Figure 1. The actions of Growth Hormone (GH) /Insulin-like Growth Factor 1 (IGF1) axis in the modulation of growth. (Source: Rosengren (2007).

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Plasma GH is detectable after embryonic day 16, increasing steeply just before hatching (Figure 2). The high level of plasma GH is maintained up till week 4 before decreasing to low levels at puberty and adulthood (Burnside and Cogburn, 1992). Hepatic GHR is present during the embryonic phase, but it decreases gradually towards hatching. It then increases significantly during the growing phase until adulthood (Burnside and Cogburn, 1992). In chicken, the plasma levels of GH are inversely related to those of GHR throughout the growth and development phases (Kim, 2010).

The thyrotropic axis has some developmental effects that are linked to the somatotropic axis. Triidothryonine (T_3), is an active form of the thyroid hormone (TH) and is partially linked to the growth rate of chicken (Kim, 2010).

IGF1 and IGF2 are regulatory factors that influence the differentiation, proliferation, growth of tissues (Kim, 2010), cell survival and maintenance of cell function (Hakuno and Takahashi, 2018). Both of them are similar peptides and structurally very similar to insulin (Dupont and Holzenberger, 2003). In terms of function, there are major differences between IGFs and Insulin. Whereas the bioactivities IGF1 and IGF2 are related to the growth, differentiation, apoptosis, survival, and maintenance of cells, on the other hand, insulin plays a key role in cell anabolism including "increases in glucose and amino acid transport, induction of glycogen, lipid and protein syntheses and inhibition of gluconeogenesis, lipolysis, and protein degradation" (Hakuno and Takahashi, 2018). In short, while IGFs predominantly controls the fate of cells in the long term, insulin, on the other hand, promotes metabolism in them.

Circulating IGF1 in chicken can be detected by embryonic day 6 with peak levels occurring by day 14 and 18 (Figure 2.) At hatch, circulating hepatic IGF1 levels are very high, plateauing between week 3 and week 7, before declining to low levels at puberty.

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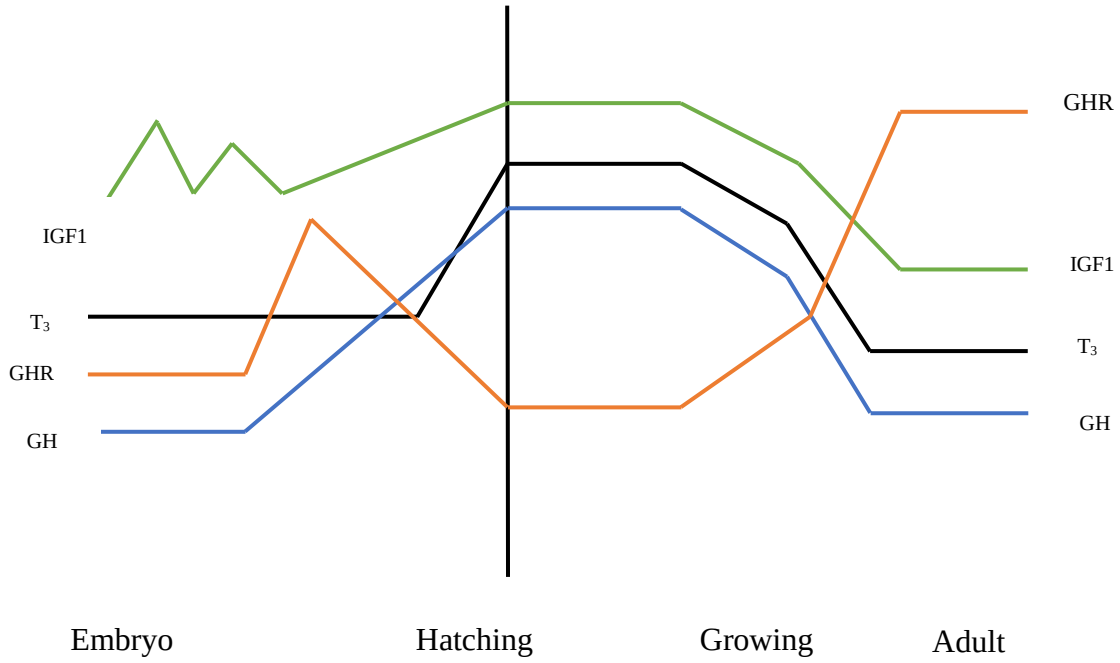

Figure 2. Developmental Pattern of Growth Hormone (GH), Insulin-like Growth Factor 1 (IGF1), Triidothryonine (T_3) and Growth Hormone Releasing Hormone (GHR). (Source: Kim, 2010)

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2.10 Molecular genetic characterization

In general terms, characterization refers to the process of identifying and differentiating an individual either by appearance or by the genetic makeup. In genetics, it refers to the detection of variations at the genic or DNA level and this is referred to as molecular characterization.

There are three types of markers, namely, morphological, biochemical and DNA/RNA markers. Morphological markers use visual observations and measurements of the external characteristics of an animal (e.g., udder shape, coat colour, body shape, skin structure, and anatomical characteristics) to identify, classify, and characterize the genetic evolution of different species (Al-Samarai and Al-Kazaz, 2015). At the genic level, biochemical markers can detect changes in the composition and structure of proteins and amino acids, while a molecular DNA marker is a transmissible DNA sequence in a specific locus of a genome (FAO, 2007).

2.10.1 Molecular Genetic Markers

In the past, the improvement of animal traits relied on breeding values of animals and the performance of their parents and sibs. However, this approach has its own limitations because environmental factors can mask phenotypic expressions of traits leading to reduced accuracy of the selection.

A genetic marker can be simply defined as any stable, quantifiable, inherited variation that can be detected and used to detect the presence of a specific phenotype or genotype which may be difficult to measure or detect. Such a variation at the DNA level amongst individuals is said to be a molecular marker.

The molecular markers are more powerful than other genetic markers in the detection of genetic variations at the DNA sequence level due to their unique genetic properties. These

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properties include their highly polymorphic nature, co-dominant inheritance (determination of homozygous and heterozygous status of diploid organisms), abundance in genomes, neutrality, availability, easy and fast assay, and high reproducibility (Yadav *et al*., 2017).

In the livestock industry, molecular markers can be placed in three major categories, based on the methods of detecting them. These are:

1. Hybridization-based DNA Markers- Restriction Fragment Length Polymorphisms (RFLPs) and Oligonucleotide fingerprinting.
2. PCR-based DNA Markers- Simple Sequence Repeats or microsatellites (SSRs), Random Amplified Length Polymorphic DNAs (RAPDs) and Amplified Fragment Length Polymorphisms (AFLPs) etc.
3. DNA Chip and Sequencing-based DNA Markers- Single nucleotide polymorphisms (Al-Samarai and Al-Kazaz, 2015).

Molecular markers can be used for genetic characterization because they provide reliable information on the genetic diversity of organisms (Perera *et al*., 2000). Molecular markers can be structural (based on the building blocks of DNA sequences) or functional (identification of genes and their functions) markers (de Vicente *et. al.,* 2005). Structural characterization helps to elucidate the genetic diversity of species.

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2.10.2 Microsatellites

Microsatellites, which are often 1 to 6 base pairs repeated sequences (up to 50 repeats) e.g., CACACACACACA. They are also known as Simple sequence repeats (SSR's), short tandem repeats (STR's), simple sequence tandem repeats (SSTR), variable number tandem repeats

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(VNTR), simple sequence length polymorphisms (SSLP), sequence tagged microsatellites (STMS).

Microsatelites have been widely used as molecular markers for studying variation within and across breeds (Civanova *et al*., 2006), in determining population substructure, in phylogenetic relationship studies among populations, in the domestication of animals, migration of breeds and in forensic science because of their high mutation rates, high abundance in genomes, high degree of polymorphism and easy detection (Naicy, T, 2008). In addition, they are also codominant markers, have a high reproducibility and accuracy.

The UTR regions of genes tend to have more microsatellite compared to the CDS (Liu et al., 2016). Microsatellites can affect gene expression if present in the promoters and intergenic regions. In the promotor region, they can lead to either an increase or reduction in the level of gene expression because of changes in transcription factor binding sites and can even culminate in gene silencing (Vieira *et al*., 2016). Tandem repeats in intergenic regions can also cause changes in the secondary structure of the DNA and affect the expression of nearby genes (Gao *et al*., 2013), as well as playing a key role in the transport and alternative splicing of mRNA, and in the regulation of transcription, acting independently or in combination with SSRs present in 5'-UTR regions (Kalia *et al*., 2011).

Microsatellites are often assumed to be neutral because they do not play any role in protein synthesis.

2.10.3 Copy Number Variation (CNV)

The phenomenon where sections of a DNA sequences (or a particular gene) in genome are repeated and the repeats differ from one entity to another within a particular species is often referred to as Copy Number Variation (Pös *et al*., 2021). The individual variants may be short or include thousands of bases and they may have come about due to duplications, deletions,

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insertions, or other changes and can affect long stretches of DNA. Such regions may or may not contain a gene(s). CNV can have serious biological implications for organisms. While it may sometimes not have serious consequences the variability of traits, it may also occasion morphological variations, (Henkel *et al*., 2019), changes in metabolic state (Elder, et al., 2018), and susceptibility to some diseases (Harteveld and Higgs, 2010). CNVs can potentially affect the genetic diversity of a population (Redon *et al*., 2006). In view of these biological roles, they can be used as markers for certain traits.

2.10.4 Mitochondrial DNA

Mitochondria are structures within cells that generate the energy that is used by cells. The Mitochondrial DNA (mtDNA) has 37 genes.

Mitochondrial DNA (mtDNA) markers are maternal markers that have found several uses in the animal and plant kingdoms. Apart from being used in forensic investigations, these markers have also been used extensively in the study of the patterns of gene flow, geographic migrations of animals, the domestication of species and in the identification of ancestors (FAO, 2011). Mitochondrial DNA markers have found use in these areas because of their neutrality, faster rate of mutation compared to nuclear DNA, technical ease of use, and the absence of recombination in them which makes the sequences of mtDNA the same over generations.

Since the thrust of this study is on single nucleotide polymorphism in the genes of local chicken ecotypes, it is important to delve deeper into them to gain a better understanding of how they affect the expression of traits in general.

2.10.5 Single Nucleotide Polymorphisms (SNPs)

A single nucleotide polymorphism (SNP) is a single base pair change at a specific locus of a DNA sequence that occurs in a significant proportion (usually more than 1%) of a large

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population (Vignal *et al.*, 2002; Emara and Kim, 2003). However, if the change in base pair occurs in less than 1% of the population, then it is a mutation (Kassam *et al.*, 2005). SNPs represent the most abundant form of genetic variation in genomes and contribute to many complex phenotypes (Nasu *et al.*, 2002; Jehan and Lakhanpaul, 2006; Slate *et al.*, 2009). SNPs are largely biallelic in nature. This is because at any site in a DNA molecule, four nucleotides can be involved, however only two nucleotides are observed at a specific locus in a gene (Jehan and Lakhanpaul, 2006). Most SNPs are in non-coding regions of genes i.e., introns, 5'UTR, 3'UTR, or exon-intron splicing sites, but they also occur in the coding regions of genes (exons), and in intergenic regions (regions between genes) as well (Ann-Christine Syvänen, 2001).

SNPs can be categorised as transition or transversion polymorphisms. Transition SNPs occur when one purine is exchanged with another purine i.e. A ⇔ G or when a pyrimidine is replaced by another pyrimidine i.e., C ⇔ T. In the case of transversions, a purine is replaced by pyrimidine (A replaces a C), or pyrimidine is replaced by a purine (T replaces G). Transition SNPs seem to occur more frequently than transversions, with a higher occurrence rate in chicken than mammals (Vignal *et al.*, 2002).

SNPs can be in the non-coding or coding regions of a gene. Non-coding SNPs may affect the timing, location, and level of gene expression by disrupting transcription factor binding sites, splice sites and other functional sites on the transcriptional level, whereas coding SNPs can change the amino acid sequence of a gene product, which could lead to an alteration of the functional or structural properties of the translated protein (Reumers *et al.*, 2008).

Coding SNPs come in two forms i.e., synonymous, and non-synonymous SNPs. Synonymous SNPs do not alter the protein sequence of gene products, whereas non-synonymous SNPs affect amino acid variation in the protein products of genes (Emara and Kim, 2003). In the past, synonymous SNPs were seen as "neutral" and so were considered not to have any bearing on

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gene expression. However, there is now a growing pool of evidence that whereas synonymous mutations do not alter protein products, they can affect the functions, structure, and expression of genes (Bailey *et al*., 2021).

There are two forms of non-synonymous SNPs i.e., Missense and Nonsense SNPs. In Missense SNPs, a change in a single nucleotide results in a codon that codes for a different protein while nonsense SNPs often lead to non-functional protein products due to early stoppage in the protein synthesis process due to a new stop codon e.g., UGA, UAA, and UAG.

Both synonymous and non-synonymous SNPs are good genetic markers for mapping studies.

SNPs have several advantages: they are numerous and widely distributed throughout the entire genome (Slate et al., 2009); they have a high genetic stability and accuracy, as well as good repeatability; they are amenable to high-throughput genotyping (Tsuchihashi and Dracopoli, 2002); and they are very useful for differentiating heterozygotic from homozygotic alleles because of their co-dominant nature. Compared to microsatellite markers, it is less expensive to genotype with SNP markers. Furthermore, SNP markers can reveal functional and neutral genetic variation leading to the identification of gene variants of specific phenotypes (FAO, 2011).

The main disadvantage of SNP markers is the relatively low amounts of information that can be obtained from them, compared with that of microsatellites (Hailu and Getu, 2015). Also, due to their bi-allelic nature which renders them less informative, larger numbers of SNPs are required to be analysed to obtain a reasonable power of discrimination to define a unique profile compared to microsatellite markers which are multi-allelic and thus more informative. As a result, surveys involving SNPs are susceptible to ascertainment bias, which must be taken into consideration (Lachance and Tishkoff, 2013).

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2.10.5.1 SNP discovery

The great strides and improvements that have been made in recent times around sequencing technology, including next generation sequencing, has now made it possible to perform SNP discovery in virtually any type of organism, even in instances where genetic information of the specimen is not available (DePristo *et al*, 2011).

Several methods are available for SNPs discovery in a high-throughput manner. These include whole-genome sequencing, exome capture, RNA sequencing, methylated DNA sequencing, restriction enzyme (RE) digestion and RNA-seq or transcriptome sequencing (Davey *et al*., 2011). According to Olson *et al*. (2015) these methods may entail either comparing reads directly to reference genomes, creating *de novo* genome assemblies from the reads (Figure 3) and before mapping them to reference genomes

In NGS methods, a whole genome, or targeted regions of the genome, is randomly digested into small fragments (or short reads) which are then sequenced before alignment to a reference genome (Flicek and Birney, 2009). After the alignment with the fragments, the SNPs can then be called or genotyped. In general, SNP calling seeks to determine the positions where there are polymorphisms or in which positions at least one of the bases differs from a reference sequence, while genotype calling aims at determining the genotype for each individual and is typically only done for positions in which a SNP has already been called (Nielsen *et al*., 2011).

NGS data is prone to high error rates due to many factors, including base-calling and alignment errors (Li *et al.*, 2013). As a result, complex algorithms have been developed that can be deployed to minimise the uncertainty and errors that are often associated with SNP calling and genotyping (Nielsen *et al*., 2011).

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Several assemblers and SNPs callers currently exist. Examples of the assemblers include Trinity, IDBA, Oases, and SOAPdenovo while Genome Analysis Toolkit (GATK), Genotyping-by-sequencing (GBS), SAMtools/BCFtools, freebayes and SOAPsnp are examples of SNP callers (Zhao *et al*., 2019).

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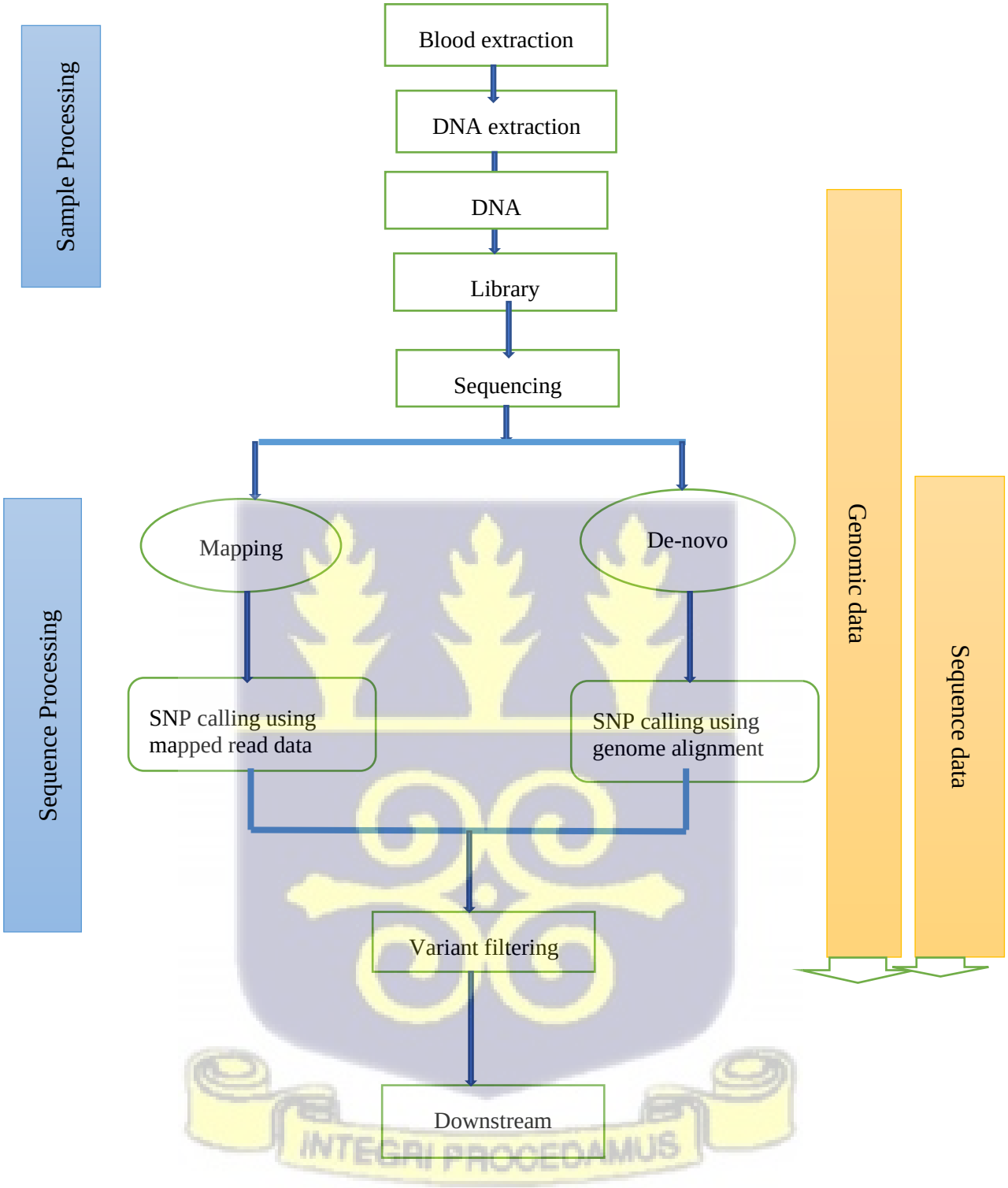

Figure 3: SNP calling workflow diagram. Source: Olson *et al*., (2015)

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2.10.5.2 Genotyping SNPs

As genetic research evolves and advances are made at a very fast pace, so too have the methods that are being used for sequencing and genotyping. Currently, for the purposes of DNA sequencing and genotyping SNPs, there are many variants of polymerase chain reaction (PCR). These include quantitative PCR (qPCR), Restriction Fragment Length Polymorphism PCR (PCR-RFLP), and Single Strand Conformational Polymorphism PCR (PCR-SSCP).

Polymerase Chain Reaction is a technique that is used to amplify genetic sequences *in vitro* (Joshi and Deshpande, 2010). It uses thermostable DNA polymerase enzymes (e.g., *Taq polymerase*) and primers that are specifically designed for a specific target region of a DNA sample. The primers are usually short sequences of nucleotides (oligonucleotides) that are complementary to the sequences of the target region and provide a starting point for DNA synthesis.

Most PCR reactions rely on thermos-cycling, a process that exposes the PCR reagents to repeated cycles of varying temperatures to allow temperature-dependent reactions to take place. Basically, PCR is a three-step process, i.e. Denaturation, where the two strands of the DNA double helix are physically separated at high temperatures (from 90 - 97°C); Annealing- a process where the forward and reverse primers hybridize to the two DNA strands at lower temperatures (from 50-60°C) and; Elongation, where the DNA polymerase attaches itself to the two DNA strands at approximately 72°C and begins to add free nucleotides to them. The whole process is repeated in a cyclic manner (between 25-35 cycles) and at the end of each cycle, the amount of product is doubled, leading to an exponential amplification of the DNA sample.

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Restriction Fragment Length Polymorphism (RFLP) is a type of polymorphism that arises due to difference in DNA sequences. RFLP can be detected after the digestion of DNA samples by restriction endonucleases (enzymes) that cleave DNA molecules when specific nucleotide sequences (restriction sites) are recognized (Chuang *et al*., 2008). RFLP analysis is based on the fact that even a single base difference, as in the case of SNPs, can result in the presence or absence of an enzyme restriction site (Ota *et al.*, 2007). Enzyme recognition sites are usually 4 to 6 base pairs in length. The presence of an enzyme restriction site will lead to the digestion of a DNA sequence into fragments while the absence of a restriction site will not lead to any fragmentation of the DNA sequence. RFLPs can be used as markers in genetic maps.

Getting sufficient DNA for RFLP analysis requires a lot of labour and time. However, with the aid of PCR, sufficient DNA can be obtained within a shorter time. Restriction Fragment Length Polymorphism PCR (PCR-RFLP) can therefore be used to rapidly detect SNPs (Ota *et al*., 2007). The resultant fragments of PCR-RFLP reactions can be separated by gel electrophoresis and visualized. It is simple, fast, and inexpensive (Chuang *et al*., 2008). The downside of the gel-based PCR–RFLP method is that it cannot detect small restriction fragments due to its low resolution (Wang *et al.*, 2010).

For PCR-RFLP SNP genotyping, two kinds of primers can be used i.e., natural, and mutagenic primers. Natural primers are used when there are enzyme restriction sites which can be cleaved by an endonuclease. Mutagenic RFLP is often used when a natural RFLP restriction enzyme for a SNP cannot be found. In this instance, with a change of a nucleotide near the SNP, some restriction enzymes can be artificially created in the mutagenic primer, i.e., the forward primer (Chuang *et al*., 2008).

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2.10.6 Haplotypes

Diploid organisms usually carry two copies of chromosomes, and so each individual organism has two haplotypes (Ong *et al*., 2011). Therefore, in the most general sense, a haplotype is a group of DNA sequences in a specific chromosome that tend to be inherited together (Becker and Knapp, 2004) and are passed down from one generation to another because they are very close together. A haplotype can also be viewed as the collection of nucleotides at the SNP sites in a locus (Judson and Stephens, 2001) or as a genomic region that is often co-inherited as a block of variation.

Haplotypes and SNPs are key markers that find use in association studies. However, haplotypes have an advantage in that they tend to carry more information about the genotype-phenotype link than do the underlying SNPs (Judson and Stephens, 2001). In instances where the arrangements of nucleotides on the chromosome is of critical importance, haplotypes may be a very important source of information. Haplotypes can be used to improve the power of genetic association studies. However, since it is inexpensive and comparatively easy to assay the position of a single base, it is better to get information from the two chromosomes of an individual than from one chromosome (Ong *et al*., 2011).

Experimental methods for haplotype determination exist, but they are currently time-consuming and expensive (Stephens and Donnelly, 2003). The alternative is the use of computational algorithms which tend to be more cost effective (Judson and Stephens, 2001). When only genotype information is available, determining the alleles arrangements on the two haplotypes for an individual requires sophisticated statistical machinery in a process known as haplotype phasing (Ong *et al*., 2011). There are several statistical procedures that can be used for haplotype phase inference. These include PHASE (Stephens and Scheet, 2005), fastPHASE (Scheet and Stephens, 2006) and Beagle (Browning and Browning, 2007).

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2.11 Application of molecular genetic markers in livestock breeding

A molecular marker is generally defined as any stable and inherited variation, which can be detected, quantified, and can be subsequently used to detect the presence of a specific genotype or phenotype (Reshma and Das. (2021).

Molecular genetic markers are an important tool in the study of variation and useful in linking mutations to traits of interest. In recent times, molecular markers have also found space in areas such as Marker-Assisted Selection, Genomic selection, Marker-Assisted Introgression and Quantitative Trait Loci mapping.

Over the centuries, breeders have always taken advantage of the fact that natural variation exists in species, breeds, and populations. This variation was used to increase the productivity of livestock by selecting superior animals to become the progenitors of future generations (Meuwissen *et al.*, 2001; Eggen, A., 2012). The traditional breeding methods were generally based on the estimation of breeding values (EBV) based or the physical appearance of phenotypes of animals (Ibtisham *et al.*, 2017). However, while these traditional breeding methods have been largely successful, they have not been very efficient in selecting for low heritability or difficult to measure traits like fertility, disease resistance, etc. (Eggen, A., 2012; Ibtisham *et al.*, 2017). With time, the traditional breeding approaches were complemented by marker-assisted selection (MAS). However, the use of MAS was limited because it requires prior knowledge of the gene alleles and markers that are associated with the traits, as well as estimates of the associations between the markers and the genes (Eggen, A., 2012).

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2.11.1 Genomic Selection

Genomic selection (GS) is a type of MAS in which genetic markers covering the whole genome are used so that all quantitative trait loci are in linkage disequilibrium with at least one marker (Ibtisham *et al.*, 2017). The concept of GS was first proposed by Meuwissen *et al.* (2001). From simulations, Meuwissen *et al*. (2001) demonstrated that from marker data alone, the breeding value could be predicted with a very high accuracy.

GS often uses both genotypic and phenotypic data of a reference population to develop predictive equations of the genetic merit of individuals within a population (de Koning, 2016). GS does not require any prior knowledge of the genes and their locations on the genome (Liu *et al*., 2014).

The GS process involves several steps: First, it is crucial to have access to a large reference population which has accurate data on the phenotypes of traits, as well as data on the genotypes. This data will then be used to develop a predictive equation for the calculation of a genomic estimated breeding value. Next, the predictive equation will be validated by using a large population of animals that have records on the traits of interest and have also been genotyped for the markers that will be used. Thereafter, the genomic breeding value of new animals can be computed using the prediction equation, based on their genotypes from the SNP array. According to Eggen, A., (2012), several factors, including trait heritability and population size have a strong bearing on the accuracy of the GEBV. GS increases the rate of genetic gain by decreasing generation interval (Meuwissen and Goddard, 2010) and increasing accuracy of prediction.

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GS has several advantages including the fact that it can be implemented very early in life; with difficult to improve traits; is not sex limited (Liu *et al.*, 2014); has high accuracy; and can reduce the generation interval, thereby increasing the intensity of selection (Eggen, A., 2012).

GS is now widely used in animal breeding because of the recent achievements in high-throughput technology development and the corresponding decrease of genotyping prices (Liu *et al.*, 2014). In the dairy cattle industry, the reliability of genomic prediction in preselecting young animals has been firmly established (Su *et al*., 2012) while in pig breeding, it has led to an increase in the number of teats of sows which ensures that sows can nurture all their piglets (Rohrer and Nonneman, 2017). The use of GS has now made it possible to reduce the generation intervals in layer chicken (Wolc *et al.* (2016).

2.11.2 Marker Assisted Selection

Most of the traits that are considered in animal genetic improvement programmes are quantitative in nature, i.e., they are controlled by many genes, alongside environmental factors, and the underlying genes have small effects on the phenotype observed (FAO, 2007). In the past, genetic improvement programmes relied on the observation of the phenotypes of the candidates to be selected without knowing the genes that are being selected. The arrival of molecular markers and marker assisted selection was therefore a major achievement as they enabled this constraint to be overcome.

Marker assisted selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker that is linked to the trait (FAO, 2007). MAS relies upon a situation of linkage disequilibrium (LD) which exists between the DNA marker and a specific gene (quantitative trait locus). LD can be exploited by selection as if the effects are caused by the marker (Ben-Ari and Lavi, 2012). The advantage of MAS results from the fact that many of

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the traits of interest to breeders are not easily assessed. Thus, selection, which is based on linked DNA markers, provides the tools to overcome this constraint.

The relationship between markers and the genes of interest is paramount to the success of MAS. According to Dekkers (2004), there are three kinds of this relationship: a) the molecular marker is located within the gene of interest. This is the best scenario for MAS but according to FAO (2007), these kinds of markers are not common. b) The marker is in linkage disequilibrium (LD) with the trait throughout the whole population and c) The marker is not in linkage disequilibrium with the trait (i.e., it is in linkage equilibrium) throughout the whole population. This is the worse situation for applying MAS.

2.11.3 Marker Assisted Introgression

Introgression or introgressive hybridization is the incorporation of alleles from one entity (species) into the gene pool of a second divergent entity (species) usually via hybridization and backcrossing (Anderson, 1949). Marker Assisted Introgression (MAI) is the introgression of some valuable genes from an inferior breed donor line to a superior breed recipient line using the information of a molecular marker linked to these genes or quantitative trait loci (QTL) (Bai, 2015).

In animal breeding, sometimes some breeds may have certain economically important traits but may be highly deficient in other equally important traits. For example, some pig breeds may have high growth and good feed conversion rates, but they may have poor meat qualities. On the other hand, there may be some pig breeds that have excellent meat qualities but have low growth and feed conversion rates. If the genes or the QTLs responsible for the good meat quality trait are known, then they can potentially be introgressed into the pig breeds that have the high growth and feed conversion ratios.

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MAI is usually done through several generations of backcrossing the population which carries the allele to be introgressed (donor line) with the recipient (recurrent) population, followed by *inter se* crossing to make the desirable allele homozygous. Molecular markers can be used in both the backcrossing and *inter se* crossing phases. The effectiveness of the backcrossing can be improved by identifying the carriers of the target gene/QTL (foreground selection) and by improving the recovery of the genetic background (background selection) of the recipient (Dekkers and Hospital, 2002). Foreground selection refers to the use of markers that are tightly linked to the gene of interest to select for the target gene or allele. On the other hand, background selection refers to the use of markers that are not tightly linked to the gene of interest to select against or eliminate donor parent alleles other than the desired ones. During the *inter se* crossing phase, markers can also be used to select individuals who are homozygous for the introgressed gene. MAI has been successfully used to introgress the naked neck gene from a donor commercial egg layer chicken line heterozygous at the naked neck locus (Na/na+), to a recipient Cornish broiler line (Yancovich *et al.*, 2009).

2.11.4 Quantitative Trait Locus Mapping

A quantitative trait locus (QTL) is a genomic region responsible for the variation of a quantitative trait. Normally, a QTL is linked to, or contains, gene(s) that control the target trait. The use of polymorphisms that are associated with QTL regions therefore helps to target key regions that contribute to the variability of the target trait. Furthermore, the identification of QTLs contributes to the discovery of the genes that control functions of interest (causative genes) and their regulation.

QTL mapping is a method that is used to define the general chromosomal position of genes or genetic variants that influence the magnitude of a measurable trait (Ferraro, 2009; Korte and Farlow, 2013). It mainly seeks to identify genetic markers that are close to the QTL (linkage

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disequilibrium markers) or the gene that the QTL is associated with (direct marker) and to use that information in MAS breeding programmes (Dekkers, 2004). QTL mapping is usually done by first constructing the population by crossing stocks that differ in the phenotype of interest after which the two stocks are characterized for genetic markers at previously mapped marker loci. The QTL mapping is then carried out by looking for correlations between the markers and the phenotype in the descendants of the original cross.

2.11.5 Candidate Gene Approach

The mapping of the genes that are associated with common diseases and quantitative traits can be broadly categorised into candidate gene studies and genome-wide studies, which include both linkage mapping and genome-wide association studies.

Candidate genes are genes with known biological function directly or indirectly regulating the developmental processes of the investigated traits, which could be confirmed by evaluating the effects of the causative gene variants in an association analysis (Zhu and Zhao, 2007).

The candidate gene approach is a genetic method for dissecting complex and quantitative traits. It is based on what is known about the phenotype of the trait that is being studied but this also happens to be one of its weaknesses because the detailed molecular anatomy of most biological traits remains unknown (Zhu and Zhao, 2007). Candidate-gene studies are usually conducted in population-based samples of affected and unaffected individuals i.e., case–control (Tabor *et al.*, 2002). This approach has been used variously to study the genetic architecture of many traits, including gene-disease research, genetic association studies, biomarker and drug target selection in many organisms from animals to humans (Tabor *et al.*, 2002). However, the candidate gene approach has also been criticized for reasons related to the fact that it is based on the ability to predict functional candidate genes and variants, but the current knowledge is insufficient to make these predictions. Secondly, many findings of association in many

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candidate-gene studies have not been replicated when followed up in subsequent association studies (Tabor *et al.*, 2002).

Many studies have reported candidate genes for growth in chicken. As a result, polymorphisms in the following candidate genes of chicken have been investigated: Growth Hormone gene (Nie *et al.*, 2005), Insulin-like Growth Factor 1 Receptor gene (Lei *et al.*, 2008), Insulin-like Growth Factor 1 gene (Tang *et al.*, 2010; Kadlec *et al.*, 2012; Anh *et al.*, 2015; Aswani *et al.*, 2015), Insulin-like Growth Factor 2 gene (Amills *et al.*, 2003; Wang *et al.*, 2005; Tang *et al.*, 2010) Insulin-like Growth Factor Binding Protein 2 gene (Lei *et al.*, 2005b; Kazemi *et al.*, 2018).

2.11.6 Genome-Wide Association Studies

A Genome-Wide Association Study (GWAS) is an approach that involves the scanning of thousands of markers (usually SNPs) across the complete sets of DNA of organisms to find genetic variations associated with a particular trait (Ahmetov and Fedotovskaya, 2015). Unlike the candidate gene approach, GWAS is hypothesis-free. GWAS has become feasible in humans as well as in domestic animals because of the development of large collections of SNPs and the development of cost-effective methods for large-scale SNP analysis (Zhang *et al.*, 2012).

Several successful GWAS have been carried out in domestic animals, including cattle, pigs, horses, dogs, sheep, and chickens, leading to the detection of SNPs with significant effects on traits of economic importance (Zhang *et al.*, 2012). Hayes *et al.* (2010) also reported a GWAS in dairy cattle regarding the response in milk production of dairy cattle to heat stress and the level of nutrition.

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2. 12 Insulin-Like Growth System

The Insulin-like Growth Factor (IGF) system consists of a family of cell signalling factors which includes two ligands (IGF1 and IGF2), two cell surface receptors (IGF1 receptor and IGF2 mannose-6 phosphate receptor) that control the action of the ligands (Le Roith *et al.*, 2001) and six different IGF binding proteins (IGFBP-1 to IGFBP-6). After the discovery and subsequent characterization of IGFs, they were termed "insulin-like" because of their ability to stimulate glucose uptake into muscle and fat cells (Wood et al., 2005).

IGF1 and IGF2 ligands are single-chain polypeptide hormones whose sequences are about 62% similar to proinsulin (Dupont and Holzenberger, 2003). IGF1 hormones have 70 amino acids and a molecular mass of 7.5 kDA (Rosengren, 2007) while IGF2 has 72 amino acids and a molecular mass of 74 kDA (Dupont and Holzenberger, 2003).

Although IGF1 is secreted mainly in the liver (Kim, 2014; Laron, 2001) and is carried to other target tissues, acting as an endocrine hormone, it is also ubiquitously present in many other types of tissues (McMurtry, 1998; Wang *et al.*, 2021;) and exerts paracrine and autocrine effects (Kim, 2010; Laron, 2001). This suggests that apart from Growth hormone, its production is also influenced by other hormones.

In the embryonic development phase of chicken, IGF1 mRNA can be detected in the tissues of the eye, skeletal muscle, and brain. After hatching, the concentration of IGF1 circulating in the blood of chicken increases significantly (Le Roith *et.al.*, 2001; Kita *et al.*, 2005; Kadlec, 2012). In their studies on IGF1 concentrations in chicken, Beccavin *et al.* (2001) concluded that IGF1 concentration in chicken was influenced by the genotype of the chickens. They arrived at this conclusion because they found out that in higher intensity growing types of chicken, the amount of circulating IGF1 increases from 1–6 weeks of age while in the slower intensity growing chicken it increases over a period of 1–12 weeks. IGF1 gene expression is also very sensitive

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to nutritional conditions as evidenced by the fact that feed restriction or starvation decreases hepatic IGF1 gene expression while refeeding restores the expression levels (Kita *et al.*, 2005).

IGF2 is associated with early embryonic and fetal development and is expressed in a wide variety of somatic tissues while the adult expression of IGF2 takes place in the liver and in the epithelial cells lining the surface of the brain (Bergman *et al.*, 2013).

The IGF1 receptor plays a major role in the signal transduction of IGF1 and IGF2 to regulate their physiological actions (Kim, 2010). IGF2 mannose-6 phosphate (IGF2/M-6-P) receptor does not mediate IGF2 signal transduction as it does not bind to it. Instead, the effects of IGF2 are mediated by the IGF1 receptor (Kim, 2010). IGF2/M-6-P plays a role in the clearance and reduction of the levels of IGF2 during embryonic development (Baker *et al.*, 1993). In addition, it also mediates the bioavailability of IGF2 (Denley *et al.*, 2005).

IGF1 is unstable in the blood and easily degrades. To achieve functional stability, it binds with Insulin-like Growth Factor Binding Proteins (IGFBP) which are a group of six secreted proteins (IGFBP1-6), which bind to IGF1 and IGF2 with high affinity and modulate their biological actions. Furthermore, they can prolong the half-life of IGFs via high affinity binding of the ligands (IGF/ IGFBP complexes). They function as simple carrier proteins to deliver IGFs to those surfaces either for the activation of specific receptors or cell responses independently of receptor activation (Kostecka and Blahovec, 1999), and also serve to regulate the endocrine and paracrine/autocrine actions of IGF by modulating the IGF available to bind to signalling IGF1 receptors (Mohan and Baylink, 2002). IGFBPs can function as growth modulators (apoptosis and cell migration) independent of IGFs (Duan, 2002). In summary, IGFBPs can act in ligand dependent and ligand independent manners, leading to the regulation of growth and muscle development of chicken (Vaccaro *et al.*, 2022).

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IGF1 signal transduction

The signal transduction of IGFs on the growth axis is mainly through the activation of the phosphoinositide-3 kinase (PI3-K) activation pathway and the Mitogen Activated Protein Kinase (MAPK) activation pathway, which transmit mitotic and metabolic signals to the nucleus of the cells (Yu *et al*., 2015).

When IGFs bind with their cell surface receptors with the aid of specific IGFBPs, a conformational change in the structure of the IGF receptor is induced, leading to the activation of the intrinsic tyrosine kinase activity. A tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to the tyrosine residues of specific proteins inside a cell. The IGF1 receptor (IGF1R) is highly homologous to insulin receptor (IR). It consists of two α subunits that are extracellular proteins binding to specific ligands and two β subunits that are transmembrane proteins possessing tyrosine kinase activity (Sarfstein and Werner, 2013; Le Roith *et al*., 2001). Binding of IGFs to the extracellular α subunits of the receptor triggers several events including autophosphorylation, phosphorylation of intracellular substrates, and activation of cellular signalling pathways involved in metabolic processes and growth regulation (Kato *et al.* 1994). The activated IGF1R then phosphorylates several substrates, including insulin receptor substrates (IRS1, IRS2) and Src homology collagen (SHC). The phosphotyrosine residues in these substrates are recognized by some Src homology 2 (SH2) domain-containing signalling molecules like the 85 kDa regulatory subunit (p85) of phosphatidylinositol 3-kinase (PI 3-kinase), the growth factor receptor-bound 2 (GRB2) and SH2-containing protein tyrosine phosphatase 2 (SHP2/Syp. Binding with these substrates leads to the activation of downstream signalling PI3-K pathway and Ras-mitogen-activated protein kinase (MAP kinase) pathway (Figure 4). Activation of these signalling pathways is required

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for the induction of various bioactivities of IGFs, including cell proliferation, cell differentiation and cell survival (Hakuno and Takahashi, 2018).

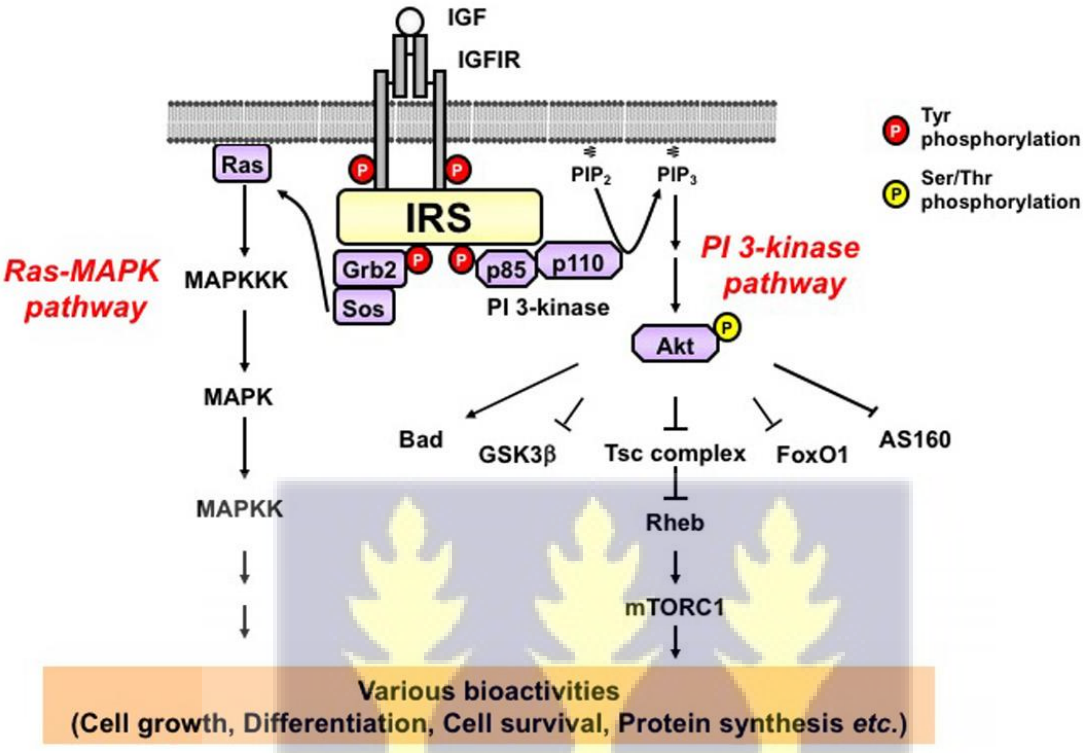

Figure 4. Intracellular signalling mechanisms of Insulin-like Growth factor /insulin. *Source*: Hakuno and Takahashi (2018).

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2.13 Insulin-like Growth Factor 1 (IGF1)

2.13.1 Structure of IGF1 proteins

Generally, IGF1 is a protein hormone whose structure consists of two polypeptide chains (A and B) which are linked by two di-sulfide bonds. The two chains all share a conserved arrangement of 4 cysteines in their A chain, the first of which is linked by a disulphide bond to the third, while the second and fourth are linked by interchain disulphide bonds to cysteines in the B chain. The structure reveals three alpha helices that are the major secondary structural elements. Helix 1 is in the B domain whereas helix 2 and helix 3 are both located in the A domain (Denley *et al.*, 2005). IGF1 is a compact globular protein with a hydrophobic core (Laajoki *et al.*, 1999).

The structure of chicken IGF1 (cIGF1) has as many as 70 amino acids and has considerable homology with human IGF1 (Kajimoto and Rotwein, 1991). Compared to the human IGF1, very little information on the tertiary structure of chicken IGF1 exists. From UniProt and the Swiss-Model databases, the proposed three-dimensional structure of chicken IGF1 is shown in Figure 5 below. The model depicts the coverage of the target sequence and the human model (Figure 5A), the 3D structure (Figure 5B), and the amino acid sequence of the mature IGF 1 protein (Figure 5C). Three disulphide bonds at amino acid residues 54-96, 66-100 and 95-100, as depicted by the yellow spots in Figure 5A, hold together the three-dimensional fold.

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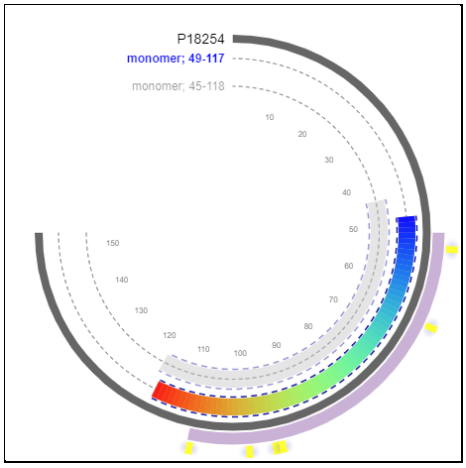
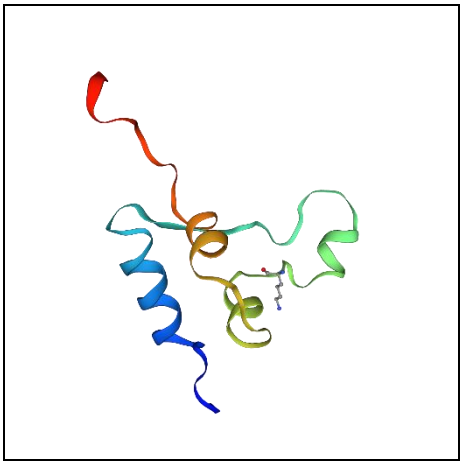


A



B

```
MODEL GPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKS 117
6rva.1.AGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLDMYCAPLKPAKS 70
```

C

Figure 5. **A)** Graphical representation of the coverage of the target sequence (Chicken IGF1 protein) with models and experimental structures. The amino acid sequence structure is indicated as grey arc with the di-sulphate bonds on the outside indicated in yellow. **B)** A cartoon image of the proposed three-dimensional structure of IGF1 protein. **C)** the amino acid sequence of matured cIGF1 in alignment with the template human homolog.

Source: https://swissmodel.expasy.org/repository/uniprot/P18254

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2.13.2 Function and mode of action of IGF1

IGF1 is a multifunctional growth factor that plays various roles in organisms including the development of chick retina during embryogenesis (Calvaruso *et al.*, 1996; Kita *et al.*, 2005), wound healing, tissue, and cartilage repair (Provenzano *et al.*,2007; Pasold *et al.*, 2015).

Several factors contribute to skeletal muscle development, including IGF1 and myogenic regulatory factors (Yu *et al.*, 2015). The role of IGF1 in skeletal muscle development is to stimulate the proliferation and differentiation of skeletal muscle cells during myogenesis (Coleman *et al.*, 1995). Apart from muscle development, it also plays a crucial role in muscle regeneration and can reduce age-related loss of muscle function in people (Engert *et al.*, 1996). These effects seem to be mediated by promoting the proliferation and differentiation of satellite cells, as well as the recruitment of proliferating bone marrow stem cells to regions of muscle tissue damage (Musaro *et al.*, 2004).

IGF 1 also plays a role in the formation of new bone through the stimulation of osteoblast differentiation (Xian *et.al.*, 2012). Xian *et al.* (2012) arrived at this conclusion after observing that mice with knockout IGF1 receptor in their pre-osteoblastic cells showed lower bone mass and mineral deposition than the wild-type mice. Zhou *et al.* (2005) also supported this observation.

During embryogenesis and at post hatch, IGF1 is involved in the stimulation of amino acid and glucose uptake, increased DNA synthesis, and stimulation of tissue growth (McMurtry, 1998).

The pattern of IGF1 expression during embryogenesis in chicken differs among the various tissues (Kita *et al.*, 2005). IGF1 mRNA transcripts can be found in the legs, eye, heart, stomach, and brain as well as in the whole embryo by days 12 and 13 (de Pablo *et al.*, 1991).

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During embryogenesis, the plasma concentration of IGF1 increases significantly from day 10 to day 14 and then gradually decreases until hatch (Lu *et al.*, 2007). After hatch, it increases again significantly, peaking at about 6 weeks before declining (Scanes *et al.*, 1997). This suggests that IGF1 plays a more important role after hatch than during embryogenesis.

2.13.3 The IGF1 Gene

The IGF1 hormone plays a major role in stimulating the differentiation, proliferation, and metabolism of several myogenic cell lines of many species. In chicken, it affects the metabolic processes by increasing the amino acid and glucose uptake, which in turn affects the synthesis of DNA and proteins (McMurtry, 1998). This hormone is encoded by the *IGF1* gene.

The *IGF1* gene maps to chromosome 1 on the chicken genome. It is made up of 4 exons and 3 introns (Figure 6) which are distributed over 50 kilobases (kb) of chromosomal DNA and are transcribed and processed into two mRNAs of 1.9 kb and 2.6 kb (Kajimoto and Rotwein, 1991; Kazemi *et al.*, 2018).

The mature 70-amino acid IGF1 molecule is encoded within exons 2 and 3, while exons 1 and 4 contain NH2-and COOH- terminal extension peptides (Kajimoto and Rotwein, 1991).

Exon 1 contains the 5'untranslated region of IGF1 mRNA and encodes the initial 21 amino acids of the long 48-residue signal peptide. Exon 2 contains the remaining 27 codons of this NH2- terminal domain, as well as the initial 25 amino acids of the B region of mature IGF1. The remaining amino acids of mature IGF 1 are found in exon 3 (182 bp), which also contains the first 16 residues of the COOH-terminal E domain. Exon 4 codes for the final 19 amino acids of the chicken homologue and a 3"untranslated region (Kajimoto and Rotwein, 1991).

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In the *IGF1* gene, exons 2 and 3 (the exons encoding the mature IGF1 protein) are separated by a large intron. Exons 1and 2 and exons 3 and 4 are also separated by relatively large introns (Rotwein, 2018a). A schematic representation of IGF 1 is shown in Figure 6.

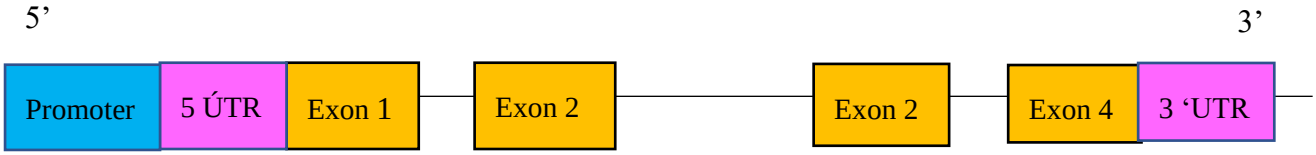

Figure 6. Structure of the chicken IGF1 gene (not drawn to scale). Exons are numbered and depicted by black boxes while introns and flanking regions depicted by the thin line. Non-coding regions are depicted by blue boxes.

2.13.4 SNPs within the IGF1 gene and their association with body weights and growth traits

Following the recent advancements in molecular technology which opened better ways of evaluating genetic variability at the DNA level, many SNPs have been discovered in the DNA of many animal species.

In an identification and characterization study of SNPs in 12 chicken growth-correlated genes by denaturing high performance liquid chromatography, Nie *et al.* (2005) reported a total of 15 SNPs in the *IGF1* gene of chicken (*cIGF1)*, of which three were in the 5'-UTR, one in an intron and 11 in the 3'-UTR.

Nagaraja *et al.* (2000) studied the association between feed intake, body weight, laying rate and egg weight and concluded that one *PstI* RFLP in the 5' end of the *cIGF1* gene was associated with egg and eggshell weight in one White Leghorn chicken population.

Amills *et al.* (2003) reported the presence of a SNP in the 5'UTR of the chicken *cIGF1* gene which consisted of an A>C transverse substitution (c.-366A>C). According to them, this SNP

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was not significantly associated with growth and feeding traits. However, suggestive associations ($P \leq 0.05$) were found between this SNP and average daily gain at 107 days and Feed Efficiency at 44, 73, and 107 days of age.

Zhou *et al*. (2005), also reported the presence of the same A>C single nucleotide polymorphism in the 5'UTR of the *cIGF1* gene and concluded that it was significantly associated with chicken growth rates, feed efficiency, breast weight, and breast yield. Similarly, Anh *et al.* (2015) also reported an A>C SNP in the promoter and 5'UTR of *cIGF1* which had a very strong influence on only the hatch weights and body weights at the early stages of growth (up to six weeks of age) in four Thai broiler populations. Furthermore, Moe *et al.* (2009) showed that the A>C SNP within the promoter region of *cIGF1* had a significant association with growth, body composition and skeletal traits in native Asian, layer and broiler chicken populations.

Sato *et al.* (2012) also found a SNP (g.570A>C) of the *cIGF1* promoter region that was significantly associated with breast muscle yields. Bennett *et al*. (2006) also reported that this SNP was significantly associated with 5-week body weight of a layer-broiler cross of chicken strain.

It has also been suggested by Lei *et al*. (2007) that a SNP in *cIGF1* (C51978309T) was related to the transversal area of the leg muscle fibre and the transversal area of the breast muscle fibre of chicken. Tang *et al*. (2010) also reported a IGF1-*PstI* C>T substitution at position 279 locating 7 kb upstream the promoter region. This SNP was found to be significantly associated with birth weights at 8, 10 and 13 weeks of age, as well as shank length and shank circumference at 13 weeks of age.

Bhattacharya *et al.* (2015) found a total of 21 SNPs in the *cIGF1* out of which, three were transverse synonymous SNPs in the 5'UTR. The 5'UTR plays a significant role in post-transcriptional splicing of the mRNA leading to the availability of mature mRNA at the

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nucleus. According to this study, exon 1 had two synonymous SNPs while exon 3 contained four synonymous and six non- synonymous SNPs. Exon 4 had two SNPs, one of which was synonymous while the other was nonsynonymous. The 3'UTR had four synonymous SNPS. The 3'UTR is known to be involved in protein expression as it plays a very important role in the splicing activity of pre-mRNA (Bhattacharya *et al.*, 2015). Polymorphisms in 3'UTR may therefore play a crucial role in protein expression.

Khadem *et al*. (2010) reported significant associations between an A>C SNP located in the 5' end of the promoter region of *cIGF1* with average egg weight and total number of eggs. Similarly, Promwatee *et al.* (2013) also noted that the polymorphic site of the *IGF1* gene in Thai synthetic chicken lines which is located in the promoter region and 5'-UTR, near a putative TATA box, and consisting of one A>C substitution was associated with some growth traits i.e., body weight (BW) and average daily gain.

In guinea fowl populations in northern Ghana, Ahiagbe (2018) reported six novel SNPs in *IGF1*, two of which were in the 5'UTR, one in exon 2 and three in the 3' UTR. According to the author, the SNPs in the 5' UTR and 3' UTRs had a significant effect on body weights and growth rates from the 2nd and 4th week respectively, suggesting the possibility of these polymorphisms to influence IGF1 synthesis at the translation level.

In Qinchuan cattle, Gui *et al.* (2018) reported three SNPs in the intronic regions of *IGF1* that are associated with growth traits i.e., body length, withers height, chest depth, chest circumference and pin bone width. According to them, even though introns do not code amino acids, SNPs in introns can also affect protein expression by altering the stability of mRNA. Mullen *et al*. (2011) also reported a significant association of a SNP in the intronic region of *IGF1* of Holstein-Friesian lactating dairy cows, with increased milk and fat yields in lactation one and two and increased protein yield in lactation two. A SNP in the promoter region of

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IGF1 of Mexican Charolais cattle breed also showed a significant effect on weaning and preweaning weights (Reyna *et al*., 2010). In Korean cattle, Chung *et al*. (2005) observed that a SNP in exon 1 of *IGF1* was significantly associated with higher body weights of calves at 3 months.

In goats, the effects of SNPs on production traits have also been studied. In their study of polymorphisms in the *IGF1* gene of Malabari and Attappady Black goats in India, Naicy *et al*. (2017) observed that a g.80 C>T SNP in exon 2 which caused a non-synonymous mutation was associated with higher body length, chest circumference and body length index. In the Nanjiang Huang goat, a SNP in intron 4 of *IGF1* gene has been reported by Zhang *et al*. (2008) to be associated with birth weight, body weight at 6 months and at 12 months, heart girth at 2 months, body length at 6 months, wither height at 6 months and at 12 months and heart girth at 12 months. Similarly, Deng *et al*. (2010) also found an association between this SNP with body size and milk yields in Xinong Saanen dairy goats. However, they argued that since this mutation is not in a coding region, it may not be a causal mutation but could be linked with another mutation in the coding or regulatory regions of a gene which is a causal mutation for production traits. In support of this argument, Rose (2019) posited that certain introns located in transcribed sequences near the 5' end of a gene, have a large effect on mRNA accumulation.

A single SNP in exon 3 of *IGF1* of Tibetan miniature pigs i.e., g. 40 T>C, was found to be associated with the growth traits of pigs aged 6-11 months (Yue *et al*., 2014). Niu *et al*. (2013) also reported a SNP in exon 3 (c. G189A) of porcine *IGF1* that was associated with higher body weight, average daily gain, and backfat thickness.

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2.14 Insulin-like Growth Factor 2 (IGF2)

2.14.1 Structure of IGF2 proteins

IGF2 is a mitogenic polypeptide with insulin-like structure modulating primary growth in chickens (Aghdam and Mardani, 2014). The predicted chicken prepro-IGF2 (precursor) protein contains 187 residues and comprises a signal peptide, a IGF2 peptide and a C-terminal peptide (Darling and Brickell, 1996). The signal peptide has 23 amino acids, while the mature peptide and propeptide have 68 and 96 amino acids, respectively. The predicted mature protein shares a high degree of homology with the human counterpart, with a 33%, 82% and 28% identity for the signal, mature and c-terminal peptides respectively (McMurtry *et al.*, 1997). The 68-amino acid chicken IGF2 protein consists of four domains, termed B, C, A, and D (Rotwein, 2018b).

Like IGF1, the structure of the IGF2 protein also consists of two polypeptide chains (A and B) which are linked by two di-sulfide bonds. The structure reveals three alpha helices that are the major secondary structural elements. Helix 1 is in the B domain whereas helix 2 and helix 3 are both located in the A domain (Denley *et al.*, 2005). The protein is compact globular with a hydrophobic core (Laajoki *et al.*, 1999).

From UniProt and the Swiss-Model databases, the proposed three-dimensional structure of chicken IGF2 is shown in Figure 7 below. The model depicts the coverage of the target sequence and the human model (Figure 7A), the 3D structure (Figure 7B), and the amino acid sequence of the mature IGF1 protein (Figure 7C). Three disulphide bonds at amino acid residues 32-71; 44-84;70-75, hold together the three-dimensional fold.

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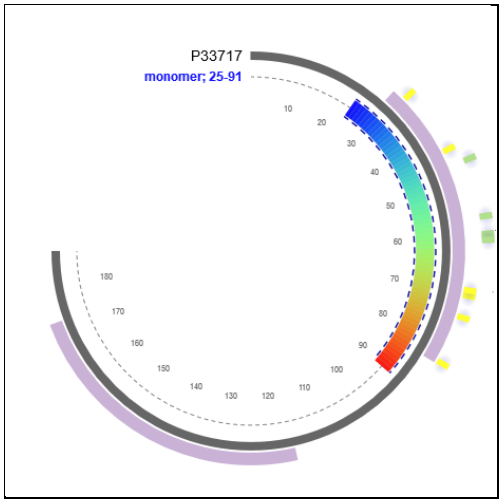
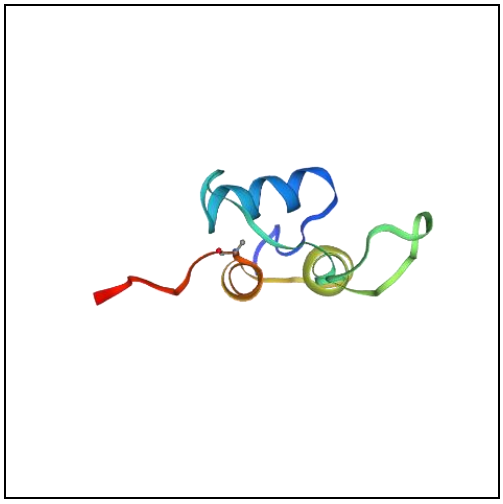


A)



B)

C)

Figure 7. **A)** Graphical representation of the coverage of the target sequence of chicken IGF2 protein with models and experimental structures. The amino acid sequence structure is indicated as a grey arc with the di-sulphate bonds on the outside in yellow. **B)** A cartoon image of the proposed three-dimensional structure of the chicken IGF2 protein. **C)** the Amino Acid sequence of matured chicken IGF2 in alignment with the template (human homolog). Source: https://swissmodel.expasy.org/repository/uniprot/P33717?csm=B301581CD4878EEC.

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2.14.2 Function and mode of action of IGF2

IGF2 is widely expressed during embryonic development (McMurtry, 1997; Chao and D'Amore, 2008). During embryogenesis, the plasma concentration of IGF2 increases significantly from day 10 to day 14 and then gradually decreases until 21 days after hatch (Scanes *et al.,* 1997; Lu *et al.,* 2007). Plasma levels of IGF2 are usually about 5- to 12-fold higher in embryos than the IGF1 levels (Lu *et al.,* 2007). This suggests that IGF2 may be a more important entity for chick embryonic development than IGF1.

Just like IGF1, IGF2 also plays several roles during embryogenesis. The biological responses attributable to IGF2 during embryogenesis includes the stimulation of amino acid and glucose uptake, increased DNA synthesis, lipid metabolism and stimulation of tissue growth (McMurtry, 1998; Beccavin *et al.*, 2001). IGF2 also promotes cell growth, apoptosis, and differentiation (Chao and D'Amore, 2008).

Despite the emphatic roles of IGF2 in embryogenesis, several studies, including Scanes *et al.* (1997) and McMurtry (1998) did not find any discernible relationships between IGF2 and growth variables in growing chicken.

2.14.3 The IGF 2 gene

The Insulin-like Growth Factor 2 gene is mapped to chromosome 5 on the chicken genome (GenBank accession number *NC_006092.5*). Unlike the *cIGF1* gene that has been comprehensively characterized, a lot remains to be found out about the *cIGF2* gene. While many authors, including Darling and Brickell (1996) submit that this is a single-copy chicken gene that consists of 3 exons and 2 introns, on the other hand, Rotwein (2018b) observed that the gene contains at least 4 exons and 3 introns, and it potentially encodes two protein precursors, but a single mature IGF2 protein. This position is in line with the latest NCBI *Gallus*

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gallus annotation release 104 (GeneBank accession number *NC_006092.5)* which assigns 4 exons and 3 introns to this gene.

The gene has one mRNA transcript variant X1 (GenBank ID: XM 0152886525.2) and one protein isoform (GenBank ID: XP_015142011.1). A schematic representation of IGF2 gene can be seen in Figure 8.

In most of the literature and databases that were reviewed for this thesis, the 5' and 3' UTRs were not defined, and neither was the promoter region characterized.

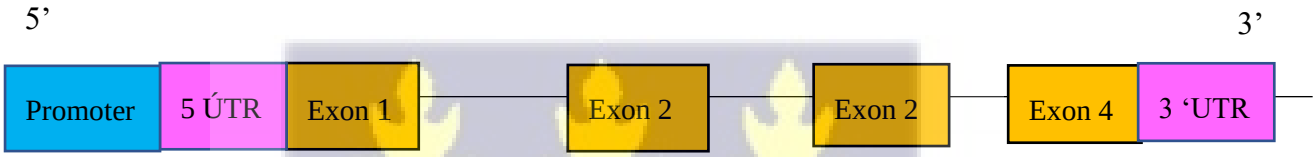

Figure 8. Structure of the chicken IGF2 gene (not drawn to scale). Exons are numbered and depicted by black boxes while introns and flanking regions by the thin line. Non-coding regions are depicted by blue boxes.

Unlike *IGF2* genes in mammals which is imprinted, the chicken *IGF2* gene (*cIGF2*) is said not to be imprinted or has a biallelic expression (O'Neill *et al.*, 2000; Wang *et al.*, 2005). However, Koski *et al.* (2000) reported that in a minority of chicken embryos, the *cIGF2* alleles were expressed monoallelically from either maternal or paternal alleles. This, according to them, suggests that the imprinting in birds is unrelated to embryonic growth.

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2.14.4 SNPs within IGF2 gene and their association with body weights and growth traits

The association between SNPs in *cIGF2* with growth and carcass traits has been reported in many animals.

Two SNPs in the *cIGF2* gene were reported by Amills *et al*. (2003). One of these was a silent substitution C >T at exon 3 while the second one was a G>A substitution in intron 2. However, they did not find significant associations between these polymorphisms with growth and feeding traits in two divergent strains of Black Penedesenca Chicken. On the other hand, Nie *et al.* (2005), reported a total of four SNPs in *cIGF2* gene of which 3 were in the introns while one was synonymous.

A SNP (C>G) within exon 2 of *cIGF2* was reported by Tang *et al.* (2010) to have a significant association with body weight and carcass weight at 17 weeks of age in native Chinese Beijing You chicken populations. Similarly, Wang *et al.* (2005) also identified the same SNP (C >G mutation) in exon 2 and reported that it was associated with production traits. In another study on the Chinese Langshan chicken, Yan *et al.* (2017) also confirmed the presence of the C >G SNP in exon 2 of *cIGF2* and concluded that it was associated with body weight at 16 weeks of age and higher egg production. Furthermore, they inferred that the C >G SNP was widely spread in chickens except for some special breeds of chicken.

Khadem *et al.* (2010) also reported significant associations between a SNP in a site corresponding to exon 2, intron 2 and most parts of exon 3 of the *cIGF2* gene with average egg weight and total number of eggs.

In a study on guinea fowl populations of Northern Ghana, Ahiagbe (2018) reported two novel SNPs within exon 3 and exon 4 of *IGF2* but these SNPs did not have any effect on body weights or growth rates.

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In the Egyptian water buffalo, a C287A non-synonymous SNP in exon 10 of *IGF2* was reported by Abo-Al-Ela *et al.* (2014) with the AA genotype significantly associated with ADG from birth to 9 months of age.

In bovine IGF2, a C292T SNP in exon 2 was found to be associated with the rib eye area and the carcass fat percent in beef cattle (Goodall and Schmutz, 2007). In another study on the association of IGF2 with growth traits in Chinese Qinchuan cattle, Huang *et al*. (2014) reported four SNPs in intron 8 that were significantly associated with body weight. Since these polymorphisms were not in a coding region, they concluded they probably indirectly contributed to the higher growth rates.

Markljung, et al. (2009) found a SNP in intron 3 of *IGF2* in pigs which abrogates a binding site for a repressor called ZBED6 and leads to a 3-fold up-regulation of IGF2 in skeletal muscle with major effects on muscle growth, size of the heart, and fat deposition.

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2.15 Gene regulation and expression

Gene regulation determines which genes are expressed, when they are expressed, and the duration of their expression. The regulation of eukaryotic gene expression occurs at the epigenetic, transcriptional, post transcriptional, translational, and post translational levels.

2.15.1 Epigenetic regulation

For a gene to be expressed, it must be physically accessible to the transcriptional machinery (RNA polymerase). In the nucleus, DNA is normally tightly packed around histone octamers that are comprised of H2A, H2B, H3, and H4 in units called nucleosome complexes (Kang *et al*., 2017). These nucleosome complexes regulate access to the DNA regions. Methylation causes tight packing of the nucleosome complexes and restricts access to DNA by the transcriptional machinery leading to gene silencing ((Roth *et al*., 2001). On the other hand, histone acetylation leads to a loose packing of the nucleosome complex, thus enabling access to the DNA and leads to gene activation or silencing depending on the location of the amino acid substrate for methylation and the valency, that is, mono-, di-, or tri-methylation (Black *et al*., 2012; Bommarito and Fry, 2019). This type of gene expression is epigenetic in nature because it is reversible and does not lead to changes nucleotide sequences.

2.15.2 Transcriptional level regulation

Genomic enhancers (*cis*-regulatory element) play a key function in the repression or activation of transcription. This is achieved by transcription factors binding to short sequences in the enhancer, reading the regulatory information contained in the enhancer and then mediating the recruitment of RNA polymerase II and activation at core-promoters (Reiter et al., 2017). Suppression of transcription binding sites could therefore have an effect in the regulation of gene expression.

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2.15.3. Post-transcriptional regulation

Post-transcriptional processing entails the splicing of introns from pre-mRNA to form mRNA, the addition of a methylated guanosine 'cap' to the 5′end of the first exon of the transcribed RNA, and the addition of adenosine residues (poly-A tail) to the last exon of the 3'end, in preparation for mRNA transport from the nucleus to the cytoplasm for translation. While methylation may cause gene silencing, the poly (A) tail plays a key regulatory role in gene expression by mediating the translocation of mRNA into the cytoplasm for translation, and also by conferring stability on it by inhibiting its decay (Subtelny, 2014). Whenever translation is completed and mRNA is no longer needed, it is degraded by the shortening of the Poly (A) tail (Chen and Shyu, 2011). Since genes contain variable numbers of introns and exons, alternative splicing (including or excluding some exons) of the messenger RNA precursor (pre-mRNA) can occur, leading to the formation of alternative mRNA isoforms and the encoding of different protein isoforms. Alternative splicing therefore plays an essential role in the regulation of gene expression and protein diversity of eukaryotes.

2.15.4 Translational level regulation.

Transcription is the primary level of the regulation of gene expression however the regulation at the translational level is also significant. At the translational level, gene expression can be regulated by a couple mechanisms including the binding of repressor proteins to sequences in the 3′ untranslated region of specific mRNAs which blocks the translation of these mRNA sequences. Another mechanism for the regulation of gene expression at the translational level involves the control of the activity of initiation factors like eIF-2 (*e*ukaryotic *i*nitiation *f*actor). eIF-2 usually binds to the initiator methionyl tRNA, bringing it to the ribosome. The binding of eIF-2 to RNA is controlled by phosphorylation by guanosine triphosphate (GTP). If eIF-2 is phosphorylated, it undergoes structural changes and is unable to bind to GTP and the translation process gets impeded.

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2.15.5. Post-translational level regulation.

Post-translational modification of proteins entails the addition of modifying groups like acetyl, phosphoryl, glycosyl and methyl, to one or more amino acids (Ramazi and Zahiri, 2021). These modifications affect the structure, stability, degradation, and functions of proteins. Except for degradation, these processes can be either reversible or irreversible (epigenetic) in nature (Wang *et al*., 2014).

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CHAPTER 3

GENERAL MATERIALS AND METHODS

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3.1 Geographical location of the experiments.

The field work was done at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana. LIPREC is located on latitude 05^0 40´N and longitude 00^0 16´W, on the Accra Plains and is part of the Coastal Savannah. The annual rainfall at LIPREC is about 785mm with a range of 128mm-1709 mm. The Centre experiences a bi-modal rainfall pattern with the major raining season occurring between March and July while the minor season occurs between September and November. The mean monthly temperature ranges from a minimum of 24.8ºC in August to a maximum of about 28.3º C in February while the relative humidity ranges from about 58% in the afternoons (15: 00 hrs.) to about 83.7% in the mornings (09:00 hrs.).

All the laboratory work, except for the sanger sequencing of the DNA samples, was carried out at the Molecular Genetics laboratory of the Department of Animal Science of the University of Ghana.

3.2 Source of experimental chicken

The experimental birds were part of the Feed the Future Innovation Lab for Genomics to Improve Poultry Project (cooperative agreement number AID-OAA-A-13-00080), located at LIPREC, University of Ghana.

In the breeder unit where three local chicken ecotypes have been reared since 2014, third generation breeder chickens of the Forest, Coastal Savannah and Interior Savannah ecotypes were grouped for natural mating to produce sire half-sib families per ecotype, using a mating ratio of 1 sire to 8 dams to produce chicks for the study. The base populations were obtained

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from randomly selected communities in the Forest, Coastal Savannah, and Interior Savannah agroecological sones of Ghana. The agroecological zones in Ghana can be seen Figure 9.

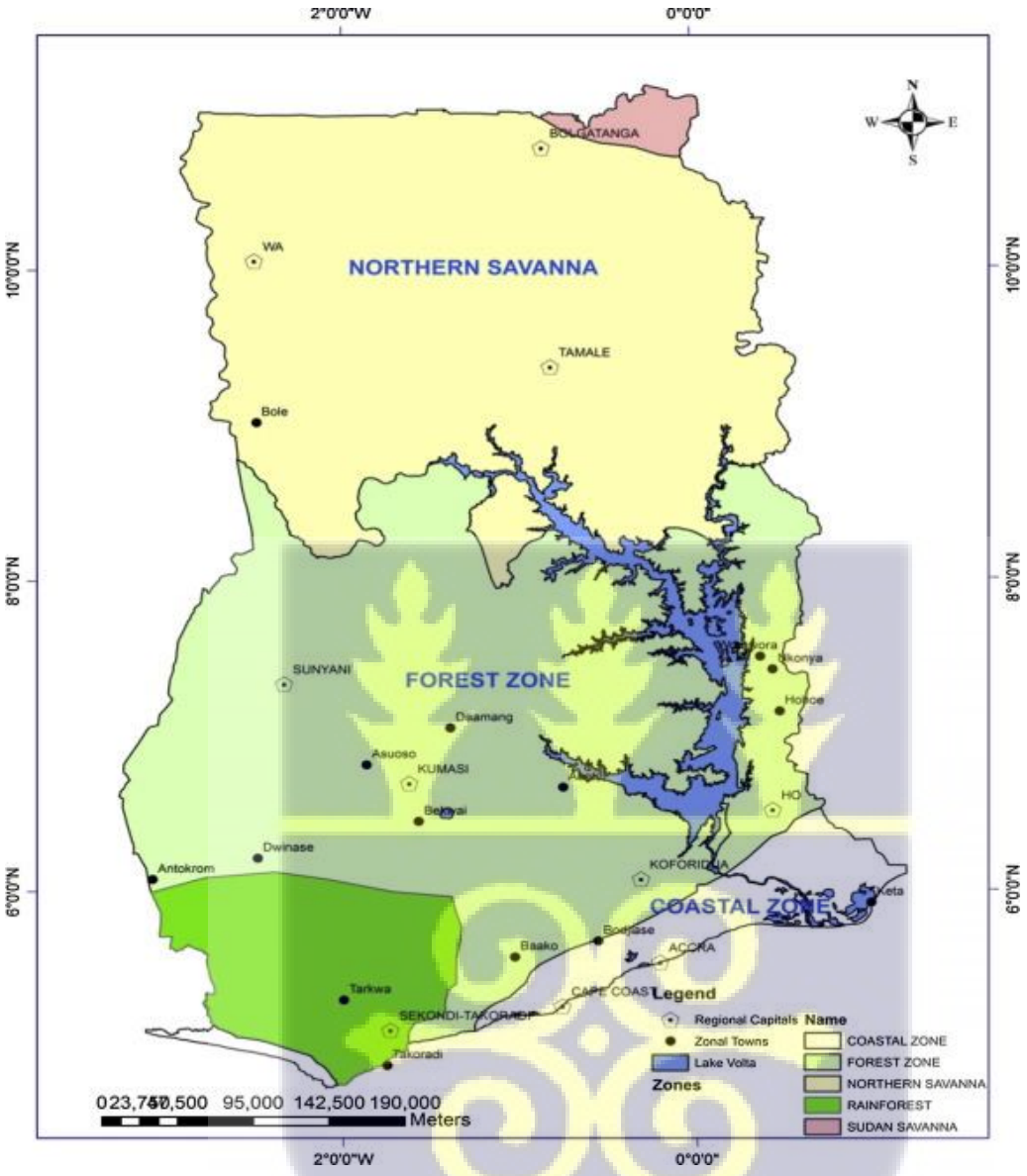

Figure 9. Agroecological zones in Ghana. Source: Osei and Stein (2017).

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The Interior Savannah agroecological zone covers about 57% of the land area of Ghana and consists of the Sudan Savannah and Guinea Savannah zones (FAO 2012). It straddles the Upper West, the North-East, Northern, Savannah, the Upper East, Bono East and Oti regions.

The Interior Savannah ecosystem has a uni-modal rainfall pattern with a mean rainfall in the range of 500-1300 mm/annum. The zone is characterized by prolonged dry seasons and a dominance of relatively short trees with grass, shrub, and scrub undergrowth.

The Forest agroecological zone covers the Ashanti, Bono, Eastern, Central, part of Volta, Western North and Western regions. It has a bi-modal rainfall pattern with a mean rainfall of about 1000-2200 mm/annum. The type and structure of the vegetation in the forest zones is quite diverse, depending on the amount of rainfall available (FAO, 2012). Humidity levels are relatively higher in the forest zones.

The Coastal Savannah agroecological zone covers about 7 % of the land area of Ghana (FAO, 2012). This zone covers the Greater Accra and part of the Volta region. It has a bi-modal rainfall pattern with the major raining season occurring between March and July while the minor one occurs between September and November. The rainfall ranges from 600-1000 mm/annum and the vegetation consists of tall grasses with scattered trees.

3.3 Handling of experimental chickens

The eggs for the experiment were collected over a ten-day period. After each day's collection, they were stored in a chiller maintained at a temperature of 15^{o}C and 75% humidity level (Fasenko, 2007). At the end of the egg collection period, all the eggs were transferred to an incubator (A.P Poultry incubator of Hyderabad, India) and incubated for 18 days under a

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temperature range of 37.2°C to 37.78°C and a humidity level of 60-65%. Subsequently, the eggs were transferred to a hatcher for 3 days at a temperature of 36 °C and 80% humidity level.

Immediately after hatching, each chick was weighed with an electronic weighing scale (OHAUS Explorer ™ Precision -0.1g readability) and then tagged. Thereafter, all the chicks were sent to a brooder house where each ecotype was reared separately in deep litter pens in four replicates. The chicks were brooded at a 34-36°C temperature range that was maintained with the aid of electric bulbs.

3.4 Management of the experimental chickens

The experiment was conducted in four replicates with a total of 1124 chickens, made up of 562 chickens of the Forest (FO) ecotype, 297 chickens of the Interior savannah (IS) ecotype, and 265 chickens of the Coastal savannah (CS) ecotype (Table 2).

Each ecotype was housed separately with a maximum of 40 birds per pen with a size of 2.54 m x 2.2 m x 2.2 m. From day 1 to week 8, all the birds were fed on a standard chick starter mash with 20.0 % crude protein and a Metabolizable Energy (ME) of 2,993 kcal/kg while from week 9 to week 22 they were fed on a standard chick grower mash with 17% crude protein and a ME of 3,015 kcal/kg. Feed and water were provided on *ad lib* basis. The diets were formulated using commercial protein concentrates following the manufacturers specifications. The vaccination, feeding and other important husbandry practices were the same for all the birds. The vaccination schedule is shown in Appendix 1. Mortalities were recorded as and when they occurred.

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Table 2. Composition of the experimental chickens by replicate, ecotype, and sex.

Replicate	Sex	Ecotype			Total
		Forest	Coastal Savannah	Interior Savannah	
1	Male	54	26	34	**114**
	Female	57	28	49	**134**
2	Male	57	32	35	**124**
	Female	67	34	45	**146**
3	Male	70	30	24	**124**
	Female	66	33	29	**128**
4	Male	96	37	44	**177**
	Female	95	45	37	**177**
Total		**562**	**265**	**297**	**1124**

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CHAPTER 4

GROWTH, CARCASS TRAITS AND SURVIVABILITY OF THREE CHICKEN ECOTYPES OF GHANA

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4.1 Summary

A total of 1124 chickens, made up of 562 chickens of the Forest ecotype (FO), 297 chickens of the Interior Savannah ecotype (IS), and 265 chickens of the Coastal Savannah (CS) ecotype were used in this study. The study was conducted in four replicates. In each replicate, the birds were housed separately by ecotype.

The body weights of the birds were measured fortnightly, while the feed intake per pen and mortality rates were recorded daily over a 22-week period. From this data, growth rates, feed conversion rates and survivability rates were calculated by ecotype, sex, and by ecotype-sex interactions. At the end of the experiment, a total of 1036 birds were euthanized and then processed using the Meat buyers' guide developed by the North American Meat Processors Association (NAMP; 2007). Several carcass traits of commercial interest were measured, including dressed weight, thigh weight, wing weight, drumstick weight, breast weight and dressing percentage. All the data collected was entered into a spreadsheet and then analysed with SAS^{TM} 9.0 software (2002). An analysis of variance (ANOVA) was carried out to isolate the various effects.

The results of this study showed that there were significant differences ($p<0.05$) in the hatch weights of the three chicken ecotypes, with the highest hatch weight being recorded in the FO ecotype. The IS ecotype had the lowest hatch weight as well as the highest chick mortality rate during the first eight weeks of life. At the end of the experiment, cocks of the IS and FO ecotypes had the highest body weights, although not significantly different from each other. On the other hand, the hens of the CS and IS had the highest body weights, although not also significantly different from each other. The growth rates of the three chicken ecotypes peaked between week 10 and week 12, after which they gradually declined. The growth rates of male chickens were significantly ($p<0.05$) higher than the growth rates of female chicken.

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From hatch to week eight, the FO ecotype had the highest daily intake of feed while the CS ecotype had the lowest daily intake of feed. This notwithstanding, there were no significant differences in the feed conversion efficiency among the three chicken ecotypes during this period.

Between week 8 and week 22, the FO ecotype had the highest daily intake of feed. Similarly, from hatch till week 22, the FO ecotype also had the highest daily intake of feed. There were no significant differences in the feed conversion efficiency among the three chicken ecotypes during these periods.

Ecotype had an influence on breast weight, with the FO ecotype having the higher breast weight. However, ecotype did not have any significant effect ($p>0.05$) on the dressing percentage, thigh weight, wing weight, drumstick weight, and dressed weight.

Sex had a significant effect on all the carcass traits with the carcass parts of the males being significantly heavier than those of the females.

Mortality is one of the key factors that can affect the profitability of any livestock enterprise. Most of the deaths among the experimental chicken occurred during the first week of life, with the IS ecotype experiencing the highest mortality rate, followed by the FO and CS ecotypes. The high mortality rates of the IS ecotype could be due to its smaller hatch weights, which in turn is a function of hen age, genetics, and environmental conditions.

The lack of significant variation in the carcass traits of the Ghana chicken ecotypes seem to suggest that they may have a similar genetic makeup. On the other hand, the lower body weights, growth rates and carcass traits of Ghana chicken ecotypes, compared with other local chicken types in Africa and Asia, reared under similar conditions, also suggests that the Ghana chicken ecotypes may not have undergone any deliberate selection to improve these traits.

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The heritability estimates of body weight of the three chicken populations were moderate to high while the heritability estimate of breast weight was moderate. This suggests that body weight and breast weight traits could be targeted in future genetic improvement programs of the three local chicken ecotypes of Ghana.

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4.2 Introduction

Three ecotypes of local chicken have been described in Ghana i.e., Interior savannah (IS), Forest (FO) and Coastal savannah (CS) ecotypes (Osei-Amponsah *et al.,* 2010a; Kayang *et al.,* 2015). An ecotype can generally be defined as a population of a species that has adapted very well to specific geographic or environmental conditions (Vallejo-Trujillo *et al*., 2021) and tend to have distinctive characteristics because of the adaptation. Most individuals who thrive in specific geographic zones or conditions tend to carry genes that confer upon them traits that facilitate their survival and fitness. This seems to suggest that to a large extent the adaptive capacity of ecotypes is influence by gene-environmental interactions (Andrew *et al*., 2010).

Some local breeds of chicken have some unique alleles that can be exploited for future breeding purposes (Kayang *et al*., 2015) but these chickens have also been reported to have low growth rates, smaller body sizes and high feed conversion ratios compared to exotic types of chicken (Minga *et al.,* 2001; Osei Amponsah *et al.,* 2011; Birteeb *et al.,* 2016; Chen *et al.,* 2019). And so, in the quest to improve the body sizes of local chickens, many farmers tend to introduce birds of unknown genetic composition into their flocks leading to the loss of important adaptive traits (Anderson, 2003).

The meat of local chickens is also reported to have a better taste than other types of chickens, thus fuelling the high preference for the meat of these types of chickens in Ghana and in other African countries (Islam, 2000; Kyarisiima *et al*., 2011; Atuahene *et al*., 2014; N'dri *et al*., 2018; Ragasa *et al*., 2020).

In a quest to improve the production parameters of local chickens, farmers, governments, and private sector actors in Africa have in the past undertaken or promoted some crossbreeding schemes that involved local and more productive exotic breeds of chicken. Most of these schemes have met with varying degrees of sustainability and success (Habimana *et al*., 2020).

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Generally, very few examples of successful genetic improvements in traits of economic importance of local chicken ecotypes in Africa exist.

Growth is generally defined as an increase in body mass while development is the progression from fertilization towards sexual maturity (Baker *et al.*, 1993; Cogburn *et al.*, 2000). Growth is a complex process involving many factors, including the neuroendocrine system, genetics, nutrition, and the environment (Kim, 2010). Growth occurs mainly through the processes of hyperplasia or hypertrophy (Christopherson, 2010). The growth rate of chicken is sexually dimorphic with males achieving higher body weights at sexual maturity than females (Osei-Amponsah *et al.*, 2012).

The ability of chicken and for that matter other farm animals to efficiently convert feed into either egg, meat, milk, or other desired animal products is of prime importance in the poultry and other livestock industries. Its importance is directly related to the cost of feeds. Feed utilization by animals is governed by several complicated biological processes and environmental interactions. It is highly positively correlated with the production level and body size (Arthur *et al.*, 2014) of an animal.

Feed conversion ratio is a function of the genetic makeup of an animal (Arthur *et al.*, 2014), the conditions under which the animal is kept, the quality of the feed, and the age and health status of the animal (Varley, 2009). Generally, younger animals tend to have lower FCRs due to their high growth rates compared to older animals. In a comparative study on the production traits of local and improved chicken varieties in India, Haunshi *et al.* (2009) reported that the local Miri chicken had lesser feed intakes and better FCRs than the improved Gramapriya and Vanaraja varieties. Differences in FCR were also reported by Youssao *et al.* (2012) in respect of the Label Rouge chicken and two local chicken ecotypes of Benin. In their study on the

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feed utilization rates of several indigenous chickens in Ethiopia, Tadelle *et al.* (2003b) reported that FCR was significantly influenced by the ecotype of chicken.

Local poultry species, because of their genetic diversity, are appropriate for rearing under various environmental, climatic, and feeding conditions. Given the economic and socio-cultural importance of local chickens, the high preference for their meat in Ghana, against the backdrop of a limited supply of these birds, it is desirable that fast-growing local chicken ecotypes be developed so that farmers can achieve more production cycles to meet the local demand. However, this must be preceded by a thorough assessment of the production, health, and survivability traits of the local chicken ecotypes of Ghana. The objective of this study was therefore to assess the growth, carcass, and survivability traits of chicken ecotypes of the IS, FO, and CS agroecological zones of Ghana.

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4.3 Materials and Methods

4.3.1 Source of experimental birds

The experimental birds were sourced from the Feed the Future Innovation Lab for Genomics to Improve Poultry project (A USAID sponsored project; cooperative agreement number AID-OAA-A-13-00080), located at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana. The location of LIPREC is described in section 3.1.

4.3.2 Management of experimental birds

The experiment was conducted in four replicates with a total of 1124 chickens, made up of 562 (277 male and 285 female) chickens of the FO ecotype, 297 (137 male and 160 female) chickens of the IS ecotype, and 265 (125 male and 140 female) chickens of the CS chicken ecotype (Table 2). The chicks were brooded at a 34-36^{o}C temperature range that was maintained with the aid of electric bulbs. Each ecotype was housed separately with a maximum of 40 birds in a pen. From day 1 to week 8, all the birds were fed on a standard chick starter mash with 20 % crude protein and a Metabolizable Energy (ME) of 2,993 kcal/kg while from week 9 to week 22 they were fed on a standard chick grower mash with 17% crude protein and a ME of 3,015 kcal/kg. Feed and water were provided on an *ad lib* basis. The diets were formulated with commercial protein concentrates following the manufacturers specification. The vaccination, feeding and other important husbandry practices were the same for all the birds.

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4.3.3 Data collection

Growth data

The body weight of each bird was recorded using a sensitive electronic weighing scale (OHAUS Explorer ^{TM} Precision -0.1g readability) at day 1 (HW), week 2 (BW 2), week 4 (BW 4), week 6 (BW 6), and week 8 (BW 8) while from week 10 (BW 10), week 12 (BW 12), 14 (BW 14), 16 (BW 16), 18 (BW 18), 20 (BW 20) and 22 (BW 22), a digital hanging scale (Kern HDN 5K5 -5.0 g readability) scale was used to weigh the birds.

These weights were then used to calculate weight gains within the periods of day 1 to week 2 (WG 2), week 2 to week 4 (WG 4), week 4 to week 6 (WG 6), week 6 to week 8 (WG 8), week 8 to week 10 (WG 10), week 10 to week 12 (WG 12), week 12 to week 14 (WG 14), week 14 to week 16 (WG 16), week 16 to week 18 (WG 18), week 18 to week 20 (WG 20), week 20 to week 22 (WG 22), and the total growth rate from hatch to week 22 (TWG). The formula used for calculation of the weight gain within every two-week period is the following:

$$\textbf{Weight Gain} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

Feed Conversion

The average feed consumed per bird was recorded daily from hatch to week 22. The feed conversion rate (FCR) was then calculated from the total feed consumed within the brooding (Day 1 to week 8) and growing (week 8-week 22) periods per bird using the following formula:

FCR=Total amount of feed consumed/Total weight gain.

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Carcass characteristics

At the end of the experimental period, a total of 1036 birds were starved of feed for 12 hours to ensure a complete emptying of the crop. They were then weighed and euthanized. Each bird was bled and weighed on a sensitive scale (OHAUS Explorer ™ Precision-0.1g) to determine the weight of the blood. Thereafter the birds were scalded in hot water, defeathered and weighed again to determine the defeathered weight.

The carcasses were stored in a cold room at 3 - 5°C for 24 hours before they were processed. The offal (gastrointestinal tract, liver, shank, lung, head, and kidney) of each bird were removed by hand from the carcass and the carcass (eviscerated) weight determined. The head, shanks, wings, thighs, drumstick, breast muscle and neck were weighed individually based on the Meat buyers' guide developed by North American Meat Processors Association (NAMP; 2007), on a sensitive scale. The heart, liver and gizzard were also weighed individually on the same scale. From these measurements, several parameters, including the dressed weight and dressing percentage, were calculated. The dressed weight and dressing percentage were calculated using the following formulae:

Dressed weight = Live weight – (offal weight + blood weight + weight of feathers)

The dressing percentage was expressed as a percentage of dressed weight over the live weight.

$$\textbf{Dressing percentage} = \left(\frac{\text{Dressed weight}}{\text{Live weight}}\right) \times 100$$

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Survivability

Daily, the number of birds dying from each ecotype group was recorded. This data was used to estimate the mortality and survival probability of the three chicken ecotypes. The percentage mortality per week was calculated as follows:

$$\textbf{Percentage mortality at week N} = \left(\frac{\text{No. of mortalities during week N}}{\text{No. of birds at hatch}}\right) \times 100$$

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4.3.4 Data analysis

Body weight and growth rate traits

Body weight data was entered into a spreadsheet and the growth rates calculated. The data was then analysed with SAS™ 9.0 software (2002). An analysis of variance (ANOVA) was carried out to isolate the effects of the main and interaction effects. The effects included ecotype, sex, and ecotype-sex interaction. Hatch weight was fitted in the model as a covariate. The following General Linear Model was used.

$$Y_{ijk} = \mu + E_i + S_j + ES_{ij} + \beta\,(X_{ij} - \bar{x}_{..}) + e_{ijk}$$

Where, Y_{ijk} = Dependent variable recorded on *i*th ecotype of *j*th sex; μ = the overall mean for a given dependent variable; E_i = the effect of *i*th ecotype; S_j = the effect of *j*th sex; ES_{ij} = the interaction between ecotype and sex; β = Slope; X_{ij}= *j*th observation of covariate for the *i*th group, $\bar{x}$ = mean of the covariate; e_{ijk} = the residual error of the *k*th observation recorded on *i*th ecotype and *j*th sex.

Least square means (LSM; Steel and Torrie 1997) were estimated and Bonferroni test was used to separate any differences among the means. The Bonferroni test is a multiple comparison test that can be used to prevent data from incorrectly appearing to be statistically significant (false positives) or type 1 error (Cleophas and Zwinderman, 2011). The standard errors were also calculated.

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Feed consumption

The total feed consumed per bird per day was entered into a spreadsheet and the data analysed with SAS^{TM} 9.0 software (2002). An analysis of variance was carried out to isolate the effects of sex, ecotype, and sex by ecotype interaction on the daily feed consumption per bird using the following General Linear Model.

$Y_{ijk} = \mu + E_i + S_j + ES_{ij} + e_{ijk}$

Where, Y_{ijk} = Dependent variable recorded on *i*th ecotype of *j*th sex; μ = the overall mean for a given dependent variable; E_i = the effect of *i*th ecotype; S_j = the effect of *j*th sex; ES_{ij} = the interaction between ecotype and sex; e_{ijk} = the residual error of *k*th observation recorded on *i*th ecotype and *j*th sex.

Least square means (LSM; Steel and Torrie 1997) were estimated and SNK test was used to separate any differences among the means. The standard errors were also calculated. The Newman–Keuls or Student–Newman–Keuls (SNK) method is a posthoc test that can be used to identify sample means that are significantly different from each other. The SNK procedure is more likely to reveal significant differences between group means i.e., very powerful but also less conservative (Batista and Ferreira, 2020).

The feed conversion ratio for each ecotype was calculated as indicated above.

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Carcass traits

The data collected on the carcass parameters was entered into an excel spread sheet and then exported to the SAS™ 9.0 software (2002) for analysis. An analysis of variance was used to isolate the effects of ecotype, sex, and ecotype- sex interactions.

Least square means (LSM; Steel and Torrie 1997) were estimated and SNK test was used to separate any differences among the means. The standard errors were also calculated.

The following General Linear model was used:

$$Yijk = \mu + Ei + Sj + ESij + eijk$$

Where, $Yijk$ = Dependent variable recorded on ith ecotype of jth sex; μ = the overall mean for a given dependent variable; Ei = the effect of ith ecotype; Sj = the effect of jth sex; $ESij$ = the interaction between ecotype and sex; $eijk$ = Residual error of kth observation recorded on ith ecotype and jth sex.

Heritability

The heritabilities for body weights and carcass traits were estimated using a univariate model which included fixed effects of replicate, sex, pen, interaction of replicate and pen, ancestral subpopulation proportions as described in Walugembe *et al.*, 2020 fitted as covariates, and random effects of the bird and residual. The animal model below was implemented in ASReml 4 (Gilmour et al., 2015).

$$\boldsymbol{y} = X\boldsymbol{b} + \boldsymbol{Z_a a} + \boldsymbol{e}$$

where **y** is the vector of phenotypic measurements; $\mathbf{X_1}$ is the incidence matrix relating the fixed effects and covariates to vector **y**; **b** is the vector of fixed effects and covariates; $\mathbf{Z_a}$ is the

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incidence matrix relating the phenotypic observations to the vector of random bird genetic effects, ***a***, with a genomic relationship matrix computed based on VanRaden (2008) to explain the (co)variance among birds; and ***e*** is the vector of random residuals.

Survival rates

The Kaplan-Meier method (Lanczky and Gyorffy, (2021) was used to estimate the survival probability of the experimental birds. In simple terms, the survival probability or survival function S(*t*) is defined as the probability of surviving at least to time *t* (Bewick *et al.*, 2004). The Kaplan–Meier method does not rely on the underlying probability distribution of the observed survival times.

The survival probability at time *ti, S(ti),* is calculated as follows:

$$S(ti) = S(ti-1)(1-\frac{di}{ni}).$$

where, *S(ti−1)* is the probability of being alive at *ti−1, ni* is the number of birds alive (at risk) just before *ti,* and *di* being the number of events at *ti.*

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4.4 Results

4.4.1 Body weights

The effect of ecotype on the body weights of the FO, CS, and IS chicken ecotypes of Ghana, measured at bi-weekly intervals, are shown in Table 3. Ecotype had a significant effect ($p < 0.05$) on the body weights of the three ecotypes at HW, BW4, BW6, BW8, BW10, BW12, BW16, BW18, BW20 and at BW22.

At hatch, the body weights of the FO and CS ecotypes were similar ($p > 0.05$), but heavier than the hatch weight of the IS ecotype ($p < 0.05$). Between week 4 and week 6, at week 14, as well as between week 18 and 20, the body weights of the FO and CS ecotypes were also similar ($p > 0.05$). However, from week 8 to week 12, the FO ecotype was heavier that the CS ecotype ($p > 0.001$). From week 4 to the end of the experiment, the IS ecotype was significantly heavier ($p < 0.05$) than the FO and CS ecotypes.

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Table 3. Least Square Means ± SE of body weights (g) of Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various ages (with hatch weight as covariate).

TRAIT	ECOTYPE			HERITABILITY (h^2)
	Forest	Coastal Savannah	Interior Savannah	
HW***	26.12^{a} ± 0.13 (560)	26.04^{a} ± 0.20 (261)	24.82^{b} ± 0.19 (289)	0.81 ± 0.06
BW2^{NS}	66.12 ± 0.48 (560)	67.90 ± 0.69 (261)	67.87 ± 0.67 (289)	0.51 ± 0.08
BW4***	147.11^{b} ± 1.11 (562)	146.35^{b} ± 1.62 (261)	154.30^{a} ± 1.57 (290)	0.50 ± 0.08
BW6***	252.09^{b} ± 1.91 (562)	251.43^{b} ± 2.78 (261)	268.79^{a} ± 2.69 (291)	0.47 ± 0.08
BW8***	377.28^{b} ± 2.53 (562)	364.09^{c} ± 3.57 (261)	396.61^{a} ± 3.46 (291)	0.45 ± 0.08
BW10***	526.67^{b} ± 3.27 (562)	504.24^{c} ± 4.61 (261)	544.62^{a} ± 4.46 (291)	0.41 ± 0.07
BW12***	674.91^{b} ± 4.04 (562)	655.46^{c} ± 5.68 (261)	695.98^{a} ± 5.50 (291)	0.37 ± 0.07
BW14***	799.56^{b} ± 4.47 (562)	788.05^{b} ± 6.49 (261)	838.89^{a} ± 6.29 (291)	0.38 ± 0.08
BW16***	927.59^{b} ± 4.97 (562)	907.96^{b} ± 7.22 (261)	961.04^{a} ± 6.70 (291)	0.64 ± 0.07
BW18***	1027.15^{b} ± 5.68 (562)	1017.30^{b} ± 8.27 (261)	1067.47^{a} ± 7.66 (291)	0.69 ± 0.07
BW20***	1098.64^{b} ± 5.82 (562)	1104.97^{b} ± 8.46 (261)	1146.23^{a} ± 8.20 (291)	0.64 ± 0.07
BW22***	1178.66^{ab} ± 6.19 (562)	1163.45^{b} ± 8.99 (261)	1196.67^{a} ± 8.71 (291)	0.58 ± 0.07

HW = Hatch weight. **BWn** = Body weight at week **n**, where **n** = 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22. Values with different superscript letters (a-c) within the same row per trait indicate significant deviation (*** =$p < 0.001$). ^{NS} = No significant deviation ($p > 0.05$) due to ecotype; The number of observations is indicated in the parenthesis.

The estimated heritabilities of body weight among the three chicken populations from hatch till 22 weeks of age, as shown in table 3, ranged from a moderate of 0.37 to a high of 0.81, with an average heritability of 0.54.

A comparison of the body weights of the hens and cocks of the three chicken ecotypes is shown in Table 4. At hatch, the body weights of the male and female chicks were similar. However,

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from BW2 till BW22, the male chickens were heavier than the female chickens, thus indicating sexual dimorphism.

Table 4: Least Square Means ± SE of body weights by sex in of Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various ages.

TRAIT	SEX	
	Male (n=541)	Female (n=573)
HW^{NS}	25.82 ± 0.15	25.51 ± 0.14
BW2***	69.86^{a} ± 0.55	65.95^{b} ± 0.53
BW4***	157.21^{a} ± 1.24	143.15^{b} ± 1.19
BW6***	271.28^{a} ± 2.10	242.62^{b} ± 2.02
BW8***	405.13^{a} ± 2.72	352.12^{b} ± 2.61
BW10***	568.36^{a} ± 3.52	479.97^{b} ± 3.38
BW12***	737.25^{a} ± 4.35	611.08^{b} ± 4.17
BW14***	891.07^{a} ± 5.01	723.47^{b} ± 4.81
BW16***	1036.71^{a} ± 5.60	824.33^{b} ± 5.37
BW18***	1156.75^{a} ± 6.12	914.15^{b} ± 5.88
BW20***	1246.76^{a} ± 6.58	982.73^{b} ± 6.31
BW22***	1311.02^{a} ± 7.02	1044.61^{b} ± 6.70

HW = Hatch weight. **BWn** = Body weight at week **n,** where **n** = week, where **n** = 2, 4, 6, 8,10, 12, 14, 16, 18, 20, 22. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =p < 0.05, ** =p < 0.01 and *** =p < 0.001). ^{NS} = No significant deviation (p > 0.05) due to sex; The number of observations is indicated in the parenthesis.

A comparison of the body weights by ecotype and disaggregated by sex is shown in Table 5. The ecotype-sex interactions were not significant from HW to BW6 and at BW22 (p > 0.05). However, at BW8, the males of the IS ecotype were significantly heavier (p < 0.05) than the males of the FO and CS ecotypes while at BW10, BW12, BW16, BW18 and BW20, the males of the IS and FO ecotypes were similar in weight, but heavier than the CS ecotype (p < 0.05).

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Among the hens, the IS ecotype was heavier than the FO and CS ecotypes at BW8 ($p < 0.05$). However, from BW10 to BW16, there were no significant variations ($p<0.05$), in body weight among the hens of three ecotypes.

Between BW18 and BW20, the IS and CS ecotypes were similar in weight and heavier than the FO ecotype ($p < 0.05$).

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Table 5. Least Square Means ± SE of ecotype-sex interactions of body weights (g) in Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various ages.

TRAIT	SEX	ECOTYPE		
		Forest	Coastal Savannah	Interior Savannah
HW ^{NS}	Male	26.25 ± 0.19 (276)	26.04 ± 0.28 (123)	25.16 ± 0.27 (141)
	Female	26.00 ± 0.19 (284)	26.05 ± 0.27 (138)	24.48 ± 0.26 (148)
BW2^{NS}	Male	68.58 ± 0.72 (276)	69.05 ± 1.07 (123)	69.02 ± 1.00 (141)
	Female	64.39 ± 0.71 (284)	67.27 ± 1.01 (138)	64.56 ± 0.98 (148)
BW4 ^{NS}	Male	154.23 ± 1.64 (277)	150.63 ± 2.42 (123)	160.83 ± 2.29 (140)
	Female	141.09 ± 1.61 (285)	142.99 ± 2.30 (138)	144.18 ± 2.21 (150)
BW6 ^{NS}	Male	267.40 ± 2.78 (277)	262.96 ± 4.11 (123)	283.48 ± 3.88 (141)
	Female	238.11 ± 2.72 (285)	241.05 ± 3.90 (138)	248.70 ± 3.75 (150)
BW8*	Male	407.06 ^{b} ± 3.59(277)	384.08^{c} ± 5.31 (123)	424.26 ^{a} ± 5.01(141)
	Female	349.20 ^{b} ± 3.52 (285)	345.68 ^{b} ± 5.04 (138)	361.49 ^{a} ± 4.85 (150)
BW10*	Male	576.43 ^{a} ± 4.65 (277)	538.38 ^{b} ± 6.88 (123)	590.28 ^{a} ± 6.50 (141)
	Female	479.02 ^{a} ± 4.5 6(285)	472.09 ^{a} ± 6.53 (138)	488.80 ^{a} ± 6.28 (150)
BW12***	Male	746.15^{a} ± 5.74 (277)	702.05 ^{b} ± 8.49 (123)	763.56 ^{a} ± 8.01 (141)
	Female	606.12 ^{a} ± 5.62 (285)	611.07 ^{a} ± 8.05 (138)	616.17 ^{a} ± 7.74 (150)
BW14***	Male	891.27 ^{b} ± 6.61 (277)	853.14 ^{c} ± 9.78 (123)	928.80 ^{a} ± 9.23(141)
	Female	710.62 ^{a} ± 6.48(285)	725.48 ^{a} ± 9.28 (138)	734.32 ^{a} ± 8.92 (150)
BW16***	Male	1048.10 ^{a} ± 7.34 (277)	990.04 ^{b} ± 10.93 (123)	1071.97 ^{a} ± 10.32 (141)
	Female	810.34 ^{a} ± 7.24 (285)	828.74 ^{a} ± 10.37 (138)	833.90 ^{a} ± 9.97 (150)
BW18***	Male	1164.90 ^{a} ± 8.08 (277)	1115.16 ^{b} ± 11.95 (123)	1190.19 ^{a} ± 11.28 (141)
	Female	892.76 ^{b} ± 7.92 (285)	992.22 ^{a} ± 11.34 (138)	927.48 ^{a} ± 10.90 (150)
BW20***	Male	1255.29 ^{a} ± 8.68 (277)	1216.56 ^{b} ± 12.84 (123)	1268.44 ^{a} ± 12.12 (141)
	Female	946.51 ^{b} ± 8.51 (285)	996.88 ^{a} ± 12.19 (138)	1004.81 ^{a} ± 11.72 (150)
BW22 ^{NS}	Male	1345.45 ± 9.27 (277)	1269.74 ± 13.72 (123)	1317.87 ± 12.95 (141)
	Female	1016.23 ± 9.09 (285)	1060 ± 13.01 (138)	1056.97 ± 12.51 (150)

HW = Hatch weight. **BWn** = Body weight at week **n**, where, where **n** = 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22. Values with different superscript letters (a-c) within the same row per trait indicate significant deviation (* =p < 0.05, ** =p < 0.01 and *** =p < 0.001). ^{NS} = No significant deviation (p > 0.05); The number of observations is indicated in the parenthesis.

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4.4.2 Weight gain

The IS ecotype was the fastest growing chicken from hatch up till week 16 after which the other ecotypes caught up with it. The variations in weight gain obtained fortnightly in the IS, FO and CS chicken ecotypes are shown in Table 6.

At WG2, WG14 and WG20, there were no variations in the growth rates of the CS and IS ecotypes, but these were significantly higher ($p<0.05$) than the growth rate of the FO ecotype but at WG16, the FO ecotype grew faster than the IS and CS ecotypes. Between WG4 and WG6, the IS ecotype had higher growth rates than the FO and CS ecotypes ($p < 0.05$) while between WG8 and WG10, the growth rates of the FO and IS ecotypes were similar, but higher than the growth rate of the CS ecotype ($p < 0.05$).

Even though the effect of ecotype on TWG was not significant amongst the three ecotypes, the IS ecotype appeared to have the highest growth rate from WG2 to WG22.

Sex had a significant effect on weight gain (Table 7), with the males having higher growth rates than the females ($p < 0.05$). Amongst both sexes of the three ecotypes, the growth rates rose steadily from hatch, peaked at WG12, and then started going down till the end of the experiment.

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Table 6. Least Square Means ± SE of weight gain (g) by ecotype in Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various periods.

TRAIT	ECOTYPE		
	Forest	Coastal Savannah	Interior Savannah
WG2*	39.97^{b} ± 0.50 (560)	42.13 ^{a} ± 0.73 (261)	41.66 ^{a} ± 0.69 (289)
WG4***	80.17 ^{b} ± 0.85 (562)	78.68^{b} ± 1.24 (261)	85.07 ^{a} ± 1.17 (290)
WG6***	104.93^{b} ± 1.14 (562)	105.63^{b} ± 1.67 (261)	113.20^{a} ± 1.58 (290)
WG8***	125.63^{a} ± 1.11 (562)	112.81^{b} ± 1.61 (261)	126.98^{a} ± 1.53 (290)
WG10***	149.21^{a} ± 1.37 (562)	140.56^{b} ± 2.00 (261)	146.20^{a} ± 1.90 (290)
WG12 NS	149.80 ± 1.57 (562)	151.91 ± 2.28 (261)	149.76 ± 2.16 (290)
WG14***	125.00^{b} ± 1.87 (562)	131.72^{a} ± 2.72 (261)	140.65^{a} ± 2.58 (290)
WG16*	127.53^{a} ± 1.81 (562)	119.64^{b} ± 2.64 (261)	120.97^{b} ± 2.51 (290)
WG18 NS	100.66 ± 2.10 (562)	108.83 ± 3.06 (261)	104.99 ± 2.90 (290)
WG20**	73.07^{b} ± 2.37 (562)	86.23^{a} ± 3.46 (261)	78.44^{ab} ± 3.28 (290)
WG22***	78.92^{a} ± 2.81 (562)	60.41^{b} ± 4.09 (261)	52.87^{b} ± 3.88 (290)
TWG NS	1155.88 ± 6.62 (562)	1138.54 ± 9.65 (261)	1160.80 ± 9.14 (290)

WG = Weight gain. **WG2** = WG between hatch and week 2; **WG4** = WG between week 2 and week 4; **WG6** = WG between week 4 and week 6; **WG8** = WG between week 6 and week 8; **WG10** = WG between week 8 and week 10; **WG12** = WG between week 10 and week 12; **WG14** = WG between week 12 and week 14; **WG16**= WG between week 14 and week 16; **WG18** = WG between week 16 and week 18; **WG20** = WG between week 18 and week 20; **WG22**= WG between week 20 and week 22; **TWG** = WG between hatch and week 22. Values with different superscript letters (a-c) within the same row per trait indicate significant deviation (* =p < 0.05, ** =p < 0.01 and *** =p < 0.001). NS = No significant deviation (p > 0.05) due to ecotype; The number of observations is indicated in the parenthesis.

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Table 7: Least Square Means ± SE of weight gain (g) by sex in Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various periods.

Trait	Sex	
	Male	Female
WG2***	43.09[a] ±0.53 (540)	39.41[b] ±0.51 (570)
WG4***	86.19[a] ±0.89 (540)	77.09[b] ±0.86 (573)
WG6***	116.14[a] ±1.20 (540)	99.71[b] ±1.16 (573)
WG8***	134.65[a] ±1.16 (540)	108.97[b] ±1.12 (573)
WG10***	163. 61[a] ±1.44 (540)	127.04[b] ±1.39 (573)
WG12***	170.06[a] ±1.64 (540)	130.92[b] ±1.58 (573)
WG14***	153.76[a] ±1.95 (540)	111.15[b] ±1.89 (573)
WG16***	147.23[a] ±1.90 (540)	98.20[b] ±1.83 (573)
WG18***	121.49[a] ±2.20 (540)	88.15[b] ±2.13 (573)
WG20***	92.80[a] ±2.48 (540)	65.69[b] ±2.40 (573)
WG22 NS	67.76[a] ± 2.94 (540)	60.38[a] ±2.84 (573)
TWG***	1296.77[a] ± 6.93 (540)	1006.71[b] ±6.69 (573)

WG =Weight gain. **WG2** = WG between hatch and week 2; **WG4** = WG between week 2 and week 4; **WG6** = WG between week 4 and week 6; **WG8** = WG between week 6 and week 8; **WG10** = WG between week 8 and week 10; **WG12** = WG between week 10 and week 12; **WG14** = WG between week 12 and week 14; **WG16** = WG between week 14 and week 16; **WG18** = WG between week 16 and week 18; **WG20** = WG between week 18 and week 20; **WG22** = WG between week 20 and week 22; **TWG** = WG between hatch and week 22. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =p < 0.05, ** =p < 0.01 and *** =p < 0.001). NS = No significant deviation (p > 0.05) due to sex; The number of observations is indicated in the parenthesis.

From hatch till week 22, male chickens of the FO ecotype had the highest growth rates while the females of the FO ecotype had the lowest growth rates. The segregation of the growth rates of the three chicken ecotypes by sex, as shown in Table 8, provides an opportunity to better perceive any subtle differences in their growth rates that may exist.

At WG2 and WG8, WG 14 and WG 18, there were no significant differences in the growth rates of the three ecotypes (p>0.05). However, from WG10 to WG12, the growth rates of the male chickens of the FO and IS ecotypes were similar and higher than the growth rates of the

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CS ecotype ($p<0.05$). At WG16, the growth rate of males of the FO ecotype was significantly ($p<0.05$) higher than those of the CS and IS ecotypes. However, by WG20, the CS ecotype had attained a similar growth rate as that of the FO ecotype. The males of the FO ecotype maintained their high growth rate up till WG22.

From HW to WG22 (TWG), the males of the FO ecotype had the highest growth rates, followed by the males of the IS and CS ecotypes ($p<0.05$).

At WG12, females of the CS ecotype had a superior growth rate than the females of the FO and IS ecotypes. At WG20, the growth rates of females of the CS and IS ecotypes were similar ($p>0.05$) but higher than those of the FO ecotype. During the rest of the experimental periods, the variations in weight gain among females of the three chicken ecotypes were not significant ($p>0.05$). The total growth rate (TWG) of the females of the CS and IS ecotypes were also similar, but higher than the total growth rate of the FO ecotype.

Based on the above results, cocks of the FO ecotype seemed to have superior growth rates than the other chicken ecotypes. On the other hand, hens of the CS and IS ecotypes which had similar growth rates, grew faster than the FO ecotype.

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Table 8: Least Square Means ± SE of ecotype-sex interactions on weight gain (g) in Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various periods.

TRAIT	SEX	ECOTYPE		
		Forest	Coastal Savannah	Interior Savannah
WG2^{NS}	Male	41.20 ± 0.71 (276)	43.08 ± 1.06 (123)	43.93 ± 1.0 (141)
	Female	37.96 ± 0.70 (284)	41.09 ± 1.0 (138)	39.41 ± 0.97 (148)
WG4 ^{NS}	Male	86.05 ± 1.2 (276)	81.81 ± 1.8 (123)	90.24 ± 1.68 (140)
	Female	76.31 ± 1.18 (284)	75.40 ± 1.7 (138)	79.94 ± 1.63 (150)
WG6 ^{NS}	Male	113.09 ± 1.62 (276)	112.75 ± 2.42 (123)	122.50 ± 2.27 (141)
	Female	96.77 ± 1.60 (284)	98.40 ± 2.29 (138)	103.99 ± 2.20 (150)
WG8 ^{NS}	Male	140.14 ± 1.57 (276)	120.92 ± 2.34 (123)	140.70 ± 2.19 (141)
	Female	111.19 ± 1.54 (284)	104.21 ± 2.21 (138)	113.31 ± 2.12 (150)
WG10*	Male	168.99^{a} ± 1.94 (276)	154.2^{b} ± 2.9 (123)	165.61^{a} ± 2.72 (141)
	Female	129.48^{a} ± 1.19 (284)	126.44^{a} ± 2.74 (138)	126.86^{a} ± 2.63 (150)
WG12**	Male	172.02^{a} ± 2.21 (276)	164.30^{b} ± 3.3 (123)	170.44^{a} ± 3.09 (141)
	Female	127.69^{b} ± 2.18 (284)	138.77^{a} ± 3.12 (138)	129.14^{b} ± 3.0 (150)
WG14 ^{NS}	Male	145.66 ± 2.65 (276)	150.24 ± 3.95 (123)	165.72 ± 3.7 (141)
	Female	104.36 ± 2.60 (284)	112.91 ± 3.73 (138)	115.81 ± 3.58 (150)
WG16***	Male	156.53^{a} ± 2.56 (276)	136.35^{b} ± 3.82 (123)	143.49^{b} ± 3.57 (141)
	Female	98.67^{a} ± 2.52 (284)	102.09^{a} ± 3.61 (138)	98.33^{a} ± 3.47 (150)
WG18 ^{NS}	Male	118.84 ± 2.99 (276)	125.34 ± 4.45 (123)	118.80 ± 4.17 (141)
	Female	82.50 ± 2.94 (284)	92.30 ± 4.21 (138)	91.0 ± 4.04 (150)
WG20***	Male	92.08^{ab} ± 3.35 (276)	99.94^{a} ± 4.99 (123)	81.03^{b} ± 4.67 (141)
	Female	54.15^{b} ± 3.29 (284)	72.46^{a} ± 4.72 (138)	75.18^{a} ± 4.53 (150)
WG22***	Male	90.26^{a} ± 3.96 (276)	55.62^{b} ± 5.91 (123)	48.88^{b} ± 5.53 (141)
	Female	67.78^{a} ± 3.90 (284)	64.25^{a} ± 5.59 (138)	56.39^{a} ± 5.37(150)
TWG***	Male	1325.65^{a} ± 9.27 (276)	1244.53^{c} ± 13.84 (123)	1291.33^{b} ± 12.95 (141)
	Female	986.87^{b} ± 9.12 (284)	1028.32^{a} ± 13.08 (138)	1029.34^{a} ± 12.56 (150)

WG = Weight gain. **WG2** = WG between hatch and week 2; **WG4** = WG between week 2 and week 4; **WG6** = WG between week 4 and week 6; **WG8** = WG between week 6 and week 8; **WG10** = WG between week 8 and week 10; **WG12** = WG between week 10 and week 12; **WG14** = WG between week 12 and week 14; **WG16** = WG between week 14 and week 16; **WG18** = WG between week 16 and week 18; **WG20** = WG between week 18 and week 20; **WG22** = WG between week 20 and week 22; **TWG** = WG between hatch and week 22. Values with different superscript letters (a-c) within the same row per trait differ significantly at * $p < 0.05$, *** $p < 0.001$

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4.4.3 Growth curves of the three local chicken ecotypes of Ghana

The growth curves of male chickens of the three local chicken ecotypes from hatch to week 22 are shown in Figure 10 while the growth curves of female chickens of the three local chicken ecotypes within the same period are shown in Figure 11.

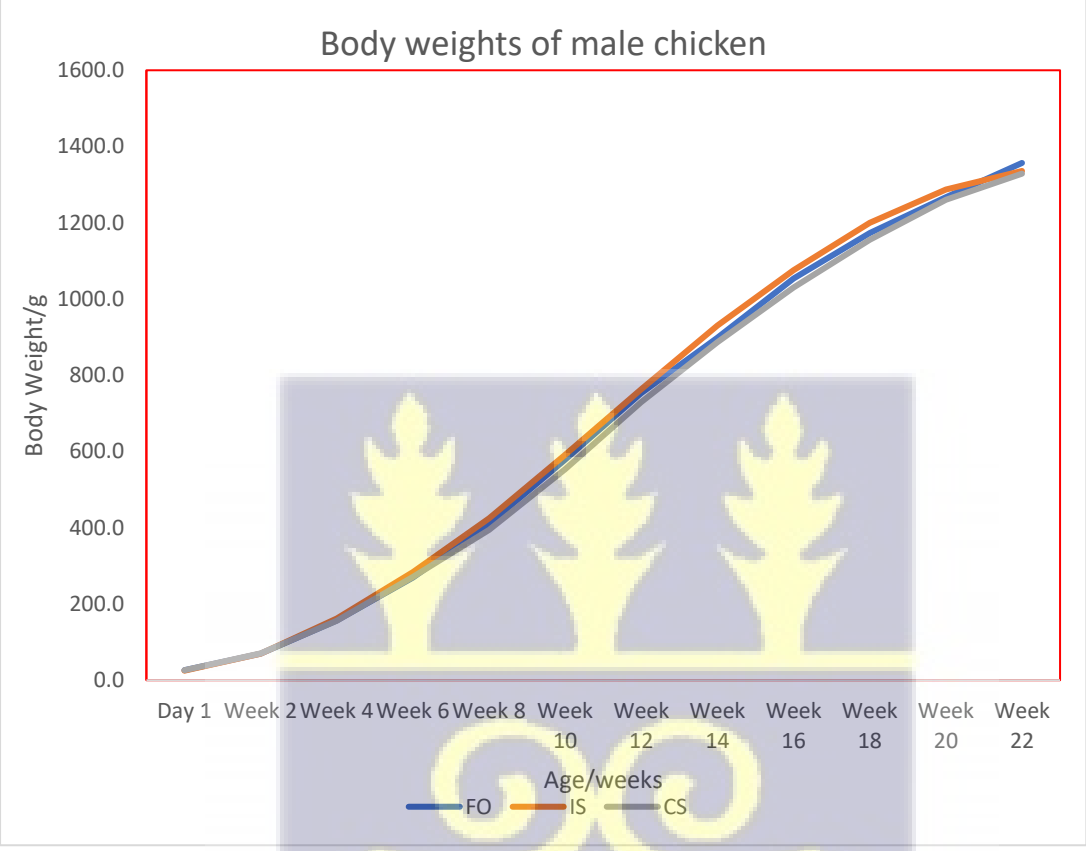

Figure 10. Growth curves of male chickens of the Interior Savannah, Forest, and Coastal Savannah chicken ecotypes

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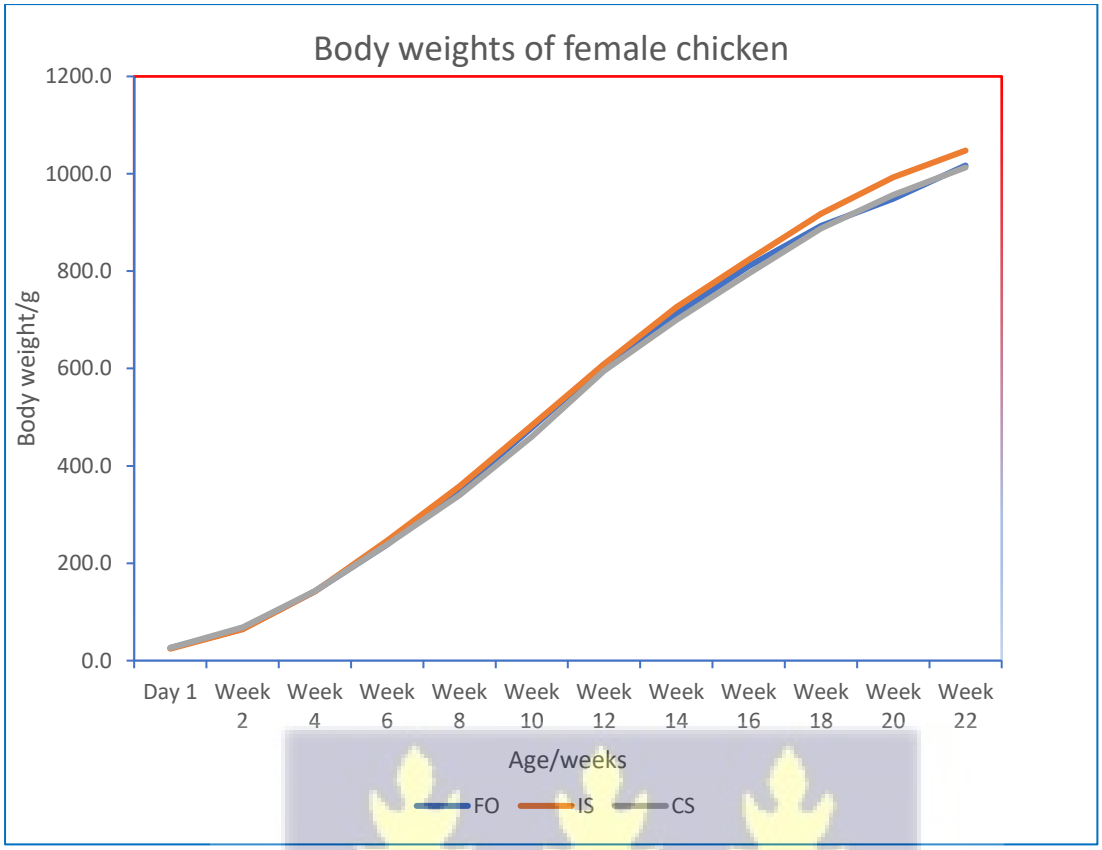

Figure 11. Growth curves of female chickens of the Interior Savannah, Forest and Coastal Savannah chicken ecotypes

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4.4.4 Feed Consumption and Conversion Efficiency

All the birds were more efficient converters of feed during the first 8 weeks of life than during the rest of the experimental period. There was a significant ($p<0.05$) variation in the daily feed intake per bird per ecotype from hatch till week 22 as shown in Table 9. From hatch up till week 8, the average daily feed intake of the FO and IS ecotypes were similar but significantly higher than the intake of the CS ecotype. However, between week 8 and week 22, the FO ecotype had the highest average daily feed intake, followed by the IS and CS ecotypes. Similarly, from hatch to week 22, the average daily feed intake of the FO was significantly higher ($p<0.05$) than the intake of the IS and the CS ecotypes, while the intake of the IS was higher than the daily intake of the CS ecotype.

The CS ecotype had a lower feed conversion rate, though not significant, than the FO and IS ecotypes during the first eight weeks of life and during the rest of the experimental period. Over the entire experimental period, though not significant, the CS ecotype had the lowest feed conversion rate, followed by the IS and the FO ecotypes.

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Table 9. Least Square Means ± SE of feed consumption and feed conversion rates of the Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana at various periods.

PARAMETER	ECOTYPE		
	Forest (n = 562)	Coastal Savannah (n = 265)	Interior Savannah (n = 297)
0-8 Weeks			
Final weight (g/bird)	$378.13^{b} \pm 2.53$	$364.88^{c} \pm 3.68$	$392.87^{a} \pm 3.5$
Initial weight (g/bird	$26.12^{a} \pm 0.13$	$26.04^{a} \pm 0.20$	$24.82^{b} \pm 0.19$
Total weight gain (g/bird)	$340.95^{c} \pm 3.68$	$354.24^{b} \pm 2.53$	$366.76^{a} \pm 3.49$
ADG (g/bird)	$6.09^{c} \pm 0.07$	$6.33^{b} \pm 0.05$	$6.55^{a} \pm 0.06$
TFI (g/bird)	$1157.43^{a} \pm 02.17$	$1109.25^{a} \pm 102.17$	$1162.40^{a} \pm 102.17$
ADFI (g/bird)	$20.84^{a} \pm 0.12$	$19.66^{b} \pm 0.20$	$20.70^{a} \pm 0.18$
FCR	**3.40**	**3.13**	**3.17**
8-22 Weeks			
Final weight (g/bird)	$1180.84^{a} \pm 6.52$	$1165.18^{a} \pm .49$	$1187.42^{a} \pm 9.04$
Initial weight (g/bird)	$378.13^{b} \pm 2.53$	$364.88^{c} \pm 3.68$	$392.87^{a} \pm 3.5$
Total weight gain (g/bird)	$806.09^{a} \pm 7.82$	$807.37^{a} \pm 5.36$	$800.69^{a} \pm 7.40$
ADG (g/bird)	$8.23^{a} \pm 0.08$	$8.24^{a} \pm 0.06$	$8.17^{a} \pm 0.08$
TFI (g/bird)	$5243.85^{a} \pm 02.17$	$5080.55^{a} \pm 102.17$	$5229.04^{a} \pm 102.17$
ADFI (g/bird)	$53.57^{a} \pm 0.12$	$51.42^{c} \pm 0.20$	$52.93^{b} \pm 0.20$
FCR	**6.51**	**6.29**	**6.53**
0-22 Weeks			
Final weight (g/bird)	$1180.84^{a} \pm 6.52$	$1165.18^{a} \pm 9.49$	$1187.42^{a} \pm 9.04$
Initial eight (g/bird)	$26.12^{a} \pm 0.13$	$26.04^{a} \pm 0.20$	$24.82^{b} \pm 0.19$
Total weight gain (g/bird)	1147.04 ± 9.10	1161.61 ± 6.24	1167.46 ± 8.61
ADG (g/bird)	7.45 ± 0.06	7.54 ± 0.04	7.58 ± 0.06
TFI (g/bird)	$6397.54^{a} \pm 34.26$	$6205.63^{b} \pm 34.26$	$6401.51^{a} \pm 34.26$
ADFI (g/bird)	$41.59^{a} \pm 0.10$	$39.86^{c} \pm 0.16$	$41.15^{b} \pm 0.16$
FCR	**5.58**	**5.34**	**5.48**

ADG = Average daily gain. TFI = Total Feed intake. ADFI = Average daily feed intake. LSM = Least square means. Values with different superscript letters (a-c) within the same row per trait differ significantly at $p < 0.05$. The number of observations is indicated in the parenthesis

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4.4.5 Carcass traits

The fasting weights of the FO and IS birds were similar, but the FO birds had heavier breast muscle weights than the IS birds. The effect of ecotype on some carcass traits, i.e., breast weight, thigh weight, wing weight, dressed weight, dressing percentage and drumstick weight of the FO, CS and IS chicken ecotypes can be seen in Table 10.

Ecotype did not have a significant effect ($p > 0.05$) on thigh weight, wing weight, drumstick weight, dressed weight and dressing percentage. However, it influenced breast weight and fasting weight. The fasting weights of the FO and IS ecotypes were similar, but higher than those of the CS ecotype ($p < 0.05$). The FO ecotype also had heavier breast muscles than the CS and IS ecotypes whose breast weights were similar ($p > 0.05$).

The estimated heritabilities of the carcass traits (Table 10) were moderate, with the heritability of breast weight being 0.29.

Table 10. Least Square Means ± SE of carcass traits (g) by ecotype of the Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana.

Trait	Ecotype			Heritability (h^2)
	Forest (n=491)	Coastal Savannah (n=249)	Interior Savannah (n=296)	
Breast weight (g)**	196.54^{a} ± 1.29	190.77^{b} ± 1.81	191.43^{b} ± 1.70	0.29 (0.07)
Thigh weight (g)^{NS}	129.90 ± 0.84	127.06 ± 1.18	128.82 ± 1.11	0.21 (0.07)
Wing weight (g) ^{NS}	98.46 ± 0.52	97.51 ± 0.73	99.32 ± 0.69	0.38 (0.08)
Drumstick weight (g) ^{NS}	117.10 ± 0.72	115.34 ± 1.01	117.26 ± 0.95	0.29 (0.07)
Dressed weight (g) ^{NS}	806.49 ±4.38	792.91 ± 6.16	812.54 ± 5.77	0.30 (0.07)
Dressing Percentage ^{NS}	70.70 ± 0.14	71.07 ± 0.19	70.80 ± 0.18	-

Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =$p < 0.05$, ** =$p < 0.01$ and *** =$p < 0.001$). ^{NS} = No significant deviation ($p > 0.05$) due to ecotype; The number of observations is indicated in the parenthesis. h^2=Heritability

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The effect of sex on some carcass traits of the three chicken ecotypes can be seen in Table 11. Sex had a significant influence ($p<0.05$) on breast weight, thigh weight, wing weight, drumstick weight, dressed weight and dressing percentage, with these traits being higher in the cocks than the hens.

Table 11. Least Square Means ± SE of carcass traits by sex of three chicken ecotypes in Ghana.

TRAIT	SEX	
	Male (n=484)	Female (n=552)
Fasting weight (g)***	$1290.10^{a} \pm 6.20$	$975.18^{b} \pm 5.77$
Breast weight (g) ***	$211.79^{a} \pm 1.36$	$174.04^{b} \pm 1.27$
Thigh weight (g) ***	$152.43^{a} \pm 0.89$	$104.76^{b} \pm 0.83$
Wing weight (g) ***	$114.29^{a} \pm 0.55$	$82.56^{b} \pm 0.51$
Drumstick weight (g) ***	$144.94^{a} \pm 0.76$	$88.20^{b} \pm 0.71$
Dressed weight (g) ***	$925.32^{a} \pm 4.62$	$682.65^{b} \pm 4.31$
Dressing percentage***	$71.7^{a} \pm 0.15$	$70.01^{b} \pm 0.14$

Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =$p < 0.05$, ** =$p < 0.01$ and *** =$p < 0.001$). ^{NS} = No significant deviation ($p > 0.05$) due to sex; The number of observations is indicated in the parenthesis.

Although ecotype was found to affect fasting weight and breast muscle weight, it is also important to find out the extent to which these effects persist, if any, at a sex disaggregated level. As shown in Table 12, the ecotype by sex interactions were not significant in the dressing percentage, fasting, breast, thigh, wing, drumstick and dressed weights among the three chicken ecotypes.

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Table 12. Least Square Means ± SE of ecotype-sex interactions of carcass traits in the Forest, Coastal Savannah, and Interior Savanah chicken ecotypes of Ghana.

TRAIT	SEX	ECOTYPE		
		Forest	Coastal savannah	Interior savannah
Fasting wt. (g)	Male	1301.99 ± 8.47	1272.01±11.87	1296. ± 11.54
	Female	974.76 ± 8.14	956.80 ± 11.45	993.97 ± 10.13
Breast wt. (g)	Male	216.91 ± 1.86	211.13 ± 2.60	207.33 ±2.53
	Female	154.87 ± 1.21	149.53 ± 1.70	152.89 ± 1.65
Thigh wt. (g)	Male	154.87 ± 1.21	149.53 ± 1.7	152.89 ± 1.65
	Female	149.40 ± 1.17	104.58±1.64	104.75 ± ^{2}.21
Wing wt. (g)	Male	114.80 ± 0.75	112.63 ± 1.05	115.45± 1.02
	Female	82.11 ± 0.72	82.38±1.01	83.18 ± 0.9
Drumstick	Male	145.46 ± 1.04	143.56 ± 1.45	154.79± 1.41
wt.(g)	Female	88.73 ± 1.0	87.13 ± 1.4	88.73 ± 1.24
Dressed wt.(g)	Male	933.22 ± 6.32	911.79 ± 8.85	930.94± 8.6
	Female	679.77 $^{\pm}$ 6.07	674.03±8.54	694.14 ± 7.56
Dressing %	Male	71.63 ± 0.20	71.68 ± 0.28	71.83 ± 0.27
	Female	69.78 ± 0.19	70.46 ± 0.27	69.78 ± 0.24

4.4.6 Survivability of the three local chicken ecotypes of Ghana

During the first week of life, the IS ecotype experienced the highest mortality rate compared to the FO and CS ecotypes (Table 13). This pattern of mortality persisted up till the time the chicks were about seven weeks of age. During the growing phase (8th to 22nd week), as well as over the entire experimental period, the IS ecotype still had the highest mortality rate, followed by the FO ecotype.

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Table 13. Percentage mortality of three chicken ecotypes at weekly intervals

PERIOD	Mortalities (%)		
	Forest (n=631)	Coastal Savannah (n=284)	Interior Savannah (n=350)
Week 1	7.765	1.761	9.143
Week 2	1.268	2.817	2.571
Week 3	0.634	0.352	0.571
Week 4	0.317	0.000	0.286
Week 5	0.000	0.000	0.000
Week 6	0.000	0.704	0.286
Week 7	0.000	0.000	0.286
Week 8	0.158	0.352	0.000
Subtotal	**10.143**	**5.986**	**13.143**
Week 9	0.000	0.000	0.000
Week 10	0.158	0.000	0.286
Week 11	0.000	0.000	0.286
Week 12	0.000	0.000	0.000
Week 13	0.000	0.000	0.000
Week 14	0.317	0.000	0.286
Week 15	0.000	0.000	0.000
Week 16	0.000	0.000	0.286
Week 17	0.158	0.000	0.286
Week 18	0.000	0.000	0.000
Week 19	0.000	0.000	0.000
Week 20	0.158	0.000	0.000
Week 21	0.000	0.000	0.000
Week 22	0.000	0.000	0.000
Subtotal	**0.792**	**0.000**	**1.429**
Total Mortality	**10.935**	**5.986**	**14.571**

The steepest decline in survival probability amongst the three ecotypes occurred during the first week of life. The survival probabilities of the three chicken ecotypes over a 22-week period are shown in the survival curves in Figure 12. From hatch to week 22, the survival probability of the CS ecotype was the highest, followed by the FO and the IS ecotype.

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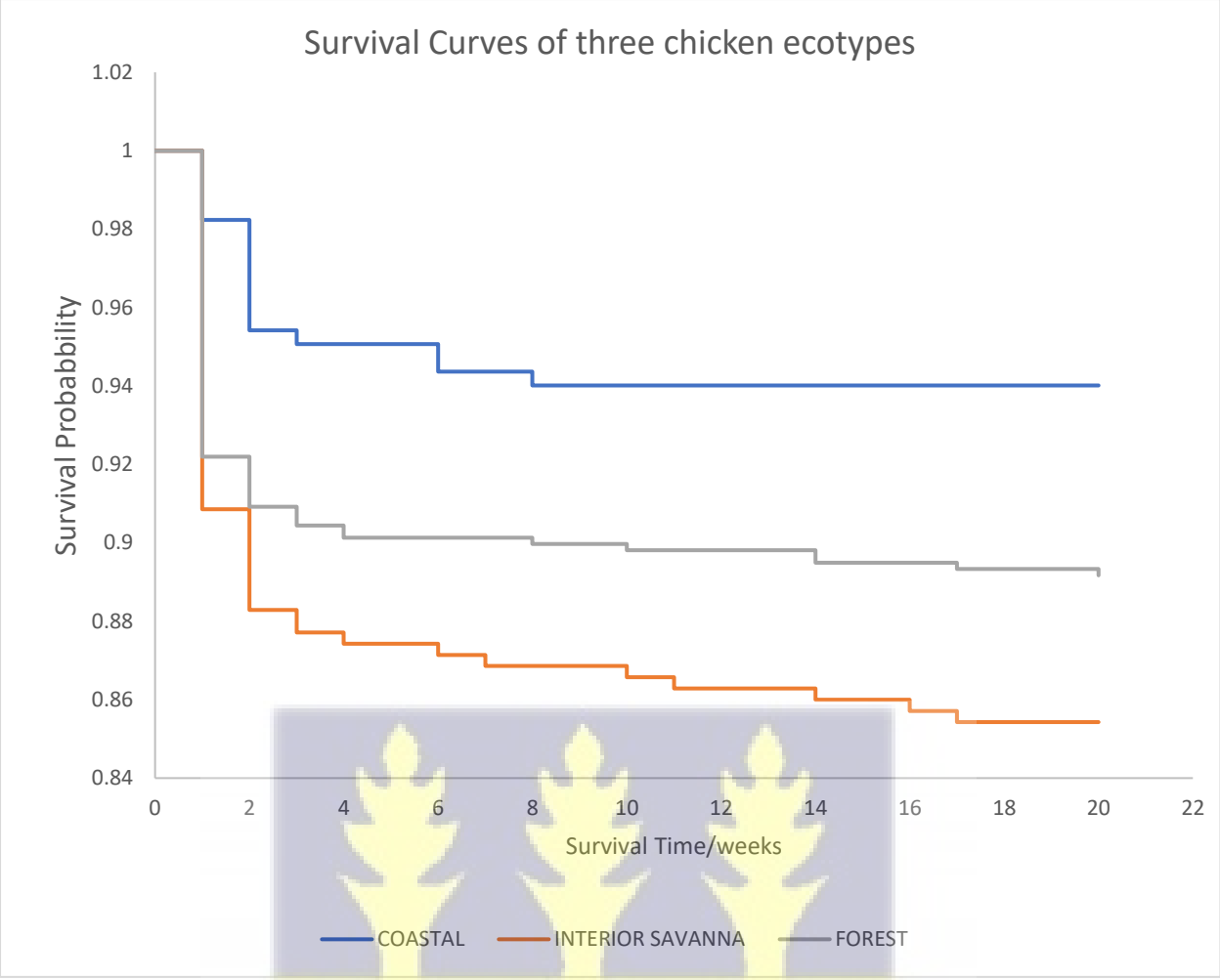

Figure 12. Survival curves of the Forest, Coastal Savannah and Interior Savannah chicken ecotypes

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4.5 Discussion

4.5.1 Body weights and growth rates

The results of the body weight measurements showed that the hatch weights of the FO and CS ecotypes were significantly higher than the hatch weight of the IS ecotype, with the highest hatch weight being recorded in the FO Ecotype (Table 3). Hatch weight is a function of egg weight (Johnsson *et al*., 2016), which in turn is determined largely by the age and weight of the dam. It is also possible that the hatch weight of the IS ecotype is reduced as an adaptation to the harsh nutritional environment in Northern Ghana where, especially during the dry season, there is hardly anything to eat. These findings are similar to the results reported by Osei-Amponsah (2010a), but different from the results of Youssao *et al.* (2012) who found no significant variations in hatch weights of intensively reared FO and IS chicken ecotypes of Benin. The findings of this study agree with N'dri *et al.* (2018) who observed that the average hatch weight of intensively reared slow growing local chickens in Cote d'Ivoire to be about 26.0 grams. Hassen *et al.* (2006) reported higher hatch weights in the range of 25.5 g to 29.3 g among seven local chicken ecotypes of Ethiopia which were reared under intensive management conditions. The hatch weights of three intensively raised Korean local chicken were also reported by Choo *et al.* (2014) to be in the range of 39.38 grams to 42.8 grams. These differences in the hatch weights of local chickens could be a result of factors such as the genetics, egg weight, age of dam and feeding conditions (Youssao *et al.*, 2012).

The results of this study also showed that at a sex disaggregated level, there was no significant difference in the hatch weights of male and female chickens. This is to be expected because at hatch, sex has no effect on the concentration of growth promoting hormones like plasma insulin, insulin-like growth factor hormones, triiodothyronine (T_3) and plasma glucose and glycogen (Lu *et al*., 2007).

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The IS ecotype was observed to be heavier than the FO and CS ecotypes from the 4th to the 20th week. Body weight is a highly heritable trait (Hanusova *et al*., 2017) and is associated with genetic factors but its expression also depends on environmental factors like nutrition and health status. Since the experimental birds were raised under similar conditions, the observed differences in body weight could be attributed to genetic differences amongst the ecotypes. These findings are similar to the observations of Youssao *et al*. (2012) that beyond the 8th week, IS ecotype was heavier than the FO ecotype. Tougan *et al.* (2009) also reported that from the 12th week onwards, IS chicken populations raised under intensive management conditions were heavier than the FO chicken populations. Similarly, Adeyinka (2010) also concluded that under intensive management conditions, the IS chicken ecotype of northern Nigeria was heavier than the FO ecotype from hatch till the 20th week.

A comparison of the body weights of the FO and CS ecotypes showed that their body weights were similar from the 4th to the 6th week as well as from the 14th to the 20th week. This supports the observation by Osei-Amponsah (2014) who did not find any variations in the body weights of the FO and CS ecotypes of Ghana between the 4th and 6th week.

On a sex disaggregated basis, the males of the IS and FO ecotypes were similar in weight, but heavier than the CS ecotype from the 10th to the 12th week and between the 16th and 20th week. Adetayo and Babafunso (2001) also observed no significant variations in the body weights of male IS and FO ecotypes of Nigeria from the 5th week onwards while Osei-Amponsah *et al*. (2014) reported that in Ghana the males of the IS ecotype were significantly heavier than the males of the FO ecotype from the 8th to the 28th week.

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Among the females, there were no significant variations in the body weights of the three ecotypes up till 6 weeks of age and then at 10 to 18 weeks of age. However, at 20 weeks of age, the IS and CS ecotypes were similar in weight but heavier than the FO ecotype. Adetayo and Babafunso (2001) observed that the females of the IS ecotypes were significantly heavier than the females of the FO ecotype from the 20th week onwards. Given the body weight superiority of the IS chicken ecotype over the other two ecotypes, it could be used as resource for the improvement of the body weight trait among local chickens of Ghana.

Sexual dimorphism was observed amongst the three chicken ecotypes from the 2nd week of age till the end of the experiment. This suggests that the three ecotypes can be separated by sex from the juvenile age onwards, but with an increasing accuracy as the birds age. Sexual dimorphism can be found in many non-feral avian species to the extent that the males are heavier than females, with some variations between species (Baeza *et al*., 2001). However, there is considerable variability in the onset of sexual dimorphism amongst local chickens (Tougan *et al.* 2009; Sola-Ojo *et al*. 2011; Youssao *et al*., 2012; Etta *et al*. 2020). Tougan *et al.* (2009) observed that sexual dimorphism in the IS ecotype of Benin was apparent after 12 weeks of age but in the FO ecotypes it was not apparent even at 24 weeks of age. Similarly, Youssao *et al*. (2012) also concluded that body size sexual dimorphism amongst the FO and IS ecotypes of Benin was evident from 8 weeks of age. Adetayo and Babafunso (2001) reported that sexual dimorphism amongst the FO ecotype of Nigeria was apparent after the 5th week with the males being heavier than the females, but this observation is at variance with Adeyinka (2010) who reported that sexual dimorphism among the IS and FO ecotypes of Nigeria was apparent from the 10th week onwards. Sola-Ojo *et al*. (2011) and Etta *et al.* (2020) concluded that sexual dimorphism in the Fulani and Cross River (Rose Comb) chicken ecotypes of Nigeria was observed from the 6th week onwards.

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In free range or semi-intensive rearing systems where there is a constraint with feed resources, dimorphism in body size may play a significant role in the struggle for dominance (Etta *et al*., 2020), to the extent that competition amongst siblings for resources could lead to sex-biased deaths (Drummond *et al*., 1991), in favour of the males who tend to have larger body sizes.

The hens and cocks of the three chicken ecotypes attained their highest growth rates at 12 weeks of age, after which the growth rates progressively declined. Over the entire 22-week period, cocks of the FO ecotype had the highest growth rates, followed by cocks of the IS and CS ecotypes (Table 8). Among the hens, the IS had overall superior growth rates, followed by hens of the CS and FO ecotypes. These observations seem to suggest that any future breeding program that seeks to improve the growth rates of the local chickens of Ghana could consider the use of males of the FO ecotype and females of the IS ecotype.

The rate of increase in body weight measured on a fortnightly basis from day old to 22 weeks in the FO ecotype ranged from a high of 60.71% to a low of 6.8%, while for the CS and IS ecotypes it ranged from 61.80% to 5.02%, and 62.79% to 4.28%, respectively. The highest rates of increase in body weight occurred during the first four weeks of life of the chicken while the lowest rates of increase occurred between the 20th and 22nd week. This observation seems to suggest that the first four weeks of life could be the best time period to make selection decisions that seek to improve the growth traits of local chicken of Ghana.

The body weights of the chicken ecotypes of Ghana as reported in this study were lower than the average body weights of Tunisian local chickens, which have been reported by Raach-Moujahed and Haddad (2013) to be about 1.25 kg (16 weeks) for cocks and 1.01 kg (16 weeks) for hens. The body weights of the Ghanaian chicken ecotypes are also lower than the Fulani (Savannah) ecotype but higher than the Yoruba (Forest) ecotype of Nigeria whose matured body weights have been reported by Olawunmi *et al.* (2008) to be 1.76 kg and 1.01 kg

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respectively. The comparatively lower matured body weights of the Ghanaian chicken ecotypes may suggest that these chicken ecotypes have over the years not undergone any serious selection with body weight as a criterion or experienced any significant infusion of genes of heavier chicken breeds. The observed superiority in matured body weights of the IS ecotype over the FO and CS ecotypes also suggests that some genetic factors may be at play instead of it being a result of differences in the management and environmental conditions since all the birds were raised under similar conditions of feed, water, vaccination, temperature, and general husbandry practices. The Interior savannah chicken ecotype could therefore be a major resource for the improvement of body weights in local chickens of Ghana. Tougan *et al.* (2009) attributed the differences in body weights of local chicken ecotypes of Benin to genetics, while Shuaibu *et al.* (2020), concluded that genotype, location, and sex had significant effects on the body weights of local chicken ecotypes.

The heritabilities estimated for body weights were moderate to high, with an overall heritability of 0.54. The estimated heritabilities of body weight decreased from hatch up to 14 weeks of age, after which they trended upwards. Similar results were reported by Osei-Amponsah et al. (2013) in their study on the phenotypic and genetic parameters of the Forest and Interior Savannah chicken ecotypes of Ghana. The high heritability rates of 0.58 to 0.69 obtained from week 16 to week 22 suggests that body weight selection among the three ecotypes could be carried out during this period.

4.5.2 Feed consumption and conversion rates

Feed conversion is a very complex trait that is influenced by the interaction of internal and external factors. The internal factors include basal metabolism, immune status, appetite, protein accretion and level of production, while the external factors include the ambient temperature, the digestibility of feed, amongst others (Patience *et al.*, 2015).

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During the first eight weeks of life, as well as during the growing phase of the experimental chicken, although not significant, the CS ecotype was a better converter of feed than the other two ecotypes (Table 9). The CS ecotype converted feed into bodyweight with a 4.3% reduction in the FCR compared to the FO ecotype and with a 2.25% reduction in the FCR compared with the IS ecotype. While feed intake increased with age, the feed conversion efficiency decreased with age. This observation agrees with the findings of Bamidele *et al*. (2020) and Tougan *et al.* (2009). This trend is expected because feed efficiency is a function of body weight and growth rate.

It has been reported by Raach-Moujahed and Haddad (2013) that at 16 weeks of age, local Tunisian chicken had a FCR of 3.97, thus making them comparatively more efficient than the Ghanaian chicken ecotypes. Mupeta *et al.* (2000) also observed that some local chickens in Zimbabwe have a FCR of 4.7, while Youssou *et al.*, (2012) reported that the slow growing Label Rouge chicken that originates from France had higher feed intakes but was more efficient than the FO and IS chicken ecotypes of Benin. Halima *et al.* (2007) also concluded that the Rhode Island Red bird was a better converter of feed than local chickens in Ethiopia. Significant differences in the FCR exists amongst improved Nigerian local chickens, with the normal feathered, frizzled feathered and naked neck genotypes having FCRs of 4.21, 4.53 and 3.84 respectively (Chimenem-Amadi *et al.*, 2021).

It appears that the three Ghanaian ecotypes are better feed converters than the local chickens of Ethiopia whose FCRs are reported by Tadelle *et al.* (2003b) and Halima *et al.* (2007) to be in the range of 13.9 to 11.0. This very high FCR, compared to the FCRs of several local chickens in Africa, could be a result of an over estimation of the feed intake, given the fact that these birds are reported to have a feed scratching behaviour (Halima *et al.*, 2007) which could lead to substantial feed losses.

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High FCRs in chicken implies high feed requirements which in turn translates into high production costs. Improvements in the FCR of the local chicken ecotypes of Ghana is therefore a desirable option from both environmental (reduction in greenhouse gas emission, reduction in land and chemicals used for the cultivation of cereals) and economic stand points. A reduction in FCRs in local chicken through appropriate strategies could in the long term catalyse a high uptake of the commercial rearing of these chickens, which in turn could contribute to closing the existing demand-supply gap of the meat of local chicken in Ghana.

4.5.3 Carcass traits

In most European and Western markets today, there is a higher demand for high quality chicken carcasses and cut-up parts, compared to many developing countries where the poultry industry is still predominantly driven by the sale of live birds and whole carcasses. It is estimated that in Europe today, over 60% of the value of chicken carcasses can be attributed to breast muscle due to the high preference for it over other chicken parts because of its low-fat content (Vervloesem, 2022).

The importation and consumption of cut-up parts of chicken in Ghana is also on the rise (USDA, 2022) and traits like carcass weight and breast muscle weight are gradually becoming very important as far as the niche markets are concerned. While this may be the case, local chickens, whether sold as whole carcasses, as cut-up parts, or as live birds, will continue to have a considerable share of the chicken market in Ghana.

Carcass traits of chicken have been reported to be affected by several factors, including the genotype of the bird, its feeding and slaughtering conditions, its live weight and sex (Brickett *et al.*, 2007; Havenstein *et al.*, 2003). In this study, there was clear evidence that ecotype

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influenced the fasting and breast weights of chicken, with the FO ecotype having a higher breast weight than the CS and IS ecotypes (Table 10). However, it did not have any significant effect on the dressing percentage, thigh weight, wing weight, drumstick weight, and dressed weight. This seems to suggest that there are some similarities in the genetic makeup of the three chicken ecotypes. However, the FO ecotype could be used as a base resource for the improvement of breast weight in local chicken ecotypes of Ghana. The moderate heritability of breast weight, as estimated among the three ecotypes (Table 10), suggests the possibility of this trait responding to within breed selection among these chicken populations.

The findings of this study disagree with Duah *et al*. (2020) who in their study on the carcass traits of crossbred chickens in Ghana, did not find any clear evidence of any genotypic effect on carcass traits like breast weight, drumstick weight, dressed weight, thigh weight and wing weight. Similarly, Patra *et al*. (2002) concluded that genotype had a significant effect on these traits. The findings of this study are also at variance with Youssao *et al*. (2012) who did not find any effect of ecotype on breast weight and carcass yield of local chickens but rather reported that wing weight and thigh-drumstick weight of the IS ecotype was significantly heavier than the FO ecotype. Nweke-Okorocha *et al.* (2020) also observed that among four local chicken breeds of Nigeria, breed had a significant effect drumstick and thigh weights, but it did not significantly influence dressed and breast weighs. These variations among the various local chickens could be a result of differences in their genetic makeup.

It was observed in this study that sex had a significant effect on all the carcass traits, with the carcass parts of cocks weighing more than those of the hens. These differences are to be expected since the cocks were heavier than the hens. These findings agree with those reported by Hussein *et al.* (2019), Youssao *et al.* (2012) and Osei-Amponsah (2010a) but are at variance

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with Nweke-Okorocha *et al.* (2020) who concluded that sex did not have any influence on breast and thigh weights.

Breast weight, as a percentage of carcass weight (i.e., breast yield), is an important criterion in broiler poultry production (Erensoy *et al*, 2020). Breast yield tends to increase in the order of slow-growing, medium-growing and high-growing chickens (Chodová *et al.*, 2021). According to Youssao *et al.* (2012), the breast yield of slow-growing chickens is in the range of 13.4 to 26%, which puts the three Ghanaian chicken ecotypes into the category of slow-growing chicken types.

In this study, the female chicken had a higher breast yield (25.50%) than the males (22.89 %). The FO ecotype had the highest breast yield followed by the CS and IS ecotypes. The females of the Forest chicken ecotype could therefore be explored as a potential candidates for the improvement of breast weight yield among the local chicken of Ghana. These findings are similar to Bongiorno *et al*. (2022) who in a study on two slow-growing Italian local dual-purpose chicken concluded that the breast yield of the females was significantly higher than the breast yield of the males. A similar conclusion was also arrived at by Erensoy *et al*. (2020), while Baéza *et al*. (2010) attributed these differences to a higher cross-sectional area of breast muscle fibres in female chicken than in the males.

4.5.4 Survivability

Mortality is a critical factor that can potentially undermine the profitability of livestock enterprises. Mortality in chicken could be a result of several factors including, predation, genetics, feeding, the quality of chicks, the management and health conditions of chicken, with Newcastle disease being the major cause of deaths amongst local chicken in Africa (Walugembe *et al.*, 2020).

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Most of the deaths that were recorded in this study occurred during the first week of life of the chicks, with the IS ecotype experiencing the highest mortality rate of 9.14%, followed by the FO ecotype (7.77%) and the CS ecotype (1.76%). This situation is to be expected because during the post hatch period, major changes often take place in the digestive, immune and thermoregulatory systems of the chick, while the yolk-based diet is also being gradually replaced by solid feeds. These factors tend to place a huge amount of stress on chicks, sometimes with fatal outcomes, particularly as they struggle to learn how to drink water and feed themselves.

Over the 22-week period, the IS ecotype had a mortality rate of 14.57%, compared to 10.94% and 5.99% among the FO and CS ecotypes, respectively. These results are higher than those reported by USDA (2015) for intensively raised chicks, layers and broilers raised in Ghana and by Adomako (2009) for local Ghanaian chicken reared under intensive, semi-intensive and extensive production systems respectively. This suggests that biosecurity and general management issues may have contributed to the high mortality rates. Improved biosecurity and strict adherence to standard poultry husbandry practices must therefore be mainstreamed to ensure higher survival rates of these chicken ecotypes when they are intensively reared.

The IS ecotype had the lowest probability of surviving while the CS ecotype had the highest survival probability. Several factors, including hatch weight (Aggrey and Marks, 2002), breed, genotype (Rodriguez *et al*., 1997), gender, season (Yerpes *et al*., 2020), and body size (Pereira *et al*., 2010; Maness *et al*., 2013) have been reported to influence growth and survival outcomes of birds. The high mortality rate that was associated with the IS ecotype during the first week of life could, in part, be attributed to the fact that it had smaller hatch weights or to its inability to fully adapt to the climatic conditions pertaining in the Coastal Savannah agroecological zone

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where this experiment was conducted. The CS ecotype which was in its natural environment recorded the lowest mortality rate over the entire experimental period.

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4.6 Conclusion

The Interior Savannah and Forest chicken ecotypes were heavier and grew at a faster rate than the Coastal savannah ecotype. However, the Forest ecotype had a higher breast weight and breast yield than the Interior Savannah and Coastal chicken ecotypes. The Coastal savannah ecotype had highest survival rate under intensive management conditions while the Interior savannah ecotype had the lowest survival rate.

The Forest, Coastal Savannah, and Interior Savannah chicken ecotypes could therefore be used jointly through selective breeding to develop a fast-growing, heavier local type of chicken that also has a high breast yield and survival rate. However, in doing so, care should be taken to ensure that the high post-hatch mortality rate associated with the Interior Savannah ecotype under intensive management conditions is taken care of by quantifying the ecotype-environmental interactions and the extent to which these interactions may affect the production levels of the birds in diverse production environments.

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CHAPTER 5

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN INSULIN- LIKE GROWTH FACTOR 1 GENE WITH GROWTH AND CARCASS TRAITS OF THREE LOCAL CHICKEN ECOTYPES OF GHANA

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5.1 Summary

The main objective of this study was to examine the diversity of some single nucleotide polymorphisms (SNPs) that are in the untranslated regions of the chicken Insulin-like growth factor 1 gene (*cIGF1)* and to determine the extent to which these polymorphisms are associated with growth and carcass traits in three local chicken ecotypes in Ghana.

The experiment was conducted in four replicates with a total of 1124 chickens, made up of 562 of the Forest ecotype, 297 of the Interior Savannah ecotype, and 265 of the Coastal Savannah ecotype.

The body weights of all the birds were taken at hatch and subsequently once every fortnight until they were 22 weeks of age. At the end of the experiment, they were euthanized, processed and various carcass measurements taken. DNA was extracted from blood sample of 300 birds and some targeted regions of *cIGF1* gene amplified by Polymerase Chain Reaction. The Restriction Fragment Length Polymorphism (RFLP) method was used to identify SNPs in the regulatory regions of the *cIGF1* gene. The RFLP analysis revealed a *HinfI* SNP in the 5' UTR, a *PstI* SNP in the promoter of the 5'flanking region and a *BstBI* SNP in the 3'UTR of the gene. The identified polymorphisms were confirmed by sanger sequencing and ClustalW sequence alignment to be *HinfI* C>A, *PstI* T>C and *BstBI* T>C. These SNPs were then used to genotype the chickens.

The genetic diversity of *cIGF1* gene at the *HinfI* C>A, *PstI* T>C and *BstBI* T>C SNP loci was investigated. This entailed an estimation of allele and genotype frequencies, observed heterozygosity, and expected heterozygosity among the three chicken populations.

An analysis of the association of the SNPs in *cIGF1* with growth and carcass traits of three local chicken ecotypes was also carried out. This was done using the General Linear Model

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procedure of SAS™ 9.0 (2002), with body weights, weight gained between specific periods being the indicators of growth, while thigh weight, breast weight, wing weight, drumstick weight, carcass weight and dressing percentage were the carcass traits.

It was observed that at the *HinfI* C>A locus, the AC heterozygote was the predominant genotype amongst the Forest and Coastal Savannah ecotypes, while the CC genotype was predominant in the Interior Savannah ecotype. The C allele had the highest frequency amongst all the three chicken ecotypes. Higher frequencies of the C allele at this locus in the three chicken ecotype populations of Ghana could be due to the fact that these chickens have not been selected for growth. High heterozygosity values were also observed in the FO and CS ecotype populations, suggesting some degree of uncontrolled mating in the FO and CS populations or substantial levels of gene flow.

This study did not find any strong evidence of an association of *HinfI* C>A with body weights, weight gain and carcass traits. However, though not significant, there was a trend of the AA genotype to have heavier body weights, breast weights and dressing percentage than the CC and AC genotypes. The results of this study were inconsistent with those reported by several authors, but these differences could have arisen due to differences in the genotype of the birds used in these studies.

At the *PstI* T>C locus, the CT heterozygote was the most predominant genotype among the three Ghanaian chicken ecotypes, with the T allele having the highest frequency. The observed heterozygosity amongst the FO and CS chicken ecotypes was higher than the expected heterozygosity thus suggesting a higher degree of diversity at this SNP locus within the FO and CS chicken ecotypes than within the IS ecotype.

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There was some evidence that *PstI* T>C was associated with the body weights at hatch and at 16 and 22 weeks of age, with the body weights of the CT and TT genotypes being higher than the body weights of the CC genotype. This study did not find any evidence of an association of this polymorphism with the carcass traits that were investigated.

At the *BstBI* T>C locus, the CT heterozygote was the predominant genotype among the three chicken ecotypes while the C allele had the highest frequency. The diversity of this polymorphism was higher in the FO and CS ecotypes than in the IS ecotype.

BstBI T>C was significantly associated with body weights at hatch, 2, 10, and 12 weeks of age, with the TT genotype being heavier than the CT and CC genotypes. This study did not find any associations between this polymorphism and the carcass traits studied.

The results of this study suggest that *PstI* T>C and *BstBI* T>C SNPs of *cIGF1* could be potential molecular markers for the improvement of body weights in these chicken ecotypes. However, *HinfI* C>A, *PstI* T>C and *BstBI* T>C SNPs of *cIGF1* may not be the ideal molecular makers for carcass traits improvement amongst the three chicken ecotypes.

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5.2 Introduction

Growth is a very complicated process that is controlled by several neuroendocrine pathways and by genes with small individual effects. The insulin-like growth factor 1 (IGF1) hormone has been implicated in the post hatch growth and development of chicken and other livestock species (Ashpole *et al.*, 2015; Hosnedlova et al., 2020). This hormone is part of the somatotropic axis which consists of Growth Hormone (GH), IGF1 and IGF2 hormones, alongside their receptors and binding proteins. IGF1 is mainly produced in the liver but is also found in other organs and tissues. In mammals, IGF1 is always sequestered in a non-covalent ternary complex which consists of one molecule each of IGF-I, IGFBP-3, and an acid labile subunit (ALS) (Kim, 2010). IGF1 mediates the actions of GH by facilitating the proliferation, differentiation, development, and growth of the targeted organs. The IGF1 hormone is coded by the insulin-like growth factor 1 gene (*IGF1*).

Several single nucleotide polymorphisms (SNPs) have been identified in the chicken insulin-like growth factor 1 gene (*cIGF1*) that have also been reported to be associated with growth and carcass traits (Zhou *et al.*, 2005; Bhattacharya *et al.*, 2015; Sinpru *et al.*, 2021; Wang *et al.*, 2021).

Various studies on the genetic diversity of local chicken ecotypes have been carried out with the aid of molecular markers like single nucleotide polymorphisms.

The insulin-like growth factor 1 gene has been reported as a candidate gene for growth and carcass traits (Tang *et al.*, 2010; Kadlec *et al.*, 2012; Anh *et al.*, 2015; Aswani *et al.*, 2015). As a result, single nucleotide polymorphisms in this gene have over the years generated some interest as far as the genetic improvement of growth and carcass traits of chickens with the aid of molecular markers is concerned. Marker-assisted selection entails the molecular analysis of individual genes and is a fast and reliable tool that can be used to speed-up the genetic

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improvement of the production traits of chickens and livestock in general (Hosnedlova et al., 2020).

Most single nucleotide polymorphisms are in the non-coding regions of genes, but they also occur in the coding and intergenic regions (Ann-Christine Syvänen, 2001) and are the most abundant form of genetic variation in genomes (Jehan and Lakhanpaul, 2006).

SNPs that are in the regulatory regions (Promoter, 5'UTR and 3'UTR) of a gene can affect the timing, location, and level of gene expression while those that are located within the coding regions of genes can change the amino acid sequences of gene products. The 5'UTR plays a very important role in post-transcriptional splicing of mRNA leading to its availability in the nucleus. On the other hand, the 3'UTR plays a major role in the splicing activity of pre-mRNA and the stability of mRNA. As a result, polymorphisms in this regulatory region can affect the splicing activity of pre-mRNA leading to variations in the levels of protein expression. Coding SNPs come in two forms i.e., synonymous (does not affect or change the protein sequence of gene products) and non-synonymous. Non-synonymous SNPs affect amino acid variation in the protein products of genes (Emara and Kim, 2003). Both synonymous and non-synonymous SNPs are good genetic markers for mapping studies (Ann-Christine Syvänen, 2001; Emara and Kim, 2003).

An extensive literature review did not find much information on the diversity of SNPs in *cIGF1* of local chicken. However, in their study on the diversity of *PstI* T>C in *cIGF1*, Amao *et al.* (2021), reported that in eight improved local chicken populations of Nigeria, the observed heterozygosity values varied from 0.36 to 0.67 while the expected heterozygosity values ranged from 0.40 to 0.50, and suggested that this could be due to either a selection for heterozygotes, null alleles or population subdivision arising from genetic drift.

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The genetic architecture of local chicken ecotypes remains largely unknown and so any study that seeks to elucidate their genetic diversity could serve as a guide to, and greatly facilitate the development of fast-growing local chickens that will meet the needs of Ghanaian farmers and consumer preferences, while also preserving the innate adaptive traits of these local chickens. Furthermore, an evaluation of the genetic architecture of local chickens may serve as a treasure trove for breeding decisions in the future.

The main objective of this study is to examine the diversity of some SNPs that are in the promoter and untranslated regions of the *IGF1* gene of chicken and determine the extent to which these polymorphisms are associated with growth and carcass traits in three local chicken ecotypes in Ghana.

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5.3 Materials and Methods

5.3.1 Geographical location and source of experimental chickens

The experimental birds were sourced from the Feed the Future Innovation Lab for Genomics to Improve Poultry Project (cooperative agreement number AID-OAA-A-13-00080), located at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana.

5.3.2 Management of the experimental chickens

The experiment was conducted in four replicates with a total of 1124 chickens, made up of 562 chickens of the FO ecotype, 297 chickens of the IS ecotype, and 265 chickens of the CS ecotype (Table 2).

Each ecotype was housed separately with a maximum of 40 birds in a pen with a size of 2.54 m x 2.2 m x 2.2 m. From day 1 to week 8, all the birds were fed on a standard chick starter mash with 20.0 % crude protein and a Metabolizable Energy (ME) of 2,993 kcal/kg while from week 9 to week 22 they were fed on a standard chick grower mash with 17% crude protein and a ME of 3,015 kcal/kg. Feed and water were provided on an *ad lib* basis. The diets were formulated using commercial protein concentrates following the manufacturers specification. The vaccination, feeding and other important husbandry practices were the same for all the birds.

5.3.3 Phenotypic measurements

Body Weights

The body weights of all the birds were taken at hatch and subsequently once every fortnight until they were 22 weeks of age. An electronic weighing scale (OHAUS Explorer ^{TM} Precision -0.1g readability) was used to weigh the birds from hatch to week 8, while from week 9 to week 22, an electronic hanging scale (Kern HDN 5K5-5.0 g readability) was used to weigh

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them. The body weights were then used to calculate the growth rates (Formula 1) up to week 22:

$$\text{Growth rate measured at bi-weekly intervals} = \frac{Final\ weight - Initial\ weight}{Time\ Interval\ (2\ weeks)} \quad (1)$$

Carcass traits

At the end of the experimental period, a total of 1036 birds were starved of feed for 12 hours to ensure complete emptying of the crop, weighed, and then euthanized. Each bird was bled and weighed on a sensitive scale (scale (OHAUS Explorer ™ Precision -0.1g readability) to determine the weight of the blood. Thereafter the birds were scalded in hot water, defeathered and weighed again to determine defeathered weight.

The carcasses were then stored in a fridge at a temperature of 3 to 5 degrees Celsius for 24 hours before they were processed. The offal (gastrointestinal tract, liver, shank, lung, head, and kidney) of each bird were removed by hand from the carcass and the carcass or dressed (eviscerated) weight determined. The head, shanks, wings, thighs, drumstick, breast muscle and neck were weighed individually based on the Meat buyers' guide developed by North American Meat Processors Association (2007), on a sensitive scale. The heart, liver and gizzard were also weighed individually on the same scale. From these measurements, several parameters, including the carcass/dressed weight and dressing percentage, were calculated (Formula 2 and Formular 3).

Dressed weight = live weight – offal weight (2)

The dressing percentage was expressed as a percentage of carcass weight over live weight.

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$$\text{Dressing percentage} = \frac{Carcass\ weight}{Live\ weight} \times 100 \quad (3)$$

5.3.4 Sample collection and DNA extraction

Blood samples were collected from the wing vein of 300 chickens aged 12 weeks, 100 of which were from each local chicken ecotype. Each sample was collected into an ETDA coated vacutainer tube, placed on ice, and eventually stored at -20^{o}C.

Genomic DNA was extracted using DNeasy® blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and guidelines. The quality of the extracted DNA was checked by resolving aliquots of 3 µl amplicons of the DNA on 1.5% agarose gel and electrophorized at 100 V for 25 min in TBE buffer (1M Tris base, 1M Boric acid, 0.02 M EDTA), and the bands visualized under a molecular imager (Bio RAD Gel Doc TM XR+). The quantity of the DNA was checked with the aid of a Qubit^{TM} 4 Fluorometer (Thermo Fisher SCIENTIFIC, Malaysia). Thereafter, working dilutions of the extracted DNA of each bird were prepared at a concentration of 50 ng/ µl.

The extraction of the DNA and subsequent Polymerase Chain Reaction (PCR) amplification of target regions of the *IGF1* gene and detection of polymorphisms were all carried out at the Molecular Genetics Laboratory of the Department of Animal Science of the University of Ghana.

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5.3.5 PCR amplification of target regions.

The PCR reactions were caried out in a 10 µl reaction volume, consisting of 1 µl of chicken genomic DNA (50 ng/ µl), 5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems,

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Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 3.0 µl of sterilized distilled water, 0.5 µl (20 µM) of the forward primer and 0.5 µl (20 µM) of the reverse primer.

The PCR was carried out in a thermal cycler (BIO-RAD C1000^{TM}) and the reaction conditions and primers used for the amplification of the respective regions of the target regions of the gene are shown below in Table 14.

Table 14. The primer sequences and cycling conditions used to amplify fragments of the *cIGF1* gene.

Gene	Primer Name	Sequence	Product size	Cycling conditions	Source
cIGF1	IGF-1PF (Forward)	5'CATTGCGCA GGCTCTATCTG 3'	813 bp	94°C (2 min) [94°C (30 s) 67°C (30 s) 72°C (30 s)] -33 cycles 72°C (5 min)	Zhou *et al.* (2005)
	IGF-1PR (Reverse)	5'TCAAGAGAA GCCCTTCAAGC 3'			
	IGF15UTRF (Forward)	5'TCATTTAAGA TCCAGCCTCCA 3'	470 bp	94°C (7min) [95°C (1min) 56°C (50 s) 72°C (50 s)] -35 cycles 72°C (7min)	Tang *et al.*, (2010)
	IGF15UTRR (Reverse)	5'AATATGAGC AGCACGGTTGA 3'			
	IGF13UTRF (Forward)	5' GTACAACGG TGCTATTT 3'	746 bp	94°C (5 min) [94°C (30 s) 55°C (30 s) 72°C (1 min)] - 35 cycles 72°C (10 min)	Bian *et al.*, (2008)

After the PCR, aliquots of 3 µl of each PCR product was resolved on 1.5% agarose gel at 100 V for 25 min by electrophoresis in TBE buffer, stained with GelRed (Biotium, California) loading buffer and visualized under a molecular imager (Bio RAD Gel Doc^{TM} XR^{+})

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5.3.6 Restriction Fragment Length Polymorphism analysis.

The Restriction Fragment Length Polymorphism (RFLP) method was used to detect polymorphisms in the *cIGF1* gene. Fragments of 813 bp, 470 bp and 746 bp which correspond with the 5' UTR, the Promoter in the 5' flanking region, and the 3' UTR regions, respectively, were targeted.

The PCR products of the 813 bp and 470 bp fragments were digested with *HinfI* and *PstI* restriction enzymes, respectively, at 37°C for 15 mins while the PCR products of the 746 bp fragment was digested with *BstBI* restriction enzyme at 65°C for 15 mins.

After the digestions, aliquots of 5 µl amplicons of the digests were resolved on 2% agarose gel and ran at 100 V for 25 minutes in TBE buffer, stained with gel red and the bands visualized under a molecular imager (Bio RAD Gel Doc^{TM} XR^{+}). The size of the bands was determined relative to 50 bp and 100 bp DNA ladders (TaKaRa, Japan).

Genotyping of the birds was then carried out based on the number of bands visualised in each sample.

5.3.7 Sequencing

The PCR products of nine samples of each genotype (36 samples in all) were purified and sanger sequenced with the aid of an ABI 3730xl.abi Genome Analyser (Thermo Fisher). The trace files of the sequences were then viewed with the aid of Finch TV (Sevindik and Okan, 2020). The chromatograms were trimmed to remove the low-quality bases found at the ends of the sequences. Thereafter, the sequences were exported in a FASTA format into MEGA 11 software (Tamura *et al.,* 2021) and sequence alignments carried out with ClustalW to confirm the presence of SNPs.

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5.3.8 Statistical analysis

Genotype and allele frequencies

The SNP genotype of each bird was entered into a spreadsheet and GenAlEx software ver. 6.51b2 (Peakall and Smouse, 2012) used to carry out the various analyses, including an estimation of the genotype and allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), and fixation index, amongst others.

Analysis of molecular variance (AMOVA) was also carried out with the aid of GenAlEx v. 6.51b2 with the objective of estimating the partitioning of the genetic variance within, among and between the populations.

Analysis of associations of growth and carcass traits

General linear model procedure of SAS^{TM} 9.0 (2002) was used to estimate the associations of SNPs in *IGF1* gene with body weights, weight gained between specific periods as an indicator of growth and the carcass traits of three local chicken ecotypes, using the following model:

$Yijk = \mu + Si + Gj + SGij + eijk.$

Where, $Yijk$ is the dependent variable per each model, μ is the overall mean for a given dependent variable, Si the effect of ith sex, Gj the effect of the jth genotype at a given SNP, $SGij$ is the effect of the interaction of sex and genotype at a given SNP and $eijk$, the random error.

The dependent variables for the body weight and growth traits were:

a) Hatch weight (HW 0), weight at week 2 (BW 2), weight at week 4 (BW 4), weight at week 6 (BW 6), weight at week 8 (BW 8), weight at week 10 (BW 10), weight at week

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12 (BW 12), weight at week 14 (BW 14), weight at week 16 (BW 16), weight at week 18 (BW 18), weight at week 20 (BW 20) and weight at week 22 (BW 22).

b) Growth rates between hatch and week 2 (WG2), week 2 and week 4 (WG4), week 4 and week 6 (WG6), week 6 and week 8 (WG8), week 8 and week 10 (WG10), week 10 and week 12 (WG12), week 12 and week 14 (WG14), week 14 and week 16 (WG16), week 16 and week 18 (WG18), week 18 and week 20 (WG20), week 20 and week 22 (WG22) and between hatch and week 22 (TWG).

The dependent variables for the carcass traits were:

a) Live weight before slaughter.
b) Breast weight.
c) Wing weight.
d) Thigh weight.
e) Drumstick weight.
f) Dressed weight
g) Dressing percentage

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5.4 Results

5.4.1 Restriction Fragment Length Polymorphism

The gel images of the restriction enzyme digestion of the PCR products of the *cIGF1* are shown in Figure 13 (*HinfI* RFLP of the 5' UTR), Figure 14 (*PstI* RFLP of the promoter region) and Figure 15 (*BstBI* RFLP of the 3'UTR).

In the *HinfI* RFLP, three restriction patterns, corresponding to the CC, AA and AC genotypes were observed. The CC genotype consisted of two fragments estimated to be about 620 bp and 193 bp in size, while the AA genotype consisted of three fragments estimated to be about 370 bp, 250 bp and 193 bp size. The AC genotype was made up of four fragments i.e., 620 bp, 370 bp, 250 bp and 193 bp.

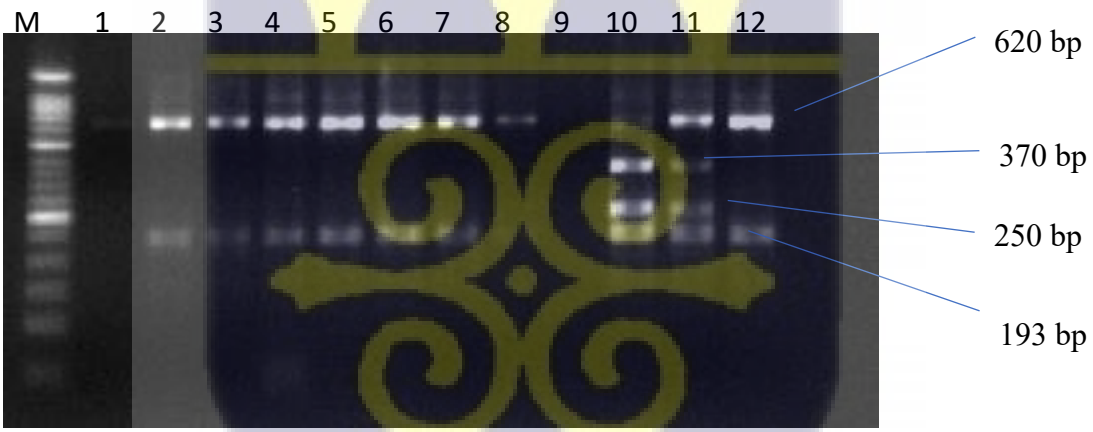

Figure 13. PCR-RFLP pattern of the promoter region of *cIGF1* with *HinfI* digestion. M = DNA ladder. Lane10 = AA genotype; Lane 11= AC genotype; Lanes 2, 3, 4, 5, 6, 8 and 12 = CC genotype.

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The digestion by *PstI* showed three restriction patterns, corresponding to the CC, TT and CT genotypes. The CC genotype consisted of two fragments estimated to be about 380 bp and 190 bp in size, while the TT genotype consisted of one fragment of about 470 bp. The CT genotype was made up of three fragments estimated to be about 470 bp, 380 bp and 190 bp in size.

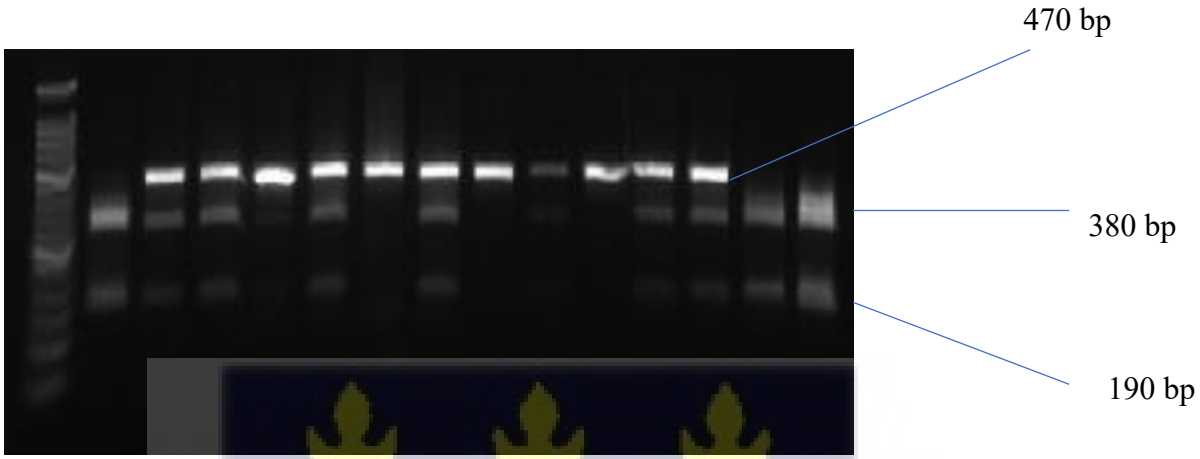

Figure 14. PCR-RFLP pattern of 5'UTR of c*IGF1* with *PstI* digestion. M = DNA ladder. Lanes 1, 13 and 14 = CC genotype; Lanes 2, 3, 4, 5, 7, 9, 11, and 12 = CT genotype; Lanes 6, 8, 10 = TT genotype

In the 3'UTR, three patterns of the restriction corresponding to the CC, TT and CT were observed. The CC genotype consisted of one fragment estimated to be about 750 bp while the TT genotype consisted of two fragments also estimated to be about 540 bp and 210 bp in size. The CT genotype was made up of all the three fragments.

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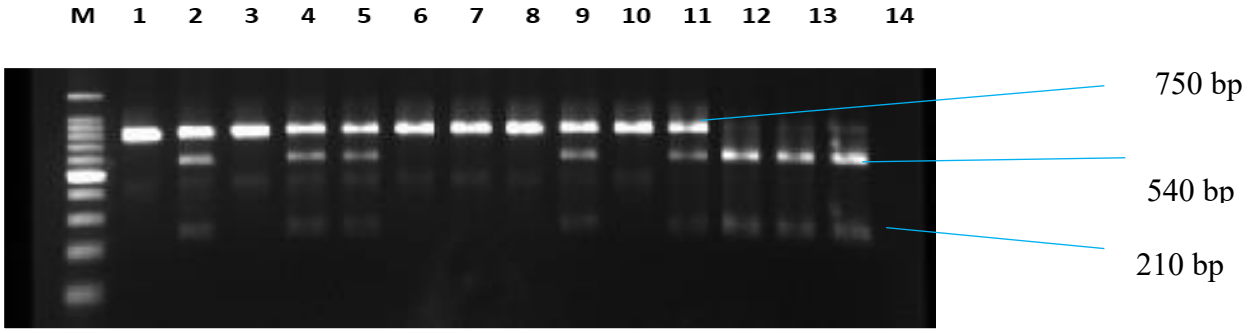

Figure 15. PCR-RFLP pattern of 3'UTR of *cIGF1* with *BstBI* digestion. M = DNA ladder. Lanes 1, 3, 6, 7, 8 and 10 = CC genotype; Lanes 2, 4, 5, 9, 10, 11= CT genotype; Lanes 12, 13 and 14 = TT genotype.

5.4.2 Sanger Sequencing

The sequences of the regulatory regions of the *cIGF1* gene were successfully amplified and sanger sequenced. Chromatographs showing mutations that were associated with *BstBI* RFLP and *PstI* RFLP of the *IGF1* gene of chicken are shown in Figure 16 and Figure 17.

A C>A SNP was found in the 5'UTR (position 537 on the consensus sequence). This mutation was associated with *HinfI* RFLP. A comparison of this consensus sequence with sequences of the annotated chicken *IGF1* gene (NC_052532.1) indicated that this nucleotide mutation occurred on chromosome 1 at position 55,326,180.

A T>C mutation that was associated with the *PstI* RFLP was found in the promoter region (position 352 on the consensus sequence). This SNP was also found in Reference Sequence EF198877.7 with the aid of the National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

A T>C mutation that was associated with *BstBI* RFLP was found in the 3'UTR (position 188 on consensus sequence). A comparison of this sequences with the sequence of the annotated

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chicken *IGF1* gene (NC_052532.1) indicated that this nucleotide mutation occurred in chromosome 1 at position 55,375,710.

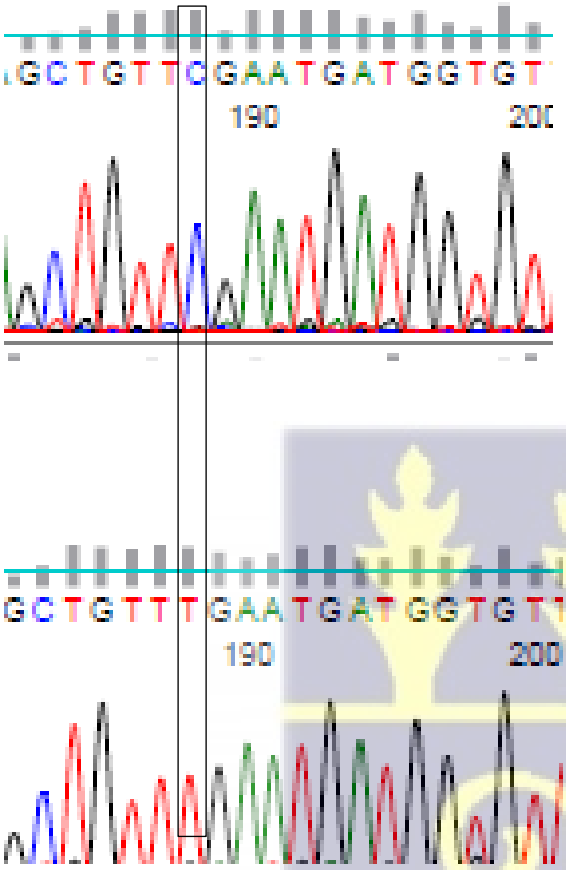

Figure 16. C>T in the 3 'UTR (position 188)

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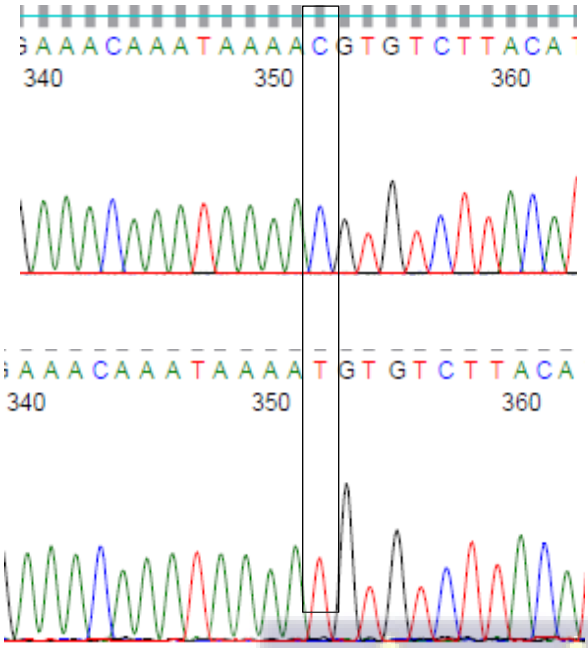

Figure 17: C>T in the 5UTR (position 352)

5.4.3 Genotype, allelic frequencies, and heterozygosity

The genotype and allele frequencies of the targeted *cIGF1* SNPS in the three chicken ecotypes are shown in Table 15.

The AC heterozygote was the predominant genotype at the *HinfI* C>A SNP locus in the FO and CS ecotypes, while the CC homozygote was the dominant genotype in the IS ecotype. The AA genotype had the lowest frequency amongst the three chicken ecotypes. The C allele had the highest frequency amongst all the three chicken ecotypes. The *HinfI* C>A SNP locus was in Hardy-Weinberg equilibrium (HWE) in all the three populations. According to Zhou *et al.* (2017), HWE is influenced by selection, the rate of recombination and mutation, genetic drift, the mating system, population structure, and genetic linkage.

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At the *PstI* T>C SNP locus, the CT heterozygote was the predominant genotype amongst the three Ghanaian chicken ecotypes, while the CC genotype had the lowest frequency. The T allele segregated at the highest frequency amongst all the three chicken ecotypes. The *PstI* T>C SNP locus was in HWE in all the three populations.

Within the *BstBI* T>C SNP, the CT heterozygote had the highest frequency amongst all the three chicken ecotypes while the TT genotype had the lowest frequency. The C allele segregated with a high frequency ranging from 0.60 to 0.51. This locus was in HWE in the three populations.

Table 15. Genotype and allele frequencies of the SNPs of the *IGF1* gene in three chicken ecotypes of Ghana.

Marker	Ecotype	N	Genotype frequency			Allele frequency		HWE
HinfI C>A			**AA**	**AC**	**CC**	**A**	**C**	
	FO	92	0.065	0.511	0.424	0.321	0.679	ns
	CS	52	0.058	0.481	0.462	0.298	0.702	ns
	IS	72	0.055	0.247	0.699	0.178	0.822	ns
			CC	**CT**	**TT**	**C**	**T**	
PstI T>C	FO	91	0.187	0.527	0.286	0.451	0.549	ns
	CS	52	0.173	0.615	0.212	0.481	0.519	ns
	IS	72	0.278	0.403	0.319	0.479	0.521	ns
			CC	**CT**	**TT**	**C**	**T**	
BstBI T>C	FO	92	0.228	0.543	0.228	0.505	0.495	ns
	CS	52	0.288	0.500	0.212	0.538	0.462	ns
	IS	72	0.375	0.431	0.194	0.604	0.396	ns

HWE = Hardy-Weinberg Equilibrium; ns = not significant.

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The diversity indices of the three single nucleotide polymorphisms in *IGF1* gene of the three Ghana chicken ecotypes are shown in Table 16.

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At the *HinfI* C>A SNP locus, the observed heterozygosity was also higher than the expected heterozygosity among the FO and CS ecotypes. However, the observed heterozygosity was lower than the expected heterozygosity in the IS ecotype.

At the *PstI* T>C SNP locus of the FO and CS ecotypes, the observed heterozygosity was higher than the expected heterozygosity. However, the observed heterozygosity was lower than the expected heterozygosity in the IS ecotype.

The observed heterozygosity was higher than the expected heterozygosity among the FO and CS ecotypes at the *BstBI* T>C SNP locus, however in the IS ecotype, the expected heterozygosity was higher than the observed heterozygosity.

Table 16. Genetic diversity indices of *cIGF1* gene in three chicken ecotypes in Ghana.

Marker	Ecotype	I	Ho	He	uHe
HinfI C>A	**FO**	0.627	0.511	0.436	0.438
	CS	0.609	0.481	0.418	0.423
	IS	0.468	0.247	0.293	0.295
	Mean	0.568 ± 0.05	0.413 ± 0.08	0.382 ± 0.05	0.385 ± 0.05
PstI T>C	**FO**	0.688	0.527	0.495	0.498
	CS	0.692	0.615	0.499	0.504
	IS	0.692	0.403	0.499	0.503
	Mean	0.691 ± 0.001	0.515 ± 0.06	0.498 ± 0.001	0.502 ± 0.002
BstBI T>C	**FO**	0.693	0.533	0.5	0.503
	CS	0.69	0.5	0.497	0.502
	IS	0.671	0.431	0.478	0.482
	Mean	0.685 ± 0.01	0.488 ± 0.03	0.492 ± 0.01	0.495 ± 0.01

I = Shannon's information index; Ho = Observed heterozygosity; He = expected heterozygosity; uHe = Unbiased heterozygosity

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5.4.4 Analysis of Molecular Variance

The partitioning of the total molecular variances at the *HinfI* C>A, *PstI* T>C, and *BstBI* T>C SNP loci of the *cIGF1* gene are shown in Table 17. At the *PstI* T>C SNP locus, all the variance emanated from within the individual while at the *HinfI* C>A SNP locus, 98% of the variance originated from within the individual and 2% from amongst the populations. At the *BstBI* T>C SNP locus, 99% of the variance was within the individual while 1% of the variance was amongst the individuals.

Table 17. Analysis of Molecular Variance

Marker	Source of Variance	Estimated Variance	Percentage
PstI T>C	Among ecotypes	0.000	0%
	Among Individuals	0.000	0%
	Within Individuals	0.253	100%
	Total	0.253	
HinfI C>A	Among ecotypes	0.005	2%
	Among Individuals	0.000	0%
	Within Individuals	0.207	98%
	Total	0.212	
BstBI T>C	Among ecotypes	0.001	0%
	Among Individuals	0.002	1%
	Within Individuals	0.245	99%
	Total	0.249	

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5.4.5 Association between *cIGF1* SNPs and Growth traits

5.4.5.1 Association between *HinfI* RFLP SNP and growth traits

The body weights of the *HinfI* C>A genotypes of the three local chicken ecotypes are shown in Table 18 while the growth rates of the *HinfI* C>A genotypes are shown in Table 19.

HinfI C>A, located in the 5'UTR of the chicken *IGF1* gene, did not significantly affect the body weights of the chicken ($p > 0.05$) from hatch till 22 weeks of age. *HinfI* C>A did not also significantly affect the growth rate of the three genotypes ($p>0.05$). However, during the first twelve weeks of life, though not significant, the AA genotype had higher body weights than the other genotypes but between week 14 to week 20, the CC genotype had the highest body weights. From week 20 till the end of the experiment, the AA genotype once again had the highest body weights.

HinfI C>A did not also significantly affect the growth rates ($p>0.05$). However, from hatch till week 4, the AA genotype had the highest growth rates, after there was no clear pattern in the growth rates. At the end of the experiment, though not significant, the AA genotype had the highest overall growth rate.

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Table 18. Least Square Means ± SE of associations of *HinfI* C>A with body weight (g) in local chicken ecotypes of Ghana.

	GENOTYPE		
Trait	AA (13)	AC (90)	CC (114)
HW	25.91 ± 1.10	25.22 ± 0.35	25.03 ± 0.32
BW2	66.95 ± 4.67	62.16 ± 1.52	62.13 ± 1.35
BW4	160.09 ± 8.77	145.63 ± 2.86	149.65 ± 2.53
BW6	267.27 ± 14.67	255.67 ± 4.79	262.56 ± 4.23
BW8	395.05 ± 17.95	380.99 ± 5.86	392.93 ± 5.18
BW10	560.27 ± 23.28	545.98 ± 7.60	553.79 ± 6.72
BW12	723.32 ± 28.62	714.57 ± 9.34	723.30 ± 8.26
BW14	823.26 ± 34.76	838.82 ± 11.35	856.62 ± 10.03
BW16	974.84 ± 39.04	968.87 ± 12.74	980.80 ± 11.27
BW18	1057.17 ± 44.09	1065.16 ± 14.39	1078.41 ± 12.72
BW20	1128.86 ± 46.05	1114,97 ± 15.03	1138.06 ± 13.28
BW22	1221.94 ± 47.33	1194.00 ± 15.45	1204.83 ± 13.76

HW = Hatch weight. **BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, BW20, BW22** = Body weight at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 weeks respectively; The number of observations is indicated in the parenthesis. All values not statistically significant at 95% confidence level ($p > 0.05$).

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Table 19. Least Square Means ± SE of associations of *HinfI* C>A with growth rates (g) in local chicken ecotypes of Ghana.

Trait	GENOTYPE		
	AA (13)	AC (90)	CC (114)
WG2	41.04 ± 3.97	36.94 ± 1.30	36.06 ± 1.14
WG4	93.15 ± 6.25	83.47 ± 2.04	88.40 ± 1.80
WG6	107.17 ± 8.87	110.04 ± 2.90	112.78 ± 2.55
WG8	127.79 ± 8.41	125.32 ± 2.75	130.11 ± 2.42
WG10	165.22 ± 9.83	164.99 ± 3.21	160.08 ± 2.82
WG12	163.04 ± 9.82	168.59 ± 3.22	169.72 ± 2.82
WG14	114.95 ± 13.76	124.25 ± 4.49	133.21 ± 3.95
WG16	136.58 ± 13.05	130.05 ± 4.26	124.32 ± 3.75
WG18	82.34 ± 18.69	96.30 ± 6.10	97.24 ± 5.37
WG20	71.68 ± 16.75	49.81 ± 5.47	59.75 ± 4.81
WG22	92.88 ± 32.59	79.03 ± 10.64	55.88 ± 9.36
TWG	1195.83 ± 55.91	1168.78 ± 18.25	1167.57 ± 16.06

WG = Weight gain. **WG2** = WG between hatch and week 2; **WG4** = WG between week 2 and week 4; **WG6**= WG between week 4 and week 6; **WG8** = WG between week 6 and week 8; **WG10** = WG between week 8 and week 10; **WG12** = WG between week 10 and week 12; **WG14** = WG between week 12 and week 14; **WG16** = WG between week 14 and week 16; **WG18** = WG between week 16 and week 18; **WG20** = WG between week 18 and week 20; **WG22** = WG between week 20 and week 22; **TWG** = WG between hatch and week 22The number of observations is indicated in the parenthesis. All values not statistically significant at 95% confidence level ($p > 0.05$).

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5.4.5.2 Association between *PstI* RFLP SNP and growth traits

In the promoter of the 5' flanking region of the *cIGF1* gene of chicken, a *PstI* C>T SNP was observed. The body weights of the *PstI* C>T genotypes of three local chicken ecotypes are shown in Table 20 while the growth rates of the *PstI* C>T genotypes are shown in Table 21.

There was clear evidence that *PstI* C>T influenced body weights at hatch, week 16 and week 22, with the body weights of the CT and TT genotypes being similar ($p > 0.05$) but significantly higher than the body weights of the CC genotype. Hatch weight as a covariate did not affect the above results.

PstI C>T had a strong influence on the growth rate of the chicken between 6 and 8 weeks of age and between 14 and 16 weeks of age ($p < 0.05$). During these periods, the growth rates of the CT and TT genotypes were similar but significantly higher than the growth rates of the CC genotype.

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Table 20. Least Square Means ± SE of body weights (g) of *PstI* C>T genotypes in three local chicken ecotypes with hatch weight as a covariate.

Trait	GENOTYPE		
	CC (46)	CT (110)	TT (57)
HW *	23.96 ^{b} ± 0.51	25.34 ^{a} ± 0.32	25.41 ^{a} ± 0.46
BW2 ^{NS}	60.983 ± 1.98	62.37 ± 1.23	63.14 ± 1.78
BW4 ^{NS}	146.62 ± 3.95	146.48 ± 2.46	151.19 ± 3.56
BW6 ^{NS}	262.19 ± 6.76	256.80 ± 4.21	262.29 ± 6.08
BW8 ^{NS}	382.41 ± 8.24	385.73 ±5.13	396.13 ± 7.42
BW10 ^{NS}	541.69 ± 10.65	550.40 ± 6.63	561.37 ± 9.58
BW12 ^{NS}	707.79 ± 13.23	720.04 ± 8.23	735.28 ± 11.90
BW14 ^{NS}	824.61± 16.35	852.62 ± 10.17	860.63 ± 14.72
BW16*	943.40^{b} ± 17.89	982.70^{ab} ± 11.13	996.72^{a} ± 16.10
BW18 ^{NS}	1050.37 ± 20.34	1079.43 ± 12.65	1093.60 ± 18.30
BW20 ^{NS}	1102.99 ± 21.31	1139.32 ± 13.26	1146.65 ±19.18
BW22*	1172.86^{b} ± 21.41	1209.94^{ab} ± 13.40	1229.77^{a} ± 19.45

HW= Hatch weight. **BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, BW20, BW22 =** Body weight at 2, 4, 6, 8,10, 12, 14, 16, 18, 20, and 22 weeks respectively. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =$p < 0.05$, ** =$p < 0.01$ and *** =$p < 0.001$). ^{NS} = No significant deviation ($p > 0.05$) due to genotypes; The number of observations is indicated in the parenthesis.

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Table 21. Least Square Means ± SE of growth rates (g) of *PstI* C>T genotypes in three local chicken ecotypes of Ghana.

Trait	GENOTYPE		
	CC (46)	CT (110)	TT (57)
WG2 ^{NS}	35.85 ± 1.73	37.38 ± 1.09	35.11 ± 1.58
WG4 ^{NS}	85.56 ± 2.86	84.45 ± 1.80	89.79 ± 2.62
WG6 ^{NS}	115.36 ± 4.12	110.40 ± 2.59	110.37 ± 3.77
WG8*	119.29^{b} ± 3.80	128.93^{a} ± 2.39	133.97^{a} ± 3.47
WG10 ^{NS}	157.15 ± 4.56	164.83 ± 2.87	165.10 ± 4.17
WG12 ^{NS}	165.51 ± 4.63	170.50 ± 2.91	174.40 ± 4.23
WG14 ^{NS}	116.19 ± 6.54	133.16 ± 4.11	125.90 ± 5.98
WG16*	116.16^{b} ± 6.02	130.52^{a} ± 3.79	137.65^{a} ± 5.51
WG18 ^{NS}	105.56 ± 8.60	96.99 ± 5.41	97.14 ± 7.87
WG20 ^{NS}	52.33 ± 7.80	59.96 ± 4.90	54.24 ± 7.13
WG22 ^{NS}	67.24 ± 15.35	57.91 ± 9.65	87.64 ± 14.05
TWG ^{NS}	1136.20 ± 25.64	1175.04 ± 16.12	1211.30 ± 23.46

WG = Weight gain. **WG2** = WG between hatch and week 2; **WG4** = WG between week 2 and week 4; **WG6** = WG between week 4 and week 6; **WG8** = WG between week 6 and week 8; **WG10** = WG between week 8 and week 10; **WG12** = WG between week 10 and week 12; **WG14** = WG between week 12 and week 14; **WG16** = WG between week 14 and week 16; **WG18** = WG between week 16 and week 18; **WG20** = WG between week 18 and week 20; **WG22** = WG between week 20 and week 22; **TWG** = WG between hatch and week 22. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =$p < 0.05$, ** =$p < 0.01$ and *** =$p < 0.001$). ^{NS} = No significant deviation ($p > 0.05$) due to genotypes; The number of observations is indicated in the parenthesis.

5.4.5.3 Association between *BstBI* RFLP SNP and growth traits

A *BstBI* C>T SNP located in the 3' untranslated region of *IGF1* gene of chicken had a significant effect on hatch weight and body weight at 2 and 10 weeks of age. The body weights of the *BstBI* C>T genotypes of the three local chicken ecotypes are shown in Table 22, while the growth rates are shown in Table 23.

The TT genotype had a significantly higher hatch weight than the CT and CC genotypes ($p<0.05$). At week 2, the body weight of the TT genotype was significantly higher than the CC

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genotype but similar to the CT genotype, while the CT and CC had similar body weights. At week 10, the body weight of the CT and CC genotypes were similar but significantly lower than the TT genotype. Within the rest of the experimental periods, though not significant, the TT genotype was also heavier than the other genotypes.

BstBI C>T had a strong influence ($p<0.05$) on growth rates between 10 and 12 weeks of age and as well as on the total growth rate. Between weeks 10 and 12, the growth rate of the CC genotype was significantly higher than the growth rates of the CC and CT genotypes, while the growth rates of the CC and CT genotypes were similar. Over the 22-week period, the growth rate of the TT genotype was significantly higher than the growth rates of the CC and CT genotypes.

Table 22. Least Square Means ± SE of body weights (g) with hatch weight as a covariate, of *BstBI* C>T genotypes in three local chicken ecotypes of Ghana.

Trait	GENOTYPE		
	CC (63)	CT (107)	TT (46)
HW**	24.59^{b} ± 0.42	24.73^{b} ± 0.32	26.67^{a} ± 0.54
BW2*	61.38^{b} ± 1.72	62.82^{ab} ± 1.30	67^{a}.00 ± 2.25
BW4 ^{NS}	148.08 ± 3.33	147.80 ± 2.52	155.90 ± 4.37
BW6 ^{NS}	262.70 ± 5.63	257.47 ± 4.25	270.49 ± 7.37
BW8 ^{NS}	386.80 ± 6.92	387.07 ± 5.22	404.94 ± 9.06
BW10*	546.01^{b}± 8.77	549.25^{b} ± 6.62	576.96^{a} ±11.49
BW12	713.32 ± 10.86	718.88 ± 8.20	747.12 ± 14.21
BW14 ^{NS}	834.36 ± 13.59	848.20 ± 10.26	872.74 ± 17.79
BW16 ^{NS}	963.65 ± 15.03	978.51 ± 11.35	997.95 ± 19.68
BW18 ^{NS}	1053.97 ± 16.90	1075.89 ± 12.76	1105.23 ± 22.13
BW20 ^{NS}	1110.88 ± 17.80	1135.16 ± 13.44	1150.97 ± 23.31
BW22 ^{NS}	1194.43 ± 18.54	1206.10 ± 14.12	1224.83 ± 24.91

BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, BW20, BW22= Body weight at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 weeks respectively. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =$p < 0.05$, ** =$p < 0.01$ and *** =$p < 0.001$). ^{NS} = No significant deviation ($p > 0.05$) due to genotypes; The number of observations is indicated in the parenthesis.

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Table 23. Least Square Means ± SE of growth rate (g) of *BstBI* C>T genotypes in three local chicken ecotypes of Ghana.

Trait	GENOTYPE		
	CC (63)	CT (107)	TT (46)
WG2 ^{NS}	34.71 ±2.69	37.62 ±1.39	33.66 ±1.75
WG4 ^{NS}	86.73 ± 4.35	84.87 ± 2.24	87.24 ± 2.84
WG6 ^{NS}	117.45 ±6.12	110.42 ±3.16	110.08 ±3.99
WG8 ^{NS}	124.37 ± 6.21	127.48 ± 3.21	126.72 ±4.05
WG10 ^{NS}	162.29 ± 7.71	162.19 ± 3.98	157.15 ±5.03
WG12*	186.93^{a} ± 8.46	163.26^{b} ± 4.37	162.72^{b} ± 5.52
WG14 ^{NS}	116.93 ± 10.46	133.63 ± 5.40	132.34 ±6.82
WG16 ^{NS}	141.01 ±9.20	123.26 ± 4.75	124.63 ± 6.00
WG18 ^{NS}	122.42 ± 11.88	91.44 ± 6.13	102.30 ± 7.75
WG20 ^{NS}	39.86 ± 10.15	58.27 ± 5.24	54.39 ± 6.62
WG22 ^{NS}	92.42 ± 23.29	52.07 ± 12.02	65.08 ± 15.19
TWG *	1131.75^{b} ± 36.29	1150.87^{b} ± 18.74	1227.95^{a} ± 23.67

WG = Weight gain. **WG2** = WG between hatch and week 2; **WG4** = WG between week 2 and week 4; **WG6** = WG between week 4 and week 6; **WG8** = WG between week 6 and week 8; **WG10** = WG between week 8 and week 10; **WG12** = WG between week 10 and week 12; **WG14** = WG between week 12 and week 14; **WG16** = WG between week 14 and week 16; **WG18** = WG between week 16 and week 18; **WG20** = WG between week 18 and week 20; **WG2 2**= WG between week 20 and week 22; **TWG** = WG between hatch and week 22. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =p < 0.05, ** =p < 0.01 and *** =p < 0.001). ^{NS} = No significant deviation (p > 0.05) due to genotypes; The number of observations is indicated in the parenthesis.

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5.4.6 Association between *cIGF1* SNPs and Carcass traits

5.4.6.1 Association between *HinfI* C>A SNP and carcass traits

The carcass traits of the *HinfI* C>A genotypes are shown in Table 24 below. There was no evidence that *HinfI* C>A affected any of the carcass traits i.e., live weight, breast weight, thigh weight, wing weight, drumstick weight, dressed weight and dressing percentage of the three local chicken ecotypes ($p>0.05$).

Table 24. Least Square Means ± SE of carcass traits (g) of *HinfI* C>A genotypes in three local chicken ecotypes of Ghana

TRAIT	ECOTYPE		
	AA (13)	AC (90)	CC (112)
Live weight	1148.32 ± 45.16	1148.59 ± 14.74	1152.08 ±13.09
Breast weight	206.46 ± 9.93	197.73 ± 3.24	198.51 ± 2.88
Thigh weight	131.42 ± 5.94	131.14 ± 1.94	131.69 ±1.72
Wing weight	97.85 ± 3.58	98.51 ± 1.70	99.19 ±1.04
Drumstick weight	115.11 ± 5.30	118.66 ± 1.73	118.64 ±1.54
Dressed weight	821.20 ± 33.03	815.22 ±10.78	823.05 ±9.57
Dressing %	71.42 ± 1.09	70.85 ±0.36	71.34 ±0.32

No significant deviations in all the values ($p < 0.05$). The number of observations is indicated in the parenthesis.

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5.4.6.2 Association between *PstI* C>T SNP and carcass traits

The carcass traits of *PstI* C>T genotypes are shown in Table 25 below. *PstI* C>T did not have any significant effect on the carcass traits of the three local chicken ecotypes ($p>0.05$). However, though not significant, the TT genotype had higher live weight, breast weight, thigh weights, wing weights, drumstick weight and dressed weights while the CC genotype had a higher dressing percentage.

Table 25. Least Square Means ± SE of carcass traits (g) of *PstI* C>T genotypes in three local chicken ecotypes of Ghana.

TRAIT	GENOTYPE		
	CC (46)	CT (108)	TT (57)
Live weight	1120.21 ± 21.21	1156.12 ± 13.46	1181.92 ± 19.40
Breast weight	197.08 ± 4.72	199.75 ± 2.99	203.70 ± 4.32
Thigh weight	129.75 ± 2.80	132.06 ± 1.78	133.91 ± 2.56
Wing weight	97.76 ± 1.70	98.92 ± 1.08	100.44 ± 1.56
Drumstick weight	116.81 ± 2.56	118.82 ± 1.62	121.47 ± 2.34
Dressed weight	805.43 ± 15.80	822. 74 ± 10.03	837.69 ± 14.46
Dressing %	71.81 ± 0.50	71.09 ± 0.32	70.67 ± 0.46

No significant deviations in all the values ($p < 0.05$). The number of observations is indicated in the parenthesis.

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5.4.6.3 Association between *BstBI* C>T SNP and carcass traits.

The carcass traits of the *BstBI* C>T genotypes are shown in Table 26. *BstBI* C>T did not have any significant effect on the carcass traits of the three local chicken ecotypes ($p>0.05$). However, the TT genotype had higher live weight, breast weight, thigh weights, wing weights, drumstick weight and dressed weights but the CC genotype had the highest dressing percentage.

Table 26. Least Square Means ± SE of carcass traits of *BstBI* C>T genotypes in three local chicken ecotypes of Ghana.

TRAIT	GENOTYPE		
	CC (62)	CT (106)	TT (46)
Live weight	1126.50 ±17.40	1149.99 ± 13.14	1187.94 ± 21.71
Breast weight	193.20 ± 3.84	198.54 ± 2.90	206.87 ± 4.79
Thigh weight	130.69 ± 2.31	131.50 ± 1.75	132.47 ± 2.89
Wing weight	98.38 ± 1.40	98.27 ± 1.05	99.80 ± 1.74
Drumstick weight	117.63 ± 2.07	117.68 ± 1.57	121.75 ± 2.59
Dressed weight	804.39 ± 12.80	818.16 ± 9.67	841.64 ± 15.98
Dressing %	71.35 ± 0.42	71.08 ± 0.32	70.52 ± 0.52

No significant deviations in all the values ($p < 0.05$). The number of observations is indicated in the parenthesis.

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5.5 Discussion

The IGF1 hormone which is mainly expressed in the liver of chicken mediates the actions of Growth hormone by facilitating the uptake of glucose and protein synthesis. The *cIGF1* gene has been widely reported to have an influence on some growth and carcass traits of several livestock species (Yue *et al.,* 2014; Naicy *et al*., 2017; Gui *et al.,* 2018; Ahiagbe, 2018; Sinpru *et al*., 2021).

5.5.1 Gene diversity

Genetic diversity is one of the major ways by which the adaptation and survival of animal populations is ensured. In populations, new alleles are constantly appearing by spontaneous mutations mainly because of DNA replication errors or DNA damage caused by environmental factors (mutagens) (Ellegren and Galtier, 2016). The rate of mutation in genomes and species is not always the same, thus giving rise to the observed genetic diversity. Genetic diversity can therefore be thought of in terms of the rate of allele loss or the rate at which a mutation spreads out in a population such that it replaces all other alleles at a site (fixation). It is also related to mating patterns, geographic range, migration, gene flow and population size.

In this study, the diversity of *HinfI* C>A, *PstI* C>T and *BstBI* C>T SNPS in the *cIGF1* gene was investigated in three local chicken ecotypes of Ghana by genotyping the birds and then calculating and analyzing the genotype and allele frequencies, the observed and expected heterozygosity values.

A RFLP analysis revealed *HinfI* C>A and *PstI* C>T single nucleotide polymorphisms in the 5' UTR and 5' flanking region while *BstBI* digestion showed a C>T single nucleotide polymorphism in the 3'UTR of the gene. These are the same SNPs that have previously been reported by Amills *et al*. (2003) and Zhou *et al*. (2005) for the *HinfI* RFLP, by Esmailnejad *et*

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al. (2017), Abbasi and Kazemi (2011) and Tang *et al.* (2010) for the *PstI* RFLP and by Bian *et al*. (2008) for the *BstBI* RFLP.

It was observed that at the *HinfI* C>A locus, the AC heterozygote was the predominant genotype amongst the Forest and Coastal Savannah ecotypes, while the CC genotype was predominant in the Interior Savannah ecotype. The frequency of the C allele was higher than the frequency of the A allele in all the three chicken ecotypes. This finding is to be expected since several authors have reported that there is a preponderance of the C allele amongst slow-growing chickens that have not undergone any selection for growth, and a higher frequency of the A allele amongst fast-growing chicken breeds (Amills *et al.*, 2003; Zhou *et al.*, 2005; Moe *et al.*, 2009; Kadlec *et al.*, 2012; Promwatee *et al.*, 2013; Ogunpaimo *et al.*, 2021). Moe *et al.* (2009) argued that the C allele segregates at a higher frequency amongst local chickens citing the case of Bhutanese, Cambodian, Chinese, Indonesian, Laotian, Nepalese, Thailand, Taiwanese, and Japanese local chickens where the frequency of this allele was in the range of 0.9 to 1.0. Piryonesi *et al.* (2013) also observed higher frequencies of the C allele in Azerbaijanian local chicken. Similarly, Khadem *et al.* (2010) observed that the Mazandaran local chicken had a higher frequency of the C allele. However, in another study on the diversity of this SNP amongst Mazandaran chicken, Sekhavati *et al.* (2017) reported a higher frequency of the A allele instead of the C allele. These differences are likely to have risen if the two studies were carried out among different populations of the Mazandaran chicken.

Amills *et al.* (2003) reported higher frequencies of the A allele and the AA genotype amongst the paternal and maternal strains of Black Panedesenca chicken while Kadlec *et al.* (2012) observed that in broiler strains (Ross 308 and Cobb 500) the allelic frequency of the A allele was higher than the C allele and ranged from 0.92 to 0.93. Similarly, Shah *et al.* (2012) also observed higher frequencies of the A allele amongst broiler chicken.

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In a comparative study amongst dual-purpose chicken i.e., Sasso, Kuroiler and FUNAAB Alpha (an improved Nigerian local chicken breed), Ogunpaimo *et al*. (2021) reported a higher frequency of the A allele (0.58) than the C allele and concluded that birds with the CC genotype for this SNP came into lay earlier than the AA and AC genotypes.

The preponderance of the C allele in the three chicken ecotype populations of Ghana could be because these chickens have not been selected for body size and growth.

It was observed that at the *PstI* C>T SNP locus, the CT heterozygote was the predominant genotype amongst the three Ghana chicken ecotypes, with the T allele having the highest frequency. Higher frequencies of the CT genotype and T allele at this SNP locus have been reported among several local chicken by various authors including Ali *et al*. (2016) in Desi chicken of Pakistan. The Desi chicken is a local nondescript bird that is very hardy and thrives under scavenging conditions. Li *et al*. (2008) and Wang *et al*. (2004) also reported similar results in the local Wenchang chicken, a small and hardy bird, and in other native chicken of China. The Korean local Ogol chicken, a dual-purpose bird, has been reported by Seo *et al.* (2001) to have higher frequencies of the CT genotype and T allele at the *PstI* C>T SNP locus. On the other hand, higher frequencies of the C allele have also been reported amongst the Silkie native chicken (dual-purpose) of China (Tang *et al.*, 2010), amongst Iranian local chicken (Abbasi and Kazemi, 2011; Esmailnejad *et al.*, 2017), in four local Indonesian chicken (Mariandayani *et al.*, 2013) and in Lohmann chicken (Wang *et al.*, 2004).

The CC genotype is reported to be associated with higher body weights (Wang *et al.*, 2004: Ali *et al.*, 2016) while the TT genotype is reported to be associated with higher total egg production, average number of days of continual egg laying and number of double yolk eggs (Li, *et al.*, 2008). The results of this study are at variance with Wang *et al.* (2004) and Ali *et al.* (2016) as the TT genotype was generally heavier than the CC genotype.

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At the *BstBI* C>T SNP locus, the CT heterozygote was the predominant genotype among the three chicken ecotypes while the C allele was the most prevalent. An extensive review of literature did not come across any findings relating to the diversity of this *BstBI* C>T SNP in chicken. However, this SNP is reported to influence body weights and some carcass traits (Lei *et al*., 2005).

Heterozygosity, also known as gene diversity, reflects the level of genetic consistency in a population. High heterozygosity values indicate low allele consistency or high genetic diversity while low heterozygosity reflects low genetic diversity or greater allele consistency.

In the *PstI* C>T SNP locus of chicken, the observed heterozygosity amongst the FO and CS chicken ecotypes was higher than the expected heterozygosity and unbiased expected heterozygosity. This suggests a higher degree of genetic diversity within the FO and CS chicken ecotypes than the IS ecotypes. The observed heterozygosity at the *PstI* C>T SNP locus in the FO and CS ecotypes is close to those of the Mazandaran chicken (Esmailnejad *et al*., 2017).

At the *HinfI* C>A and *BstBI* C>T SNP loci, the FO and CS ecotypes were also genetically more diverse than the IS ecotype, as higher heterozygosity values were observed in them than in the IS ecotype.

Based on the estimates of the average expected heterozygosity of the three chicken ecotypes across the three SNP loci, the FO ecotype appears to be genetically more diverse than the CS and IS ecotypes, while the CS ecotype also appears to be genetically more diverse than IS ecotype. The higher diversity of the *cIGF1* gene at these SNP loci amongst the FO and CS ecotypes, compared to the IS ecotype, seems to suggest a higher degree of random mating and gene flow into and between the FO and CS ecotypes. This study therefore hypothesizes that

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the higher concentration of small to large sized commercial poultry enterprises, as well as the proliferation of many backyard poultry farming operations in Forest and Coastal Savannah agroecological zones where different types of chicken are reared, provides an opportunity for higher levels of gene flow to occur here. In addition, the provision of various types of chicken breeds to resource constrained households by Government Institutions and Agencies and by Non-Governmental Organizations in the past as part of their livelihood security enhancement projects and programmes, could have also facilitated this suggestive gene flow.

The genetic similarity of the CS and FO chicken ecotypes at the three SNP loci of the c*IGF1* gene relative to the IS ecotype agrees with Walugembe *et al.* (2020) who concluded that there is some overlap between the local chicken ecotypes of Ghana with the FO and CS ecotypes being closer to each other than the IS ecotype.

An analysis of molecular variance of among the three chicken ecotypes showed that most of the total variance was within individuals and ranged from 82 to 100%, thus suggesting that the overall genetic diversity of the three chicken ecotypes can be attributed to heterogeneities of individual birds within the populations and not by differences between the chicken ecotypes.

By way of a summary, it was observed in this study that at the *HinfI* C>A SNP locus, the AC heterozygote was the predominant genotype amongst the Forest and Coastal Savannah ecotypes, while the CC genotype was predominant in the Interior Savannah ecotype. However, the C allele had the highest frequency amongst all the three chicken ecotypes. Higher frequencies of the C allele at this locus of the three chicken ecotype populations of Ghana seems to suggest that these chickens may not have been selected for growth. Higher observed heterozygosity values among the FO and CS ecotypes also suggests uncontrolled mating in the FO and CS ecotypes or high gene flow.

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At the *PstI* C>T, SNP locus, the CT genotype was the most predominant amongst the three Ghana chicken ecotypes, with the T allele having the highest frequency. The observed heterozygosity amongst the FO and CS chicken ecotypes was higher than the expected heterozygosity thus suggesting a higher degree of genetic diversity within the FO and CS chicken ecotypes than the IS ecotypes.

At the *BstBI* C>T SNP locus, the CT genotype was predominant amongst the three chickens while the C allele segregated at the highest frequency. The FO and CS ecotypes were more genetically diverse than the IS ecotype.

Between 98-99% of the total molecular variances emanated from individuals rather than the ecotypes or the populations.

5.5.2 Association of Single Nucleotide Polymorphisms with growth traits

Growth is a very complicated process that is controlled by several neuroendocrine pathways and by genes with small individual effects. Growth rates vary in populations, genotypes and even among individuals. Variations in the growth rate among individual chickens of the same breed could be caused by differences in the environmental conditions, by the amounts of growth-related hormones in circulation in the tissues or by differences in the expression of the genes involved in myogenesis (Xiao et al., 2017). Gene expression and regulation are very important processes that allows cells to respond to changing environments. The regulation of genes can be carried out at the transcriptional or translational levels and mainly occurs in the non-coding regions of genes (Gebauer and Hentze, 2004).

The regulation of protein expression at the translational level is important in proteins that are involved in growth, especially in embryonic development, cell differentiation and metabolism (*Pesole et al*., 2001). Since the translation efficiency of mRNAs varies, the amount of protein

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produced needs to be modulated. Therefore, structural features located in the UTRs (i.e., the 7-methyl-guanosine (cap), hairpin-like secondary structures, upstream open reading frames (uORFs), internal ribosome entry sites (IRES) which are in the 5′ UTR, RNA-protein interactions, cytoplasmic polyadenylation elements (CPE) and poly-A tail located in the 3′ UTR) play critical functional roles in the modulation processes (Pesole *et al*., 2001; Gebauer and Hentze, 2004). The 5′ and 3′ UTR are therefore key genomic regions that modulate several processes including the transport of mRNAs out of the nucleus, translation efficiency, subcellular localization, stability, and protein degradation in cells (Mignone *et al*., 2002; Schuster and Hsieh, 2019). Therefore, polymorphisms in these UTRs can affect gene regulation and the growth rates of chicken by altering the affinity for transcript factors (Savinkova et al., 2013).

The *cIGF1* gene has been widely implicated in the regulation of growth in animals, to the extent that it plays critical roles in the differentiation, proliferation, development, growth, and apoptosis of cells (Kim, 2010; Hakuno and Takahashi, 2018).

5.5.2.1 Association of *HinfI* C>A with growth and carcass traits

In this study, a *HinfI* C>A transition mutation was observed in chromosome 1 located at 55, 326,180 bp within the 5'UTR of the *cIGF1* gene of Ghanaian chicken ecotypes. This SNP was not significantly associated with body weights and weight gain. This polymorphism may therefore not be a good marker for use in a marker-assisted selection for the improvement of body weight and growth traits in local chicken ecotypes of Ghana. This finding agrees with those of Khadem *et al*. (2010) and Attarchi *et al*. (2017) who did not also find any significant associations between this SNP with body weights in Mazandaran chicken. Similarly, Sato *et*

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al. (2012) did not find any significant associations of this SNP with the body weight of Plymouth Rock chicken at 6 and 9 weeks of age. In another study on the effects of *HinfI* C>A on the growth and feeding traits of paternal and maternal strains of Black Panedesenca chicken, Amills *et al*. (2003) also concluded that this SNP was not significantly associated with growth traits although there were some suggestive associations ($P \leq 0.05$) between this SNP with average daily gain at day 107. According to Amills *et al*. (2003), these suggestive associations could have been produced by linkage disequilibrium with other mutations at this SNP locus or with another linked gene directly involved in the regulation of the growth and feeding traits. On the other hand, they suggested that the suggestive associations could also be viewed in the light of differences in the transcriptional rate of both alleles since *HinfI* C>A SNP was involved in the suppression of the CdxA transcription factor binding site, thus affecting protein expression. Furthermore, according to Bian *et al.* (2008), birds with the CdxA binding allele A increased the efficiency of *cIGF1* in the small intestine. The *HinfI* C>A SNP has also been reported by Wang *et al*. (2021) to have an altering effect on the affinity levels of transcription factors, to the extent that the luciferase activity of the allele A promoted by the Activator Protein 1 transcription factor (AP1) and Octamer transcription factor 1 (OCT1) was higher than that of allele C. In addition, the allele A binding to the transcription factor AP1 and OCT1 was also stronger than that of allele C, thus suggesting that this SNP may affect *cIGF1* transcription.

The results of this study are however inconsistent with Zhou *et al*. (2005), who in their study on broiler crosses, generally concluded that this SNP was significantly associated with body weight and average daily gain. However, whereas the authors of this study observed a significant association between this SNP and growth traits in broiler-Leghorn crosses, they also reported that in another population of broiler-Fayoumi crosses, significant effects of this SNP did not appear in some of the traits studied. These differences reflect the differences in genetic backgrounds and underscore the importance of interactions between this SNP and the genetic

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background (Zhou *et al*., 2005). Bennett *et al*. (2006) also reported a significant association of this SNP with the body weight of layer-broiler crosses at 5 weeks of age. Sinpru *et al*. (2021) reported significant associations between this SNP and body weight at hatch, 2 and 4 weeks of age among the Korat slow growing chicken, with the AA genotype having higher body weights. Similarly, Anh *et al.* (2015), also found significant associations of this SNP with body weights at hatching and at the early stages of growth (up to six weeks of age) in four Thai broiler populations, with the AA genotype being heavier than the CC or the heterozygote. In another study on Thai synthetic chicken lines (crosses between Thai local chicken and commercial layers), Promwatee *et al*. (2013) also reported significant associations with body weight at hatch, 4, 8, 12 and 14 weeks of age and with average daily gain. Furthermore, Moe *et al.* (2009) also demonstrated that the *HinfI* C>A SNP within the promoter region of *cIGF1* had a significant association with the body weight and average daily gain in native Asian, layer and broiler chicken populations. Bian *et al*. (2008) also observed that haplotypes of *cIGF1* were suggestively associated with body weight at 2 and 12 weeks of age of a broiler layer cross.

This study also investigated the association of *HinfI* C>A SNP with some carcass traits of the three chicken ecotypes of Ghana i.e., live weight, breast weight, wing weight, thigh weight, drumstick weight, dressed weight and dressing percentage. Carcass weight and breast muscle weight are very important traits in poultry industry today.

This study did not find any significant associations of *HinfI* C>A SNP with any of the above-mentioned carcass traits (Table 23). This polymorphism may therefore not be a good molecular marker for use in marker-assisted selection for the improvement of carcass traits in local chickens of Ghana. This finding agrees with that of Attarchi *et al*. (2017) who did not observe any associations of this SNP with carcass traits in Mazandaran chicken, and that of Kadlec *et al*. (2012) who did not also find associations with carcass traits of broilers. Similarly,

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Promwatee *et al*. (2013) did not find any significant associations of this SNP with carcass traits in three out of four Thai synthetic broiler chicken strains. However, in one of the strains, the authors reported significant associations with dressing percentage and breast muscle weight, with the AA genotype having a higher dressing percentage and breast muscle weights than the AC and CC genotypes.

The findings of this study differ from Sato *et al*. (2012) as well as from Zhou *et al.* (2005) who found significant associations with the breast muscle and dressing percentage of broiler crosses and concluded that this SNP may have contributed to a higher level of circulating cIGF1 hormone in the AA genotype compared with the CC genotype. Bian *et al*. (2008) also observed that haplotypes of *cIGF1* were suggestively associated with the carcass weight of a broiler layer cross.

The differences between the results of this study with those cited above could have arisen due to differences in the genotype of the birds in question. While this study was carried out amongst slow-growing local chicken ecotypes which had a higher frequency of the C allele or the Leghorn allele (Zhou *et al*., 2005), the other studies were carried out mainly among broilers or broiler crosses where the frequency of the A allele or broiler allele (Zhou *et al*., 2005) was high due to the selection of these birds for growth.

These differences seem to suggest that the *HinfI* C>A may not be a good molecular marker for the improvement of growth and carcass traits in local chickens.

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5.5.2.2 Association of *PstI* C>T with growth and carcass traits

A transition *PstI* C>T SNP was detected at the 5' flanking region of *cIGF1* of three local chicken ecotypes of Ghana. This SNP has been widely studied amongst various categories of chicken including local chickens, layers, broilers and broiler-layer chicken crosses (Tang *et al.*, 2010; Ali *et al.*, 2016). In this study, the *PstI* C>T SNP was found to have a significant effect on the body weights of the Ghana chicken ecotypes at hatch and at 16 and 22 weeks of age (Table 20), with the body weights of the CT and TT genotypes being higher than the body weights of the CC genotype. The TT genotype was also heavier that the CC genotype during the rest of the experimental periods, but these differences were not significant.

The 5'flanking region does not code amino acids, but it plays an essential role in the regulation of post-transcriptional pre-mRNA splicing, hence in the determination of when, where and the amount of protein that is expressed. Polymorphisms in this region could therefore affect the splicing activity of pre-mRNA leading to different outcomes of efficiency of *cIGF1* gene transcription (Wang *et al*. 2021).

The *PstI* C>T SNP has been reported to be associated with body weight amongst Chinese native Silkie chicken at 8, 10 and 13 weeks of age, with the CT genotype being heavier than the TT and CC genotypes which were similar in weight (Tang *et al.*, 2010). However, the authors of this study suggested that these differences could probably have been a result of linkage disequilibrium. The *PstI* C>T SNP has also been reported to influence the body weights of Wanzhai and Nindu yellow chickens at 8 and 16 weeks of age with the CC genotype being heavier than the TT variant (Wang *et al.*, 2004; Seo *et al*., 2001), on the other hand, they also observed that this polymorphism was only associated with the body weight of male Korean Ogol chickens but not with the body weights of the female chickens.

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The results of this study are also similar to those of Ali *et al.* (2016) who reported a significant effect of this SNP on body weights from hatch to 24 weeks of age. In another study on Leghorn chicken, Nagaraja *et al*. (2000) observed that this SNP did not have any significant effect on body weight during the laying period, but the effect was significant during the pre-laying period. Similarly, Al-Hassani *et al.*, (2015) did not find any significant association between this SNP and the body weight and growth rates of broilers.

This study did not find any strong evidence of an association of this SNP with some carcass traits of local chicken ecotypes in Ghana. This finding is in line with Tang *et al.* (2010) who also observed that the *PstI* C>T SNP was not significantly associated with many carcass traits, including slaughter weight, carcass weight, breast muscle weight, wing weight and drumstick weight amongst Thai broilers. While this SNP could be used as a molecular marker in marker-assisted selection for growth traits, it may not a good molecular marker for the carcass traits of the local chickens of Ghana.

5.5.2.3 Association of *BstBI* T>C with growth and carcass traits

A *BstBI* RFLP digestion of genomic DNA of three local chicken ecotypes of Ghana and subsequent sequencing revealed a T>C mutation in chromosome 1 located at 55,375,710 bp within the 3'UTR of *cIGF1*. This SNP was found to significantly affect body weight at hatch, and at 2 and 10 weeks of age, with the TT genotype being heavier than the CT and CC genotypes. During the other experimental periods, though not significant, there was a trend of the TT genotype to be heavier than the CT and CC genotypes while the CT genotype was also observed to be heavier than the CC genotype. This finding seems to suggest that the T allele disproportionately contributes to higher body weights in chicken than the C allele.

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In a similar study, Lei *et al*. (2005) also reported that this SNP significantly affected hatch weight and body weight at 12 weeks of age. In a study on guinea fowls in northern Ghana, Ahiagbe (2018) reported two SNPs in the 3, UTR that were significantly associated with the growth from the fourth to the eleventh week.

BstBI T>C also had a significant effect on growth rate between weeks 10 and 12 and on the total growth rate from hatch to the end of the experiment. Between 10 and 12 weeks of age, the CC genotype had the highest grow rate however, over the entire experimental period, the TT genotype had the highest growth rate followed by the CT and then the CC genotype. In view of the above, the best period for selection for body weight among these three chicken ecotypes would be between week 10 and week 12, given the fact that the highest growth rate also occurred within this period.

BstBI T>C could have exerted its effect on the body weights and growth rate via the modulation of gene expression. Indeed, the control of mRNA stability is another way of facilitating gene expression. In eukaryotes, 3′ UTRs are longer than 5′ UTRs and are key elements of the overall post-transcriptional regulation (Pesole *et al*., 2001). In the 3′ UTRs there are several regulatory motifs that are recognized by microRNAs (miRNAs) and RNA binding proteins to control mRNA stability, localization, and translation (Menendez-Gil, and Toledo-Arana, 2021). These motifs include the adenylate- and uridylate-rich element (ARE) which is characterised by repeats of the pentanucleotide AUUUA and is known to constitute a major mRNA destabilization determinant. This class of ARE controls the cytoplasmic deadenylation of mRNAs by the degradation of all parts of the poly(A) tail at the same rate, generating intermediates with poly(A) tails of 30-60 nucleotides, which are then completely degraded (Mignone *et al*., 2002). In summary, 3′-UTRs determine protein levels through regulation of mRNA stability and translation mediated largely by AU-rich elements and miRNAs. In view

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of this, polymorphisms in ARE motifs and binding sites could contribute in no small manner to gene expression and protein levels. It is therefore hypothesized that a) one of the alternative alleles at the *BstBI* T>C SNP locus could have biased the post-transcriptional process of *cIGF1* mRNA leading to the observed effects on body weights and growth, and b) that the association of this SNP marker with body weights and growth could be a result of linkage disequilibrium with another mutation in the 3'UTR of *cIGF1* that contains trans-acting regulatory sequences on the same chromosome.

BstBI T>C did not have any significant effect ($p>0.05$) on the carcass traits of the three local chicken ecotypes. These findings disagree with those of Lei *et al.* (2005) who reported a significant association between *BstBI* T>C and the carcass weight of Chinese broiler/layer crossbred chicken. These differences may be due to differences in the genotype or ages of the birds evaluated.

The *BstBI* T>C SNP locus could be useful in future marker-assisted selection for the improvement of body weight traits in the local chicken ecotypes of Ghana, but it may not be a good marker for carcass traits.

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5.6 Conclusions

It is the conclusion of this study that the preponderance of the C allele of the *HinfI* C>A locus in the three chicken ecotype populations of Ghana indicates that they may not have been subjected to any selection for growth. Furthermore, based on the *HinfI* C>A, *PstI* C>T and *BstBI* C>T SNP loci of *cIGF1*, the Forest chicken ecotype seems to be genetically more diverse than the Coastal Savannah and Interior Savannah chicken ecotypes, while the Coastal Savannah chicken ecotype also appears to be genetically more diverse than the Interior Savannah chicken ecotype.

The *HinfI* C>A locus of *IGF1* was not associated with body weight, growth, and carcass traits of local chicken ecotypes of Ghana. It may therefore not be a good molecular marker for use in marker-assisted selection that seeks to improve these traits in local chicken ecotypes of Ghana.

The *PstI* C>T locus of *IGF1* had an influence on the growth traits of chicken, but it did not influence some carcass traits of local chicken ecotypes of Ghana. This SNP could be used as a molecular marker in marker-assisted selection for growth traits but not for carcass traits.

The *BstBI* C>T was also associated with the growth traits of local chicken ecotypes of Ghana, but it did not influence any of the carcass traits studied. This SNP may therefore not a good molecular marker for carcass traits, but it could be useful in marker-assisted selection for the improvement of growth traits at 12 weeks of age.

Given the small sample sizes used in this study, it is recommended that further investigations be carried out to determine the full extent to which *PstI* C>T and *BstBI* C>T polymorphisms in *cIGF1* can be used as molecular markers in marker-assisted selection for growth traits in local chicken ecotypes.

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CHAPTER 6

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DIVERSITY OF CHICKEN INSULIN-LIKE GROWTH FACTOR 2 GENE AND THE ASSOCIATION OF ITS POLYMORPHISMS WITH GROWTH AND CARCASS TRAITS OF THREE LOCAL CHICKEN ECOTYPES OF GHANA.

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6.1 Summary

This study aimed to investigate the genetic variation of *cIGF2* gene and the association between c*IGF2* gene polymorphisms with growth and carcass traits in three chicken ecotypes of Ghana.

This experiment was carried out in four replicates with a total of 1124 chickens, made up of 562 chickens of the Forest ecotype (FO), 297 chickens of the Interior Savannah ecotype (IS), and 265 chickens of the Coastal Savannah (CS) ecotype. The body weights of all the birds were taken at hatch and subsequently once every fortnight until they were 22 weeks of age. At the end of the experiment all the birds were euthanized, processed and various carcass measurements taken.

Blood samples were also collected from a total of 300 chickens, with the Forest, Coastal Savannah, and Interior Savannah chicken ecotypes each contributing 100 birds. DNA was extracted from the blood samples and PCR used to amplify it. The Restriction Fragment Length Polymorphism (RFLP) method was used to detect polymorphisms in a large fragment of part of exon 2, intron 2 and part of exon 3 of the c*IGF2* gene using the *NlaIII* restriction enzyme. Sanger sequencing and ClustalW sequence alignment with MEGA11 software identified this mutation to be a *NlaIII* C>T single nucleotide polymorphism (SNP). Sequence alignment with annotated sequences of the *cIGF2* confirmed this to be a C>T single nucleotide polymorphism (SNP) in exon 3 of the gene.

An analysis of the diversity of the *NlaIII* C>T SNP in three chicken ecotype populations showed that the BB homozygote was the predominant genotype amongst the FO and IS ecotypes while the AB genotype was predominant in the CS ecotype. The B allele had the highest frequency among the three chicken ecotypes.

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The CS ecotype had a higher observed heterozygosity value (Ho) than the expected heterozygosity (He) value. However, in the FO and IS ecotypes, the observed heterozygosity was lower than the expected heterozygosity. These findings suggest that at the *NlaIII* C>T SNP locus, the CS ecotype is genetically more diverse than the FO and IS ecotypes. At this SNP locus, about 41% of the total molecular variance was found to reside among individuals in the populations, while 59% was within the individual, thus suggesting that the expression levels of *cIGF2* at this SNP locus and its effects on some growth and carcass traits does not differ greatly among the populations but rather differs within individuals or among individuals within the populations. The comparatively higher genetic diversity of the CS ecotype at this SNP locus could be a result of gene flow that could have been occasioned by several factors including livestock trade and government policies on poultry production.

In this study, a *cIGF2 NlaIII* C>T SNP which is a neutral substitution was detected in exon 3 of *cIGF2*. This SNP was not associated with body weights of the local chicken ecotypes. However, though not significant, the AB genotype was heavier than the AA and BB genotypes from hatch till 22 weeks of age. Between hatch and week 2, the growth rates of the AB and AA genotypes similar, but significantly higher than the growth rate of the BB genotype. Between week 20 and week 22, the growth rate of the AA genotype was significantly higher than the growth rates of the BB and AB genotypes. Since this single nucleotide polymorphism is a neutral substitution and does not lead to a different amino acid at this locus, the observed effect on growth rate could be attributed to linkage disequilibrium between this SNP and other SNPs on this chromosome.

The results of the study did not show any significant association of *cIGF2 NlaIII* C>T SNP with the carcass traits studied, i.e., breast weight, drumstick weight, wing weight, thigh weight, dressed weigh and dressing percentage. The lack of significant associations with the carcass

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traits could probably be due to smaller contributions of the SNP genotypes to muscle development or that the SNP genotypes did not have any effect on the number of muscle fiber or muscle fiber size.

The results of this study showed that *cIGF2 NlaIII* C>T may not be a good molecular marker for the selection of chicken on body weight and carcass traits in future marker-assisted breeding programmes of local chicken ecotypes in Ghana.

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6.2 Introduction

Insulin-like growth factors (IGF1 and IGF2) are a family of hormones that are structurally and functionally homologous to insulin (Dupont and Holzenberger, 2003; Wang *et al*., 2021). Insulin-like growth factor 2 (IGF2) hormone is coded by the *IGF2* gene. It is produced mainly in the embryo while its adult expression takes place in the liver and in the epithelial cells lining the surface of the brain (Bergman *et al.,* 2013). IGF2 is reported to influence embryonic development and post hatch growth of chicken by enhancing glucose and amino acid uptake, and DNA and protein synthesis (McMurtry, 1998). IGF2 also promotes cell survival, migration, and differentiation via the IGF1 receptor tyrosine kinase (Chao and D'Amore, 2008). Through the insulin receptor isoform A, this hormone also induces mitogenic responses and is important for foetal growth and development. In mice, the *IGF2* gene knockout leads to a significant reduction in body size (Gicquel and Le, 2006).

The *IGF2* gene is located on chromosome 5 of the chicken genome and has 4 exons and 3 introns. Many polymorphisms have been detected in the *IGF2* gene and found to be associated with several reproductive and productive traits of livestock. For example, a C>G SNP within exon 2 of chicken *IGF2* was reported by Tang *et al.* (2010) to have a significant effect on the body and carcass weight of the native Chinese Beijing You chicken at 17 weeks of age, however they also concluded that this SNP was not associated with these traits in Silkie chicken. In contrast, Yan *et al*. (2002) concluded that this SNP was associated with the growth and carcass traits of Silkie chicken. The same polymorphism has been reported by Wang *et al.* (2005) to be associated with body weight at 3 weeks of age and carcass weight. Furthermore, in another study on the Chinese Langshan chicken, Yan *et al.* (2017) also confirmed the same SNP in exon 2 of *cIGF2* and concluded that it was associated with body weight at 16 weeks of

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age and higher egg production. This polymorphism has also been linked with abdominal fat weight and percentage of abdominal fat of broiler chicken (Zhihui *et al*., 2004).

In a study on the effects of polymorphisms of the *cIGF2* on the growth and feeding traits in two divergent strains of Black Penedensca Chicken, Amills *et al*. (2003) found 2 SNPs, one of which was a neutral substitution C>T at exon 3 while the second one was a G>A substitution at intron 2. However, they did not find significant associations between these polymorphisms with growth and feeding traits in the two strains of Black Penedensca Chicken. On the other hand, Molee *et al.* (2018) reported a strong association between this C>T polymorphism with body weight at 16 weeks of age of Leung Hang Khao local chicken of Thailand, but they did not find any significant effect of it on the carcass traits. Similarly, Kuadsantia *et al*. (2015) did not find any significant effect of this polymorphism on carcass traits of Leung Hang Khao local chicken. This SNP has also been reported by Khadem *et al*. (2010) as not having any significant influence on body weights but that it was rather strongly associated with egg weight and the number of eggs laid. In contrast, Aghdam *et al*. (2014) concluded that in West Azerbaijan native chicken, this polymorphism was significantly associated with body weight at 8 and 12 weeks of age. This observation is however at variance with Amills *et al*. (2003) who also studied the same polymorphism.

In guinea fowl populations, Ahiagbe (2018) reported two novel SNPs within exon 3 and exon 4 of *IGF2* but these SNPs were found not to be associated with body weights or growth rates. The *IGF2* gene has been reported to be associated with growth and carcass traits of local turkeys of Nigerian (Ewuola, 2021). In Japanese quails, a neutral A>G SNP at exon 2 was found to be negatively associated with body weight, egg weight and egg number (Ali et al., 2021).

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Associations of polymorphisms of *IGF2* with growth and carcass traits in other animals have also been reported. In bovine, a C>T SNP in exon 2 of *IGF2* was observed to be associated with the rib eye area and the carcass fat percent in beef cattle (Goodall and Schmutz, 2007) while in the Egyptian water buffalo, a C>A non-synonymous SNP in exon 10 of *IGF2* is reported to be associated with ADG from birth to 9 months of age (Abo-Al-Ela *et al.*, 2014). Markljung *et al.* (2009) reported a SNP in intron 3 of pigs IGF2 which abrogates a binding site for a repressor called ZBED6 and leads to a 3-fold up-regulation of IGF2 in skeletal muscle with major effects on muscle growth, size of the heart, and fat deposition.

Based on the effects of *IGF2* gene polymorphisms on growth and carcass traits, this gene has been recommended as an appropriate molecular marker for the improvement of growth and carcass traits of chicken (Tang *et al*., 2010; Agdam *et al*., 2014; Molee *et al*., 2018).

This study therefore sought to analyse the diversity of the *cIGF2* gene and to estimate the extent to which its polymorphisms are associated with growth and carcass traits of three chicken ecotypes of the Interior Savannah, Forest and Coastal agroecological zones of Ghana.

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6.3 Materials and Methods

6.3.1 Geographical location and source of experimental chickens

The experimental birds were sourced from the "Feed the Future" Innovation Lab for Genomics to Improve Poultry Project (cooperative agreement number AID-OAA-A-13-00080), located at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana.

In the poultry breeder unit of LIPREC, a third generation of local breeder chickens of the forest, coastal savannah and interior savannah ecotypes were grouped for natural mating to produce sire half-sib families per ecotype, using a mating ratio of 1 sire to 8 dams to produce chicks for the study.

The eggs for the experiment were collected over a ten-day period. After each day's collection, the eggs were stored in a chiller maintained at a temperature of 13^{o}C. At the end of the egg collection period, all the eggs are transferred to an incubator (A.P Poultry incubator of Hyderabad, India) and incubated for 18 days under a temperature range of 37.2 to 37.78^{o}C and a humidity level of 60-65%. After incubation, the eggs were transferred to a hatcher for hatching. Immediately after hatching, each chick was weighed with an electronic weighing scale (OHAUS Explorer ^{TM} Precision -0.1g readability) and then tagged. The chicks were brooded in a 34-36^{o}C temperature range that was maintained with the aid of electric bulbs.

6.3.2 Management of the experimental chickens

The experiment was conducted in four replicates with a total of 1235 chickens, made up of 622 chickens of the FO ecotype, 330 chickens of the IS ecotype, and 283 chickens of the CS chicken ecotype (Table 2).

Each ecotype was housed separately with a maximum of 40 birds in a pen with a size of 2.54 m x 2.2 m x 2.2 m. From day 1 to week 8, all the birds were fed on a standard chick starter

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mash with 20.0 % crude protein and a Metabolizable Energy (ME) of 2,993 kcal/kg while from week 9 to week 22 they were fed on a standard chick grower mash with 17% crude protein and a ME content of 3,015 kcal/kg. Feed and water were provided on an *ad lib* basis The diets were formulated using commercial protein concentrates following the manufacturers specification. The vaccination, feeding and other important husbandry practices were the same for all the birds.

6.3.3 Phenotypic measurements

Body Weights

The body weights of all the birds were taken at hatch and subsequently once every fortnight until they were 22 weeks of age. An electronic weighing scale (OHAUS Explorer ™ Precision -0.1g readability) was used to weigh the birds from hatch to week 8, while from week 9 to week 22, an electronic hanging scale (Kern HDN 5K5-5.0 g readability) was used to weigh them. The body weights were then used to calculate the growth rates up to week 22 (Formula 1).

$$\text{Growth rate measured at bi-weekly intervals} = \frac{Final\ weight - Initial\ weight}{Time\ Interval\ (2\ weeks)} \quad (1)$$

Carcass traits

At the end of the experimental period, a total of 1036 birds were starved of feed for 12 hours to ensure complete emptying of the crop, weighed, and then euthanized. Each bird was bled and weighed on a sensitive scale (scale (OHAUS Explorer ™ Precision -0.1g readability) to determine the weight of the blood. Thereafter the birds were scalded in hot water, defeathered and weighed again to determine defeathered weight.

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The carcasses were then stored in a cold room at a temperature of 3 to 5 ^{o}C for 24 hours before they were processed. The offal (gastrointestinal tract, liver, shank, lung, head, and kidney) of each bird was removed by hand from the carcass and the carcass or dressed (eviscerated) weight determined. The head, shanks, wings, thighs, drumstick, breast muscle and neck were weighed individually based on the Meat buyers' guide developed by North American Meat Processors Association (2007), on a sensitive scale. The heart, liver and gizzard were also weighed individually on the same scale. From these measurements, several parameters, including the carcass/dressed weight and dressing percentage, were calculated (Formular 2 and Formular 3).

Dressed weight = live weight – offal weight (2)

The dressing percentage was expressed as a percentage of carcass weight over live weight.

$$\textbf{Dressing percentage} = \left(\frac{\text{Dressed weight}}{\text{Live weight}}\right) \times 100 \quad (3)$$

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6.3.4 Sample collection and DNA extraction

The extraction of DNA, Polymerase Chain Reaction (PCR) amplification of target regions of the chicken Insulin-like Growth Factor 2 gene (c*IGF2)* and the subsequent detection of polymorphisms using a restriction enzyme were all carried out at the Molecular Genetics Laboratory of the Department of Animal Science of the University of Ghana.

Blood samples were collected from the wing vein of 300 chickens aged 12 weeks, 100 of which were from each local chicken ecotype. Each sample was collected into an ETDA coated vacutainer tube, placed on ice, and eventually stored at -20^{o}C.

Genomic DNA was extracted using DNeasy® blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and guidelines. The quality of the extracted DNA was checked by resolving aliquots of 3 µl of the DNA on 1.5% agarose gel and electrophorized at 100 V for 25 min in TBE buffer (1M Tris base, 1M Boric acid, 0.02 M EDTA), and the bands visualized under a molecular imager (Bio RAD Gel Doc TM XR+). The quantity of the DNA was checked with the aid of a Qubit^{TM} 4 Fluorometer (Thermo Fisher SCIENTIFIC, Malaysia). Thereafter, working dilutions of the extracted DNA of each bird were prepared at a concentration of 50 ng/ µl.

6.3.5 PCR amplification of target regions.

The PCR reactions were caried out in a 10 µl reaction volume, consisting of 1 µl of chicken genomic DNA (50 ng/ µl), 5 µl of AmpliTaq Gold 360 Master Mix (Applied Biosystems, Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 3.0 µl of sterilized distilled water, 0.5 µl (20 µM) of the forward primer and 0.5 µl (20 µM) of the reverse primer.

The PCR was carried out in a thermal cycler (BIO-RAD C1000^{TM}) and the reaction conditions and primers used for the amplification of the target region of the gene are shown in Table 27.

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Table 27. The primer sequences and cycling conditions used to amplify fragments of the c*IGF2* gene.

Gene	Primer Name	Sequence	Product size	Cycling conditions	Source
IGF2	IGF2E2I2E3R IGF2E2I2E3F	TTC CTG GGG GCC GGT CGC TTC A CCA GTG GGA CGA AAT AAC AGG AGG A	1300 bp	94°C for 4 min. 94°C, 1 min-40 cycles 67°C, 1.5 min 72°C, 1.5 min 72°C for 10 min	Shah *et al.* (2012)

After the PCR, aliquots of 3 µl of each PCR product were resolved on 1.5% agarose gel at 100 V for 25 min by electrophoresis in TBE buffer, stained with GelRed (Biotium, California) and visualized under a molecular imager (Bio RAD Gel Doc^{TM} XR^{+}).

6.3.6 Restriction Fragment Length Polymorphism

The Restriction Fragment Length Polymorphism (RFLP) method was used to detect polymorphisms in the c*IGF2* gene. A large fragment which straddles exon 2, intron 2 and exon 3 was targeted. The PCR products of the fragment were digested with *NlaIII* restriction enzyme at an incubation temperature of 37°C for 15 minutes.

After the digestions, aliquots of 5 µl of the digests were resolved on 2% agarose gel and electrophorized at 100 V for 25 minutes in TBE buffer, stained with gel red and the bands visualized under a molecular imager (Bio RAD Gel Doc^{TM} XR^{+}).

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Genotyping of the birds was then carried out based on the number of bands visualised in each sample.

6.3.7 Sequencing

To confirm the polymorphism observed in the RFLP, the PCR products of three samples of each genotype were purified and sanger sequenced with the aid of an ABI 3730xl.abi Genome analyser. The trace files of the sequences were then viewed with the aid of Finch TV (Osman *et al.*, 2022). The chromatograms were trimmed to remove the low-quality bases found at the ends of the sequences. Thereafter, the sequences were exported in a FASTA format into MEGA 11 software (Tamura *et al.*, 2021) and sequence alignments carried out with ClustalW to confirm the presence of SNPs.

The location of the confirmed polymorphism in the gene was determined by means of a comparison of the sequences with the annotated sequence of chicken *IGF2* gene (NC_052536.1) obtained from NCBI database (https://www.ncbi.nlm.nih.gov). The comparison was made using the National Center for Biotechnology information (NCBI) Basic Local Alignment Search Tool (BLAST).

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6.3.8 Statistical analysis

Genotype and allele frequencies

The SNP genotype of each bird was entered into an Excel spreadsheet and GenAlEx software ver. 6.51b2 (Peakall and Smouse, 2012) used to carry out the various analyses, including an estimation of the genotype and allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), and fixation index, amongst others. The probability of random mating in each population was also estimated using Chi-square (χ^2) test to examine Hardy-Weinberg equilibrium (HWE). An analysis of Molecular Variance (AMOVA) was also carried out with the aid of GenAlEx v. 6.503 with the objective of estimating the proportion of the total genetic variance that exists within, amongst and between the populations.

Analysis of the association of polymorphisms with growth and carcass traits

A general linear model procedure of SAS^{TM} 9.0 (2002) was used to estimate the associations of a SNP in *cIGF2* gene with body weights and weight gained between specific periods as indicators of growth and carcass traits of three local chicken ecotypes, using the following model:

$$Yijk = \mu + Si + Gj + SGij + eijk.$$

Where, $Yijk$ is the dependent variable per each model, μ is the overall mean for a given dependent variable, Si the effect of ith sex, Gj the effect of the jth genotype at a given SNP, $SGij$ is the effect of the interaction of sex and genotype at a given SNP and $eijk$, the random error.

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The traits used as dependent variables were:

Body weight and growth traits

a) Hatch weight (HW 0), weight at week 2 (BW 2), weight at week 4 (BW 4), weight at week 6 (BW 6), weight at week 8 (BW 8), weight at week 10 (BW 10), weight at week 12 (BW 12), weight at week 14 (BW 1W), weight at week 16 (BW 16), weight at week 18 (BW 18), weight at week 20 (BW 20) and weight at week 22 (BW 22).

b) Growth rates between hatch and week 2 (WG2), between week 2 and week 4 (WG4), between week 4 and week 6 (WG6), between week 6 and week 8 (WG8), between week 8 and week 10 (WG10), between week 10 and week 12 (WG12), between week 12 and week 14 (WG14), between week 14 and week 16 (WG16), between week 16 and week 18 (WG18), between week 18 and week 20 (WG20), between week 20 and week 22 (WG22) and between hatch and week 22 (TWG).

Carcass traits

a) Live weight before slaughter,
b) breast weight,
c) wing weight,
d) thigh weight,
e) drumstick weight,
f) dressed weight
g) dressing percentage.

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6.4 Results

6.4.1 Restriction Fragment Length Polymorphism

The image of the *NlaIII* digestion of the PCR products of exon 2, intron 2 and part of exon 3 of the c*IGF2* gene is shown below in Figure 18. Three patterns were observed. One pattern named A, had five fragments, estimated to be about 1140 bp, 750 bp, 640 bp, 480 bp, and 280 bp in size. The second pattern named B, had six fragments estimated to be about 750 bp, 640 bp, 480 bp, 280 bp and 160 bp in size. The third pattern was a combination of pattern A and B and had seven fragments.

Figure 18. PCR-RFLP pattern of exon 2, intron 2 and part of exon 3 region of *cIGF2* gene. All Lanes are AB heterozygotes. M=DNA ladder. Lanes 1 to 10 and lanes 12 to 14=AB heterozygotes; Lane 11=BB genotype

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6.4.2 Sanger Sequencing

The sequences of the target region of the *cIGF2* gene were successfully amplified and sanger sequenced using genomic DNA samples. Chromatographs showing mutations that were associated with the *NlaIII* RFLP SNP of the *cIGF2* gene are shown in Figure 19.

A T>C SNP was found in exon 3 (position 902 of my sequence). This mutation was associated with the *NlaIII* RFLP. A comparison of the sequences of exon 2, intron 2 and exon 3 with sequences of the annotated chicken *IGF2* gene (NC_052536.1) with the aid of NCBI BLAST indicated that this polymorphism is in c*IGF2* at position 13,389,296.

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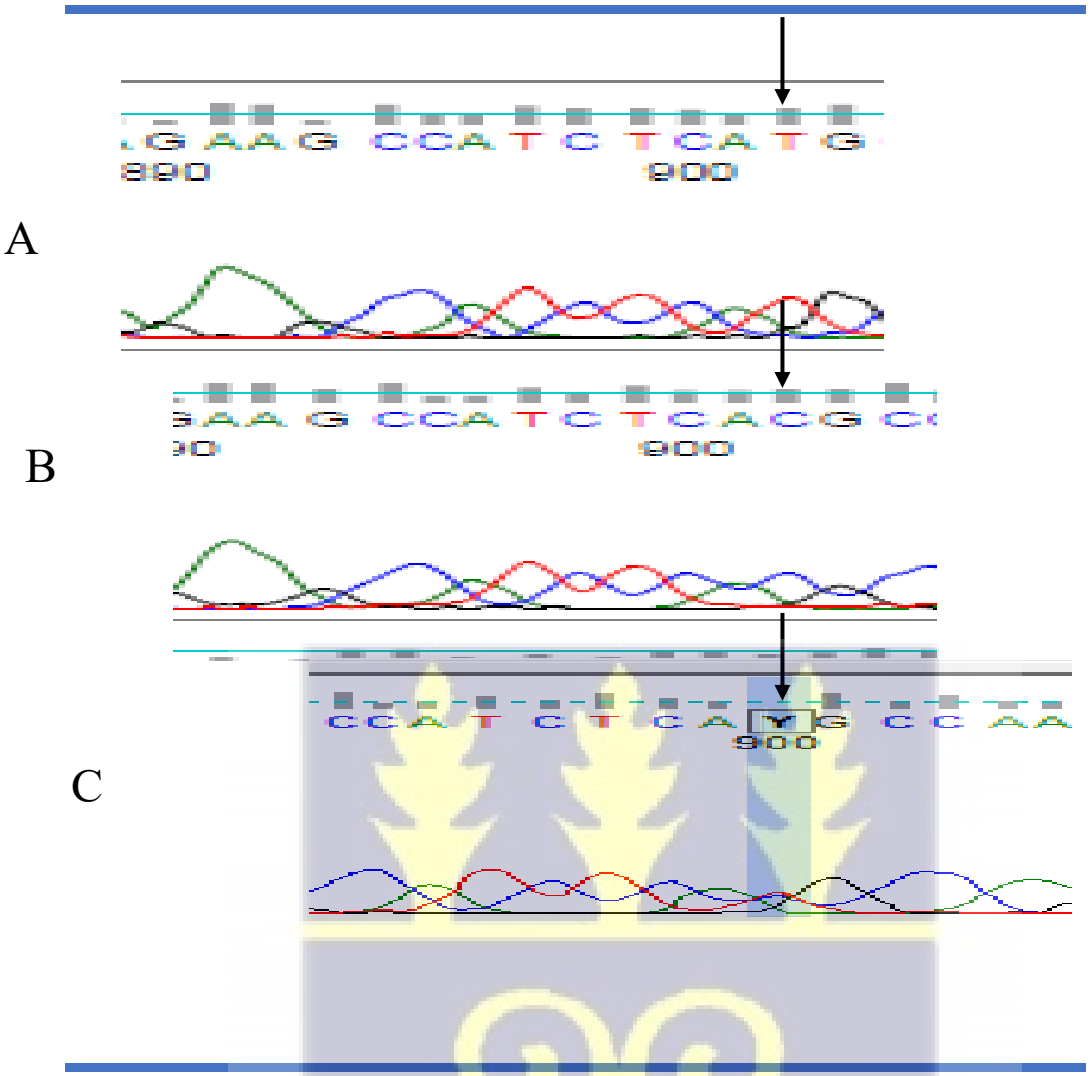

Figure 19. A T>C SNP in exon 3 of the chicken *IGF2* gene. A=homozygote (T allele). B=homozygote (C allele). C=Heterozygote

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6.4.3 Genotype diversity

The genotypic and allelic frequencies of the *cIGF2-NlaIII* T>C SNP in the three chicken ecotypes is shown in Table 28. The AB genotype had the highest frequency amongst the CS ecotype while the BB genotype was predominant in the FO and IS ecotypes. The B allele had the highest frequency among all the three chicken ecotypes. The genotype frequencies of all the three chicken ecotypes were in Hardy-Weinberg Equilibrium (HWE).

Table 28: Genotype and Allele frequencies of IGF2-*NlaIII* SNP in three chicken ecotypes of Ghana

Marker	Ecotype	N	Genotype frequency			Allele frequency		HWE
			AA	AB	BB	A	B	
IGF2-*NlaIII*	Forest	86	0.388	0.035	0.576	0.406	0.594	***
	Coastal Savannah	40	0.050	0.825	0.125	0.463	0.537	***
	Interior Savannah	73	0.219	0.274	0.507	0.356	0.644	***

HWE= Hardy-Weinberg Equilibrium. *** P<0.001.

The population diversity indices at the *IGF2-NlaIII* T>C locus of the three Ghana chicken ecotypes are shown in Table 29. The observed heterozygosity values were higher than the expected heterozygosity in the CS ecotype. However, among the FO and IS ecotypes, the observed heterozygosity was lower than the expected heterozygosity values.

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Table 29. Diversity of IGF2-*NlaIII* SNP in three chicken populations

Marker	Ecotype	N	Ho	He	uHe
IGF2-*NlaIII*	**FO**	86	0.035	0.482	0.485
	CS	40	0.825	0.497	0.503
	IS	73	0.274	0.459	0.462
	Mean	66.0	0.378	0.479	0.483
	SE	13.45	0.23	0.01	0.01

N=Number of observations. Ho=Observed heterozygosity; He=expected heterozygosity; uHe=Unbiased heterozygosity

The partitioning of total molecular variances at the *IGF2-NlaIII* T>C locus is shown in Figure 20. At this locus, 59% of the total molecular variance resided within individuals in the populations, while 41% of the total molecular variance resided amongst individuals.

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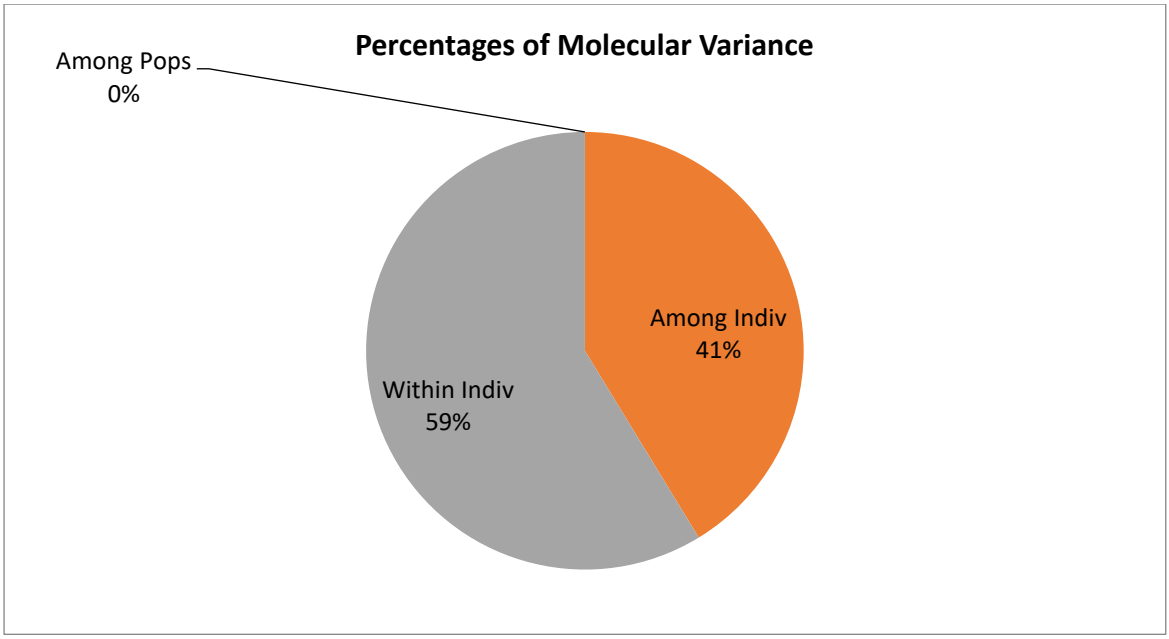

Figure 20: Partitioned molecular variance in three chicken populations

6.4.4 Association between *cIGF2-NlaIII* T>C with growth and carcass traits

6.4.4.1 Association between c*IGF2-NlaIII* T>C with growth traits

Sequence alignment with the annotated chicken *IGF2* gene (NC_052536.1) showed that the *NlaIII* SNP was a C>T mutation which is in exon 3 of the *cIGF2* gene.

The association of this mutation with body weight and growth rate, measured fortnightly, are shown in Table 30 and 31 respectively. There was evidence that the C>T mutation did not influence body weights however it influenced growth rates between hatch and week 2 and between week 20 and week 22.

The body weight of the AB genotype, though higher than the body weights of the AA and BB genotypes throughout the experimental period, was not significantly different from them.

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Between hatch and week 2, the growth rate of the AB genotype was significantly higher than the growth rate of the BB genotype ($p<0.05$) but similar to the growth rate of the AA genotype. There were no significant variations in the growth rates of the AA and BB genotypes within this period. On the other hand, the growth rate of the AA genotype was significantly ($p<0.05$) greater than the growth rates of the AB and BB genotypes between weeks 20 and 22, while the growth rates of the AB and BB were similar to each other. This mutation had no significant effect on the total growth rate measured from hatch till 22 weeks of age.

Table 30. Least Square Means ± SE of association of *cIGF2-NlaIII* SNP genotypes with body weight (g) in local chicken ecotypes in Ghana.

TRAIT	GENOTYPE		
	AA (50)	AB (54)	BB (90)
HW	25.59 ± 0.61	25.98 ± 0.42	24.71 ± 0.41
BW2	61.99 ± 2.68	66.58 ± 1.90	63.79 ± 1.83
BW4	146.61 ± 5.20	154.37 ± 3.68	146.96 ± 3.54
BW6	255.39 ± 8.58	267.99 ± 6.07	254.84 ± 5.84
BW8	386.36 ± 10.58	390.14 ± 7.48	385.11 ± 7.19
BW10	548.85 ± 13.43	556.09 ± 9.49	546.88 ± 9.13
BW12	717.51 ± 16.67	731.47 ± 11.78	712.25 ± 11.34
BW14	826.76 ± 20.93	862.29 ± 14.80	841.81 ± 14.24
BW16	965.61 ± 23.28	998.12 ± 16.46	966.13 ± 15.84
BW18	1067.93 ± 25.83	1100.16 ± 18.26	1068.37 ± 17.57
BW20	1108.25 ± 27.42	1164.42 ± 19.38	1112.94 ± 18.65
BW22	1209.18 ± 28.77	1226.76 ± 20.50	1170.94 ± 20.06

HW= Hatch weight. **BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, BW20, BW22=** Body weight at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 weeks respectively. No significant deviations in all the values ($p < 0.05$). The number of observations is indicated in the parenthesis.

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Table 31. Least Square Means ± SE of association of *cIGF2-NlaIII* SNP genotypes with growth rate (g) in local chicken ecotypes in Ghana.

TRAIT	GENOTYPE		
	AA (51)	AB (56)	BB (91)
WG2*	36.59 ± 2.29^{ab}	39.99 ± 1.59^{a}	33.80 ± 1.56^{b}
WG4	85.21 ± 3.83	87.24 ± 2.66	85.38 ± 2.61
WG6	109.03 ± 5.23	113.45 ± 3.63	105.91 ± 3.56
WG8	130.59 ± 4.88	122.14 ± 3.39	130.12 ± 3.33
WG10	161.50 ± 5.68	165.81 ± 3.95	160.77 ± 3.87
WG12	168.85 ± 5.78	175.04 ± 4.01	165.68 ± 3.94
WG14	108.74 ± 8.54	130.40 ± 5.92	130.39 ± 5.81
WG16	138.65 ± 7.91	135.52 ± 5.49	124.97 ± 5.39
WG18	101.76 ± 10.48	99.68 ± 7.27	102.68 ± 7.14
WG20	39.88 ± 9.42	63.73 ± 6.54	46.46 ± 6.42
WG22*	100.43 ± 12.98^{a}	60.80 ± 9.09^{b}	66.56 ± 8.84^{b}
TWG	1181.24 ± 28.79	1196.09 ± 20.16	1152.72 ± 19.61

WG=Weight gain. **WG2**= WG between hatch and week 2; **WG4**= WG between week 2 and week 4; **WG6**= WG between week 4 and week 6; **WG8**= WG between week 6 and week 8; **WG10**= WG between week 8 and week 10; **WG12**= WG between week 10 and week 12; **WG14**= WG between week 12 and week 14; **WG16**= WG between week 14 and week 16; **WG18**= WG between week 16 and week 18; **WG20**= WG between week 18 and week 20; **WG22**= WG between week 20 and week 22; **TWG**= WG between hatch and week 22. Values with different superscript letters (a-b) within the same row per trait indicate significant deviation (* =$p < 0.05$, . ^{NS} = No significant deviation ($p > 0.05$) due to genotype.

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6.4.4.2 Association between *cIGF2-NlaIII* T>C with Carcass traits

The least square means of the carcass traits of *cIGF2-NlaIII* T>C genotypes of the three local chicken ecotypes of Ghana are shown in Table 32. There was no evidence of any association of *cIGF2-NlaIII* T>C with the carcass traits of any of the genotypes (p>0.05). However, there was a clear tendency of the AB genotype to have higher values of live weight, breast weight, thigh weight, wing weight, drumstick weight, dressed weight and dressing percentage.

Table 32. Least Square Means ± SE of carcass traits (g) of *cIGF2-NlaIII* SNP genotypes in three local chicken ecotypes

TRAIT	GENOTYPE		
	AA (50)	AB (53)	BB (91)
LW	1156.82 ± 27.95	1167.45 ±18.95	1134.03 ± 18.03
Breast wt.	200.908 ± 6.15	203.37 ±4.17	198.87 ± 3.97
Thigh wt.	133.10 ± 3.68	134.62 ±2.49	127.50 ± 2.37
Wing wt.	99.05 ±2.31	100.76 ±1.57	97.93 ± 1.49
Drumstick wt.	119.32 ± 3.53	119.93 ±2.39	117.53 ± 2.28
Dressed wt.	828.18 ± 20. 63	835.31 ±13.98	809.21 ± 13.31
Dressing %	71.45 ± 0.67	71.46 ±0.45	71.32 ± 0.43

No significant deviations in all the values ($p < 0.05$). The number of observations is indicated in the parenthesis.

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6.5 Discussion

The IGF2 protein hormone is reported to influence embryonic development and post hatch growth of chicken (McMurtry, 1998; Zhao *et al*., 2004; Rotwein, 2018b), and to play a key role in cell survival, migration, and differentiation via the IGF1 receptor tyrosine kinase (Chao and D'Amore, 2008). This hormone is produced mainly in the embryo but is also present in the liver and other tissues. IGF2 is coded by the *IGF2* gene. Some polymorphisms have been detected in this gene and reported to be associated with some production and reproduction traits of chicken and other livestock species (Abo-Al-Ela *et al.*, 2014; Ahiagbe, 2018; Ali *et al*., 2021; Ewuola, 2021).

In this study, the genetic variability of *cIGF2* was explored in three chicken ecotypes of Ghana. In the process, the Restriction Fragment Length Polymorphism (RFLP) method was used to detect polymorphisms in exon 2, intron 2 and part of exon 3 of the c*IGF2* gene and subsequently to SNP-genotype a total of 196 birds. The RFLP digestion revealed fragments whose sizes are quite different from those reported by several authors including Amills *et al*. (2003). Variations in the sizes of the fragment of the *cIGF2-NlaIII* digestion have also been reported by Khadem *et al.* (2010) and Amills *et al*. (2003). Further investigations may therefore be required to find out if there has been an insertion in the DNA sequence of targeted region of the samples analyzed.

The results showed that there was a polymorphism in exon 3 of the gene. Sanger sequencing and sequence alignment with an annotated chicken *IGF2* gene (NC_052536.1) showed that this was a T>C SNP located in exon 3 of *IGF2*. This polymorphism has also been reported by other authors including Amills *et al*. (2003), Khadem *et al.* (2010) and Shah *et al*. (2012).

An in-depth review of literature revealed that several other SNPs have been reported within the *cIGF2* gene. A C>G transition mutation within exon 2 has been reported by several authors

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including Yang *et al*. (2017) in Langshan chicken, by Tang *et al.* (2010) in Beijing You chicken and by Wang *et al*. (2005) in broiler and Silky chicken and found to be associated with growth and carcass traits. Amills *et al*. (2003) also reported two SNPs including G>A substitution within intron 2 which were not associated with growth and feeding traits of chicken.

IGF2-*NlaIII* SNP diversity

Genetic diversity analysis is an important step towards the implementation of genetic improvement or germplasm conservation programmes of local chicken ecotypes of Ghana.

The diversity of *cIGF2-NlaIII* T>C in three chicken ecotype populations was analysed based on the genotype and allele frequencies, the observed heterozygosity, the expected heterozygosity, and the fixation index. The partitioning of the total molecular variance was also carried out.

The B allele had the highest frequency in all the three chicken ecotypes. The allele frequencies in all the three chicken ecotypes were in Hardy-Weinberg Equilibrium (HWE).

The allele frequencies of all the three Ghana chicken ecotypes were within the range of those reported by Khadem *et al*. (2010) among Mazandaran local chicken but are different from those of the West Azerbaijan local chicken as reported by Aghdam and Karin (2014) and by Koski *et al*. (2000). Among Leung Hang Khao chicken of Thailand, the A allele is reported to have a frequency of 0.501 while the B allele had a frequency of 0.499 (Molee *et al*., 2018), which are quite high but different from the allele frequencies of any of the three local chicken ecotypes of Ghana.

The CS ecotypes had a higher observed heterozygosity value than the expected heterozygosity value. However, among the FO and IS ecotypes, the observed heterozygosity was lower than

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the expected heterozygosity. These findings suggest that at the *cIGF2-NlaIII* T>C locus, the CS ecotype is genetically more diverse than the FO and IS ecotypes. The comparatively higher genetic diversity of the CS ecotype could be a result of gene flow that could have been occasioned by both livestock trading activities and government policies. The Coastal areas of Ghana have high human populations and for that matter an extremely high demand for chicken meat. As a result, there is a considerable movement of local chickens from northern Ghana and the neighbouring countries to markets in the Coastal areas (Kamuanga *et al*., 2008; Rudloff and Schmieg, 2017; Valerio *et al*., 2020). Furthermore, the abundance of commercial exotic chicken production enterprises, including hatcheries selling cockerels, layers, and broilers in the CS areas of Ghana (Aning, 2008) could also be a significant source of gene flow in these areas. Non-governmental Organizations could also, to some extent, have contributed to these gene flows through the projects they implemented here. For example, in the past, some Non-Governmental Organizations promoted small-scale poultry production enterprises as means of livelihoods enhancement for the poor and resource constrained households in the rural parts of the Country These interventions included cockerel and layer production schemes. Unfortunately, in some places, though not an intended purpose, some exotic cockerels ended up being used to cross local chickens (Aning, 2008).

Based an analysis of the total molecular variance associated with the *cIGF2-NlaIII* T>C locus, 59% of the total molecular variance resides within individuals while 41% was among individuals in the populations.

By way of a summary, the AB heterozygous was the predominant genotype amongst the CS ecotype while the BB genotype was predominant in the FO and IS ecotypes. The B allele had the highest frequency in all the three chicken ecotypes. Furthermore, at the *cIGF2-NlaIII* T>C locus, the CS ecotype was found to be genetically more diverse than the FO and IS ecotypes.

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Association of IGF2-*NlaIII* SNP with Growth traits

Growth is a complex process that is modulated by several neuroendocrine pathways including the somatotropic axis. The somatotropic axis consists of Growth Hormone Releasing Hormone, Growth Hormone, Insulin-like Growth Factors and their receptors and binding proteins. IGF2 is one of the ligands of the Insulin-like Growth Factors.

In this study, a *NlaIII* C>T SNP was detected in exon 3 of *cIGF2*. The effects of this transition SNP, which is reported to be a synonymous polymorphism, on the growth, carcass and feeding traits of chicken, has also been studied by Amills *et al*. (2003), Kuadsantia *et al*. (2015) and Molee *et al*. (2018).

The results of the study showed that *cIGF2 NlaIII* C>T SNP did not significantly affect body weight, but it influenced growth rates between hatch and week 2 and between week 20 and week 22. Between hatch and week 2, there were no significant variations in the growth rates of the AA and AB genotypes, but the AB genotype had a significantly higher growth rate than the BB genotype while between week 20 and week 22, the AA genotype had a significantly higher growth rate than the AB and BB genotypes.

The cIGF2 hormone has been widely reported to have significant effects on growth during the embryonic and early post hatch periods (Kim, 2010). However, the results of this study showed that *cIGF2 NlaIII* C>T was not associated with body weight at hatch and at the early stages of growth. The results of this study are similar to Amills *et al*. (2003) who did not find any effect of this polymorphism on the body weight of two Penedesenca chicken strains. Similarly, Khadem *et al*. (2010) did not also find any significant effect of this polymorphism on the body weight of Mazandaran chicken. These observations are to be expected given the fact that this

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polymorphism has been reported to be a synonymous substitution in the coding region of *cIGF*2 and should therefore not lead to changes in the coded protein sequence.

The results of this study are however at variance with Molee *et al*. (2018) who observed that this polymorphism significantly influenced body weights of Leung Hang Khao chicken at 16 weeks, with the BB genotype being heavier that the AA and AB genotypes.

The inconsistencies in these results could be due to differences in genetic makeup of the chickens used in the various experiments. Alternatively, it is possible that in some of the populations, this SNP could be in linkage disequilibrium with polymorphisms in other genes on the same chromosome which influence growth traits. Furthermore, this polymorphism could have affected the folded structure of the secondary mRNA leading to changes in its stability and translation efficiency (Sauna and Kimchi-Sarfaty, 2013) or altered the codon and resulted in either increased or decreased translation rates depending on the relative abundance of the corresponding tRNA molecules in the cell. It is also possible that this polymorphism led to the addition or reduction of miRNA binding sites, leading to altered levels of expression (Gotea, *et al*., 2015).

Other studies (Yan *et al*., 2002; Wang *et al*. 2005; Tang *et al*., 2010; Aghdam *et al*., 2014; Yan *et al*., 2017; Ahiagbe, 2018; Ewuola, K.M. 2021) have investigated the effects of *cIGF2* and reported significant associations of its polymorphisms with the growth traits of chicken, guinea fowls and turkeys.

The results of this study do not suggest the existence of significant variations in the body weights of the *NlaIII* C>T SNP genotypes. This SNP may therefore not be a good molecular marker for the improvement of growth traits of local chicken ecotypes of Ghana in future marker-assisted breeding programmes.

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Association of *cIGF2-NlaIII* SNP with Carcass traits

The results of this study showed that the *NlaIII* C>T SNP did not have a significant effect on body weight as well as the with the carcass traits studied, i.e., breast weight, drumstick weight, wing weight, thigh weight, dressed weigh and dressing percentage. The lack of significant associations with the carcass traits, despite the significant associations with some growth rates, could probably be due to smaller contributions of the SNP genotypes to muscle development or that the SNP genotypes did not have any effect on the number of muscle fiber or muscle fiber size. If the differences in the growth rates of the SNP genotypes were large enough (Table 31), then this could have influenced the number of muscle fibers or their sizes. Chicken with high growth rates tend to have higher muscle weight than chicken with low growth rates (Berri *et al*. 2007).

The findings of this study agree with those of several authors, including Molee *et al*. (2018) who reported that this polymorphism was not associated with the carcass traits of Leung Hang Khao chicken at 16 weeks of age. Similarly, Kuadsantia *et al*. (2015) did not also find any significant effect of this polymorphism on carcass traits of the Leung Hang Khao chicken. Amills *et al*. (2003) also concluded that the *NlaIII* C>T SNP was not associated with the carcass traits of Penedesenca chicken strains.

Some studies have also analyzed the effects of other polymorphisms in the *cIGF2* gene on carcass traits. Wang *et al*. (2005) analyzed a C>G polymorphism in exon 2 of *cIGF2* and reported significant associations with some carcass traits. Tang *et al*. (2010) reported a significant association of the C>G polymorphism of *cIGF2* with carcass weight of Beijing You chicken but they did not observe similar associations in the Chinese Silkie chicken.

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In summary, the findings of this study agree with some previously reported observations that the *cIGF2 NlaIII* C>T SNP polymorphism in exon 3 of the *cIGF2* gene is not associated with body weight and carcass traits of chicken.

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6.6 Conclusion

A *cIGF2 NlaIII* T>C SNP was detected in exon 3 of *cIGF2*. It was observed that the BB genotype had the highest frequency among the Forest and Interior Savannah chicken ecotypes while the AB was the predominant genotype in the Coastal Savannah ecotype. The B allele had the highest frequency among all the three chicken ecotypes. At the *cIGF2 NlaIII* T>C SNP locus, the Coastal Savannah chicken ecotype had higher observed heterozygosity values than the expected heterozygosity values. However, in the Forest and Interior Savannah ecotype, the observed heterozygosity was lower than the expected heterozygosity. These findings suggest that at this SNP locus, the Coastal Savannah ecotype is genetically more diverse than the Forest and Interior Savannah chicken ecotypes.

The *cIGF2 NlaIII* T>C SNP locus was not associated with body weights and carcass traits, but it affected some growth rates of the local chicken ecotypes of Ghana.

The results of this study showed that *cIGF2 NlaIII* T>C SNP may not be a good molecular marker for the selection of chicken on body weight and carcass traits in future marker-assisted breeding programmes of local chicken ecotypes in Ghana.

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CHAPTER 7

GENERAL DISCUSSION

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7.0 General discussion

Chicken is one of the most consumed animal sources of protein in Ghana, and it has been estimated that in 2022, the national demand for chicken will be about 460,000 metric tons while the national production (broilers and spent layers) will be about 58,000 metric tons (USDA, 2022). Unfortunately, there is no reliable data on the volumes of chicken meat that is locally produced from local chickens in Ghana. This notwithstanding, there is a huge demand-supply gap of chicken that needs to be filled either from imports or by local production. The high consumption rate of chicken in Ghana is driven mainly by increased population growth, a growing middle class, rapid urbanization, more disposable incomes, and the low cost of chicken compared to fish and red meats. The meat of local chicken is generally preferred by most Ghanaian consumers to frozen imported chicken (Asante-Addo and Weible, 2020; Ragasa *et al.*, 2020). Local chickens are generally hardy, easy to manage and are well adapted to tropical climates. Therefore, local chickens can play a significant role in reducing the demand-supply gap if the barriers to the commercial production of these chickens, i.e., slow growth rates and lack of adequate protection of the local poultry industry by the Ghana government which has led to unfair competition with cheap imported poultry products (Banson, *et al.*, 2015), are addressed.

Growth is a complicated process that is regulated by several neuroendocrine pathways and their genes. The Insulin-like growth factor 1 (cIGF1) hormone and Insulin-like growth factor 2 hormone (cIGF2), which are coded by the *cIGF1* and *cIGF2* genes, respectively, have been implicated in the embryonic and post-hatch growth and development of chicken. As a result, single nucleotide polymorphisms (SNPs) in these genes have been studied in various types of chickens with the objective of exploring their suitability as molecular markers for growth and development of chicken.

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This research sought to examine the growth, carcass, and survivability traits of three chicken ecotypes of Ghana, determine the diversity of some targeted SNPs in the 5' flanking, untranslated and the 5' UTR of the *cIGF1* and some exonic regions of the *cIGF2* and to analyze the association of SNPs in these genes with the growth and carcass traits of the three chicken ecotypes.

The results observed during the appraisal of growth in the three chicken ecotypes showed that the hatch weights of the FO and CS ecotypes were significantly ($p<0.05$) higher than the hatch weight of the IS ecotype, with the highest hatch weight being recorded in the FO ecotype (Chapter 4).

Hatch weight is influenced mainly by egg size, weight loss during incubation and genetic factors (Tullett and Burton, 1982; Hanusova *et al*., 2017; Chimezie *et al*., 2020). Hatch weight is a very important production trait in the poultry industry. It influences the subsequent growth and body weights of chicken to the extent that heavier chicks tend to grow faster and have heavier body weights (Atteh *et al.,* 1994; Sola-Ojo *et al*., 2011; Ng'ambi *et al.,* 2013). However, in this study, even though the IS ecotype recorded the lowest hatch weight, it was heavier than the FO and CS ecotypes from the 4th to the 20th week. The difference between these results and those of Ng'ambi *et al.* (2013), Sola-Ojo *et al*. (2011) and Atteh *et al.* (1994) could have arisen due to several factors, including differences in the genotype of the birds and differences in the management practices.

In this research, there was clear evidence that ecotype influenced the fasting and breast weights of chicken, with the FO ecotype having a higher breast weight than the CS and IS ecotypes. Breast muscle yield and body size are very important traits in the broiler industry today, to the extent that small differences in breast yield can have a huge impact on the bottom lines of poultry production enterprises. This is therefore one of the main reasons why a lot of resources

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are being channelled by large commercial poultry companies towards research into these two important variables. In Ghana, most of the imported chicken comes in as cut-up parts while most of the locally produced chicken are sold in the form of whole carcasses or live birds. The consumption of cut-up chicken parts is on the rise (USDA, 2022) and is likely to become a main feature in the culinary landscape of Ghana in the near future. This development is driven by the fact that these days most families tend to hold full time day jobs and often do not have enough time to butcher whole chickens prior to cooking them. The improvement of carcass traits of local chickens such as drumstick weight, breast muscle yield and carcass weight will therefore be highly desirable as this will align quite well with the shifting demand for processed chicken in Ghana.

Feed conversion efficiency has a direct relationship with the production costs and profitability of poultry enterprises. It is influenced by factors such as basal metabolism, immune status, appetite, protein accretion, levels of production, ambient temperature, and the digestibility of feeds. This study did not find any significant differences in the feed conversion efficiency of the three chicken ecotypes of Ghana. However, the local chicken ecotypes of Ghana appear to be more efficient convertors of feed than local chickens of Ethiopia but are less efficient than Tunisian local chickens reared under intensive management systems (Tadelle *et al.* (2003b); Raach-Moujahed and Haddad (2013).

In Ghana, between 60 and 70% of the production costs of intensively reared chicken emanates from feed costs (Banson, *et al.*, 2015). Unfortunately, in recent times, poultry industries all over the world, and in Ghana in particular, have been facing spiralling high costs of inputs, especially maize, which is also in high demand for human consumption. Consequently, the production costs have gone up considerably, to the extent that some farms have had to either

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scale down their operations or close. It is therefore a desirable option to select birds that are efficient convertors of feed to either meat or eggs.

High mortality rates can undermine the profitability of any livestock production enterprise. Most of the mortalities that were recorded among the three chicken ecotypes in this research occurred during the first week of life of the hatchlings, with the IS ecotype experiencing the highest mortality rate followed by the FO ecotype and the CS ecotype. First week mortality is an important performance criterion in poultry production with several internal and external factors accounting for it. The internal factors include breeder age, as older breeders tend to produce larger eggs which require less incubation time leading to lower risks of dehydration; chick gender due to differences in the rate of development of immune responses between male and female chicks with the male chicks being more susceptible to pathogens, leading to higher first week mortality rates in the males; and breed which is a function of egg size and chick quality (Yerpes, *et al*., 2020). The external factors include the density of chicken in a pen, season, ventilation in pens, feeding, quality of chicks, standards of hygiene and biosecurity, predation, and disease outbreaks with Newcastle disease being one of the major causes of deaths amongst local chickens in Africa (Enahoro *et al*., 2021).

The high mortality rate among the IS ecotype could partly be attributed to the lower hatch weights and its inability to fully acclimatize to the climatic and weather conditions of the coastal agroecological zone where this research was carried out. In view of this, in the development of a fast-growing local chicken type for Ghana, care should be taken to quantify the ecotype-environmental interactions and to fully understand the extent to which these interactions may affect the production levels of the birds in diverse production environments. From this analysis, appropriate strategies can be developed to mitigate these effects if they are substantial and significant.

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Several single nucleotide polymorphisms (SNPs) have been identified in the chicken Insulin-like growth factor 1 gene (*cIGF1*) that have been reported to be associated with growth and carcass traits (Bhattacharya *et al.*, 2015; Sinpru *et al*., 2021; Wang *et al*., 2021).

In this research, the Restriction Fragment Length Polymorphism (RFLP) method was used to identify SNPs in the 5' flanking and regulatory regions of the *cIGF1* gene. The RFLP analysis revealed a *HinfI* C>A SNP in the 5' UTR, a *PstI* T>C SNP in the promoter of the 5' UTR and a *BstBI* T>C SNP in the 3'UTR of the gene.

The diversity of the *cIGF1* gene at the *HinfI* C>A, *PstI* T>C and *BstBI* T>C SNP loci was investigated. This entailed an estimation of allele and genotype frequencies, observed heterozygosity, expected heterozygosity, and molecular variance among the three chicken populations. Genetic diversity is very important for the survival and fitness of animal species as it enables living organisms to resist diseases, pests, changes in the climate and other stresses. Therefore, it gives flexibility and allows populations to adapt quickly to changing environmental conditions. Reduced genetic diversity or increased homozygosity often results in higher frequencies of deleterious alleles, leading to recessive disorders in animals.

The results of this research showed that at the *HinfI* C>A locus, the AC heterozygote was the predominant genotype amongst the Forest and Coastal Savannah ecotypes, while the CC genotype was predominant in the Interior Savannah ecotype. The C allele had the highest frequency amongst all the three chicken ecotypes. The A allele has been reported to be associated with high growth rates (Zhou *et al*., 2005) and so higher frequencies of the C allele at this locus in the three chicken ecotype populations of Ghana could be because these chickens may not have been selected for growth. High observed heterozygosity values were also found in the FO and CS ecotype populations, which suggests some degree of uncontrolled mating in

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the FO and CS populations or substantial levels of gene flow. This SNP was not significantly associated with body weight, weight gain and carcass traits (Chapter 5).

At the *PstI* T>C locus, the CT heterozygote was the predominant genotype among the three Ghanaian chicken ecotypes, with the T allele having the highest frequency (Chapter 5). The observed heterozygosity amongst the FO and CS chicken ecotypes was higher than the expected heterozygosity thus suggesting a higher degree of diversity at this SNP locus within the FO and CS chicken ecotypes than within the IS ecotype. *PstI* T>C was associated with body weight at hatch and at 16 and 22 weeks of age but there was no evidence of its association with the carcass traits that were investigated.

With regards to the *BstBI* T>C locus, CT was dominant genotype amongst the three chicken ecotypes while the C allele had the highest frequency. The diversity of this polymorphism was higher in the FO and CS ecotypes than in the IS ecotype. This SNP influenced body weight at hatch, 2, and 10 weeks of age but it had no effect on the carcass traits studied (Chapter 5).

Based on the expected heterozygosity values of the three chicken ecotypes across the three SNP loci, the FO ecotype appears to be genetically more diverse than the CS and IS ecotypes, while the CS ecotype also appears to be genetically more diverse than IS ecotype. This seems to suggest a higher degree of random mating and gene flow into and between the FO and CS ecotypes. The FO ecotype could therefore contribute significantly to the development of a fast-growing local chicken breed in Ghana.

While the *HinfI* C>A SNP had no effect on body weight, growth rate and carcass traits, *PstI* T>C influenced body weight and growth rate, but it had no effect on the carcass traits studied. *BstBI* T>C also influenced body weight and weight gained but it was not associated with any

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of the carcass traits studied. In the development of a fast-growing local chicken breed in Ghana, *BstBI* T>C could be a useful molecular marker for growth traits.

In the *cIGF2* gene, a Restriction Fragment Length Polymorphism digestion with *NlaIII* restriction enzyme revealed a T>C SNP in exon 3 of the gene (Chapter 6).

At this SNP locus, the BB genotype had the highest frequency among the FO and IS chicken ecotypes while in the CS ecotype, the AB heterozygote was the dominant genotype. The B allele had the highest frequency in all the three chicken ecotypes. The amongst the CS population, the observed heterozygosity values were higher than the expected heterozygosity values while in the FO and IS ecotypes the observed heterozygosity values were lower than the expected heterozygosity values. The allele frequencies in all the ecotypes were in HWE. This seems to suggest that at *NlaIII* T>C SNP locus of the *cIGF2* gene, the FO and IS ecotypes are genetically similar but less diverse that the CS ecotype. These differences could be a result of different rates of gene flow in these agroecological zones.

The *cIGF2 NlaIII* C>T SNP did not have any significant influence on body weight or any of the carcass traits that were studied.

The results of this study suggest that all the analysed SNPs i.e., the *cIGF1 HinfI* C>A, *cIGF1 PstI* T>C, *cIGF1 BstBI* T>C and *cIGF2 NlaIII* T>C may not be ideal molecular makers for carcass improvement amongst the three chicken ecotypes. However, *cIGF1 PstI* T>C and *cIGF1 BstBI* T>C could be potential molecular marker for the improvement of body weights in these chicken ecotypes in future marker-assisted breeding programmes.

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CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

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8.1 Conclusions

This is the first study to report on SNPs in the *IGF1* and *IGF2* genes in the local chicken ecotypes of Ghana.

The Interior Savannah and Forest chicken ecotypes were heavier and grew at a faster rate than the Coastal Savannah ecotype. However, the Forest ecotype had a higher breast weight and breast yield than the Interior Savannah and Coastal chicken ecotypes. The Coastal Savannah ecotype had the highest survival rate under intensive management conditions while the Interior Savannah ecotype had the lowest survival rate. All the three chicken ecotypes could therefore be used jointly through selective breeding to develop a faster growing, heavier local type of chicken that also has a high breast yield and survival rate.

The preponderance of the C allele of the *HinfI* C>A locus in the three chicken ecotype populations of Ghana indicates that they may not have been subjected to any selection for growth.

Based on the heterozygosity values at the *HinfI* C>A, *PstI* C>T, *BstBI* C>T and *NlaIII* T>C loci in the *cIGF1* and *cIGF2* genes, the Forest and Coastal Savannah ecotypes seem to be genetically more diverse than the Interior Savannah chicken ecotype.

The *HinfI* C>A locus of *IGF1* was not associated with body weight, growth, and carcass traits of local chicken ecotypes of Ghana. As a result, it may not be a good molecular marker for use in a marker-assisted selection that seeks to improve these traits in local chicken ecotypes of Ghana.

The *PstI* C>T locus of *IGF1* had an influence on the growth traits of chicken, but it did not influence some carcass traits of local chicken ecotypes of Ghana. This SNP could be a good

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molecular marker of body weight in future marker-assisted genetic improvement efforts of the local chicken of Ghana, but it may not be a suitable molecular marker for carcass traits.

The *BstBI* C>T was associated with the growth traits of local chicken ecotypes of Ghana, but it did not have any effect on their carcass traits. This SNP may therefore not a good molecular marker for carcass traits, but it could be a potential molecular marker for use in marker-assisted selection for the improvement of the growth traits of local chicken ecotypes in Ghana.

The *cIGF2 NlaIII* T>C SNP locus did not influence body weight or any of the carcass traits of the local chicken ecotypes of Ghana. It may therefore not be a good molecular marker for the selection of chicken on body weight and carcass traits in future marker-assisted improvement programs for these chicken ecotypes.

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8.2 Recommendations

On account of the body weight, growth rate, breast muscle yield and survivability variables, it is recommended that the Forest, Coastal Savannah and Interior Savannah ecotypes be used to develop a fast-growing, heavy chicken type that will maintain a high adaptability to our harsh environmental conditions and have an ability to thrive under conditions of minimal feed and health care. However, in doing so, care should be taken to ensure that the high post-hatch mortality associated with the Interior Savannah ecotype under intensive management and different agroecological conditions is taken care of. This can be done by doing a thorough assessment of the genotype-environmental interactions to fully understand the extent to which these interactions may affect the production levels of the birds under diverse production environments.

It is recommended this research be replicated at on-farm levels to explore further the differences in body weight and carcass traits among the three local chicken ecotypes.

Given the relatively small sample size of this research, it is recommended that further research be conducted to validate the potential use of *PstI* C>T and *BstBI* C>T as molecular markers for growth traits selection in local chickens of Ghana using larger sample sizes.

The observations from this study underscored the high diversity of IGF1 and IGF2 genes in local chicken ecotypes of Ghana and the possibility to improve their growth traits using within breed selection. It is therefore recommended that steps are taken to improve the body weight of the local chicken ecotypes of Ghana through within breed selection, a process that can also conserve local chicken genetic resources so that their desirable adaptive traits may not be lost through indiscriminate crossbreeding. In line with this, the Government of Ghana should provide the needed resources for the genetic improvement of these chickens. A fast-growing local chicken type will spur a high uptake of commercial production of local chicken ecotypes

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which will in turn create many jobs along the poultry value chain and save the country huge foreign exchange that will otherwise go into the importation of frozen chicken.

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APPENDICES

Appendix 1. Vaccination schedule

Type	Age at vaccine administration	Mode of administration
Mareks vaccine	Day old	In water
Gumboro vaccine	week 1	In water
Newcastle vaccine (1st)	week 3	In water
Gumboro vaccine	weeks 4	In water
Newcastle vaccine (2nd)	weeks 6	In water
Fowl Pox vaccine (1st)	week 7	Injected
Fowl Pox vaccine (2nd)	week 11	Injected
Newcastle vaccine (3rd)	week 15	In water

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Appendix 2. The breeder unit of local chicken ecotypes at LIPREC, University of Ghana

INTEGRI PROCEDAMUS

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Appendix 3. Chick brooding and rearing facility at LIPREC, University of Ghana.

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Appendix 4. Local chicken ecotypes in the brooder unit.

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Appendix 5. Experimental chickens in a pen.

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Appendix 6. Consensus Sequences of *cIGF1* gene with SNPs obtained from local chicken ecotypes of Ghana.

Target Region	Sequence
Promoter Region	ACTCTATCTGCTCTGATTTTAGCAGTGACAGTGAGATTTAG CAAACAGAAGAGGGATTTAGAGAAAATCCTCACATTTATC TACATTACACAGACACTGTAGACAGGAAACAGCTGGGGGA GCATTTGCCTTCTCTCTCTCTCCCTCTTCTGGCAAAGTTACC GAGTAAGGACTTTTTTGGGCATGGTGACAAATAACATCATA CCTTTGCATTTTAAAACTAGAGCACAGAAGCATATTTTTTCC CCTTTAAAAGAATGTGAATTAGTGACTGAGGGGTTAGCAGG CAAAAAAGCTTACGCTGCCACGGAAAATAAGGGAATGTATT CTGGTTAACTTTCGGGTGGCTGTGTGTATATTTGCATTTTTGT GTGTATGTGTCTGCTTTTCAAATAGACAAAAACTTCCATAGG TGAAGACATTGTCTGTATACCTTTATATTCCTGTGTACATCTG TGCACATTTCATTCATGCAGAGACACAGGTATTTTATTTATTT CATTTTTTTTTTTAACCTAGAGAGACAGGCAGGCAGTTTACTT TGTTTTAAATGCATCTTTACGTTATTAAACTTGGCACGCCTAC ATGCTGACTAACAATTAAAACCTCTGACTCTCTGTGCTATTAA CCCTAAAATAGCAGTTTGTAATTTGCTAAAAGTAAAAGAGTTG TTGAGCACTGCTTGTAAATAGAGCAAAACAGCGCTGCGATCCT TTAGCAACCACACAGAAGTCATGCAATTTCCTAGAATTTACAG TACTATGAATGTAGTAACTCAAGTAGCCCAGCTTGAAGG
5'UTR	TCTATATATCCCTAGGATATAGCAACTATTTCTGCAACTGTAG TCCTAAGAAGTATTGGCTTGTCAGATGACCAAATCACATTTTC TTTTCTTCTTTCCAAATAATTTCTAAAATAAATAGTCCCTTGGA CTGTAGCAGAATTAGGCCCTGCATTTAGAATACTAGGTGCAGA TCTACCCTAACTCATCCATGGATTCTATAGTTCTGAACAATAAC TGTTTATTAAGTGCAATGTATTTTTCACCTTTTATTTTCATCAGC TTTTTTATTTTCATAGCATATGTTTTTAAGTTTTCCAAACAGTGA TGGTAGCTTAGGTCATTAAGACTGTTTTTACAGTTTCAGATTTTG ATGCATCCTGTATTTGAATGCATTGAAACAAATAAAACGTGTCTT ACATTTCTAATAGATACTAAATACATCTGTGCTCAACCGTGCTGC TCATATTA
3ÚTR	AAAAAAATTCAGATATTTTGCTTTTTTTTCTTTTAGCTGTTYGA ATGATGGTGTTTTCCCCTAGTTACATGAAACACAGATTTATAG ATGACTAGGTTCAATTAATAAAARGCAATTAATAAAAGAGTT- TTCAAATAACTTCAAAAGGTAGCACATTGAGGCCTAAATCTTTC TGAAATACCAGAATTCTATTCAAATATAACTGAGGGTTAATCAG GTCCTCTGCCATTTTAACTCTTCCCAAAATCTGATGTGACTGAAT TGACTGTTAATCAGCCCACTGCATCTCCCTGTAAATCAGAAAAG CTAAGCTAATCACCAAATTACAGTGTAGAAAGTCTTCAGGAAGAG ATAAACCACAATGTCCTTCAAACTGCTACTTAAAACAGCTTTTCAT ATACTCTTGTAGGAGACATTTAATGTATTTCCAGGTAATATCTGAC ATATAAACTTGATATGTAATGCATTGATTCTAAGATTAATAACACT TTCTTCTGTTTCATTTTTCTAAATCCA

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Appendix 7. Consensus Sequences of *cIGF2* gene with SNPs obtained from local chicken ecotypes of Ghana.

Target Region	Sequence
Part Exon 2, Intron 2, part Exon 3	TCAGAGCGTGACCTCTCCGCCACCTCCCTCGCGGGCCTCCC AGCCCTCAACAAGGTAGGGCTGCACTCGGGCTGCTAGCTCC CTGAAGCAAGAAAGAGGAGAAAAGGGAAACCATTGGTGGG GGAGGACTGCTGCATGCTCTATGATCTGCTGCTTCTGCAGCC AAGGCAGCATGCAGAGCCTTGAAGTGTTTATAGCTGTTGAGG AGTCAAAGAAATTGCAGAAAACAAACTTCTAACTGTTGAATC ATAGAACAATTTGGGTTGGAAGAGCTCCCTAGTGATTATCTTG TCCATCCCTCCACCATGGGCAGGGACACATCCCACTCCTATGT CATGTTGCCCAAAGGCCCATCCAGCCTGGTCTTGAATGCCTCC AGAGAGCGGGCACCCACAGCTCCTCTGGACAGCCTGTCCCAG CATCTTGCCACTTTGTTGTGGAAAACTTCCTCCTTATGTGCAGT TTCCTTCAGCTTAAAACCATTGCCCCTTGGCCTGTCCCTACAGT TTTGATAAAAGGTTTTACTCCAGCTGTAGTATATACTGCAAAG CTGCAGCAAGGTCTCCCTGGAACCTTTTCCACGCTCAGTGACC CCAACTCTCCCTGCCTTTCCTCATAGAAGAGGTGTTCCAGCTTG CTAATAATTCTTGTGGCCTTTCTCTGTCCACGTCCTCCTTGTGCT GGGGACCCAATAGAACCTGTAAAATGGGCGAGCAGCAATGAG TAGAGGAAGCCCCAGGGCAGTGTCAGTAACCCACCTTGTGTTG ACACTGTGTTGTTCTCCCTTCCCCAG