DIVERSITY AMONG LOCAL CHICKEN ECOTYPES IN TANZANIA

BY

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ABSTRACT

This study was conducted in order to establish the genetic and phenotypic diversity among nine local chicken ecotypes of Tanzania namely *Ching'wekwe, Kuchi, Mbeya, Morogoro-medium, N'zenzegere, Pemba, Singamagazi, Tanga* and *Unguja* in a series of five experiments. The first experiment determined the genetic diversity among the ecotypes using manual microsatellites typing. Allele numbers, genetic distancing, differentiation (G_{ST}), and individual associations were established between the nine local chicken ecotypes and phylogenetic trees were constructed. In total, 152 alleles were detected across all loci ranging between 4 and 15 per loci. Up to 100% (n = 13) of individuals in some ecotypes clustered together. The phylogenetic trees grouped the chickens by geographical and historical origin. The second experiment was on productivity and reproductive performance of the ecotypes. Weekly weight measurements and growth rates were evaluated for each ecotype and sex of the birds as was egg weight, fertility and hatchability. Significant differences ($P<0.05$) existed between ecotypes in the parameters studied. *Ching'wekwe* showed consistently low productivity contrary to *Morogoro-medium* and *Tanga* ecotypes often showing higher values. The third experiement assessed the disease resistance potential by challenging one-week old chicks with 2.54×10^{-8} CFU of virulent *S. gallinarum* strain. For 14 days, clinical signs, necropsy findings, antibody titres, haematocrit, leukogram, growth rate and viable bacterial cell counts in the liver and spleen were recorded. There were differences in the leukogram; viable bacterial cell counts as well as the effect on growth

rate between the different ecotypes. Based on clinical signs and mortality, *N'zenzegere* and *Mbeya* ecotypes as well as the commercial layer strain appeared to be the most severely affected. The fourth experiment studied the responses of ecotypes to Newcastle disease vaccine. Parents (hens) were vaccinated and HI titres measured after two weeks on hens, eggs and chicks hatching from the eggs. Some chicks were monitored until their HI titres were zero and then were vaccinated against Newcastle disease. HI titres were monitored weekly for 28 days; 70 days later, the chicks were re-vaccinated and titres were observed for a further 28 days. The HI titres in chicks were higher than those of hens and eggs. *Tanga* ecotype showed early protective immunity while *Morogoro-medium* and *Mbeya* ecotypes showed persistently higher responses. The fifth experiment assessed the productivity and reproductive performance of the local chickens under free-range system. Wide ranges were detected in the parameters investigated and there were significant differences in productivity between the different farmers. The overall hatchability was 80% with ranges between 33 and 100%. It was concluded that genetic and phenotypic diversity exists in the local chicken ecotypes of Tanzania. The diversity constitutes a valuable resource for use in breeding programmes for improvement of the health and productivity of the local chickens and in designing proper conservation strategies. Further studies are required to identify genetic markers associated with productivity and disease resistance within the local chicken ecotypes. In depth studies on the performance of the Tanzanian medium ecotype (*Morogoro-medium* and *Tanga*) is required to ascertain their suitability for promotion throughout the country.

DECLARATION

I, PETER LAWRENCE MAKENGA MSOFFE, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and that it has not been submitted for a degree award in any other University.

Signature.....

Date.....12. 11. 2003.....

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To my beloved wife Grace, my child Gloria-Modesta and my parents Lawrence and Modesta who always encouraged me to believe in myself and search for the best.

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LIST OF ABBREVIATIONS AND SYMBOLS

μg – Microgramme

μl – Microlitre

μM – Micromolar

^{0}C – Degrees Celcius

A – Adenine

AAFC – Agriculture and Agri-Food Canada

ABO – Surface antigens on the human erythrocytes, basis for human blood typing.

B-F – Chicken MHC class I

B-G – Chicken MHC class IV

BoLA – Bovine Lymphocytic Antigen

bp – Base pair

BP – Before Present

C – Cytosine

CFU – Colony forming Unit

cm – Centremetre

DANIDA – Danish International Development Agency

DNA – Deoxyribonucleic acid

dNTP – Deoxynucleoside tri-phosphate

EDTA – Ethylene Diamine Tetraacetic Acid

ENRECA – Enhancement of research capacities in developing countries

FAO – Food and Agriculture Organisation

G – Guanine

g- Gramme

HI – Haemaglutination Inhibition

IFN – Interferon

IL – Interleukin

Kg – Kilogramme

ml – Millilitre

mM – Millimolar

MHC – Major Histocompatibility Complex

ng – Nanogramme

nm – Nanometre

Nramp – Natural resistance associated macrophage protein

OIE - Office International d'Epizooties

PCR – Polymerase Chain Reaction

PFGE – Pulse Field Gel Electrophoresis

SDS – Sodium Dodecyl Sulphate

SUA – Sokoine University of Agriculture

T – Thymine

TE – Tris EDTA

TES – Tris EDTA SDS buffer

Tris- Tizma base

CHAPTER 1

1.0 INTRODUCTION

1.1 Background:

The domestic fowl or chicken is one of the most utilised poultry type in the world. Based on the polyphyletic ancestry theory, the domestic fowl is believed to originate from four wild species of Southeast Asian jungle fowls (*Gallus lafayette*, *G. sonnerati*, *G. varius* and *G. gallus*) and is therefore referred to as *Gallus domesticus* (Crawford, 1984). The other theory (monophyletic ancestry) refers the domestic chicken as *G. gallus* assuming the red jungle fowl and its five subspecies as the only ancestors.

The world chicken population stands at 14.14 billion, of which Africa contributes over 1.1 billion equivalent to 8% (FAO, 2001). Based on ownership and utilization, domestic fowl can be classified into three broad categories; industrial chickens, middle-level stock and native or indigenous stocks (Crawford, 1984).

The industrial or commercial chickens are the multi-cross hybrids bred by the multinational breeding companies for production of white or brown-shelled eggs and chicken broiler (Crawford, 1984; Payne, 1990). The middle level stocks are mostly the pure breeds earlier utilised for industrial production and the fancier breeds. This category includes the traditionally kept chickens in the developed countries (Crawford, 1984).

The third category is the native or indigenous stock also known as local chickens/village chickens /scavenging chickens or free-ranging local chickens. These lead a very precarious existence as scavengers with minimum human care (Crawford, 1984). This type of production is attractive to the rural population of the developing world owing to the ever-increasing cost of energy and feeds necessary for the industrial chicken production (Payne, 1990). There are no reports on artificial selection on the local chickens, strongly supporting the view that they are surviving through the process of natural selection, the result of which is a diverse genetic pool (Katule, 1990).

The diversity of the local chickens has mostly been expressed in terms of phenotypic characteristics. Earlier studies have shown that the chickens differ in terms of adult body weight, egg weight, reproduction performance and immune responses to various diseases (French, 1942, Trail 1963, Kabatange and Katule, 1989, Minga *et al.*, 1989, Gwakisa *et al.*, 1994a, Gueye, 1998, Msoffe *et al.*, 2001). Only very few attempts have been made to study the genetic make-up and diversity of the local chickens (Okada *et al.*, 1987; Horst, 1988; Lawrence, 1998, van Marle-Koster and Nel, 2000; Wimmers *et al.*, 2000). For instance, the first attempt to type genetically local chicken in Tanzania was based on blood or serological MHC typing using alloantisera from White Leghorn (WL) chickens (Lawrence, 1998). It was a labour intensive undertaking with encouraging results but which also indicated that it was not proper to use WL alloantisera to type local chickens. In Bangladesh, Okada and others, (1987) calculated the genetic distances of the local Bangladesh chickens using four blood groups and eight plasma proteins concluding that the genetic

distances were narrow hence the local chickens in Bangladesh can be regarded as one breed.

DNA based typing methods provide a rapid and reliable method for differentiating individuals in a genetically diverse population (Bidwell, 1994; Parham and Ohta, 1996). The genetic differences obtained in DNA typing can be linked with some traits of economic importance such as disease resistance (Yonash *et al.*, 1999). Several DNA typing methods exist including, amplified fragment length polymorphisms (AFLP), variable number of tandem repeat (VNTR especially microsatellites), restriction fragment length polymorphisms (RFLP), single nucleotide polymorphisms (SNP), mitochondrial DNA (mtDNA) typing and random amplified polymorphic DNA (RAPD) (Akishinonomiya *et al.*, 1996, Juul-Madsen, 1996; Smith *et al.*, 1996; Weigend and Romanov, 2000). Among the DNA based methods used for genetic typing in local chickens, microsatellite typing has provided better and reliable results (van Marle-Koster and Nel, 2000; Wimmers *et al.*, 2000). In the current study, microsatellite DNA typing was attempted based on the success of the method in typing local chicken elsewhere (van Marle-Koster and Nel, 2000; Wimmers *et al.*, 2000).

Microsatellites are highly polymorphic and abundant in all vertebrate genomes and can easily be typed using polymerase chain reaction (PCR) and scored on an electrophoresis gel (Rincon *et al.*, 2000). As a consequence, microsatellites have emerged as markers of choice in a number of genetic areas such as genome mapping, medical, ecological and evolutionary genetics (Primmer *et al.*, 1997).

The productivity of the local chickens has always been considered to be low (Katule, 1988; Minga *et al.*, 1989; Payne, 1990; Sonaiya 1990; Mwalusanya, 1998; Kitalyi, 1998). However, more recent studies have reported wide ranges in productivity parameters signifying the potential in the local chickens for high productivity that has not been tapped through selective breeding (Minga *et al.*, 1996; Msoffe *et al.*, 1998). For instance Msoffe and others (1998) reported adult body weights of 800g to 2300g in hens and 1000g to 3500g in cocks, whereas in a similar study Minga *et al.* (1996) reported adult body weight ranging between 800g–2450g (hens) and 1650g–3800g (cocks), respectively. On egg weight, Minga *et al.* (1996) reported a range of 25g–56g per egg, while weights ranging from 27g to 72g were reported in another study (Msoffe *et al.*, 1998). It might therefore be the lack of selection and the in-depth knowledge of the genetic potential of the local chicken ecotypes that accounts for the apparent low productivity.

It is generally conceived that the local chickens are naturally resistant to diseases (Kulube, 1990; Chrysostome *et al.*, 1995). However, only a few studies have been conducted to ascertain the natural resistance to diseases in local chickens. In one study, five local chicken ecotypes in Tanzania were described and one ecotype was shown to have some resistance to experimental fowl typhoid (Lawrence, 1998; Msoffe *et al.*, 2002). Nevertheless, the study did not involve all the possible ecotypes found in the region. In another study, the Nigerian local chickens were found to be more susceptible to Gumboro disease than the exotic chickens (Okoye and Aba-Adulugba, 1998). Disease resistance is a trait often controlled by multiple genes as well as interactions between several factors (Hartmann, 1997). However, some

specific gene loci such as the major histocompatibility complex (MHC) and the natural resistance associated macrophage protein 1 (Nramp1) have been proven to be directly linked with resistance to different diseases (Bacon, 1987; Lamont, 1998).

The major bottlenecks facing the local chicken sector are the low genetic potential, diseases and poor management practices (Minga and Nkini, 1986; Payne 1990; Mwalusanya; 1998, Kitalyi, 1998). Most of the previous efforts to improve the productivity of the local chickens were based on the improvement of the genetic potential through crossbreeding with the exotic breeds (Katule, 1990; Payne 1990; Kitalyi, 1998). It is unfortunate to note that all those efforts could not be able to solve the problem of low productivity in local chickens and to date the productivity remains low. This in part might be compounded by the fact that very little is known about the genetic potential of the local chicken.

Therefore, this study was aimed at establishing the phenotypic and genetic diversity of the local chicken ecotypes in Tanzania based on productivity data (body weight, egg weight and reproductive data), disease resistance (fowl typhoid and Newcastle disease) and genetic diversity at DNA level. The identification of diverse chicken ecotypes will be a crucial step in the quest to increase productivity among the free-range local chickens. Identification of disease resistant ecotypes will reduce the need for drugs used in treatment (which is usually expensive) and will compliment vaccination programmes and improved biosecurity measures. This will in turn increase local chicken population, the off-take rate and hence improve the human nutrition and the income of the rural poor.

1.2 Justification

The local chickens (kept under the free range management system) are essential for the livelihood of the resource-poor farmers in terms of provision of animal protein, manure and a quick source of income through the sale of live chickens and eggs (Spradbrow, 1997; Gueye, 1998; Kitalyi, 1998). The chickens also serve for some traditional and social functions (Sonaiya, 1990). Nevertheless, productivity of the local chickens is low, morbidity and losses due to diseases and predation is high while the cost of medicines and vaccines for disease control is high and supplementary feeds are scarce (Kitalyi, 1998). However, the local chicken survive amidst all these constraints with virtually no or very little input from the owners (Crawford, 1984). Variations within the Local chickens in productivity parameters and disease resistance (French, 1942; Kabatange and Katule, 1989; Minga *et al.*, 1989; Lawrence, 1998; Msoffe *et al.*, 2001) justify the need to study them in details as a prelude to purposeful selection. Studies on productivity of different ecotypes found in Tanzania will serve as a benchmark in selection strategies aimed at increased productivity. Identification and breeding of disease resistant ecotypes of high productivity will ensure lower mortality, increased off-take rate, reduce the need for costly drugs used in the treatment of diseases and will complement vaccination programmes. The nutrition and income of the rural poor will hence be improved.

1.3 Objectives

1.3.1 Main objective

To identify the local chicken ecotypes in Tanzania and assess their genetic and phenotypic diversity.

1.3.2 Specific objectives

a. To optimise a reliable molecular (DNA based) typing method for the local chickens.

b. To genetically characterise local chicken ecotypes based on the DNA typing methods.

c. To compare the productivity of the identified local chicken ecotypes.

d. To compare the identified local chicken ecotypes in terms of disease resistance using Newcastle disease virus and *Salmonella gallinarum*.

e. To assess the productivity of local chicken ecotypes under free-range management conditions.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The domestic fowl (*Gallus domesticus/G.gallus*)

There are four wild species of fowl (Southeast Asian jungle fowls) from which the domestic fowl is believed to originate. The four species are: the red jungle fowl (*G. gallus*) distributed throughout India, China and the Pacific islands, the green or Javan jungle fowl (*G. varius*), of Java and Indonesia, the grey jungle fowl (*G. sonnerati*) of Southern India and the Singalese jungle fowl (*G. lafayetti*) of Sri Lanka (Payne, 1990).

The literature is divided on the origin of the domestic fowl with one group believing in the monophyletic and another on polyphyletic theories of origin.

The monophyletic theory deem the domestic fowl as originating from the red jungle fowl (*Gallus gallus*) and its five subspecies namely *G. gallus gallus*, *G. gallus spadiceus*, *G. gallus jabouillei*, *G. gallus murghi* and *G. gallus bankiva* (Simonsen, 1996 citing Crawford, 1990). There is strong evidence supporting the monophyletic origin of domestic fowl in the form of mitochondrion DNA sequences (Akishinonomiya *et al.*, 1996). Akishinonomiya and others (1996), showed that most of the subspecies of the red jungle fowl (*G. gallus*) were very close to the present day domestic fowl. The subspecies status of *G. g. spadeceus* was questioned owing to its closeness to *G. g. gallus* (Akishinonomiya *et al.*, 1996). In an earlier study, Akishinonomiya and others (1994), reported a 60-bp unit in the mitochondrion D-loop whose distribution within *G. gallus* was similar to that in domestic fowl, but

different from the other three jungle fowl species. Another evidence supporting the single species origin is the fact that although all the four wild species hybridise with the domestic fowl only the red jungle fowl produce fully viable and reproductive off-springs (Crawford, 1984). In this case the domestic fowl is referred to as *Gallus gallus.*

On the other hand, the polyphyletic theory consider that all the four wild species of fowl contributed some genes to the domestic fowl although the red jungle fowl may have been the main contributor, the chicken is hence referred to as *G. domesticus* (Crawford, 1984, Simonsen, 1996). It is important to note that, although the two names (*G. gallus* and *G. domesticus)* are accepted in literature, there is more evidence in support of the monophyletic origin than there is for the polyphyletic theory. For that reason, the domestic fowl in this text will be referred to as *Gallus gallus.*

Through its history of domestication, the domestic fowl has undergone several changes in its external appearance, such as body weight (dwarfs and giant breeds), colourings (uni or multivariate), feather cover (naked-necks), and posture (horizontal and upright) (Stern, 1988). The centre for domestication or the person who first tamed the fowl is unknown (Payne, 1990; Simonsen, 1996). The earliest evidence of domestication, which is conclusive, is that of the Indus valley over 4000 years before present (BP) (Crawford, 1984, Payne, 1990, Simonsen, 1996). However, archaeological findings in China have recently dated chicken domestication to 8000 years BP, prompting Simonsen, (1996) to conclude that the history of domestication

of fowl is as old as that of man. It is believed that from the Indus valley chickens were spread to Iran, then westward to Mesopotamia and Asia Minor (Crawford, 1984 citing Zeuner, 1963). Chickens arrived in Europe through Iran and the Mediterranean and probably from China and Russia (Crawford, 1984 citing Brown, 1929). There is much controversy as to the migration of chickens to the America. Some evidence points to the pre-Columbian era (most likely from Asia) while others ascribe the chickens in America to the colonial conquests (Crawford, 1984).

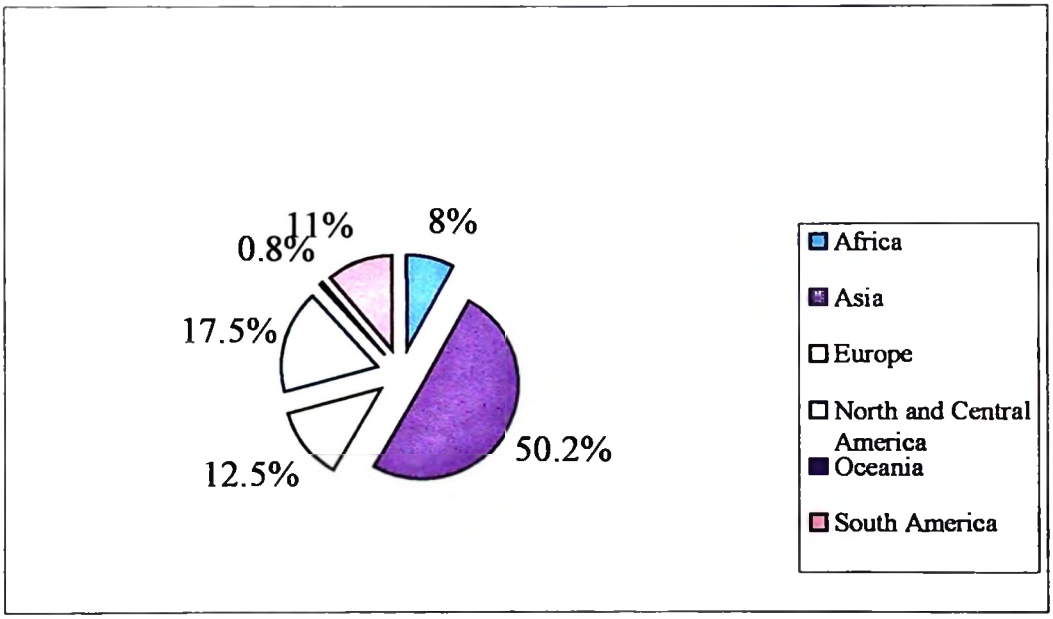
Chickens in Africa apart from Egypt were probably brought from India as there is evidence of their presence at the time of first colonial contacts and the chickens described in Mozambique in 1635 were similar to those described in India (Crawford, 1984 citing Sauer, 1969). Egyptian chickens were distributed directly from the Indus valley but not from India as is the case for the rest of Africa (Crawford, 1984). It is widely accepted that initially chickens were not kept for food rather for cockfighting and for cultural and religious functions but later the people began to eat eggs and finally the meat (Crawford, 1984; Payne, 1990; Simonsen, 1996).

Chicken adaptability has proved to be immense both in climatic and to the demands of man chiefly good egg laying, good meat production and good fights in cock fighting (Simonsen, 1996). In Tanzania, chickens are kept mainly for food, but they also serve as a quick source of income through the sale of live chickens and occasionally eggs. Local chickens in Tanzania have some specific social uses such as

payments of dowry, gifts and are also known to be important in some traditional religious functions.

2.2 The Chicken Industry

According to FAO (2001), the number of chickens in the world stands at over 14 billion with over half (7.1 billion) being found in Asia and less than 1% found in Oceania (107 million). Africa has over one billion (8%), Europe 1.8 billion (12.5%), North and Central America 2.5 billion (17.5%), and South America 1.6 billion (11%).



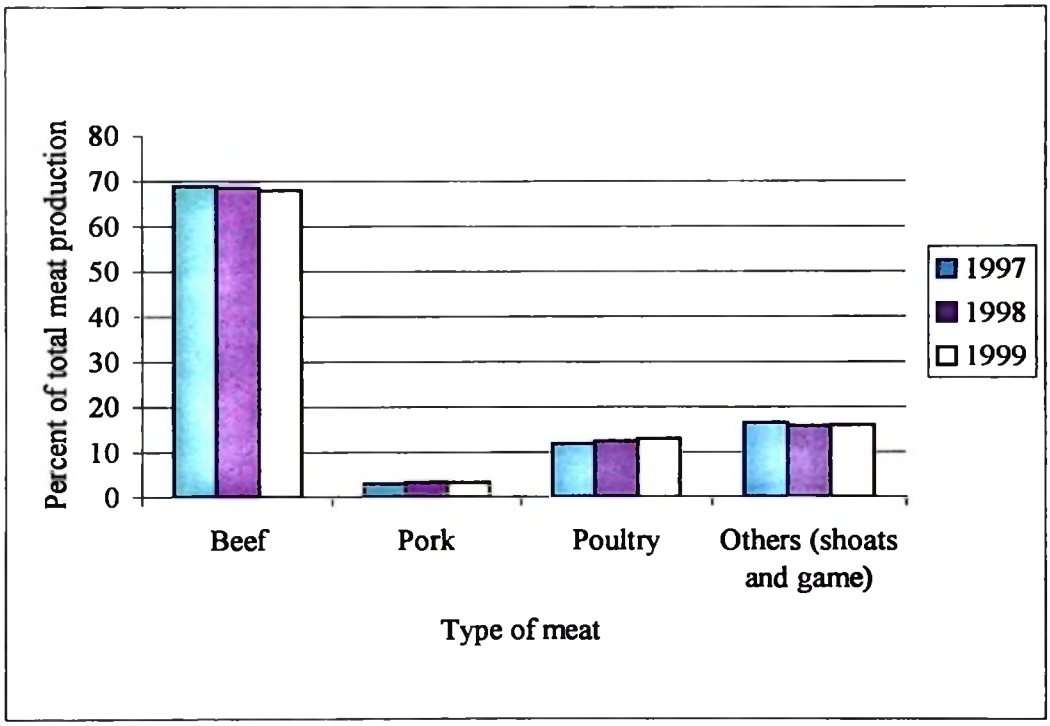
Source: FAO, 2001.

Figure 1: Distribution of chickens in the world (shown as %).

The world egg production has increased tremendously from 45.8 million metric tonnes in 1997 to 47.6 million metric tonnes in 1999 (FAO, 2001). During the same period of time, the world's poultry meat production also increased from 59 million

metric tonnes to 63.2 million metric tonnes (FAO, 2001). Expressed as a percentage of the total meat production in the world, poultry meat accounted for 27.5% and 28% in 1997 and 1999, respectively.

In Tanzania, beef continue to contribute a large percentage of the total meat produced annually (FAO, 2001). However, poultry meat contribution to the total meat production has increased from 11.8% in 1997 to 13% in 1999 (Figure 2).



Computed from FAO, 2001.

Figure 2: Poultry meat production in Tanzania (1997-1999) plotted with other major meat sources.

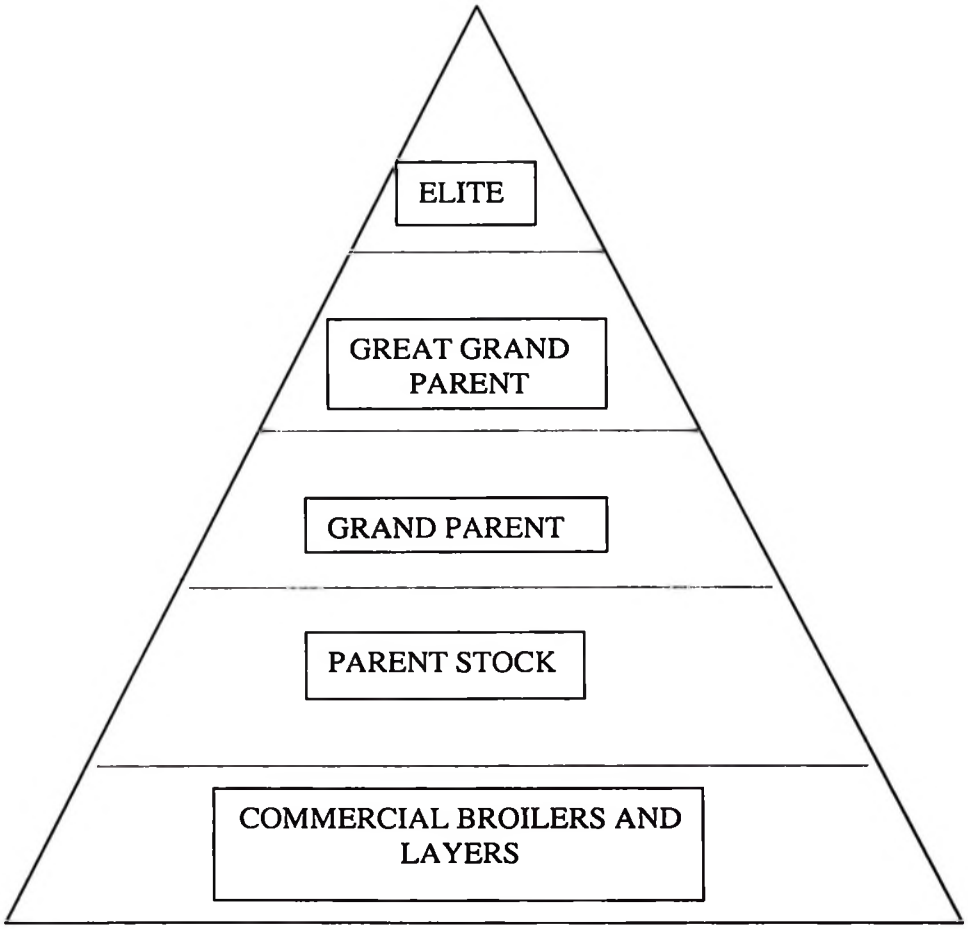
The world's poultry industry can be divided into three main groups: the industrial or commercial chickens, the middle stock and the native or indigenous chickens (Crawford, 1984).

2.2.1 The industrial/commercial chickens

The success of the industrial or commercial chicken industry is based on consumer demand, healthy flocks and least cost production (Dekich, 1998). The introduction of intensive production systems utilizing cutting edge technologies coupled with efficient disease control strategies has enhanced the continuing growth of the industry (Law and Payne, 1996). The relative ease with which new technologies can be transferred between countries, acceptability of the meat to all religions as well as their competitive costs are some of the key factors in the continued growth of the industry (Law and Payne, 1996).

The basic structure of the chicken industry forms a breeding pyramid with the elite stock at the apex and the commercial layers and broilers at the base (Figure 3). All the intensive, careful breeding and selection at the elite to grandparent level is done by multinational corporations referred to as primary or basic breeders (Law and Payne, 1996, AAFC, 2000). The primary breeders' main functions are to maintain pure bloodlines, expand pure designated bloodlines and develop crossbred bloodlines (AAFC, 2000). Primary breeders require high capital investments in research to be competitive as a consequence; there are only a few multinational corporations that have resulted from amalgamations and acquisitions between companies. Over the last two decades, the number of primary breeders has decreased from 11 (each for broiler

and layers) in 1984 to eight (layers) and ten (broilers) in 2000 (Crawford, 1984, AAFC, 2000). Breeding and selection work at the primary breeder is based on four purebred grand parents lines A, B, C and D which are mated to form two-way cross male parent AB and a different two-way cross female parent CD (Crawford, 1984).



Source: Law and Payne 1996

Figure 3: Industrial poultry breeding pyramid.

The parent stock are then utilised by franchised hatcheries throughout the world as multiplier flocks where a four-way cross ABCD is produced for table egg and broiler meat production (Crawford, 1984). The chickens are selected for high genetic potential and uniformity in phenotypic characters hence their performance worldwide is very similar (Crawford, 1984). Selection in the layer chickens has been towards increasing the number of eggs per annum and reducing the weight of the chickens. Under ideal industrial settings, a white-shelled layer will produce 250-300 eggs annually weighing an average of 62 g (Crawford, 1984; Payne, 1990; Ansah, 2000). In broiler production, the continuous genetic selection is responsible for fast growth, better feed conversion and high meat output on annual basis (Dekich, 1998). Broilers attain up to 1.9 kg live weight in just five weeks with very high feed conversion ratio (Crawford, 1984; Payne, 1990).

The commercial chicken sector in the developing world is invariably located in the urban and peri-urban areas where exotic hybrid chickens are reared in form of small commercial projects among the urban dwellers (Sonaiya, 1990; Kitalyi, 1998). Availability of feeds, drugs and vaccines, labour, markets and capital required for housing and equipment, are some of the limitations that prevent rearing of exotic hybrid chickens in the rural areas (Kitalyi, 1998). The location of the commercial chicken production near the urban centres ensures the availability of the market for meat and eggs produced in this sector (Mbugua, 1990).

2.2.2 The indigenous/local chickens

The contribution of the local chickens to the livelihood of the poor people in the developing countries cannot be over emphasised. It is a sector appropriate to feed the fast growing human population, provide income for the rural poor (particularly women) and make best use of the locally available resources (Sonaiya, 1990; Gueye, 1998; Branckaert *et al.*, 2000). Researchers across Africa, Asia and Latin America have consistently stated the importance of local chickens to the life of the rural poor (Sonaiya, 1990; Barua and Yoshimura, 1992, Kitalyi, 1998; Gueye, 1998; Samang, 1998; Kyvsgaard *et al.*, 1999; Mallia, 1999; Farooq, 2000; Ndegwa, 2001). For instance, although the number of exotic poultry farms in Bangladesh is substantial poultry keeping is still a rural household activity (Barua and Yoshimura, 1992). Furthermore, in Tanzania the commercial chicken sector has had very little impact on the economy and nutrition of the majority rural poor (Minga *et al.*, 2000). In Cambodia, even the poorest family will have at least 5-6 hens and a cockerel (Samang, 1998).

The local chicken production system in Tanzania as in many other developing countries is the free-range type (Katule, 1988; Kabatange and Katule, 1989). It is not known exactly when chicken were first domesticated in Tanzania, but worldwide the domestication of chickens may be as old as civilisation of man (Simonsen *et al.*, 1989) and that in Tanzania, local chickens have been kept since time immemorial (Kabatange and Katule, 1989). According to Melewas (1989), the local chickens are found almost in every place with human settlement in Tanzania. The fact that chickens are not affected by vector borne diseases such as Trypanosomosis, which

have restricted ruminants distribution, that they have a short generation interval and can survive on kitchen leftovers, eat grass and insects and convert these cheap and easily available materials into high quality proteins, favours their wide distribution (Kabatange and Katule, 1989; Melewas, 1989). Under this traditional management system, the local chickens are allowed freedom to scavenge around the homesteads in the daytime, to pickup whatever feed is available in the environment, usually ranging from insects, seeds, leftovers to grass (Kabatange and Katule, 1989; Melewas, 1989; Williams, 1990).

Breeding under this traditional management system is random and non-selective (Horst, 1990). It is usually the most aggressive, strong and dominant cock, which sires most offsprings in the neighbourhood (Williams, 1990). The lack of controlled breeding program is responsible for the genetic heterogeneity found in the local chicken populations (Minga *et al.*, 1996).

In Tanzania, the average household flock size was reported to be 2-20 birds (Melewas, 1989), 23-30 (Minga *et al.*, 1996) and more recently 2-58 (Mwalusanya, 1998). There is lack of proper shelter for the chickens, in some places they share the house with the people (usually the kitchen) or live in makeshift shelters some are even left to roost on trees (Yongolo, 1996; Mwalusanya, 1998). Little effort has been directed to the control and treatment of various diseases and hence exposing the chickens to great health risks (Melewas, 1989).

Diseases have been cited as the major drawback in the existence of the local chicken industry (Sonaiya, 1990; Gueye, 1998; Kitalyi, 1998). Newcastle disease is reported to be the most important disease (Melewas, 1989; Minga *et al.*, 1989; Awan *et al.*, 1994). Other diseases reported include, Gumboro, coccidiosis, fowl pox, fowl typhoid, fowl cholera, infectious coryza, chronic respiratory disease (CRD) and both internal and external parasites (Melewas, 1989; Yongolo, 1996). There is usually high rate of predation from wild animals, birds, as well as stray dogs and cats (Melewas, 1989; Mwalusanya, 1998). The above-mentioned factors together with the un-exploited genetic potential in the local chickens account for the low production and productivity (Katule, 1988; Melewas, 1989; Minga *et al.*, 1989). To date only scanty information is available on the local chickens' phenotypic and genotypic characteristics, productivity and natural disease resistance to diseases endemic in Tanzania (Lawrence, 1998).

2.2.1 Local chicken Breeds/Varieties/Ecotypes:

The term breed refers to a group of animals that have similar appearances and usually developed by deliberate selection (Crawford, 1984). Breeds in chickens have their description carefully laid down and each breed has its own standards and register (Crawford, 1984). Variations particularly in colour or colour patterns or comb resulted in classification of varieties within breeds (Crawford, 1984). Used in this context, it becomes difficult to ascertain the legitimacy of classifying local chickens into breeds, as their characteristics are not well known, neither are the standards and register available. Sonaiya, (1990) pointed out that most of the previously reported local chicken breeds were probably the phenotypic descriptions

of the same breed. Indeed apart from Fayoumi breed developed in Egypt, no record exists in Africa for instance of tropical adapted breed developed from local chickens (Gueye, 1998; Kitalyi, 1998). Most local chickens are therefore better described as ecotypes meaning they originated from different eco-climatic regions of a country (Gwakisa *et al.*, 1994a; Malia, 1999; Rodriguez and Preston, 1999; Msoffe *et al.*, 2001). Table 1 gives a list of names for some local chickens from different parts of the world. However, when no specific description of local chickens into breeds or ecotype exists, the chickens have taken the name of the country or location. Examples are Kenyan local chickens, Malawian local chickens or Senegalese local chickens (Safolouh *et al.*, 1997; Gueye *et al.*, 1998; Ndegwa *et al.*, 2001). There might be other breeds and ecotypes of local chickens that are yet to be identified and characterised.

Table 1: Some of the local chicken breeds/ecotypes of the world

Country	Breed/ecotype	Characteristics
Morocco	Beldi	Fibre handling ability
	Roumi	-
Egypt	Fayoumi	-
	Dandarawi	-
Comoros	Dokky	-
Sudan	Baladi	Medium size, meat type (1.6 kg Bw). Small comb, low egg production
	Betwil	Medium size, Egg type
Cameroon	Dzaye	White feathers
	Tsabatha	Grey, black and white feathers, meat type
	Dongwe	Black feathers, Layer strain
	Zarwa	
Mali*	Kokochie	Black and white feathers
	Balachie	Frizzled feathers
	Kolokochie	Naked body
	Touloukenechie	Red spotted comb
	Centicrochie	Five digits
Botswana	Tswana	
S. Africa	Lebowa venda	
Namibia	Ovambo	

Table 1: Contd.

Country	Local breed/ecotype	Characteristics
Tanzania	*Ching'wekwe*	Very short shanks, single comb, small eggs
	Kuchi	Long shanks, vertical stance, rose comb
	Mbeya	Medium sized, black plume, single comb
	Morogoro-medium	Medium sized, single comb,
	Singamagazi	Tall and heavy cocks, single comb
Japan	Iwate-Jidori	
	Aizu-Jidori	
	Sodahige-Jidori	
	Siba-Tori	
	Onaga-Dori	
	Echigo-nankin	
	Hinai	
	Kinpa	
	Koeyoshi	
	Tomaru	
Bangladesh	Desi	
Denmark	Danish landrace	
Finland	Finnish landrace	

Table 1: Contd.

Country	Breed/ Ecotype		Characteristics
India	Aseel		
	White Chittagong		High number of eggs (130 per year)
Nigeria	Yoruba names	Hausa names	
	Alaradidan	Ja	Smooth feathers, basically red with some black at the tip
	Adiye dudu	Beki	Smooth feathers black all over
	Adiye funfun	Ferri	Smooth feathers white
	Abolorum	Pingi	Bare neck
	Asa	Shazumana	Frizzled feathers
	Arupe	Durugu	Dwarf
	Opipi	-	Without flight
	Goloba	Godogodo	Legged
		Wakewake	Mottle coloured
		Danya Ferri	Light brown with some of mottling of white and black
		Kwoi Makera	Layer strain. Mottle colour of silver, black and white.

*Kassambra (1990), described 15 breeds of chicken and four breeds of guinea fowl based entirely on feather colour.

Source: Payne, 1990; Sonaiya, 1990, Barua and Yoshimura, 1997; Takahashi *et al.*

1998; Vanhala *et al.* 1998; Aganga *et al.* 2000; van Marle-Koster and Nel, 2000; Permin and Ravnig, 2001; Msoffe *et al.* 2001.

2.2.2 Productivity in local chickens

Local chicken breeds and ecotypes show great variations in productivity as assessed by adult body weight, egg production per annum and egg weight (Payne, 1990; Zaza, 1992; Barua and Yoshimura, 1997; Mwalusanya, 1998; Aganga *et al.*, 2000; Msoffe *et al.*, 2001). The variations in productivity parameters for some local breeds or ecotypes is given in Table 2 below:

The productivity of the local chickens has been reported to be low (Katule, 1988; Minga *et al.*, 1989; Sonaiya, 1990; Kitalyi, 1998; Mwalusanya, 1998). The low productivity has been attributed to the low genetic potential and poor husbandry (Katule, 1988; Kitalyi, 1998). In Tanzania, daily growth rate for chicks under the free-range production system from day old to ten weeks of age has been reported to be 4.6g/day for females and 5.4g/day for males (Mwalusanya, 1998). Katule (1988), reported juvenile body weights for female local chickens to be 61.8g at 4 weeks and 731g at 16 weeks, figures which are lower compared to chickens of the same age but from commercial egg type breed used in the study (117g and 1025.5g respectively).

Table 2: Productivity of some local chickens breeds/ecotypes

Breed/ecotype	Adult body weight (kg)		Eggs/year	Egg weight (gm)	Reference
	Male	Female			
Aseel	4.5	3.6	35	-	Payne, 1990
White Chitaagong	-	-	130	-	Payne, 1990
Fayoumi	1.95	1.65	160	42	Zaza, 1992
Dandrawi	2.46	1.78	153	48	Zaza, 1992
Tswana	2.2	2.0	28-38	38-60	Aganga *et al.*, 2000
Desi		1.0-1.2	35-40	37	Barua and Yoshimura, 1997
Ching'wekwe	2.1	1.4		37.6	Msoffe *et al.*, 2001
Kuchi	2.7	1.8		45	Msoffe *et al.*, 2001
Singamagazi	2.9	2.0		45.6	Msoffe *et al.*, 2001
Mbeya	1.6	1.4		41	Msoffe *et al.*, 2001
Morogoro-medium	1.85	1.2		38	Msoffe *et al.*, 2001

Adult body weights ranging from 800g to 2300g for females and 1000g to 3500g for males have been reported in the local chickens (Kabatange and Katule, 1989; Minga *et al.*, 1989, Lawrence, 1998; Mwalusanya, 1998). The local chicken begins laying at 6 to 9 months of age and may lay 66 to 78 eggs per annum in two or three laying cycles (Minga *et al.*, 1989; Mwalusanya, 1998). Eggs per clutch averaged 12 (ranging 6 to 28 eggs), averaging 38g to 46g in weight and hatchability was recorded at 83.6% (Lawrence, 1998; Mwalusanya, 1998). Chick survival up to eight weeks of age was recorded at around 60% (Mwalusanya, 1998).

2.3 Genetic characterisation of animals

Animal genetic resources are populations that show a wide range of genetic characteristics resulting from domestication (Weigend and Romanov, 2000). Major forces behind creation of genetic differences between breeds and populations are mutation and recombination together with genetic drift, selection and migration (Falconer and Mackay, 1996; Weigend and Romanov, 2000). As a consequence of genetic ability and differences in management, variations in production, health, reproduction and conformational traits are seen between animals (Oldenbroek, 1999). Accurate description of genetic variations within animal species is important for conservation purposes as well as other purposes (Oldenbroek, 1999). Conservation of animal genetic resource is important in order to cater for possible future market demands, insurance against future production environment, present socio-economic values, opportunities for research as well as cultural, historical and ecological values (Gandini and Oldenbroek, 1999; Oldenbroek, 1999).

To establish the extent to which a breed is different from all others (i.e. its uniqueness), the diversity in a set of breeds must be studied (Eding and Larval, 1999). The diversity between populations can be studied by means of mathematical tools that translate the difference to a measure of distance between population pairs (Eding and Larval, 1999). In most studies, genetic distances are measured as differences in allele frequencies in different populations using specific markers (genetic markers). The genetic markers commonly used include, biochemical markers (blood types, allozymes) and DNA polymorphic molecular markers (AFLP, mtDNA, RAPD, RFLP, VNTRs, SNP) (Eding and Larval, 1999). In animal genetics and production, DNA typing methods are now used extensively in search for quantitative trait loci (QTLs), which are useful in marker assisted selection (Wei *et al.*, 1997; Spelman and Bovenhuis, 1998).

2.3.1 Biochemical markers

2.3.1.1 Blood typing

The classical examples of using blood typing to determine variations between individuals are the human ABO blood grouping and the chicken B-complex (Briles *et al.*, 1982; Janeways *et al.*, 1999). The principle behind ABO and B-complex typing (serological typing) is the presence of surface antigens on erythrocytes which on binding to the corresponding antibodies result in alteration of their physical state specifically agglutination (Janeways *et al.*, 1999).

Although haemaglutination reactions are faster, and less laborious, they lack the precision provided by DNA based molecular markers. For instance, serological B-

complex typing in chickens is greatly affected by the cross-reactivity between alloantisera and it follows that alloantisera raised against one breed of chickens cannot be used effectively in typing another breed (Simonsen *et al.*, 1989; Kroemer *et al.*, 1990; Lawrence, 1998). The former is due to the complexity of antibodies that are directed against both B-F and B-G antigens of the erythrocytes (Lanqing *et al.*, 1999). Nonetheless, serological B-complex is responsible for the discovery of chicken MHC that is linked to a number of useful traits including disease resistance and productivity (Briles *et al.*, 1950, Plachy *et al.*, 1992, Lunden *et al.*, 1993, Kauffman and Wallny, 1996). Haplotype B21 of White Leghorn breed has been reported to be resistant against Marek's disease while B19 is a susceptible haplotype (Simonsen, 1987; Lamont, 1998). Blood grouping has also been used to determine the genetic distance between local chickens in Bangladesh (Okada *et al.*, 1987).

2.3.1.2 Allozymes

Allozymes are one of a number of forms of the same enzyme with different electrophoretic mobilities (Lawrence, 1995). For many years allozymes were the main nuclear genetic markers used in evolutionary genetics in most species (Hedrick, 1999). Allozymes may give valuable insights into the general patterns of evolution (Hedren *et al.*, 2001). Allozymes have recently been used in studies of genetic diversity in Chinese native chickens (Zhang *et al.*, 2000). However, they lack the polymorphisms inherent in DNA based typing techniques (Hedrick, 1999; Hedren *et al.*, 2001; Sun and Wong, 2001).

2.3.2 DNA molecular markers

2.3.2.1 Mitochondrial DNA analysis

Mitochondrial DNA (mtDNA) and genes have been used extensively in molecular evolution studies and have high potential to demonstrate the power of evolutionary genomics (Pollock *et al.*, 2000). The advantages of these markers include their presence in high concentrations in many tissues, ability to be amplified by PCR, and the ease with which they can be enriched by purification of mitochondria prior to DNA extraction (Pollock *et al.*, 2000). Mitochondrial genomes are highly conserved as exemplified by the fact that all vertebrate mtDNA analysed carried the same 37 genes (Mindell *et al.*, 1998). mtDNA has no recombination, hence the number of nucleotide differences between mitochondrial genomes is a direct portrayal of the genetic distances separating them (Toro and Maki-Tanila, 1999).

mtDNA sequence variations have been used to study the genetic variation and evolutionary patterns in giant Galapagos tortoises (Caccone *et al.*, 1999), domestic goats (MacHugh and Bradley, 2001), Japanese black cattle (Mannen *et al.*, 1998), house mice (Prager *et al.*, 1998), birds (Mindell *et al.*, 1998) and has provided evidence for monophyletic ancestry of domestic fowl (*G. gallus*) (Akishinonomiya *et al.*, 1996).

However, mtDNA analysis has a major disadvantage in that it is based on only one segregating locus that may not always represent the ancestry of the whole genome (MacHugh and Bradley, 2001).

2.3.2.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP is one of the most powerful PCR-based markers enabling discrimination of organisms by DNA analysis (Quagliaro *et al.*, 2001). It is a relatively new DNA marker technology based on the selective amplification of restriction fragments to simultaneously produce multiple polymorphic markers that can be tested on a single PCR reaction (Vos *et al.*, 1995; Otsen *et al.*, 1996). It has proved convenient and reliable in generating highly polymorphic molecular markers that facilitate the construction of genetic linkage maps (Qi *et al.*, 1997). In this technique, a number of restriction fragments (50 – 100) from a total digest of genomic DNA are amplified in PCR using oligonucleotide adapters and restriction site DNA sequences as target for primer annealing (Vos *et al.*, 1995). AFLP is advantageous in that no prior information on the genomic DNA sequence is required and the markers are highly polymorphic (Otsen *et al.*, 1996). It also enables quick and efficient sampling of a very large array polymorphisms (Tan *et al.*, 2001). AFLP patterns are reproducible and polymorphic fragment segregates in Mendelian fashion (Ajmore-Marson *et al.*, 1997).

AFLP has been employed successfully in building maps and characterisation for various economically important organisms including chickens (Herbergs, *et al.*, 1999; Knorr, *et al.*, 1999), rat (Otsen *et al.*, 1996), Italian goat populations (Ajmore-Marsan *et al.*, 2001) and cattle (Ajmore-Marsan *et al.*, 1997).

2.3.2.3 Random amplified polymorphic DNA (RAPD)

RAPD is a procedure based on random amplification by PCR of DNA segments using short oligonucleotide primers that anneal at low temperatures (Williams *et al.*, 1990). Primers for RAPD are chosen randomly, hence no prior knowledge of the DNA sequence is necessary and each primer or combination of primers will yield low-molecular-weight DNA fragments that can be resolved on agarose gels (Rothuizen and Van Wolferen, 1994; Wei *et al.*, 1997). RAPD markers are dominant-recessive in that a band may be present (dominant) or absent (recessive) (Williams *et al.*, 1990). This marker system is attractive to researchers and appropriate for screening genetic variations in out-bred populations due to the ease with which many polymorphic RAPD markers can be generated and the direct scoring of the results on agarose gels (Gwakisa *et al.*, 1994b). RAPD analysis can provide a higher number of reproducible marker loci than allozymes, is easier and faster to perform than microsatellites analysis (Sun and Wong, 2001).

This method has been used in the construction of the autosomal linkage map in chickens (Levin *et al.*, 1993, 1994), characterisation of Zebu cattle breeds in Tanzania (Gwakisa *et al.*, 1994b), differentiation of *Bos taurus* and *B. indicus* (Kemp and Teale, 1994), distinguishing inbred lines of chickens (Wei *et al.*, 1997) and developing suitable genetic markers for dogs (Rothuizen and Van Wolferen, 1994). RAPD analysis is increasingly being used in studies of natural plant populations (Busell, 1999; Sun and Wong, 2001).

There are several pitfalls associated with RAPD analysis including the complexity of the resultant fingerprints, inability to distinguish heterozygotes from homozygotes and the problem with reproducibility of the patterns (Gwakisa *et al.*, 1994b; Kemp and Teale, 1994; Rothuizen and Van Wolferen, 1994; Sun and Wong, 2001).

2.3.2.4 Restriction Fragment Length Polymorphism (RFLP)

RFLP is a method entailing the digestion by restriction endonucleases (restriction enzymes) of purified or partially purified DNA to yield an array of segments called restriction fragments (Dowling *et al.*, 1996; Tang *et al.*, 1997; Arens, 1999; Weaver, 1999). The endonucleases recognises specific palindromic sequences in the DNA and cleave on or close to these sites also called restriction sites (Weaver, 1999). The resulting restriction fragments can be presented directly on stained electrophoresis gels or on solid media (such as nitrocellulose paper) after Southern blotting and hybridisation with specific DNA probes (Arens, 1999; Soll, 2000). The variations in the number of restriction fragments within species is due to mutations that remove or add a restriction site (Arens, 1999; Roizes, 2000). RFLP were the first markers available for genetic analysis (Roizes, 2000).

RFLP has been used in different genetic studies including the construction of genetic linkage maps for human and chicken (Donis-Keller *et al.*, 1987; Bumstead and Palyga, 1992). RFLP has also been instrumental in MHC typing in the chicken (Juul-Madsen, 1993, 1997; Uni *et al.*, 1993). Using RFLP, six groups of *Mycobacterium ulcerans* were classified (Chemlal *et al.*, 2001a, 2001b). Furthermore, this technique was applied in characterisation of *M. tuberculosis* from eight countries in Western

pacific (Park *et al.*, 2000). RFLP was also among the methods used in the epidemiological investigations of *M. bovis* infection (Costello *et al.*, 1999).

However, most RFLP studies requires large quantities of good quality DNA, is labour intensive with hybridisation steps being difficult to automate. However, the use of PCR-RFLP techniques in genetic studies has reduced the problem of using genomic DNA. The informativeness of RFLP markers is low compared to other DNA based methods.

2.3.2.5 Single nucleotide polymorphisms (SNPs)

SNP is a very promising novel molecular marker system that enables the assessment of genetic diversity in farm animals by examining the mode and extent of changes in specific genomic positions (Weigend and Romanov, 2000). SNPs are useful for gene mapping and it is expected that these markers may replace microsatellite markers as informative markers in linkage studies (Kryglyak, 1997). These markers are particularly advantageous to use in genetic studies because they are more frequent than microsatellites hence are nearer or in the loci of interest (Sayers *et al.*, 2000; Zhao and Zarbil, 2000). SNPs are stable mutationally and the genotyping can be automated with high throughput enabling the identification of even the subtle genetic risks (Sayers *et al.*, 2000; Weigend and Romanov, 2000). SNPs have been used in molecular genetic epidemiology of diseases (Sayers *et al.*, 2000) and also on detection and genotyping of cytokine genes (Keen, 2000). The potential disadvantages of these novel markers includes low polymorphisms, generation of large amount of data that requires careful handling and interpretations and also that most SNPs are in non-coding regions of the genome (Zhao and Zarbil, 2000).

2.3.2.6 Variable numbers of tandem repeats (VNTRs)

Genomes, particularly eukaryotic often carry regions of DNA with a single base or a short sequence that is tandemly repeated (Debrauwere *et al.*, 1997). These repeats are classified into three main types namely, satellites, minisatellites and microsatellites (Amour *et al.*, 1999).

2.3.2.6.1 Satellites

Satellite repeats can occupy a large percent of the total genome and the individual array may be as big as 5Mbp (Amour *et al.*, 1999). Satellites are only used occasionally for genotyping individuals, and may be typed by means of Southern blot hybridisation after PFGE, restriction digests or PCR with primers detecting locus-specific unit variants (Amour *et al.*, 1999).

2.3.2.6.2 Minisatellites

Minisatellites entails repeat length of over 10bp with repeat blocks of 0.5 to 30 kb and the number of loci per genome may be as many as hundreds or thousands (Amour *et al.*, 1999). Minisatellites can be typed by multi-locus fingerprinting a hybridisation-based method using low-stringency probes or single-locus genotyping that utilises high stringency probes or PCR (Amour *et al.*, 1999).

2.3.2.6.3 Microsatellites

Microsatellites are DNA sequences containing one sequence motif of up to six bases long, arranged head-to-tail without interruption by any other base or motif i.e. tandemly repeated (Hancock, 1999). They show high levels polymorphisms, are

highly informative, abundant and uniformly distributed in different eukaryotic genomes (Tautz, 1989; Rapley and McDonald, 1992; Susol *et al.*, 2000). Within the first decade of their introduction, microsatellites have developed into markers of choice in a host of genetic areas such as genome mapping as well as medical, evolutionary and ecological genetics (Primmer *et al.*, 1997; Ritz *et al.*, 2000). Microsatellites are also favoured as genetic markers of choice because of their neutrality i.e. they are neutral markers (Schloterer and, 1999).

Microsatellites are described according to the number of repeats in a unit hence there are mononucleotide repeats (for example A), dinucleotide (AT), trinucleotide (CAC), tetranucleotide (TTTA), pentanucleotide (AAGAG), and hexanucleotide repeats (AACAGG) (Rapley and McDonald, 1992; Gábor *et al.*, 2000). In human genome poly (A)/poly (T) are the most prevalent mononucleotide repeats with CA/TG and CAG/AAT the most common di-and tri-nucleotides repeats respectively (Hancock, 1999). Microsatellites are found on both the protein coding and non-coding regions of the genome (Gábor *et al.*, 2000).

There is considerable variation between microsatellite frequencies in coding and non-coding regions with the latter exhibiting the highest frequency (Metzgar *et al.*, 2000). The low frequency of microsatellites in coding regions is attributed to selection against frameshift mutations especially for non-triplet motifs (Metzgar *et al.*, 2000). When the distribution of microsatellites was compared between exons, introns and intergenic regions of different taxa (mammalian and non-mammalian) striking difference were observed (Toth *et al.*, 2000). Dinucleotide repeats for

instance, were found to be more frequent in rodents than in fungi and these repeats were not often seen on exons. In an earlier study, Primmer and others (1997), reported a general low frequency of microsatellites in the avian genome. The authors ascribed this phenomenon to the small size of the avian genome. It appears therefore that microsatellites frequency varies between taxa and between coding and non-coding regions of the genome.

Several drawbacks are associated with microsatellites typing and use in genetic diversity studies. These include difficulty in interpretation especially due to high frequency of back-mutations (Wilson and Balding, 1998). Difficulty in obtaining microsatellites in groups of organisms such as lepidopteras, some dipteras, a number of avian groups and many plant species (Beaumont and Bruford, 1999). Some of its strengths such as the ability to utilise PCR also account for some of the shortfalls. PCR problems, for instance non-amplification of certain alleles as a result of mutations within the priming site leading to apparent null alleles as well as Taq polymerase slippage that causes band scoring problems (Beaumont and Bruford, 1999).

2.3.2.6.3.1 Microsatellites as genetic markers for population studies

The usefulness of microsatellites loci in evolution and genetic studies lies on their inherent instability which is brought about by changes in the number of copies of the microsatellite repeats (Eisen, 1999). This change in copy number is believed to result from either of the two events namely strand slip mis-pairing (SSM) errors and recombination (Hancock, 1999; Eisen, 1999).

Slippage during replication usually occurs when the lagging DNA strand dissociates from the template strand and re-anneal out-of-phase (Fig. 4). At the end of the replication involving such mis-annealing, the formed lagging strand will be shorter or longer than the template strand. If the looped-out bases are in the template strand, the product will be shorter and longer if the looped bases are on the lagging strand (Hancock, 1999). There is correlation between the number of copies of the repeat and SSM events with high rate of slipped on increasing number of repeat (Wierdl *et al.*, 1997; Eisen, 1999). The long motifs are more likely to incur large multi-repeat deletions than shorter ones (Wierdl *et al.*, 1997). In case of mutations resulting in single repeat change, long motifs are biased towards additions while in short motifs additions and deletions occur with almost similar frequencies (Wierdl *et al.*, 1997; Eisen, 1999).

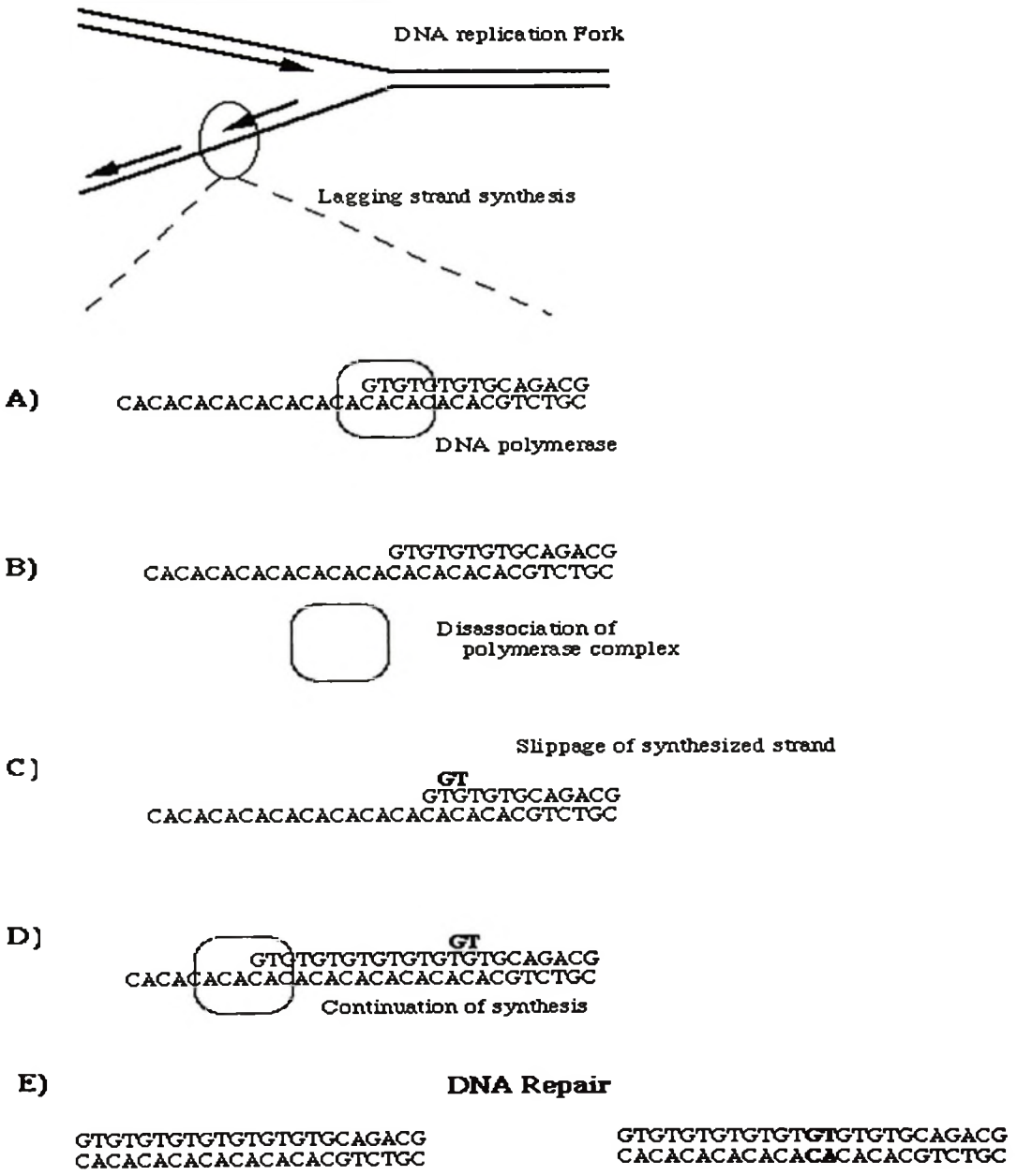


Figure 4: Strand Slip-mis-pairing (SSM) involving a GT repeat.

Recombination may change the length of microsatellites by either unequal crossing-over between mis-aligned chromosome strands or by gene conversion (Hancock, 1999). Unequal crossing-over can occur both between chromatids in the same

chromosome or between different chromosomes. Gene conversion involves the uni-directional transfer of information by recombination probably in answer to DNA damages and can transfer sequences from one allele to the other in an out-of phase fashion. However, more evidence on microsatellites instability is available on SSM than recombination (Hancock, 1999).

Studies on evolution of microsatellites have shown that mutations at these loci involve addition or loss of one repeat or less often several repeats (Estoup and Cornuet, 1999). Two extreme theoretical mutational models have been described; the infinite allele model (IAM, Kimura and Crow, 1964) in which each mutation yields a novel allele and a stepwise mutation model (SMM, Kimura and Ohta, 1978) where mutation results in gain or loss of one allele. In between these two extremes is the two-phase model (TPM, Di Rienzo *et al.*, 1994) where mutation causes a gain or loss of X alleles and the k-allele model (KAM, Crow and Kimura, 1970), postulating the existence of k possible alleilic state in each mutation. However, studies have also shown that mutation of repeat motifs will depend on allele size and purity, is biased towards gain of alleles and due to selection or mutation process some constraints on length exists (Estoup and Cornuet, 1999). Consequently, none of the theoretical mutation models for microsatellites evolution is deemed adequate.

2.3.2.6.3.2 Functional roles of microsatellites

There is accumulating evidence that microsatellites as well as minisatellites code as functional elements of protein molecules and work as regulatory rudiments of

transcription (Kashi and Soller, 1999). Kashi and Soller (1999), pointed out that the conservation of sequences across species is an indication of biological function.

Microsatellites have also been shown to act as enhancer elements in transcription as exemplified by reduced transcriptional activity in sequences lacking (through deletion) the microsatellite (Lafyatis *et al.*, 1991; Ramalingham *et al.*, 1995). Microsatellite repeat number variations may directly affect the phenotype of an individual if the repeat is part of the coding region (Kashi and Soller, 1999). Some examples to this effect are mostly inferred from human genetic disorders such as fragile X syndrome associated with mental retardation, autosomal dominant spastic paraplegia and Friedreich's ataxia (Reviewed by Sutherland and Richards, 1995). Most of these abnormalities result from abnormal expansions of tri-nucleotide repeats (Kashi and Soller, 1999).

2.3.2.6.3.3 Applications of microsatellites

Microsatellite typing has been used in various genetic relatedness studies on different species such as chickens (Takahashi *et al.*, 1998; Vanhala *et al.*, 1998; van Marle-Koster and Nel, 2000; Wimmers *et al.*, 2000), cattle (Hanslik *et al.*, 2000; Rincon *et al.*, 2000), dogs (Koskinen and Bredbacka, 2000), Japanese quail (Pang *et al.*, 1999), sheep (Saberivand *et al.*, 1998) and Eurasian otter (Dallas *et al.*, 1999). Microsatellite typing has also been used to elucidate the history of human population establishing firmly the existence of higher genetic diversity of African population, which is consistent with the hypothesis that they represent the oldest human population (Linares, 1999). Microsatellites are useful in forensic science replacing minisatellites

and multilocus DNA fingerprinting (Balding, 1999). These markers have also been used to identify a number of disease-associated genes mostly those of monogenic inheritance (Carrington *et al.*, 1999).

Microsatellite typing has been used to assess the extent and effect of hybridisation in endangered species. For example it was shown that the world's most endangered canid, the Ethiopian wolf (*Canis simensis*) has significantly hybridised to domestic dogs (i.e. female wolves to male dogs) (Beaumont and Bruford, 1999). This may help to explain why the number of the Ethiopian wolf is still on the decline. Other studies utilizing microsatellites have shown that another endangered wolf, the red wolf of the South East region of USA was actually a hybrid between coyotes and grey wolf (Roy *et al.*, 1994). These two examples show the power of microsatellites in conservation genetics.

2.4 Natural Disease Resistance Phenomenon

Resistance to disease, particularly infectious disease operates at various levels. Innate resistance reflects properties in animals that are fixed and that protect the animal against certain disease (Wakelin, 2000). On the other hand, acquired resistance are those immune and immunologically mediated responses that protect an initially susceptible host once it has experienced an infection (Wakelin, 2000). The genetic make-up of an individual determines its disease resistance potential (Lamont, 1998). Individuals will therefore differ greatly in their ability to resist, control and or reject infections and their susceptibility to diseases in general (Doenhoff, 2000). Genes controlling disease resistance can be grouped into three categories: those controlling

innate immunity, those determining the specificity of the adaptive immunity and others that control the quality of the acquired immune response (Doenhoff, 2000). Disease resistance is generally a polygenic phenomenon (Hartman, 1997; Lamont, 1998; Hill, 2001).

However, some genes or gene families have singly been responsible for resistance in some host species. The genes of the major histocompatibility complex (MHC), the natural resistance-associated macrophage protein (Nramp), T-cell receptor, cytokines, growth hormone and the immunoglobulins are but a few such examples (Doenhoff, 2000).

Innate resistance is contributed by such factors as barriers to penetration (tails, hair, acidic secretions, lysozymes) and the presence of harmless resident bacteria in the intestines and skin. At molecular level, carbohydrate- and lipopolysaccharide-binding lectins, complement activating and acute-phase proteins, interferon and other cytokines are some of the controlling factors (Medzhitov and Janeway, 1997). The genetic control of the mechanisms of innate immunity is very complex. Indeed in cattle, over 30 genes are known to be coding for type 1 interferon that have pronounced effect on severity of herpes-1 virus infection (Ryan and Womark, 1993).

Much studied determinants of innate resistance include the gene for Nramp and MHC (Govoni *et al.*, 1996; Hu *et al.*, 1997; Doenhoff, 2000; Hill *et al.*, 1991). In the host, Nramp confers resistance against intracellular pathogens such as Mycobacteria, Leishmania and Salmonella (Govoni *et al.*, 1996; Hu *et al.*, 1997; Doenhoff, 2000).

Likewise, MHC genes have been associated with a number of well-documented incidences of natural disease resistance. In humans some HLA haplotypes have been proven to be associated with resistance to malaria in West Africa (Hill *et al.*, 1991). In chickens the haplotype B21 of the MHC is strongly linked with resistance to Marek's disease (Kauffman *et al.*, 1995). Other diseases of chicken whose resistance has been linked to the MHC include Rous sarcoma virus tumours (Schierman and Collins, 1987), fowl cholera (Lamont *et al.*, 1987), Coccidiosis (Lillehoj *et al.*, 1989; Caron *et al.*, 1997), and Salmonellosis (Cotter *et al.*, 1997). Other studies have shown association between disease resistance and MHC in other farm animals including cattle, swine, sheep and horse (Doenhoff, 2000). It is therefore, appealing for researchers to try to select for specific MHC alleles known to confer resistance to different diseases. Nevertheless, the only authentic marker assisted selection involving the MHC is the B21 and Marek's disease (Doenhoff, 2000).

Three approaches can be employed in studying the genetic disease resistance phenomenon (Biozzi *et al.*, 1982; Bumstead and Barrow, 1993; Siegel and Gross, 1980; Prescot *et al.*, 1982; Gyles *et al.*, 1986; Cheng and Lamont, 1988; Gwakisa *et al.*, 1994a; Max *et al.*, 1997). The first approach is to use animal models such as genetically manipulated laboratory mice and in-bred lines of chickens (Biozzi *et al.*, 1982; Bumstead and Barrow, 1993). The results obtained with the models can then be inferred to the host of interest.

Another approach is the indirect measurement of immune response parameters such as antibody production following challenge with non-pathogenic highly

immunogenic substances such as sheep red blood cells (SRBC) or bovine serum albumin (BSA) (Siegel and Gross, 1980; Prescot *et al.*, 1982). A similar approach can be used but with disease causing microorganisms of reduced virulence in place of the non-pathogenic substances (Gyles *et al.*, 1986; Cheng and Lamont, 1988; Gwakisa *et al.*, 1994a; Max *et al.*, 1997). The hosts can then be divided between those with higher immune responses and those with lower immune responses. The high responders will have a higher immunological response when vaccinated (or during exposure to low virulence agents) compared to the low responders and hence be expected to be highly immune when challenged by virulent agents.

The final approach is the actual challenge of the host using the disease causing organisms and following the host's response over a period of time (Pinard *et al.*, 1996; Okoye and Aba-Adulgba, 1998; Permin and Ranvig, 2001, Gauly *et al.*, 2002; Mdegela *et al.*, 2002). This involves bacterial pathogens, helminths, viral pathogens and protozoan pathogens (Pinard *et al.*, 1996; Okoye and Aba-Adulgba, 1998; Permin and Ranvig, 2001, Gauly *et al.*, 2002; Mdegela *et al.*, 2002).

2.4.1 The use of rodent models in studying genetic disease resistance

The fact that disease resistance is a genetically variable trait of all animals brings in prospects of selective breeding for increased resistance or in introduction of the resistance genes into the existing flocks (Wakelin, 2000). By applying laboratory animal models (especially mice) one can be able to elucidate the underlying mechanisms of resistance and manipulate host responses to infection exploiting controlled genotype (Vassiloyanakoupoulos *et al.*, 1998; Wakelin, 2000).

Laboratory mice have the advantage of being the best defined (genetically and immunologically) species and the most manipulatable experimentally (Biozzi *et al.*, 1982). Mice are now available with some genes inserted (transgenic) or with deleted genes (gene "knock-out" mice) making it easy to study the effect of the individual genes (Wakelin, 2000). Currently, there are several rodent models covering a number of diseases due to bacteria, coccidia, trypanosome, nematodes and arthropods (Vassiloyanakopoulos *et al.*, 1998; Wakelin, 2000). The model systems are useful because of their closeness to the situation modelled, their predictive value and their capacity to provide fundamental insights to the problem under investigation.

2.4.2 Indirect measurements of genetic disease resistance

In most instances, the relevant rodent model for specific disease and direct inferences from the host may be unavailable. Under such circumstances, the indirect measurements that apply immunological and genetic markers become practical. Indirect parameters of resistance include physiological and immunological values (haematological and serological responses) as well as genetic markers (Gasbarre and Miller, 2000).

The total immunocompetence of an individual is a foundation in selection for immune response and disease resistance (Cheng and Lamont, 1988). It has been suggested that the use of harmless easily administered antigens in genetic selection for immune responsiveness may supplement vaccination (Parmentier *et al.*, 1996). To gauge the immunocompetence of an individual, humoral and cellular responses may be investigated. Serum antibody levels and serum complement levels can be

used as measures of the humoral immune response while delayed hypersensitivity reactions can be used to determine the cellular mediated response (Prescot *et al.*, 1982; Siegel and Gross, 1980; Shen *et al.*, 1984). Over the years Sheep Red Blood Cells (SRBC), Bovine Serum Albumin (BSA), Glutamic acid-Alanine-Tyrosine (GAT) and different parts of microorganisms have been used to challenge the humoral immune response (Siegel and Gross, 1980; Gross *et al.*, 1980; Cheng and Lamont, 1988). It has also been shown that there is a genetic variation in chickens for antibody response to Sheep Red Blood Cells (SRBC) (Siegel and Gross, 1980). It has been shown that in a population of chickens, there are those with persistently high antibody response to SRBC and those with low response (Gwakisa *et al.*, 1994a; Max *et al.*, 1997; Msoffe *et al.*, 2001). Gross *et al* (1980) and Siegel and Gross (1980) reported that the high antibody producers to SRBC were more resistant to parasitic and viral infections but less so to bacterial infections. In studying the immunocompetence of local chicken ecotypes in Tanzania, all the five ecotypes studied showed similar immunological responses (Msoffe *et al.*, 2001).

Apart from these non-pathogenic compounds, the different vaccines available on the market have been used to achieve similar ends (Cheng *et al.*, 1991). Newcastle disease, *Mycoplama gallisepticum*, *Pasteurella multocida*, infectious bronchitis disease and *Salmonella pullorum* vaccines are among a few that are regularly used in testing the chicken humoral immune response (Gyles *et al.*, 1986; Cheng and Lamont, 1988; Gwakisa *et al.*, 1994a; Max *et al.*, 1997). On the other hand the use of different types of mitogens (such as plant derived mitogens) and purified protein derivatives (PPDs) from various microorganisms have been used for assessing the

cell-mediated immunity to organisms (Prescot *et al.*, 1982). The skin thickness index or the wattle thickness index is employed to judge the cellular immune response and the most commonly used mitogen is Phytohaemagglutins-P (PHA-P) (Goto *et al.*, 1978; Cheng and Lamont 1988; Corrier and De Loach, 1990; Cheng *et al.*, 1991). Apart from using immunological methods, typing for specific gene loci responsible for disease resistance such as MHC and Nramp1 may serve as indirect methods as well. The indirect methods provide rapid results and use less invasive methods in obtaining the samples.

2.4.3 Direct inferences from the host

Laboratory animal models are not always available for all diseases whose resistance is of interest neither is indirect measurements always applicable. Under these circumstances direct inferences from the host become yet another alternative (Gasbarre and Miller, 2000; d'leteren *et al.*, 2000). The host animals may be allowed to acquire natural infection or can be challenged artificially and a set of direct or indirect parameters for resistance is observed (d'leteren *et al.*, 2000; Okoye and Aba-Adulgba, 1998; Permin and Ranvig, 2001; Gauly *et al.*, 2002). Direct parameters of resistance may include recovery of the pathogens, determination of faecal worm egg or coccidian count and quantification of lesions at necropsy. The major advantage of this method lies on the fact that results from such experiments present the true picture of the situation in real life.

2.4.4 Mechanisms of resistance to bacterial infections

Animals have innate mechanisms to protect themselves from different invading microorganisms. Using bacteria of the genus *Salmonella* as model different immunological mechanisms are reviewed. A number of mechanisms have been reported that are responsible for resistance to *Salmonellae* infections. The most important of these include cellular domains (polymorphonuclear cells (PMN) and macrophages) and the sub-cellular domains (major histocompatibility complex (MHC) and Nramp). For convenience Nramp will be discussed together with macrophages.

2.4.4.1 Polymorphonuclear cells (PMN)

It has been shown that PMNs kill Salmonellae effectively and the mechanism that protects the bacteria in the macrophages does not work in PMNs (Vassiloyanakopoulos *et al.*, 1998). PMNs play a major role in host resistance against a number of pyogenic bacteria and it has been reported that neutropaenic mice are increasingly susceptible to a number of facultative intracellular pathogens such as *Salmonella*, *Listeria* and *Francisella* (Conlan and North, 1992; Conlan, 1996, 1997). In a study by Conlan (1997), it was shown that PMNs help to resist early bacterial proliferation by helping the initial destruction by fixed macrophages of the liver and spleen. However, the exact contribution of PMNs in this process is not known (Conlan, 1997).

In chickens, heterophils have been reported to prevent establishment of overt infections by many potential pathogens (Stabler *et al.*, 1994). In that study, a large

number of non-opsonised *Salmonella enteritids* were killed by heterophils. In a different study, Kogut and others (1994) concluded that heterophils were the decisive components in the early responses of chickens to *S. enteritidis* infections.

2.4.4.2 Macrophages and Nramp

These cells belong to the mononuclear phagocytic system and provide early immunological response against infective agents (Qureshi, 1998). Macrophages phagocytosise foreign particles, kill bacteria and tumour cells and secrete prostaglandin and cytokines that regulate lymphocytes as well as other macrophages (Qureshi, 1998). Macrophages also play a central role in the acquired immunity through processing and presentation of antigenic fragments to T-lymphocytes in relation to both class I and class II major histocompatibility complex (MHC) cell antigens (Unanue and Allen, 1987). These functions make the macrophages crucial effectors in both innate and acquired immune responses (Klasing, 1998; Qureshi, 1998). More specifically, these cells have been established as mediators of resistance to extra-intestinal salmonella infections (Vassiloyanakopoulos *et al.*, 1998).

Macrophages express the natural resistance associated macrophage protein 1 (Nramp1) genes that contribute significantly to host resistance against salmonellosis (Govoni *et al.*, 1995; Govoni and Gross, 1998; Vassiloyanakopoulos *et al.*, 1998). Nramp1 influences the rate of replication of salmonella in macrophages (Govoni and Gross, 1998). Nramp1 has been identified in mice, humans, chickens, sheep and cattle (Cellier *et al.*, 1994; Pitel *et al.*, 1994; Feng *et al.*, 1996; Hu *et al.*, 1997; Bussmann *et al.*, 1998; Govoni and Gross, 1998). The protein coded by this gene is

an integral membrane protein expressed in macrophages/monocytes and polymorphonuclear cells (Cellier *et al.*, 1995; Govoni and Gross, 1998). The protein is located in the lysosomal/endosomal compartment of the macrophage and upon phagocytosis it is rapidly recruited to the membrane of the particle containing phagosome (Govoni and Gross, 1998; Govoni *et al.*, 1999). However, the exact mechanisms by which Nramp1 influences the biochemical properties of phagosome to control the replication of the microbes is still unknown (Govoni *et al.*, 1996; Govoni and Gross, 1998).

2.4.4.3 Lymphocytes

The exact function of the B and T-lymphocytes in the modification and outcome of Salmonella infection in different hosts is still debatable (Lo *et al.*, 1999; Mittrucker *et al.*, 2000). Lo and others (1999), have shown that CD8+ T-lymphocytes contributed significantly in the immunity to Salmonella infections. But other studies have shown that Salmonella infection tend to prevent optimal cell-mediated immunity by inhibiting the expression of IL-6, IL-12, IFN-γ receptors thereby making the cell mediated immunity of less importance (Elhofy *et al.*, 2000; Kaiser *et al.*, 2000). Nevertheless, compelling evidence exists that the B-lymphocytes have a role in the protective immunity against Salmonella infection (Mittrucker *et al.*, 2000).

2.4.4.4 The major histocompatibility complex (MHC):

The genes of the major histocompatibility complex (MHC) encode class I and class II glycoproteins with the function of binding peptide fragments from degraded

proteins and presenting them to the circulating T-lymphocytes as an essential step in specific immune response (Rudesky *et al.*, 1991; Germain, 1994). The relationship between MHC haplotypes in resistance or susceptibility to specific diseases in different animal species including humans has been studied (Bacon, 1987; Apanius *et al.*, 1997). The most concrete evidence on this relationship is portrayed in resistance to malaria to people bearing specific MHC haplotypes in West Africa and Marek's disease in B21 containing haplotypes of White Leghorn chickens (Briles *et al.*, 1983; Bacon, 1987; Hill *et al.*, 1991). Evidence is gathering that the human MHC haplotype HLA-B27 is associated with resistance against bacterial spondyloarthropathies (Kingsley, 1993; Ringrose *et al.*, 1996a; 1996b; Laitio *et al.*, 1997). In chickens, the outcome of experimental infection with *S. enteritidis* was shown to be influenced by the B-complex (Cotter *et al.*, 1998). In that study, Cotter and others, (1998) reported increased susceptibility in haplotypes B18 and B15 as opposed to the resistant haplotype BC. In swine, the swine lymphocytic antigen (SLA), had a high influence on the degree of O-polysaccharide specific lymphocyte proliferation following immunisation and later oral challenge with *S. typhimurium* (Lumsden *et al.*, 1993). The murine MHC has also been linked to resistance against autoimmune reactivity following experimental infection with *S. typhimurium* and *Escherichia coli* (Hol, 1993). It is important to note that none of the mechanisms employed by animals against *Salmonellae* is well understood or sufficient.

2.4.5 Disease resistance in chickens

Specific gene loci such as the Major Histocompatibility Complex (B-Complex), the cytokines, the T-cell receptor, Natural resistance associated macrophage protein 1,

growth hormone and immunoglubulins have been demonstrated to influence natural disease resistance in chickens (Lamont 1998). It is now known that Nramp1 gene is responsible for resistance of chickens to *Salmonella* infections (Hu *et al.*, 1997).

Recently, two studies on genetic differences in resistance to helminth (*Ascaridia galli*) have been conducted in Denmark and Germany, respectively (Permin and Ranvig, 2001; Gauly *et al.*, 2002). In both studies it was concluded that selection for *A. galli* resistance in chicken should be possible and relevant especially now that more chickens are kept under organic farming. However, in both studies the mechanisms governing the genetic resistance was not investigated.

It is worth mentioning that resistance to diseases is not controlled by a single factor rather it involves an interaction of factors or genes (Hartman, 1997). Hartman (1997), pointed out that resistance to avian leucosis which is controlled by genes *Tv-A* and *Tv-B*, involve also the genetic characteristics of the line in question. In fact, the negative effects on immune response to the virus by another gene *ev* 6, may be involved as well. It is also true that disease resistance is under the influence of environmental factors (Zijjp and Niewland, 1989). Genetic disease resistance should therefore be considered to be as a component in a comprehensive disease management strategy including high level of biosecurity, sanitation and vaccination (Lamont, 1998).

2.4.6 Disease resistance in other animals

The phenomenon of natural disease resistance has been expressed in different animal species including cattle, swine, sheep and horse (Rothschild *et al.*, 2000; Gasbarre and Miller, 2000; Raadsma, 2000a; 2000b; Owen *et al.*, 2000).

In cattle, genetic resistance has been reported against bovine leukaemia virus, bovine mastitis, ticks, trypanosomes and helminths (Rothschild *et al.*, 2000; Gasbarre and Miller, 2000; d'leteren *et al.*, 2000; Owen *et al.*, 2000; Minjaw and de Castro, 2000). Cattle of the BoLA A14 haplotype have been shown to be resistant to the bovine leukaemia virus infection (Rothschild *et al.*, 2000). Resistance to bovine mastitis measured by somatic cell counts (SCC) in milk has been associated with some MHC haplotypes in particular BoLA DRB3.2*16 (Sharif *et al.*, 1998; Owen *et al.*, 2000). However, like in many MHC related resistance, the same allele (BoLA DRB3.2*16) was associated with high SCC hence increased susceptibility to mastitis (Kelm *et al.*, 1997).

Resistance to ticks has been shown by several studies to be associated with the presence of zebu genes (Wambura *et al.*, 1998; Minjaw and de Castro, 2000). Evidence for genetic resistance against tick-borne diseases is not available. The classical example of natural disease resistance in cattle is presented by the trypanotolerant N'Dama breed of West Africa cattle. This phenomenon of trypanotolerance has been attributed to superior humoral responses and maintenance of higher complement levels (d'leteren *et al.*, 2000).

In sheep, there are reports on natural disease resistance against helminths, cutaneous myasis and foot rot (Gasbarre and Miller, 2000; Raadsma, 2000a; 2000b). Breed resistance to helminths has been correlated with haemoglobin type (sheep have haemoglobin A and B) with haemoglobin type AA (HbAA) being more resistant than Hb AB; and Hb BB was the least resistant (Gasbarre and Miller, 2000). The genes of the MHC have also been linked to helminths resistance in sheep (Rothschild *et al.*, 2000). Genetic resistance to ovine myasis is thought to be controlled by immunological and skin-based factors (Raadsma, 2000a). Sheep from resistant line showed high skin inflammatory response to intradermal injection with larval products as compared to those from a susceptible line (O'Meara *et al.*, 1993; O'Meara and Raadsma, 1995). However, both the resistant and susceptible lines did not differ significantly in antibody titres and specificity directed against *Lucilia cuprina* (a blowfly) larval antigens (O'Meara and Raadsma, 1995). There is a paucity of information on breed differences in resistance to footrot in sheep. There is however, a difference between strains and bloodlines with the Merino sheep breed (Raadsma, 2000b). The specific mechanisms responsible for resistance to foot rot in sheep and differences between sheep strains are not known (Raadsma, 2000b).

Studies in swine have revealed the involvement of the MHC (SLA) in resistance against *Salmonella typhimurium* and *Staphylococcus aureus* infections (Lacey *et al.*, 1989; Lumsden *et al.*, 1993). Evidence from these studies showed that SLA haplotypes had a significant influence on the uptake and killing of each bacterium. The role of SLA in resistance against *Trichnella spiralis* has been studied. It has been demonstrated that there is lower larvae burden on muscles in SLA haplotype cc as

compared to SLA haplotype aa or dd (Lunney and Murrel, 1988). Recently, Lunney and Buttler (1998) have cited possible associations between SLA and skin diseases. Resistance to *E. coli* causing neonatal diarrhoea and post-weaning oedema disease in pigs is controlled by specific receptors (Edfors-Lilja and Wallgren, 2000). Receptor molecules are not involved in resistance to *Salmonella* infections rather the antimicrobial activity of macrophages are most probably aided by the Nramp1 genes (Tuggle *et al.*, 1997; Edfors-Lilja and Wallgren, 2000).

Although there is ample evidence on natural disease resistance in different farm animals, most of the mechanisms involved are still not understood or are poorly understood. It is therefore important that more efforts should be directed towards the elucidation of the mechanisms involved in natural resistance by identifying relevant genetic markers. The identified markers can then be used in marker-assisted selection (MAS) to select the resistant genotypes.

From the above review, it is evident that both phenotypic characterisation and genotypic typing are important in order to assess the diversity in chickens. Both these methods were applied in the current study, the outcome of which is presented in the results chapter of this work.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Preamble

Data used in this study was obtained from five experiments, four of which were conducted at the Sokoine University of Agriculture and the fifth was conducted at Mkundi village situated about twenty kilometres from Morogoro town along the Morogoro-Dodoma highway.

The first experiment was done to investigate the genetic diversity of the free-range local chicken ecotypes using microsatellite DNA polymorphisms. The second experiment, looked into the productivity and reproductive performance of the local chicken ecotypes under deep litter management system where data on weekly weight measurements, daily growth rates, egg weights, fertility and hatchability were recorded. The third and fourth experiment tested disease resistance of the local chicken ecotypes through experimental infection with *Salmonella gallinarum* and Newcastle disease vaccination, respectively. The fifth experiment investigated the productivity and reproductive potential of the local chickens under free-range management system. Parameters recorded in this fifth experiment were similar to those of experiment two except for fertility studies.

3.2 Study areas and chickens

3.2.1 Study areas

Sampling was done from five Tanzania mainland regions namely Mbeya, Morogoro, Mwanza, Tabora and Tanga representing the southern highlands, eastern, lake,

western and northern geographical zones, respectively. Figure 5 shows the administrative map of Tanzania indicating the regions where local chickens were purchased. Due to logistical reasons, sampling was not done in the central and southern zones. In the Isles, chickens were purchased from two regions in Zanzibar Island namely Central and Western and in Pemba Island, the Southern region.

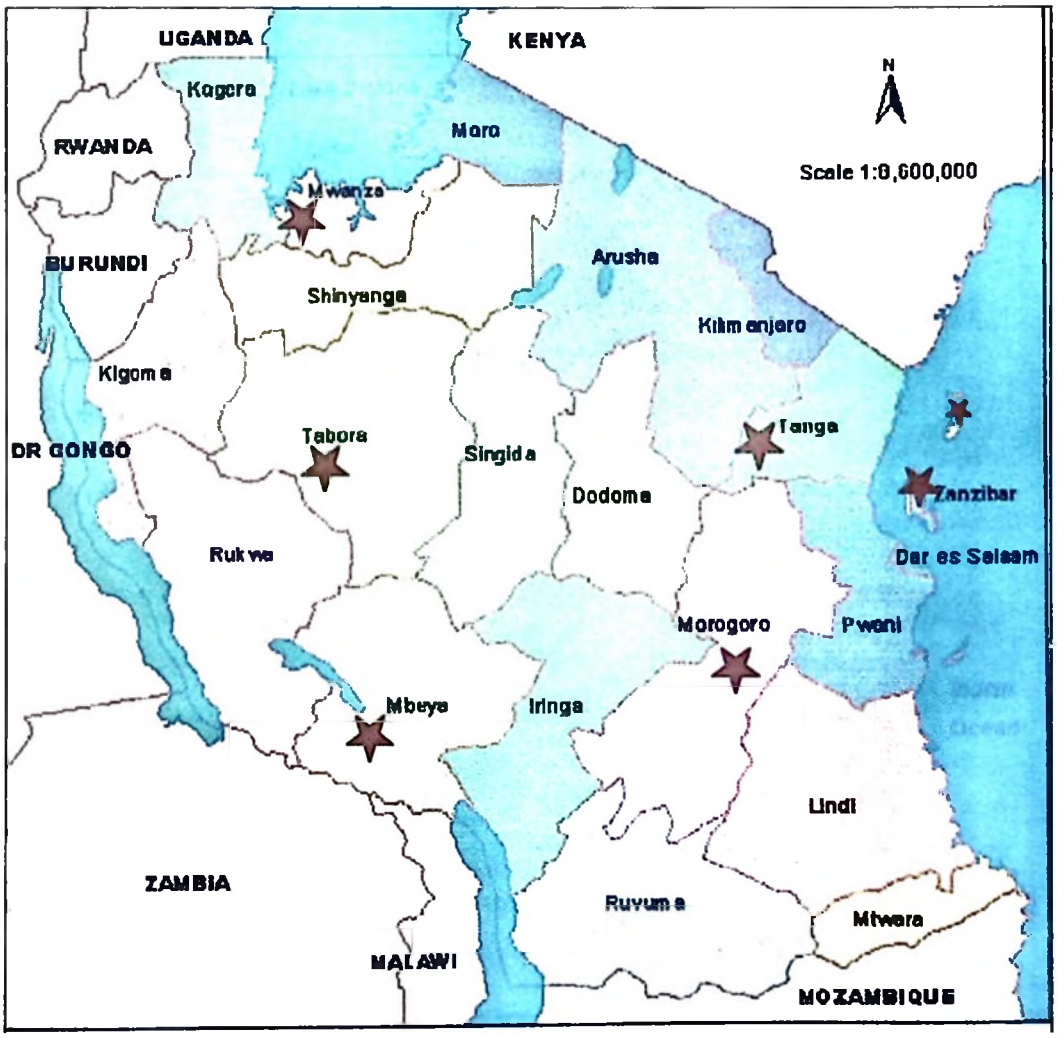


Figure 5: The map of Tanzania showing the regions where local chickens were purchased (red stars).

In the sampled regions, previous information on the existence of unique local chickens was used to select areas for sampling. This was the case in purchasing local chickens from Mbeya, Morogoro, Mwanza and Tabora regions. In areas where no such information existed consultations were made with the livestock officers in the region on where samples could be obtained. This second approach was applied for sampling in Tanga and the Isles regions.

In Mbeya region, sampling was done at Ileje district, which borders the Republic of Malawi. The chickens referred to as *Mbeya* ecotype are either purebred or crosses of the Black Australorp breed imported from Malawi. The chickens are popularly known as *Mikolongwe* referring to the name of the breeding station in Malawi. They are popular among the farmers because of their good scavenging ability and good egg laying capacity. In Morogoro region, sampling was done in two districts, Kilosa district where the *Ching'wekwe* and *Morogoro-medium* ecotypes were acquired and Mvomero district where the *N'zenzegere* ecotype was purchased. In Mwanza region, the *Kuchi* ecotype chickens were bought from Sengerema district where they are widely kept. In Tabora region, the chickens (*Singamagazi* ecotype) were bought mostly from dealers and farmers in Tabora Rural district. Handeni district in Tanga region was the area where the majority of the chickens of the Tanga ecotype were bought. A few of these chickens were bought from Tanga Urban district and the owners ascribed the origin of these chickens to Handeni district. In the Isles, it was more convenient to sample through a region rather than districts due to the small geographical size of the landmass. In Zanzibar Island, samples were obtained from the Western and Central regions leaving out the Northern region, which is in close

geographical proximity to Pemba. In Pemba, due to logistical problems local chickens were obtained from villages in the Southern region only.

3.2.2 Experimental chickens and their management

A total of 182 adult free-range local chickens divided between nine ecotypes were purchased from villages in Tanzania mainland and isles. Five of the ecotypes used in this study were described in an earlier study by Msoffe and others, (2001). These included the *Ching'wekwe*, *Mbeya*, *Morogoro-medium*, *Kuchi* and *Singamagazi* ecotypes. In addition, local chickens were procured from villages in Pemba Island, Tanga and Zanzibar Island and were named *Pemba*, *Tanga* and *Unguja* ecotypes respectively. The ninth ecotype in the study was a frizzled variant locally known as *N'zenzegere* purchased from villages in Mvomero district Morogoro region. The ecotypes used in this study and their phenotypic characteristics are summarised in Table 3.

Criteria used in selecting the variety of chickens for this study were as follows:

1. The widest possible coverage of Tanzania that was made possible by the inclusion of five out of seven geographical zones of Tanzania as well as the Isles.
2. That the villages selected had to be without a history of cross-breeding programmes involving exotic chickens. (The existence of Black Australorp in Mbeya was considered not to contravene this criterion since they were not on official government programme).

3. The chickens had to have unique and easily described phenotypic characteristics such as size, plumage properties and comb types (that was the basis for choosing *Ching'wekwe*, *Mbeya*, *Kuchi*, *Singamagazi*, and *N'zenzegere* ecotypes).
4. Where chickens lacked any unique phenotypic characteristics, the sole criterion for selection was the area of origin (*Morogoro-medium*, *Pemba*, *Tanga* and *Unguja* ecotypes were acquired this way).
5. The willingness of the owner of the chickens to sell the birds at a reasonable price.

Throughout the collection of chickens, the sampling strategy was purposeful, that is when a chicken fulfilled the characteristics of an ecotype and the above criteria it was purchased. However, since the chicken owner had the mandate to sell or not to sell, the sample (chickens) drawn was fairly randomly distributed. Caution was also taken not to buy more than three chickens from the same homestead to avoid bias.

Table 3: The local chicken ecotypes used in the current study

Ecotype	Number purchased	Origin	Brief description
Ching'wekwe	N = 21 M = 4, F = 17	Chakwale, Kilosa district Morogoro region	Very short and compact chickens with multi-variety plumage colour, single combed
Mbeya	N = 20 M = 4, F = 16	Itumba, Ileje district, Mbeya region	Medium sized birds, black bluish in colour, single combed
Kuchi	N = 19 M = 3, F = 16	Sengerema, Sengerema district, Mwanza region	Tall and heavy birds with an upright posture, multi-variety plumage colour, rose or walnut combed
*Morogoro-M**	N = 23 M = 5, F = 18	Msolwa, Kilosa district, Morogoro region	Medium sized birds (the commonly kept local chicken in Tanzania), multi-variety plumage, single combed
N'zenzegere	N = 18 M = 3, F = 15	Mtamba, Mvomero district, Morogoro region	Medium sized birds, multi-variety frizzled plumage, single combed

**Morogoro-medium* ecotype; N = sample size; M = Males; F = Females.

Table 3: Contd.:

Ecotype	Number purchased	Origin	Brief description
Pemba	N = 25 M = 5, F= 20	Southern region	Small sized birds, multi-variety plumage, mixed comb types
Singamagazi	N = 25 M = 5, F =20	Ndala, Tabora district, Tabora region	Tall (cocks) and heavy birds, multi-variety plumage, single combed
Tanga	N = 18 M = 4, F = 14	Handeni, Handeni district, Tanga region	Medium sized birds, multi-variety plumage, single combed
Unguja	N = 13 M = 3, F = 10	Western and Southern regions	Small sized birds, multi-variety plumage, rose, walnut or single combed

Purchased chickens were transported to Sokoine University of Agriculture where they were maintained on floor pens in a deep litter system. Chickens of the same ecotype were placed in the same floor pen each having two cocks and all the purchased hens. These chickens formed the parental population from which studies on genetic diversity were conducted. Their offspring were used in the on-station productivity, reproduction and disease resistance potential experiments. Throughout the study period the chickens were maintained on a nutritionally balanced commercial feed preparation (Layers mash). Both the feeds and water were given *ad libitum*.

A comprehensive disease management strategy was followed that involved regular vaccination against Newcastle disease using La Sota strain, (Laboratorios Hipra SA, Spain), deworming using Kukuzole®, (Interchem Pharma Ltd., Moshi Tanzania), ectoparasites control using Sevin dudu dust ® (Rhone-Poulenc (K) Ltd. Nairobi, Kenya) prophylactic use of the anticoccidial drug Amprolium 200® (Diocare Vet Ltd., AV Lab. Syva, South Africa) antibiotics (Chlortetracycline CTC®) (ANUPCO, Ipswich, England) at three months interval and regular testing for *S. gallinarum* antibodies using the rapid plate agglutination test utilizing a locally prepared antigen (OIE, 1996) at six months interval.

From the parental populations data on egg weight, fertility and hatchability were collected separately for each ecotype. The chicks hatched from eggs collected from the parental populations were used for studies on productivity, reproductive and disease resistance potential as detailed below. Unfortunately, the chickens of the *Kuchi* and *Singamagazi* ecotypes died of Newcastle disease on arrival hence were not included in the productivity, reproductive performance and disease resistance potential experiments.

3.2.3 Blood samples for DNA isolation and antibody detection

From each of the 182 local chickens, 3ml of blood was collected through brachial venipuncture using a 21-gauge needle attached to a 3ml syringe. Immediately after collection, 1ml of the blood was transferred into 5ml vacutainer tube containing EDTA and mixed gently. This blood was stored immediately in a refrigerator before further processing for DNA extraction. The remaining 2ml was left in the syringe,

slanted overnight on the laboratory table for serum preparation. The serum was collected into duplicate 0.5ml eppendorf tubes; one sample was used for the rapid plate agglutination test (*S. gallinarum* antibodies) and the other was stored in a freezer (-20^0 C) for future uses. This procedure was done during the first week after the arrival of the chickens.

3.3 Diversity among local chicken ecotypes in Tanzania

3.3.1 Microsatellites DNA polymorphisms

3.3.1.1 Blood sampling and processing

Stabilised blood collected from the 182 local chickens as described above (3.2.3) was washed three times in Phosphate buffered saline (PBS pH 7.4) within 48 hours after collection. PBS (about twice the volume of blood) was added into the tube containing blood, mixed gently and centrifuged at 3000 rpm for 5 to 10 minutes. The supernatant was discarded and the procedure was repeated twice. The resulting sample of packed blood cells (mostly red blood cells) was transferred into a 1.5ml eppendorf tube and stored in a -20^0 C freezer. These samples were used for extraction of DNA.

3.3.1.2 DNA extraction, quantification and quality determination

3.3.1.2.1 DNA extraction

DNA extraction was done according to the procedure described by Sambrook *et al.*, (1989) with few modifications to suit the laboratory conditions.

A total of 50 μl packed red blood cells was drawn into a 1.5 ml eppendorf tube. 500 μl of TES buffer (0.2M Tris, 0.1M EDTA and 1% SDS) was added into the tube containing RBCs followed by 50μl of 20mg/ml Proteinase K (Sigma-Aldrich

chemicals, St. Louis, USA). The mixture was incubated overnight in a 60^0C waterbath with occasional hand shaking. An aliquot of 0.4ml 5M Potassium Acetate per ml of the solution was added and this mixture was placed on ice for at least 30 minutes. The mixture was centrifuged at 12,000*g* for 10 minutes and the supernatant was transferred into a new tube. An equal volume of Phenol: Chloroform: Isoamyl alcohol (24: 23: 1; Sigma-Aldrich chemicals, St. Louis, USA) was added to the supernatant mixed gently until there was a formation of emulsion. Centrifugation at 12,000*g* for ten minutes was done and the mixture separated into lower organic phase and upper aqueous phase. The aqueous phase was carefully removed using a 1ml micropipette and transferred into a new tube. These two steps were repeated until no proteins (debris) were seen at the interphase. An equal volume of Chloroform: Isoamylalcohol (23:1) was added to the clear aqueous phase, mixed and centrifuged at 12,000*g* for 10 minutes and the aqueous phase was obtained as above. An equal volume of Isopropanol was added very slowly at an angle, and then mixed by inversion.

The DNA was spooled using closed Pasteur pipette and was transferred into a new tube where the DNA was washed gently using 70% ethyl alcohol. In cases where DNA yield was not seen as a precipitate, the tube was centrifuged at 13,000*g* for 15 minutes, the supernatant was discarded (carefully without dislodging the DNA pellet) and the pellet was washed with ethyl alcohol as above. The DNA was allowed to dry at room temperature by leaving the tubes open for about one hour. Then the DNA was re-suspended in 50μl TE buffer (0.2M Tris, 0.1M EDTA) and was stored in the refrigerator at 4^0C.

3.3.1.2.2 Determination of DNA quality and quantity

The quality and quantity of the DNA was estimated by the use of a spectrophotometer (Cecil Instruments, Cambridge, U.K). From the DNA samples extracted above (3.3.1.2.1) 10μl was taken and mixed with 990μl TE buffer to make a 1000μl of a 1% DNA solution. Using a spectrophotometer, the optical density (OD) of this solution was determined at 260 and 280 nm wavelengths. OD_{260} measures the DNA concentration while OD_{280} measured the protein content in the DNA sample.

The quantity of DNA was calculated by the formula:

Concentration = OD_{260} x 50 x dilution

Where Concentration was the DNA concentration in μg/ml, OD_{260} was the Optical density of the DNA solution at 260 nm wavelength, 50 was a constant (one optical density value at 260 nm wavelength is equivalent to 50μg/ml of double stranded DNA) and dilution was the dilution factor for the DNA (this experiment used 100 as the dilution factor). After quantification of the DNA, all samples were standardized to 25ng/μl using the formula:

$C_1V_1 = C_2V_2$ Where C_1 and C_2 were the initial and final DNA concentration respectively and V_1 and V_2 were the initial and final DNA volume respectively. The DNA stock solutions were stored in the -20^0C freezer, the diluted solutions were stored in the refrigerator.

The quality of the DNA was calculated from the ratio between OD at 260 nm and 280 nm wavelengths (OD_{260}/OD_{280}). A good quality DNA must have $OD_{260}/OD_{280} \geq$ 1.8.

3.3.1.3 Microsatellites loci, PCR settings and genotyping

3.3.1.3.1 Microsatellite loci

A total of 20 microsatellite loci were employed in this experiment. Eleven microsatellites loci were chosen from the chicken genome database maintained at the Roslin Institute (http://www.ri.bbsrc.ac.uk/cgi-bin/microsatellite/microsearch.pl). The other nine loci were from the population tester kit (http://poultry.mph.msu.edu) a gift from the National Animal Genome Research Program (NAGRP courtesy of Dr. Hans Cheng). The properties of the 20 microsatellite loci are shown in Table 4. The primers were synthesised in Denmark (DNA technology, Aarhus, Denmark) and the USA (Operon technologies, Michigan, USA).

3.3.1.3.2 PCR settings

The PCR was performed in 500μl reaction tubes (Sigma-Aldrich chemicals, St. Louis, USA) on a PTC-100 programmable thermal controller (MJ research Inc, USA). The composition of each PCR reaction mixture is shown on Table 5. After all the ingredients were in place, about 50 – 100μl of mineral oil (Sigma-Aldrich, St. Louis, USA) was overlaid to prevent evaporation. A blank (a tube with all the ingredients except the template DNA) was each time included as a negative control.

For the ADL primer pair series all PCR amplifications were subjected to an initial denaturing step at 94°C for 5 minutes followed by 30 cycles at 94°C for 1 minute, appropriate annealing temperature (Table 4) for 1 minute and 72°C for 1 minute. There was a final 10 minutes extension at 72°C. For the LEI primer pair series, the initial denaturing step was at 96°C for 1 minute, followed by 30 cycles at 96°C for 1 minute, appropriate annealing temperature (Table 4) for 30 seconds, 72°C for 30

seconds and the final extension at 72°C for 10minutes. For the MCW primer pair, the initial denaturation step was at 94°C for 1minute, followed by 35 cycles of 94°C for 1 minute, then 62°C for 2 minutes, 72°C for 3 minutes with the final extension at 72°C for 10 minutes. Each local chicken DNA sample was run through all the 20 primer pairs. In addition, 13 standard White Leghorn DNA samples (Courtesy of Dr. Helle R. Juul-Madsen, Research Centre Foulum, Denmark) were included as reference samples.

3.3.1.3.3 Genotyping

PCR products were analysed by electrophoresis on 6% native polyacrylamide gels (Sigma-Aldrich, St. Louis, USA) using a Mini-Protean II® (Bio-Rad Italy,) electrophoretic equipment. The PCR products (15-20ul) were mixed with ficol loading buffer at the ratio of 1:6 (loading dye: PCR product) and run for 40 minutes at 20v/cm. A standard size marker, step ladder (Sigma-Aldrich, St. Louis USA) was included in each run to enable the correct determination of the size of the PCR products (in bp). The PCR products were then visualised by Silver staining (Rapley and McDonald, 1992). The gels were sealed in polythene bags and stored in the refrigerator until they were analysed. The size (bp) of the PCR products was determined using TotalLab® version 1.10 computer software (Nonlinear dynamics, Newcastle, UK). The gels were scanned into the software (Plate 1), which then calculated the PCR product size relative to the standard DNA size marker. The data on PCR product size were then entered into Microsoft Excel® spreadsheet programme where a macros programme (Microsatellite toolkit kindly supplied by Steven Park) was used to organise the data for analysis. The organised data was then

entered into population genetics analysis software namely DISPAN, GENEPOP, MICROSAT, PHYLIP and TREEVIEW for determination of allele frequencies, genetic distances, deviations from Hardy-Weinberg equilibrium and the construction of phylogenetic trees. A four-letter (three for reference breed) code system was designed to identify each population to fulfil the requirements of the population genetics programmes used. The coding was as follows:

DAN for the White Leghorn reference population, CHING for *Ching'wekwe*, FRIZ for *N'zenzegere*, KUCH for *Kuchi*, MBEY for *Mbeya*, MORO for *Morogoro-medium*, PEMB for *Pemba*, SING for *Singamagazi*, TANG for *Tanga* and UNGU for *Unguja* ecotypes. In the results presented in tables and figures on the genetic diversity study populations are represented by the said letter codes.

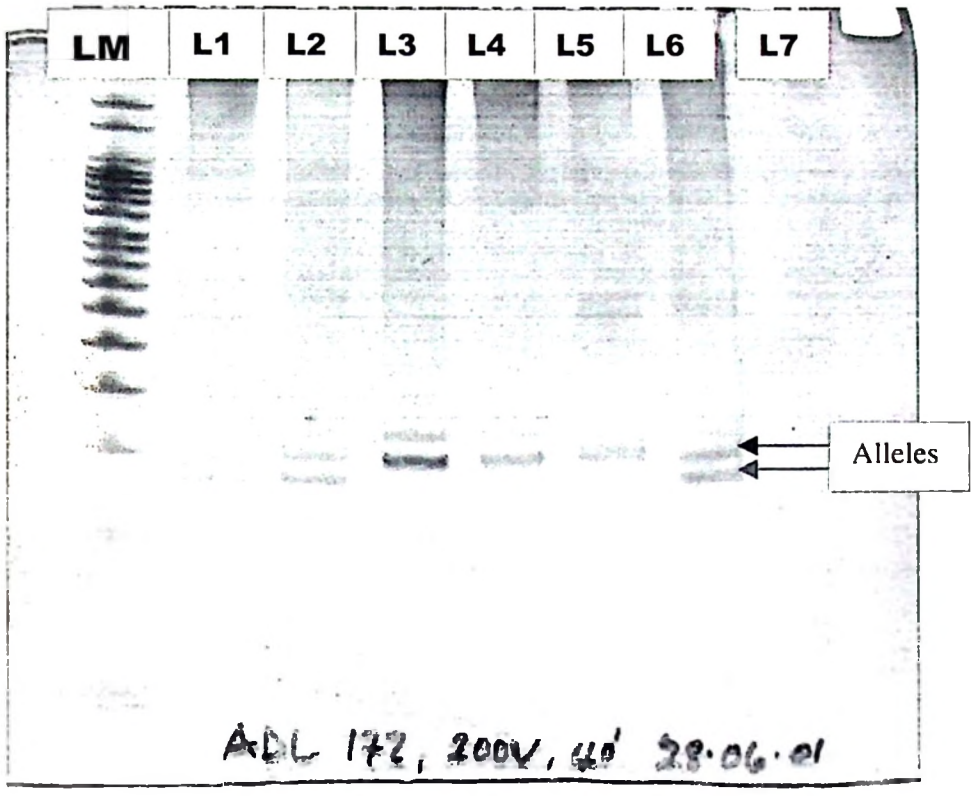


Plate 1: Scanned gel image for determination of allele sizes (bp).

Note:

LM - DNA size marker lane.

L1 to L7 – individual sample lanes (with L7 showing no amplification).

Table 4: The properties of the 20 microsatellite loci used in the current experiment

Locus name	Primer sequence (5'-3')	PCR Temp*	Mg++ conc. (mM)[§]	Size (bp)[¤]
LEI0217	[†]F-GATGACTGAGAGAAATAACTTG	51	1.5	198
	R-AAATTACTGAGGCACAGGAG			
LEI0234	F-ATGCATCAGATTGGTATTCAA	55	1.5	289
	R-CGTGGCTGTGAACAAATATG			
LEI0214	F-TGCCT GT TT CT AG GA	50	2.5	159
	R-GATCAAGCACTGTATTTTATTC			
LEI0248.	F-TTGCACTGAGACCAAATGTC	51	1.0	250
	R-CATAGATTTTCCTTAGTAGGTAACTTG			
LEI0237	F-GTTAAGTGTTCTCTGATGTAGC	53	1.0	224
	R-CTTCAACTATAAAGCATAGCTG			
LEI0193	F-AGCTGGAGGTAGAATCCTGAA	52	1.0	258
	R-ACAATCCCTTCCAACACACC			
LEI0040	F-GCATTGCAGGTGGTGATAGGG	60	0.625	198
	R-TCAGCGCTCTTGAACTCCAGC			
LEI0093	F-TCCTTGAAGTATTCCAAAGCTCA	60	0.625	143
	R-TCTCCTACTCCAGTGCCTTCA			
LEI0043	F-CTTCCATGGCAGCTCAGCCT	60	0.625	139
	R-ATCACTCGCGGCCATTAGGA			
ADL0102	F-TTCCACCTTTCTTTTTTATT	47	1.5	122
	R-GCTCCACTCCCTTCTAACCC			

Table 4: Contd.

ADL0136	F-TGTCAAGCCCATCGTATCAC	52	1.5	145
	R-CCACCTCCTTCTCCTGTTCA			
ADL0158	F-TGGCATGGTTGAGGAATACA	52	1.5	216
	R-TAGGTGCTGCACTGGAAATC			
ADL0171	F-ACAGGATTCTTGAGATTTTT	46	1.5	104
	R-GGTCTTAGCAGTGTTTGTTT			
ADL0172	F-CCCTACAACAAAGAGCAGTG	49	1.5	154
	R-CTATGGAATAAAATGGAAAT			
ADL0176	F-TTGTGGATTCTGGTGGTAGC	52	1.5	192
	R-TTCTCCCGTAACACTCGTCA			
ADL0181	F-CCAGTGAAATTCATCCTTTT	48	1.5	178
	R-CAATCTTTTGTGGGGTATGG			
ADL0210	F-ACAGGAGGATAGTCACACAT	46	1.5	130
	R-GCCAAAAAGATGAATGAGTA			
ADL0267	F-AAACCTCGATCAGGAAGCAT	50	1.5	117
	R-GTTATTCAAAGCCCCACCAC			
MCW005	F-ACCTCCTGCTGGCAAATAAATTGC	62	1.5	253
	R-TCACTTTAGCTCCATCAGGATTCA			
LEI0258	F- GGAATGGTTCAAGACGACGCAC	54	2.5	205
	R- AGCTGTGCTCAGTCCTCAGTGC			

*PCR temp = the annealing temperatures of the PCR; [$]Mg++ con. = The Magnesium ion concentration; [¤]Size = the expected size (in base pairs) of the PCR product.

[l] F and R are the forward and reverse primers respectively.

Table 5: Composition of each PCR reaction mixture

Ingredient	Quantit, μl
dNTPs 200μM (dATP, dCTP, dGTP and dTTP)[1]	5
10X PCR buffer (100 mM Tris-HCl,50 mM KCl, 0.01% gelatin and 0.25% Tween 20)	2.5
$MgCl_2$ XmM	Y*
Primer 1 1 μM[2]	0.5
Primer 2 1 μM[2]	0.5
Double distilled water	Z§
Taq DNA polymerase (5units/μl)[1]	0.1
Template DNA 25ng/μl	2
Total	25

*Quantity of $MgCl_2$ depended on the concentration required by individual primer pairs

§Double distilled water was added to make the final volume of 25μl.

[1]Taq DNA polymerase, buffers and dNTPs (Armesham, Copenhagen, Denmark)

[2]Primer pairs (DNA Technology, Aarhus, Denmark)

3.3.2 Productivity and reproductive performance of the local chicken ecotypes

The productivity and reproductive performance of the local chicken ecotypes was studied by determining the weekly live body weight measurements, daily growth rate, egg weights, the fertility and the hatchability of the incubated eggs.

3.3.2.1 Weekly weight measurements and growth rate for chickens up to 20 weeks of age

The weekly live weight measurements for the local chicken ecotypes were assessed by measuring the live weight from hatch until 20 weeks of age using a sensitive digital weighing balance (Tefal ® France). On the day of hatch the chicks were given special identity in the form of a coloured string (corresponding to each ecotype) sewn onto its wing. This was necessary to avoid confusion of which chick belonged to which ecotype during the off-loading of the chicks from the incubator.

On arrival to the brooder, the chicks were wing-tagged, weighed and the hatch weight as well as subsequent weekly weight measurements was recorded. The chicks involved in this experiment were hatched in 19 batches and the chicks reported in the results section were those that made it to 20 weeks of age. The number of chicks included to the end of the experiment was 194 females and 189 males. Sex differentiation was done retrospectively.

For each ecotype, the daily growth rate was calculated from the weekly live weight measurements data separately for each of the five months of the experiment. The daily growth rate was calculated based on the formula below:

Daily growth rate = $W_2 - W_1$ /28; Where W_1 was the initial weight in the month; W_2 was the final weight in the month and 28 was the number of days in the month. Therefore, for the first month W_1 was the hatch weight and W_2 was the weight at four weeks. In the subsequent months, the final weight of the previous month became the initial weights. Therefore, the weight at four weeks became the initial weight during the second month and that at eight weeks during the third month.

3.3.2.2 Reproductive performance of the local chicken ecotypes

The reproductive performance of the local chicken ecotypes were assessed by taking the weights of all eggs produced, as well as determining the fertility and hatchability of the eggs set for incubation. Egg weights were determined separately for each ecotype after every three days for a period of one calendar year. The weights were taken using a sensitive digital balance and afterwards the average for each three-day measurement were calculated. The three-day averages were used to generate the results on this parameter and 100 entries were used for each ecotype.

A total of 4072 eggs were incubated in 19 batches to determine the fertility and hatchability of the local chicken ecotypes. Five to seven days after incubation, the eggs were candled to determine the number of eggs that were fertile with viable embryos. A second candling was done after 14 days to determine early embryo death. For the purpose of this experiment, early embryo death was considered as eggs that did not hatch. The percentage fertility of the eggs was calculated as follows:

% Fertility = $(T_e - I_e / T_e) \times 100$; Where T_e was the total number of eggs incubated and I_e the total number of infertile eggs.

The percentage hatchability of the eggs was calculated as follows:

% Hatchability = (H_e /V_e) x 100; Where H_e was the total number of hatched eggs and V_e the total number of viable eggs (after the first candling).

3.3.3 Disease resistance potential in the local chicken ecotypes

To assess the disease resistance potential in the local chickens ecotypes, a challenge experiment with a local virulent strain *S. gallinarum* and Newcastle disease vaccine were used.

3.3.3.1 Experimental infection with *Salmonella gallinarum*

3.3.3.1.1 Experimental chickens

This experiment was conducted using a total of 120 chicks aged one week and divided between six local chicken ecotypes and an exotic commercial layer chicken group. All the chicks were hatched on station from the parental local chicken population except the commercial layer strain whose eggs were purchased from a local breeding farm. From each of the seven groups, 15 chicks were selected and transferred separately to wooden boxes (one cubic metre in volume) covered with chicken wire on four sides with a slatted floor 30cm off the ground. The boxes were kept at the University chicken experimental house, which had provision for electricity that supplied heat and light to chicks throughout the experiment.

The chicks were wing-tagged and given prophylactic doses of Kukuzole® antihelmintic (Interchem Pharma, Moshi Tanzania) and Amprolium 200® anticoccidial (Diocare Vet Ltd., AV Lab. Syva, South Africa) in accordance with the manufacturers recommendations. Another group of 15 chicks were maintained on

floor brooders housed at SUA control unit and these served as a negative control group.

3.3.3.1.2 Experimental design

Each of the 15 chicks from the seven groups was inoculated orally by passage into the crop with 100μl of an overnight culture containing 2.5 x 10^8 CFU/ml of *S. gallinarum* grown in Laurie Bertani broth (LB broth) incubated at 37^0C. Each of the 15 negative control birds was given 100μl of uninoculated LB broth that was incubated overnight but with no bacteria. The strain used for infection was RD8 (Mdegela *et al.*, 2000) that was isolated from a cloacal swab of a naturally infected layer and through plasmid profiling it was revealed to contain the virulence associated heavy (85kb) plasmid (Mdegela *et al.*, 2000).

The chickens were observed twice daily for clinical signs and mortalities. On the day of infection (day 1) about 200μl of whole blood was taken by brachial venipuncture from three randomly selected chicks from each of the eight groups. The blood was taken directly into capillary tubes coated with heparin due to the small size of the chicks. Samples were again taken on days 3, 6, 10 and 14 pi. On each of these days, three chicks were selected randomly at day 3 pi and on subsequent days, chicks showing marked clinical signs were selected from each group and were killed by cervical dislocation.

Two blood samples were collected from each bird for PCV (stabilized by EDTA) determination and for serology. Some of the stabilized blood was used to make thin

microscopic blood films for leukocyte enumeration. The killed chicks had their liver and spleen asceptically removed for determination of viable bacterial cell counts. All chicks that died or those killed were subjected to a thorough post-mortem examination as described by Fowler, (1996).

3.3.3.1.3 Determination of PCV and antibody titres to *S. gallinarum*

PCV was determined by centrifugation of the stabilised blood in a microhaematocrit centrifuge (Hawksley and Sons Ltd. Marlborough Rd, Lancing UK) then read on a haematocrit reader. The serum rapid plate agglutination test (SRPT) and serum agglutination test (SAT) were conducted on all sera in order to detect anti-*S. gallinarum* antibodies as according to the OIE guidelines (OIE, 1996).

3.3.3.1.4 Bacterial re-isolation and determination of viable bacterial cells

Bacterial re-isolation was done on all chicks that died in the course of the experiment. The procedure followed was to sterilise by heat the surface of the liver and spleen from the dead bird. A piece of flat short metal bar was heated on an open flame until red hot, then was placed on the surface of the liver or spleen for about ten seconds. A sterile, wire loop was introduced deep into the organ then streaked on the surface of McConkey agar (ADSA, MICRO, Copenhagen, Denmark). The plates were incubated at 37^0C overnight. Colourless, non-lactose fermenting colonies with seminal odour were regarded as *S. gallinarum.* The colonies were tested for their agglutination properties to poly 'O', poly 'H' and O: 9 antisera (State Serum Institute, Copenhagen, Denmark) where a positive poly 'O' and O: 9 but negative poly 'H' was taken as a further confirmation of *S. gallinarum.*

The viable bacterial cell counts from the liver and spleen was conducted on the birds that were killed during the experiment. The organs that had been removed asceptically as described above were dipped in 95% ethyl alcohol and flamed for sterilization. The organs were then separately placed in sterile Stomacher bags (Struers Kebo Lab, Alberslund, Denmark), weighed and homogenised with 10ml of sterile PBS using a Stomacher laboratory blender (Seaward Medical London, UK) for about 2 minutes. One millilitre of the homogenate was serially diluted (ten-fold) in sterile PBS. One ml of the different dilutions was spread onto MacConkey agar plates and incubated for 24 hours at 37^0C. Counts of viable bacteria were made manually on plates with 30-300 colony-forming units (CFU).

3.3.3.1.5 Enumeration of selected leukocytes

The thin blood films made were air-dried and fixed on absolute methanol for 30 seconds. The blood films were then stained by Wright's staining method (Boon and Drijver, 1986). The films were then observed under a light microscope (x1000 magnification) and the cells were enumerated according to their morphology (100 cells were counted from each slide). The cells, which were counted, were the heterophils, lymphocytes and monocytes and the results were expressed as percentage distribution.

3.3.3.2 Experimentation with Newcastle disease vaccine

The haemaglutination inhibition (HI) test was conducted on sera and egg yolk of local chicken ecotypes that were innoculated with a live Newcastle disease vaccine (La Sota strain, Hipra Loborotorios, SA, Spain). A total of 103 sera from hens, 103

from chicks and 103 yolk materials were collected and used in the experiment. These samples were collected from seven local chicken ecotypes namely *Ching'wekwe* (N =16), *Mbeya* (14), *Morogoro-medium* (16), *N'zenzegere* (16), *Pemba* (15), *Tanga* (12) and *Unguja* (14). The sample number was the same for hens, chicks and eggs. In addition HI titres were determined in a total of 105 sera from chicks whose HI status was zero (naïve) that were vaccinated against Newcastle disease.

3.3.3.2.1 HI titres in Hens, Eggs and Chicks

The hens from the parental stock were vaccinated against Newcastle disease using a live La Sota vaccine strain by eye drop method. A fortnight later the chickens were bled and sera were prepared separately for each ecotype. Eggs laid on the third week after vaccination were collected separately for each ecotype and some were incubated while others were stored in the refrigerator for preparation of yolk material. Some of the chicks hatched from these eggs were killed within the first three days of their life and sera were prepared separately for birds in each ecotype.

HI titres for sera obtained from hens and chicks were determined following a procedure described by Allan and Gough, (1974). The standard antigen used in HI was locally prepared in the virology laboratory SUA. From the eggs, the yolk material was carefully prepared by breaking each egg into a separate petri dish, removing the albumen and incising the yolk membrane. One millilitre of yolk material was taken and diluted into 9ml PBS (making a 10% yolk solution). HI procedure similar to that performed in the sera was conducted.

3.3.3.2.2 HI titres in Naïve chicks immunized against Newcastle disease virus

The rest of chicks from 3.3.3.2.1 above (15 from each local chicken ecotype) were monitored once a week until their maternal HI titres were down to zero. It took about four weeks to achieve this naïve state; the chicks were then immunized using live ND La Sota vaccine and the seroconversion monitored using the HI test at weekly intervals for four weeks (28 days) then on day 63 after immunisation. On day 70 the chicks were re-immunized with the ND vaccine and the HI titres were monitored for a further 28 days.

3.3.4 Productivity and reproductive performance of the local chickens under free-range management

This experiment was similar to the study in 3.3.2 in the type of parameters studied *viz*-weekly weight measurements, daily growth rate, egg weight and hatchability. Due to the experimental settings, it was not realistic to determine the fertility since candling was not performed on incubated eggs. The major differences between the two experiments was the fact that in the on-farm experiment, unlike the on-station experiment there was no control on the feeds or disease management and there was no guarantee of getting data as planned for instance at times the farmers were not at home at the time of field visits.

In this experiment, 12 farmers from Mkundi village, Morogoro municipality (about 20km from Morogoro town) were included. Each farmer had to agree to weekly visits that required him or her to lock up the chicks, collect all eggs laid for weighing and marking. Due to some inconsistencies from some farmers, three farmers had to

be dropped from the experiment. The farmers were coded A to I without any particular preference.

3.3.4.1 Weekly live weight measurements and daily growth rate for free-range local chickens

All chicks from the selected farmers were weighed from hatch and followed to 20 weeks of age. Hatch weight was regarded as the weight of a chick from hatch to up to three days, beyond three days a chick was considered to be in its first week. The weighing procedure, and determination of the daily growth rate was similar to that described under 3.3.2 above. There were many disparities in the results beyond the third month; hence the results reported were for up to three months of age.

3.3.4.2 Reproductive performance for free-range local chickens

All eggs laid over the course of the week were weighed and marked with a pencil to avoid repetition in the subsequent weeks. A total of 1243 eggs were weighed and 684 incubated during the 20-week observation period. The formula for calculating the percent hatchability was modified in the absence of candling; hence,

%Hatchability = $(H_e/I_e)*100$. Where H_e was the total number of hatched eggs, and I_e the total number of incubated eggs.

3.4 Statistical Analysis

The data collected from these experiments were subjected to statistical analyses to determine meaningful associations between them. For microsatellites data, special population genetics statistical packages were used after processing with the

Microsoft Excel macros (3.3.1.3.3 above). All other data were analysed using Statistix ® statistical package version 4.1 (1996, Analytical software).

3.4.1.1 Genetic differentiation within populations and test for Hardy-Weinberg equilibrium

The mean number of alleles per locus and the average observed and expected homozygosities were used to determine the within population genetic diversity. The number of alleles per locus per population was obtained through direct counting. The mean observed and expected heterozygosities were calculated using the GENEPOP population genetics statistical package version 3.3 updated from v.1.2 (Raymond and Rousset, 1995). Deviations from the Hardy-Weinberg equilibrium (HWE) were tested either by an exact test for each locus per population using Guo and Thompson's (1992) Markov chain Monte Carlo algorithm that is conducted by the GENEPOP package or for all loci and populations using Fisher's method also in the GENEPOP package.

3.4.1.2 Genetic variation and relationships between populations

Two genetic distance measures Nei's, (1972) standard genetic distance (D_S) and Nei *et al.*, (1983) angular distance (D_A) were used to measure the genetic diversity among populations. The D_S is said to be more appropriate to estimate evolutionary time (it is based on the infinite allele model) while the D_A is efficient in recovering the true topology of an evolutionary tree of closely related populations. Both distance measures were calculated using the DISPAN programme (Ota, 1993). The phylogenetic trees of ecotype as well as individual relationship were constructed

using the Neighbour Joining (NJ) method (Saitou and Nei, 1987). The bootstrap re-sampling of 100 was used to test the reliability of the tree topology.

3.4.1.3 Measurement of population sub-division

The coefficient of gene differentiation (G_{ST}) (Nei, 1973) was used to measure the genetic differentiation among the local chicken ecotypes. This measure was chosen because it is based on genetic drift that is considered to be the main factor in genetic differentiation among closely related populations (Tekazaki and Nei, 1996). This value was estimated using DISPAN programme.

The genetic structure of the local chicken ecotypes of Tanzania was evaluated by looking at the similarity between individual birds. This was done by determining the proportion of alleles shared over all loci and a distance measure between pairs of individuals using the MICROSAT program (Minch, 2000). A distance matrix for all individuals was generated by MICROSAT program, processed by PHYLIP program (Felsentein, 1993) and a Neighbour-Joining tree (Saitou and Nei, 1987) using individual birds as operational taxonomic units was constructed. The TREEVIEW program (Page, 1996) was used to build the tree.

3.4.2 Productivity and reproductive performance data

All the data on weekly weight measurements, daily growth rates, egg weights, fertility and hatchability were analysed using Statistix ® analylitical package (v.4.1). Before analysis all data were tested for normality (a function present in the Statistix ® program). All the normally distributed data were analysed by parametric tests

while those not following the normal distribution were analysed using non-parametric tests. All data were subjected to descriptive statistics that gave the means, standard errors of the means, medians as well as the range of the data.

Data from experiments 3.3.2 and 3.3.3 above were normally distributed hence were analysed by parametric tests mainly one-way analysis of variance (ANOVA) and the comparison of means was done by the least significant difference (LSD) at 5% level of significance.

Data for experiment 3.3.4 were not normally distributed hence were analysed by Kruskal-Wallis one-way analysis of variance (a non-parametric test) and the mean ranks were tested for variations at 5% significance level. Graphical presentations were made using either the Statistix ® programme or Microsoft Excel® spreadsheet programme.

CHAPTER 4

4.0 RESULTS

4.1 Genetic diversity in Tanzanian local chicken ecotypes

The genetic diversity of nine local chicken ecotypes from Tanzania and one reference breed (White Leghorn) was assessed by analysing 20 microsatellite loci. Out of the 20 loci, 16 showed amplification and the remaining four loci could not be amplified and hence were not included in the analysis. Table 6 shows the number of alleles per locus and the allele size ranges for all the 16 loci analysed. All amplified loci were polymorphic and the total number of alleles detected was 152. The allele sizes ranged from 80 to 380 bp while the number of alleles per locus ranged from 4 (LEI214) to 15 (ADL176). Table 6 also shows a measure of genetic differentiation (G_{ST}). The G_{ST} value for all loci was 0.134 while the G_{ST} values for individual loci ranged from 0.071 (ADL181) to 0.313 (LEI258).

4.1.2 Test for Hardy-Weinberg Equilibrium (HWE)

The results for the test to determine the deviations from HWE for the different microsatellite loci are shown in Table 7. A total of 40 out 160 (25%) locus-ecotype combinations did not deviate significantly to HWE ($P > 0.05$). The remaining 75% of the locus-ecotype combinations significantly deviated from the HWE ($P \leq 0.0001$ to $P \leq 0.05$). At loci LEI214 and LEI217 five ecotypes conformed to the HWE while at loci ADL158, ADL176, ADL176, LEI258 and LEI234 four ecotypes conformed. At the remaining loci except ADL267, three or less ecotypes did not deviate from the HWE. All local chicken ecotypes deviated significantly from the HWE at locus ADL267 (Table 7). The *Kuchi* ecotype showed a significant departure from HWE in

14 out of 16 loci. On the other hand 13 out of 16 loci in the *Ching'wekwe, Morogoro-medium, Pemba and Unguja,* ecotypes showed significant departure from the HWE. The HWE test for all loci, all ecotypes combinations revealed a high significant deviation. The inbreeding coefficients (*f*) were shown to be both positive and negative (Table 7).

4.1.3 Genetic variation within ecotypes

Table 8 shows the mean number of alleles per ecotype and the mean heterozygosity values that are measures of genetic variability within ecotypes. The mean number of alleles per ecotype ranged from 4 (White Leghorn) to between 7 (*Pemba, N'zenzegere, Singamagazi, Kuchi* and *Morogoro-medium* ecotypes). Except for the White Leghorn all other ecotypes had mean allele number of above 5. The lowest average observed heterozygosity value was 0.464 seen in White Leghorn and the highest average value was 0.688 seen in *Singamagazi.* Only three ecotypes (*Singamagazi, Pemba* and *N'zenzegere*) showed average heterozygosity values above 0.6, while the other ecotypes had values below 0.6. In all cases the average expected heterozygosity values were higher than the observed. The lowest average expected heterozygosity value was 0.621 (White Leghorn) while the highest value was 0.794 (*Ching'wekwe*).

Table 6: Allele numbers and size ranges and measure of genetic differentiation (G_{ST}) for the 16 loci examined.

Locus	Allele size range (bp)	Number of alleles	G_{ST}
ADL267	92 – 120	8	0.114
ADL102	96 – 138	11	0.194
ADL158	174 – 228	13	0.111
ADL171	80 – 108	9	0.097
ADL172	130 – 188	14	0.089
ADL176	170 – 230	15	0.090
ADL181	164 – 196	9	0.071
ADL210	116 – 150	9	0.096
ADL136	124 – 200	12	0.086
LEI43	140 – 176	9	0.169
LEI40	180 – 264	9	0.223
MCW005	216 – 276	6	0.133
LEI217	184 – 280	11	0.140
LEI214	144 – 176	4	0.134
LEI234	288 – 380	5	0.108
LEI258	192 – 324	8	0.313
All loci	80 - 380	152	0.134

Table 7: Test for HWE deviation and the inbreeding coefficients (*f*) in the local chicken ecotypes of Tanzania.

Ecotype	Loci															
	ADL267	ADL102	ADL158	ADL171	ADL172	ADL176	ADL181	ADL210	ADL136	LEI40	LEI43	LEI214	LEI217	LEI234	LEI258	MCW05
DAN (9)	0.60 **	0.46 ***	0.73 ****	-0. 43 ****	-0.26 ns	-0.37 ns	0.29 ns	0.81 ****	-0.00 ns	1 **	1 *	-0.494 ns	0.49 ns	0.82 *	-0.41 ns	1 ****
CHIN (13)	0.71 ****	0.70 ****	0.93 ****	-0.07 ****	0.40 ****	0.11 ****	0.27 ***	0.45** **	-0.08 ****	0.09 **	0.88 ****	0.17 *	-0.14 ns	0.35 ns	0.24 **	0.16 ns
KUCH (14)	-0.03 ****	0.10 ****	-0.21 **	-0.17 **	0.10 ****	0.16 *	-0.09 ns	-0.12 **	0.36 ****	1 ns	0.54 ***	0.71 *	0.36 *	0.58 ***	0.35 ****	0.25 ****
SING (10)	-0.29 ****	-0.19 ns	0.03 ns	-0.43 ***	0.48 ****	-0.10 ns	0.12 ****	-0.06 ns	0.19 ****	-0.14 *	0.48 ****	0.52 *	0.37 ****	0.03 ns	0.44 ***	0.26 ns
UNGU (13)	-0.06 *	0.02 **	0.12 ns	0.06 *	-0.00 ***	0. 49 **	-0.14 *	-0.20 ****	0.40 ****	0.47 *	0.78 ****	-	0.20 ns	0.72 **	-0.13 **	0.72 **
PEMB (13)	-0.13 **	0.30 ****	0.37 ****	-0.07 **	0.01 ***	0.32 ***	-0.48 ***	0.02 **	0.13 ****	0.65 ****	0.79 ****	1 ns	0.12 ns	0.49 **	0.14 ns	0.40 **
TANG (11)	-0.17 **	0.27 ***	-0.05 *	-0.51 **	0.05 *	0.03 ns	0.13 ***	-0.35 *	0.03 **	1 ns	0.66 ****	0.11 ns	0.76 ****	0.26 ns	0.06 ns	0.6 **
FRIZ (10)	0.10 **	-0.01 ns	-0.17 ns	-0.59 ***	0.06 ***	0.03 ns	-0.05 ****	-0.01 **	-0.11 ns	0.22 *	0.58 ****	0.57 ns	0.61 ****	0.65 ****	-0.11 ns	1 ****
MBEY (12)	0.36 ****	0.23 ****	-0.14 ns	-0.45 *	0.15 ****	0.44 ****	0.58 ****	-0.09 ns	0.36 ****	0.93 ****	0.77 ****	-0.15 ns	0.46 ****	0.36 ns	0.20 *	1 ****
MORO (13)	-0.31 **	0.54 ****	-0.06 ***	-0.39 ns	0.40 ****	0.05 ****	0.95 ****	-0.24 ****	0.51 ****	0.14 ns	0.54 ****	0.75 *	0.17 ns	0.0 *	0.24 **	0.73 ***

DAN = White Leghorn, CHING=*Ching'wekwe*, KUCH=*Kuchi*, SING=*Singamagazi*, UNGU=*Unguja*, PEMB=*Pemba*, TANG=*Tanga*, FRIZ=*N'zenzegere*, MBEY=*Mbeya*, MORO=*Morogoro-medium*; Numbers in brackets indicate loci deviating from HWE; * P≤0.05, ** P≤0.01, *** P≤.001, **** P≤0.0001; ns not significant

Table 8: Mean allele number and heterozygosity values for local chicken ecotypes of Tanzania.

Ecotype	Mean number of alleles (Mean ± SD)	Expected heterozygosity (Mean ± SD)	Observed heterozygosity (Mean ± SD)
White Leghorn	4.31 ± 2.02	0.621 ± 0.0432	0.464 ± 0.0363
Ching'wekwe	5.94 ± 2.14	0.794 ± 0.0164	0.545 ± 0.0296
Kuchi	6.56 ± 1.93	0.789 ± 0.0251	0.594 ± 0.0304
Singamagazi	6.75 ± 2.72	0.775 ± 0.0244	0.688 ± 0.0254
Unguja	5.06 ± 1.65	0.736 ± 0.0509	0.579 ± 0.0379
Pemba	7.06 ± 2.46	0.788 ± 0.0236	0.602 ± 0.0276
Tanga	6.38 ± 3.20	0.771 ± 0.0304	0.064 ± 0.0316
N'zenzegere	6.69 ± 2.77	0.789 ± 0.0235	0.660 ± 0.0304
Mbeya	6.50 ± 1.71	0.757 ± 0.0294	0.532 ± 0.0298
Morogoro-medium	6.94 ± 2.95	0.778 ± 0.0248	0.586 ± 0.0288

4.1.4 Genetic distances between the local chicken ecotypes of Tanzania

The genetic distances between the local chicken ecotypes measured by the two measures D_A and D_S are presented in Tables 9 and 10. From the D_A genetic distances, the lowest value was between *Kuchi* and *Unguja* ecotypes (0.1606) while the highest distance value was seen between White Leghorn and *Unguja* ecotype (0.5352). The genetic distances between the three ecotypes from Morogoro region (*Ching'wekwe*, *N'zenzegere* and *Morogoro-medium*) were below 0.3 similarly for *Mbeya* ecotype and the White Leghorn breed. The D_S genetic distance showed that the lowest distance (0.0671) was between *Unguja* and *Kuchii* ecotypes with *Unguja* and White Leghorn presenting the highest (0.9149) value. Apart from the distance between Unguja and Kuchi, those between Pemba/Unguja, Pemba/Kuchi, N'zenzegere/Singamagazi, Mbeya/White Leghorn were also below 0.3 (Table 10).

4.1.5 Phylogenetic relationships among the local chicken ecotypes

The D_A based NJ phylogenetic tree for the local chicken ecotypes is presented in Figure 6. The phylogeny of all ecotypes indicated three main clusters. The first cluster can be further divided into two sub-clusters the first comprised the two isle ecotypes (*Pemba* and *Unguja*) and *Kuchi* ecotype and the second formed by the *Morogoro-medium* and *Ching'wekwe* ecotypes. The second main cluster was made up of the *Singamagazi* and *N'zenzegere* ecotypes and third cluster comprised of the *Tanga* ecotype together with White Leghorn and *Mbeya* ecotype.

For the NJ phylogenetic tree constructed based on the D_S distance, three clusters were evident (Figure 7). The first cluster comprised of the *Ching'wekwe* ecotype together with

Pemba, *Unguja* and *Kuchi* ecotypes. The second cluster was made up of the *Morogoro-medium* ecotype together with the *Singamagazi* and *N'zenzegere* ecotypes. The third cluster was formed by the *Tanga* ecotypes together with the White Leghorn and *Mbeya* ecotype. The bootstrap values ranged from 29 to 100 in the D_A based phylogenetic tree and 41 to 98 in the D_S based phylogenetic tree.

4.1.6 Genetic structure of the local chicken ecotypes of Tanzania

The genetic structure of the Tanzanian local chicken ecotypes was evaluated by the phylogenetic analysis of individual chickens. The phylogenetic tree using individual chickens from the different ecotypes as operational taxonomic units is shown in Figure 8 Nine clusters marked A to I were formed with most chickens from same ecotypes clustering together. Table 11 shows the distribution of chickens into these nine clusters. It was shown that 100% (13/13) of chickens from the *Ching'wekwe*, *Mbeya*, *Tanga* ecotypes and the White Leghorn clustered in the A, F, B and E clusters respectively. It was also observed that 77% (10/13) of chickens from the *Kuchi* ecotype clustered together (cluster C), with the remaining 23% clustering in cluster I. Chickens from the *Morogoro-medium* and *Pemba* ecotypes had each 69% (9/13) of individuals clustering together (cluster G and D respectively) with the other four located in other clusters. Individuals from the *N'zenzegere* ecotype were located on clusters B (54%) and G (46%) while those from the *Unguja* ecotype were mainly found in clusters C (54%) and D (38.5%). Table 11 also shows that more individuals were found in cluster G (21)

while cluster I (3) had the fewest individuals. Clusters E and I had each individuals belonging to only one breed/ecotype.

Table 9: Nei's D_A Genetic distances for nine local chicken ecotypes of Tanzania and the White Leghorn breed.

	DAN	CHIN	KUCH	SING	UNGU	PEMB	TANG	FRIZ	MBEY
CHIN	0.4229								
KUCH	0.4074	0.3242							
SING	0.4255	0.3531	0.3491						
UNGU	0.5352	0.4032	0.1606	0.4483					
PEMB	0.4435	0.2907	0.2149	0.3487	0.1827				
TANG	0.3651	0.3582	0.3388	0.3183	0.3873	0.2922			
FRIZ	0.4026	0.3879	0.2876	0.2304	0.4045	0.3226	0.3261		
MBEY	0.2923	0.3934	0.3245	0.3161	0.4122	0.3315	0.3261	0.2661	
MORO	0.4445	0.2904	0.3258	0.2610	0.4280	0.3225	0.3183	0.2830	0.3261

Table 10: D_S Genetic distances for nine local chicken ecotypes of Tanzania and the White Leghorn breed.

	DAN	CHIN	KUCH	SING	UNGU	PEMB	TANG	FRIZ	MBEY
CHIN	0.6416								
KUCH	0.6296	0.4697							
SING	0.7108	0.4962	0.5758						
UNGU	0.9149	0.6127	0.0671	0.7670					
PEMB	0.6452	0.3738	0.2063	0.5802	0.1163				
TANG	0.5347	0.5431	0.5400	0.4905	0.5959	0.4324			
FRIZ	0.6252	0.5841	0.4723	0.2650	0.6879	0.5213	0.4583		
MBEY	0.2997	0.5563	0.5343	0.4882	0.6365	0.4888	0.4524	0.4267	
MORO	0.7506	0.3702	0.5211	0.3134	0.6795	0.5543	0.5681	0.4096	0.4787

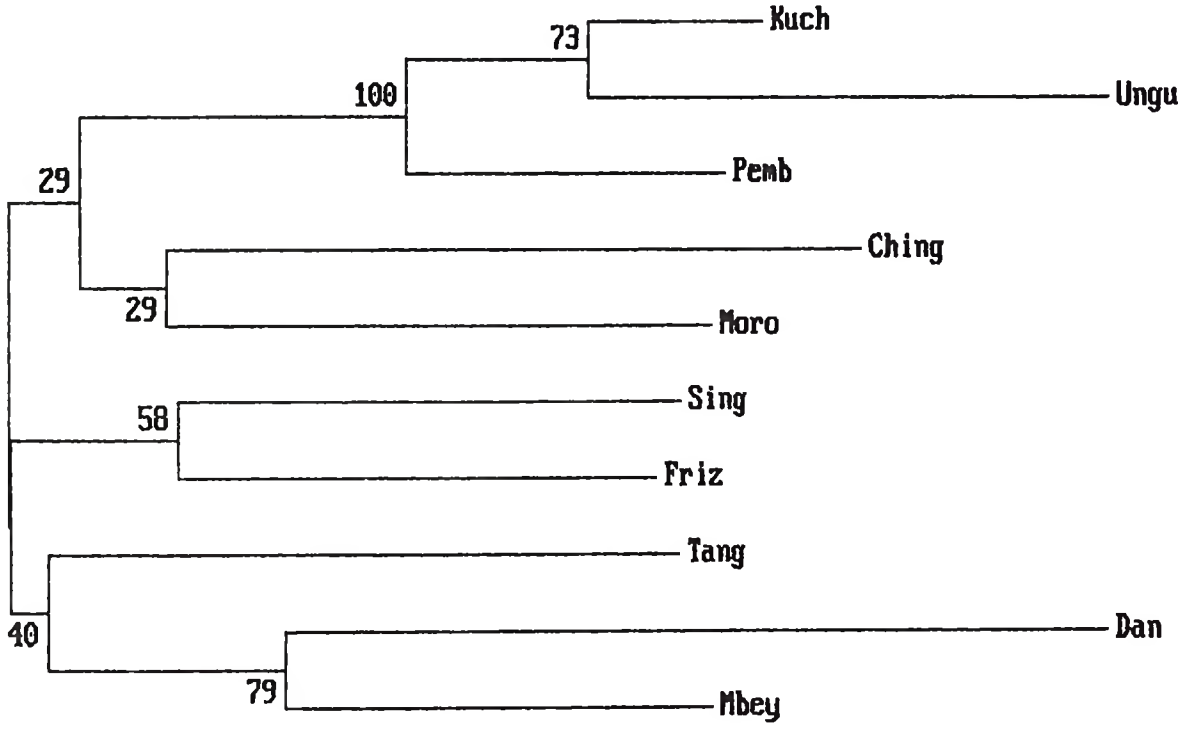


Figure 6: Unrooted neighbour-joining tree displaying the genetic relationship of nine local chicken ecotypes of Tanzania and a White Leghorn breed based on D_A genetic distances. (Bootstrap values x 100 re-sampling).

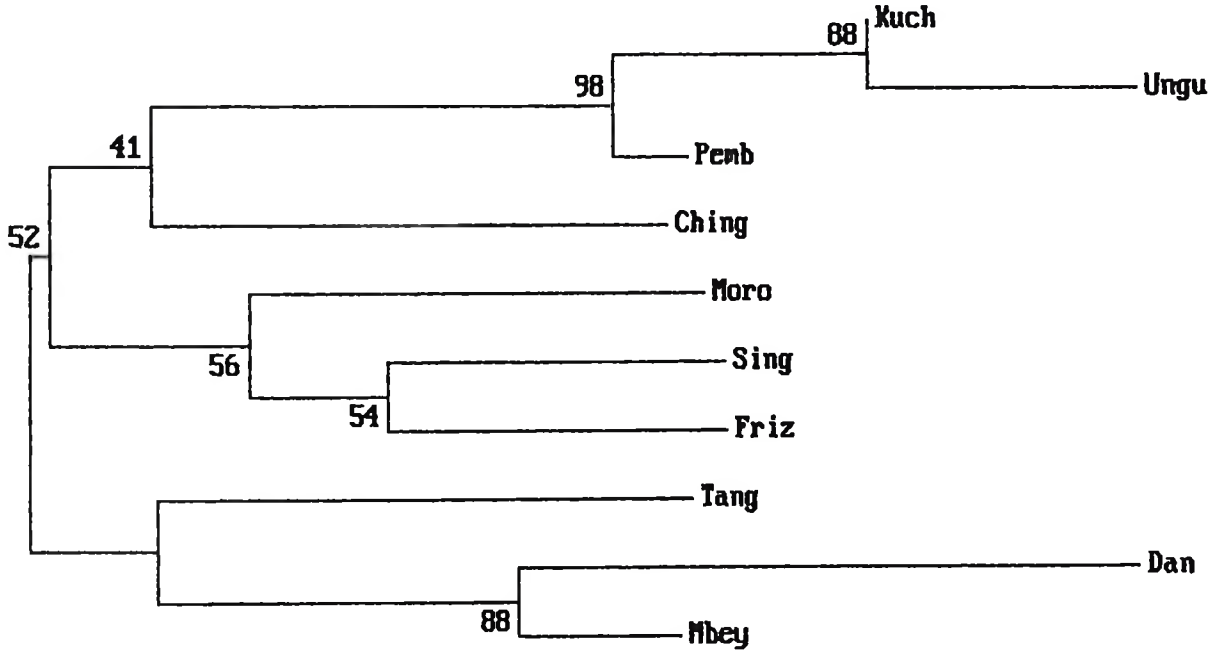


Figure 7: Unrooted neighbour-joining tree displaying the genetic relationship of nine local chicken ecotypes of Tanzania and a White Leghorn breed based on D_s genetic distances. (Bootstrap values x 100 re-sampling).

Table 11: Placement of individual local chickens into clusters resulting from individual chicken dendogram for the local chicken ecotypes of Tanzania.

Ecotypes	Clusters									
	A	B	C	D	E	F	G	H	I	Total
Ching'wekwe	13	0	0	0	0	0	0	0	0	13
Kuchi	0	0	10	0	0	0	0	0	3	13
Singamagazi	0	0	0	0	0	0	6	7	0	13
Unguja	0	0	7	5	0	0	0	1	0	13
Pemba	3	0	0	9	0	0	0	1	0	13
Tanga	0	13	0	0	0	0	0	0	0	13
N'zenzegere	0	7	0	0	0	0	6	0	0	13
Mbeya	0	0	0	0	0	13	0	0	0	13
Morogoro-M*	2	0	0	0	0	2	9	0	0	13
White Leghorn	0	0	0	0	13	0	0	0	0	13
Total	18	20	17	14	13	15	21	9	3	130

*Morogoro-medium ecotype.

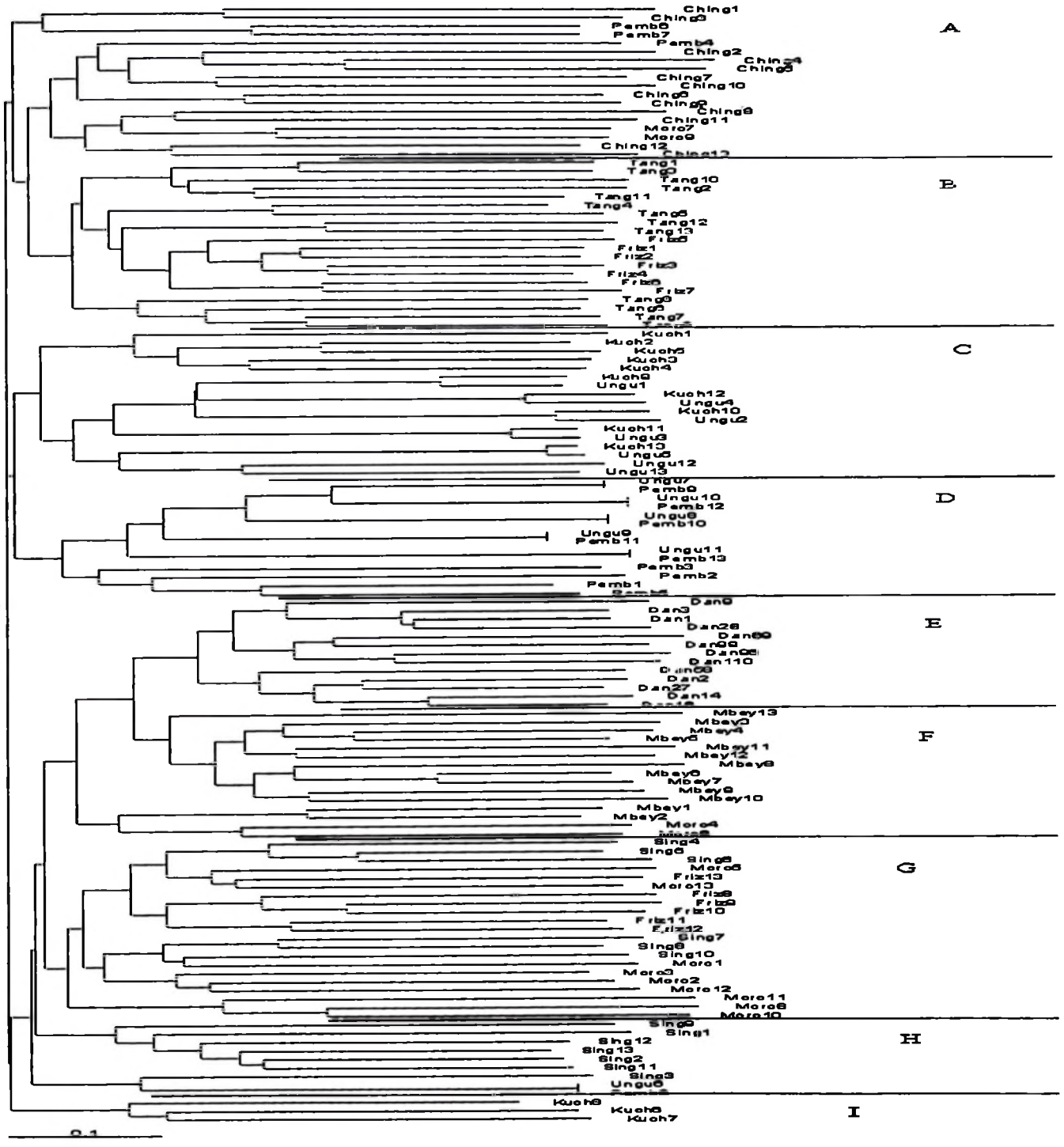


Figure 8: A Neighbour-Joining tree based on allele sharing distances among 130 individual local chicken ecotypes of Tanzania and White Leghorn breed.

Note: A – I represent different clusters

4.2 Productivity and reproductive performance of the local chicken ecotypes

4.2.1 Weekly weight measurements

Data on the overall mean weight measurements for the local chicken ecotypes at hatch, four, eight, twelve, sixteen and twenty weeks of age are presented in Table 12. As expected the weight measurements in cocks were higher compared to those of hens throughout the study period. Wide ranges were observed between the groups signifying the diversity of the chicken ecotypes studied. It was also noted that, attainment of 1kg body weight did not occur until the chickens were 16 and 20 weeks of age for cocks and hens, respectively.

Tables 13 and 14 show the mean weekly weight measurements for the local chickens separated by ecotype and sex. With regard to hatch weight in hens, the *Ching'wekwe* and *Mbeya* ecotypes showed the lowest and the highest mean values (18.8 and 30.8g), respectively. When the means were compared it was seen that except for *N'zenzegere* and *Unguja* ecotypes all other means were significantly different from each other ($P<0.05$).

Throughout the five-month period, the *Ching'wekwe* ecotype showed consistently low mean weight measurements and with the exception of *Pemba* ecotype in the fourth and fifth month this low mean was significantly different from the other ecotypes. The highest mean weight measurements were shared between *Mbeya* and *Morogoro-medium* ecotypes (4^{th} and 12^{th} week) and between *Morogoro-medium* and *Tanga* ecotypes (8^{th},

12th, 16th and 20th week). In the fourth week, the mean weight measurements for *Mbeya* and *Morogoro-medium* ecotypes were significantly higher than the rest of the ecotypes ($P<0.05$).

Table 12: The overall mean weekly body weight measurements (g) for the local chicken ecotypes.

Sex	Trait	Mean ± SE	Range
Hens (N = 194)	Hatch weight	25.7 ± 0.3	14 – 34
	Weight at week 4	89 ± 1.6	47 - 134
	Weight at week 8	239.3 ± 4.2	118 – 358
	Weight at week 12	381.1 ± 6.5	205 – 568
	Weight at week 16	540.9 ± 8.1	316 – 776
	Weight at week 20	741.7 ± 9.2	518 - 1036
Cocks (N = 189)	Hatch weight	29.9 ± 0.3	22 – 36
	Weight at week 4	136.6 ± 3.2	60 – 238
	Weight at week 8	357.9 ± 4.2	237 – 870
	Weight at week 12	579 ± 7.5	375 – 870
	Weight at week 16	826.5 ± 10.5	560 – 1234
	Weight at week 20	1088.8 ± 13.3	698 - 1524

In the eighth week, the mean weight measurements for *Morogoro-medium* was significantly higher compared to the other ecotypes. Comparison of means at the twelfth

week, showed three ecotypes (*Mbeya*, *Morogoro-medium* and *Tanga*), to have significantly higher mean values than the other ecotypes. The last two months of the studies showed the *Morogoro-medium* and *Tanga* ecotypes to have significantly higher mean weight measurements compared to the other ecotypes. It is also seen from the results (Table 13 and Table 14) that, the average weight measurements for *Ching'wekwe* ecotype was consistently lower than the overall mean values (Table 12). *N'zenzegere*, *Pemba* and *Unguja* ecotypes showed mean weight measurements close to the overall means. The other two ecotypes (*Mbeya* and *Morogoro-medium*) had mean weight measurements consistently higher than the overall mean values calculated for all ecotypes.

Mean hatch weights for cocks showed the *Ching'wekwe* and the *Mbeya* ecotypes to have the lowest and the highest values, respectively (23.3g and 35.2g). Comparison of means showed these two extreme values to be significantly different from each other and to the means of all other ecotypes ($P<0.05$). The mean hatch weight for *N'zenzegere*, *Tanga* and *Unguja* ecotypes was significantly different from *Morogoro-medium* and *Pemba* ecotypes. Weekly mean weight measurements trend for the *Ching'wekwe* ecotype was similar to that observed in hens (was significantly lower than other ecotypes except *Pemba* ecotype at 12^{th} and 16^{th} week). Mean weight measurements in the fourth week showed that the *Mbeya* ecotype had a significantly higher mean compared to the other ecotypes ($P<0.05$). The means for *N'zenzegere* and *Unguja* were similar but significantly different from the other ecotypes. Similarly, the means for *Morogoro-*

medium, *Pemba* and *Tanga* ecotypes were equal but significantly different from the other ecotypes. During the eighth week, *Mbeya*, *Morogoro-medium*, *Pemba* and *Tanga* ecotypes showed similar mean weight measurements that were significantly different from the rest of the ecotypes. Similarly, the *N'zenzegere* and *Unguja* ecotypes had similar mean weights that were different from the other ecotypes. Mean weight measurements at 12^{th} and 16^{th} week depicted three groups with means that differed significantly with each other. The groups were *Ching'wekwe* and *Pemba*; *N'zenzegere*, *Mbeya* and *Unguja*; and *Morogoro-medium* and *Tanga* ecotypes. At the 20^{th} week, *N'zenzegere* and *Unguja* ecotypes showed means that differed significantly from each other and from *Morogoro-medium* and *Tanga* ecotypes ($P<0.05$).

Compared to the overall mean hatch weight, the *Ching'wekwe* and *Pemba* ecotypes had lower mean values. On the mean weekly weight measurements, the *Ching'wekwe*, *N'zenzegere* and *Pemba* ecotypes had lower values compared to the overall mean throughout the study period. *Unguja* ecotype showed lower mean values in comparison with the overall means during the first two months, but the means were comparable to the overall means in the third and fourth months. The mean weight measurements in the fifth month were above the overall mean. *Mbeya* ecotype's mean weight measurements at the fifth month dropped below the overall mean although the means were consistently higher on the previous four months. Mean weight measurements for *Morogoro-medium* ecotype was consistently higher than the overall means throughout the experimental period.

Table 13: Mean weekly weight measurements up to 20 weeks for hens (Mean ± SE)

Weekly weight measurements in grams						
Ecotype	Hatch weight	Week 4	Week 8	Week 12	Week 16	Week 20
Ching'wekwe (N = 33)	18.8 ± 0.5^{a}	55.3 ± 0.7^{a}	163.6 ± 5.7^{a}	294 ± 4.7^{a}	443.7 ± 14^{a}	592.3 ± 7.8^{a}
N'zenzegere (N = 33)	27.7 ± 0.3^{d}	83.4 ± 1.9^{b}	234 ± 4.3^{bc}	382.4 ± 6^{b}	557.5 ± 10^{c}	731.5 ± 12.9^{bc}
Mbeya (N = 24)	30.8 ± 0.4^{f}	113.9 ± 2.8^{d}	246.9 ± 11.9^{cd}	440.8 ± 17.3^{c}	592.1 ± 22.6^{cd}	772 ± 19.2^{cd}
Morogoro-M* (N = 28)	28.7 ± 0.2^{e}	109.3 ± 2.2^{d}	298.5 ± 8.4^{f}	456.7 ± 15^{c}	648.4 ± 19.9^{de}	853.6 ± 22.3^{e}
Pemba (N = 30)	24.7 ± 0.3^{c}	93.6 ± 1.5^{c}	255.4 ± 7.8^{de}	310.7 ± 6.4^{a}	458 ± 13^{a}	754.7 ± 23.2^{c}
Tanga (N = 23)	23.4 ± 0.4^{b}	96.8 ± 4.8^{c}	277.6 ± 12^{ef}	441.4 ± 24.2^{c}	615.1 ± 22.1^{de}	829 ± 29.8^{de}
Unguja (N = 23)	27.1 ± 0.3^{d}	81.2 ± 1.9^{b}	216.4 ± 4.8^{b}	381.1 ± 15.4^{b}	506.3 ± 12.3^{b}	698.5 ± 16.3^{b}

Means in column with common superscripts are not significantly different from each other (One way analysis of variance, $P<0.05$)

Table14: Mean weekly weight measurements up to 20 weeks for cocks (Mean ± SE)

Weekly weight measurements in grams						
Ecotype	Hatch weight (g)	Week 4 (g)	Week 8 (g)	Week 12 (g)	Week 16 (g)	Week 20 (g)
Ching'wekwe (N = 21)	23.3 ± 0.2^{a}	61.6 ± 0.4^{a}	259.9 ± 4.3^{a}	462.4 ± 6.3^{a}	650.9 ± 15^{a}	780.6 ± 17.5^{a}
N'zenzegere (N= 21)	30.1 ±0.2^{c}	119.7 ± 3.7^{b}	339.1 ± 13.2^{b}	571.3 ± 20.9^{b}	844.4 ± 27^{b}	1066.4 ± 18.3^{b}
Mbeya (N = 33)	35.2 ± 0.3^{e}	186.4 ± 6.1^{d}	379.6 ± 3^{c}	604.4 ± 10.7^{b}	824.2 ±19^{b}	1047.5 ± 22.7^{bc}
Morogoro-M* (N = 28)	31.7 ± 0.2^{d}	154.1 ± 5.8^{c}	397 ± 6.3^{c}	645.2 ± 16.9^{c}	957.3 ± 26.2^{c}	1229.2 ± 32.5^{d}
Pemba (N = 24)	27 ± 0.1^{b}	148.9 ± 5.1^{c}	374.6 ± 9.3^{c}	473.9 ± 17.2^{a}	695.8 ± 20.8^{a}	1031.4 ± 13.1^{bc}
Tanga (N = 31)	29.5 ± 0.4^{c}	144.5 ± 2.4^{c}	379.4 ± 6.8^{c}	657.4 ± 17.4^{c}	919.1 ± 10.3^{c}	1216.7 ± 17^{d}
Unguja (N = 31)	29.7 ± 0.2^{c}	113 ± 6.5^{b}	344.4 ± 10.9^{b}	579.5 ± 12.9^{b}	826.6 ± 22.5^{b}	115.4 ± 34.4^{c}

Means in column with common superscripts are not significantly different from each other (One way analysis of variance, P<0.05)

4.2.3 Daily mean growth rate.

Figure 9 shows the overall daily mean growth rate for chicks up to five months of age for both hens and cocks. The results show that in hens, the mean growth rate during the first month was 2 grams/day with a sharp increase of up to 5.6 g/d in the second month. A slight depression in growth occurred during the third month, but a positive trend (a less sharp increase) resumed for the fourth and fifth month. In cocks, the mean daily growth rate for the first month was 3.3 g/d increasing sharply to 7.4g/ during the second month. In contrast to the hens, there was a plateau on the third month then a gentle increase in the fourth and fifth month.

Results for mean daily growth rates for the various local chicken ecotypes are presented in Figures 10 and 11. It was observed that each ecotype had a decrease in mean daily growth rate at a certain point in the study period. This period was between the second and third month in *Morogoro-medium*, *N'zenzegere*, *Pemba* and *Tanga* ecotypes but was between the third and fourth month in the *Mbeya* and *Unguja* ecotype. Between the fourth and fifth month, *Ching'wekwe* and *N'zenzegere* ecotypes showed a small decrease in the mean daily growth rate. The lowest mean growth rates were recorded in the *Ching'wekwe* ecotype hens during the first, second and fifth month of the experiment. Hens from *Pemba* and *Unguja* ecotypes showed the lowest mean growth rates for the third and fourth months respectively. The highest daily mean growth rate was shared between *Mbeya*, *Morogoro-medium*, *Pemba* and *Tanga* ecotypes at different times in the

study period. However, it was the *Morogoro-medium* ecotype that had a high frequency on this parameter (three out of the five month).

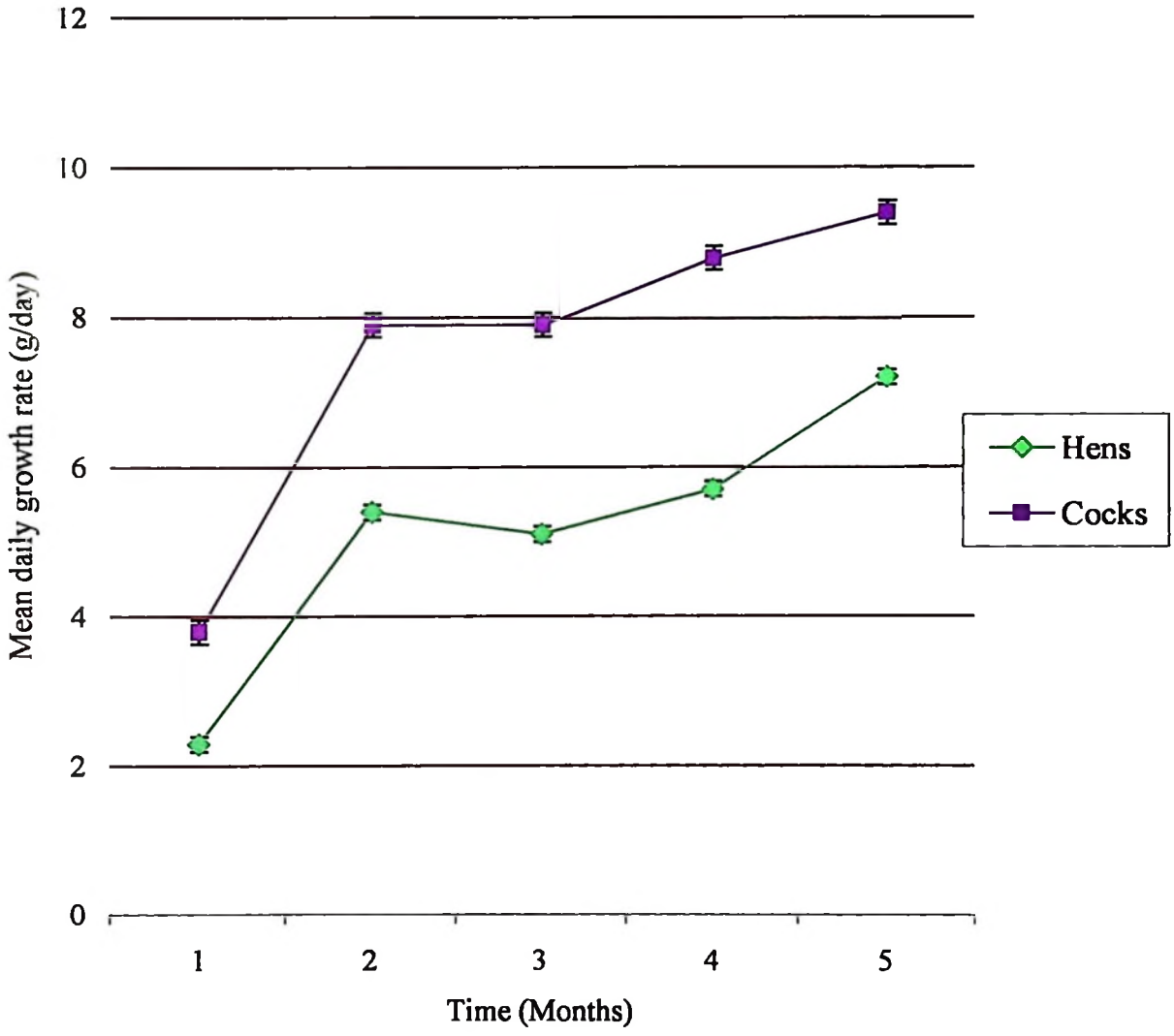


Figure 9: Overall mean daily growth rate for local chicken ecotypes in Tanzania.

The daily mean growth rate in cocks showed a more or less similar trend as in hens with only a few disparities. In general, each ecotype showed a sharp increase in the daily mean growth rate in the second month of the study period. In the third month, *Pemba* ecotype showed an obvious drop in the daily mean growth rate (comparable to what was experienced in hens) although a sharp rise growth rate was recorded in the fourth and fifth months of the study period. *Mbeya*, *Morogoro-medium* and *Ching'wekwe* ecotypes had their daily mean growth rates levelling during the third month; followed by an increase in the fourth month and a further decrease in the fifth month (the decrease in *Ching'wekwe* ecotype started on the fourth month). *N'zenzegere* ecotype showed an increasing mean daily growth rate up to the fourth month followed by a decrease in fifth month. *Mbeya* and *Tanga* ecotypes showed an increasing mean daily growth rate up to the third month, then there was a levelling (*Mbeya* ecotype) and a decrease (*Tanga* ecotype) in the growth rate in the fourth month. The fifth month saw an increase in the growth rate in both *Mbeya* and *Tanga* ecotypes.

Comparison of means between the various local chicken ecotypes over the five-month study period resulted in significantly different values ($P<0.05$). For instance for the greater part of the five-month trial (three months for hens; four months for cocks), daily mean growth rates for the *Ching'wekwe* ecotype was significantly lower (in both cocks and hens) compared to the other ecotypes. None of the ecotypes showed a persistently significantly higher mean daily growth rate. Of interest is the *Pemba* ecotype, which after a significantly lower growth rate in the third month, showed a significantly higher

growth rate starting from the fourth month. *Morogoro-medium* and *Tanga* ecotypes showed comparable means from the second to the fifth month in hens and the first two months and the fifth month in cocks. *N'zenzegere* and *Unguja* ecotypes showed persistently similar mean growth rate in cocks and only differed significantly in the fourth month for hens. The Zanzibar ecotypes (*Pemba* and *Unguja*) showed significantly different daily mean growth rates in all occasions except during the second month (cocks) and fourth month (both cocks and hens).

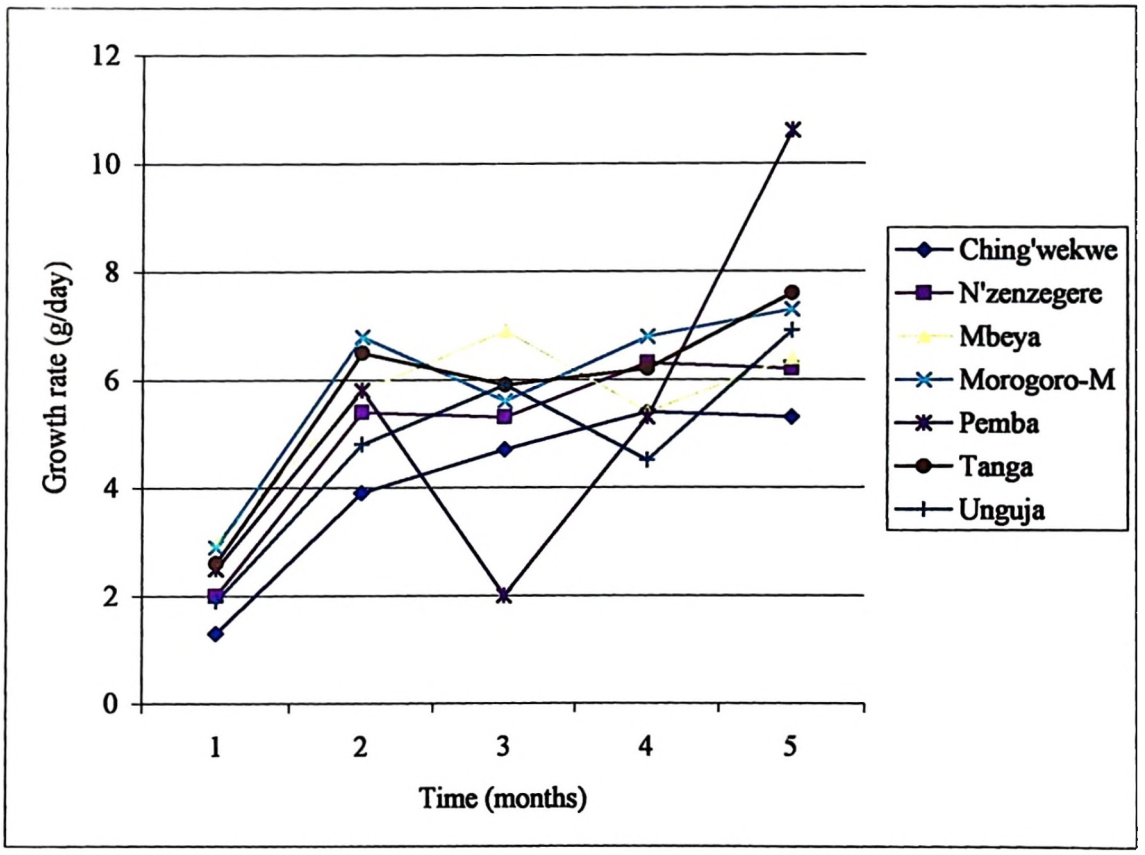


Figure 10: Mean growth rate (g/day) for local chicken ecotypes in Tanzania (hens).

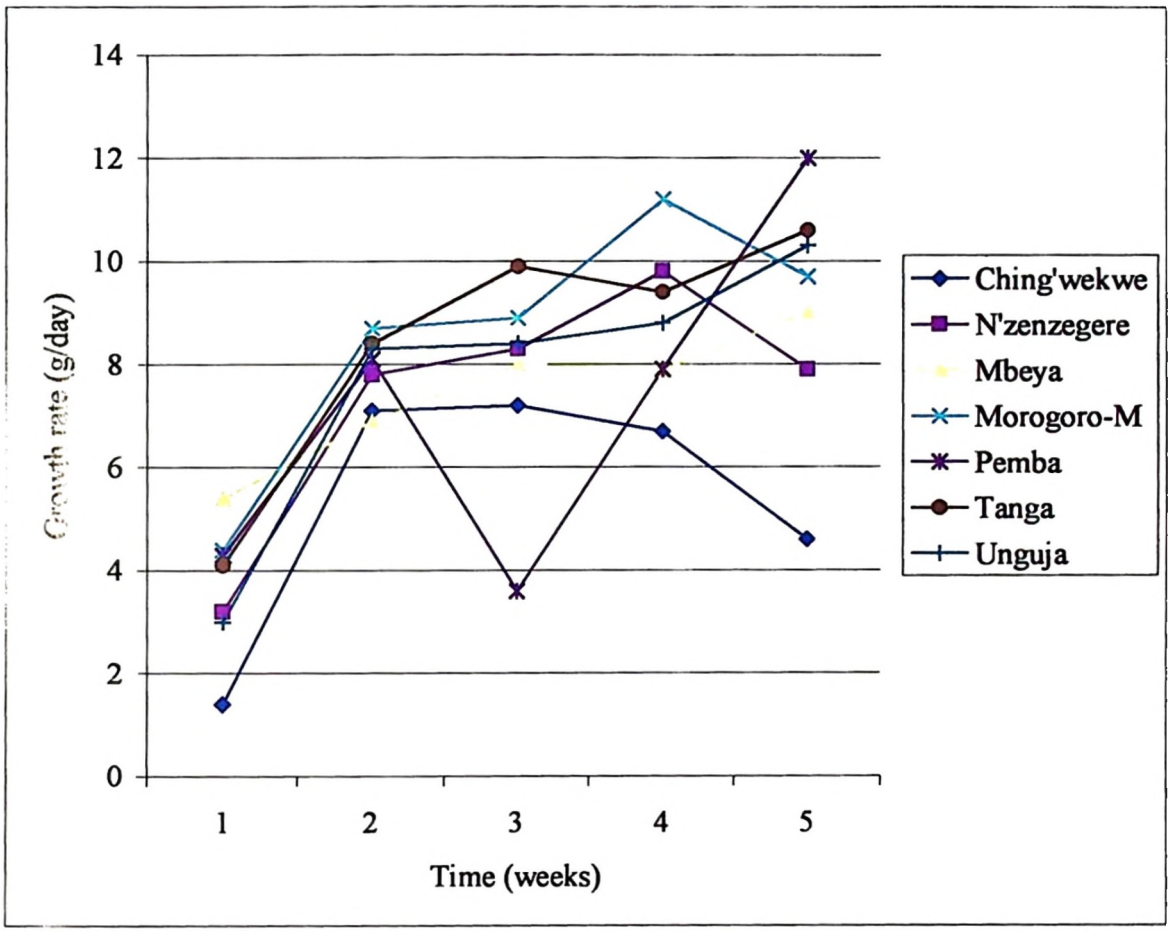


Figure 11: Mean growth rate (g/day) for local chicken ecotypes in Tanzania (cocks).

4.2.4 Egg weights, fertility and hatchability for the local chicken ecotypes

The overall reproductive performance for the local chicken ecotypes measured by mean egg weight, fertility and hatchability is shown in Table 15. The mean values were greatly influenced by the range within the different groups. For instance, the mean egg weight of 42.5g with a range of 20.8g to 55g indicate that most of the ecotypes had their mean egg weight towards the right side of the range. Similarly, fertility and hatchability showed wider ranges.

Table 16 shows the average egg weights, proportion mean fertility and proportion mean hatchability by ecotypes. The mean egg weight for *Ching'wekwe* ecotype was the lowest (37.2g) while that for *Mbeya* ecotype was the highest (49.3g). The other ecotypes had mean egg weights falling between these two extremes albeit with a tendency towards the lower extreme. When the means were compared, it was revealed that the mean egg weights for *Ching'wekwe* and *Mbeya* ecotypes were significantly different from each other and from the other ecotypes ($P<0.05$). The mean egg weight for *N'zenzegere* and *Pemba* were different from each other and from the other ecotypes. Mean egg weight for *Morogoro-medium*, *Tanga* and *Unguja* ecotypes were similar but significantly different from the rest of the ecotypes.

The mean fertility indicated that *Morogoro-medium* and *Pemba* ecotypes had the lowest mean value (0.64) while *Unguja* ecotype had the highest mean value (0.8). In comparing the means, it was seen that *Morogoro-medium* and *Pemba* ecotypes had significantly

lower mean values compared to the other ecotypes ($P<0.05$). However, the high mean value for *Unguja* ecotype was not significantly different from those of *Ching'wekwe*, *Mbeya*, *N'zenzegere* and *Tanga* ecotypes. There was a great variation in the between ecotype ranges. For example, the range for proportion fertility for *Morogoro-medium* ecotype was 0.2 to 0.9 (20 – 90%) while that for *Mbeya* ecotype was 0.5 to 0.9 (50 – 90%). Except for *N'zenzegere* ecotype (0.3), the lowest value for proportion fertility was 0.4 (40%) and the highest value was 1.0 (100%).

The proportion hatchability showed two groups with significantly different means. The group with the lowest mean including the *Ching'wekwe*, *N'zenzegere* and *Morogoro-medium* ecotypes and that with the highest mean encompassing the *Pemba* ecotype. The other ecotypes had means that were not significantly different from the two groups. Again there were great variations between ecotype ranges with recorded data being as low as 0.1 (10%).

Table 15: Overall mean reproductive performance of the local chicken ecotypes.

Trait	Mean ± SE	Range
Egg weight (g)	42.5 ± 0.6	20.8 - 55
Fertility	0.7 ± 0.01	0.15 – 1.0
Hatchability	0.62 ± 0.01	0.11 – 1.0

Table 16: Mean reproductive performance of the local chicken ecotypes

Ecotype	Proportion mean fertility (Range)	Proportion mean hatchability (Range)	Mean egg weight (grams)
Ching'wekwe	$0.7 \pm 0.03^{bc*}$	0.55 ± 0.04^{a}	37.2 ± 0.3^{a}
	(0.4 – 1.0)	(0.2 – 0.9)	
N'zenzegere	0.75 ± 0.04^{bc}	0.57 ± 0.05^{a}	41.1 ± 0.3^{b}
	(0.3 – 1.0)	(0.2 – 0.9)	
Mbeya	0.69 ± 0.03^{bc}	0.64 ± 0.04^{ab}	49.3 ± 0.2^{c}
	(0.5 – 0.9)	(0.4 – 1.0)	
Morogoro-medium	0.64 ± 0.06^{a}	0.55 ± 0.06^{a}	42.6 ± 0.6^{d}
	(0.2 – 0.9)	(0.2 – 0.9)	
Pemba	0.64 ± 0.03^{a}	0.74 ± 0.05^{b}	41.9 ± 0.2^{c}
	(0.4 – 0.9)	(0.3 – 1.0)	
Tanga	0.68 ± 0.03^{bc}	0.63 ± 0.06^{ab}	43 ± 0.3^{d}
	(0.4 – 0.8)	(0.1 – 1.0)	
Unguja	0.8 ± 0.03^{c}	0.66 ± 0.03^{ab}	42.6 ± 0.3^{d}
	(0.4 – 1.0)	(0.4 – 0.9)	

*Means in column with common superscripts are not significantly different from each other (One way analysis of variance, $P<0.05$)

4.3 Responses of the local chicken ecotypes to experimental infection with *Salmonella gallinarum*

The PCV values for all the infected local chickens as well as the non-infected controls were within the normal range. No further analysis was therefore performed on this parameter. The antibody response measured by both SRPAT and SAT revealed that only a few birds seroconverted. It was evident for instance that on day 3 post infection (pi) only one bird from *Mbeya* ecotype seroconverted out of 120 chicks. It was further observed that no bird serocoverted on day 6 pi while four birds (one each from *Morogoro-medium* and *Pemba* and two from *Tanga* ecotypes) showed positive response on day 10 pi. No response was observed on day 14 pi.

4.3.1 Clinical signs, mortality and pathological lesions

Table 17 shows scoring of the clinical signs for the local chickens infected with *S. gallinarum*. The clinical signs for fowl typhoid were seen on the challenged chicks from day 3 pi. The chicks appeared drowsy and occasionally stood with eyes closed. Chicks from all experimental groups were affected. From the fifth to seventh day pi, the severity of the clinical signs increased hence apart from drowsiness the chicks also showed marked decrease in feed intake and were reluctant to move. From the clinical signs it would seem that the mostly affected group was the *N'zenzegere* ecotype while the *Morogoro-medium* ecotype was the least affected. Throughout the experiment diarrhoea was not observed in any of the groups. Beyond nine days pi clinical signs disappeared from all the infected groups. The uninfected control group did not show any clinical

signs of the disease. Throughout the course of infection, only four chicks died at eight and nine days pi.

The post mortem picture of the dying chicks or those killed on day 6 pi onwards revealed varying degrees of congestion in liver and spleen. The surface of the liver often had some whitish pinpoint foci of necrosis. Occasionally there were whitish areas of necrosis on the myocardium. Chicks killed on day 3 pi did not show any notable changes in their internal organs.

Table 17: Clinical signs for the local chickens infected with *S. gallinarum*

DPI	Ecotypes (n = 15 for each)						
	Commercial layer strain	*Unguja*	*Tanga*	*Morogoro -medium*	*Mbeya*	*N'zenzegere*	*Pemba*
3	+ (5)	+ (3)	+ (5)	+ (5)	+(5)	+ (8)	+ (3)
4	+(2)++(2)	+ (3)	+ (5)	+ (3)	+(2)++(3)	+(2)++(4)	+ (2)
5	++ (3)	++ (1)	+(2)++(1)	-	++ (4)	++(5)	-
6	++ (3)	++ (2)	++ (2)	-	++ (3)	++ (4)	++(1)
7	++ (2)	-	-	-	++ (1)	++ (2)	-
8	++ (2)*	-	-	-	++ (1)*	++ (2)*	-
9	-	-	-	-	-	++ (1)*	-
10	-	-	-	-	-	-	-
14							

+ Drowsiness, occasional closure of the eyes; ++ drowsiness, closure of the eyes and reluctance to move; - no clinical signs; () number of chicks presenting the signs; * chicks died.

DPI = days post infection

4.3.3 Cellular dynamics

Figure 12 shows the overall changes in the percent distribution of heterophils, lymphocytes and monocytes in the local chickens infected with *S. gallinarum*. The mean percent distribution of heterophils increased steadily from day 1 to day 3-post infection (pi), then slowly from day 3 to day 6 pi. A sharp decrease in the mean percent distribution was experienced from day 6 to day 10 pi while it increased from day 10 to day 14 pi. Lymphocytes showed a similar trend to that of heterophils with the exception of a drop in the mean percent distribution between day 3 and day 6 pi. The mean percent distribution of monocytes showed a sharp decrease from day 1 to day 3 pi followed by a period of very minimal changes from day 6 to day 14 pi.

Comparison of the overall mean percent distribution indicated that for heterophils, the day 10 pi values were significantly lower than the day 3 and day 6 pi values (which were the highest values). The day 1 and day 14 pi values were not significantly different from each other. There was a significant increase on lymphocyte values from day 1 to day 10 pi. The day 1 value was not significantly different from that of day 6 pi; similarly for values at day 3 and day 14 pi. The overall mean percent distributions for monocytes indicated that the day 1 value was significantly higher and different from the rest of the days whose means were not significantly different from one another ($P< 0.05$).

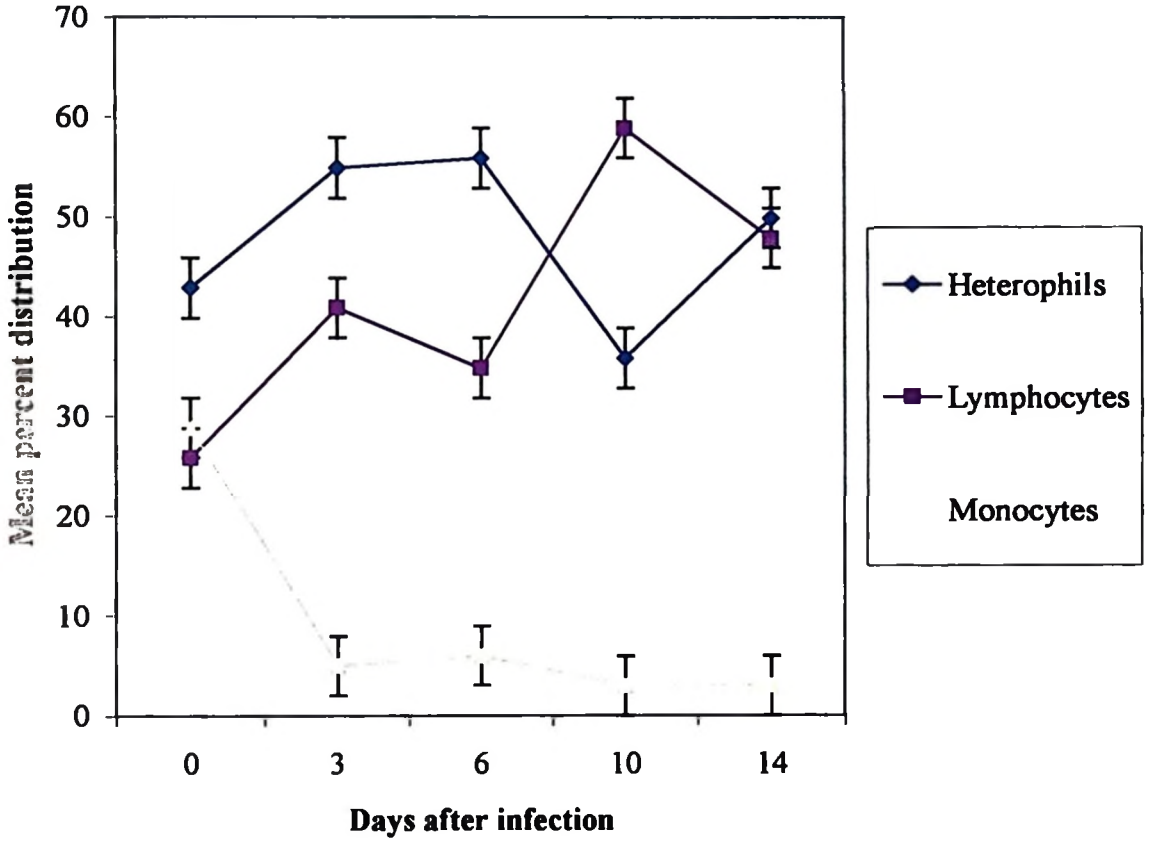


Figure 12: Selected leukocyte changes in local chickens infected with *S. gallinarum*.

4.3.3.1 Heterophils

Figure 13 shows the heterophils changes over the 14 days experimental period separately for each local chicken ecotype. The *N'zenzegere* ecotype showed the lowest mean percent distribution for heterophils at all times except on day 10 pi. The trend of change in the *N'zenzegere* and *Pemba* ecotypes was similar in that after an increase on day 3 and day 6 pi, there was a decrease on both day 10 and day 14 pi. This trend was in contrast to what was shown by other ecotypes where the values on day 14 pi were higher than those on day 10 pi (with the exception of the *Morogoro-medium*). The *Morogoro-medium* ecotype showed a higher value on day 1, day 3 and day 6 pi followed by a sharp decrease on day 10 pi and a plateau on day 14 pi. The commercial layer strain birds and the *Unguja* ecotype showed a peculiar trend where they had higher values on day 1, a negligible change on day 3 and day 6 pi, a slight decrease on day 10 pi and a marked increase on day 14 pi. The mean percent distribution trend on the *Tanga* ecotype showed a sharp increase by day 3 pi, then decreased through day 6 to day 10 pi after which there was an increase to day 14 pi.

When the means were compared, significant differences were detected between the various ecotypes. For instance while the *N'zenzegere* ecotype had a consistently and significantly lower mean percent distribution throughout the experiment, the highest significant mean changed from day to day. On day 1 the highest significant mean was shown by the Commercial layer strain, *Mbeya* and *Unguja* ecotypes; *Tanga*, *Mbeya* and

Morogoro-medium ecotypes (day 3 pi), *Morogoro-medium* ecotype (day 6 pi), *Unguja* ecotype (day 10 pi) and Commercial layer strain and *Mbeya* ecotypes (day 14 pi).

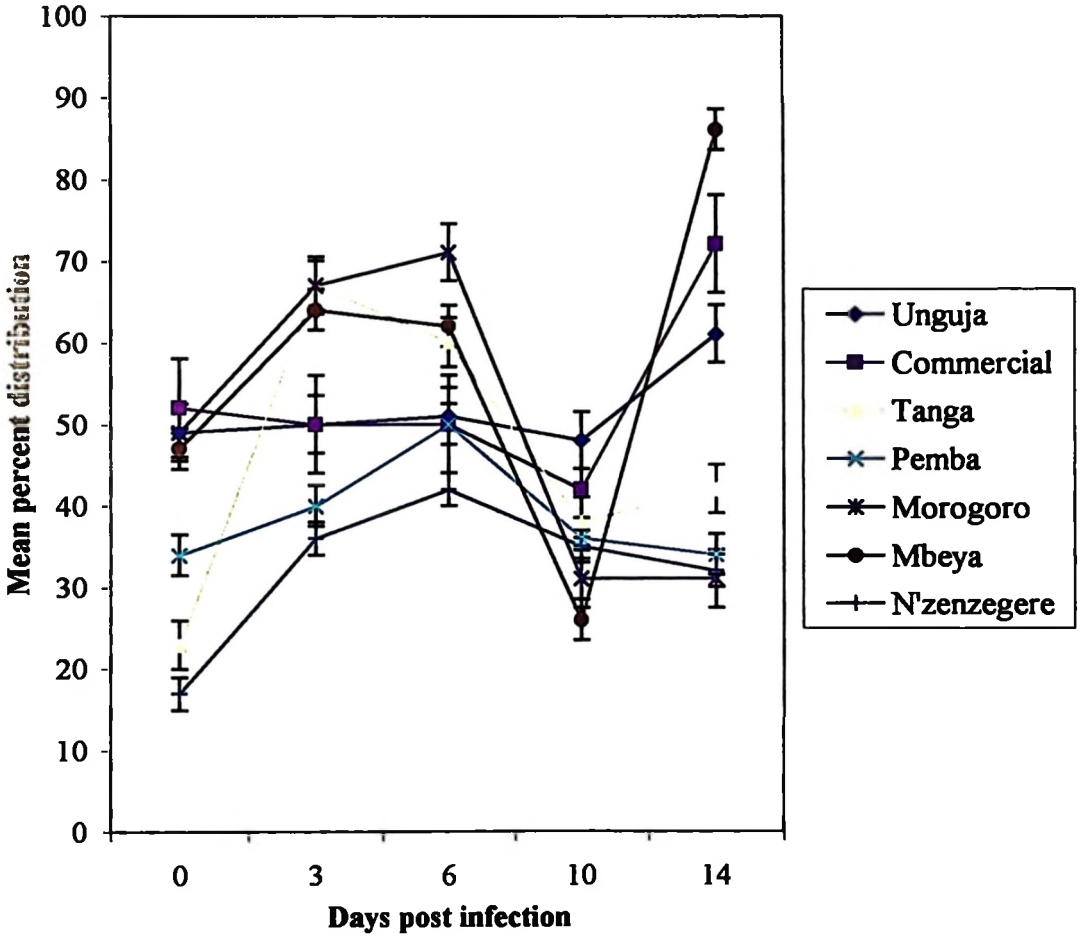


Figure 13: Mean percent distribution for heterophils in local chicken ecotypes infected with *S. gallinarum* (mean ± SE)

4.3.3.2 Lymphocytes

The mean percent distribution for lymphocytes is presented in Figure 14. The *Mbeya* and *Morogoro-medium* ecotypes showed only a slight change on day 3 and day 6 pi values a feature that was not seen in the other ecotypes. Another peculiar feature was that shown by the *Tanga* ecotype where the mean values increased until day 10 pi, then decreased on day 14 pi. Generally, most ecotypes presented two peaks that coincided with their highest means. These peaks were seen on day 3 pi (all ecotypes except for *Mbeya*, *Morogoro-medium* and *Tanga*) and on either day 10 pi (Commercial layer strain and *Unguja* ecotype) or day 14 pi (*N'zenzegere* and *Pemba* ecotypes). However, other ecotypes showed only one peak on either day 10 pi (*Mbeya* and *Tanga* ecotypes) or day 14 pi (*Morogoro-medium* ecotype). Peaks were mostly followed by a nadir (except day 14 pi peaks). Comparison of means showed that some means were significantly different from each other. However, no one ecotype had a consistently lower or higher mean that was significantly different from others.

4.3.3.3 Monocytes

The variation in the mean percent distribution for monocytes is presented on Figure 15. The means for all ecotypes decreased sharply from day 1 to day 3 pi, then there was a small increase on day 6 pi (*Morogoro-medium*, *Tanga*, *Unguja*, *Pemba*, Commercial layer strain and *Mbeya* ecotypes) that was followed by a decrease to day 14 pi. Only *N'zenzegere* showed a continuous decrease and *Tanga* showed an increasing trend on day 14 pi.

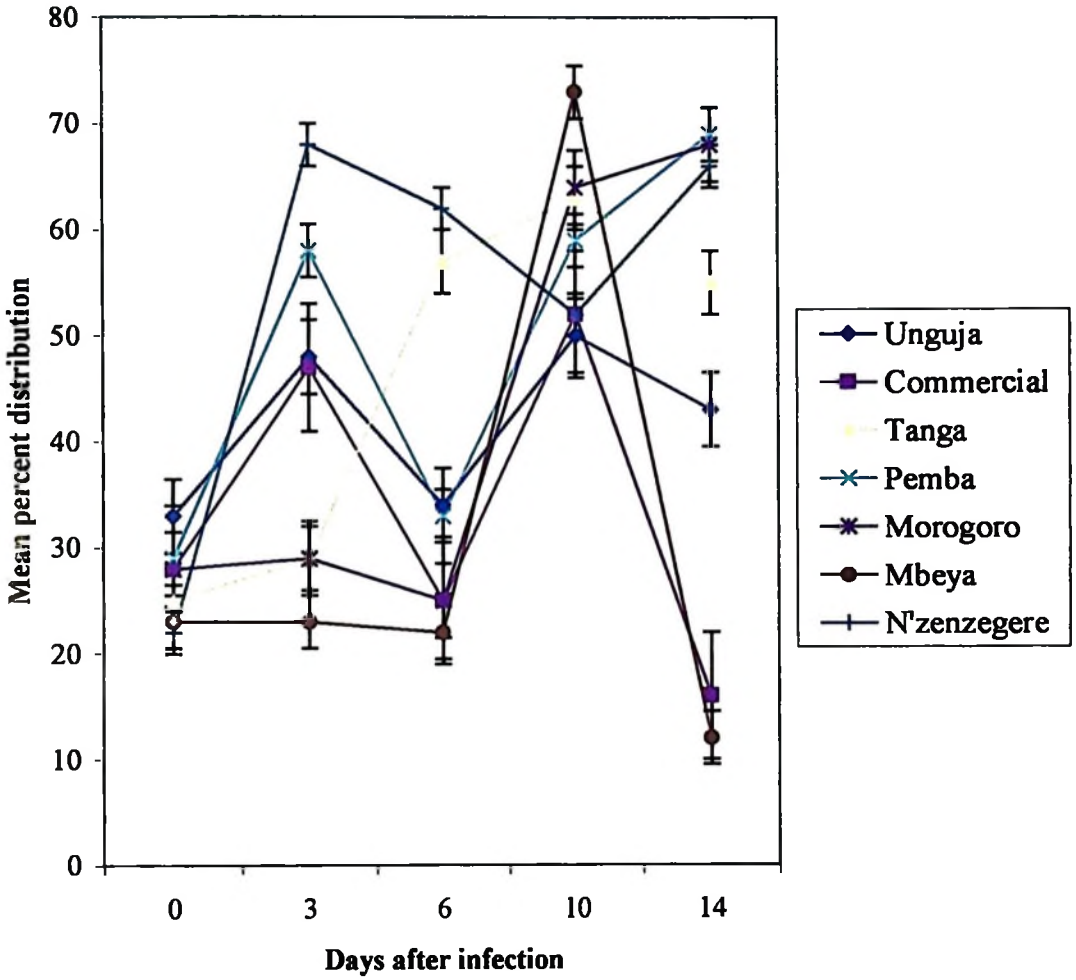


Figure 14: Mean percent distribution for lymphocytes in local chicken ecotypes infected with *S. gallinarum* (Mean ± SE)

Comparison of means on this parameter indicated that means on day 10 pi were not significantly different from each other. It was also seen that on day 6 pi only *Morogoro-medium* and *Unguja* ecotypes had means that were significantly different from the other ecotypes. The day 1 mean for *N'zenzegere* was the highest and significantly different

from all other ecotypes. On day 14 pi, the mean for *Tanga* ecotype was the highest and significantly different from the other ecotype whose means were similar.

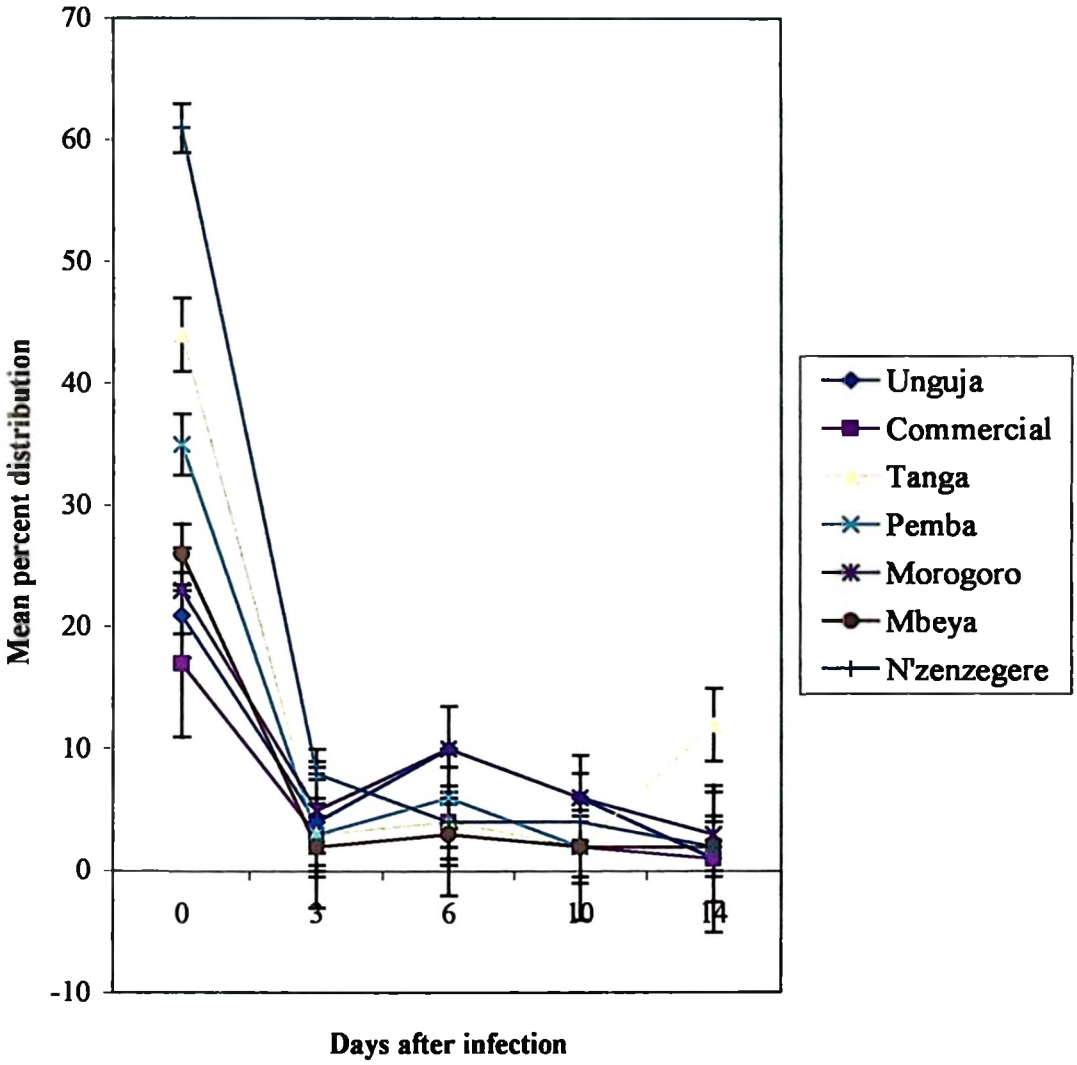


Figure 15: Mean percent distribution for monocytes in local chicken ecotypes infected with *S. gallinarum* (Mean ± SE)

4.3.4 Viable bacterial cell counts

The overall mean viable bacterial cell counts (log_{10}) in the liver and spleen of local chickens inoculated with *S. gallinarum* is shown in Table 18. The lowest viable bacterial cell counts were obtained on day 14 pi while the highest mean values were recorded on day 10 pi for both liver and spleen. From the range of the data, it was seen that the lowest values were 10 and 79 bacterial cells for spleen and liver, respectively. On the other hand, the highest values were 1.6×10^4 and 3.98×10^6 (for spleen and liver, respectively). The spleen counts were therefore consistently lower compared to those from the liver.

The mean log_{10} live bacterial counts from the liver of the local chicken ecotypes infected with *S. gallinarum* are presented in Table 19. It was seen that on day 3 pi the commercial layer strain group and the *N'zenzegere* ecotype had no bacterial growth from the liver. The highest mean live bacterial count in the liver was shown by the *Morogoro-medium* ecotype.

With the exception of *Mbeya* and *Pemba* ecotypes, the peak viable bacterial cell count was attained on day 10 pi. The peak for *Mbeya* and *Pemba* ecotypes were attained on days 6 and 14, respectively. It was noted that the highest mean value attained from the liver was 2.5×10^6 (day 10 pi value for the commercial layer strain group). It was also noted that the mean values on day 14 pi was lower than that of day 10 pi indicating that the viable bacterial counts were diminishing. The only exception to this trend was

Pemba ecotype, which peaked on day 14 pi. Viable bacterial cell counts from the spleen presented a less conspicuous trend with only *Tanga* ecotype showing values on each of the 4-day points (Table 20). The highest value obtained from the spleen was 1.6×10^4 (day 10 pi *Unguja* ecotype). The *Mbeya* and *N'zenzegere* ecotypes did not show any viable bacterial cells over the entire period of the experiment.

Table 18: The overall mean Log_{10} viable bacterial cell counts in the liver and spleen of local chickens inoculated with *S. gallinarum.*

Days after inoculation	Liver (n = 3 for each ecotype in each day)		Spleen (n = 3 for each ecotype in each day)	
	(Mean ± SE)	Range	(Mean ± SE)	Range
3	3.88 ± 0.33	1.9 – 5.4	1.86 ± 0.37	1.2 – 3.2
6	3.62 ± 0.20	2.4 – 4.9	2.72 ± 0.49	1.3 – 3.5
10	4.02 ± 0.46	2.5 – 6.6	3.6 ± 0.24	2.9 – 4.2
14	3.51 ± 0.26	2.6 – 5.9	1.82 ± 0.35	1 – 2.5

Table 19: Mean log_{10} liver bacterial cell count from local chicken ecotypes infected with *S. gallinarum* (Mean ± SE).

Ecotypes (n = 3 for each day)	Days after inoculation			
	3	6	10	14
Commercial	-	3 ± 0.5	6.4	2.9 ± 0.3
Unguja	2.85 ± 0.4	3.65 ± 1.8	3.7	3 ± 0.4
Tanga	4.6 ± 0.6	3.8 ± 0.6	4.6 ± 2.8	3.8 ± 1.3
Morogoro	5 ± 0.6	3.1 ± 0.4	4.4	4.1
Mbeya	3.6 ± 2.3	4 ± 0.4	-	2.6
N'zenzegere	-	1.9	3.9 ± 0.9	2.9
Pemba	3.6 ± 0.5	4.5 ± 0.2	3.2 ± 0.2	4.7 ± 1

Table 20: Mean $\log_{10}$ spleen bacterial cell count from local chicken ecotypes infected with *S. gallinarum* (Mean ± SE).

Ecotypes	Days after inoculation			
(n = 3 for each day)	3	6	10	14
Commercial	1.7	-	2.9	-
Unguja	-	-	4.2	1.5
Tanga	2.3 ± 1	3	4.1	2.5
Morogoro-medium	-	-	3.7 ± 0.6	2.3
Mbeya	-	-	-	-
N'zenzegere	-	-	-	-
Pemba	1.2	2.2 ± 1.3	-	-

4.3.5 Effect on growth rate

The mean growth rates for the first and second week of infection are shown on Table 21. The range of the dataset gave a more realistic situation on the effect of the infection on the local chicken ecotype than the mean. It was seen that the depression of growth rate was as high as –2.7g (*Mbeya* ecotype) during the first week and –1.3g (*Tanga* ecotype) in the second week of the experiment. The first week of the experiment produced more pronounced growth reduction than the second week. During this time marked depression of growth rate were observed in *Mbeya*, *Pemba* and *Tanga* ecotypes. During the second

week the *Morogoro-medium* ecotype showed a growth rate that was less influenced by the infection, similarly for the commercial layer group.

Table 21: Mean growth rates for local chicken ecotypes infected with *S. gallinarum*

Ecotype	Growth rate 1st week (g/day)		Growth rate 2nd week (g/day)	
N = 15	Mean ± SE	Range	Mean± SE	Range
Commercial	1.5 ± 0.3	0.04 to 2.7	5.1 ± 0.4	3.7 to 6.1
Unguja	1.6 ± 0.4	0.1 to 3.4	3.9 ± 0.9	2.7 to 5.8
Tanga	1.7 ± 0.5	-0.9 to 5.1	2.3 ± 1	-1.3 to 4.1
Pemba	0.7 ± 0.3	-0.8 to 2.3	2.7 ± 0.5	1.2 to 4.4
Morogoro medium	1.5 ± 0.4	-0.1 to 3.3	6 ± 1.8	2.5 to 8.6
Mbeya	1.7 ± 0.9	-2.7 to 4.4	3.1 ± 1.3	1.8 to 4.4
N'zenzegere	0.7 ± 0.2	-0.3 to 1.2	2.8 ± 0.7	1.6 to 4.1

4.4 Responses of the local chicken ecotypes to immunization with Newcastle disease (ND) vaccine

4.4.1 HI titres in hens, eggs and chicks from the local chicken ecotypes

Table 22 shows the overall mean HI titres as measured in hens, eggs and chicks. It is seen that the mean HI titres in chicks were significantly higher than those in hens and eggs ($P<0.05$). However, the range of the three datasets was not different.

Table 22: Overall mean HI titres in hens, eggs and chicks

Parameter	Category		
	Hens (n = 103)	Eggs (n = 103)	Chicks (n = 103)
Mean ± SE	$6 \pm .14^{a}$	6.2 ± 0.16^{a}	7.1 ± 0.13^{b}
Range	4 - 9	3 - 10	4 - 9

Means in a row that share the same superscript are not significantly different ($P<0.05$)

Figure 16 shows the mean HI titres in the three categories separately for each local chicken ecotype. It is observed that *Pemba* ecotype hens had the lowest mean (5.4), which differed significantly from that of *Tanga* and *N'zenzegere* ecotypes. *Tanga* ecotype had the highest mean HI titre (7.2) that was significantly different from *Ching'wekwe*, *Mbeya*, *Morogoro-medium* and *Unguja* ecotypes with HI titres means of 5.6, 6.2, 5.9, and 5.6, respectively. *N'zenzegere* also had a mean HI titre similar to all ecotypes except the *Pemba* ecotype. Mean HI titres in eggs revealed *Morogoro-medium* and *Unguja* ecotypes as having the lowest values that were significantly different from

Ching'wekwe, *Mbeya*, *N'zenzegere* and *Tanga* ecotypes. The highest HI mean titre was shown by the *N'zenzegere* ecotype and was significantly different from all the other ecotypes. *Pemba* ecotype shared mean HI titre values that were similar to all other ecotypes except the *N'zenzegere*. In chicks, the mean HI titres were the lowest in the *N'zenzegere* and highest in the *Pemba* ecotypes and these two were significantly different from each other. The mean HI titre for *Morogoro-medium* was significantly different from that of *N'zenzegere* and *Pemba* ecotypes but similar to other ecotypes.

4.4.2 Dynamics of HI titres in naïve local chicken ecotypes immunized with Newcastle disease vaccine

The overall HI mean titres for the local chicken ecotypes following two consecutive immunizations with ND vaccine are shown in Figure 17. It was observed that there was a two-fold increase in the overall mean HI titre from day 7 to day 14 after immunization. There was only a slight increase between day 14 and day 28 after immunisation. The mean HI titres decreased from 5.6 to 3.7 between day 28 and day 63. After the second vaccination, the mean HI titre rise was slower than in the first vaccination and by day 28 after re-immunisation there was already a decreasing trend. Comparison of the overall HI titre means shows that in the first immunisation, the day seven value was significantly lower than other values. The mean HI titre for day 28 was significantly higher than mean day 7, day 14 and day 63 but not day 21.

Following the second immunisation on day 70, the mean HI titres for day 1 were significantly lower ($P<0.05$) compared to other days. The mean HI titres on days 14, 21 and 28 were similar and significantly higher than those of other days.

Viewing each ecotype separately (Figure 18) it is seen that seven days after the first immunisation, *Morogoro-medium* ecotype had not yet sero-converted, whilst *Tanga* ecotype had a mean HI titre above 3 (with a range between 2 – 7). In the subsequent days (days 14, 21, 28 and 63) the *Mbeya* ecotype showed a high and consistent mean HI titre. There was a difference on the peaking of the mean HI titres in the different local chicken ecotypes. *Pemba*, *Tanga* and *Unguja* ecotypes showed their peak mean HI titres on day 21 while the other ecotypes peaked on day 28 after immunisation. Except for *Tanga* ecotype, all other ecotypes had a mean value at day 63 that was above the day 7 HI mean.

On re-immunization (Figure 19), most chicken ecotypes showed an increase in the mean HI titres up to day 21, followed by a decrease on day 28 after re-immunization. Deviations from this trend were shown by the *Mbeya* ecotype whose mean remained constant from day 14 onwards and *Morogoro-medium* ecotype whose mean peaked on day 21 after immunisation. The existence of wide ranges within the dataset indicates the presence of chickens with very low HI titres and some with very high titres.

ıparing the means for the re-immunisation indicated significant variation on the n HI titres between the ecotypes except on the day 1 values. *Tanga* ecotype showed ;istently lower mean HI titres for the whole experimental period. This lower mean itre was significantly different from that of *Ching'wekwe* ecotype (throughout the :riment), *Morogoro-medium* and *Pemba* ecotypes (on day 7 and 14), and the rest of ecotypes (on day 7). The *Ching'wekwe* ecotype showed a high mean HI titre ughout the experimental period. However, the high mean HI titres were only ificantly different to (apart from that of *Tanga* ecotype) the *Unguja* ecotype on days and 21 after re-immunisation. Other ecotypes showed means that were not ificantly different from each other.

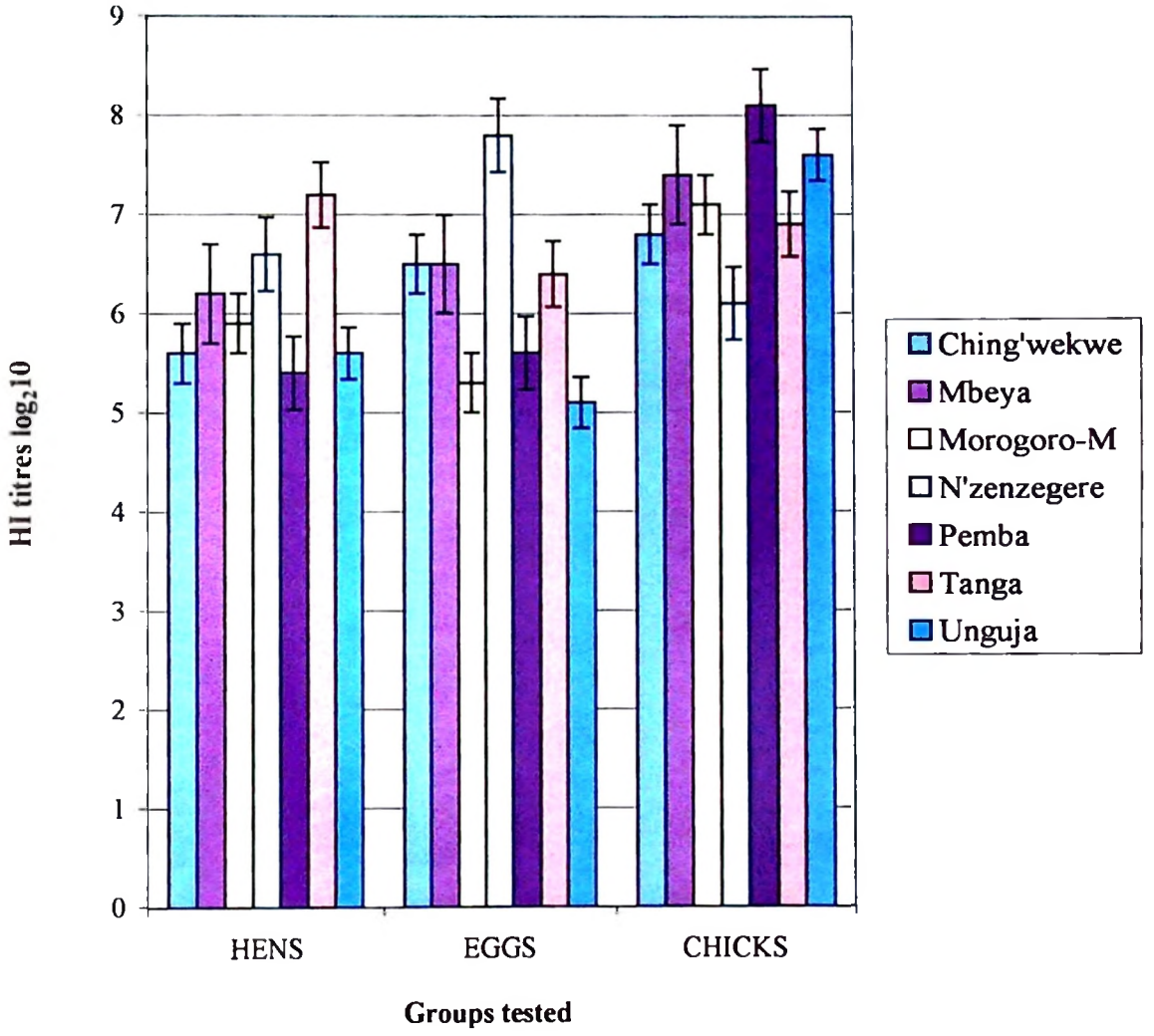


Figure 16: HI titres to Newcastle disease virus in local chicken ecotypes' hens, eggs and chicks following immunization of the hens with ND vaccine (La Sota strain).

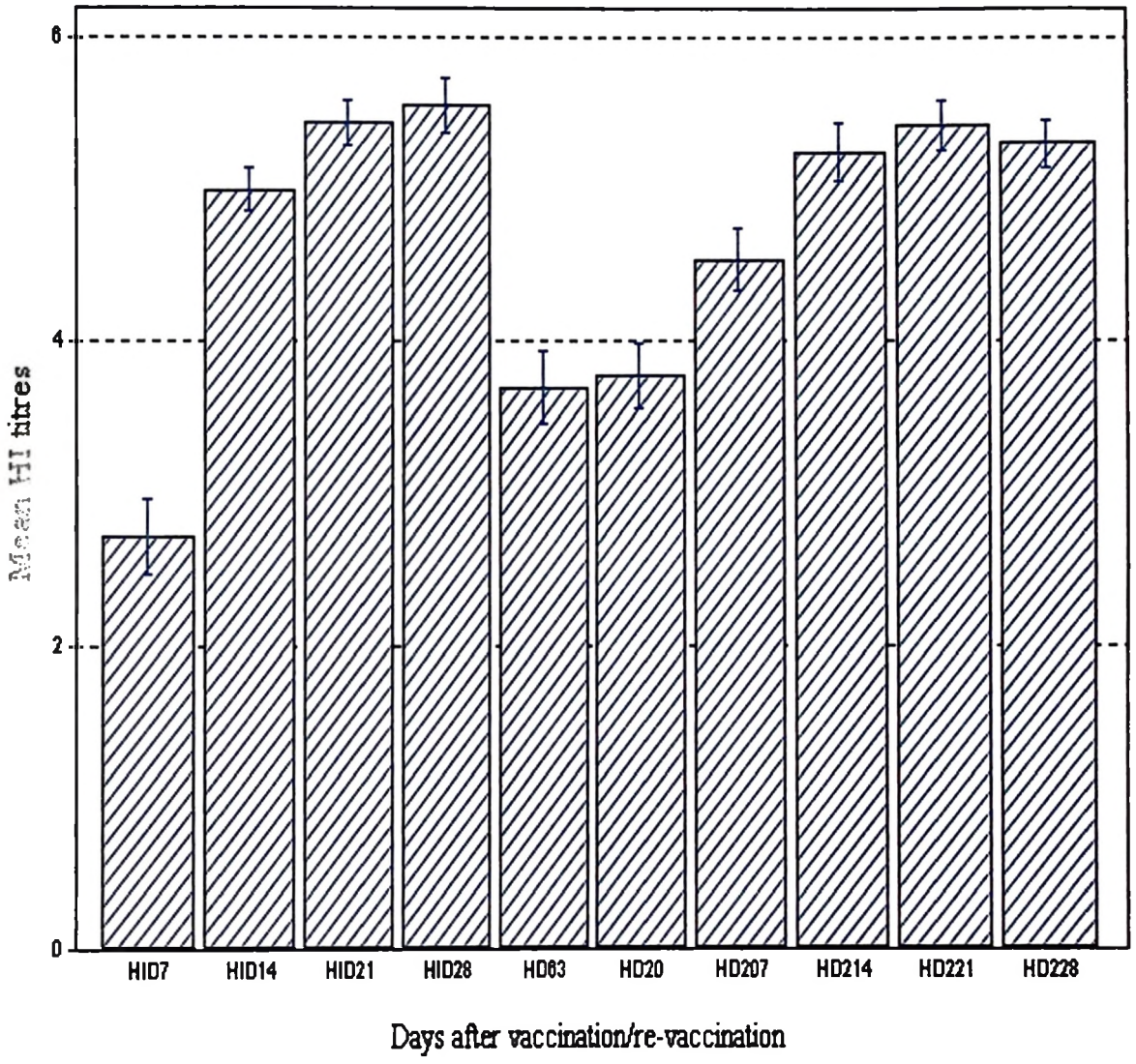


Figure 17: Overall mean HI titres for NDV in local chicken ecotypes vaccinated (HID7 to HID 63) and re-vaccinated (HD20 to HD228) with ND vaccine

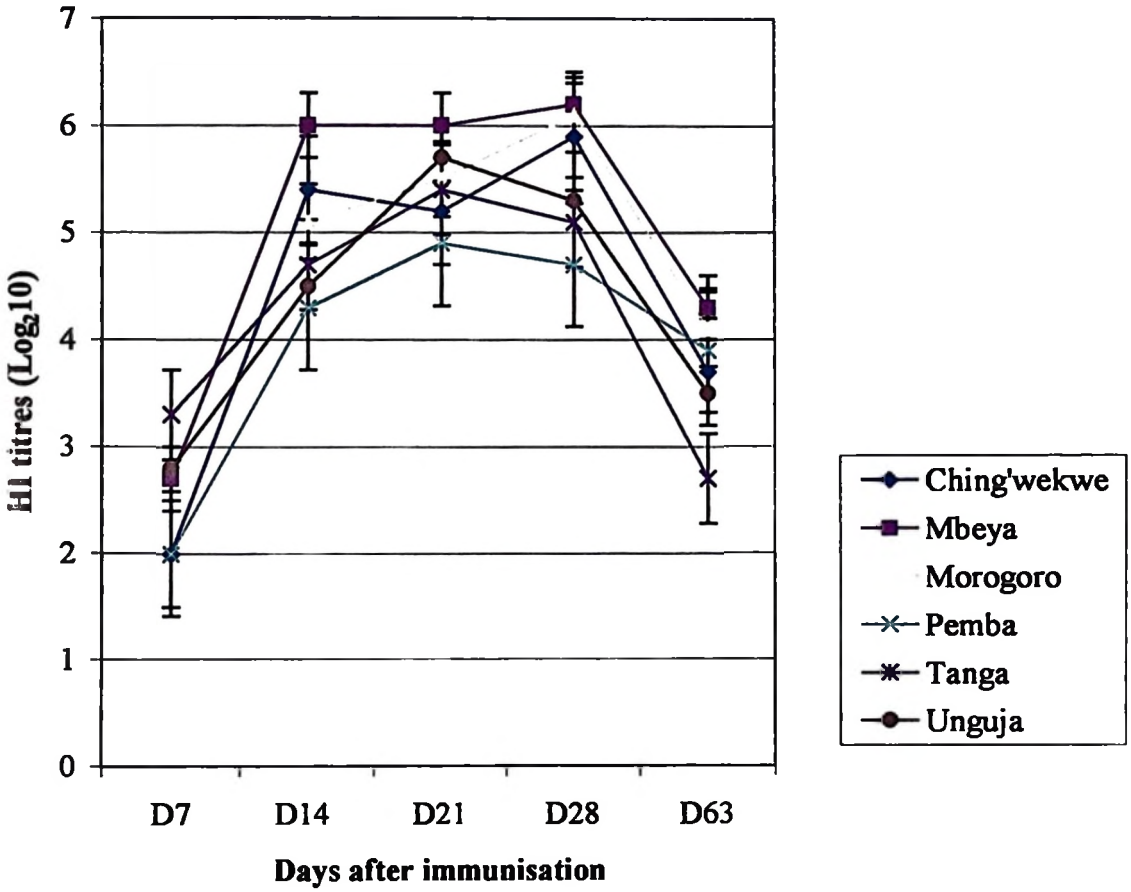


Figure 18: HI titres for naïve local chicken ecotypes immunized with Newcastle disease vaccine.

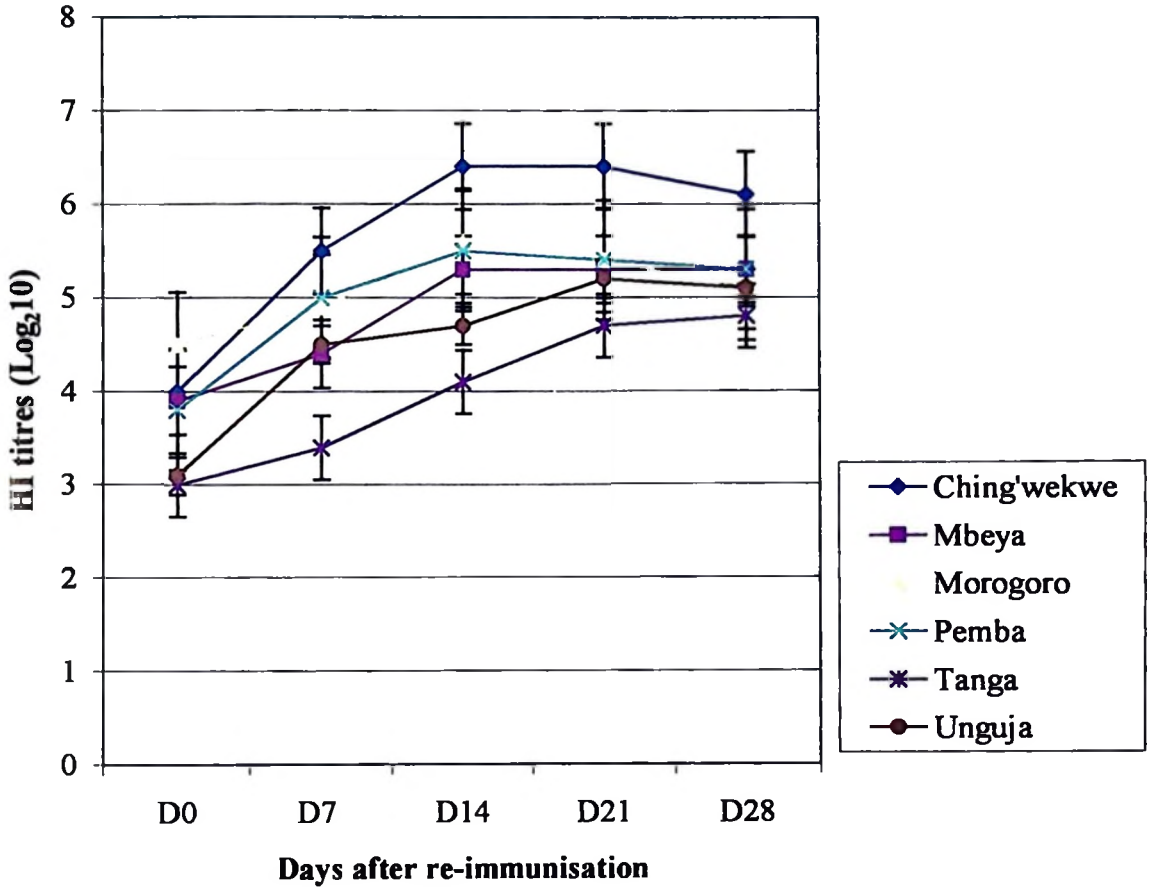


Figure 19: HI titres for local chicken ecotypes re-vaccinated with Newcastle disease virus.

4.5 Productivity and reproductive performance of free range local chickens

4.5.1 Productivity of local chickens under free-range management system

The results on the overall weekly weight measurements for the free-range local chickens are presented in Table 23. There were very wide ranges on the data indicating the extent of variability of the individuals included. This was particularly seen from the fourth week onwards. However, it was evident from the mean values that there were only a few individuals with very low or high mean weight measurements. Mean weekly weight measurements for free-range local chickens by farmers are presented in Table 24. The results indicate some variations in the weight measurements between the different farmers. Farmers C and G for instance presented the lowest mean hatch weight while E and H showed the highest hatch weight. During the first month (week 4) chicks from three farmers (A, E and I) had average weight measurements above 100g. The chicks that showed the lowest hatch weights also had the lowest mean weight during the first month. Similarly for those that showed high weekly weight measurements.

The results also showed that in the second month, chicks from farmer H had the lowest mean weekly weight measurements while farmer I chicks showed the highest mean value. It was interesting to note that the highest mean value in the third month was shown by chicks of farmer G whose hatch weights were among the lowest. Chicks from two farmers, A and E had their mean value below 400g and only chicks from one farmer (G) had mean values above 500g. When the means were compared statistically, it was observed that the differences between farmers were not significant for hatch weight and

weight measurements for the first month. During the second month, the means were partitioned into two groups each with a mean that was significantly different from each other ($P<0.05$).

Table 23: The overall weekly weight measurements for the free-range local chickens.

	Weight measurements (g)			
	Hatch weight	Week 4	Week 8	Week 12
Mean ± SE (n = 150)	25.3 ± 0.4	95 ± 3.1	233.8 ± 8.5	418.1 ± 13.9
Range	16 - 38	36 - 282	104 - 518	104 - 756

Table 24: Weekly weight measurements up to 12 weeks of age of local chickens under free range management system

Weight measurements (g)				
Farmer	Hatch weight	Week 4	Week 8	Week 12
A (n = 19)	25.2 ± 0.7	105.7 ± 7.5	216.2 ± 12.7^{a}	353.8 ± 21.6^{a*}
B (n = 20)	26.1 ± 0.8	97.4 ± 8.7	241 ± 18.1^{ab}	437 ± 17.2^{ba}
C (n = 9)	22 ± 0.6	74.2 ± 2.9	229.1 ± 27.7^{ab}	491 ± 99^{ba}
D (n = 15)	25.4 ± 1	80.1 ± 5.5	181.7 ± 13.7^{a}	426.3 ± 73.3^{ba}
E (n = 19)	27.2 ± 1.8	103.4 ± 23.3	315.3 ± 16.3^{b}	397.2 ± 26.3^{ba}
F (n = 18)	25.4 ± 1.3	96.2 ± 5.5	202 ± 12.2^{a}	459.2 ± 49.1^{ba}
G (n = 14)	22.3 ± 1.4	70.5 ± 9.2	244.8 ± 13.3^{ab}	602.5 ± 62^{b}
H (n = 16)	27.1 ± 1.1	97 ± 6.9	152.3 ± 12.1^{a}	406 ± 33.1^{ba}
I (n = 20)	25.4 ± 1.6	107.2 ± 5.2	440.4 ± 31.6^{b}	443.5 ± 10^{ba}

Means in a column with different superscripts are significantly different from each other (*Kruskal-Wallis One Way analysis of variance, $P<0.05$)

It followed that mean weekly weight measurements for the local chickens from farmers A, D, F and H were similar but significantly lower than those from farmers E and I, which were also similar. In the third month, local chicks from farmers A showed a significant lower mean weight measurements compared to chicks from farmer G. The rest of the chicks had means that were not significantly different from one another.

4.5.2 Daily growth rates for free range local chickens

Table 25 presents the overall growth rate (g/d) for the free-range local chickens. There was an almost two-fold increase in growth rate from the first to the second month (2.45g/d to 5.1g/d). The increase in growth rate from the second to the third month was less pronounced (5.1g/d to 6.6g/d). The range on the overall dataset was very wide on the third month there was a decreasing growth in some of the chickens. In Figure 21, data are presented on the daily growth rate (g/d) of the free-range local chickens separately for each of the nine participating farmers. It was observed that in the first month, the lowest mean growth rate recorded was 1.9g/d and was from three farmers C, G and H. On the other hand, the highest mean growth rate (3g/d) was seen on chicks from farmer I. However, there was no significant difference between the means. In the second month, the lowest mean growth rate increased to 2.85g/d and was from chicks of farmer H. The highest mean growth rate was 12g/d and was shown by chicks from farmer I. The two extreme values were significantly different from each other ($P<0.05$). There were other groups of means that showed significant difference (Table 25). During the third month, the lowest mean growth rate was 0.8g/d obtained in chicks from farmer

I. The highest mean of 13.1g/d was shown by chicks from farmer G. When these means were compared, significant differences were observed between the extreme values as well as between other groups (Table 26). It is observed that chicks from all farmers experienced a rise in growth rate from the first to the second month (Figure 21). From the second to the third month, chicks from two farmers experienced an obvious drop in growth rate (E and I) while two others experienced a very low increase (A and B) but the rest had a sharp increase.

Table 25: Overall growth rate (g/d) for local chickens under free-range management system

	Daily Growth Rate (g/d)		
	Month 1	Month 2	Month 3
Mean ± SE (N = 150)	2.45 ± 0.12	5.15 ± 0.26	6.61 ± 0.52
Range	0.64 – 9.10	1.57 – 14.71	-0.71 – 19.10

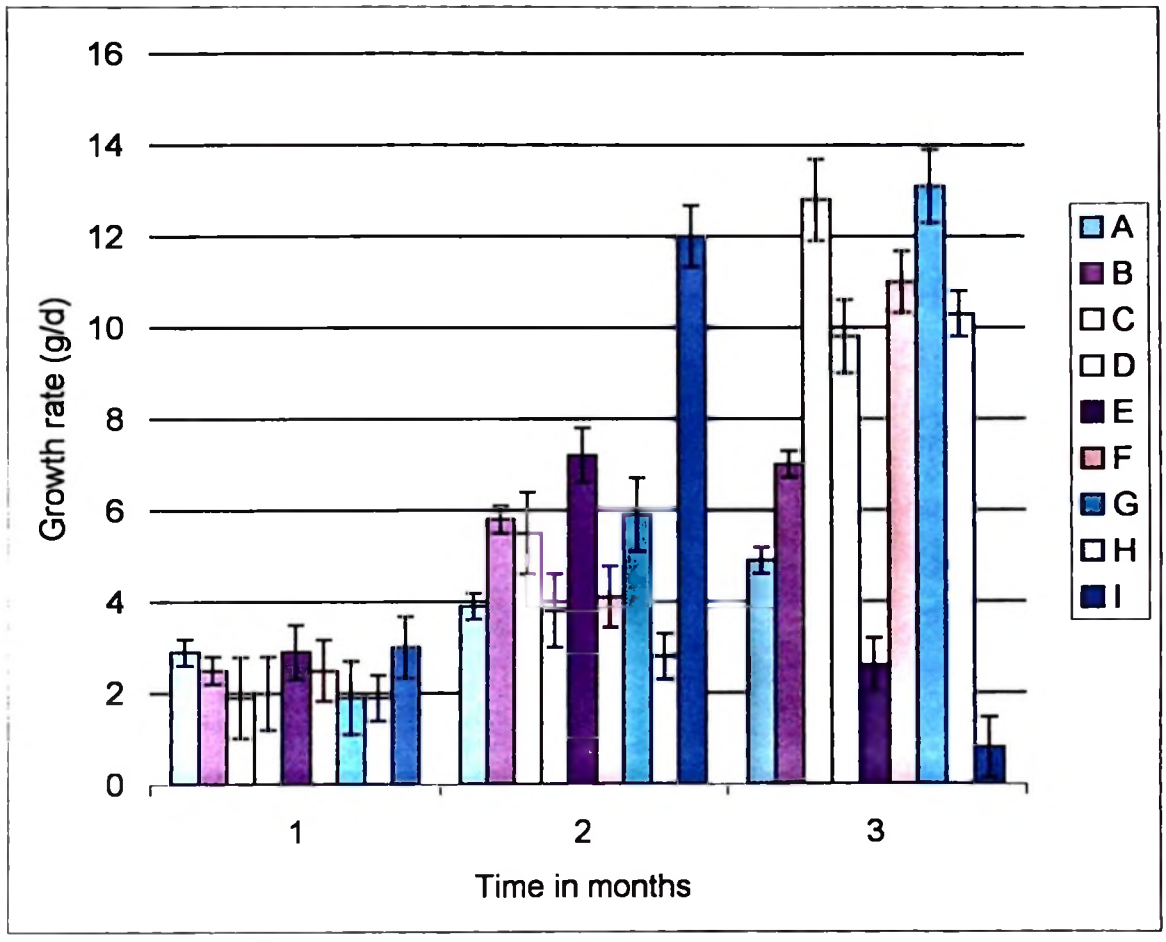


Figure 20: Growth rate for local chickens (mixed sex) under free-range management system during their first three months of life.

Table 26: Comparisons of mean ranks for growth rate in the second month by farmer

Farmer	Mean	I	E	G	B	C	F	A	D	H
I	92.6									
E	76.7	1.05								
G	67.25	1.37	0.58							
B	62.18	2.12	1.27	0.32						
C	53.17	2.56	1.86	0.85	0.77					
F	38.57	3.76*	3.34*	1.83	2.27	1.24				
A	35.29	4.14*	3.84*	2.11	2.77	1.60	0.34			
D	31.54	4.16*	3.83*	2.24	2.82	1.78	0.65	0.37		
H	15.37	4.91*	4.69*	3.07	2.83*	2.82	1.90	1.71	1.28	

*Means significantly different ($P<0.05$) Kruskal-Wallis one-way ANOVA.

Table 27: Comparisons of mean ranks of growth rate in the third month by farmer

Farmer	Mean	G	C	H	F	D	B	A	ED	I
G	62.7									
C	61.25	0.09								
H	58.00	0.31	0.18							
F	57.10	0.42	0.25	0.06						
D	50.43	0.98	0.67	0.55	0.57					
B	41.89	1.83	1.28	1.26	1.45	0.92				
A	24.92	3.43*	2.44	2.65	3.19	2.88	2.40			
E	13.00	4.25*	3.13	3.44*	4.08*	3.86*	3.57*	1.57		
I	6.50	3.97*	3.15	3.36*	3.76*	3.49*	3.11	1.67	0.55	

*Means significantly different ($P<0.05$) Kruskal-Wallis one-way ANOVA.

4.5.3 Reproductive performance of the local chickens under free-range management system

The overall reproductive performance of the free-range local chickens (as assessed by mean egg weight and hatchability) indicated mean egg weight of 41g (29 – 53g) and a hatchability of 80% (33 – 100%). The results were skewed towards the right although there were wide ranges on both egg weight and hatchability. Results on the reproductive performance of the free-range local chickens are presented on Table 27. The results indicated some variations between farmers in terms of the average egg weights. When compared statistically, these variations in mean egg weights showed some significant differences ($P<0.05$). The mean egg weight for chickens from farmers C, D and E were similar but significantly different from those obtained from farmers A, B, G and I. In addition, the mean egg weight for chickens from farmer E were also significantly higher than those from farmers F and H, which were in turn similar but significantly higher than those from farmers A and I.

Mean hatchability was 70% in chickens from farmer E and in 7 out the 9 farms it was over 75%. The maximum value was 100% in 7 out 9 farms falling short only twice 0.92 (92%; farmer E) and 0.8 (80%; farmer I).

Table 28: Reproductive performance of local chickens under free-range management system

Farmer	Egg weight Mean ± SE (Range)	Number brooding eggs	Number hatched	Hatchability	
				Proportions	Percentages
A	37.7 ± 0.6 (31.7 – 44.7)	182	145	0.8 (0.42 - 1.0)	80
B	39.4 ± 1.3 (29.2 – 44.7)	57	47	0.819 (0.5 – 1.0)	82
C	44 ± 2.3 (30.3 – 51.3)	65	57	0.889 (0.7 – 1.0)	89
D	45 ± 1.4 (38 – 47.7)	49	36	0.727 (0.33 – 1.0)	73
E	44.9 ± 0.8 (39.8 – 53)	54	33	0.636 (0.43– 0.92)	64
F	41.3 ± 1.1 (36.3 – 46.8)	103	79	0.764 (0.5 – 1.0)	76
G	39.4 ± 1.1 33 – 47.2	65	54	0.823 (0.67 – 1.0)	82
H	41.5 ± 1.1 (33 – 46.8)	74	64	0.866 (0.67 – 1.0)	87
I	38 ±1.1 (30 – 48)	35	27	0.773 (0.75 - 0.8)	77

CHAPTER 5

5.0 DISCUSSION

5.1 Genetic diversity in the Tanzanian local chicken ecotypes

5.1.1 Variation in allele numbers

The present study looked into the genetic diversity within and between the local chicken ecotypes of Tanzania using allele frequency information derived from microsatellite DNA analysis. It was observed that four out of the 20 selected microsatellite loci (20%) could not be amplified in the local chicken ecotypes. Similar observations were made by van Marle-Koster and Nel, (2000), when characterizing the native Southern African chicken populations. In that study, four out of the 27 primer-pairs used (15%) did not produce specific products in the native chickens (Marle-Koster and Nel, 2000). This might indicate that different microsatellite markers exist in the exotic chicken breeds and the local chicken populations. Nevertheless, the results from these two studies are evidently different from a study by Pang and others (1999), who used chicken specific-primers to amplify Japanese quail microsatellite loci. In that study, 37 out 48 (77%) primer-pairs used could not produce specific products.

From the results, all the 16 loci were highly polymorphic with allele numbers ranging from 4 to 15 per locus (mostly >8). Similar studies by Wimmers *et al.* (2000), van Marle-Koster and Nel (2000), Vanhala *et al.* (1998) and Takahashi *et al.* (1998) showed polymorphisms in the loci used but with fewer numbers of alleles per locus. For instance, in examining the genetic distinctiveness of African, Asian and South American local chickens using 22 microsatellite markers, Wimmers *et al.* (2000) detected two to

11 alleles per locus. Studies on genetic characterisation of the native Southern African chicken populations using 23 microsatellite markers (van Marle-Koster and Nel, 2000) showed the number of alleles per locus to range between two and 17. Vanhala *et al.* (1998) evaluated the genetic distance and variability between eight chicken lines using nine microsatellite markers and found four to 13 alleles per locus. Applying eight microsatellite markers to determine the genetic relatedness of the native Japanese breeds of chickens, Takahashi *et al.* (1998) detected two to 10 alleles per locus. All these other studies are in general agreement with the current study although the methodology used was different. While the current study applied a manual genotyping method, all the other mentioned studies applied automated methods.

The existence of >8 alleles per locus in the current study may be an indication of the variability among the local chickens. In a different study on genetic characterisation of indigenous goat populations from the sub-Saharan Africa a sufficiently high number of alleles per locus (8 to 23) were detected (Chenyambuga, 2002). These results support the view of high polymorphisms in indigenous animal genetic resources.

5.1.2 Test for Hardy-Weinberg Equilibrium (HWE)

The exact test for the HWE showed that 120 out of 160 locus-ecotype combinations deviated significantly from the equilibrium ($P \leq 0.0001$ to 0.05). Departure of this magnitude (75%) could not occur by chance alone. Similar observations were made by Chenyambuga (2002) where over 67% of locus-population combinations deviated significantly from the HWE. However, results from the current investigation differed

from those by Vanhala *et al.* (1998) and that by Koskinen and Bredbacka, (2000) who reported deviations from HWE of 17 and 16%, respectively.

Several factors may influence the deviation from the HWE including inbreeding or outbreeding, selection, migration or gene flow from external population, population sub-division and the presence of null alleles (Falconer and Mackay, 1996). It was noted in this study that the estimate for inbreeding coefficient (*f*) was mostly positive (113 out of 160). Positive (*f*) values are associated with heterozygosity deficiency and hence evidence of inbreeding. However the mostly likely cause of heterozygosity deficiency in this study was genotyping errors due to poor resolutions of alleles in some gels. Vanhala *et al.* (1998) had similar observations and associated wrong genotyping to the observed deviations from HWE observed in their study. The presence of null alleles cannot be discounted as a factor linked to the deviations from HWE (Chenyambuga, 2002).

The other factors such as outbreeding, migration and population sub-division may also have contributed to the observed deviations from the HWE. However, it is unlikely that selection played any part because the samples were drawn from outbred populations and all ecotypes showed both positive and negative (*f*) values.

5.1.3 Diversity within ecotypes

The mean number of alleles in different populations observed over a number of loci can be taken as a reliable indicator of genetic variability within the populations (MacHugh *et*

al., 1997). There was high genetic variability within the local chicken ecotypes as shown by mean number of alleles per ecotype that ranged from 4.31 (White Leghorn) to 7.06 (*Pemba* ecotype). The mean allele numbers per ecotype in this experiment were higher compared to those reported by van Marle-Koster and Nel, (2000) (2.3 to 4.3) or those reported by Vanhala *et al.* 1998 (2.4 to 5.7) indicating more diversity within the local chicken ecotypes of Tanzania. The main reason for the difference would be the inclusion of well-established chicken breeds (Vanhala *et al.*, 1998), native chickens derived from exotic chicken crosses or local chickens whose crossbreeding status was not immediately known (van Marle-Koster and Nel, 2000). The high genetic diversity within population in indigenous animal genetic resources is further exemplified by two studies on genetic characterisation of sheep and goats (Stephen, 2000; Chenyambuga, 2002). In the first study, Stephen (2000), observed mean allele numbers ranging from 5.5 to 7.17 in five local sheep ecotypes of Tanzania using six microsatellite markers. In the second study, Chenyambuga (2002), found mean allele numbers of between 5.26 and 7.05 using 19 microsatellite markers on sub-Saharan goat populations.

According to Nei (1987), the expected heterozygosity (gene diversity) is a more appropriate measure of within population genetic variations. The expected heterozygosity values ranged from 62.1% in the White Leghorn to 79.4% in the *Ching'wekwe* ecotype. These were high values compared to those observed by Wimmers *et al.* 2000 (45 to 67%) and Vanhala *et al.* 1998 (37.8 to 67.1%). These values were

much closer to those obtained by Bowcock *et al.* (1994) when examining the genetic variations between human populations.

5.1.4 Genetic relationships between the local chicken ecotypes

Two measures of genetic distance (D_A and D_S) were employed because of their proven better performance in phylogeny reconstruction using microsatellites data (Takezaki and Nei, 1996). The D_A distance measure has been particularly useful in recovering the true topology on closely related populations. On the other hand, the D_S distance measure gives best estimate of the evolutionary time.

The genetic distance between *Pemba* and *Unguja* ecotypes was short on both measures. This is consistent with their close geographical association and the physical isolation from the mainland ecotypes. The geographical proximity argument would also be valid when the genetic distance between the three ecotypes from the Morogoro region (*Ching'wekwe*, *Morogoro-medium* and *N'zenzegere*) are examined. The short genetic distance between *Singamagazi* ecotype and four other ecotypes (*Mbeya*, *Morogoro-medium*, *N'zenzegere* and *Tanga*) cannot be explained by geographical proximity. However, the high observed heterozygosity in the *Singamagazi* ecotype indicate the likelihood of massive number of shared alleles between this ecotype and the others mentioned.

The phylogenetic relationships between the local chicken ecotypes showed that the two distance measures used (D_A and D_S) were able to separate the local chicken ecotypes according to their geographical origin and historical background. This is consistent with work by Wimmers *et al.* (2000) who used a different distance measure (chord distance) that is also known to provide correct tree topology (Takezaki an Nei, 1996) to separate local chickens from different continents according to their geographical origins. These results are also in agreement with work by Chenyambuga (2002), who by using the two distance measures managed to separate the indigenous goat breeds from the sub-Saharan Africa according to their geographical origins. Similarly, a study by Hanslik and others (2000), showed that using Nei's genetic distance corrected for population size (Nei, 1978), Holstein Friesian cattle could be separated according to their geographical origins. A study by Bowcock *et al.* (1994) that showed geographical associations between human populations using the D_S distance measure is also in support of the current results.

The presence of the *Kuchi* ecotype in the same cluster with the *Pemba* and *Unguja* ecotypes was an unexpected finding. Their current geographical location does not explain their phylogenetic relatedness. However, it is historically known that during the slave trade era some inhabitants from the Mwanza and Tabora were taken to the isles en-route to the Middle East and America. It is possible therefore, that some of the freed slaves managed to take home some chickens from the isles when the slave trade was abolished. The argument that chickens were taken from the isles to mainland and not

otherwise is based on the idea that slaves were most likely not allowed to carry any of their possessions. Secondly, even today in Zanzibar a local chicken called *Kuchi* exists and it is mostly used for cockfighting, bringing the idea that probably these chickens were brought from Asia by the early traders. Takahashi *et al.* (1998), were able to use microsatellite markers to infer to the natural history of some Japanese native breeds of chicken. As seen in this study, the *Mbeya* ecotype and White Leghorn appeared on the same cluster regardless of the distance measure used. As indicated in the materials and method section, the *Mbeya* ecotype was composed of purebred or crosses of Black Australorp breed. Their genetic relatedness is most likely based on the two being selected egg laying breeds.

It was observed that when individual chickens from the nine local ecotypes and the reference breed were compared for shared alleles between 38.5% and 100% of allele sharing was within ecotypes. However, it was only the White Leghorn that occupied the whole clade. All other ecotypes showed sharing of clades with others even those that clustered together at 100%. This finding provides an indication that although the local chicken ecotypes are probably independent genetic groups, there is still sharing of genetic materials between them. This may also indicate that there is limited interbreeding between the different ecotypes due to their geographical separation. It is possible that most chickens are moved only within a limited geographical location and that those transported far are not usually kept for breeding. Chenyambuga (2002),

attributed the distinctness between one local breed of goat (Newala) and the rest to the lack of communication between Newala and other parts of Tanzania.

This study supports the findings by other workers that microsatellite polymorphism data are useful in genetic differentiation between closely related populations (Vanhalla *et al.*, 1998; Wimmers *et al.*, 2000). It was also observed that microsatellite data could be used in evaluating geographical and natural historical backgrounds of different chicken populations.

Accurate determination of the genetic variations within animal species is a fundamental step towards conservation of the animal genetic resources (Oldenbroek, 1999). Conservation of animal genetic resources would safeguard the mankind's future opportunities for such diverse issues as market demands, production environment, cultural, historical as well as research (Gandini and Oldenbroek, 1999; Oldenbroek, 1999).

The current study has established the genetic uniqueness between different local chicken ecotypes. This is a very basic step towards rational decision-making on the modalities of selective breeding without compromising the existence of each unique genetic resource. In the later stages, it would be more informative to use genetic markers to locate the genes that are responsible for some economical traits; the quantitative trait loci (QTL). These QTL may range from those associated with productivity to those involved in disease resistance (Weigend and Romanov, 2000).

5.2 Productivity and reproductive performance of the local chicken ecotypes.

5.2.1 Weekly weight measurements

Sexual dimorphism could be followed to hatch weights where the mean weights for male chicks were higher than those of females. Similar observations were reported by Katule (1990), who showed sexual dimorphism on weight measurements from the fourth week of age. Other studies have reported sexual dimorphism on body weight to occur after 13 weeks of life (Wilson *et al.*, 1987). The overall mean weekly weight measurements for the local chicken ecotypes showed that after 20 weeks of life, hens had an average weight below 1kg. Katule, (1990) had earlier shown that at 16 weeks of age, the average juvenile body weights for the local chickens were 731.6g and 946.3g for females and males, respectively. In the current observations the overall mean weekly body weight measurements at 16 weeks were 540.9g and 826.5g for females and males respectively. The probable reason for this discrepancy would be the fact that the chickens used in the previous experiment were not selected based on specific criteria (as was done in the current experiment) for selection hence chickens from different ecotypes or crosses with exotic chickens could have been included.

When the results were scrutinised by ecotype, significant departure from the overall mean were observed in each parameter. For instance the mean values in all parameters for the *Ching'wekwe* ecotype (regardless of sex) were consistently below their respective overall mean values. On the contrary, the mean values in all parameters measured in the study in the *Morogoro-medium* ecotype were consistently above their respective overall

mean values. It was observed that the *Morogoro-medium* ecotype performed better in weekly weight measurements compared to the other ecotypes. This was shown by the fact that the ecotype had the highest or shared the highest mean values on all parameters at all times except hatch weighs for hens and body weight for cocks at week four of age. The *Morogoro-medium* chicken ecotype is what is generally referred to as the ordinary local chicken of Tanzania with the widest geographical distribution and very few unique distinctive features. The *Tanga* local chicken ecotype also by large shares this description and it is therefore not surprising that the two ecotypes although from different eco-climatic regions shared most values on the phenotypic parameters. These results are in agreement with those by Adedokun and Sonaiya (2001), who showed that local chickens from different agro-ecological zones might show similarities in their productivity parameters. It can therefore be assumed that, the local chicken ecotypes that showed significant variations in the productivity parameters suggest the possibility of genetic differences. Katule (1990), demonstrated that different genetic groups of chickens showed consistently significant different weight measurements over a period of up to 39 weeks. Safaloah (1998), made similar observations regarding body weights at eight weeks of age where different genetic groups showed statistically significant mean values. In that study, the groups compared included the Malawi local chickens, Starbro broilers and Black Australorp. In a different study, Adedokun and Sonaiya (2001), having failed to show any statistical significant difference on a score of productivity parameters concluded that the Nigerian local chickens from three agro-ecological zones studied were of the same genetic group. These observations fully support the assumption

that the local chickens of Tanzania are composed of different genetic groups, with different genetic potentials.

5.2.2 Mean daily growth rates

The overall mean growth rates varied between sexes and between the times of measurements. The overall mean growth rate in the second month was higher compared to what was observed in earlier studies (Wilson *et al.*, 1987; Mwalusanya, 1998). In that study Wilson *et al.* (1987) observed daily growth rates in Malian local chickens for the first 10 weeks of life to be 4g/day. Mwalusanya (1998), reported mean growth rates per day of 4.1 to 5.11g/day for females and 5.06 to 5.66g/day for males in the Tanzanian local chickens from three ecological zones. The discrepancies observed in the current study and the other studies may be due to the difference in the experimental settings. While the current study was based on-station, the other two studies obtained data from field observations. Lack of control of various factors such as feeds (availability and quality), and disease problems under the field situation may be responsible for preventing the local chickens from expressing their full genetic potential. However, the results from the current experiment are lower compared to a study in Malawi by Safalouh (1998), who reported mean daily growth rates of 10.7g/day at eight weeks of age for the Malawian local chickens. Nevertheless, as earlier stated, the genetic status of the Malawian local chicken was not known.

Marked variations were observed when the mean daily growth rate was compared between ecotypes further suggesting the possibilities of ecotypes being distinct genetic groups. The absence of clear-cut and consistent differences does not mean that the chickens are of a homogenous genetic group. Safalouh (1998), showed that the mean growth rate at eight weeks of age was not significantly different between the Black Australorp and the Malawian local chickens.

5.2.3 Mean egg weight, fertility and hatchability

The overall mean egg weight in the current experiment was 42.5g. This value was higher compared to 36.8g from Nigerian local chickens (Adedokun and Sonaiya, 2001), 37g from Bangladesh desi chickens (Barua and Yoshimura, 1997), 38.2g from Tanzanian local chickens (Katule, 1990) and 34.4g from Malian local chickens (Wilson *et al.*, 1987). However, there are other studies that have reported higher mean egg weights compared to the current experiment. Mwalusanya (1998), reported overall mean egg weight of 43.6g from local chickens from three ecological zones of Tanzania. In a different study, Zaza (1992) reported mean egg weight of 48g in Dandrawi, a local Egyptian chicken.

Significant differences were observed when comparing mean egg weight across ecotypes. While the *Ching'wekwe* ecotype showed the lowest mean value at 37g, the *Mbeya* ecotype showed the highest mean value of 49.3g. The remaining local chicken ecotypes had mean egg weights ranging from 41.1 to 43g. The existence of such

differences in the mean egg weights between the *Mbeya* ecotype and the other ecotypes can be explained by their divergent genetic disposition. As described earlier, the *Mbeya* ecotype belongs the Black Australorp breed (pure bred/cross bred), which had been specifically selected for egg production. It is observed that except for the *Mbeya* ecotype, mean egg weights for the other local chicken ecotypes were lower compared to those reported for *Kuchi* and *Singamagazi*; the two relatively large local chicken ecotypes from Tanzania (Msoffe *et al.*, 2001). Katule (1990), had earlier shown that different genetic groups of chickens laid eggs of significantly different weights. Such distinctions could not be seen when local chickens from different agro-ecological zones of Nigeria (Adedokun and Sonaiya, 2001) or different climatic zones of Tanzania (Mwalusanya, 1998) were studied. This gives a strong indication that the differences observed in the current study are genetical in nature.

An earlier study by Msoffe *et al.* (2001), reported mean egg weights values for *Ching'wekwe*, *Mbeya* and Morogoro-medium ecotypes that were lower compared to values from the same ecotypes in the current experiment. The reason for this difference may be due to the fact that while only fifty eggs (taken once) were used to calculate the mean egg weight in the previous study, the current mean was an average of eggs produced over one calendar year. Therefore, the current data represent a more realistic picture on this parameter.

The overall fertility (70%) reported in the current experiment fell short of the figure (95%) on the same parameter reported by Wilson, (1979). It is possible that the difference in the two experiments was attributed to the differences in the experimental settings. The low fertility in the on-station experiment was probably caused by confinement stress that prevented the cocks from expressing their optimal reproductive performance. There is also a possibility that there were some deficiencies in the commercial feeds that was given to the chickens since no attempt was made to evaluate the nutritional status of the feeds. As it was discussed elsewhere in this thesis, the choice of mate is linked to the genetic make-up (at the MHC locus) of the individuals (Wittzell *et al.,* 1999). It is possible that the selected cocks were "compatible" to only a few hens hence affecting the mean fertility. Another reason for the low fertility in the current experiment might be the fact that eggs had to be stored for up to one week prior to the incubation. Although all the necessary storage precautions were taken, there was a chance that fertility was lost during storage especially due to diurnal temperature variations. Between ecotypes, fertility varied significantly possibly due to differences in the inherent ability of each ecotype to cope with the factors mention above.

The hatchability values obtained in the current experiment were rather low (overall hatchability of 62%) compared to some previous studies. For instance Wilson (1979), observed mean hatchability values of 90% in the Malian local chickens. Barua and Yoshimura (1992), reported hatchability of 75% on the local Bangladesh chickens. Similarly, Mwalusanya (1998), reported hatchability in the free-range local chickens of

Tanzania to be over 80%. The overall hatchability in the current experiment was probably affected by the frequent electric power interuptions that impeded the optimal performance of the egg incubator. Furthermore, it is possible that some chicks might have required some assistance during hatching (known to be provided by the brooding hen).

Individual ecotypes showed a wide variation in hatchability ranging from 55% (*Ching'wekwe* and Morogoro-medium) to 74% (*Pemba* ecotype). Since eggs from all the local chicken ecotypes were subjected to similar conditions (prior to and during incubation) it could be concluded that within the problems stated above the values obtained here may represent genetic variations between the different ecotypes.

The productivity and reproductive parameters assessed in the current study have strongly suggested the presence of genetic variations within the local chicken ecotypes. This is particularly supported by other studies that used only the ecological or climatic region as the criterion for differentiating the local chickens and found the means of the different phenotypic parameters to be insignificant (Mwalusanya, 1998; Adedokun and Sonaiya, 2001). It is therefore more appropriate and meaningful to describe the local chicken ecotypes based not only on their geographical origin, but also using observable physical parameters such as size of the adult bird, shape of the comb or the plumage characteristics (Msoffe *et al.*, 2001). This would increase the repeatability between experiments done at different intervals and will reduce the variations between laboratories. Finally, it would be possible to standardise these phenotypic characters

within countries or regions and come up with some criteria for characterisation of the local chickens into breeds.

Further studies involving more parameters such as age at sexual maturity, number of eggs per clutch and per year, nutritional composition of the eggs, eggshell strength and coloration will be necessary before the ecotype status is elevated to variety or even breed. In the mean time, the ecotype status can be useful in planning for future improvement programmes through selective breeding within and between ecotypes.

5.3 Productivity and reproductive performance of local chickens under free-range management system

5.3.1 Weekly weight measurements and daily growth rate up to twelve weeks of age

The overall mean for weekly weight measurements showed very wide ranges indicating the extent of variation between the individuals in the different farmers. These observations are in agreement with previous studies, which have shown great variability between individual chickens especially with regard to adult body weights (Minga *et al.*, 1989; Katule, 1990; Msoffe *et al.*, 1998; Mwalusanya, 1998). The study by Minga *et al.*, (1989) and Mwalusanya (1998), were field observations while that of Katule (1990), and Msoffe *et al.* (1998) were on-station experiments. The convergence of the results from field observations and on-station experiments supports the view that the phenotypic variations observed was under genetic control rather than the environment. For instance, in their study, Minga *et al.*, (1989) observed average adult body weight of 1200g (500 to

1300g) and 2200g (1500 to 2800g) for hens and cocks, respectively. In a later study, Mwalusanya (1998), reported average adult body weight of 1348g (900 to 2250g) for hens and 1948g (1150 to 3150g) for cocks. Both studies exemplify the wide ranges inherent of regarding the local chickens as one genetic group. It has been suggested that the grouping of the local chickens according to their eco-climatic or geographical regions and phenotypic characters reduces significantly the range between the physical parameters hence providing more realistic results (Msoffe *et al.*, 1998; 2001). For example the overall mean adult body weight for the local chickens of Tanzania were reported to be 1471g (800 to 2300g) and 2261.5g (1000 to 3500g) for hens and cocks respectively (Msoffe *et al.*, 1998). As shown, the ranges were very wide and comparable to the previous studies mentioned above. However, when the chickens were grouped as ecotypes the ranges were reduced substantially. For example, the adult body weight for the *Morogoro-medium* ecotype was 1107.7g (800 to 1600g) and 1850g (1000 to 2350g) for hens and cocks respectively.

Comparison of the performance of the free-range local chickens from different farmers was done to underscore the influence of varying management practices between the participating farmers. The weekly weight measurements to 12 weeks showed significant variations on week 8 and 12, grouping the free-range local chickens into three groups; high, intermediate and low performers. This observation indicates that the level of performance in the free-range local chickens will also depend on the level of management practised by the individual farmer.

The overall mean daily growth rate for the second month (8^{th} week) was comparable to the ten weeks values reported by Mwalusanya, (1998). On the other hand the 12 weeks value was lower than the 14 weeks value from an earlier study. The difference observed between the two experiments cannot be explained by the disparity in the time at which the measurements were taken. This is because of the agreement between the week eight and 10 of the two experiments. The other explanation for the difference would be the number of birds included in the previous experiment where only seven birds were included. This low number of birds might have caused bias because the chosen individuals might have been selected for size.

When the free-range local chickens were compared between the different farmers, significant differences in the mean daily growth rate were seen in the second and third month. Nevertheless, none of the chickens from any particular household had consistently low or high rates. For instance, it was observed that chickens from farmer I had the highest daily growth rate in the second month, but the lowest value in the third. This strongly suggests that the differences in management practices between the different farmers could have attributed to the observed variations. Although the possibility that, different farmers might keep chickens of different genetic groups cannot be completely discounted. On the other hand, lack of differences between farmers in the first month and the lack of consistency could support the idea that the between household variation was purely due to the management. Also the trends of growth rate where chickens from some farmers showed an increasing rate in the first two months

and decrease in the third supports the change in management (in the third month) as the cause of variations rather than the existence of different genetic groups.

5.3.2 Reproductive performance

The overall mean egg weight of 41g observed on the free-range local chickens was comparable to the overall mean reported by Msoffe *et al.* (1998) on the scavenging local chickens of Tanzania. Data from both studies indicated wide ranges between the minimum and the maximum values. Minga *et al.* (1989), reported mean egg weight of 41.4g that was in agreement with the present study. Other studies have shown mean egg weights that were lower than the current values. For instance, Katule (1990), reported mean egg weights in the local chickens of Tanzania of 38.2g. Similarly, separate studies on Nigerian local chickens revealed mean egg weights of 38g and 38.6g (Omeje and Nwosu, 1984; Adedokun and Sonaiya, 2001). There are other studies where the mean egg weights were higher compared to the current experiments. Mwalusanya (1998), reported mean egg weight of 44.1g. Likewise Aganga *et al.* (2000) observed mean egg weight of 48g for the Tswana chickens. All these results have clearly shown the amount of variations existing within the local/indigenous chickens. It is most likely therefore that the differences between the results of the current study and the others would depend on the diversity among the individual chickens. The diversity could be mainly affected by the amount of crossbreeding within the populations involved.

The current study revealed overall hatchability of 80%. This value contrasts the mean hatchability (61.8%) exhibited in the Tswana chickens (Aganga *et al.*, 2000). In

Pakistan, (Farooq *et al.*, 2002), the reported mean hatchability was 61.2%. Results from Tanzania (Minga *et al.*, 1989), reported hatchability of 70% in the local chickens from Morogoro area. Other studies have reported hatchability values that were higher than the current values. Mwalusanya (1998), reported mean hatchability of 83.6% in the local chickens from Morogoro Tanzania. In a different study, Farooq (2000), reported mean hatchability of 84% in the local chickens of Pakistan. The reasons for variations between the current study and the previous ones would be the level of understanding on the pre-incubation practices by the different farmers. Farooq (2000), exemplified this in an experiment where farmers were educated on the optimal interval of storage of hatching eggs prior to incubation. The result from this effort was an increase in hatchability from 63 to 84%. Wilson *et al.* (1987), attributed variations in hatchability to the seasonal effects with hatchability being low during the dry season. However, the current study was conducted mostly during the dry season hence the seasonal effect although not studied was less likely to have influenced the results. The more likely reason for the high hatchability would be the level of awareness on farmers resulting from their participation in an on-going project aimed at improving the health and productivity of the rural chickens.

The farmer-to-farmer variations in the current study could be attributed to the level of adoption of the different husbandry practices offered through the said project. The most likely practices that would negatively influence the hatchability and that was seen in the current study included allowing the brooding hens to sit on more eggs than it would

optimally handle. Another practice was the storage of eggs for more than a week in a closed vessel (a tin or box) while waiting for the hen to go broody. The level of hygiene on the brooding nest was another factor, with some farmers not paying much attention to whether the nest was dry and clean. The positive response from most of the participating farmers is encouraging and one can project that after some years the hatchability will increase even further.

The results from the present study have shown that great variations exist in phenotypic parameter in the free-range local chickens. It was seen also that there were differences in levels of understanding between the different farmers regarding the different husbandry practices. It was observed that farmers were willing to adopt or improve on practices that they find useful. More efforts are therefore required in helping the farmers improve the productivity of their chickens. More research can be directed into enabling farmers to select superior stock in terms of productivity and disease resistance amongst their chickens for breeding purposes.

Farmers can also be helped through research on setting up some indices that can be used to determine the productivity potential in their stock even at a young age. Research is also required in evaluating the effect of season on such parameters as growth rate, egg production, fertility and hatchability under the free range management system.

5.4 Response to *S. gallinarum* infection for the local chicken ecotypes

5.4.1 PCV, humoral response, clinical and gross pathological features

From the results above, it was observed that neither the PCV nor the antibody response provided any clue on the variability between the local chicken ecotypes following experimental infection with virulent *S. gallinarum*. While the PCV remained within the normal range throughout the experiment, there were minimal antibody reactions. This is in contrast with studies by Mdegela *et al.* (2002) who found significant decrease in the PCV among the local chickens of Tanzania as well as marked changes in the antibody responses. In a different study, Waihenya *et al.* (2002a) also reported obvious changes in the antibody responses. The main reason for the difference between the current study and previous studies would probably be due to the experimental settings. While the current study used one-week old chicks, both other studies used adult chickens. Since it is known and accepted that fowl typhoid is a disease of adult chickens (Pomeroy and Nagaraja, 1991), it is possible that the chicks were mildly affected. However, by using young chicks it is possible to follow the pathogenesis of the disease without heavy mortalities in the chicks. The failure to mount a reasonable humoral response may be attributed to the immaturity of the immune system for the young chicks. However, Christensen (1996) was able to show significant drop in the PCV in chicks of the same age as the current experiment but of a different breed (White Leghorn). It is possible that the breed predisposition was responsible for the variations observed between these two studies. Furthermore, the two studies utilised different strains of *S. gallinarum*, a factor

that could also lead to the observed difference, as was shown in the study by Christensen (1996).

Clinical signs and post-mortem findings were consistent with some previous work (Pomeroy and Nagaraja, 1991; Christensen 1996; Mdegela *et al.*, 2002), where experimental infection with virulent strains of *S. gallinarum* resulted into Fowl typhoid. The severity and persistence of the clinical signs showed a bias towards three ecotypes; mainly the commercial layer strain, *Mbeya* and *N'zenzegere* ecotypes. Mortality also occurred only in the three mentioned groups among all the challenged groups. With the increasing evidence of ecotypes representing different genetic groups, it might be possible that the differences observed are genetical in nature. This would be in agreement with earlier work by Bumstead and Barrow (1993), who reported genetic variations in susceptibility to some *Salmonella enterica* serovars in in-bred lines of chickens. Although direct comparison between the two studies might be misleading (in-bred lines as compared to out-bred local chickens), but the main comparative feature (genetic predisposition) is supportive of such comparisons.

5.4.2 Leukocytic dynamics

The overall mean pre-challenge percent distribution for heterophils, lymphocytes and monocytes in the local chickens used in this experiment showed deviations from the established mean values (Jain, 1986). Whereas the mean percent distribution for heterophils and monocytes were well above the standard values, lymphocytes values

were below the standard. The deviations may be partly due the genetic differences between the birds from which the standard values were derived and the local chickens in this experiment. The diversity between the local chickens might be another factor for the deviations (the overall means were averages between the different ecotypes). The trend for the leukogram dynamics for both heterophils and lymphocytes were consistent with reports by Allan and Duffus (1971), albeit with higher values in the current experiment. Based on the standard chicken haematological values (Jain, 1986), the mean percent distribution for the lymphocytes was below the normal range except on day 10 pi and 14 of the experiment. The trend in the mean percent distribution for monocytes showed a very subtle change from day 3 pi onwards. However, the pre-challenge value was very high and much different from the normal values (Jain, 1986) and those reported by Allan and Duffus (1971), following experimental challenge. The day 3 pi to 14 values were consistent with the two previous observations.

The results from the leukocytic dynamics as examined by ecotypes revealed some significant difference. For instance, the day 3 pi mean percent distribution for heterophils showed that *N'zenzegere* ecotype had significantly lower measurements when compared to *Mbeya*, Morogoro-medium and *Tanga* ecotypes that had the highest means at that day. Heterophils have been well established as crucial effectors in the control of infection by *Salmonella* (Stabler *et al.*, 1994; Kogut *et al.*, 1994; 1998; Harmon, 1998). These cells (heterophils) have been reported to be of great importance in the restriction of the bacterial growth in the early stages of infection before the acquired

immunity takes over (Kogut *et al.*, 1998; Harmon, 1998; Henderson *et al.*, 1999). Heterophils are especially important in young chicks where the acquired immunity to pathogens is not yet fully developed (Kogut *et al.*, 1994). In mammalian models, neutrophils have been shown to play a similar role in the control of *Salmonella* and other intracellular pathogens (Conlan, 1996; 1997). It has been further suggested that both heterophils and neutrophils play an important role in eradicating the lumenal pathogens thereby limiting their dissemination to other organs (Henderson *et al.*, 1999). It follows that an immunocompetent bird will have higher numbers of heterophils in the first few days pi. The differences that were observed in the local chicken ecotypes especially on day 3 pi were suggestive of a differential immunocompetence with regard to this pathogen.

The results on the percent distribution for lymphocytes also showed some significant difference between the local chicken ecotypes. It is possible that the difference seen might reflect the variation in the adaptive immune capabilities between the different ecotypes. However, the exact role of lymphocytes (both B and T) in the modification of *Salmonella* infection in different hosts is still debatable (Lo *et al.*, 1999; Mittrucker *et al.*, 2000). The study by Lo *et al.* (1999), showed that CD8+ T-lymphocytes contributed significantly in the immunity to Salmonella infections. On the other hand, it has been shown that Salmonella infection tends to prevent optimal cell-mediated immunity by inhibiting the expression of IL-6, IL-12, IFN-γ receptors hence rendering the cell mediated immunity of less importance (Elhofy *et al.*, 2000; Kaiser *et al.*, 2000). There is

compelling evidence that the B-lymphocytes have a role in the protective immunity against Salmonella infection (Mittrucker *et al.*, 2000).

The crucial role of monocytes/macrophages in the control of *Salmonella* through phagocytosis and enhancement of inflammation has long been established (Stabler, 1994; Qureshi, 1998). Although monocytes are naïve precursors of macrophages, they differentiate upon activation into competent macrophage hence their presence provides a clue on immune competence of the individual (Klasing, 1998). The results from this experiment revealed some significant differences between the local chicken ecotypes that might imply differential immunocompetence. However, the results must be taken with caution because the mean percent distribution for monocytes after day 3 pi was within the normal range (Jain, 1986). Furthermore, the pre-infection mean percent distribution was very high and outside the normal range bringing some confusion as to whether the normal values for the local chickens were higher or that the chickens were responding to an infection by another pathogen.

Overall the results on the leukocytic dynamics showed significant variations between the local chicken ecotypes. The differences observed could be utilised in selective breeding where within each ecotype, chickens with high and low cellular responses would be identified. This will in turn provide a better model for the study of disease resistance mediated through cellular (leukocytes) changes.

5.4.3 Viable bacterial counts

The overall mean viable bacterial cell count for the liver and spleen peaked on day 10 pi and was lowest on day 14 pi indicating that the infection was being cleared. These findings are in agreement with a study by Mdegela *et al.* (2002), who reported peak viable bacterial cell counts from the liver and spleen of the local chicken on day nine pi. In that study, a similar trend where bacteria were being cleared after the peak counts was observed. Other studies (Christensen, 1996; Waihenya *et al.*, 2002a) have shown peak counts to occur on day seven and six respectively, contrasting the current results. However, the clearance of the bacteria from the two organs followed a trend similar to that obtained in the current experiment. The differences between the current observations and those made by Christensen (1996), may be due to the genetic difference between the strains of bacteria used as well as the chickens used in the study. The most probable reason for the discrepancies on the peak bacterial counts between the current experiment and that of Waihenya *et al.* (2002a), would be the fact that the current overall results was influenced by the presence of different ecotypes. As will be seen below, there were differences in the mean viable bacterial cell counts in the different local chicken ecotypes.

The mean viable bacterial cell counts from different ecotypes showed different peaking times; although as expected (the overall performance) most ecotypes peaked on day 10 pi. Two ecotypes (Morogoro-medium and *Tanga*) showed peak viable bacterial cell counts from the liver on day 3 pi. On the other hand, the *Mbeya* ecotype peaked on day 6

pi and the *Pemba* ecotype on day 14 pi. It was seen therefore that between the different ecotypes, some were in agreement with the previous studies (Christensen, 1996; Mdegela *et al.*, 2002; Waihenya *et al.*, 2002a). The results may suggest the existence of different pathogenetic mechanisms between the different local chicken ecotypes studied. There was no correlation between the viable bacterial cell counts and the clinical signs in the current experiment. This is in contrast to the observation by Christensen (1996), who reported that the peak viable bacterial cell counts coincided with severe anaemia and other clinical signs. However, as seen in the current experiment there was no anaemia and the clinical signs were not observed beyond nine days pi. The results from the current experiment were challenging because although the bacteria were detected from the liver and spleen as early as day 3 pi and persisted up to 14 days the clinical signs or the pathological picture did not reflect this fact.

It is well established that the clearance of *Salmonella* from the tissues is dependent on the cell-mediated immune responses (Lee *et al.*, 1983 include more ref). From the current results, the lymphocytes dynamics especially from day 6 pi and beyond provided some evidence in support of this earlier observation. It would appear that a two to three-fold increase in the percent distribution of the circulating lymphocytes was necessary to reduce the bacterial load. For instance, in *Mbeya* ecotype the lymphocyte distribution changed from 22 to 73% (day 6 and 10 pi respectively) and this change was associated with a complete clearance of the bacteria from the liver. On a similar note, a change from 62 to 52% in the *N'zenzegere* ecotype from day 6 to 10 pi seemed to have resulted

in increase in the viable bacterial cell counts from 79 to 7943 cells. Since there were no detectable antibodies, it appears that it was mostly the T-lymphocytes that were involved. These results support some earlier observations that CD8+ T-lymphocytes contribute to the host defence against Salmonella (Lo *et al.*, 1999).

5.4.4 Effect on growth

Fowl typhoid causes, apart from other clinical signs, poor growth in chicks (Pomeroy and Nagaraja, 1991). The current results have shown that infection with *S. gallinarum* negatively affected the pattern of growth in the infected chicks. For instance although the mean growth rate in the first week was 1.7g/d in the *Mbeya* ecotype, the range was –2.7g/d to 4.4g/d indicating that most chicks were actually losing weight. This is consistent with observations by Waihenya *et al.* (2002a), who reported significant drop in body weights in local chickens infected with *S. gallinarum*. Although there were no statistical significant differences between the ecotypes with regard to weight changes, the variations observed can still be used as an indication of biological differences between them. However, statistically significant differences may exist but wer not detected by the small number of birds that were included in this experiment.

This study has provided some insights into the responses of the different local chicken ecotypes to the experimental challenge with *S. gallinarum*. Differential responses between the ecotypes had been indicated and some mechanisms involved in the host defences have been evaluated. There is a need for focused research on the role played by

each mechanism (heterophils, macrophages/monocytes and lymphocytes; bacterial establishment and persistence) in chickens of different genetic backgrounds. It would be interesting to study the role of the different cellular secretions such as lymphokines including the molecular aspects of their secretion.

5.5 Response of the local chicken ecotypes to immunization with Newcastle disease vaccine.

5.5.1 HI titres in hens, eggs and chicks

The overall mean HI titres for the three categories showed that the titres were significantly higher in chicks compared to either hens or eggs. This implies that the maternal antibodies transmitted to the chicks undergo concentration in the yolk sac to afford them the highly required protection during the early days of life. Although there were significant differences in mean HI titres between the different categories of the local chicken ecotypes, chicks had high titres across all the ecotypes (with exception of *N'zenzegere* ecotype). The differences between ecotypes with regard to antibody responses to ND vaccine are consistent with earlier reports by Gwakisa *et al.* (1994a). In that study, divergent immune responses were observed between four local chicken ecotypes of Tanzania.

The results of the current work showed that the mean HI titres for chicks across all ecotypes were above $6Log_2$. This indicated that regardless of the ecotype, chicks hatched from eggs of recently vaccinated hens had protective maternal antibodies. This

observation is in agreement with an earlier hypothesis that day old chicks from immunised parents possessed high level of maternal antibodies to prevent a haematological spread of the virulent virus during the first seven days of life (Kouwenhoven, 1993). Allan and Gough, (1974), have reported that HI titres of $3\log_2$ were protective hence the current experiment has shown that vaccination of hens would offer protection to the offsprings. The maternally derived protection waned gradually and was down to zero in four weeks after hatch. This is consistent with previous studies that reported maternally derived antibodies for up to five weeks (Awan *et al.*, 1994).

5.5.2 HI titres in immunised chicks

The results on the overall responses of the chicks whose HI status was zero at the time of immunization showed that chicks were barely protected at day seven after immunisation. This observation is based on protection afforded by HI titres of $3\log_2$ (Allan and Gough, 1974), although birds with HI titres below $3\log_2$ may also be protected probably by cell mediated or mucosal immunity (Beard and Hanson, 1984). However, the range of 1 to $7\log_2$ was wide enough to suspect between ecotype variations. Results from day 14 pi to 28 and the subsequent immunization on day 70 showed that the chickens had protective HI titres.

When the local chicken ecotypes were compared on their response to ND vaccine, significant differences in mean HI titres were observed. It was clear that only a few individuals responded seven days after immunisation and in one ecotype (Morogoro-

medium) there was no response at all. The mean and the range for the responding ecotypes indicated that some chickens had already attained the protective HI titre within the first week and could hence be protected. For instance, mean HI titre for *Mbeya* ecotype was 3.3 (ranging from 2 to 7) while that of Morogoro-medium ecotype was zero. Early response to immunization would be of great advantage when it comes to survival during ND outbreaks. In two separate studies, Mtambo *et al.* (1999) and Waihenya *et al.* (2002b), reported that high levels of HI antibodies contributed towards the recovery of the infected birds. Early responders to immunisation will most probably attain high HI titres before the disease outbreak when strategic immunisation is applied.

It was shown in the current study that two months after immunization although the mean HI titres indicated protective levels, but the range indicated that some chickens within each ecotype were no longer protected. The lack of significant differences between ecotypes was an indication that the waning of the HI antibodies was common to all ecotypes. Earlier studies have shown differences in opinion on whether susceptibility to ND was genetically controlled. While Higgins and Shortridge (1988), were of the opinion that there were no host genetic influences, Ratanasethakul (1989), believed the native chickens to be more resistant. Nevertheless, recent observations suggest that limited evidence exists to support genetic resistance to ND in chickens (Schat and Davies, 2000). However, the possibility of selection for increased antibody responses to live or killed ND vaccines could exist.

The current study is increasingly supportive of selection for high antibody responses to vaccine as well as for early responses. It has been observed in earlier studies that the response of the local chickens to sheep red blood cells could be divided into high and low responders (Msoffe *et al.*, 2001). The existence of birds with high and low, early and late response to ND vaccine in each ecotype will provide the best starting point. It would be of great interest to study the response of the high/low responders or the early/late responders to infection with the virulent virus after immunisation. It would also be of interest to find genetic markers that are associated with the named categories of chickens.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The current study has revealed the existence of genetic and phenotypic diversity among the local chicken ecotypes of Tanzania. More specifically the following was shown:

1. Microsatellite DNA variations have been shown to provide useful markers for evaluating the genetic relatedness within and between local chicken ecotypes. Furthermore, reliable results have been achieved using the manual typing method thereby establishing a possibility for technology transfer to laboratories in the developing countries.
2. Uniqueness in phenotypic characters coupled with geographical separation has been successfully used as a fair guide in describing local chickens of different genetic groups.
3. Local chicken ecotypes are better models for studies on productivity and disease resistance due to the low variability between their genetypes and phenotypes. The problem of high variability in different parameters is common when local chickens are treated as one genetic group.
4. There exists in each ecotype, individuals with extreme performances (low and high) in productivity and disease resistance. This situation can be utilized in selective breeding programmes to establish within each ecotype chickens of optimal productivity and disease resistance potential.

5. It appears that within each ecotype there is preferential mate selection with chickens of the same ecotype having a greater chance to reproduce than otherwise. This explains the existence of unique ecotypes in only certain locations but amidst other ecotypes. For instance the existence of the *Ching'wekwe* ecotype amidst the *Morogoro-medium* ecotype.
6. The *Morogoro-medium* and *Tanga* ecotypes showed good performance in weight measurements, growth rates and egg weights. Apparently these ecotypes are distributed all over the country hence promising a big impact in the livelihood of the rural poor if improvement packages are directed at these chickens.

Recommendations

It is recommended that the search for more unique ecotypes should be continued to enable the documentation of as much local chicken genetic resources as possible. It is further recommended that the designing and execution of conservation programmes involving local chickens should be centred on the individual ecotypes rather than the local chickens as one genetic group. It is also recommended that genetic characterisation be continued using more markers especially those linked with productivity and disease resistance collectively known as quantitative trait loci (QTL).

It is recommended that steps be taken to establish a database that will contain information on different local chicken breeds/ecotypes that have been characterised. This will eventually lead to the establishment of African local chicken breeds/ecotype standards and hence uniform nomenclature.

7.0 REFERENCES

AAFC (Agriculture and Agri-Food Canada) (June 2000). Primary poultry breeders of egg layers and broilers. [http://www.agr.gc.ca/misb/aisd/poultry/breedpro.htm] site visited on 25/03/2002.

Adedokun, S. A. and Sonaiya, E. B. (April 2001). Comparison of the performance of Nigerian indigenous chickens from three agro-ecological zones. *Livestock Research for Rural Development* 13: [http://www.cipav.org.co/lrrd/lrrd13/2/aded132.htm], site visited on 09.08.2002.

Aganga, A. A., Omphile, V. J., Malope, P., Chabanga, C. H., Motsamai, G. M and Motsumi, L. G. (August 2000). Traditional poultry production and commercial broiler alternatives for small holder farmers in Botswana. *Livestock Research for Rural Development* 12: [http//www.cipav.org.co/lrrd/lrrd12/4/Agal24a.htm]. Site visited on 09.08.02.

Ajmore-Marsan, P., Negrini, R., Crepaldi, P., Milanes, E., Gorni, C., Valentini, A. and Gcogna, M. (2001). Assessing genetic diversity in Italian goat population using AFLP markers. *Animal Genetics* 32: 281-288.

Ajmore-Marsan, P., Valentini, A., Cassandro, M.,Vecchiotti,-Antaldi, G., Bertoni, and Kuiper, M. (1997). AFLP makers for DNA fingerprinting. in cattle. *Animal Genetics* 28: 418-426.

Akishinonomiya, F., Miyake, T., Sumi, S., Takada, M., Ohno, S. and Kondo, N. (1994). One subspecies of the red jungle fowl (*Gallus gallus gallus*) suffices as the

metriachic ancestor of all domestic breeds. *Proceeding of the National Academy of Science in USA* 91: 12505-12509.

Akishinonomiya, F., Tetsuo, M., Masaru, T., Ryosuke, S., Toshinoru, E., Takashi, G., Norio, K. and Susumu, O. (1996). Monophyletic origin and unique dispersal patterns of domestic fowls. *Proceeding of the National Academy of Science in USA* 93: 6792-6795.

Allan, D. and Duffus, W. P. (1971). The immunopothalogy in fowls (*G. domesticus*) of acute and sub-acute *Salmonella gallinarum* infections. *Research in Veterinary Science* 12: 140-151.

Allan, W. H., and Gough, L. E. (1974). A standard haemaglutination inhibition test for Newcastle disease: A comparison of macro and micro-methods. *Veterinary Record* 95: 120-123

Ansah, G. A. (2000). Matching genetic potential for performance with field conditions: The layer industry worldwide. In: *Proceedings of the XXI World's Poultry Congress* 20-24, August, Montreal, Canada.

Apanius, V., Penn, D., Slev, P. R., Ruff, L. R. and Potts, W. K. (1997). The nature of selection of the major histocompatibility complex. *Critical Reviews in Immunology* 17: 179-224.

Arens, M. (1999). Methods of subtyping and molecular comparison of human viral genomes. *Clinical Microbiology Reviews* 12: 612-626.

Armour, J. A. L., Alegre, S. A., Miles, S., Williams, L. J and Badge, R. M. (1999). Minisatellites and mutation processes in tandemly repetitive DNA. In:

Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C) Oxford University Press, New York. pp. 24-33.

Awan, M. A., Otte, M. J and James, A. D. (1994). The epidemiology of Newcastle disease in rural poultry: A review. *Avian Pathology* 23: 405-423.

Bacon, L. D. (1987). Influence of the major histocompatibility complex on disease resistance and productivity. *Poultry Science* 66: 802-811.

Balding, D. (1999). Forensic applications of microsatellite markers. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press, New York. pp. 198-210.

Barua, A. and Yoshimura, Y. (1997). Rural poultry keeping in Bangladesh. *World's Poultry Science Journal* 53: 387-394.

Beard, C. W., and Hanson, R. P. (1984). Newcastle disease. In: *Diseases of Poultry (Edited by Hofstad, M. S.)*, 8th edition, Iowa University Press, Ames, USA. pp. 452-470.

Beaumont, M. A. and Bruford, M. W. (1999). Microsatellites in conservation genetics. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press, New York. pp. 165-182.

Bidwell, J. (1994). Advances in DNA-based HLA-typing methods. *Immunology Today* 15: 303-307.

Biozzi, G., Mouton, D., Hermann, A. W. and Bouthilier, N. (1982). Genetic regulation of immunoresponsiveness in relation to resistance against infectious diseases. In:

Proceedings of the 2nd World Congress on Applied Livestock Production, Madrid, Spain. pp. 150 - 163

Bowcock, A. M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J. R. and Cavalli-Sforza, L. L. (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455-457.

Branckaert, R. D. S., Gaviria, L., Jallade, J. and Seiders, R. W. (2000). Transfer of technology in poultry production for developing countries. In: *Proceedings of the XXI World's Poultry Congress* 20-24, August, Montreal, Canada.

Briles, W. E., Briles, R. W., Pollock, D. L and Pattison, M. (1982). Marek's disease resistance of MHC heterozygotes in a cross of purebred Leghorn lines. *Poultry Science* 61: 205-211.

Briles, W. E., Briles, R. W., Taffs, R. E. and Stone H. A. (1983). Resistance to a malignant lymphoma in chickens is mapped to subregion of major histocompatibility (B) complex. *Science* 219: 977-979.

Briles, W. E., McGibbon, W. H. and Irwin, M. R. (1950). On multiple alleles affecting cellular antigens in the chickens. *Genetics* 35: 633-652.

Bumstead, N. and Barrow, P. B. (1993). Resistance to *Salmonella gallinarum*, *S. pullorum* and *S. enteritidis* in inbred lines of chicken. *Avian Diseases* 37: 189-193.

Bumstead, N. and Palyga, J. (1992). A preliminary linkage map of the chicken genome. *Genomics* 13: 690-697.

Bumstead, N., Huggins, M. B. and Cook, J. K. A. (1989). Genetic differences in susceptibility to a mixture of avian infectious bronchitis virus, *Escherichia coli*. *British Poultry Science* 30: 39-48.

Bussman, V., Lantier, I., Pitel, F., Patri, S., Nau, F., Gross, P., Elsen, J. M. and Lantier, F. (1998). cDNA cloning, structural organisation and expression of the sheep NRAMP1 gene. *Mammalian Genome* 9: 1027-1031.

Caccone, A., Gibbs, J. P., Ketmier, V., Suatoni, E., and Powell, J. R. (1999). Origin and evolutionary relationships of giant Galapagos totoises. *Proceedings of the National Academy of Science in USA* 96: 13223-13228.

Caron, L. A., Abplanalp, H. and Taylor, R. L. J. (1997). Resistance susceptibility and immunity to *Eimeria tenella* in major histocompatibility (B) complex congenic lines. *Poultry Science* 76: 677-682.

Carrington, M., Marti, D., Wade, J., Kiltz, W., Barcellos, L., Thomson, G., Chen, J., Truedsson, L., Sturfelt, G., Alper, C., Awdeh, Z. and Huttley, G. (1999). Microsatellites markers in complex disease: mapping disease-associated regions within the human major histocompatibility complex. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press; New York. pp. 225-237.

Cellier, M., Govoni, G., Vidal, S., Kwan, T., Groulx, N., Liu, J., Sanchez, F., Skamene, E., Schurr, E and Gros, P. (1994). The human NRAMP gene cDNA cloning, chromosomal mapping, genomic organization, and tissue specific expression. *Journal of Experimental Medicine* 180: 1741-1752.

Cellier, M., Prive, G., Belouchi, A., Kwan, T., Rodrigues, V., Chia, W. and Gros P. (1995). Nramp defines a new family of membrane proteins. *Proceedings of the National Academy of Science in USA* 92:10089-10093.

Chemlal, K., De Ridder, K. Fonteyne, P. A., Meyers, W. M., Swings, J. and Portaels, F. (2001a). The use of IS2404 restriction fragment length polymorphisms suggests the diversity of *Mycobacterium ulcerans* from different geographical areas. *American Journal of Tropical Medical Hygiene* 64: 270-273.

Chemlal, K., Huys, G., Fontyene, P. A., Vincent, V., Lopez, A. G., Rigouts, L., Swings, J., Meyers, W. M. and Portaels, F. (2001b). Evaluation of PCR-restriction profile analysis and IS2404 RFLP and AFLP fingerprinting for identification and typing of *Mycobacterium ulcerans* and *M. avium*. *Journal of Clinical Microbiology* 39: 3272-3278.

Cheng, S. and Lamont, S. J. (1988). Genetical analysis of immunocompentence measures in a White Leghorn chicken line. *Poultry Science* 67: 898-995.

Cheng, S., Rothchild, M. F. and Lamont, S. J. (1991). Estimates of quantitative genetic parameters of immunological traits in chickens. *Poultry Science* 70: 2023-2027.

Chenyambuga, S. W. (2002). Genetic characterisation of indigenous goat population of Sub-Saharan Africa using microsatellite DNA markers. Unpublished Thesis for Award of PhD Degree at Sokoine University of Agriculture, Morogoro, Tanzania, pp. 78-119.

Christensen, J. P. (1996). Phenotypic and genotypic characterisation of *Salmonella enterica* serovar Gallinarum biovars *gallinarum* and *pullorum* in relation to

typing and virulence. Unpublished Thesis for Award of PhD Degree the Royal Veterinary and Agriculture University, Copenhagen, Denmark. Pp. 99

Chrysostome, C. A. A. M., Bell, J. G., Demey, F. and Verhulst, A. (1995). Seroprevalence to three diseases in village chickens in Benin. *Preventive Veterinary Medicine* 22: 257-261.

Conlan, J. W. (1996). Neutrophils prevent extracellular colonisation of liver microvasculature by *Salmonella typhimurium*. *Infection and Immunity* 64: 1043-1047.

Conlan, J. W. (1997). Critical role of neutrophils in host defence against experimental systemic infections of mice by *Listeria monocytogenes, Salmonella tyhpimurium* and *Yersinia enterocolita*. *Infection and Immunity* 65: 630-635.

Conlan, J. W. and North, R. J. (1992). Early pathogenesis of infection in the liver with facultative intracellular bacteria *Listeria monocytogenes, Francisella tularensis* and *Salmonella typhimurium* involve the lysis of infected hepatocytes by leukocytes. *Infection and Immunity* 60: 5164-5171.

Corrier, D. E. and De Loach, J. R. (1990). Evaluation of cell-mediated cutaneous basophil hypersensitivity in young chicks by an inter-digital skin test. *Poultry Science* 69: 403-408.

Costello, E., O'Grady, D., Flynn, O., O'Brien, R., Rogers, M., Quigley, F., Egan, J. and Griffin, J. (1999). Study of restriction fragment length polymorphisms analysis and spoligotyping for epidemiological investigation of *Mycobacterium bovis*, infection. *Journal of Clinical Microbiology* 37: 3217-3222.

Cotter, P. F., Taylor Jr, R. L. and Abplanalp, H. (1998). B-complex associate immunity to *Salmonella enteritidis* in congenic chickens. *Poultry Science* 77: 1846-1851.

Crawford, R. D. (984). Domestic fowl. In: *Evolution of Domestic Animals (Edited by I. L. Meson)*, Longman, London UK. pp. 298-310.

Crow, J. F. and Kimura, M. (1970). *An introduction to population genetics theory.* Harper and Row, New York, Evanston and London.

d'leteren, G. D. M., Authié, E., Wissocq, N and Murray, M. (2000). Exploitation of resistance to Trypanosomes and trypanosomosis. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 195-216.

Dallas, J. F., Bacon, P. J., Carss, D. N., Convoy, J. W. H., Green, R. Jefferries, D. J., Kruuk, H., Marshall, F., Piertney, S. B and Racey, P. A. (1999). Genetic diversity in the Eurasian Otter, *Lutra lutra,* in Scotland. Evidence from microsatellites polymorphism. *Biological Journal of the Linnean Society* 68: 73-86.

Debrauwere, H., Gendrel, C. G., Lechant, S. and Dutreix, M. (1997). Differences and similarities between various tandem repeat sequences: Minisetallites and microsatellites. *Biochemie* 79: 577-586.

Dekich, M. A. (1998). Broiler industry strategy for control of respiratory and enteric diseases. *Poultry Science* 77: 1176-1180.

Di Rienzo, A., Peterson, A.C., Garza, J. C., Valdes, A. M., Stalkin, M. and Freimer, N. B. (1994). Mutational processes of simple sequence repeat loci in human

populations. *Proceedings of the National Academy of Science in USA* 91: 3166-3170.

Doenhoff, M. J. (2000). The immune system. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 47-72.

Donis-Keller, H., P. Green, C. Helms, S. Cartinhour, B. Weiffenbach, K. Stephens, T. P. Keith, D. W. Bowden, D. R. Smith, E. S. Lander, D. Botstein, G. Akots, K. S. Rediker, T. Gravius, V. A. Brown, M. B. Rising, C. Parker, J. A. Powers, D. E. Watt, E. R. Kauffman, A. Bricker, P. Phipps, H. Muller- Kahle, T. R. Fulton, S. Ng, J. W. Schumm, J. C. Braman, R. G. Knowlton, D. F. Barker, S. M. Crooks, S. E. Lincoln, M. J. Daly and Abrahamson, J. (1987). A genetic linkage map of the human genome. *Cell* 51: 319-337

Drijver, J. S. and Boon, M. E. (1986). Staining methods. In: *Routine cytological staining techniques* Theoretical background and practice. MacMillan Education, Ltd. London, UK. pp.145-146.

Edfors-Lilja, I and Wallgren, P. (2000). *Escherichia coli* and *Salmonella* diarrhoea in pigs. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 253-268.

Eding, J. H. and Laval, G. (1999). Measuring genetic uniqueness in livestock. In: *Genebanks and Conservation of Farm Animal Genetic Resource*, DLO, Institute for Animal Science, Lelystad, the Netherlands. pp. 33-58.

Eisen, J. A. (1999). Mechanistic basis for microsatellites instability. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press, New York. pp. 34-48.

Elhofy, A., Marriot, I. and Bost, K. (2000). *Salmonella* infection does not increase expression and activity of the high affinity IL-12 receptor. *Journal of Immunology* 165: 3324-3332.

Estoup, A and Cornuet, J. (1999). Microsatellites evolution: inferences from population data. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press, New York. pp. 49-65.

Falconer, D. S. and Mackay, T. F. C. (1996). *Introduction to quantitative genetics. 4th edition*, Longman group Ltd. Essex, England. 463pp.

FAO (2001). *FAO Production yearbook Volume 53, 1999.* FAO, ROME ITALY. pp. 207-229.

Farooq, M., Gul, N., Chaud, N., Durran, F. R., Khurshid, A., Ahmed, J., Asghar, A. and Zahir-ud-Din. (February 2002). Production performance of backyard chickens under the care of women in Charsadda, Pakistan. *Livestock Research for Rural Development* 14: [http://www.cipav.org.co/lrrd/lrrd14/1/faro141.htm] site visited on 23/06/2002.

Farooq, M., Shonkat, K., Asrar, M., Mussawar, S. Durrani, F. R., Asghar, A. and Faisal, S. (August 2000). Impact of female livestock extension workers on rural household chicken production. *Livestock Research for Rural Development* 14: [http://www.cipav.org.co/lrrd/lrrd12/4/faro124.htm] site visited on 23/06/2002.

Felsentein, J. (1993). PHYLIP-Phylogeny inference package, version 3.5. Department of genetics, Washngton University, Seattle, Washington, USA.

Feng, J., Li, Y., Hashad, M., Schurr, E., Gross, P., Adams, L. G. and Templeton, J. W. (1996). Bovine natural resistance-associated macrophage protein1 (Nramp1) gene. *Genome Research* 6: 956-964.

Fowler, N. G. (1996). How to carry out field investigations. In: *Poutry Diseases (Edited by Jordan, F. T. W. and Pattison, M.), 4th edition*, W. B. Saunders Company Ltd London, UK. pp. 428-432.

French, M. A. (1942). The productivity of native poultry. *East African Agricultural Journal* 7: 175.

Gandini, G. C. and Oldenbroek, J. K. (1999). Choosing the conservation strategy. In: *Genebanks and Conservation of Farm Animal Genetic Resource*, DLO, Institute for Animal Science, Lelystad, the Netherlands. pp. 11-31.

Gasbarre, L. C and Miller J. E. (2000). Genetics of helminths resistance. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 129-152.

Gauly, M., Bauer, C., Preisinger, R. and Erhardt, G. (2002). Genetic differences of *Ascaridia galli* egg output in laying hens following a single dose infection. *Veterinary Pasitology* 103: 99-107.

Germain, R. N. (1994). MHC-dependent antigen processing and peptide presentation: Providing ligands for T-lymphocytes activation. *Cell* 76: 287-293

Goto, N., Kodama, H., Okada, K. and Fujimoto, Y. (1978). Suppression of Phytohaemagglutinin skin response in thymectomized chickens. *Poultry Science* 57: 246-250.

Govoni, G. and Gross, P. (1998). Macrophages NRAMP1 and its role in resistance to microbial infections. *Inflammation Research* 47: 277-284.

Govoni, G., Cannone-Hergaux, F., Pfeifer, S. G., Marcus, S. L., Mills, S. D., Hackam, D. J., Gristein, S., Malo, D., Finlay, B. B., and Gross, P. (1999). Functional expression of Nramp1 in-vitro in the murine macrophage line, RAW264.7. *Infection and Immunity* 67: 2225-2232.

Govoni, G., Vidal, S., Cellier, M., LePage, M., Malo, D., and Gross, P. (1995). Genomic structure, promoter sequence and induction of expression of the mouse Nramp1 gene in macrophages. *Genomics* 27: 9-19.

Govoni, G., Vidal, S., Gauthier, S., Skamene, E., Malo, D. and Gross, P. (1996). The Bcg/Ity/Lsh locus: genomic transfer of resistance to infections in C57BL/6J mice transgenic for the Nramp^{Gly169} allele. *Infection and Immunity* 64: 2923-2929.

Gross, W. B., Siegel, P. B., Hall, R. W., Domermuth, C. H. and Dubose, R. T. (1980). Production and persistence of antibodies in chickens. 2. Resistance to infectious diseases. *Poultry Science* 59: 205-210.

Guèye, E. F (1998). Village egg and fowl meat production in Africa. *World's Poultry Science Journal* 54: 73-86.

Guo, S. W. and Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48: 361-372.

Gwakisa, P. S., Katule, A. M. and Rugaimukamu, E. A. (1994a). Divergent immune responses to Newcastle disease virus vaccination of rural chicken in Tanzania. *Tanzania Veterinary Journal* 43: 171-176.

Gwakisa, P. S., Kemp, S. J. and Teale, A. J. (1994b). Characterisation of Zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. *Animal Genetics* 25: 89-94.

Gyles, N. R., Fallah-Moghaddam, L. T., Skeeles, J. K., Whtfill, C. E. and Johnson, L. W. (1986). Genetic aspects of antibody response in chickens to different classes of antigens. *Poultry Science* 65: 223-232.

Hancock, J. M. (1999). Microsatellites and other simple sequences: Genomic context and mutational mechanics. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press; New York. pp. 1-9.

Hanslik, S., Harr, B., Brem, G. and Schlötterer, C. (2000). Microsatellites analysis reveals substantial genetic differentiation between contemporary new world and old world Holstein Friesian populations. *Animal Genetics* 31: 31-38.

Harmon, B. G. (1998). Avian Heterophils in inflammation and disease resistance. *Poultry Science* 77: 972-977.

Hartman, W. (1997). Evaluation of major genes affecting resistance to diseases in poultry. *World's Poultry Science Journal* 53: 231-252.

Hedren, M., Fay, M. F. and Chase, N. W. (2001). Amplified fragment length polymorphisms reveal details of polyploid evolution in *Dactylorhiza*, (Orchidaceae). *American Journal of Botany* 88: 1868-1880.

Hedrick, P. W. (1999). Perspectives: highly variable loci and their interpretation in evolution and conservation. *Evolution* 53: 313-318.

Henderson, S. C., Bounous, D. I. and Lee, D. M. (1999). Early events in the pathogenesis of avian Salmonellosis. *Infection and Immunity* 67: 3580-3586.

Herbergs, J., Siwek, M., Crooijmans, R. P., van der Poel, J. J. and Groenen, M. A. (1999). Multicolour fluorescent detection and mapping of AFLP markers in chicken (*Gallus domesticus*). *Animal Genetics* 30: 274-285.

Higgins, D. A. and Shortridge, K. F. (1988). Newcastle disease in tropical and developing countries. In: *Newcastle Disease (Edited by Alexander D. J.)* Kluwer Academic Press, Dordrecht, the Netherlands. pp. 273-302.

Hill, A. V. S. (2001). The genomics and genetics of human infectious disease susceptibility. *Annual Reviews on Genomics and Human Genetics* 2: 373-400.

Hill, A. V. S., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumwasi, P., Rowe, P. A., Bennet, S., Brewster, D., McMichael, A. J., and Greenwood, B. M. (1991). Common West African HLA antigens are associated with protection against severe malaria. *Nature* 352: 595-600.

Hol, C., Bloembergen, P., and van Dijk, H. (1993). Bacterial induced autoimmune reactivity. *Autoimmunity* 15: 49-54.

Horst, P. (1988). Native fowl as reservoir for genomes and major genes with direct and indirect effects on production adaptability. In: *Proceedings of 18th World's Poultry Congress*, 4-9th September, Nagoya, Japan, pp.105.

Horst, P. (1990). Research and development perspectives. In: *Proceedings of the CTA Seminar on Small holder Rural Poultry Production* 9-13th October, Thessaloniki, Greece volume 1 pp. 61-69.

Hu, J., Bumstead, N., Barrow, P., Sebastian, G., Olien, L., Morgan, K. and Malo, D. (1997). Resistance to salmonellosis in chickens is linked to NRAMP1 and TNC. *Genome Research* 7: 693-704.

Jain, N. C. (1986). *Schalm's Veterinary Haematology*, 4th edition. Lea and Febiger, Philadelphia, USA. pp. 527.

Janeway, C. A., Travers, P., Walport, M. and Capra, J. D. (1999). *Immunobiology and Immunlobiology: The immune system in Health and Diseases.* 4th edition, Elsevier Science Ltd/Garland Publishing, New York, USA.

Juul-Madsen, H. R., Hedemand, J. E., Salmonsen, J. and Simonsen, M. (1993). Restriction fragment length polymorphism analysis of the chicken B-F and B-L genes and their association with serologically defined B haplotypes. *Animal Genetics* 24: 243-247.

Juul-Madsen, H. R., Zoorob, R., Auffray, C., Skjodt, K. and Hedemand, J. E. (1997). New chicken RFP-Y haplotypes on the basis of MHC class II RFLP and MLC analysis. *Immunogenetics* 45: 345-352.

Kabatange, M. A and Katule, A. M. (1989). Rural poultry production systems in Tanzania. In: *Proceedings of an International Workshop on Rural Poultry in Africa, (Edited by Sonaiya, E. B)* Ile-Ife, Nigeria, pp. 171-176.

Kaiser, P., Rothwell, L., Galyov, E. E., Barrow, P. A., Burnside, J. and Wingley, P. (2000). Differential cytokine expression in avian cell in response to invasion by *Salmonella typhimurium*, *S. enteritidis* and *S. gallinarum*. *Microbiology* 146: 3217-3226.

Kaling, J. K. (1998). Avian macrophages: Systemic immune responses. *Poultry Sicence* 77: 983-989.

Kashi, Y and Soller, M. (1999). Functional roles of microsatellites and minisatellites. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press; New York. pp. 10-23.

Katule, A. M. (1988). Cross breeding as a tool for genetic improvement of chickens in less optimum environments. In: *Proceedings of the 15th Scientific Conference of the Tanzania Society of Animal Production*, 27-30 September, Arusha, Tanzania, pp. 86-111.

Katule, A. M. (1990). Studies on the prospects of improving the performance of local chicken populations in Tanzania by cross breeding. Unpublished Thesis for Award of PhD Degree at Sokoine University of Agriculture.

Kauffman, J. Volk, H. and Wallny, H. J. (1995). A "minimum essential Mhc" and "Unrecognised Mhc": Two extremes in selection for polymorphism. *Immunological Reviews* 143: 63-88.

Keen, L. J. (2000). Conformational analyses in the detection and genotyping of polymorphisms: Application to cytokine genes. In: *SNP and Microsatellites*

Genotyping Markers for Genetic Analysis. (Edited by Hajeer, A., Worthington, J and John, S.), Bio Techniques Books Publication, London, U.K. pp. 1-12.

Kelm, S. C., Detilleuh, J. C., Freeman, A. E., Kehrli, M. E., Dietz, A. B., Fox, L. K., Buttler, J. E., Kasekovics, I. and Kelley, D. H. (1997). Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. *Journal of Dairy Science* 80: 1767-1775.

Kemp, S. J. and Teale, A. J. (1994). Randomly primed PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiates *Bos indicus* and *B. Taurus. Animal Genetic* 25: 83-88.

Kimura, M. and Crow, J. F. (1964). The number of alleles that can be maintained in a finite population. *Genetics* 49: 725-738.

Kimura, M. and Ohta, T. (1978). Stepwise mutation model and distribution of allelic frequencies in a finite population. In: *Proceeding of the National Academy of Science in USA*. 75: 2868-2872.

Kingsley, G. H. (1993). Reactive arthritis: A paradigm for inflammatory arthritis. *Clinical Experimental Rheumatology* 11: S29-36.

Kitalyi, A. J. (1998). Village chicken production systems in rural Africa: Household food security and gender issues. *FAO Annual Production and Health paper 142* Rome, 81pp.

Klasing, K. C. (1998). Avian macrophages: Regulator of local and systemic immune responses. *Poultry Science* 77: 983-989.

Knorr, C., Cheng, H. H. and Dodgson, J. B. (1999). Application of AFLP markers to genome mapping in poultry. *Animal Genetics* 30: 28-35.

Kogut, M. H., Lowry, V. K., Moyes, R. B., Bowden, L. L., Bowden, R., Genovese, K., and Deloach, J. R. (1998). Lymphokine-Augmented activation of avian heterophils. *Poultry Science* 77: 964-971.

Kogut, M.H., Tellez, G. I., McGruder, E. D., Hargis, B. M., Williams, J. D., Corrier, D. E. and DeLoach, J. R. (1994). Heterophils are decisive components in early responses of chickens to *Salmonella enteritidis* infections. *Microbial Pathogenesis* 16: 141-151.

Koskinen, M. T. and Bredbacka, P. (2000). Assessment of the population structure of five Finnish dog breeds with microsatellites. *Animal Genetics* 31: 310-317.

Kouwenhoven, B. (1993). Newcastle disease. In: *Virus Infection of birds, (Edited by McFerran, J. E. and McNulty, M. S.)* Elsevier Science Publishers, Amsterdam the Netherlands, pp. 341-361.

Kroemer, G., Guillemont, F. and Auffray, C. (1990). Genetic organisation of the chicken MHC. *Immunological Research* 9: 8-19.

Kryglyak, L. (1997). The use of a genetic map of biallelic markers in linkage studies. *Nature Genetics* 17: 21-24.

Kulube, K (1990). Smallholder rural poultry production in Zimbabwe. In: *Proceedings of CTA Seminar on Smallholder Rural Poultry Production,* 9-13 October Thessaloniki, Greece, pp. 263-270.

Kyvsgaard, N. Chr., Luna L. A. and Nansen, P. (September 1999). Analysis of a traditional grain-and scavenge-based poultry system in Nicaragua. In: *Proceedings of the Tune Workshop*: p*oultry as a tool in poverty eradication and promotion of gender equality* [http://www.husdyr.kvl.dk/htm/php/tune99/9-Kyvsgaard.htm] site visited on 03/01/2002.

Lacey, C., Wilkie, B. N., Kennedy, B. W. and Mallard, B. A. (1989). Genetic and other effects on bacterial phagocytosis and killing by cultures of peripheral blood monocytes of SLA-defined miniature pigs. *Animal Genetics* 20: 371-382.

Laitio, P., Virtala, M., Salmi, M., Pelliniemi, L. J., Yu, D., and Granfors, K. (1997). HLA-B27 modulates intracellular survival of *Salmonella enteritidis* in human monocytic cells. *European Journal of Immunology* 27: 1331-1338.

Lamont, S. J. (1998). Impact of genetics in disease resistance. *Poultry Science* 77: 1111-1118.

Lamont, S. J., Bolin, C., and Cheville, N. (1987). Resistance to fowl cholera is linked to the major histocompatibility complex. *Immunogenetics* 25: 284-289.

Lanqing L., Johnson, W. L., Livant, E. J and. Ewald, S. J. (1999). The MHC of a broiler chicken line: serology, B-G genotypes, and *B-F/B-LB* sequences. *Imuunogenetics* 49: 215-224

Law, W. A. and Payne, L. N. (1996). The poultry industry. In: *Poultry Diseases (Edited by Jordan, F. T. W. and Pattison, M.).* 4th edition, W. B. Saunders Company Ltd, London, UK. pp. 1-8.

Lawrence, P. (1998). Ecotypes and natural disease resistance among scavenging local of Tanzania. Unpublished Dissertation for Award of MVSc. degree at the Royal Veterinary and Agriculture University, Copenhagen, Denmark.

Lee, G. M., Jackson, D. F. and Cooper, G. N. (1983). Infection and immune responses in chickens exposed to *Salmonella typhimurium*. *Avian Diseases* 27: 577-583.

Levin, I., L. B. Crittenden, and Dodgson, J. B. (1993). Genetic map of the chicken Z chromosome using random amplified polymorphic DNA (RAPD) markers. *Genomics* 16: 224-230.

Levin, I., L. Santangelo, H. Cheng, L. B. Crittenden, and J. B. Dodgson. (1994). An autosomal genetic linkage map of the chicken. *Journal of Heredity* 85: 79-85.

Lillehoj, H. S., Ruff, M. D., Bacon, L. D., Lamont, S. J. and Jeffers, T. K. (1989). Genetic control of immunity to *Eimeria tenella*: interaction of MHC genes and genes within the genetic background influence levels of disease susceptibility. *Veterinary Immunology and Immunopathology* 20: 135-148.

Linares, A. R. (1999). Microsatellites and the reconstruction of the history of human populations. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press; New York. pp. 183-197.

Lo, W-F., Ong, H., Metcalf, E. S. and Soloski, M. J. (1999). T cell responses to Gram-negative intracellular bacterial pathogens: A role for CD8+ in immunity to *Salmonella* infection and the involvement of MHC class Ib molecules. *Journal of Immunology* 162: 5398-5406.

Lumsden, J. S., Kennedy, B. W., Mallard, B. A., and Wilkie, B. N. (1993). The influence of the swine major histocompatibility genes on antibody and cell-mediated immune responses t o immunisation with an aromatic-dependent mutant of *Salmonella typhimurium*. *Canadian Journal of Veterinary Research* 57: 14-18.

Lunden, I., Edifors-Lilja, Johnson, K. and Liljedahl, E. (1993). Association between the Major Histocompatibility Complex and production traits in White Leghorns. *Poultry Science* 72: 989-999.

Lunney, J. K. and Buttler, J. E. (1998). Immunogenetics. In: *The Genetics of the Pig, (Edited by Rothschild, M. F. and Ruvinsky, A.)*, CAB International, Wallingford, U.K. pp. 163-197.

Lunney, J. K. and Murrel, K. D. (1988). Immunogenetic analysis of *Trichnella spiralis* infections in swine. *Veterinary Parasitology* 29: 179-199.

MacHugh, D. E. and Bradley, D. G. (2001). Livestock genetic origins: Goats buck the trend. *Proceedings of the National Academy of Science in USA*. 98: 5382-5384.

MacHugh, D. E., Shriver, M. D., Loftus, R. T., Cunningham, P. and Bradley, D. G. (1997). Microsatellites DNA variation and evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics* 146: 1071-1086.

Mallia, J. G. (June 1999). Observations on family poultry units in parts of Central America and sustainable development opportunities. *Livestock Research for Rural Development*. 11: (http//www.cipav.org.co/lrrd/lrrd11/3/mall13.htm) site visited on 23/06/2002.

Mannen, H., Tsuji, S., Loftus, R. T. and Bradley, D. G. (1998). Mitochondrial DNA variation and evolution of Japanese black cattle (*Bos Taurus*). *Genetics* 150: 1169-1175.

Max, R. A., Gwakisa, P. S., Balthazar, S. T. and Rugaimukamu, E. 91997). Concerted immune response to Newcastle disease virus and sheep red blood cells in chickens supplemented with different levels of vitamin C and E. *Tanzania Veterinary Journal* 17: 104-113.

Mbugua, P. N. (1990). Rural smallholder poultry production in Kenya. In: *Proceedings of CTA Seminar on Smallholder Rural Poultry Production,* 9-13 October Thessaloniki, Greece, pp 263-270.

Mdegela, R. H., Msoffe, P. L. M., Waihenya, R. W., Kasanga, J. C., Mtambo, M. M. A., Minga, U. M. and Olsen, J. E. (2002). Comparative pathogenesis of experimental infection with *Salmonella gallinarum* in local and commercial chickens. *Tropical Animal Health and Production* 34: 195-204.

Mdegela, R. H., Yongolo, M. G.S., Minga, U. M. and Olsen, J. E. (2000). Molecular epidemiology of *Salmonella gallinarum* in Tanzania. *Avian Pathology* 29: 457-463.

Medzhitov, R. and Janeway, C. A. (1997). Innate immunity impact on the adaptive immune responses. *Seminars in Immunology* 10: 351-353.

Melewas, J. N. (1989). The contribution of poultry industry to the national economy. In: *Proceedings of the 7th Tanzania Veterinary Association Scientific Conference, Arusha, Tanzania*, TVA volume 7: pp 13-35.

Metzgar, D., Bytof, J. and Willis, C. (2000). Selection against frameshift mutations limits microsatellites expansion in coding DNA. *Genome Research* 10: 72-80.

Minch, E. (2000). MICROSAT, the microsatellite distance programme [http://lotka.Stanford.edu./microsat/microsat.htm] site visited 23/04/2002.

Mindell, D. P., Sorenson, M. D. and Dimcheff, D. E. (1998). Multiple independent origins of mitochondrial gene order in birds. *Proceedings of the National Academy of Science in USA*. 95: 10693-10697.

Minga U. M., Katule, A. M., Maeda, T and Musasa, J. (1989). Potential of the traditional chicken Industry in Tanzania. In: *Proceedings of the 7th Tanzania Veterinary Association Scientific Conference, Arusha, Tanzania*, pp 207-215.

Minga, U. M. and Nkini, R. P. (1986). Disease as a major constraint to chicken production in Tanzania. In: *Proceedings of the 13th Scientific Conference of the Tanzania Society of Animal Production,* 26-29 November, Arusha, Tanzania, pp 284-291.

Minga, U. M., Katule, A. M., Maeda, T. and Mwanjala, T. (1996). The rural chicken industry in Tanzania: does it make economic sense? *Tanzania Veterinary Journal* 16 (supp. 2): 25-28.

Minga, U. M., Yongolo, M. G. S., Mtambo, M. M. A., Mutayoba, S. K., Lawrence, P., Mwalusanya, N. A., Katule, A. and Mlozi, M. R. S. (2000). The potential for rural poultry production and health in Africa. In: *Proceedings of the Workshop on the Possibilities for Smallholder Poultry Projects in Eastern and Southern Africa,* 22-25 May, Morogoro, Tanzania, pp 83-94.

Minjaw, B and de Castro, J. J. (2000). Host resistance to ticks and tick-borne diseases: Its role in integrated control. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 153-170.

Mittrucker, H-W., Raupach, B., Kohler, A. and Kaufman, S. H. E. (2000). Cutting edge: Role of B lymphocytes in protective immunity against *Salmonella typhimurium* infection. *Journal of Immunology* 164: 1648-1652.

Msoffe, P. L. M., Mtambo, M. M. A., Minga, U. M., Gwakisa, P. S., Mdegela, R. H., and Olsen, J. E. (June 2002). Productivity and natural disease resistance potential of free-ranging local chicken ecotypes in Tanzania. *Livestock Research for Rural Development 14:* [http://www.cipav.org.co/lrrd/lrrd14/3/msof143.htm] site visited on 15/08/02.

Msoffe, P. L. M., Minga, U. M., Olsen, J. E., Yongolo, M. G. S., Juul-Madsen, H. R., Gwakisa, P. S and Mtambo, M. M. A. (2001). Phenotypes including immunocompetence in scavenging local chicken ecotypes of Tanzania. *Tropical Animal Health and Production* 33: 341-354.

Msoffe, P. L. M., Mtambo, M. M. A., Minga, U. M., Gwakisa, P. S., Katule, A. M., Mutayoba, S. K., Mdegela, R. H., Mwalusanya, N. A., Yongolo, M.G. S. and Olsen, J. E. (1998). Scavenging local chickens in Tanzania: Is there room for improvement in productivity? *Tanzania Veterinary Journal*, 18 (Suppl. 4): 117-126.

Mtambo, M. M. A., Mushi, E. J., Kinabo, L. D. B., Maeda-Machang'u, A., Mwamengele, G. L. M., Yongolo, M. G. S. and Temu, R. P. C. (1999). Evaluation of the efficacy of the crude extracts of *Capsicum frutescens, Citrus limoni* and *Opuntia vulgaris* against Newcastle disease in domestic fowl in Tanzania. *Journal of Ethnopharmacology* 68: 55-61.

Mwalusanya, N. A. (1998). Productivity and nutritional status of local chickens under village management conditions. Unpublished Dissertation for Award of MVSc. Degree at the Royal Veterinary and Agriculture University, Copenhagen, Denmark. 56pp.

Ndegwa, J. M., Mead, R., Norrish, P., Kimani, C. W and Wachira, A. M. (2001). The growth performance of indigenous Kenyan chickens fed diets containing different levels of protein during rearing. *Tropical Animal Health and Production* 33: 441-448.

Nei, M. (1972). Genetic distance between populations. *American Naturalist* 106: 283-292.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science in USA* 70: 3321-3323.

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press, New York, USA. 506pp.

Nei, M., F. Tajima, and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular* Evolution 19: 153-170.

O'Meara, J. J., Egerton, J. R. and Raadsma, H. W. (1993). Recombinant vaccines against ovine footrot. *Immunology and Cell Biology* 71: 473-488.

O'Meara, T. J., and Raadsma, H. W. (1995). Phenotypic and genetic indicators of resistance to ectopathogens. In: *Breeding for Resistance to Infectious Diseases in Small Ruminants, (Edited by Gray, G. D., Woolaston, R. R., and Eaton, B. T.).* ACIAR monograph series, No. 34, pp 187-218.

OIE (Office International des Epizooties, Geneva) (1996), Fowl typhoid and pullorum disease. In: *Manual for Standards for DiagnosticTests and vaccines* pp 532-538.

Okada, I., Maeda, Y., Ota, K., Namikawa, T., Amano, T. and Kurosawa, Y. (1987). Genetical studies on breed differences of the native chickens in Bangladesh. In: *Genetic studies on Breed Differences of the Native Domestic Animals in Bangladesh*, Part 2, Hiroshima University, pp 11-26.

Okoye, J. O. A and Aba-Adulugba, E. P. (1998). Comparative study of the resistance or susceptibility of local Nigerian and exotic chickens. *Avian Pathology* 27: 168-173.

Oldenbroek, J. K. (1999). Introduction. In: *Genebanks and Conservation of Farm Animal Genetic Resource*, DLO, Institute for Animal Science, Lelystad, the Netherlands. pp 1-9.

Ota, T. (1993). DISPAN: Genetic distance and phylogenetic analysis, Pennsylvania State University, University Park, PA, USA.

Otsen, M., de Bieman, M., Kuiper, M. T. R., Pravenec, M., Krens, V., Kurtz, T. W., Jacob, H. J., Lankhorst, E. and van Zutphen, B. F. M. (1996). Use of AFLP markers for gene mapping and QTL detection in the rat. *Genomics* 37: 289-294.

Owen, J. B., Axford, R. F. E and Bishop, S. C. (2000). Mastitis in dairy cattle. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 243-252.

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Application in Biosciences,* 12: 357-358.

Pang, S. W. Y., Ritland, C., Carlson, J. E and Cheng, K. M (1999). Japanese quail microsatellites loci amplified with chicken specific primers. *Animal Genetics* 30: 195-199.

Parham, P. and Ohta, T. (1996). Population biology of antigen presentation by MHC class I molecules. *Science* 272: 67-74.

Park, Y-K., Bai, G-H. and Kim, S-J. (2000). RFLP analysis of *Mycobacterium tuberculosis* isolated from countries in Western Pacific regions. *Journal of Clinical Microbiology* 38: 191-197.

Parmentier, H. K., Nieuwland, M. G. B., Rijke, E., De Vries, R. R. P. and Schramer, J. W. (1996). Divergent antibody response to vaccines and divergent body weights in chicken lines selected for high and low humoral responsiveness to sheep red blood cells. *Avian Diseases* 40: 634-644.

Payne, W. J. A. (1990). Domesticated birds. In: *An Introduction to Animal Husbandry in the Tropics*. 4th edition, Longman group Ltd., Essex, U.K. pp. 684-726.

Permin, A. and Ranvig, H. (2001). Genetic resistance to *Ascaridia galli* infections in chickens. *Veterinary Parasitology* 102: 101-111.

Pitel, F., Lantier, I., Riquet, J., Lanneluc, I., Table-Aoul, K., Saidi-Mehtar, N., Lantier, F. and Gellin, J. (1994). Cloning, sequencing and localisation of an ovine fragment of NRAMP gene, a candidate for the ITY/LSH/BCG gene. *Mammalian Genome* 5: 834-835.

Plachy, J., Pink, J. R. L., Hala, K. (1992). Biology of the chicken MHC (B-complex). *Critical Reviews in Immunology* 12: 47-79.

Pollock, D. D., Eisen, J. A., Doggett, N. A. and Cummings, M. P. (2000). A case for evolutionary genomics and comprehensive examination of sequence biodiversity. *Molecular Biology and Evolution* 17: 1776-1788.

Pomeroy, B. S. and Nagaraja, K. V. (1991). Fowl typhoid. In: *Diseases of Poultry (Edited by Calneck, B. W., Barnes, H. J., Beard, C. W., Reid, W. M.* and *Yoder, H. W, Jr.)*, 9th edition, Wolfe Publishing Ltd, London, UK. pp. 87-99.

Prager, E. M., Orrego, C. and Sage, R. D. (1998). Genetic variation and phylegeography of Central Asian and other house mice, including a major new mitochondrial lineage in Yemen. *Genetics* 150: 835-861.

Prescot, C. A., Wilkie, B. N., Hunter, B. and Julian, R. J. (1982). Influence of purified grade pentachlorophenol on immune response in chickens. *American Journal of Veterinary Research* 43: 481-487.

Primmer, C. R., Randsepp, T. Chowdhary, B. P., Moller, A. P. and Ellegren, H. (1997). Low frequency of microsatellites in the avian genome. *Genome Research* 7: 471-482.

Qi, X., Stam, P. and Lindhout, P. (1997). Use of locus specific AFLP markers to construct a high density molecular map in barley. *Theoretical and Applied Genetics* 96: 376-384.

Quagliaro, G., Visch, M., Tynka, M., and Olivieri, M. (2001). Identification of wild and cultivated sunflower for breeding purposes by AFLP markers. *Journal of Heredity* 92: 38-42.

Qureshi, M., A. (1998). Avian macrophages : Regulation of local and systemic immune responses. *Poultry Science* 77: 983-989.

Raadsma, H. W. (2000a). Genetics aspects of resistance to ovine cutaneous myiasis. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 171-194.

Raadsma, H. W. (2000b). Genetics aspects of resistance to ovine foot rot. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York. pp. 219-242.

Rapley, E. and McDonald, B. (1992). Microsallites: The genomic road map. *Today's Life Science* 62-65.

Ratanasenthakul, C. (1989). Disease problems of importance in the Thai village poultry. In: *Proceedings of International Seminar on Animal Health and Production Services for Village Livestock,* 2-9 August, Khon Kaen, Thailand, pp 113-115.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248249.

Rincón, G., D'Angelo, M., Gagliardi, R., Kelly, L., Llambi, S. and Postiglioni, A. (2000). Genomic polymorphism in Uruguayan Creole cattle using RAPD and microsatellite markers. *Veterinary Science* 69: 171-174.

Ringrose, J. H., Yard, B. A., Muijsers, A., Boog, C. and Feltikamp, T. E. (1996b). Comparison of peptides eluted from the groove of HLA-B27 from *Salmonella* infected and non-infected cells. *Clinical Rheumatology* 15: 74-78.

Ringrose, J. H., Yard, B. A., Verjans, G. M. and Boog, C. (1996a). Immunisation of HLA-B27 transgenic and non-transgenic mice with *Salmonella typhimurium* results predominantly in the generation proliferative T-cells responses. *Clinical Rheumatology* 15: 79-85.

Ritz, L. R., Glowatzki-Mullis, M. L., MacHugh, D. E. and Gaillard, C. (2000). Phylogenetic analysis of the tribe Bovini using microsatellites. *Animal Genetics* 31: 178-185.

Rodriguez, L and Preston, T. R. (February 1999). Observations on scavenging local (indigenous) and Tam Hoang (exotic) chickens given free access (when confined at night) to duckweed (Lemnaceae) offered alone or mixed with rice bran. *Livestock Research for Rural Development* 11: (http://www.cipav.org.co/lrrd/lrrd11/1/lyl111.htm) site visited on 23/05/2002).

Roizés, G. (2000). Identification of microsatellites markers: screening for repeat sequences and mapping polymorphisms. In: *SNP and Microsatellites Genotyping*

Markers for Genetic Analysis. (Edited by Hajeer, A., Worthington, J and John, S.), Bio Techniques Books Publication, London, U.K pp. 35-48.

Rothschild, M. F., Skow, L and Lamont, S. J. (2000). The major histocompatibility complex and its role in disease resistance and immune responsiveness. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York pp. 73-106.

Rothuizen, J. and Van Wolferen, M. (1994). Randomly Amplified polymorphic DNA, polymorphisms in dogs are reproducible and display Mendelian transmission. *Animal Genetics* 25: 13-18.

Roy, M. S., Geffen, E., Smith, D., Ostrander, E. A. and Wayne, R. K. (1994). Patterns of differentiation in North American Wolf-like canids, revealed by analysis of microsatellites loci. *Molecular Biology and Evolution* 11: 553-570.

Rudesky, A., Preston-Hubert, P., Hong, S. C., Barlow, A. and Janeway, C. J. (1991). Sequence analysis of peptides bound to MHC class II molecules. *Nature* 353: 622

Ryan, A. M. and Womack, J. E. (1993). Type-1 interferon genes in cattle: Restriction fragment length polymorphisms, gene numbers and physical organisation on bovine chromosome-8. *Animal Genetics* 24: 9-16.

Saberivand, A., Sackey, D., Hohenhaus, M. A. and Outteridge, P. M. (1998). Microsatellite genotyping of MHC-DRB genes in sheep and goats. In: *Proceedings of 6th World Congress on Genetics Applied to Livestock Production*, January, Armidale, USA, pp. 319-322.

Safaloah, A. C. L. (1998). Response of the Malawi local chickens to commercial feed up to eight weeks of age. *Bulleting of Animal Health and Production in Africa* 46: 245-249.

Saitou, N. and Nei, M. (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular cloning: A loboratory manual 1st edition, Cold Spring Harbour Laboratory Press, volume2, pp. 9.16-9.23.

Samnang, H. (June 1998). Pasture versus integrated farming system as scavenging source for local and exotic chickens. *Livestock Research for Rural Development* 10: [http://www.cipav.org.co/lrrd/lrrd10/3/sam103p.htm] site visited 27/12/2001.

Sayers, I., Chen, X-H., Ye, S., Beghé, B and Day, I. N. M. (2000). MADGE and other single nucleotide polymorphisms analysis methods: application to the molecular genetic epidemiology of asthma and cardiovascular disease. In: *SNP and Microsatellites Genotyping Markers for Genetic Analysis. (Edited by Hajeer, A., Worthington, J and John, S.)*, Bio Techniques Books Publication, London, U.K. pp. 13-34.

Schat, K. A. and Davies, C. J. (2000). Viral diseases. In:*Breeding for Disease Resistance in Farm Animals (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W. and Owen, J. B)*, 2nd Edition, CABI Publishing, New York, USA. pp. 283.

Schierman, L. W. and Collins, W. M. (1987). Influence of the major histocompatibility complex on tumour regression and immunity in chickens. *Poultry Science* 66: 812-818.

Schlöterer, C and Wiehe, T. (1999). Microsatellites: a neutral marker to infer selective sweeps. In: *Microsatellites Evolution and Applications. (Edited by Goldstein, D. B and Shlöterer, C)* Oxford University Press; New York. pp. 238-248.

Shen, P. F., Smith, E. J. and Bacon, L. D. (1984). The ontogeny of blood cells, haemolytic complement and immunoglobulins in 3 to 12 weeks old $15I_5$·B congenic White Leghorn chickens. *Poultry Science* 63: 1083-1093.

Siegel, P. B. and Gross, W. B. (1980). Production and persistence of antibodies in chickens to sheep erythrocytes. *Poultry Science* 57: 1-5.

Simonsen, M. (1996). Why I think the chicken should survive in immunology and developmental biology. *Immunology and Developmental Biology of the Chicken*: 212: 1-6.

Simonsen, M., Arnul, M. and Sorensen, P. (1989). The chicken MHC and its importance. In: *Improving Genetic Resistance in Farm Animals (Edited by Zijjp, A. J. and Sybesma, W.)* Kluwer Academic Publishers, London, UK, pp. 42-62.

Smith, E.J., Jones, C. P., Bartlett, J and Nestor, K. E. (1996). Use of randomly amplified polymorphic DNA markers for the genetic analysis of relatedness and diversity in chickens and turkeys. *Poultry Science* 75: 579-584.

Sneath, P. H. A. and Sokal, R. R. (1973). *Numerical taxonomy.* W. H. Freeman and Co. San Francisco, California, USA, pp. 573.

Soll, D. (2000). The Ins and Outs of DNA fingerprinting in the infectious fungi. *Clinical Microbiology Reviews* 13: 332-370.

Sonaiya, E. B. (1990). The context and prospects for development of small holder rural poultry production in Africa. In: *Proceedings of the CTA Seminar on Small holder Rural Poultry Production* 9-13th October, Thessaloniki, Greece volume 1 pp. 35-52.

Spelman, R. and Bovenhuis, H. (1998). Genetic response from marker assisted selection in an outbred population for differing marker bracket sizes and with two identified quantitative trait loci. *Genetics* 148: 1389-1396.

Spradbrow, P. B. (1997). Policy framework for smallholder rural poultry development. In: *Sustainable Rural Poultry Production in Africa. Proceedings of an International Workshop*, Addis Ababa, Ethiopia, pp. 30-39.

Stabler, J. G. McCormick, T. W., Powell, K. C. and Kogut, M. H. (1994). Avian heterophils and monocytes; Phagocytic and bactericidal activities against *Salmonella enteritidis*. *Veterinary Microbiology* 38: 293-305.

Stephen, J. (2000). Genetic characterisation of five local Tanzanian sheep ectypes using microsatellite and RAPD DNA markers. Unpublished Dissertation for Award of MSc. Degree at University of Malawi, Malawi.

Stern, A. (1988). *Poultry and poultry keeping. (Edited by Duin, N.)* Merehurst Press, London, U.K pp. 144.

Sun, M. and Wong, K. C. (2001). Genetic structure of three orchid species with contrasting breeding systems using RAPD and allozyme markers. *American Journal of Botany* 88: 2180-2188.

Susol, E., Eyre, S and John, S. (2000). High throughput genotyping of microsatellites markers. In: *SNP and Microsatellites Genotyping Markers for Genetic Analysis. (Edited by Hajeer, A., Worthington, J and John, S.)*, Bio Techniques Books Publication, London, U.K pp. 49-66

Takahashi, H., Nirasawa, K.,Nagamine, Y., Tsudzuki, M and Yamamoto, Y. (1998). Genetic relationships among Japanese native breeds of chickens based on microsatellite DNA polymorphisms. *Journal of Heridity* 89: 543-546.

Takezaki, N. and Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144: 389-399.

Tan, Y-D., Wan, C., Zhu, Y., Lu, C., Xiang, Z. and Deng, H-W. (2001). An amplified fragment length polymorphism map of Silk worm. *Genetics* 157: 1277-1284.

Tang, Y-W., Procop, G. W. and Persing, D. H. (1997). Molecular diagnostics of infectious diseases. *Clinical Chemistry* 43: 2021-2038.

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acid Research* 17: 4127-4138.

Toth, G., Gaspari, Z. and Jurka, J. (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. *Genome Research* 10: 967-981.

Trail, J. C.M. (1963). The indigenous poultry. 2. Egg production. *Empire Journal of Experimental Agriculture* 31: 323-326.

Tuggle C.K., Schmitz, C.B. and Gingerich-Feil, D. (1997). Rapid communication: cloning of a pig full-length natural resistance associated macrophage protein (NRAMP1) cDNA. *Journal of. Animal. Science* 75: 277.

Uni, Z., Gutman, M., Leitner, G., Landesman, E., Heller, D. and Cahaner, A. (1993). Major histocompatibility complex class IV restriction fragment length polymorphism markers in replicated meat type chicken line divergently selected for high or low early immune response. *Poultry Science* 72: 1823-1831.

Van Marle-Koster, E. and Nel, L. H. (2000). Genetic characterisation of native Southern African chickens populations: Evaluation and selection of polymorphic microsatellites markers. *South African Journal of Animal Science* 30: 1-8.

Van Oppen, M. J. H., Rico, C., Turner, G. F. and Hewitt, G. M. (2000). Extensive homoplasy, non-stepwise mutations and shared ancestral polymorphisms at a complex microsatellite locus in lake Malawi Cichlids. *Molecular Biology and Evolution* 17: 489-498.

Vanhala, T., Tuiskula-Haavisto, M., Elo, K., Vilki, J. and Mäki-Tanila, A. (1998). Evaluation of genetic variability and genetic distances between eight chicken lines using microsatellites markers. *Poultry Science* 77: 783-790.

Vassiloyanakoupoulos, A. P., Okamoto, S. and Fierer, J. (1998). The crucial role of polymorphonuclear leukocytes in resistance to *Salmonella Dublin*, infection in genetically susceptible and resistance mice. *Proceedings of National Academy of Science in USA* 95: 7676-7681.

Vos, P., Hogers, M., Bleeker, M., Reijans, M. and van de Lee, T. (1995). A new technique for DNA fingerprinting. *Nucleic Acid Research* 23: 4407-4414.

Waihenya, R. K., Mtambo, M. M. A. and Nkwengulila, G. (2002b). Evaluation of the efficacy of the crude extracts of *Aloe secundiflora*, in chickens experimentally

infected with Newcastle disease virus. *Journal of Ethnopharmacology* 79: 299-304.

Waihenya, R. K., Mtambo, M. M. A., Nkwengulila, G. and Minga, U. M. (2002a). Efficacy of crude extracts of *Aloe secundiflora* against *Salmonella gallinarum* in experimentally infected free-range chickens in Tanzania. *Journal of Ethnopharmacology* 79: 317-323.

Wakelin, D. (2000). Rodent models of genetic resistance to parasitic infections. In: *Breeding for Disease Resistance in Farm Animals. 2nd Edition (Edited by Axford, R. F. E., Bishop, S. C., Nicholas, F. W and Owen, J. B)*, CABI publishing, New York, USA. pp. 107-126.

Weaver, R. F. (1999). *Molecular Biology.* McGraw-Hill, Company, Inc. New York, USA. pp. 64-65.

Wei, R., Dentine, M. R. and Bitgood, J. J. (1997). Random amplified polymorphic DNA markers in crosses between inbreed lines of Rhode Island red and White Leghorn chickens. *Animal Genetics* 28: 291-294.

Weigend, S. and Romanov, M. N. (2000). Current strategies for the assessment and evaluation of genetic diversity in chicken resources. In: *Proceedings of the XXI World's Poultry Congress* 20-24, August, Montreal, Canada.

Wierdl, M., Domiska, M. and Peters, T. D. (1997). Microsatellites instability in yeast: Dependence on the length of the sequence of the microsatellite. *Genetics* 146: 769-779.

Williams, G. E. S. (1990). Smallholder rural poultry production in Ghana. In: *Proceedings of the CTA Seminar on Small holder Rural Poultry Production* 9-13th October, Thessaloniki, Greece 2, pp. 89-97.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful genetic markers. *Nucleic Acid Research* 18: 6531-6547.

Wilson, I. and Balding, D. J. (1998). Genealogical inferences from microsatellite data. *Genetics* 150: 499-510.

Wilson, R. T. (1979). Studies on the livestock of Southern Darfur, Sudan. VII Production of poultry under simulated traditional conditions. *Tropical Animal Health and Production* 11: 143-150.

Wilson, R. T., Traore, A., Traore, A., Kuit, H.G. and Slingerland, M. (1987). Livestock production in central Mali: Reproduction, growth and mortality of domestic fowl under traditional management. *Tropical Animal Health and Production* 19: 229-236.

Wimmers, K., Ponsuksili, S., Hardge, T., Valle-Zarate, A., Mathur, P. K. and Horst, P. (2000). Genetic distinctness of African, Asian and South American local chickens. *Animal Genetics* 31: 159-165.

Wittzell, H., Madsen, T., Westerdahl, H., Shine, R. and von Schautz, T. (1999). MHC variations in birds and reptiles. *Genetica* 104: 301-309.

Yonash, N., Bacon, L. D., Witter, R. L. and Cheng, H. H. (1999). High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. *Animal Genetics* 30: 126-135.

Yongolo, M. G. S. (1996). Epidemiology of Newcastle disease in village chickens in Tanzania. Unpublished Dissertation for Award of MVM degree at Sokoine University of Agriculture, Morogoro, Tanzania. 230pp.

Zaza, G. H. M. (1992). Poultry and rural development in Egypt. In: *Proceedings of the Introductory Seminar on Poultry Development Policy*, Barnveld, the Netherlands pp. 115-118.

Zhang, X., Leung, F. C., Chan, D. K.O., Yang, G. and Wu, C. (2000). Genetic diversity of Chinese native chickens based on allozymes, randomly amplified polymorphic DNA and microsatellites polymorphisms. In: *Proceedings of XXI World's Poultry Congress*, 20-24 August, Montreal, Canada.

Zhao, L. P. and Zarbl, H. (2000). Single Nucleotide polymorphisms and future applications. In: *SNP and Microsatellites Genotyping Markers for Genetic Analysis. (Edited by Hajeer, A., Worthington, J and John, S.)*, Bio Techniques Books Publication, London, U.K. pp. 117-138.

Zijjp, A. J. and Neuwland, M. G. B. (1989). *Improving Genetic Disease Resistance in Farm Animals (Edited by Zijjp, A. J and Sysbesma, W.)*, Kluwer Academic Publishers, London, UK. 342pp.