GENETIC DETERMINATION OF IMMUNE RESPONSES TO NEWCASTLE DISEASE VIRUS VACCINE IN CHICKENS

SNAL - SUA / O
056959 0

BY

JAMES LWELAMIRA

SOKOINE UNIVERSITY OF AGRICULTURE LIBRARY

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN TROPICAL ANIMAL PRODUCTION OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

2002

ABSTRACT

A study was conducted at Sokoine University of Agriculture to investigate the genetic control of immune response of chickens against Newcastle disease virus (NDV) vaccine. The study involved local chickens from Tanzania (LL), Rhode Island Red derivative hybrids (EE), and their reciprocal crosses (LE and EL). The local ecotype had the highest mean antibody titres following primary vaccination, as well as before (residual) and after secondary vaccination, while the exotic breed had the lowest titres in all three measurements. The crossbred chicks were found to be intermediate between the two parental genotypes, but the differences between the local ecotype and the crosses were not significant except for the primary vaccination antibody titre. Furthermore, there were no significant differences between the reciprocal crosses. Differences between sexes were not significant. Despite the observed superiority of the local ecotype for antibody titre, there were no significant differences among genotypes with respect to mortality rate. Heritability estimates for antibody titre ranged from 0.27 ± 0.06 to 0.39 ± 0.09, 0.17 ± 0.03 to 0.32 ± 0.11, and 0.18 ± 0.08 to 0.28 ± 0.08 for antibody titre following primary vaccination, before, and after secondary vaccination, respectively. While the contribution of additive breed merit and heterosis effects in antibody titre before and after secondary vaccination were both significant, only the contribution of additive breed merits were significant for primary immune response. There were no significant correlation coefficients between antibody titres at different periods of measurement, nor between antibody titres and body weights. It was concluded that substantial improvement in immunological status of a stock against Newcastle disease could be

achieved through both selection and crossbreeding.

DECLARATION

I, JAMES LWELAMIRA, do hereby declare to the Senate of Sokoine University of Agriculture that the work presented in this dissertation is my own original work and has not been submitted for a degree award in any other University.

Signature.....

Date.....22/11/2002.....

COPYRIGHT

No part of this dissertation may be reproduced, stored in any retrieval system, or transmitted in any form or by any means; electronic, mechanics, photocopying, recording or otherwise, without the prior written permission of the author or Sokoine University of Agriculture in that behalf.

ACKNOWLEDGEMENTS

I wish to express my gratitude to many people who have helped me to accomplish this work. Foremost, sincere thanks are due to my supervisor Dr. A. M Katule of the Department of Animal Science and Production, Sokoine University of Agriculture for his encouragement, guidance and constructive comments without which the completion of this study would have been extremely difficult.

Thanks are also due to Prof. P. Gwakisa of the Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture for his invaluable guidance and assistance in the laboratory work.

Thanks are also due to other members of staff and my graduate student colleagues in the Department of Animal Science and Production for their encouragement and assistance during the whole study period.

Thanks are extended to Mr. Nyange and Mr. Mangachi of Sokoine University Farm for making incubation facilities available to me during the experimental period, and also to Mr. Moses for his day to day care of the birds during the experimental period.

I'm most indebted to the Norwegian Agency for International Development (NORAD), for granting the scholarship.

DEDICATION

To my father, late ELIAS LWELAMIRA, and

my mother, EUSTINA RWIZA.

TABLE OF CONTENTS

ABSTRACT ii

DECLARATION iv

COPYRIGHT v

ACKNOWLEDGEMENTS vi

DEDICATION vii

TABLE OF CONTENTS ix

LIST OF TABLES xii

LIST OF APPENDICES xiii

LIST OF ABBREVIATIONS AND SYMBOLS xiv

CHAPTER ONE 1

1.0 INTRODUCTION 1

2.0 LITERATURE REVIEW 5

2.1 The avian immune system 5

2.2 Genetic and non genetic factors influencing immune response in chickens 8

2.2.1 The role of genetics in conferring immune response 8

2.2.1.1 The Major Histocompatibility Complex (MHC) and immune response 8

2.2.1.2 Non-MHC genes and immune response 10

2.2.2 Environmental factors influencing immune response in chickens 10

2.2.2.1 Nutrition 10

2.2.2.1.1 Protein and energy 10

2.2.2.1.2 Minerals and vitamins 11

2.2.2.1.3 Fats 13

2.2.2.2 Antibiotics, feeds and drinking water contaminants 13

2.2.2.3 Environmental temperature 14

2.2.2.4 Social environment 14

2.3 Parental influence on immune response in young chicks 15

2.4 Selection for immune response in chickens 15

3.0 METHODOLOGY 17

3.1 Study site 17

3.2 Experimental materials 17

3.3 Vaccination and antibody assessment scheme 18

3.4 Antibody assay 19

3.5 Body weight and mortality measurements 19

3.6 Statistical analysis 19

4.0 RESULTS 25

4.1 Antibody titres 25

4.2 Mortality rates 26

4.3 Variance components and heritability estimates 27

4.4 Relative contribution of additive breed merit and heterosis on antibody titres 29

4.5 Correlation coefficients among traits 30

5.0 DISCUSSION 31

5.1 Antibody titres 31

5.2 Mortality rates 33

5.3 Heritability estimates 35

5.4 Relative contributions of additive breed and heterotic effects on antibody titres 37

5.5 Correlations among traits 38

CHAPTER SIX 40

6.0 CONCLUSIONS AND RECOMMENDATIONS 40

6.1 Conclusions 40

6.2 Recommendations 41

7.0 REFERENCES 42

APPENDICES 58

LIST OF TABLES

Table1: Design matrix for estimating constants for merit components..... 24

Table 2: Least square means + SE for antibody levels (log_2) by genetic groups and sex in chicks challenged with Newcastle disease virus vaccine..... 26

Table 3: Mortality rates of chicks by genetic groups and sex..... 27

Table 4: Heritability estimates + SE of antibody titres in chick serum by genetic group and vaccination phase. 28

Table 5: Constant estimates for additive breed differences and heterosis for immune response..... 29

Table 6: Genetic and phenotypic correlations (above and below diagonal, respectively) among traits studied. 30

LIST OF APPENDICES

Appendix Table 1a. Analysis of variance for primary response (i.e. antibody titres after the first ND vaccination). 58

Appendix Table 1b. Analysis of variance for residual titre (i.e. antibody titres prior to the second ND vaccination). 58

Appendix Table 1c. Analysis of variance for secondary response (i.e. antibody titres after the second ND vaccination)..... 58

Appendix Table 2. Components of variance for antibody titres at different stages of vaccination..... 59

LIST OF ABBREVIATIONS AND SYMBOLS

CBH	Cutaneous basophil hypersensitivity
DF	Degree of freedom
EE	Exotic chickens
EL	Crossbred chickens produced through mating of exotic cocks to local hens
GLM	General linear model
HA	Haemagglutination
HI	Haemagglutination inhibition
LE	Crossbred chickens produced through mating of local cocks to exotic hens.
LL	Local chickens
MANOVA	Multivariate analysis of variance
ME	Metabolizable energy
MHC	Major histocompatibility complex
ND	Newcastle disease
NDV	Newcastle disease virus
NORAD	Norwegian agency for international development
PHA-P	Phytohemagglutinin-P
PUPHA	Polyunsaturated fatty acids
REML	Restricted maximum likelihood
SAS	Statistical analysis system

SRBC	Sheep red blood cells
VARCOMP	Variance components
h^2	Heritability coefficient
S.E	Standard error
σ_s^2	Sire variance component
σ_e^2	Error variance component
t	Intraclass correlation
%	Percentage
P	Probability of significance to a referred level
*	Significant at $P< 0.05$
**	Significant at $P< 0.01$
***	Significant at $P< 0.001$
ns	Non significant at $P > 0.05$

CHAPTER ONE

1.0 INTRODUCTION

The poultry industry in Tanzania has emerged as a profitable enterprise. The industry can be divided into the traditional and commercial sectors. The traditional sector is dominated by indigenous chickens kept under the free range system, while the commercial sector is based on genetically improved exotic breeds of chickens raised under the high-input deep litter system (Yongolo, 1996).

Local or village chickens play an important role in the livelihood of the rural population. Of the 30 million people in Tanzania, about 84% live in rural areas where all of the eggs and chicken meat consumed are supplied from local chickens. In addition, village chickens cater for part of the demand of the urban population. They supply about 20% of the chicken products to urban consumers (Melewas, 1989; Yongolo, 1996).

In spite of the apparent importance of the chicken industry in Tanzania, factors such as nutrition and diseases have constrained its growth. However, among these factors, diseases rank the highest (Melewas, 1989; Minga *et al.*, 1989). Among many diseases of poultry endemic in Tanzania, Newcastle disease (ND) has been reported (Minga *et al.*, 1989) to be the most important. Newcastle disease is an acute rapidly spreading viral disease of domestic poultry characterised by respiratory signs, nervous manifestations and diarrhoea. This disease is an obnoxious killer and is of economic importance in the poultry industry since it affects all categories of chickens. The prevalence of this disease

has been demonstrated by epidemiological studies in scavenging village chickens in Tanzania (Awan *et al.*,1994; Yongolo, 1996). These studies confirmed its occurrence through serological and Newcastle disease virus characterisation techniques. Furthermore, it was noted that the disease exhibits seasonal pattern of outbreaks, and many factors are associated with the occurrence of the disease.

Although the strategy of mass vaccination has largely been an effective control of Newcastle disease, high vaccination costs and losses in performance of birds due to the disease are substantial (Nestor *et al.*, 1996). Furthermore, vaccination programmes have not been able to adequately cope with the disease in young chicks. This is because young chicks have poor responses to vaccination due to their underdeveloped immune systems and the interference between vaccine virus replication and maternal antibodies (Giambrone, 1981; Schwartz, 1994). Therefore, combining vaccination programs and development of genetically resistant stocks may offer an opportunity for maximising protection of the chickens from the disease with a consequence of increased productivity and returns from the poultry industry.

Studies conducted by several workers (Peleg *et al.*, 1976; Gwakisa *et al.*, 1994; Leitner *et al.*, 1994) have demonstrated the existence of differences in immunocompetence to Newcastle disease within strains/breeds, as well as between strains/breeds of chickens. Thus, Leitner *et al.* (1994) reported that both genetic and environmental factors might influence an animal's resistance to infectious disease. Further, using meat and egg type

chickens, several workers (Siegel and Gross, 1980; Soller *et al.*, 1981; Pitcovski *et al.*, 1987) have suggested the possibility of improving resistance of a population to several infectious diseases through genetic improvement. The existence of a strong relationship between antibody levels in the blood and the tolerance of an individual against the pathogen that provoked its production is known in animals. The antibody level following antigenic challenge has therefore been used as an indicator of immunocompetence of an individual against the disease under study (Siegel and Gross, 1980; Soller *et al.*, 1981; Pitcovski *et al.*, 1987). Furthermore, results from these studies indicate that both additive and non additive genetic effects, as well as maternal influences might be involved in conferring resilience against diseases. However, the relative contributions of the various factors have not been documented.

The overall objective of the present study was therefore to investigate the relative importance of genetic make up in conferring resistance of chickens to Newcastle disease, and hence investigate prospects for establishing genetically resistant stocks. The specific objectives were :

(i) To determine the relative importance of heritable and non heritable factors on immunocompetence of chickens against Newcastle disease;

(ii) To determine the effects of crossbreeding on immunocompetence to Newcastle disease;

(iii) To determine the correlation between immune response to Newcastle disease virus vaccine and growth, and survival rates of chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The avian immune system

The life of every organism is constantly threatened by other destructive organisms e.g infectious agents. For their continual battle against harmful microorganisms, vertebrates have evolved a very elaborate set of protective measures, collectively called the immune system. The word immune (Latin *immunis*, meaning "exempt") implies freedom from burden. An animal that is immune to a specific infecting agent will remain free of infection by the agent. Therefore, the immune system of an individual enables it to resist and overcome infection and also helps an individual to get rid of abnormal body cells (Klein, 1982; Cheng and Lamont, 1988).

Similar to mammalian species, the avian immune system is divided into two broad structural categories of lymphoid and non-lymphoid systems. Among lymphoid components, the Bursa of Fabricus, a site of B-lymphocyte development and differentiation, and the thymus, site of T-lymphocyte development and differentiation, are considered primary lymphoid organs, whereas the spleen is known to be a secondary lymphoid organ. In addition, lymphoid structures distributed throughout the intestinal tract represent the 'intestinal arm' of the immune system. Intestinal immune structures are a crucial barrier to external pathogens because many economically important pathogens replicate in the intestinal epithelium (Quereshi, *et al.*, 1998).

Usually, The B (bursal produced) lymphocytes are associated with humoral response

and the T (thymus produced) lymphocytes are associated with the cell mediated response.

Humoral immunity is the adaptive function of the immune system through which antibodies are produced in response to an antigenic challenge. Cell mediated immunity involves immune mechanism (s) through which cells infected with a foreign agent, such as a virus, are destroyed, and is accomplished via a direct effector (e.g. an activated T cell) and the target cell contact. Characteristics of both of these responses include specificity and memory (Weinstock *et al.*, 1989; Qureshi, 1998). The T cells also function in a regulator capacity. Helper T cells cooperate with B cells for antibody production. Other subclasses of T cells are responsible for cytotoxic or suppresser functions (Norkdsorg, 1987).

The non-lymphoid components of the immune system include cells that provide a non specific immunological defence to the host. The cells include blood monocytes, tissue macrophages, heterophils (counterparts of mammalian neutrophils) and thrombocytes. Being phagocytic in nature, these cells bind, internalise, and degrade foreign antigen, such as bacteria (Quereshi *et al.*, 1986; Harmon *et al.*, 1992).

Therefore, as in mammalian species, there are two major types of response in a bird's immune system: the specific, consisting of the humoral and cell mediated response, and non-specific. Usually coordination of specific and non specific immunity enables an individual to resist infection and disease (Cheng and Lamont, 1988).

Both cell mediated and humoral immune responses, either separately or in combination have been widely used as indicators of immunocompetence of an individual and hence its resistance to diseases in poultry. A number of studies (Siegel and Gross, 1980; Soller *et al.*, 1981; Van der Zijpp, 1983; Takahashi *et al.*, 1984; Pitcovski *et al.*, 1987) have reported the strong relationship between levels of antibodies circulating in the blood stream of an individual at a given time and its tolerance against pathogens responsible for provoking antibody production.

Humoral immune response to non-specific multi- determinant complex antigens e.g sheep red blood cells (SRBC) has also been used as an indication of natural immunity status in poultry. A study conducted by Gavora (1990) showed guinea fowls with high response to SRBC to be immunologically more sensitive to certain bacterial, viral and parasitic disease causing agents. Similar observations in chickens were reported by Siegel and Gross (1980).

Cutaneous basophil hypersensitivity (CBH) elicited in chickens by intradermal injection of phytohemagglutinin-P (PHA-P) has been shown to be a thymus-dependent response mediated by thymic (T) cells (Edelman *et al.*, 1985). Due to this fact, CBH response has been used in assessing cell mediated immunocompetence in individual chickens. CBH response can be evaluated on the skin of the wattle (wattle skin test), featherless skin, wing web, and on the skin of the thoracic region after removal of feathers (Corrier, 1990). This is achieved by measuring the magnitude of changes of skin or wing web

thickness.

In addition to immune response, persistence of antibody titre following antigenic challenge has also been noted to play a significant role in immunocompetence of an individual against the disease caused by the antigen (i.e. microorganism) those caused production of the antibodies (Gross et al., 1980; Siegel and Gross, 1980).

Furthermore, several studies have reported that the immunocompetence of an individual is influenced by factors that range from genetic to non-genetic ones (Gross and Siegel 1980; Hassan *et al.*, 1990; Lamont, 1998; Quereshi *et al.*, 1998).

2.2 Genetic and non genetic factors influencing immune response in chickens

2.2.1 The role of genetics in conferring immune response

2.2.1.1 The Major Histocompatibility Complex (MHC) and immune response

Genetic and environmental factors influence animal resistance to diseases. The genetics of a bird or a flock define the disease resistance potential (Lamont, 1998).

The best characterised genetic control of disease resistance and immune response is that associated with the major histocompatibility complex (MHC). The MHC is a group of genes coding for cell surface antigens. In chickens, the MHC codes for three classes of cell surface antigens. These include B-F, B-L and B-G antigens. B-F and B-L antigens

are equivalent to the mammalian class I and class II antigens. The B-G antigen is absent in mammalian MHC (Lamont and Dieter, 1990; Yoo Sheldon, 1990; Lamont, 1993; Kaufman and Lamont, 1996). The MHC was earlier recognised as the B blood group (Briles *et al*, 1950), because the B-F and B-G antigens expressed on erythrocytes determine the blood group. Since the chicken MHC is relatively small and compact and recombination is rare between the genes coding for these antigens, the B blood group has been used extensively to type the MHC haplotype in chickens (Yoo and Sheldon, 1990; Nordskog *et al.*, 1987). The MHC genes have a profound effect on the ability of animals to respond to specific antigens.

Several workers (Vaiano and Toivanen, 1987; Vaiano *et al.*, 1987; Loudovaris *et al.*, 1990; Schat, 1994) have demonstrated that the T cell-B cell interaction in antibody response, the antigen presentation by macrophages to T cells, and the antigen recognition by T cells are all MHC-dependent. Immune response to synthetic polypeptides such as (T,G)-A-L, GAT and GT are linked to the MHC in the chicken (Gunther *et al.*, 1974;Benedict, *et al.*, 1975; Pevzner *et al.*, 1979). Antibody titres to other soluble antigens (e.g. bovine serum albumin) and to cellular antigens (e.g. *Salmonella pullorum* bacterin and sheep red blood cells) are also linked to chicken MHC (Pevzner *et al.*, 1975; Loudovaris *et al.*, 1990)

The most prominent viral diseases of chickens for which resistance have been associated with the MHC, include Marek's disease (Cole, 1985), Rous sarcoma (White *et al*,

1994), *v-src* tumours (Taylor *et al.*, 1992), Avian leucosis (Yoo and Sheldon, 1990), and Newcastle disease (Dunnington *et al.*, 1992). Resistance to non viral pathogens also has been associated with the MHC. These diseases include spontaneous autoimmune thyroditis (Rose, 1994), coccidiosis (Lillehoj *et al.*, 1989; Caron *et al.*, 1997) and fowl cholera (Lamont *et al.*, 1987).

2.2.1.2 Non-MHC genes and immune response

The most important observation from studies on the MHC and chicken diseases is that there is no single haplotype (perhaps with the exception of B^{21} and resistance to Marek's disease) that responds optimally in all genetic backgrounds to all disease challenges. The repeated associations with the MHC suggest that there are genes for disease response within the MHC. However, the lack of consistent association with specific haplotypes suggests that MHC- linked genes other than the classical MHC class I (B-F) and II (B-L) genes may have a major influence in chicken immune systems (Lillehoj *et al.*, 1986;Dunnington *et al.*,1992; Caron *et al.*, 1997; Lamont, 1998).

2.2.2 Environmental factors influencing immune response in chickens

2.2.2.1 Nutrition

2.2.2.1.1 Protein and energy

Protein requirements in poultry are actually requirements for amino acids contained in dietary protein. Amino acids are the building units of tissues, organs, and other molecules in the body such as immunoglobulins (antibodies). Amino acids are also

important for cellular responses in cell mediated immunity (Chandra, 1980). Therefore, protein deficiency can impair immune responses in chickens. Tsiagbe *et al.* (1987) reported enhanced cell mediated and antibody mediated immune response associated with supplemental methionine and cysteine in broiler diets. Similarly supplements of valine have been reported to result in increased HI- titres (antibody titres) to Newcastle disease virus in chicks (Bhargava *et al.*, 1970).

Energy in the form of metabolizable energy (ME) must be available to chickens for production and maintenance of vital functions such as respiration, transport, communication and homeostasis including temperature regulation and antibody production (Kabasa *et al.*, 1995). A significant reduction in antibody production associated with protein-energy deficient diets has been reported (Bruce *et al.*, 1981).

2.2.2.1.2 Minerals and vitamins

Both minerals and vitamins have been reported to have a significant influence on immune function of birds. Thus, Flicken *et al.* (1986) reported that low concentrations of dietary zinc led to decreased macrophage activation for killing of *Escherichia coli*, while supplemental zinc has been reported (Kidd *et al.*, 1993) to improve cellular immunity in chicks. In chickens, supplemental selenium improves immune response against SRBC (Marsh *et al.*, 1981) and coccidiosis (Colnago *et al.*, 1985). On the other hand, reduced immune response against SRBC induced by sodium or chloride deficient diets in chickens has been reported (Pimental and Cook, 1986).

Results from numerous studies (Tengerdy and Nockels, 1975; Tengerdy and Brown 1977; Corwin and Shloss, 1980) have demonstrated increased susceptibility to infections in chickens with vitamin E deficiency. Vitamin E has been shown to favour proliferation of macrophages and to promote cooperation between T and B lymphoytes, resulting in antibody production (Gebremichael *et al.*, 1984). Colnago *et al.* (1984) reported a significant increase in immune response as measured by plaque forming cell test or by haemagglutination in chicks and hens fed supplemental vitamin E.

Many individual B-complex vitamins are also known to have unique effects on immunocompetence. For example, pyridoxine has been shown to inhibit cell mediated immune function and humoral responsiveness to a variety of test antigens (Biesel *et al.*, 1981). Biesel (1982) observed that chickens fed riboflavin deficient diets had reduced immune response against *Salmonella pullorum* antigens.

Submarginal levels of vitamin A, D and C also have been shown to impair immunocompetence. Vitamin A deficiency and long-term excess in chickens impairs antigen-specific antibody production and T lymphocyte proliferation *in vitro* (Friedman and Sklan, 1989). Aslam *et al.* (1998) noted vitamin D deficiency to depress cellular immune response in young chicks but not humoral or antibody mediated response. A study conducted by Pardue and Thaxton (1984) showed that steroid (corticosterone) mediated immunosuppression induced by a stress could be ameliorated by supplemental vitamin C.

2.2.2.1.3 Fats

Apart from their role in supplying calories, dietary fats are being recognised as important modulators of the immune response. In birds, it has been shown that fat sources, fatty acid composition and amounts have effects on immune cells. A higher antibody production by chickens fed fish oil based diets compared to those on linseed oil based diets was noted by Fritsche *et al.* (1991). Fish oil and linseed oil are both rich in n-3 polyunsaturated fatty acid (PUFA). On the other hand, the direct effects of PUFA intake on antibody production in avians has been demonstrated by Friedman and Sklan (1995). Low and high intakes were associated with decreased antibody production and lymphocyte proliferation.

2.2.2.2 Antibiotics, feeds and drinking water contaminants

Immunosuppression associated with antibiotics also has been reported in chickens. Hassan *et al.* (1990) observed reduced immune response and high mortality of chickens treated with Spiramycin adipique following a challenge with virulent ND virus compared to the untreated control group.

Contamination of poultry feeds by aflatoxins (potent mycotoxins produced by *Asperigillus parasiticus*) is not uncommon (Hegazy *et al.*, 1991). Several studies have reported the toxic nature of aflatoxins in both avian (Dieter *et al.*, 1985; Ghost *et al.*, 1991; Potchinsky and Bloom, 1993) and mammalian (Cusumo *et al.*, 1990: Raisuddin *et al.*, 1990) species.

Water contamination with heavy metals and volatile organic compounds has been reported to be common in many countries (Lanciotti *et al.*, 1989; Schumacher *et al.*, 1990; Srikahn *et al.*, 1993). Toxic effects of both heavy metals such as Arsenic, Cadmium, Lead and Mercury and volatile organic compounds such as Benzene, Trichloroethylene in chickens and other animal species is well documented (Bridger and Thaxton, 1982; Blakley *et al.*, 1980; Vodela *et al.*, 1997). These have been shown to have the effect of lowering humoral and cell mediated immune responses.

2.2.2.3 Environmental temperature

High environmental temperatures appear to affect the development of specific immune responses in chickens. Continuous exposure to elevated temperatures has been reported to reduce the immune response in chickens. For instance, Miller and Quereshi (1991) observed a depression of circulating antibodies and cell mediated immunity when birds were exposed to high environmental temperatures. Cold stress, i.e. chilling, has also been associated with reduced antibody production in chickens (Gross and Siegel, 1980).

2.2.2.4 Social environment

Social stresses are among the factors that have been noted to have significant influence on productivity and immunological status of chickens. A positive socialisation of chickens with their human associates was reported (Gross and Siegel, 1982; Gross and Siegel, 1988) to result in two-fold antibody production levels. Relatively high levels of

social stress inhibits cell mediated immunity defence against Marek's disease tumour and, coccidiosis (Gross and Siegel, 1988).

2.3 Parental influence on immune response in young chicks

The variance components of dams may contain, in addition to the chromosome transmitted factors, other specific maternal effects i.e. vertical transmission of maternal immunity through egg yolk (Gwakisa *et al.*, 1994). An estimate of heritability of serum gamma globulin levels in chicks was reported by Garnett and Roberts (1972) to be 2.5 times higher for dams (0.67) than for sires (0.26). Maternal effects on immune response were also found in reciprocal crosses between White Leghorn chicken lines selected for high or low levels of antibody response to sheep red blood cell (SRBC) antigen (Gross *et al.*, 1988). These authors found that high-sire x Low-dam F_1 chicks were more susceptible than reciprocal cross chicks. Leitner *et al.* (1994) studied the parental effects on immune response to *Escherichia coli* and NDV vaccines in young chicks, using meat type chicken lines divergently selected for either early or late maturation of immune system. From this study, it was noted that the level of the offspring humoral immune response was more of a dam than a sire effect.

2.4 Selection for immune response in chickens

Many researchers have investigated the genetic control of humoral immune response in egg-type chickens. Siegel and Gross (1980) and Gross *et al.* (1980) selected egg-type chickens for antibody production to SRBC and tested the resistance of selected lines to

infectious diseases. The high-antibody production line was more resistant to parasites and viruses, but not bacteria, than the low antibody line. Similarly, Pitcovski *et al.* (1987) selected for high and low early antibody response to NDV vaccine and *Escherichia coli*, and after four generations found that average antibody production of the high line exceeded that of the low line. Selection of egg type chickens for resistance to *Salmonella pullorum* antigen (Pevzner *et al.*, 1981b) and response to Marek's disease (Pinard *et al.*, 1993) was also successful. Selection for high and low antibody response in meat type chickens also yielded similar results (Leitner *et al.*, 1992; Yonash *et al.*, 1996). Cell mediated immunity of chickens has also been examined and demonstrated to be of genetic origin (van der Zijpp, 1983; Lamont and Smyth, 1984). Multitrait immune response selection in egg-type chickens has also been successful (Cheng and Lamont, 1988; Cheng *et al.*, 1991). Thus, many studies have demonstrated the feasibility of altering immunophysiology by genetic selection and crossbreeding.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study site

The study was conducted at the Sokoine University of Agriculture Poultry Research Unit, Morogoro, Tanzania. The place is located at an altitude of about 525m., above sea level. The relative humidity at the location is about 81%, while the monthly mean and maximum temperatures are 18.7 and 30.1^0 C respectively. The area has a mean annual rainfall of 846mm.

3.2 Experimental materials

The birds for the study consisted of sire and dam families from two genetically diverse breeds of chickens, i.e. a Tanzanian local ecotype, an improved commercial hybrid of Rhode Island Red descent, and reciprocal crosses of these stocks.

The breeding stock for producing the experimental birds consisted of 10 local cocks and 30 local hens, and equal numbers of exotic cocks and hens, respectively. Each cock was mated to 6 hens (i.e. 3 local and 3 exotic hens). The mating of the birds was done in two phases. In phase I, 10 local (LL) cocks were mated to 30 exotic (EE) hens, and 10 exotic cocks were mated to 30 local hens for about one week in floor pens. Then the mated hens were transferred to individual battery cages where the eggs produced by each hen were marked and collected for hatching for a period of two weeks.

After the end of Phase I the hens were again placed in mating pens and the cocks were interchanged, i.e. local cocks were mated to local females, and exotic cocks were mated to exotic hens. After one week of mating the hens were placed in battery cages again and collection of eggs for incubation resumed for another two weeks.

At hatching, the chicks were tagged and housed intermingled by sire family groups in floor pens. Altogether there were about 153 chicks, of which 39, 40, 36, 38 were local (LL), exotic (EE), and reciprocal crosses (LE and EL), respectively.

The feeding, health management, and other husbandry procedures were in accordance with standard practices for growing commercial egg-type chickens.

3.3 Vaccination and antibody assessment scheme

The presence and level of NDV specific antibodies were determined in eggs from the breeding hens during the egg collection period, as well as in offspring (Chick) serum. Chicks were vaccinated twice intraocularly with live inactivated NDV vaccine (La Sota strain). They were vaccinated first, when they were about 3 weeks, and then when they were 8 weeks of age, for primary and secondary stimulation, respectively. Blood samples for determination of antibody levels were collected from the wing vein just prior to, and then 2 weeks post vaccination for both vaccination cycles.

3.4 Antibody assay

The egg and serum samples were titrated for NDV specific antibodies by the microtitre method of the haemagglutination inhibition (HI) test (Allan and Gough, 1974) using NDV antigen. Four haemagglutination (HA) units were used and twofold serial dilutions of sera added with a starting dilution of 1:2. The titres were expressed in $\log_2$ form of the highest dilution causing HI.

3.5 Body weight and mortality measurements

Body weights were recorded on all individuals at 3, and 10 weeks of age, and mortality was recorded up to 10 weeks of age.

3.6 Statistical analysis

Data for titre values and body weights were checked for skewness using the SAS (1995) UNIVARIATE procedure and found to conform to normal distribution. The data were then subjected to the least squares analysis of variance using the SAS (1995) General Linear Model (GLM) procedures using models 1, 2, and 3 shown below for antibody titres following primary vaccination, prior to secondary vaccination i.e residual antibody titre, and after secondary vaccination, respectively.

$Y_{ijkl} = \mu + G_i + S_{ij} + C_k + (GC)_{ik} + e_{ijkl}$ ..... model 1

Y_{ijkl} = titre value of the l^{th} individual in the i^{th} genetic group, j^{th} sire and k^{th} sex;

μ = population mean;

G_i = fixed effect of the i^{th} genetic group;

S_{ij} = random effect of the j^{th} sire within the i^{th} genetic group;

C_k = fixed effect of the k^{th} sex;

(GC)ik = interaction between the i^{th} genetic group and k^{th} sex;

e_{ijkl} = random effect peculiar to each individual distributed as $N(0, I\sigma_e^2)$.

$$Y_{ijkl} = \mu + G_i + S_{ij} + C_k + (GC)_{ik} + b(x_{ijkl} - \overline{X}) + e_{ijkl} \quad \text{..... model 2}$$

Y_{ijkl} = titre value of l^{th} individual in the i^{th} genetic group, j^{th} sire and k^{th} sex;

μ = population mean;

G_i = fixed effect of the i^{th} genetic group;

S_{ij} = random effect of the j^{th} sire within the i^{th} genetic group;

C_k = fixed effect of the k^{th} sex;

(GC)ik = interaction between the i^{th} genetic group and k^{th} sex;

b = linear regression coefficient of residual antibody titre (i.e. before secondary vaccination) on antibody titre following primary vaccination;

$\overline{X}$= mean titre following primary vaccination;

x_{ijkl} = observed titre value following primary vaccination;

e_{ijkl} = random effect peculiar to each individual distributed as $N(0, I\sigma_e^2)$.

$$Y_{ijkl} = \mu + G_i + S_{ij} + C_k + (GC)_{ik} + b_1(x_{1(ijkl)} - \overline{X}_1) + b_2(x_{2(ijkl)} - \overline{X}_2) + e_{ijkl} \quad \text{..... model 3}$$

Y_{ijkl} = titre value of l^{th} individual in i^{th} genetic group, j^{th} sire and k^{th} sex;

μ = population mean;

G_i = fixed effect of i^{th} genetic group;

S_{ij}= random effect of the j^{th} sire within i^{th} genetic group;

C_k = fixed effect of k^{th} sex;

(GC)ik = interaction between i^{th} genetic group and k^{th} sex;

b_1 = linear regression coefficient of antibody titre following secondary vaccination on antibody titre following primary vaccination;

b_2 = linear regression coefficient of antibody titre following secondary vaccination on residual antibody titre (i.e before secondary vaccination);

$\overline{X}_1$= Mean titre following primary vaccination;

$x_{1(ijkl)}$ = observed titre value following primary vaccination;

$\overline{X}_2$= mean residual titre (i.e. before secondary vaccination);

$x_{2(ijkl)}$ = observed titre value before secondary vaccination;

e_{ijkl} = random effect peculiar to each individual distributed as $N(0, I\sigma_e^2)$.

In addition, the SAS (1995) VARCOMP procedure was used to calculate variance components between and within sire groups for the purpose of calculating heritability coefficients within each genetic group. In this aspect the following mathematical model was used: -

$Y_{ijkl} = \mu + C_i + S_j + D_{k(j)} + e_{ijkl}$ ..... model 4

Y_{ijk}= titre value of the l^{th} individual from the i^{th} sex, and k^{th} dam mated to j^{th} sire;

μ = population mean;

C_i = fixed effect of i^{th} sex;

S_j = random effect of j^{th} sire;

$D_{k(j)}$ = random effect of k^{th} dam mated to j^{th} sire;

e_{ijkl} = random effect peculiar to each individual distributed as $N(0, I\sigma_e^2)$.

The variance components were estimated by using the REML method of the VARCOMP procedure. Furthermore model 1 was used for deriving variance components for estimating the overall heritability and genetic correlations.

Partial phenotypic correlations controlled for main effects in Model 1 were determined using the MANOVA method of the GLM procedures of SAS (SAS, 1995).

Heritability estimates were calculated according to Falconer (1989) and their standard error were calculated according to Becker (1967) using equations 1 and 2 below.

$$h^2 = \frac{4(\sigma_s^2)}{(\sigma_e^2 + \sigma_s^2)} \quad \text{.....Equation (1)}$$

Where:

h^2 = heritability coefficient;

σ_s^2 = Sire variance component;

σ_e^2 = error variance component.

$$S.E = 2\sqrt{\frac{2(n-1)(1-t)\{1+(k_1-1)t\}^2}{k_1^2(n-s)(s-1)}} \quad \text{.....Equation (2)}$$

Where:

S.E = Standard error;

n = number of individuals;

t = intraclass correlation;

s = number of sires;

k_1 = number of offspring per sire.

Since the number of offsprings per sire were unequal, k_1 was calculated according to equation 3 as suggested by Becker (1967).

$$k_1 = \frac{1}{s-1}\left(n-\frac{\Sigma n_i^2}{n}\right) \quad \text{.....Equation (3)}$$

Where: Σn_i^2 = Sum of squares for number of individuals per sire;

Additive genetic standard deviations for each measure were estimated as $\sqrt{4 \times \sigma_s^2}$. These were then used to estimate additive genetic correlations as shown in equation 4.

$$\frac{covs_i s_j}{(\sigma_{si} x \sigma s_j)} \quad \text{.....Equation (4)}$$

Where s_i and s_j refer to sire effects for traits i and j, while σ_{si} and σ_{sj} refer to square roots of sire component of variance for trait i and j.

A design matrix was used to estimate additive genetic and heterosis constants through linear regression in order to determine the relative importance of additive genetic effects and heterosis on immune response as shown in Table 1.

Table1: Design matrix for estimating constants for merit components

Sire breed	Dam breed	Offspring Genotype	Incidence matrix (contribution to phenotypic expression)		
			A_L	A_E	H
Local	Local	LL	1.0	0.0	0.0
Local	Exotic	LE	0.5	0.5	1.0
Exotic	Local	EL	0.5	0.5	1.0
Exotic	Exotic	EE	0.0	1.0	0.0

A_L = Additive gene effect due to local breed;

A_E = Additive gene effect due to exotic breed;

H = Heterosis effects

Maternal influences were not included in this analysis because antibody titres in eggs and chicks before primary vaccination were seen to be all zero.

The frequency procedure of SAS (1995) was used to obtain Chi-square tests for independence between mortality rate and genotypes, and sex.

CHAPTER FOUR

4.0 RESULTS

4.1 Antibody titres

Antibody titres in eggs and in chick blood serum before primary vaccination were negative, while those for serum extracted after vaccination were all positive. The analyses of variance for antibody levels are summarized in Appendix Tables 1a, 1b and 1c. The least square means for the titres are shown in Table 2.

The results reveal that genetic groups were the only important source of variation for antibody titres. All other effects did not have any significant effects on this trait. The residual levels of antibodies prior to the secondary vaccination, which are a measure of persistence for antibody titre also did not have any significant influence on antibody levels after the secondary vaccination.

The results also show that the local ecotype (LL) had the highest antibody titres, while the exotic stock (EE) had the lowest titres in all three phases of measurements. The mean antibody titres for the reciprocal crosses (EL and LE genotypes) were intermediate between the two parental genotypes (LL and EE). However, differences between the reciprocal crosses in all three antibody measurements were not significant. The results further reveal that the crossbred birds did not differ significantly from the local ecotype except for the response following the primary vaccination.

Table 2: Least square means ± SE for antibody levels (log_2) by genetic groups and sex in chicks challenged with Newcastle disease virus vaccine

Factor		Antibody titres in various phases		
		Primary vaccination	Residual	Secondary vaccination
Genetic group	Local	6.46 ± 0.24^{a}	4.50 ± 0.18^{a}	8.24 ± 0.21^{a}
	Local x Exotic	6.04 ± 0.25^{b}	4.28 ± 0.19^{a}	8.00 ± 0.21^{a}
	Exotic x Local	5.74 ± 0.28^{b}	4.24 ± 0.21^{a}	8.13 ± 0.23^{a}
	Exotic	5.12 ± 0.25^{c}	2.91 ± 0.20^{b}	6.26 ± 0.26^{b}
Sex	Male	5.78 ± 0.23	3.90 ± 0.17	7.78 ± 0.19
	Female	5.90 ± 0.15	4.06 ± 0.11	7.54 ± 0.12

Least square means with no superscripts in common within a factor and column are significantly different ($P<0.05$).

4.2 Mortality rates

With the exception of a few chicks which died from drowning in drinking water, all chicks survived up until one week prior to the secondary vaccination, thereafter a substantial loss of chicks occurred due to the outbreak of coccidiosis. However most chicks recovered after treatment with the Amprolium® coccidiostat.

The mortality rates in the different genetic groups and sexes are shown in Table 3. The results reveal that the exotic stock had a slightly higher, but non significant mortality

rate than other genetic groups. Effects of sex on mortality rate were also found to be non- significant (P>0.05)

Table 3: Mortality rates of chicks by genetic groups and sex

Factor		Sample size	Mortality		Chi-square Test*
			Number	%	
Genetic group	Local	37	5	13.5	0.11^{ns}
	Local x Exotic	36	5	13.9	
	Exotic x Local	37	5	13.5	
	Exotic	38	6	15.8	
Sex	Male	47	6	12.8	0.12^{ns}
	Female	101	15	14.9	

* ns = non significant (P>0.05)

4.3 Variance components and heritability estimates

The components of variance for antibody titres are shown in Appendix Table 2. Table 4 shows estimates of heritability coefficients for antibody titres. The results show that most of the phenotypic variation (90% and above) with respect to antibody titres existed within sire family groups and the remaining (10% and below) was between sire family groups. Furthermore, as for the titres before secondary vaccination i.e. residual levels,

the contribution of the between sire variance component to the total phenotypic variation tended to be lower in secondary response than in primary response.

The results also indicate that, all of the heritability estimates were moderate in magnitude ranging from 0.17 ± 0.03 to 0.39 ± 0.09. Heritability estimates for antibody titre after the primary vaccinations were consistently higher in all genotypes than those for titres prior to (residual) and after the secondary vaccination. The results further show that heritability estimates in local ecotypes and crosses were relatively higher than those in exotics. Most of the heritability estimates for titres prior to the secondary vaccination were close to those for the secondary vaccination.

Table 4: Heritability estimates ± SE of antibody titres in chick serum by genetic group and vaccination phase.

Genetic group	Primary Vaccination	Residual	Secondary vaccination
Local	0.36 ± 0.07	0.25 ± 0.04	0.26 ± 0.05
Local x Exotic	0.38 ± 0.10	0.32 ± 0.11	0.28 ± 0.08
Exotic x Local	0.39 ± 0.09	0.28 ± 0.04	0.23 ± 0.03
Exotic	0.27 ± 0.06	0.17 ± 0.03	0.18 ± 0.02
Pooled	0.31 ± 0. 05	0.21 ± 0.02	0.24 ± 0.04

4.4 Relative contribution of additive breed merit and heterosis on antibody titres.

Table 5 shows the constant estimates for additive breed differences and heterosis with respect to antibody titres. The results reveal that both additive breed differences and heterosis effects were highly significant (P<0.001), except for the heterosis component in the primary response.

Table 5: Constant estimates for additive breed differences and heterosis for immune response

Measurement phase	Additive breed		Heterosis	
	Estimate	Significance	Estimate	Significance
Primary vaccination	-1.34	***	0.06	ns
Residual	-1.60	***	0.54	**
Secondary vaccination	-1.75	***	0.72	***

*** = significant at (P<0.001)

** = significant at (P<0.01)

ns = non-significant (P>0.05)

4.5 Correlation coefficients among traits

Genetic and phenotypic correlation coefficients among antibody levels and body weights are presented in Table 6. The results reveal that all genetic and phenotypic correlations examined were small and non significant.

Table 6: Genetic and phenotypic correlations (above and below diagonal, respectively) among traits studied.

	Primary titre	Residual titre	Secondary titre	3wk bwt.	10wk bwt.
Primary titre	.	0.18	0.10	-0.14	-0.09
Residual titre	0.17	.	-0.15	0.17	-0.14
Secondary titre	0.08	-0.12	.	0.10	-0.11
3wk bwt.	-0.13	0.15	0.07	.	0.47
10wk bwt.	-0.06	-0.10	-0.08	0.35	.

3wk bwt = 3 week body weight.

10wk bwt = 10 week body weight

CHAPTER FIVE

5.0 DISCUSSION

5.1 Antibody titres

The results of this study indicated that antibody titres for secondary immune response were higher than to those for primary immune response in all genetic groups. This observation was not unexpected, as it is well known in avian and mammalian species that the immune system of an individual has an ability to learn. Usually, the first encounter of the immune system with an antigen leads to a slowly rising synthesis of antibodies, dominated by IgM. A second encounter with the same antigen leads to a more rapid and greater response, dominated by IgG (Klein, 1982).

This observation shows the importance of repeated vaccinations at specific intervals in boosting the immunological status of an individual.

The results of this study have also demonstrated the existence of significant differences between genetic groups in primary immune responses, persistence of titres (residual titre), and secondary immune responses, in which local and crossbred chicks outperformed the commercial egg type exotic chicks in all three traits. Since the parent stocks which produced the experimental chicks originated from genetically diverse stocks i.e. local chickens, which are well adapted to many diseases and stressful conditions (Katule, 1988; Chrysostome *et al.*, 1995), and the Rhode Island Red hybrid stock which had been developed for egg production, the differences in genetic background could account for the observed differences in antibody titres. Genetic

differences especially in the MHC region have been demonstrated to have a strong influence on antibody production against antigens in chickens (Gyles *et al.*, 1986). The results from this study support the earlier findings from other studies in which similar results have been reported in different lines of other avian species such as turkeys (Tsai *et al.*, 1992; Sharaf *et al.*, 1988), and Japanese quails (Takahashi *et al.*, 1984) with respect to antibody responses. As observed in this study, differences between genetic groups in persistence of antibody titres also had been noted by other workers in chickens (Siegel and Gross.,1980; Gross *et al.*, 1980).

The results from this study also showed that there were no significant differences between reciprocal crosses in primary antibody responses, persistence of the antibody titres, and secondary immune responses against NDV vaccine. These observations suggest that the inheritance of genes that are involved in conferring immunocompetence to ND in chickens are not sex –linked.

It is evident from the results of this study that the exotic chickens were less resistant to ND than to the local chickens. The immunocompetence level in exotic birds may possibly be improved through crossbreeding. Since there was no reciprocal effects on immunocompetence against ND, then either of the two parental breeds may be used as the sire or dam.

Furthermore, results from this work showed that there was no significant difference between sexes in all three antibody measurements, i.e primary immune response, persistence of antibody titre, and secondary immune response against ND. This observation implies that immunocompetence of chickens against ND is not sex influenced. These results are contrary to those reported by Sarker *et al.* (1999), in which female birds were noted to have high antibody titres following antigenic challenge compared to their male counterparts. The discrepancy in the results of this study from those of other researchers could be explained by the differences in ages of the chickens involved in the different experiments. In this experiment, young chicks were used while in the study conducted by Sarker *et al.* (1999) mature birds were used. Several studies (Leitner, 1992; Ahmed *et al.*, 1985; Grossman, 1989) demonstrated that female sex hormones have a tendency to enhance immune response. Therefore because this study involved immature birds, differences between sexes could not be manifested.

5.2 Mortality rates

Despite the significant differences between genotypes in antibody production against NDV vaccine observed in this study, mortality rates among genotypes following the natural outbreak of coccidiosis were not significant. This implies that the mortality rates of the high responding and high persistent genotypes against NDV vaccine was not different from that of the low responding and low persistent genotypes. This result implies that genes involved in conferring immunocompetence to ND are different from those for coccidiosis. i.e they are genetically independent. A number of studies have

demonstrated the lack of correlation between responses to viral infectious diseases especially ND and to non-viral infectious diseases. Sacco *et al.* (1994), working with turkeys, noted genetic correlation of humoral immune response to ND and *P. multocida* to be not different from zero. A non-significant genetic correlation between humoral immune response against ND and that against *E.coli* was also reported in chickens by Soller *et al.* (1981). Therefore these results also confirmed that individuals resistant to certain diseases are not necessarily resistant to others.

The results from this study on heritability coefficients showed moderate heritability estimates for primary immune responses, persistence of antibody titres, and secondary immune responses against NDV vaccine, suggesting the possibility of improving these traits through conventional selection. Therefore, in view of the lack of correlation between humoral immune responses against NDV vaccine and diseases caused by other pathogens, selection for increased antibody production and persistence of antibody titres may not influence the ability of chickens to resist diseases caused by other pathogens. That means, no correlated response is expected with regard to resistance to diseases caused by other pathogens. These observations suggest that the tandem method of selection can be applied if, in addition to ND, genetic improvement for resistance against other common diseases i.e. coccidiosis, fowl typhoid, and fowl cholera, is to be achieved.

5.3 Heritability estimates

In this study, heritability estimates ranged from 0.17 ± 0.03 to 0.39 ± 0.09. According to Herald's (1994) classification, these estimates fit in the category of moderate heritabilities. The moderate heritability coefficients obtained in this study are attributed to the observed large proportion of variance on antibody titres existing within sires, thus leaving only a small proportion of the variance between sires. The heritability estimates reported in this study for humoral immune response are close to those reported by other workers using different types of antigen, including NDV vaccine, different strains of chickens, and other avian species whereby heritability estimates were based on sire components of variance. Gyles *et al.* (1986) reported heritability estimates for primary immune response to NDV live vaccine (B1 type Lasota) to range between 0 and 37% in different haplotyes of the B blood group of White leghorn chickens. On the other hand, Soller *et al* (1981) reported heritability value of 0.41 ± 0.17 in White rock chickens. Heritability values of 0.38 ± 0.07 and 0.29 ± 0.063 estimated from sire variance components were reported by Sacco *et al.* (1994) for primary and secondary immune response, respectively, against NDV vaccine in turkeys. The same authors reported heritability estimates for antibody titres against *Pasteurella multocida* vaccine in turkey to be 0.46 ± 0.075 and 0.33 ± 0.07 for primary and secondary antibody response, respectively.

The moderate heritability estimates obtained from this study indicate the existence of distinct additive genetic effects for immune response and persistence of antibody titres

against ND. From this observation it can be suggested that there is a possibility of improving these traits and hence the immunocompetence of a population against ND through conventional selection.

Furthermore, from this study, higher estimates of heritability were observed for primary immune response than for secondary immune response and persistence of antibody titres. Relatively higher heritability estimates were also obtained from local and crossbred chicks than from exotic chicks.

The observed differences in heritability estimates between primary and secondary immune response may be attributed to differences in the types of antibodies in these two responses. Whereas the primary response is characterized by a predominance of IgM (a type of antibody), secondary response is characterized by a predominance of IgG (Sacco *et al.*, 1994). Therefore the heritability estimates for primary and secondary antibody response may reflect a difference in genetic regulations of the responses.

Relatively high heritability estimates for primary immune response compared to secondary immune response, and persistence of antibody titre implies that higher genetic gain upon selection will be realized in primary immune response than in secondary immune response and persistence of antibody titre. Since the heritability estimates for all three attributes were relatively higher in local chicks than in exotic chicks, much higher

gain is expected to occur when it is carried out within local chickens than within the exotic chicken population.

5.4 Relative contributions of additive breed and heterotic effects on antibody titres

The observed significant effects of additive breed effects in this study for primary immune responses, persistence, and secondary immune responses to NDV vaccine further indicate that additive genetic determination is an important factor in conferring immune responses in chickens. The significant effect of heterosis for persistence of antibody titre and, secondary immune responses observed in this study reveals that in addition to additive genetic determination, heterosis effects are also important for these traits in chickens.

The difference in genetic control between primary immune response and the other two antibody measurements is also reflected by the relatively higher heritability values for primary immune response and low genetic correlation between primary response on one hand and persistence and secondary response on the other. These findings are consistent with those reported in turkeys (Sacco *et al.*, 1994), and in mice (Sant'anna *et al.*, 1979) for humoral immune response. Sant'anna *et al.* (1979) working with mice noted heterotic effects to be more pronounced in secondary immune response than in primary immune response. Similarly, Siegel and Gross (1980), and Gross *et al.* (1980), also

observed both additive and heterotic effects to be equally important for the persistence of antibodies.

These results suggest that, while selection alone may be adequate for realizing progress in genetic improvement of primary immune responses, selection coupled with crossbreeding may be important for genetic improvement in persistence of antibody titres and secondary immune responses.

5.5 Correlations among traits

The results from this study revealed that both genetic and phenotypic correlations between antibody titres against NDV vaccine and body weight measurements were generally low. The lack of significant correlations between immune responses against NDV vaccine and body weights has also been observed in other studies conducted with turkeys (Sacco *et al.*, 1994) and broiler chicks (Van der Zijpp., 1984). Furthermore, results from this study indicated that genetic and phenotypic correlations between primary immune response, persistence of antibody titres and secondary immune responses against NDV vaccine were all low. The lack of significant genetic correlations between traits observed in this study suggests that these attributes are inherited independently of each other. Since the immune responses, including persistence of the antibody titre against ND, are genetically independent of the body weights of the birds, it is obvious that selection for improving these immunocompetence traits against ND would not have a negative effect on the growth performance of birds. This observation

further justifies the use of selection in improving immunocompetence of chickens against ND.

Furthermore, results for genetic and phenotypic correlations suggest that selection based on either of the antibody measurements (i.e primary response, persistence of the antibody titre or secondary immune response) against ND would not be expected to improve other attributes. i.e no correlated response is expected. This observation stresses the need for separate and independent breeding programmes for these traits.

In summary, the results from this study indicated a possibility of improving immunocompetence of a population against ND through genetic improvement. However, care must be taken as the genetic approach to ND control may be further complicated by the following factors:-

i) The disease agent may mutate or a new strain may move in from elsewhere and the established resistance may not be effective against the new strain.

ii) The virulence of a given strain may vary depending on a number of other factors, thus reducing the prospects of attaining genetic resistance under different circumstances.

Furthermore, the suitability of this approach (i.e. genetic improvement) will depend on the economic benefits to be derived from the breeding ventures compared to those from conventional ND control methods.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study it can be concluded that;

- Genetic factors play a significant role in the immunocompetence of chickens against Newcastle disease;
- Additive gene action is the most important genetic factor for primary immune responses, while both additive and heterosis effects are important for persistence of antibody titres and for secondary immune responses against the disease;
- There are no significant correlations between primary immune responses, persistence of antibody titres, secondary immune responses against NDV vaccine, and body weights.
- Substantial improvement in the immune response status of a stock can be achieved through both conventional selection and crossbreeding.

6.2 Recommendations

- A breeding programme should be instituted to improve genetic resistance against ND in endemic areas such as Tanzania. The most prospective way of going about this matter is to first, screen populations for differences in immunocompetence, followed by selection of the most prospective populations. The next step would be to embark on intensive within line selection for immune response in two to four lines. Crossing among the lines should follow this in order to produce commercial chicks for distribution to farmers;
- The economic benefits of such a breeding venture should be compared with those of conventional ND control methods on short a term, medium term, and long term basis.

7.0 REFERENCES

Ahmed, A.S., Penhale, W. and Talal, N. (1985). Sex hormones, immune response, and autoimmune diseases. *American Journal of Pathology.* 125: 531-551.

Allan, W.H. and Gough, R.E. (1974). A standard haemagglutination inhibition test for Newcastle disease. (2) Vaccination and challenge. *Veterinary Record.* 95: 147-149.

Aslam, S.M., Garlich, J.D. and Qureshi, M.A. (1998). Vitamin D deficiency alters the immune responses of broiler chicks. *Poultry Science.* 77: 842-849.

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

Becker, W.A. (1967). *Manual of quantitative genetics.* Academic Enterprises, Washington. pp 180-200.

Beisel, W.R. (1982). Single nutrient and immunity. *American Journal of Clinical Nutrition.* 35: 412-468.

Beisel, W.R., Edelman, R., Nauss, K. and Suskind, R.M. (1981). Single nutrient effects on immunological functions. *Journal of American Medical Association.* 254: 53-58.

Benedict, A.A., Pollard, L.W., Morrow, P.R., Abplanalp, H.A., Mauer, P.H. and Briles, W.E. (1975). Genetic control of immune responses in chickens.I. Response to ter-polymer of poly ($glu^{60}ala^{30}tyr^{10}$) associated with the major histocompatibility complex. *Immunogenetics.* 2: 313-324.

Bhargava, K.K., Hanson, R.P. and Sunde, M.L. (1970). Effects of methionine and Valine on antibody production in chicks infected with Newcastle disease virus. *Journal of Nutrition.* 100: 241-248.

Blakley, B.R., Sisodia, C.S. and Mukkur, T.K. (1980). The effect of methyl-mercury, tetraethyl lead, and sodium arsenite on the humoral immune response in mice. *Toxicology and Applied Pharmacology.* 52: 245-254.

Blankert, J.J., Albers, G.A.A., Briles, W.E., Vrielink-van Ginkel, M., Groot, A.J.C., Winkel, G.P., Tilanus., M.G.J. and Van der Zijpp, A.J. (1990). The effect of serologically defined major histocompatibility complex haplotypes on Marek's disease resistance in commercially bred White Leghorn chickens. *Avian Diseases.* 34: 818-823.

Bridger, M.A. and Thaxton, P. (1982). Cell-mediated immunity in the chicken as affected by mercury. *Poultry Science.* 61: 2356-2361.

Briles, W.E., McGibbon, W.H. and Irwin, M.R. (1950). On multiple alleles affecting cellular antigens in the chicken. *Genetics.* 35: 633-640.

Bruce, G., Day, E.J. and Doris, T. (1981). Calorie-protein deficiencies and immune response of the chicken. I. Humoral immunity. *Poultry Science.* 60: 2494-2500.

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

Chandra, R.K. (1980). *Immunology of nutritional disorders.* Edwards Arnold Publishers Ltd., 41 Bedford Square, London. UK: pp 1.

Cheng, S. and Lamont, S.J. (1988) Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Science.* 67: 989-995.

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

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

Cole, R.K. (1985). Natural resistance to Marek's disease. In: *International symposium on Marek's disease.* (Edited by Calnek, B.W; Spencer, J.L.). American Association of Avian Pathologist Inc., Kenneth Square. PA. pp 318-329.

Colnago, G.L., Jensen, L.S. and Long, P.L. (1984). Effect of selenium and vitamin E on the development of immunity to Coccidiosis in chickens. *Poultry Science.* 63:1136-1143.

Corrier, D.E. and DeLoach, J.R. (1990). Evaluation of cell mediated , cutaneous basophil hypersensitivity in young chickens by interdigital skin test. *Poultry Science.* 69:403-408.

Corwin, L.M and Shloss, J. (1980). Influence of Vitamin E on the mitogenic response of lymphoid cells. *Journal of Nutrition.* 110:916-923.

Cusumo, V., Costa, G,B. and Seminara, S. (1990). Effect of aflatoxin on rat peritoneal macrophages. *Applied Environmental Microbiology.* 56:3482-3484.

Dieter, R.R., Qureshi, M.A., Nanna, U.C. and Bloom, S.E. (1985). Embryonic exposure to aflatoxin-B1: Mutagenicity and influence on development and immunity. *Environmental Mutagen.* 7: 715-725.

Dunnington, E.A., Larsen, C.T., Gross, W.B. and Siegel, P.B. (1992). Antibody responses to combination of antigens in White Leghorn chickens of different background genomes and major histocompatibility complex genotypes. *Poultry Science* 71:1801.

Edelman, A.S., Sanches, P.L., Robinson, M.E., Hochwald, G.M and Thorbecke. G.J. (1985). Primary and secondary wattle swelling response to phytohegglutinnin as a measure of immunocompetence in chickens. *Avian Diseases.* 30:105-111.

Falconer, D.S. (1989). *Introduction to quantitative genetics.* 3rd ed. John Wiley and Sons Inc., New York, NY. USA. pp 123.

Flicken, M.D., Edwards,J.F. and Lay, C.J. (1986). Induction, collection and spartial characterization of induced respiratory macrophages of the turkey. *Avian Diseases.* 30: 766-771.

Friedman, A. and Sklan, D. (1989). Antigenic-specific immune response impairment in the chick as influenced by dietary vitamin *A. Journal of Nutrition* 119:790-795.

Friedman, A. and Sklan, D. (1995). Effect of dietary fatty acids on antibody production and fatty acid composition of lymphoid organs in broiler chicks. *Poultry Science.* 74: 1463-1469.

Fritsche, K.L., Cassity, N.A. and Huang, S. (1991). Effect of Dietary Fat Source on Antibody Production and Lymphocyte Proliferation in Chickens. *Poultry Science*. 70: 611-617.

Garnett, I. and Roberts, C.W. (1972) The heritability of percent gamma globulin level in the New Hampshire chicks. *Poultry Science*. 51: 1087-1094.

Gavora, J.S. (1990) Disease genetics. In: *Poultry Breeding and Genetics* (Edited by Crowford, R.E) New York, Elsevier.

Gebremichael, A., Levy, E.M. and Corwin, L.M. (1984). Adherent cell requirement for the effective of vitamin E on in vitro antibody synthesis. *Journal of Nutrition* 114: 1297-1305.

Ghosh, R.C., Chauhan, H.V.S. and Jha, G.J. (1991). Suppression of cell mediated immunity by purified aflatoxin B_1 in broiler chicks. *Veterinary Immunology and Immunopathology.* 28:165-172.

Giambrone, J.J. (1981). Laboratory evaluation of the immune response of young broiler chickens vaccinated against Newcastle disease under field conditions. *Poultry Science*. 60: 1204-1208.

Glick, B., Day, E.J. and Thompson, D. (1981). Calorie-Protein deficiencies and the immune response of the chicken I. Humoral immunity. *Poultry science.* 60: 2494-2500.

Gross , W.B. and Siegel, P.B. (1988). Environment-genetic influences on immunocompetence. *Journal of Animal Science*. 66: 2091-2094.

Gross, W.B. and Siegel, P.B. (1980). Effects of early environmental stress on chicken body weight, antibody response to RBC antigens, feed efficiency and response to fasting. *Avian Diseases*. 24:569.

Gross, W.B. and Siegel, P.B. (1982). Socialization as a factor to resistance infection, feed efficiency, and response to antigens in chickens. *American Journal Veterinary Research*. 43: 2010.

Gross, W.B., Domermuth, C,H., and Siegel, P.B. (1988). Genetic and environmental effects on the response of chickens to avian adenovirus group II infection. *Avian Pathology*. 17: 767-774.

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

Grossman, C.J. (1989). Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *Journal of Steroid Biochemistry*. 34: 241-251.

Gunther, E., Balcoravo, J., Hala,K., Rude, E. and Hraba, T. (1974). Evidence for an association between immune responsiveness of chickens to (T,G)-A-L and the major histocompatibility system. *European Journal of Immunology*. 4: 548-553.

Gwakisa, P.S., Katule, A.M. and Rugaimukamu, E. (1994). Divergent immune responses to NDV vaccination of rural chickens in Tanzania. *Tanzania Veterinary journal*. 14: 198-203.

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

Harmon, B.G., Glisson, J.R. and Nunnally, J.C. (1992). Turkey macrophage and heterophil bactericidal activity against *Pasteurella multocida. Avian Diseases.* 36: 986-991.

Hassan, A.B; Atta, H.A; Zohdi, M.S.E. and Afify, N. (1990). Spiramycin adipique-induced immunosuppresion in chickens . *Bulletin of Animal Health.and Production in Africa* 38: 27-31.

Hegazy, S.M., Azzam, A. and Gabal, M.A. (1991). Intercaction of naturally occurring aflatoxins in poultry feed and immunization against fowl cholera. *Poutry Science* 70: 2425-2428.

Heller,D., Soller, M., Peleg, B.A., Ron-Kuper, N. and Hornstein, K (1981) Immune response to NDV vaccine, Fowl Pox Vaccine and Escherichia coli vaccine in Bedouin and white leghorn chickens. *Poultry Science*. 60: 34-37.

Herald, W. (1994). *The tropical Agriculturist. Animal breeding*, 3rd ed. University of Edinburgh, U.K., London. pp 250-370.

Johnston P.V. (1988). Lipid modulation of immune responses.In; *Nutrition and Immunology*. (Edited by Alan R.K.) Liss, Inc, New York, NY.USA. pp 37-86.

Kabasa, J.D; Kugler, W. and Opuda-Asibo, J. (1995). Effects of protein-energy malnutrition on serum immunoglobulins G (IgG) responses of broiler chickens in Uganda. *Bulletin of Animal Health and Production in Africa*. 43:133-141.

Katule, A.M. (1988). Crossbreeding as a tool for genetic improvement of chickens in less optimum environments. In:*Proceeding of the 15th Scientific Conference of the Tanzania Society of Animal Production*, Arusha International Conference Centre, 86-111. Arusha, Tanzania.

Kaufman, J.F. and Lamont, S.J. (1996). The chicken major histocompatibility complex. In: *The Major Histocompatibility Complex in Domestic Animal Species* (Edited by Lamont, S.J. and Schook, L.B.) CRC Press, Boca Raton, FL. USA. pp 35-64.

Kidd, M.T.; Anthony, N.B. and Lee, S.R. (1993). Progeny performance when dams and chicks are fed supplemental zinc. *Poultry Science.* 71:1201-1206.

Klein, J.(1982) *Immunology. The science of Self and Non self Discrimination.* A Wiley – Interscience publication. John Wiley and Sons. pp 1-23.

Lamont, S.J. (1993). The major histocompatibilty complex. In: *Manipulation of the avian Genome*. (Edited by Etches, R.J. and Verrinder, A.M.) CRC Press, Boca Raton, FL. USA. pp 185-103.

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

Lamont, S.J. and Smyth, J.R. Jr (1984) Effect of selection for delayed amelanosis on immune response in chickens. 2. Cell-mediated immunity. *Poultry Science.* 63: 440-442.

Lamont, S.J.; and Dieter, R.R. (1990). Immunogeteitcs. In:*Poultry Breeding and Genetics.* (Crowford, R.) Elsevier, Amsterdam, The Netherlands. pp 497-541.

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

Lanciotti, E., Comodo, N., Gamabssini, L., Cerbi, E., Vallone, G., Griffini, E. and Mugelli, A. (1989). Survey of Barium in Italian drinking water supplies. *Bulletin of Environmental Contamination and Toxicolology*. 43: 833-837.

Leitner, G., Gutman, M., Heller, E.D., Yonash, N. and Cahaner, A. (1994) Parental effect on the Humoral immune response to Escherichia coli and Newcastle Disease Virus in young Broiler chicks. *Poultry Science*. 73: 1534-1541.

Leitner, G.Z., Uni, Z., Cahaner, A., Gutman, M. and Heller, E.D. (1992). Replicated divergent selection of broiler chickens for high or low early antibody response to *Escherichia coli* vaccination. *Poultry Science*. 71:27-37.

Leitner, G., Uni, Z., Cahaner, A., Gutman, M. and Heller, E.D. (1992). Replicated divergent selection of broiler chickens for high or low early antibody response to Escherichia coli vaccination. *Poultry Science*. 71:27-37.

Lillehoj, H.S; Ruff, M.D; Bacon, L.D; Lamont, S. and Jeffers, T. (1986). Disease susceptibility to coccidial infection in congenic inbred strains of chickens. *Poultry Science*. 65 (Suppl. 1):80. (Abstr.).

Lillehoj, H.S., Ruff, M.D., Bacon, L.D.,Lamont, S. and Jeffers, T. (1986). 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.

Loudovaris, T.; Brandon, M.R. and Fahey, K.J. (1990). The major histocompatibility complex and genetic control of antibody response to sheep red blood cells in chickens. *Avian Pathology.* 19: 89-99.

Marsh, J.A., Dieter, R.R. and Combs, G.F. (1981). Influence of dietary selenium and vitamin E on humoral immune response of the chick. *Proceedings of Society of Experimental Biology and Medicine.* 66: 228-236.

Melewas, J.N. (1989) Contribution of poultry industry to the National Economy. In: *Proceeding of the 7th TVA scientific conference. Tanzania Veterinary association. December, 1989, Arusha, Tanzania* 7, 13-36.

Miller, L. and Qureshi, M.A. (1991). Induction of heat shock proteins and phagocytic function of chicken macrophage following *in vivo* heat exposure. *Veterinary. Immunology and Immunopathology.* 30:179-191.

Minga, U.M., Katule, A., Maeda, T. and Musasa, J (1989) Potential and problems of the traditional chicken industry in Tanzania. In: *Proceeding of the 7th TVA scientific conference. Tanzania Veterinary association. December, 1989, Arusha, Tanzania* 7, 207-215.

Nestor, K.E., Saif, Y.M., Zhu, J. and Noble, D.O (1996). Genetics of disease resistance in Turkeys. *Research and reviews.* Pp 203-207.

Nordskog, A.W., Pevzner, I. Y. and Lamont, S.J. (1987). Subregions and functions of the chicken major histocomaptibilty complex. A review. *Poultry Science.* 66:790-794.

Pardue, S.L. and Thaxton, J.P. (1984). Evidence for ameriolation of steroid-mediated immunosuppresion by ascorbic acid. *Poultry Science*. 63:1262-1268.

Pardue, S.L. and Thaxton, J.P.(1984). Evidence of ameriolation of steroid-mediated immunosuppresion by ascorbic acid. *Poultry Science*. 63: 1262-1268.

Peleg, B.A., Soller, M., Ron, N., Hornstein, K., Brody, T. and Kalmer, E. (1976) familial differences in antibody response in broiler chickens to vaccination with attenuated and inactivated NDV vaccine. *Avian Diseases*. 20: 661-668.

Pevzner, I.V., Nordskog, A.W. and Kaeberle, (1975). Immune response and the B blood group locus in chickens. *Genetics* 80: 753- 759.

Pevzner, I.V.; Trowbridge, C.L. and Nordskog, A.W. (1979). B-complex genetic control of immune response to GAT, (T,G)-A-L, GT and other substances in chickens. *Journal of Immunogenetics*. 37: 381-385.

Pevzner, I.Y., Trowbridge, C.L., Nordskog, A.W and Crittenden, L.B (1978). Mortality differences associated with divergent selection for immune response to *salmonella pullorum* with B blood group genotype in chickens. *Poultry Science*. 57: 1180.

Pevzner, I.Y., Stone, H.A. and Nordskog, A.W (1981b) Immune response and disease resistance in chickens. 1. selection for high and low titre to *Salmonella pullorum* antigen. *Poultry Science*. 60: 920-926.

Pimental, J.L. and Cook, M.E. (1987). Suppressed humoral immunity in chicks fed diets deficient in Sodium Chloride, or both Sodium and Chloride. *Poultry Science*. 66:2005-2010.

Pinard, M.H; Janss, L.L.G; Maatman, R; Noordhuizen, J.P.T.M. and Van der Zijpp, A.J. (1993). Effect of divergen selection for immune responsiveness and major histocompatibility complex on resistance to Marek's disease in chicikens. *Poultry Science*. 72: 391-402.

Pitcovski, J., Heller, E.D., Cahaner, A. and Peleg, B.A. (1987) selection for early responsiveness of chicks to Escherichia coli and New castle disease virus. *Poultry Science*. 66:1276-1282.

Potchinsky, M.A. and Bloom, S.E. (1993). Selective aflatoxin B1-induced sister chromatid exchanges and cytotoxicity in differentiating B and T lymphocytes *in vivo*. *Environmental Molecular. Mutagen*. 21:87-94.

Qureshi, M.A; Dieter, R.R. and Bacon, L.D. (1986). Genetic variation in the recruitment and activation of chicken peritoneal macrophages. *Proceedings of Society of Experimental Biology and Medicine*. 181: 560-568.

Qureshi, M.A; Hussain, I. and Heggen, C.L. (1998). Understanding immunology in disease development and control. A review. *Poultry science*. 77:1126-1129.

Raisuddin, K;Singh, P;Zaidi, S.I.A; Saxena, A.K. and Ray, P.K. (1990). Effects of aflatoxin on lymphoid cells of weaning rats. *Journal of Applied Toxicology*. 10: 245-250.

Rose, N.R. (1994). Avian models of autoimmune disease: lessons from the birds. *Poultry Science*. 73:984-990.

Sacco, R.E, Nestor., K.E, Saif., Y.M., Tsai, H.J., Anthony, N.B. and Patterson, R.A. (1994). Genetic analysis of of antibody response of turkeys to Newcastle Disease virus and *Pasteurelle multocida* Vaccines. *Poultry Science.* 73:1169-1174.

Sant'anna, O.A., Bouthillier, Y., Siqueira, M. and Biozzi, G. (1979). Differences in genetic control of primary and secondary antibody responses. *Immunology.* 37:849-856.

Sarker,N., Tsudzuki, M., Nishibori, M. and Yamamoto, Y. (1999). Direct and correlated response to divergent selection for serum Immunoglobulin M and G levels in chickens. *Poultry Science.* 78:1-7.

SAS (1995). Statistical analysis system (SAS) users guide. Version 6.12. Statistical Analysis Institute, Inc, Carry, NC. USA.

Saxena, V.K., Sigh, H., Pal, S.K. and Kumar, S (1997). Genetic studies on primary antibody response to sheep erythrocytes in guinea fowls. *British Poultry Science.* 38: 2, 156-158.

Schat, K.A. (1994). Cell mediated immune effector functions in chickens. *Poultry Science.* 66: 812-818.

Schumacher, M., Bosque, M.A., Domingo,J.L. and Corbella, J. (1990). Lead and Cadmium concentreations in marine organisms from the terregona coastal water, Spain. *Bulletin of Environmental Contamination and. Toxicology.* 44:784-789.

Schwartz, L.D. (1994). *Poultry health handbook.* University Park, PA, Pennsylvania State College of Agriculture. USA.pp 214.

Sharaf, M.M., Nestor, K.E., Saif, Y.M., Sacco, R.E and Havenstein, G.B (1988). Antibody response to Newcastle disease virus and *Pasteurella Multocida* of two strains of turkeys. *Poultry Science*. 67:1372-1377.

Siegel, P.B and Gross, W.B (1980) Production and persistence of antibodies in chickens to sheep erythrocytes, I. Directional selection. *Poultry Science*. 59:1-5.

Soller, M., Heller, D., Peleg. B., Ron-Kuper, N. and Hornstein, K. (1981). Genetic and phenotypic correlations between immune response to Escherichia coli and to Newcastle disease virus vaccines. *Poultry Science*. 60:49-53.

Srikanth, M., Madhumohan, A.R., Shravan, C.K. and Khanum, A .(1993). Lead, Cadmium, Nickel and Zinc contamination of ground water around Hussain sagar lake, Hyderabad, *Indian. Bulletin Environmental Contamination and Toxicology* 50:138-143.

Takahashi, S., Inooka, S. and Mizuma,Y. (1984) Selective breeding for high and low antibody response to inactivated Newcastle disease virus in Japanese Quails. *Poultry Science*. 63: 595-599.

Taylor, R.L; Ewert, J; England, M. and Halpern, M..S. (1992). Major histocompatibility (B) complex control of growth pattern of *v-scr* DNA-induced primary tumours. *Virology*. 191:477-479.

Tengerdy, R.P. and Brown, J.C (1977). Effect of vitamins E and A on humoral immunity and phagocytosis in *E.Coli* infected chickens. *Poultry Sci*. 56:957-963.

Tengerdy, R.P. and Nockel, C.F. (1975). Vitamin E or Vitamin A protects chickens against E.coli infection. *Poultry Science*. 54:1292-1296.

Tsai, H.J., Saif,M.Y., Nestor, K.E., Emmerson, D.A. and Patterson, R.A. (1992). Genetic variation in resistence of turkeys to experimental infection with Newcastle Disease virus. *Avian Diseases* 36: 561-565.

Tsiagbe, V.K., Cook, M.E., Harper, A.E. and Sunde, M.L. (1987). Efficacy of cysteine in replacing methionine in the immune responses of broiler chicks. *Poultry Science*. 66:1138-1146.

Vaiano, O. and Toivanen, A. (1987). Cellular cooperation in immunity. In: *Avian immunology. Basis and Practice*. (Edited by Toivanen, A. and Toivanen, P.) CRC Press, Boca Raton, FL .USA. 2:1-12

Vaiano, O.; Toivanen, P. and Toivanen, A. (1987). Major histocompatibility complex and cell cooperation. *Poultry Science*. 66: 795-801.

Van der Zijpp, A.J. (1983) The effect of genetic origin, source of antigen and dose of antigen on the immune response of Cockreals, *Poultry Science*. 62: 205-211.

Van der Zijpp, A.J. (1984). Breeding for immune responsiveness and disease resistance in poutry. In: *Proccedings of 33rd Annual National Poultry Breeders Roundtable*, St. Louis, Amsterdam, The Netherlands. pp 1-11.

Vodela, J.K; Renden, J.A., Lenz, S.D., Mcelhenney, W.H. and Kemppainen, B.W. (1997).Drinking water contaminants (Arsenic, Cadmium, Lead, Benzene, and Trichloroethylene). 1. Interaction of contaminants with nutritional status on general performance and immune function in broiler chickens. *Poultry Science*. 76:1474-1492.

Weinstock, D. and Schat, K.A (1987). Virus specific syngeneic killing of reticuloendotheliosis virus transformed cell line target cells by spleen cells.In: *Progress in Clinical and Biological Research , Avian Immunology*. 238:258-263.

White, E.C; Briles, W.C; Briles, R.W. and Taylor, R.L. (1994). Response of six major histocompatibility (B) complex recombinant haplotypes to rous sarcoma. *Poultry Science*. 73:836-842.

Yonash, N., Leitner, G., Waiman, R; Heller, E.D. and Cahaner, A. (1996). Genetic differences and heritability of antibody response to Escherichia coli vaccination in young meat type chicks. *Poultry Science*. 75:683-690.

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

Yoo, B.H. and Sheldon, B.L. (1990). Association of the major histocompatibility complex with Avian Leucosis Virus infection in chickens. *British poultry Science*. 33:613-620.

APPENDICES

Appendix Table 1a. Analysis of variance for primary response (i.e. antibody titres after the first ND vaccination).

Source	DF	Significance[1]
Sex	1	ns
Genotype	3	**
Genotype x Sex interaction	3	ns
Sires within genotype	36	ns
Residual	93	

Appendix Table 1b. Analysis of variance for residual titre (i.e. antibody titres prior to the second ND vaccination).

Source	DF	Significance[1]
Primary titre	1	ns
Sex	1	ns
Genotype	3	***
Genotype x Sex interaction	3	ns
Sires within genotype	36	ns
Residual	86	

Appendix Table 1c. Analysis of variance for secondary response (i.e. antibody titres after the second ND vaccination).

Source	DF	Significance[1]
Primary titre	1	ns
Residual titre	1	ns
Sex	1	ns
Genotype	3	***
Genotype x Sex interaction	3	ns
Sires within genotype	36	ns
Residual	84	

1 *** = very highly significant ($P < 0.001$)

** = highly significant ($P < 0.01$)

* = significant ($P < 0.05$)

ns = non-significant ($P > 0.05$)

Appendix Table 2. Components of variance for antibody titres at different stages of vaccination.

Genotype	Vaccination phase	Variance components			
		Between sires (σ_s^2)		Within sires (σ_e^2)	
		Value	%	Value	%
Local	Primary	0.154	9.0	1.552	91.0
	Residual (intervaccinations)	0.038	6.1	0.580	93.9
	Secondary	0.065	6.6	0.923	93.4
Local x Exotic	Primary	0.121	9.6	1.146	90.4
	Residual (intervaccinations)	0.082	8.0	0.942	92.0
	Secondary	0.086	7.0	1.134	93.0
Exotic x Local	Primary	0.204	9.8	1.872	90.2
	Residual (intervaccinations)	0.076	7.0	1.009	93.0
	Secondary	0.109	5.6	1.831	94.4
Exotic	Primary	0.136	6.8	1.880	93.2
	Residual (intervaccinations)	0.038	4.4	0.820	95.6
	Secondary	0.031	4.6	0.650	95.4
Pooled	Primary	0.140	7.6	1.695	92.4
	Residual (intervaccinations)	0.049	5.4	0.864	94.6
	Secondary	0.071	6.0	1.114	94.0