GENETIC DIFFERENCES IN RESISTANCE TO NEWCASTLE DISEASE VIRUS IN THREE LOCAL CHICKEN ECOTYPES OF GHANA

BY

GODWIN KWAKU AGBENYEGAH

(10085316)

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DEDICATION

I dedicate this work to God Almighty for His Mercy and Grace which has brought me this far.
DECLARATION

I hereby declare that this thesis which is submitted to the Department of Animal Science, College of Basic and Applied Sciences, University of Ghana, for the award of Master of Philosophy in Animal Science degree is the result of my own investigation. This thesis has not been submitted or presented for another degree elsewhere, either in part or in whole, except for other people’s work which was duly cited and acknowledged.

....................................

Godwin Kwaku Agbenyegah
(STUDENT)

This work has been submitted for examination with our approval as supervisors:

....................................

Prof. B.B. Kayang
(PRINCIPAL SUPERVISOR)

..................................

Prof. Augustine Naazie
(CO-SUPERVISOR)
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# TABLE OF CONTENTS

**DEDICATION**............................................................................................................................ i

**DECLARATION**.......................................................................................................................... ii

**ACKNOWLEDGEMENT** ................................................................................................................ iii

**LIST OF TABLES** ........................................................................................................................ vi

**LIST OF FIGURES** ....................................................................................................................... vii

**ABSTRACT** ........................................................................................................................ viii

**CHAPTER ONE** ........................................................................................................................ 9

1.0. INTRODUCTION .............................................................................................................. 9

**CHAPTER TWO** ..................................................................................................................... 14

2.0 LITERATURE REVIEW ........................................................................................................ 14

2.1 Importance of local chickens .............................................................................................. 14

2.2 Challenges facing local chicken production in Ghana ....................................................... 15

2.3 Newcastle Disease ................................................................................................................. 16

2.3.1 Newcastle Disease Virus Nomenclature ............................................................................ 17

2.3.3 The ND viral genome ........................................................................................................ 20

2.4 Interventions to combat Newcastle disease .......................................................................... 20

2.4.1 Vaccines and vaccination regimes .................................................................................. 21

2.5 Factors affecting NDV antibody titre in local chicken ......................................................... 22

2.5.1 Maternal antibody ................................................................................................................. 22

2.5.2 Vaccination ......................................................................................................................... 23

2.5.3 Season of year ..................................................................................................................... 24

2.5.4 Other infections .................................................................................................................. 24

2.5.5 Genetic factors .................................................................................................................... 25

2.6 Mechanism of disease resistance ......................................................................................... 28

2.7 The major histocompatibility complex .................................................................................. 30

2.8 Genetics of disease resistance .............................................................................................. 33

2.9 Correlation between disease resistance and growth ......................................................... 34

2.11 Growth rate of local chicken ............................................................................................... 37

**3.0 MATERIALS AND METHODS** ..................................................................................... 38

3.1 Sampling area and breeding stock constitution................................................................. 38

3.2 NDV animal challenge ........................................................................................................ 39

3.3 Quantification of NDV antibody with ELISA ...................................................................... 40

3.4 Calculations .......................................................................................................................... 41

3.5 Validity criteria ..................................................................................................................... 41
LIST OF TABLES

Table 1: Least squares mean of growth rate before pre-infection ..................45

Table 2: Least squares mean estimates for post-infection growth rate ..........47

Table 3: Least squares means (IU) for average antibody titres ..................48

Table 4: Heritability estimate for growth rate and antibody titre ..................49

Table 5: Genetic correlation between growth rate (g/day) and antibody titre (IU) .......50

Table 6: Phenotypic correlation between growth rate and antibody titre ........50

Table 7: Summary of distribution of body weights .................................74
LIST OF FIGURES

Figure 1: Schematic representation of the viron structure of NDV (Source: Viral zone, Swiss Institute of Bioinformatics, 2014) ................................................................. 19

Figure 2: Negative-stranded RNA linear genome, about 15 kb in size. Encodes for seven proteins (Source: Viral zone, Swiss Institute of Bioinformatics, 2014) ....................... 20

Figure 3: Growth curve for Interior savannah, Forest and Coastal savannah ecotypes showing point of NDV challenge ....................................................................................... 46

Figure 4: Distribution of S/P ratio at ten days- post-infection ........................................ 74

Figure 5: Distribution of normalised S/P ratio ............................................................. 75

Figure 6: Normalised Average titre ............................................................................. 76

Figure 7: Log10 titres for ten days-post infection ....................................................... 77
ABSTRACT

The potential benefits of local chickens to the rural economy is compromised annually by regular outbreaks of Newcastle Disease (ND). Every year over 80% of the local chicken population is lost to ND, depriving the rural people of the much needed protein and income from chicken. In an effort to reduce the effect of Newcastle Disease on local chicken production, this study was conducted to determine if there is variation in the genetic resistance to NDV in three local chicken ecotypes of Ghana. Local chicken sampled from the Forest, costal savanna and Interior savanna eco-zones were bred and their offspring challenged with the La Sota strain of the Newcastle Disease Virus (NDV) at four weeks old. Antibody titre levels were determined using ELISA before infection and ten days post-infection. Chickens from the Forest ecozone were found to have significantly higher antibody titres (p<0.05) against the La Sota strain of the virus. Chickens from the Interior Savannah ecozone had significantly higher body weight and growth rate than the other ecotypes. Heritability for growth rate (before and after challenge) and antibody titres (Log_{10} titre and average titre) were found to be 0.9322 ± 0.1448, 0.8909 ±0.1437 and 0.2096 ± 0.0850 0.2635 ± 0.0960, respectively. Genetic correlation between both traits was 0.37. From the study, local chicken ecotypes showed genetic differences in immune response to NDV. This study provides evidence substantial enough for selection for improved antibody production against NDV and higher growth rates among local chicken ecotypes. Thus, selection for improved growth rate will lead to a correlated in response ND resistance.
CHAPTER ONE

1.0. INTRODUCTION

Rural poultry remains an important element of the socio-economic and cultural aspect of people living in rural areas (Alders and Spradbrow, 2001; Smith, 2013). Local chickens, being a major component of rural poultry, contribute to nutrition by providing the much needed protein and minerals in the form of egg and meat. Local chicken is sold to generate income for women and vulnerable groups, providing about 30% of rural household incomes (Adeyemo et al., 2012). Culturally rural poultry is used for traditional sacrifices, fines and dowries. They are also involved in pest control as they feed on pests such as termites, ants, geckos, etc., thereby controlling the population of such pests (Blakie, 2014).

Diseases, most especially Newcastle disease, have been the major threat to rural poultry depriving the rural economy of the much needed economic benefits (FAO, 2009). For many decades Newcastle Disease (ND) has remained the major threat to local poultry production (Copland, 2005). The disease which is of viral origin occurs yearly in many countries across the world with a mortality rate of 80% (Alders and Spradbrow, 2001).

Newcastle disease is caused by virulent strains of avian paramyxovirus type 1 (APMV-1) of the genus Avulavirus which belongs to the family Paramyxoviridae (OIE, 2014). NDV infects over 200 species of birds, but the severity of disease produced depends largely on the host and strain of virus.

Several efforts have been made to control the disease. The most successful method which is vaccination is faced with challenges such as availability of the vaccine,
accessibility and the maintenance of the cold chain in rural areas (Alders et al., 2003).

Reports since the 1940s have indicated the existence of genetic variations in susceptibility of chicken to the Newcastle Disease Virus (Hutt and Cole, 1947). Certain variants of chickens such as the Egyptian Fayomi have been observed to be more resistant to NDV (Lamont, 1998). Certain ecotypes of the Nigerian local chicken have also been observed to show genetic variations in susceptibility to the NDV (Adeyemo et al., 2012). Despite these observations, reasons to explain the variations were not available.

Recent studies using advanced genomics have attributed the variations in resistance or susceptibility to diseases to variations in the major histocompatibility complex (MHC) of the chicken (Zakarias et al., 2002). The underlying factor is a single nucleotide polymorphism (SNP) associated with the MHC. It is believed that the SNP influences the expression of genes associated with the MHC (Zhang et al., 2015). SNPs are basically variations in gene sequence at single nucleotide positions, i.e. changes in a single base pair of a particular gene that tends to influence the amino acid that gene codes for. SNPs are responsible for the different forms a particular trait and also continuous variations in a particular trait. Specifically, a number of SNPs have been associated with resistance or susceptibility to NDV (Luo et al., 2013). Local or indigenous chicken are a pool of genes that have not been artificially selected for any purpose. They have survived the harsh environmental conditions and disease burdens over several generations and even though they are mostly small in size they are more efficient than their commercial counterparts (Gueye, 2000). Msofe et al. (2001) suggested that rural chicken ecotypes which have evolved and adapted to stressful environments are likely to carry valuable genes and gene
combinations controlling specific behavioural, physiological and disease as well as parasite resistance traits. Indeed, variations in immunological responsiveness have been reported among various chicken ecotypes challenged with a strain of NDV (Gwakisa et al., 1994; Adeyemo et al., 2012). Since it is possible to map genes responsible for disease resistance, such genes could be identified among rural chickens. One significant application of this would be selective breeding for a chicken type which would be resistant to important diseases such as ND and has high productivity potential under village management or low input systems.

1.1 Problem statement
Local chicken populations play an important role in the rural economy (Gueye, 2000; Blackie, 2014). However, ND is devastating, killing over 80% of the local chicken population every year (Gwakisa et al., 1994). Although vaccines are available and effective in controlling the disease, ND vaccination programmes have only been successful with commercial producers and have largely failed among smallholder farmers in Africa due to inadequate agricultural extension services and lack of cold chain facilities to maintain the vaccine in a viable state. Even in situations where thermostable vaccines are available, unreliable production and distribution have restricted their use. These constraints have led to the need for harnessing the innate potential of resistance to NDV as an alternative control strategy. Considering the important value of local chicken, harnessing the innate potential of resistance to ND would be crucial to ensuring the efficient control of the disease.
1.2 Justification

Although the virus is currently controlled effectively by vaccination and mass slaughtering, it remains a potential threat to commercial or backyard production, as is proven by the regular outbreaks (Westbury, 2001).

Development of local breeds of chicken that are resistant to NDV would be vital to the realisation of the full socio-economic potential of the local chicken. However, information on the various Ghanaian chicken ecotypes with regard to disease resistance is not available thereby making it difficult if not impossible to select and breed chickens that can survive ND outbreaks. An assessment of the disease resistance potential of the Ghanaian local chicken is a necessary first step to provide important information that could be utilised to develop a resistant strain of chicken to enhance the contribution of local chicken to the rural economy.

1.3 Hypothesis

It is hypothesised that:

1. There are genetic variations in resistance to ND among Ghanaian local chicken ecotypes.

2. There are genetic variations in growth rate among Ghanaian local chicken ecotypes.
1.4 Objectives

The overall aim of this project was to determine the genetic variations in resistance to NDV among Ghanaian local chicken ecotypes. The specific aims are:

1. To determine variations in resistance to NDV between and within three local chicken ecotypes of Ghana.
2. To examine the growth rate among the three chicken ecotype of Ghana.
3. To determine the heritability of resistance to ND in the three local chicken ecotypes of Ghana.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of local chickens

Indigenous chickens comprise about 80% of the national flocks in Africa and Asia (Dana et al., 2011). Despite their low growth rates and egg production, indigenous chickens are generally better in disease resistance and could maintain higher level of performance under poor nutrition and high environmental temperatures compared to commercial strains under village systems (Horst, 1989).

Local chickens are the most widely produced chicken in Ghana, forming 80% of the total national poultry population (MoFA, 2007; FAO, 2014) and are well adapted to the local environmental conditions (Gyening, 2006). Their production system is classified as the village or backyard production system and is characterised as “low input low output” (Blackie, 2014). This system of production is most prevalent in Ghana where local chickens play a very important role in the livelihoods of farmers. Local chickens mainly scavenge for their feed, however in some areas, supplementary feed including maize, agro-by products and insects such as termites, are provided (FAO, 2014). While scavenging, they also feed on worms and other organisms that are considered as pests thereby controlling their population (Blackie, 2014).

Local chickens play a significant role in the rural economy by providing income, food in the form of meat and eggs and thereby contributing to enhanced food security. Women who keep rural poultry are able to pay school fees and buy school uniforms for their children thereby serving as a means of sustaining and improving livelihoods (Msoffe et al., 2002; Aboeet al., 2006; Karbo et al., 2003). Local chickens are also of high cultural
value as they are used for sacrifices, gifts or dowries in some traditional settings. Further, local chickens have been observed to possess important economic traits such as disease resistance and good carcass qualities (Aboe et al., 2003). They therefore serve as a local pool of genetic resources providing genetic diversity since they have not been selectively bred for any specific purposes (Osei-Amponsah et al., 2012). Indeed, studies have shown that local chickens have varying degrees of resistance to important poultry diseases (Adeyemo et al., 2012).

2.2 Challenges facing local chicken production in Ghana

In spite of the importance of local chickens to the rural economy of Ghana, their production is beset with a number of challenges. These include poor husbandry practices such as poor feeding, poor housing, diseases, low growth rate and small body size (Kperegbeyi et al., 2009). Since the local chicken production system is a low input low output system (FAO, 2009), little or no attention is paid to the welfare of the chickens (Aboe et al., 2006). This deprives the rural economy of the full benefits of rural poultry production (Padhi, 2016). In most rural areas there is little or no supplementary feeding for local chicken (Onyimba et al., 2014) and this limits their growth as they cannot meet their nutritional requirements (Padhi, 2016). Further, in many cases, the inappropriate or inadequate housing facilities leaves the chickens vulnerable to theft and predators such as snakes, hawks, and wild cats and dogs (Kusina et al., 1998). When left to roost under trees, they are also exposed to severe weather conditions such as rainstorms and extreme temperature conditions which could be fatal.
Diseases remain the most important challenge to rural chicken production resulting in 80-100% mortality in some cases (Padhi, 2016). As chickens scavenge for feed they may be exposed to disease causing organisms. The major diseases that affect poultry production in Ghana include Newcastle disease, infectious bursal disease, fowl pox, coccidiosis and bacterial diseases such as *E. coli* infection (Awuni, 2002). Among these diseases, Newcastle disease ranks number one, causing up to 80% mortality annually (MoFA, 2007; FAO, 2014).

2.3 Newcastle Disease

Newcastle disease is a highly contagious viral disease that attacks many species of domestic and wild birds. The earliest outbreaks were reported in poultry in Java, Indonesia and later Newcastle-Upon-Tyne, Britain in 1926 (Doyle, 1927), hence the name Newcastle Disease. Currently the disease is distributed worldwide (Alexander, 1997). A total of 27 out of the 50 orders of birds, are affected by the NDV (Kaleta and Baldauf, 1988). This infectious virus may be transmitted by ingestion or inhalation, thus forming the basis for the route used in mass NDV vaccination procedures for poultry (Alexander, 1997). Isolates of NDV may be categorised into three main pathotypes depending on the severity of disease in chickens produced by the isolate (Ababneh *et al.*, 2012). According to Cary *et al.* (2011) the lentogenic isolates do not usually cause disease in adult birds and are considered mildly virulent. Viruses of intermediate virulence that causes respiratory disease are termed mesogenic, while virulent viruses that cause high mortality are termed velogenic. Neurotropic (characterised by nervous and respiratory
symptoms) and viscerotropic (characterised by enteric symptoms) forms of velogenic
viruses have been reported worldwide (OIE, 2014).

ND is reported as the most important viral disease of poultry in the world including
developing countries (Spradbrow, 2009). In developing countries, ND is a major
constraint to the development of both industrial and village poultry production (Alders et
al., 2001). NDV infections of poultry range from latent to rapidly fatal depending on the
pathotype of virus involved (Alexander, 2000). The transmission of NDV occurs through
newly introduced infected birds, selling or giving away sick birds, exposure to faecal
matter and other excretions from infected birds. It can also be transmitted through contact
with contaminated feed, water, equipment and clothing (Adeyemo et al., 2012). The
disease causes high economic losses due to high mortality, morbidity, stress, decreased
egg production and poor hatchability (Alexander, 2000).

2.3.1 Newcastle Disease Virus Nomenclature

The NDV belongs to the virus order Mononegavirales, (which comprises single stranded,
non-segmented, negative-sense RNA viruses showing helical capsid symmetry), and the
family Paramyxoviridae(Alexander, 2000). The family Paramyxoviridae has two
subfamilies namely Paramyxovirinae and Pneumovirinae(Lamb et al., 2005). NDV
belongs to the subfamily Paramyxovirinae and the genus Avulavirus. The International
Committee on the Taxonomy of Viruses rearranged the order of the Paramyxovirus genus
in 1993 and placed NDV within the Rubulavirus genus among the Paramyxovirinae(Rima
et al., 1995). Subsequently, since the virus does not have the small hydrophobic gene
(Lamb and Kolakofsky, 1996) which occurs in all of the other rubulaviruses, Peeters et al.,(2000) proposed that it be considered as a separate member of the Paramyxovirinae.

Previously, nine serogroups of avian paramyxoviruses namely Avian Paramyxovirus types 1 to 9 (APMV-1 to APMV-9) were recognised (Alexander, 1997). A tenth serotype, APMV-10 has now been recognised. Newcastle disease is caused by virulent strains of APMV-1 (OIE, 2014), which remains the most important pathogen for poultry, but APMV-2, APMV-3, APMV-6, and APMV-7 are known to also cause disease in poultry (Peeters et al., 2000).

2.3.2 Structural and biological characterization of Newcastle disease virus

The structural and biological characterization of Newcastle disease virus has seen a lot of advancement since the 1970s (Yusoff and Tan, 2001). The complete sequence of the viral genome has been determined and clearer understanding of the viral proteins and their respective roles in the life cycle has also been achieved (Yusoff et al., 1987).

The viron is enveloped with a lipid bilayer membrane derived from the host cell membrane. The enveloped virus has a negative sense single-stranded genome of approximately 15 kb which codes for six proteins. These include an RNA directed RNA polymerase (L), hemagglutinin-neuraminidase (HN) protein, fusion (F) protein, matrix (M) protein, phosphoprotein (P) and nucleoprotein (N). HN and F proteins are glycoproteins that are embedded in the wrapper and appear as tiny spines sticking out from the outer surface of the membrane when viewed under an electron microscope (Yusoff and Tan, 2001). These comparatively complex proteins interact with one another.
and are involved in viral infectivity and virulence. Under this lipid membrane is a layer of comparatively hydrophobic non-glycosylated matrix (M) protein, which is not only linked with the membrane but also with the N-terminal segment of the HN protein positioned in its inner surface. The M protein is assumed to interact with the nucleocapsid (NP) that resembles the classical herringbone morphology that can be clearly seen when the viral membrane is removed or disrupted (Figure 1). This structure consists of many NP subunits that are associated strongly with several copies of phosphoprotein (P) and large protein (L). The non-segmented, single-stranded negative-sense RNA genome 15 186 bases.

Figure 1: Schematic representation of the viron structure of NDV (Source: Viral zone, Swiss Institute of Bioinformatics, 2014)
2.3.3 The ND viral genome

The RNA genome (Figure 2) is made up of six major genes that encode the structural proteins in the order 3’-NP-P-M-F-HN-L-5’ as well as two non-structural proteins, W and V. These are non-structural proteins known to produce differential initiation or transcriptional editing of the P gene mRNA (Yusoff and Tan, 2001). Studies from the RNA sequencing revealed that the 3’ and 5’ ends of the genomic RNA respectively contain a leader sequence and a trailer sequence of about 50 nucleotides (Kurilla et al., 1985). Transcription of the leader sequence results in the fabrication of the leader transcript, which is the smallest but commonest mRNA (Peeples, 1988). Phillips et al. (1998) indicated that the trailer region of Beaudette C has 114 nucleotides. The 5’ terminus of the trailer sequence shares a high degree of complementarity with that of the 3’ terminal leader sequence (Peeters et al., 2000).

![Figure 2: Negative-stranded RNA linear genome, about 15 kb in size. Encodes for seven proteins (Source: Viral zone, Swiss Institute of Bioinformatics, 2014)](image)

2.4 Interventions to combat Newcastle disease

In Ghana, various interventions have been developed to mitigate the effect of Newcastle disease on rural or village chicken production. These include the use of husbandry or
management practices that can reduce the exposure of the chickens to the disease but this has been ineffective mostly because of the production system used by rural dwellers (Conan et al., 2012). The use of vaccines which has proven to reduce the incidence of outbreak of Newcastle disease is fraught with challenges such as the maintenance of the cold chain to keep the vaccine viable before it reaches the rural areas (Awuni, 2002). Unreliable electricity supply and the absence of storage facilities for preserving the vaccine till it is ready to be administered also pose a challenge to the use of vaccination. This has led to the advancement of ‘I-2’, a thermostable ND vaccine which can be stored at room temperature for a considerable period of time. The I-2 vaccine can also be easily administered by the farmer without any complications (FAO, 2014).

2.4.1 Vaccines and vaccination regimes

With the intensification of large scale commercial production, there has been an increase in the incidence of poultry diseases and the severity with which they affect flocks (Alders and Spradbrow, 2001). The most devastating diseases are those of viral origin, because they have no direct cure and cause high mortality. Viral diseases such as Newcastle Disease, Infectious Bursal Disease, Fowl Pox and Marek’s Disease can be prevented by vaccination (FAO, 2004).

Vaccines produced by weakening these viruses have been very successful in reducing the incidence of these diseases. In spite of this losses still occur, and there is evidence of the occurrence, if not the evolution, of highly pathogenic variants of numerous viruses, which are able to overcome previously effective vaccines (Alexander, 2000). While improved vaccines have been produced, there are limits to the extent to which attenuation
can be reduced before the vaccine itself becomes ineffective (Bumstead, 1998), and other means of controlling infection or enhancing the effect of vaccines can only be helpful. Differences have been noted in the vulnerability of chickens to many of the principal viral pathogens of poultry, and resistance to Marek's disease virus and Avian leukosis virus. In developing countries however, there have been challenges with the production, storage and distribution of vaccines, a situation which makes the vaccine harmful before it reaches the farmer (Copland, 2005). An alternate means of controlling chicken diseases is the production of breeds or strains that are resistant to the pathogens (virus).

According to Bishop and Mackenzie (2003), the use of vaccines in viral disease management and vaccination programmes for domesticated animals is faced with constant challenges since every new generation of vaccination protocols results in the discovery of new more virulent viral strains, as has been observed in the case with Marek’s disease in poultry.

2.5 Factors affecting NDV antibody titre in local chicken

Some of the factors that have been identified to affect antibody titres in chickens include maternal antibody, vaccination, season of the year, genetic factors and other infections.

2.5.1 Maternal antibody

Maternal antibody is sustained in the chick for about the first 14 days of the chick’s life and begins to recede or decay beyond this period (Saad and Kamel, 2013). Studies have
shown that the maternal antibody titres are not correlated to the immune response of the chick later in life (King et al., 2010).

2.5.2 Vaccination

Vaccination is meant to offer protection to an animal by triggering the production of antibodies to specific antigens. This is done by introducing live attenuated disease causing agents or parts of viruses to induce specific immune response (Alders and Spradbrow, 2001).

The causative virus depends on the mode of transmission to chickens. In the 1930s, it was shown that the virus could be cultivated in embryonated eggs and that the virus could be easily quantified by measuring its hemagglutinin (Spradbrow, 2009).

Vaccines produced for commercial flocks were made by the standard contemporary methods for the control of serious veterinary diseases (Alders and Spradbrow, 2001). Crude inactivated vaccines were produced first, then virulent virus was applied together with antiserum. However, a later refinement was the use of viable attenuated vaccines. These have served the commercial industry well but they have found little use in village chickens. The commercial vaccines have been too expensive and insufficiently robust for rural flocks (Gwakisa et al., 1994).

A virulent strain of ND virus was recognised in Australia in 1966. The first isolate, strain V4, was later developed as a commercial vaccine. When the Australian Centre for International Agricultural Research (ACIAR) was founded, an initial project (in 1984) was to develop a ND vaccine suitable for use in village chickens (Spradbrow, 2009). The first trials, conducted by the University of
Queensland used variants of strain V4, artificially selected for enhanced heat resistance but this was not found to be efficacious (Aning and Brewoo, 2001). ACIAR sponsored the development at the University of Queensland of a new vaccine master seed. The result was strain I-2, another Australian avirulent virus that had properties, including heat resistance (Spradbrow, 2009) conducive for use in local chickens in rural areas.

2.5.3 Season of year

Keemboi et al. (2013) observed higher NDV titres in village chickens of Mbeere District, Eastern Province, Kenya during the rainy season than during the dry season. This presupposes that immunity to NDV during the wet season is higher and declines towards the dry season. This is because thermal stress reduces the concentration of circulating antibodies (Thaxton and Siegel, 1970) and suppresses the cell mediated immune response in birds (Regnier and Kelley, 1980) and also due to scarcity of feed for scavenging poultry. In a similar study, Kperegbeyi et al., (2009) observed that ND is most prevalent during the dry season. For this reason, they recommended that vaccinations be done at the beginning of the dry season to boost immunity against NDV.

2.5.4 Other infections

The existence of other infections such as *Escherichia coli* and Infectious Bursal Disease vaccine has also been shown to influence the antibodies against NDV. A study conducted by Cardoso et al. (2006) showed that when NDV vaccine was administered...
with Infectious Bursal Disease vaccine, the response to the NDV vaccine is greatly decreased. In a similar study on the interaction between NDV and *Escherichia coli*, Tayeb and Hanson (2001) observed that when certain *E. coli* strains were administered before birds were exposed to NDV the virus titres were lowered. This could be due to the suppression of the immune response by *E. coli* endotoxins. However when the NDV was administered prior to exposure to *E. coli*, the virus titres were significantly higher.

### 2.5.5 Genetic factors

Since the early 1920s, studies have shown that there are genetic factors that confer resistance to Newcastle disease virus but this was not completely understood till the advent of technologies that could be used to study and compare the genomes of organisms (Hutt and Cole, 1947). Msofe *et al.* (2002) suggested that rural chicken ecotypes which have evolved and adapted to stressful environments are likely to carry valuable genes and gene combinations controlling specific behavioural, physiological and disease as well as parasite resistance traits. Studies have been done quite recently to identify resistant breeds and develop a more rapid means of identifying the resistant breeds and this has resulted in the identification of the Egyptian Fayomi (Zhou and Lamont, 2003; Muhammad *et al.*, 2004).

According to Zakarias *et al.* (2002), genetic variation for disease resistance is ubiquitous and selecting for resistance is possible. A good example in poultry is the Egyptian Mandarah chickens that were observed to be resistant to both Infectious Bursal disease virus and NDV (Mohamed *et al.*, 2004).

Recent advancements in genomics have ushered-in a golden age of genomic exploration that is identifying markers linked to genes for resistance. Zhou and Lamont
suggested that selection for resistance will involve maintenance of genes rather than simply increasing the frequency of genes conferring resistance on an animal. Genetic resistance to diseases is a multigenic trait governed mainly by the immune system and its interactions with many physiologic and environmental factors (Zakarias et al., 2002). As observed by Allison (1961), when a population of animals is repeatedly exposed to an infectious disease producing significant mortality, the progeny of surviving animals shows through several generations increasing resistance to the disease. This could be due to natural selection as only those that are able to survive the infection make it to the next generation. According to Davies and Grange (2001) the genetic factors affecting resistance to disease are complex, depending upon many genes affecting antibody synthesis and other reactions. Recent studies in Nigeria (Adeyemo et al., 2012) and Tanzania (Msofe et al., 2004) show that ecotypes of local chicken from regions in the study area have genetic resistance to Newcastle Disease Virus.

Throughout the world, a great deal of effort and resources have been expended in the quest to understand the genetics of disease resistance and genetically improve disease resistance in domestic animals (Shook, 1989). Immune capacity associated with specific diseases has been a useful indicator for indirect selection for general disease resistance, because such traits can be evaluated and quantified in live animals (Luo et al, 2013). Traits associated with immune response such as antibody titre have been shown to be heritable. Quantitative trait locus (QTL) and genes associated with immunity or disease resistance have also been discovered recently (for example as is the case for Marek’s Disease) (Zhang et al., 2015).
Antibody production has been used to measure the magnitude of immune response and hence the level of resistance to specific disease agents. Variations in antibody production have been attributed to the variations (polymorphism) in the MHC of chicken. Several studies have been carried out using genome wide association studies (GWAS), to linking the antibody production to Single Nucleotide Polymorphisms (SNPs) of the Major Histocompatibility complex (MHC).

Zhang et al. (2015) in a GWAS identified a region located approximately 100 Mb from the proximal end of GGA1, which was most significantly associated with rs15354805 and accounted for 5% of the phenotypic variation of the antibody response to NDV. They suggested that there are probably only two divergent QTLs for this trait on GGA1 and GGA10, while other SNP effects might result from linkage disequilibrium.

Zhang et al. (2015) in an effort to identify major genomic regions (loci) and candidate genes associated with the immune response using GWAS, reported three SNPs associated with six immune traits (total serum IgY level, numbers of, and the ratio of heterophils and lymphocytes and antibody responses against AIV and SRBC). Five SNPs, associated with the serum total IgY level (which were clustered within a 0.26 Mb region on chromosome 16) and three SNPs associated with Lymphocyte heterophils ratio (on chromosome 13) were also discovered.

For the first time the power of GWAS was demonstrated as a tool for mapping genes associated with Marek’s Disease (MD) resistance. Li et al. (2013) investigated host genetic resistance to Marek’s Disease in a genome-wide association study and reported two SNPs associated with host resistance to MD. The expression patterns of these two genes in spleens were detected by qPCR.
Zhou and Lamont (2003) suggested that gene polymorphism might be utilised to improve antibody production by marker assisted selection (MAS), if the allele effect associated with antibody response can be estimated.

Yonash et al., (2000) in a study using DNA microsatellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens, reported that several microsatellite markers were identified to be associated with QTL for immune response traits in young broilers. None of these markers was linked to the MHC, supporting the suggestion by Zakarias et al., (2002) that something other than the MHC also affects the immune response.

2.6 Mechanism of disease resistance
A disease occurs if the immune system fails to protect the body from the injuries inflicted by the invading pathogen, due to insufficient-, misdirected- or aberrant immune responses (Gavora, 1996). The immune response against infectious pathogens is an elaborate process and varies among individuals. Animals and birds for that matter depend on various protective mechanisms to promote survival. These mechanisms could be classified as specific and non-specific. The non-specific mechanism, also known as innate immunity works to keep invaders or pathogens away. It is made up of mechanical and chemical barriers and cells that attack invading pathogens (Alters, 2000). The innate immunity is the first line of defence against invading pathogens. It includes inflammatory reaction, phagocytosis, acute phase reaction and complement proteins, whereas the specific immunity works against specific pathogens (Zakarias et al., 2002).
The adaptive immune response is antigen-specific and requires the recognition of specific "non-self" antigens during a process called antigen presentation. Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells. The ability to mount these tailored responses is maintained in the body by memory cells. Should a pathogen infect the body more than once, these specific memory cells are used to speedily eliminate it (Jacob et al., 2000).

Specific immunity includes two features of immunity. These are humoral immunity, and cell-mediated immunity. Lymphocytes are the cellular constituents of specific immunity (Erf, 1997). There are various subpopulations of lymphocytes which are morphologically not clearly identifiable but differ in site of growth, tissue location, phenotypic expression of cell surface molecules, and useful abilities (Kaufman et al., 1999). Chicken lymphocytes consist of B cells, which are produced in the Bursa of Fabricius and T cells, which are produced in the thymus. Both B cells and T cells express antigen receptors on for a given antigen (Kronenberg et al., 1986). Collectively, the B cell and the T cell compartment each have a repertoire of nearly 10^9 different antigen-specificities (Jacob et al., 2000).

When it first encounters an antigen, for example a pathogen A, there are comparatively few T and B cells with receptors specific for the antigen. Before pathogen A can be eliminated by specific immune components, T and B cells with pathogen A- specific receptors have to proliferate and differentiate into effector cells (e.g. antibody producing plasma cells). However, proliferation and differentiation take time, often giving pathogen “A” the opportunity to cause disease. Instead of becoming effector cells, cells recognising pathogen A can also differentiate into long-lived, fast-reacting memory cells.
Hence, during a T or B cell in reaction to pathogen A, the number of pathogen A-specific cells increases, resulting in large numbers of pathogen A-specific effector cells and memory cells. The effector cells will participate in the elimination of pathogen A, whereas the memory cells will be set aside, ready to effectively respond to pathogen A – upon a repeated counter- before pathogen A can cause disease. However, pathogen A-specific memory cells will not protect the individual from a different pathogen (e.g. pathogen B). This concept of expanding the pool of antigen-specific cells and producing antigen-specific memory cells is directly utilised in vaccination programmes, whereby a non-pathogenic form of a pathogen is introduced to an individual's immune system (Jeanway et al., 2001). The immune system will mount a reaction to target components of the pathogen. When it later encounters with the pathogenic form of the pathogen, the immune system will be ready to react and remove the pathogen before it can cause disease (Jacob et al., 2000). The immune competence of the host can be measured using certain immune parameters such as the antibody production, lymphocyte proliferation, phagocyte activity, parasite load (Bumstead, 1998).

2.7 The major histocompatibility complex

The Major Histocompatibility Complex (MHC) is a group of genes that codes for proteins located on the surfaces of cells that help the immune system recognize foreign substances (Lamont, 1998). The term histocompatibility, derived from the Greek word *histo* (meaning tissue) and the English word compatibility, was applied to the MHC molecules to describe their function in transplantation reactions and does not reveal their
Hosomichi et al. (2008) described the MHC as a highly conserved gene region especially interesting to geneticists because of the rapid evolution of gene families found within it. High levels of MHC genetic diversity often exist within populations. There are two major types of MHC protein molecules—class I and class II. Class I MHC molecules span the membrane of almost every cell in an organism, while class II molecules are restricted to cells of the immune system called macrophages and lymphocytes (Zhou & Lamont, 2003).

The MHC also contains a variety of genes that code for other proteins—such as complement proteins, cytokines (chemical messengers), and enzymes—that are called class III MHC molecules. MHC molecules are important components of the immune system because they allow T lymphocytes to detect cells, such as macrophages, that have ingested infectious microorganisms (Zakarias, et al., 2002). When a macrophage engulfs a microorganism, it partially digests it and displays peptide fragments of the microbe on its surface, bound to MHC molecules. The T lymphocyte recognizes the foreign fragment attached to the MHC molecule and binds to it, stimulating an immune response. In uninfected healthy cells, the MHC molecule presents peptides from its own cell (self-peptides), to which T cells do not normally react.

Of the two components of the immune response, the adaptive immunity is most involved in individual differences in resistance to infectious pathogens. The adaptive immune response mainly operates through communications of the antigen-presenting cells (APC), T cells and B-cells by direct cell-to-cell contact using MHC, TcR and immunoglobulins and or by secreted proteins such as cytokines in a paracrine way (Roitt, 1993). The quantity and quality of the immune response depends on the nature of the interaction of
these molecules. According to Zakarias, et al. (2002), the B complex in the chicken, is composed of heterodimeric transmembrane glycoproteins that are essential in the presentation of antigens to T-lymphocytes. The MHC molecules (class I, class II and class IV or B-F, B-L and B-G, respectively), belong to the immunoglobulin superfamily with extracellular regions composed of two N-terminal antigen binding domains and two constant domains that bind to the analogous receptor molecules on the T cell membrane.

The chicken MHC is located on chromosome 16 (Jacob et al., 2000). The MHC molecules play important roles in the regulation of the immune response by communicating among different cellular components of the immune system: T cells, B cells, and antigen-presenting cells (Lamont, 1998). The crucial function of the MHC in the immune response therefore makes this a promising candidate region for genetic selection to improve avian immunity. The chicken MHC contains the three classes of MHC molecules identified by alloantisera: B-F (class I), B-L (class II), and B-G antigens (Kaufman and Lamont, 1996). There are two B-F, and two B-L loci (designated as the “major” and “minor” B-F and B-L loci, respectively) within a single haplotype encoding chicken class I and II proteins (Kaufman et al., 1999).

The chicken MHC is the focus of considerable interest because of the strong, reproducible infectious disease associations found with particular MHC-B haplotypes (Hosomichi, 2008). It is generally difficult to find associations between MHC polymorphism and infectious disease outcome in vertebrates. In this regard, chicken MHC-B region is the exception. Strong, reproducible associations exist between MHC-B haplotypes and their response to diseases caused by several different pathogens (Ababneh et al., 2012)
Miller et al. (1996) divided the chicken MHC region into two major parts, linkage group B (MHC-B) and linkage group Y (MHC-Y). MHC-B and MHC-Y are inherited independently of each other even though they are physically linked on microchromosome 16 (GGA16) (Miller, et al., 1996). In the chicken, the MHC is closely associated with resistance or susceptibility differences to a number of diseases (Lamont, 1989) such as resistance to viral infections (Zacharias et al., 2002), resistance to bacterial infections (Yoo et al., 1992), resistance to protozoal infection (Lillehoj et al., 1989) and autoimmune diseases (Rose, 1994).

2.8 Genetics of disease resistance

Genetic resistance to diseases is a great resource for the control and prevention of diseases and to the improvement of productivity in poultry (Bumstead, 1998) in the face of increasing virulence and emergence of drug resistant pathogens. The FAO (2007) defined resistance as the ability of the host to resist infection. Disease resistance is more important than disease tolerance in the genetic management of animal diseases where the objective is to prevent the spread of the disease to other populations (as in the case of zoonotic diseases).

Genetic resistance is a trait controlled by many genes governed through the immune system and its interactions with many physiologic and environmental factors (Zakarias et al., 2002). In any outbred population, there exist differences in genetic resistance or susceptibility to disease agents (Cole and Hutt, 1961). Genetic diversity of the immune
system is the major cause of variability in immune response. According to the FAO’s State of the World’s Animal Genetic Resources for Food and Agriculture (FAO, 2007), there is genetic variation in the extent to which host animals are susceptible. As far back as 1960, it has been noted that there exist differences in the resistance or susceptibility to NDV between strains and among sire families but could not establish any clear cut evidence (Cole and Hutt1, 961).

2.9 Correlation between disease resistance and growth

Any two characters that are associated have a correlation. In animals, this association may be what is observable (phenotypic) or at the genetic level influenced by the same genes as in the case of Pleiotropy. Correlation between two traits, X and Y is the association between the two and in animal genetics, measures the extent to which selection for X impacts Y (Falconer and MacKay, 1996). The value ranges between 1 and -1. Genetic correlations are classified by the strength of the relationship (low from 0 to ± 0.2, moderate from > ± 0.2 to ± 0.6, and high > ± 0.6 to ± 1.0) between two traits and its directionality. These two things, strength and directionality, determine if a genetic correlation is advantageous or not (Roy and Kirchener, 2000). A negative genetic correlation indicates that as one trait increases, the other trait tends to decrease while a positive genetic correlation simply means that as one trait increases, the other trait also tends to increase. The correlation between two traits can be partitioned into genetic (the degree of relation between changes in two traits) and phenotypic correlations. The phenotypic correlation is a reflection of the effect of the environment on the genotype.
The correlation between growth rate and disease resistance has been an issue for researchers for decades. Some researchers found no correlation between the two traits, whilst others reported negative correlation between the two traits. van der Most et al. (2011) argued that selection for growth rate redirects nutrients towards growth rate at the expense of resistance. Saif(2012), and other workers (Roy and Kirchner 2000; Mangel and Stamps 2001; Hansson and Westerberg 2002) however suggest that overall genetic diversity and heterozygosity at the major histocompatibility locus plays a role in relative fitness in wild populations concerning their ability to respond to various pathogens and therefore expect a positive correlation. However, some studies have shown positive correlation between growth rate and disease resistance to specific pathogens. van der Most et al. (2011) reported that selection for rapid growth strongly and significantly decrease the response to a variety of immune challenges, however selection for increased immune response results in increased growth rate but to a lesser extent. This suggests that selection for disease resistance can both inhibit and promote growth rate, but usually has no effect, which means it may be possible to select for better immuneresponse in commercial settings without affecting the growth rate.

2.10 Heritability of disease resistance in chicken and livestock populations

Understanding heritability of disease resistance and tolerance is important in breeding or selection of animals that can survive the challenges posed by diseases in the livestock industry. Infectious diseases are of major importance to livestock breeders for many reasons as they impose a large cost on livestock production systems in the area of prevention, treatment and losses due to mortality and morbidity (FAO, 2007). According
to Bennett et al. (2005), total disease costs have been estimated to be up to 20% of turnover in developed countries and as high as 35–50% of turnover within the livestock sector. In the developing world extensive research has been done in the area of breeding and selection for disease resistance and the interpretation of data arising from such studies and the utilisation of research outcomes to breed for disease resistance (FAO, 2007).

A number of livestock species including poultry have been found to be resistant to some diseases. For instance, the Egyptian Fayomi chicken is resistant to ND (Lamont, 1998) and some breeds of chicken are resistant to Marek’s disease and fowl typhoid while some breeds of cattle are resistant to mastitis (FAO, 2007). Heritability of resistance of such infectious diseases has been a major objective of research in breeding and genomics as this will provide the needed quantification of the extent to which such important traits are transmissible to the next generation. Heritability estimates for immune response of chicken to sheep red blood cell have ranged from 0.28 to 0.38 (Lamont 1998) suggesting that the trait is under moderate genetic control. Genetic control of disease resistance may be as a result of the presence or absence of some receptors that are simply heritable. For instance, resistance to a specific subgroup of Leukosis virus in chicken is seen to be simple and may be as a result of not having receptors for the virus (Crittenden, 1975).

Payne (1973) grouped the control of inheritance of disease resistance into two types: single gene control and polygenic (multiple genes) control. It was noted that when a trait is controlled by more than a few pairs of genes it becomes difficult to identify distinct phenotypes and individuals tend to fall into a continuously graded series. This is due to
the cumulative effect of many genes and the blending effect of environmental factors to determine the extent of expression of such traits. Disease resistance is a multigenic trait and tends to be controlled by many genes (Zakarias et al., 2002).

2.11 Growth rate of local chicken

For many years, local chickens have been noted to exhibit lower growth rate and have smaller body size compared to the commercial chickens (Aboe et al., 2006). Numerous indigenous chicken breeds and their economic traits and genetic potential remain largely unknown (Hegan et al., 2013). Recently, much attention has been focused on indigenous chickens as meat or layer strains because of increasing consumer demand and environmentally viable characteristics of local ecotypes. Several works have been done to evaluate the economic traits such as disease resistance (Adeyemo et al., 2002) growth rate (Osei-Ampomsah et al., 2012), carcass characteristics and reproductive performance (Hegan et al., 2013). Local chickens have genetically smaller body size, lower growth rate and lower feed conversion efficiency. Adomako et al. (2009) reported a body weight of 1.55kg, 1.13kg for matured cocks and hens, respectively, in the Ashanti Region of Ghana. Osei-Ampomsah et al. (2012) reported a growth rate of 5.557 to 7.80g/day for the Ghanaian local chicken. However, for local chicken in Benin, Youssao et al. (2012) reported an average daily gain of 4.29 g/day and 1.47 g/day for savannah and forest ecotypes, respectively. Comparatively, 8.29g/day has been reported for local chicken in Korea (Choo et al. 2014). The lower growth rate of the local chicken compared to the commercial chicken is due mainly to genetics and environment.
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling area and breeding stock constitution

Sampling of local chickens was carried out from the major agro-ecological zones of Ghana (ecotypes), namely, Interior Savannah, Forest and Coastal Savannah. In all 225 chickens (25 males and 200 females) per ecotype were purchased from each agro-ecological zone (Ecozone). For each ecotype, 25 sire half sib families were constituted with a mating ratio of one male to eight (8) females. The chickens were identified at the point of purchase with wing bands.

All 675 birds collected were quarantined at various locations within each ecozone for 10 days. Within the quarantine period, prophylactic treatments against ND, IBD, ecto-parasites and endo-parasites were administered to clear the birds of any pathogen that they may be carrying. After the tenth day, the birds were transported to the breeding facility at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana.

Each ecotype represents a treatment and each hatch (batch of chick per ecotype) represented a replicate. In all there were three treatments and four replicates. Chicks from each ecotype were randomly allocated to pens.

For each replicate, an average of 500 eggs from the breeders were collected per ecotype, labelled and incubated. The chicks hatched were tagged and transported to the challenge facility and randomly allocated to pens by ecozone at LIPREC. Measurement of body weight was done at the day of hatch (day 0), days 7, 14, 21, 28, 34 and 38.
Four replicates were designed and run consecutively, each lasting for a period of 38 days. The challenge facility was sterilised in between replicates.

3.2 NDV animal challenge

About 700 µl of blood was collected on the 27th day from all chicks, into 1.5ml tubes and labelled as pre-challenge blood samples. The samples were transported on ice to the Molecular Genetics Laboratory at the Department of Animal Science. Serum was then extracted from the blood samples by centrifuging at 1500 RPM for 5 minutes in a Thermo Scientific centrifuge (MEGAFUGE 16R). The serum was then collected into newly labelled 1.5 ml tubes and stored at -30°C.

At 28 days old, about 100µl of the La Sota virus (10^7 EID_{50}) was administered to each chick through the ocular and nasal route. This was recorded as the day of infection whilst the subsequent days were noted as the ‘days post-infection’ (dpi). For each replicate 30 chicks (10 chicks per ecotype) were randomly selected and isolated as controls. These 30 birds were not challenged with the virus.

At 10 dpi, (38th day), blood samples were again collected and labelled. Serum was extracted from the blood samples and stored at -30°C. All serum samples were used in antibody analysis adopting the Enzyme Linked Immunosorbent Assay (ELISA) technique.
3.3 Quantification of NDV antibody with ELISA

Antibody response to NDV La Sota was assessed by ELISA using Newcastle Disease Virus Test kit according to the protocol recommended by the manufactures, IDDEX (IDEXX Laboratories, Inc. Maine, USA).Briefly, the serum samples were thawed at room temperature for 30 minutes. Subsequently, 2µl of each sample was added to labelled 1.5 ml tubes containing 998µl of liquid diluent and mixed thoroughly by vortexing.

Sample positions on a 96-well NDV antigen coated plate were recorded on a test sheet. The first two wells on the 96-well NDV antigen coated plate were filled with 100µl each of undiluted negative control and each of the next two wells were dispensed with 100µl of undiluted positive control. The rest of the wells had 100µl of diluted sample dispensed in duplicates. The last two wells of the plate were filled with 100µl of diluent to serve as blank and the plate was incubated for 30 minutes at room temperature.

The plates were flipped, emptied and washed by adding 350µl of distilled water to each well. The washing was repeated four times to remove uncaptured antibodies. Residual fluid was removed by tapping the plate onto absorbent tissue paper. To each well was then added 100µl of conjugate and the plate incubated for 30 minutes at room temperature. During this step, the conjugate reacted with the antibody that had previously been captured by the NDV antigen which coated the wells. The solution was removed and each well washed with 350µl of distilled water. The washing was repeated four times. Residual fluid was removed by tapping the plate onto absorbent tissue paper. Next, to each well was added 100µl of TMB and then incubated for 15 minutes at room temperature. Finally, 100µl of stop solution was dispensed into each well to stop the
reaction. The absorbance values were measured and recorded at 630 nm using a microplate reader (BioTek Instruments, Inc., WA, USA).

3.4 Calculations
Average absorbance was calculated for the positive control (PC) as:
\[
\text{Posit } PC\bar{x} = \frac{PC1 A(630) + PC2 A(630)}{2}, \text{ where } PC1 \text{ and } PC2 \text{ are the duplicate positive control samples.}
\]

(IDEXX NDV ELISA Kit manual, 2014)

Average absorbance was calculated for the negative control (NC) as:
\[
\text{NC}\bar{x} = \frac{NC1 A(630) + NC2 A(630)}{2}, \text{ where } NC1 \text{ and } NC2 \text{ are the duplicate negative control samples.}
\]

(Source: IDEXX NDV ELISA Kit manual, 2014)

3.5 Validity criteria
The assay was said to be valid if the difference between the mean positive control and mean negative control was greater than 0.075 and the mean negative control less than or equal to 0.15 (PC\bar{x} – NC\bar{x} > 0.075 and NC\bar{x} \leq 0.15)

The sample to positive (SP) ratio of each sample was calculated as
\[
\text{SP} = \frac{\text{sample mean} - NC\bar{x}}{PC\bar{x} - NC\bar{x}}
\]

For each sample, the log_{10} titre was calculated as
\[
\log_{10} \text{Titre} = 1.09(\log_{10} \text{SP}) + 3.36
\]
A plot of the raw sample to positive (S/P) ratios (Figure 5, Appendix) and raw antibody titres (Figure 6) against the number of birds to assess the distribution of the S/P ratio shows a skewed distribution. To normalise the distribution, the square root of the S/P ratio and antibody titres were plotted as normalised S/P ratio and normalised antibody titres respectively. This is shown in Figure 7 and Figure 8. In the raw values more birds recorded lower S/P ratio values.

3.6 Interpretation of results
A sample is negative if the S/P ratio is less than or equal to 0.2 (Negative sample $S/P \leq 0.2$) and positive if the S/P ratio is greater than 0.2 (Positive sample $S/P >0.2$). A positive result (titre ≥396) indicates vaccination or exposure to NDV.

3.6.1 Genetic parameters
Genetic parameters (heritability and genetic correlation) were used to evaluate ecotype differences using growth rate and average antibody titres before and after inoculation. Differences in growth rate, body weight and average antibody titres were determined using ASReml version 4 (VSNi, 2015).

ANOVA was run for the model $y = s + p + r + e$

Where $y$ = genetic parameter, $s$ = sire, $p$ = pen, $r$ = replicate and $e$ the error term.
where \( t \) is the intra-class correlation, \( k \) is the coefficient of variance component being estimated and \( S \) is the number of sires (Falconer and Mackay (1996)). Heritability is a proportion, the value ranges from zero where genes do not contribute at all to individual differences to one where genes are the only reason for individual differences. A heritability value in the range of 0-0.3 (0-30%) is low, 0.3-0.6 (30-60%) is considered moderate and that of 0.6 and above is ranked high (Robinson et al., 1949).

### 3.4.3 Correlation

Genetic correlation was estimated using the formula

\[
\gamma_G = \frac{\text{Cov}G_{t1,t2}}{\sqrt{\sigma^2_{G_{t1}} \times \sigma^2_{G_{t2}}}},
\]

where \( \gamma_G \) is the genotypic correlation between growth rate (t1) and antibody titre (t2), \( \text{Cov}G_{t1,t2} \), the covariance for the two traits and \( \sigma^2_{G_{t1}} \) and \( \sigma^2_{G_{t2}} \) are the genotypic variance for growth rate and antibody titre, respectively (Falconer and Mackay, 1996).

The phenotypic correlation was estimated using the formula

\[
\gamma_P = \frac{\text{Cov}P_{t1,t2}}{\sqrt{\sigma^2_{P_{t1}} \times \sigma^2_{P_{t2}}}},
\]

where \( \gamma_P \) is the phenotypic correlation between growth rate (t1) and antibody titre (t2), \( \text{Cov}P_{t1,t2} \), the phenotypic covariance for the two traits and \( \sigma^2_{P_{t1}} \) and \( \sigma^2_{P_{t2}} \) are the phenotypic variance for growth rate and antibody titre, respectively (Falconer and Mackay, 1996).
CHAPTER 4

4.0 RESULTS

4.1. Growth rate

Least squares means of pre-infection growth rate are shown in Table 1. The Interior Savannah ecotype recorded a significantly (p < 0.05) higher mean pre-infection growth rate compared to the Forest and Coastal savannah ecotypes. The means for Forest and Coastal savannah ecotypes were however not significantly (p < 0.05) different.

<table>
<thead>
<tr>
<th>ECOTYPE</th>
<th>LSMEAN*</th>
<th>SE</th>
<th>DF¹</th>
<th>95% CL</th>
<th>LOW</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interior Savannah</td>
<td>6.401890</td>
<td>0.112259</td>
<td>82.81</td>
<td>6.158685</td>
<td>6.645096</td>
<td></td>
</tr>
<tr>
<td>Forest</td>
<td>5.937841</td>
<td>0.1117432</td>
<td>74.78</td>
<td>5.715226</td>
<td>6.160456</td>
<td></td>
</tr>
<tr>
<td>Coastal Savannah</td>
<td>5.959042</td>
<td>0.1222736</td>
<td>82.65</td>
<td>5.735749</td>
<td>6.182335</td>
<td></td>
</tr>
</tbody>
</table>

*Means in the same column with different subscripts are significantly different (p<0.05)

¹Effective degrees of freedom as calculated by the Welch-Satterthwaite correction
4.2 Growth curves

The growth curve for the chicks from day of hatch to the 38th day shows the trend of growth for the three ecotypes. The black vertical line shows the point of infection (Figure 3). Initially, all the three ecotypes had similar growth curves until after day 14 when the Interior Savannah ecotype exhibited superior growth compared to the Forest and Coastal Savannah ecotypes. The growth curve of the Forest and Coastal Savannah ecotypes did not seem to differ until the end of the experiment at day 38. From the graph the exposure to ND vaccine did not appear to have any negative effect on the growth rate or trend of growth. The Interior savannah ecotype had higher weights than the other ecotypes at each point in time.

Figure 3: Growth curve for Interior savannah, Forest and Coastal savannah ecotypes showing point of NDV challenge
Least Squares estimates of the means for post infection growth rate (Table 2) also show that the Interior Savannah ecotype had significantly (p < 0.05) higher mean post-infection growth rate compared to the Forest and Coastal savannah ecotypes. Again the means for Forest and Coastal savannah ecotypes were not significantly (p < 0.05) different.

### Table 2: Least Squares Mean estimates for post-infection growth rate of local chicken from three ecozones of Ghana

<table>
<thead>
<tr>
<th>ECOTYPE</th>
<th>LS MEAN*</th>
<th>SE</th>
<th>DF¹</th>
<th>95% CL LOW</th>
<th>95% CL HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interior Savannah</td>
<td>6.8123a</td>
<td>0.1361</td>
<td>82.7779</td>
<td>6.5417</td>
<td>7.0830</td>
</tr>
<tr>
<td>Forest</td>
<td>6.1709b</td>
<td>0.1252</td>
<td>75.7800</td>
<td>5.9212</td>
<td>6.4206</td>
</tr>
<tr>
<td>Coastal Savannah</td>
<td>6.1935b</td>
<td>0.1251</td>
<td>65.0000</td>
<td>5.9444</td>
<td>6.4424</td>
</tr>
</tbody>
</table>

*Means in the same column with different subscripts are significantly different (p<0.05)

¹Effective degrees of freedom as calculated by the Welch-Satterthwaite correction
4.3 Antibody titres

Table 3 shows estimates of the Least Squares Means for average antibody titres per ecotype. The estimates of mean antibody titres showed significantly (p < 0.05) higher titres for the Forest ecotype than for the Interior and Coastal Savannah ecotypes. However, there were no difference between the estimates for Interior and Coastal Savannah ecotypes.

Table 3: Least squares means (IU) for average antibody titres per ecotype of local chickens from three ecozones of Ghana

<table>
<thead>
<tr>
<th>ECOTYPE</th>
<th>LSMEAN</th>
<th>SE</th>
<th>DF(^1)</th>
<th>LOWER.CL</th>
<th>UPPER.CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interior Savannah</td>
<td>3,929.94(_b)</td>
<td>516.31</td>
<td>177.67</td>
<td>2,911.04</td>
<td>4,948.84</td>
</tr>
<tr>
<td>Forest</td>
<td>4,868.86(_a)</td>
<td>346.20</td>
<td>91.09</td>
<td>4,181.19</td>
<td>5,556.53</td>
</tr>
<tr>
<td>Coastal Savannah</td>
<td>3,320.06(_b)</td>
<td>438.95</td>
<td>147.87</td>
<td>2,452.64</td>
<td>4,187.48</td>
</tr>
</tbody>
</table>

\*Means in the same column with different subscripts are significantly different (p<0.05)

\(^1\)Effective degrees of freedom as calculated by the Welch-Satterthwaite correction
4.4 Heritability

Heritability estimates for pre- and post-infection growth rate and average antibody titre are shown in Table 4. Heritability estimates for the growth rates were relatively high. Heritability estimate for antibody titre was 0.21 and that for the Log10 titre was 0.26. The heritability estimate for growth rate was thus high while that for antibody response was low to moderate.

### Table 4. Heritability estimates for growth rate and antibody titre of local chickens from three ecozones of Ghana

<table>
<thead>
<tr>
<th>Trait</th>
<th>Additive Genetic Variance</th>
<th>Phenotypic variance</th>
<th>n</th>
<th>h²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate (pre-infection)</td>
<td>1.24</td>
<td>1.33</td>
<td>1815</td>
<td>0.93 ± 0.14</td>
</tr>
<tr>
<td>Growth rate (post-infection)</td>
<td>1.50</td>
<td>1.69</td>
<td>1815</td>
<td>0.89 ± 0.14</td>
</tr>
<tr>
<td>Average Antibody titre</td>
<td>0.26 E+07</td>
<td>0.13 E+08</td>
<td>993</td>
<td>0.21 ± 0.09</td>
</tr>
<tr>
<td>Log10 antibody titre</td>
<td>0.56 E-01</td>
<td>0.21</td>
<td>993</td>
<td>0.26 ± 0.10</td>
</tr>
</tbody>
</table>

4.5 Correlations between growth rate and antibody titre

4.5.1. Genetic correlation

The genetic correlation between growth rate and antibody titre are shown in Table 5. Genetic correlation between growth rate and antibody titres was found to be 0.37±0.19...
Correlation with average antibody titres had high standard error (695.60) and therefore the normalised Log_{10} titres were preferred. Genetic correlation between growth rate and antibody titres is positive and moderate.

Table 5: Genetic correlation between growth rate (g/day) and antibody titre (IU)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genetic correlation (SE.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log_{10} Titre</td>
</tr>
<tr>
<td>Growth rate before infection</td>
<td>0.37 (0.19)</td>
</tr>
<tr>
<td>Growth rate after infection</td>
<td>0.37 (0.19)</td>
</tr>
</tbody>
</table>

4.3.2 Phenotypic correlation

Phenotypic correlation between growth rate and antibody titre is shown in Table 6. The phenotypic correlation recorded for this study was 0.1, which is low but positive.

Table 6 Phenotypic correlation between growth rate and antibody titre

<table>
<thead>
<tr>
<th>Traits</th>
<th>Phenotypic correlation (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log_{10} Titre</td>
</tr>
<tr>
<td>Growth rate before challenge</td>
<td>0.08 (0.04)</td>
</tr>
<tr>
<td>Growth rate after challenge</td>
<td>0.10 (0.04)</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 DISCUSSION

5.1 Growth

5.1.1 Body weight
The distribution of weekly body weight gain was generally normal from hatch to day 38. Hence, any statistical analyses of these are valid since normality is usually a basic requirement. Some of the birds showed lower bodyweight while others had higher bodyweight at each week. This may be due to the genetic make-up of the birds and also as an adaptation for surviving with very little food (Overfield, 1995) and escaping from predators as these birds are derived from chickens raised under extensive conditions. Awuni (2002) in a study of the management systems in of Ghana noted that the majority of household poultry live as scavengers as minority of farmers (41.7%) supplement the diet of their chickens with prepared feed.

5.1.2 Growth rate
The Interior Savannah chicken ecotype showed a higher growth rate than the Forest and Coastal Savannah ecotypes. Estimates of the Least Squares Means for pre-infection growth rate and post-infection growth rate show significantly higher values for the Interior Savannah ecotype. This result is similar to that obtained by Youssao et al. (2012) who determined whether the differences in growth performance between the forest and Savannah ecotypes of Benin were due to environmental effects or to
differences in the genetic make-up and breeding mode of local chickens. Osei-Amponsah et al. (2012) in a similar study on two ecotypes reported higher growth rate for Interior Savannah ecotypes over the Forest ecotype. Furthermore, there was no deviation of the growth trend from the point of infection with NDV. This shows that the viral challenge had little effect on the trend of growth for the three ecotypes and is an indication that the dosage and low virulence of the La Sota strain of virus used for the challenge had very little adverse effect on growth rate; the birds were more or less vaccinated.

5.1.3 Growth curve

The growth curve shows the Interior Savannah birds being significantly heavier than the Coastal Savanna and the Forest ecotypes. Similarly, Osei-Amponsah et al. (2012) observed that Interior Savannah chickens grew significantly faster than their Forest counterparts. There was however no significant difference between the Forest and Coastal Savannah ecotypes, even though the Coastal Savannah ecotypes recorded slightly higher weights than the Forest ecotypes. This implies that it will be more beneficial to use the Interior Savana ecotype for improving growth rate in the local chicken ecotypes. Youssao et al. (2012) also observed a similar growth trend in local Benin chicken indicating that their Savanna ecotypes had significantly higher weights than their Forest ecotypes. The growth rate recorded from this study were however higher than that recorded by Youssao et al. (2012) but the values are similar to those recorded by Osei-Amponsah et al. (2012). This is an indication that Savannah ecotype chickens are better suited for selection for higher growth rate.
5.2 Heritability of growth rate

Heritability estimate for growth rate was high for both the pre-infection and post-infection (0.9322 ± 0.14 and 0.8909 ±0.14, respectively). Kaylee et al. (2016) in a similar study estimated heritabilities for growth rate of 0.38 for hatch weight (day 0) and 0.29 for day 31 for Hy-line commercial layer chicken lines. Osei-Amponsah et al. (2013) also recorded heritability estimates of body weight of 0.54 for broilers. Also heritability measurement for post infection growth rate was 0.89 ± 0.14 meaning that 89% of the parental differences in growth rate in these chickens will be transferred to offspring, i.e., selection progress will be fast. The high heritability for growth rate makes improving growth rate easier and faster.

Heritabilities recorded by Kaylee et al. (2016) are lower than that estimated in this study because the local chicken population has not been selected for any specific trait and hence genetic diversity is high resulting in higher additive genetic variance and hence higher heritability whereas the Hy-line commercial layers used by Kaylee et al. (2016) had been selected and bred for egg laying characteristics resulting in a lower genetic diversity and hence lower additive genetic variance and therefore a lower heritability.

5.3 Antibody titres

The antibody titre is a test that detects the presence and measures the level of antibodies within an animal’s blood. The amount and diversity of antibodies correlates to the
strength of the body's immune response. The Forest ecotype recorded higher antibody titres which is an indication of a superior immune response.

The CostalSavannah ecotype chickens had lower titres compared to both the Forest and InteriorSavannah ecotype chickens. Banu et al. (2009) reported a Least Squares Mean titre of 861.66±140.21 antibody titres for ISA commercial layers inoculated with La Sota vaccine at 31 days old. Even though this study had not been able to measure the viral excretion in the birds, the higher antibody levels could be due to genetic differences. This is because the birds were subjected to the same treatment and the only difference in the treatment was that they came from different agro-ecological zones. In a similar study, Ayman et al. (2012) attributed the significantly high antibody titres of the Dokki4 to the genetic potential it inherited from the Fayoumi breed which is noted for its high immunogenic ability. The Forest ecotype may therefore be genetically superior with regard to NDV antibody response and hence will be likely to better tolerate NDV challenge or exhibit higher resistance. This observation could also be due to the possibility that forest zone ecotype chickens are exposed to higher ND in their natural environment and therefore have adapted to this higher challenge and had a higher response to infection (challenge).
5.4 Heritability estimates of antibody titres

Wijga et al. (2009) reported on humoral immune parameters in chicken lines selected for humoral immunity. However, heritability estimates reported by Kaylee et al. (2016) in a similar study were 0.04, and 0.07 for 0dpi and 10dpi antibody levels, respectively. This difference could have been due to the fact that Kaylee et al. (2016) with Hyline layers, were able to adequately account for the effect of sex on the antibody titres. Also, their birds were reared in controlled environments with very high disease control. So disease challenge was low and so genetic difference in disease resistance was not developed or expressed, it could also be due to differences in the breeds of chicken used in the experiment. Kaylee et al. (2016) used commercial strains selected for egg production. The heritability measurement for antibody titre post infection was 0.21 ± 0.09 meaning that 21% of the difference in response to NDV between the chickens in the study was as a result of genetic diversity within the local chicken ecotype. This heritability measurement is substantial enough for selection of improved antibody production to be successful.

Variability with respect to the commercial strains was expected to be lower and hence lower heritability than that obtained in this study as shown in the result. Local chickens have not been selected for any specific trait apart from being able to survive the local environment conditions and management and hence are genetically diverse.
5.5 Correlation between growth rate and antibody titres

5.5.1 Genetic correlation

Genetic correlation between growth rate and antibody titre was found to be positive in this study. This is contrary to many observations in literature (Roy and Kirchner 2000; Mangel and Stamps, 2001; Hansson and Westerberg 2002; Saif, 2012). It is likely that there is some threshold in body weight beyond which the relation is negative (more nutrients transferred to support growth rather than disease resistance) and below which it is positive (enough nutrients for both growth, which is very low, and development of disease resistance (Saif, 2012)). The correlation was positive and moderate; this therefore means that a selection for an improvement in one trait (antibody titre) will result in an improvement in the other trait (growth rate). For instance, a number of studies have consistently shown that rapid growth rate is negatively correlated with disease resistance. Selection for higher growth rate is believed to redirect nutrient towards growth rate at the expense of resistance (Saif, 2012). However, the result from this study is in agreement with that found by Sacco et al. (1994) who observed a positive genetic correlation between the antibody titre against the Newcastle disease virus and growth rate. Nevertheless, Tsai et al. (1992) in another study found NDV mortality higher in the same selection line despite higher antibody levels. Overall genetic diversity and heterozygosity at the major histocompatibility locus have been proposed to play a role in relative fitness in wild populations concerning their ability to respond to various pathogens (Roy and Kirchner, 2000; Mangel and Stamps, 2001; Hansson and Westerberg, 2002). The result of this study could be due to fact that local chickens have a higher genetic diversity (Osei-
Amponsah et al., 2012) and hence heterozygosity at the MHC leading to higher genetic correlation than that recorded in the commercial chicken lines.

5.5.2 Phenotypic correlation

Phenotypic correlation between growth rate and antibody titre was found to be low. A low phenotypic correlation and a high genetic correlation is an indication of a negative environmental correlation, meaning the environment could mask the expression of a particular trait (Saif, 2012). This is an indication that one trait cannot be used as a measure or direct indicator for the other. In other words, an improvement in growth rate cannot be assumed to result in an improvement in antibody titre (Thaxton, 1970) with this result.

This finding is quite encouraging as NDV resistance will not be antagonistic to improved growth; thus, both traits can be improved simultaneously.
CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study, the Forest ecotype chicken showed higher immune response or resistance to the La Sota virus challenge than the Costal Savannah and Interior Savannah ecotypes. The heritability measurement for immune response post-infection is substantial enough for selection for improved antibody production to be successful. Growth rate was higher in the Interior Savannah ecotype as compared to the Forest and Coastal Savannah ecotype and hence this trait would be suitable for selection for faster growth in local chickens. Overall, the heritability of the growth rate was very high, implying that selection for improved growth would result in higher gains.

6.2 Recommendations

It is recommended that further studies be conducted using Genome Wide Association Study to estimate the heritability of growth rate and antibody response to account for the effect of the dams on the heritability of these traits. Also, genes responsible for the growth rate and antibody response can be identified and possible variations studied. Further studies could also be carried out to determine the viral load of each sample so as to confirm whether the chicken has been able to suppress the viral replication or not.
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### Appendix

**Table 7: Summary of distribution of body weights (g) from day old to day 38**

<table>
<thead>
<tr>
<th>Statistic</th>
<th>BWD0</th>
<th>BWD7</th>
<th>BWD14</th>
<th>BWD21</th>
<th>BWD28</th>
<th>BWD34</th>
<th>BWD38</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>29.39</td>
<td>46.01</td>
<td>79.76</td>
<td>123.15</td>
<td>170.51</td>
<td>205.76</td>
<td>240.44</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>29.30</td>
<td>45.93</td>
<td>79.70</td>
<td>123.54</td>
<td>169.067</td>
<td>204.00</td>
<td>236.80</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>21.34</td>
<td>54.50</td>
<td>92.50</td>
<td>145.30</td>
<td>224.90</td>
<td>324.30</td>
<td>371.80</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>19.30</td>
<td>16.20</td>
<td>36.60</td>
<td>54.00</td>
<td>71.30</td>
<td>66.30</td>
<td>86.10</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>40.64</td>
<td>70.70</td>
<td>129.10</td>
<td>199.30</td>
<td>296.20</td>
<td>390.60</td>
<td>457.90</td>
</tr>
</tbody>
</table>

**Figure 4:** Distribution of S/P ratio at ten days- post-infection
Figure 5: Distribution of normalised S/P ratio
Figure 6: Normalised Average titre
Figure 7: Log10 titres for ten days-post infection