PERFORMANCE OF THREE NIGERIAN INDIGENOUS CHICKEN STRAINS VACCINATED AGAINST NEWCASTLE DISEASE *IN OVO* AND POST-HATCH

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DEDICATION

To the glory of God Almighty

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ABSTRACT

Indigenous Chickens (IC) population in Nigeria is threatened due to high Mortality Rate (MR) from Viral Diseases (VD). Vaccination is currently the most effective preventive measure against VD such as the Newcastle Disease (ND). Vaccination during embryonation could help to mitigate susceptibility to ND post-hatch. Information on *in ovo* and post-hatch vaccinations of IC in Nigeria is scanty. Therefore, performance of three strains of IC vaccinated against ND *in ovo* and post-hatch were investigated.

Parent stocks of IC comprising five cocks and 25 hens each of Normal Feathered (NF), Frizzle Feathered (FF) and Naked Neck (NN) strains were obtained from a reputable source to produce Hatchable Eggs (HE). Two hundred and forty HE of each of NF, FF and NN chickens were incubated for 18 days. At day 18, HE were candled and 60 Fertilised Eggs (FE) from each strain were drilled and injected with 0.2 mL Injection Water-IW ($T1_1$) or B1 Lentogenic Strain-B1LS ($T1_2$) ND vaccine using *in ovo* procedures. At hatch, 10 chicks (r=6) each from the strains were injected subcutaneously using 0.2 mL IW ($T2_1$) or B1-LS ($T2_2$) in a randomised complete block design. On days 1, 21, 42, 63 and 84, blood (2 mL) was sampled (r=5) for Geometric Mean Antibody Titre (GMAT), and at week 16, blood (5 mL) was sampled (r=5) for haematological profile using standard procedures. Body Weight Gain (BWG), mortality and hen-day production were recorded during early (22-42 weeks), mid (43-59 weeks) and late (60-72 weeks) phases of egg production. Data were analysed using ANOVA at $\alpha_{0.05}$

At days 21, 42 and 84, GMAT of birds vaccinated *in ovo* ($T1_2$) and post-hatch ($T2_2$) and their controls ($T1_1$ and $T2_1$, respectively) were similar. At days 1 and 63, GMAT differed significantly across all treatments and were 3.2±0.1 and 3.3±0.2 ($T1_1$), 4.0±0.1 and 6.0±0.2 ($T1_2$), 2.0±0.1 and 3.1±0.2 ($T2_1$), 2.5±0.1 and 4.5±0.2 ($T2_2$), respectively. At day 63, GMAT was significantly higher in FF (4.8±0.2) than NF (4.4±0.2) and NN (3.6±0.2), while at day 84, GMAT in FF (3.2±0.1) was similar to NF (3.0±0.1) but significantly higher than NN (2.4±0.1). Monocytes and lymphocytes in $T1_2$ (3.41±0.03%, 64.1±0.1%) and $T2_2$ (3.36±0.03%, 64.2±0.1%) were significantly higher than in $T1_1$ (3.31±0.03%, 63.2±0.1%) and $T2_1$ (3.28±0.03%, 63.1±0.1%, respectively). Similarly, heterophil and white blood cell count ($x10^3/mm^3$) in $T1_2$ (29.6±0.1%, 3.47±0.02) and $T2_2$ (29.5±0.1%, 3.47±0.02) were significantly higher than in $T1_1$ (29.2±0.1%, 3.41±0.02) and $T2_1$ (29.1±0.1%, 3.42±0.02, respectively). The B1-LS had no effect on BWG, while it significantly reduced mortality from 2.8±0.0% ($T1_1$ and $T2_1$) to 1.8±0.0% ($T1_2$ and $T2_2$). Hen-day production was significantly improved by vaccination and ranged from 53.63±0.00% ($T1_1$) to 54.62±0.00% ($T2_2$), 68.56±0.00% ($T1_1$) to 69.63±0.00% ($T2_2$), and 50.48±0.00% ($T1_1$) to 51.43±0.00% ($T2_2$) for early, mid and late laying phases, respectively.

In ovo vaccination against Newcastle disease was superior to post-hatch vaccination. Hen-day production was enhanced and mortality was reduced using the B1 Lentogenic strain vaccine in the Nigerian Indigenous Chicken strains for both *in ovo* and post-hatch vaccinations.

Keywords: Indigenous chicken, Newcastle disease, Geometric mean antibody titre, Chicken vaccination, Lentogenic strain vaccine.

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CHAPTER ONE

1.0 Introduction

Native fowls are mainly found in the non-urban areas of the tropics where they are reared by a large percentage of the non-urban dwellers. Native fowls of Africa are generally strong, blending with ever changing weather conditions. Native fowl accounts for about 96 million of over 120 million fowl population reared in the non-urban communities of Nigeria (RIM, 1992).

They have traits such as the capacity to incubate, produce and nurse chicks on their own, and scavenge for significant parts of their feed and possession of appreciable level of protection from endemic diseases. The meat and eggs are readily available to non-urban dwellers and the literates, hence serving as good sources of protein and income. (Horst, 1989)

The native fowls symbolize important assets for small-holders livestock farmers and can be nurtured under different ecological circumstances presenting a variety of goods and purposes. Thus, huge genetic assets stored in the native fowls are awaiting full utilization that are foundation for genetic enhancement and development to generate strains and genotypes that are unique to local environments for utilization by producers in a growing economy.

In Nigeria, native fowls were classified along hereditary lines of colour of plumage and feather (normal feather or frizzle feathered), body structure (naked neck, dwarf types and colour variations (brown, white, black, spotted etc.).

Frequency of distribution of the normal feathered fowls was close to 91.8% while frizzle feathered and naked neck fowls were 5.20 and 3.00% respectively in Bayelsa State of South-South Nigeria (Ajayi and Agaviezor, 2009).

Native fowls are again classified in line of geographical locations. Researchers have categorized various ecotypes of the native fowls according to Nigeria's varied agro-ecological regions. Many of the differentiations by the varied agro-ecological regions

evaluated majorly the normal feathered native fowls since they are the most famous while the naked neck and frizzle feathered are uncommon and nearly becoming endangered and the genetic material they stand for may disappear if not differentiated and preserved.

Olori (1992) observed 2 different Ecological-Types (ET) classified as the savannah and rainforest or Fulani and Yoruba ET, respectively. Olori (1992) equally gave differences in several attributes of the native fowls from the Southern part of Nigeria which were identified to vary from those of other regions of the Nigeria.

Nigerian native chickens belong to the light breed, with single comb, black and mottling from brown plumage laced with different colours. Recently, the various ecotypes have been categorized into 2 major groups on the ground of life weight and heaviness as weighty ET and small ET (Momoh *et al*., 2007).

The weighty ET (Fulani ET) is located in the drier Savannahs (Sahel and Guinea), Montane vegetations and Kraals of cattle in Northern Nigeria and weigh between 0.90 to 2.50 kg when they mature.

The small ET (Yoruba ecotypes) are the native fowls from the Rain-forest and Derived savannah regions with a body-weight ranging between 0.68 to 1.50 kg at maturity.

1.0.1 *In-ovo* immunization

In-ovo immunization is a substitute approach to post-hatch immunization of poultry birds, when chicks are vaccinated at the embryo stage. Embryo vaccination (day18) assists in closing the windows of vulnerability and immune defenselessness Parmentier., *et al.* (1996). *In-ovo* immunization facilitates both the inherent and non-inherent immune-reactions with the advantages of protection that follows as a result of prenatal vaccination.

1.0.2 Immunity

Poultry birds, like all other animals, have very tough, in-built defenses (immunity) against diseases as a result of invasion of the body by various microbes and toxins (jointly called ‘antigens’). These defenses include:

- **The body surface (skin):** The body surface is a barrier that protects the body against invasion from microorganisms. Invasion through the skin barrier occurs when the body surface is broken.
- **Mucous membranes:** These are special linings of the digestive and respiratory tracts and other systems of the body which provide a good barrier against invasion by microbes. If anything should damage the mucous membranes invasion occurs. A very good example is when diets that lack vitamin A cause damage to the mucous membranes which results in a rise in the out-break of contagious diseases.
- **The defense system:** In spite of the competence of the body surface and the mucous membranes as defense mechanisms, microbes usually find their ways into the body. Although, many of these do not cause any harm, while others cause various infections, usually specific infections are caused by specific microbes.

The recognition of microbes and substances that are considered foreign (antigens), which entered the body are the primary responsibility of the defense system. The defense system initiates and manages the appropriate physiological reactions to neutralize and/or remove these foreign agents and substances.

Different mechanisms are employed towards achieving these goals, including inactivation of foreign agents, lyses (bursting) of foreign bodies, agglutination/clumping or precipitation of cells, or phagocytosis (engulfing and inactivating) of biological agents (Davison *et al.,* 1996).

For a very effective defense response, a correct mechanism, or a combination of mechanisms, must be activated. On the other hand, for each animal species there are several diseases for which the term ‘protection’ does not come into play. Moreover, at times, these usually defensive responses can result in a considerable tissue damage, which normally results into immune-mediated infections.

The defense system is a greatly complicated physiological system which is yet to be comprehensively understood. New findings are still on-going regarding the function of various organs and physiological compounds in defense responses. Hence, this section

contains only a short and straightforward explanation of the major components of the defense system and the defense system responses.

Development of a disease by a bird after such an invasion from a pathogenic agent depends on the following factors:

1. Health status of the bird , that is, state of well-being and level of protection
2. Amount of invading pathogenic microbes, called 'the state of being challenged'
3. Virulence/Potency or Strength of the invading pathogenic microbes.

1.1 Diseases of Poultry

A disease represents a departure from the regular psychological and anatomical growth of an animal that could be as a result of pathogenic agents or contaminated environment.

Smith, (1990) showed that the majority of important health related infections are caused by respiratory diseases, salmonellosis, infection of the yolk sacs and Myco-toxicosis,

There are so many diseases, which affect poultry birds, which may result in death or poor performance level. These include: Coccidiosis, Chronic Respiratory Disease, Newcastle disease, Fowl cholera, Infectious bronchitis, Salmonella, Fowl Pox, etc

1.2 Newcastle Disease

Newcastle disease virus (NDV) normally results into one of the mainly essential contagious poultry diseases. Newcastle disease (ND), which is global in nature, usually results into huge financial losses resulting from death and disapproval of carcasses.

In 2010, about 70 states recorded ND in household stocks to the World Animal Health Organization (OIE) and several states have prevalent NDV, with disease incidence happening almost every year. Otherwise called Avian Paramyxovirus Serotype1 (APMV-1) virus, genus: Abulavirus and family: Paramyxoviridae (Mayo, 2002).

Newcastle disease can be prevented through the use of vaccines. Several Newcastle disease vaccines which are suitable for use in commercial flocks are available. They

are available even on the Global markets. The 1-2 Newcastle disease vaccine has been produced for non-urban or regional use in controlling Newcastle disease in rural community flocks.

Several Newcastle disease vaccines depreciate after storage for 1 or 2 hours at room temperature, and this renders them unfit for utilization in rural communities where the vaccine requires transportation for hours or at times days at ambient temperature.

The 1-2 Newcastle disease vaccine is more vigorous and is called a thermo stable vaccine. Thermo stable vaccines still need lasting storage space in a cold chain. Nevertheless, while transporting the vaccine to the farm, the vaccine will not depreciate as fast as the local vaccines.

Provision of evaporative cooling through the packaging of vaccine in a wet clothing will assist in the maintenance of the viability of the vaccine at period of movement to local places.

A NDV strains show protective similarities and an isolate will cross-protect against an enchanter with another isolate of NDV. It is this stimulation which is immunological in nature serving as the starting point of immunization using live less potent NDV (leNDV) to offer protection against virulent NDV (vNDV). From the genetic point of view, there are various types of ND viruses and with no fewer than 16 various genotypes are established (Diel *et al.*, 2012).

Earlier research works did show antigenic dissimilarities among various types of NDV by means of virus neutralization analysis, haemagglutination inhibition (HI) tests with monoclonal immunoglobulins, and also through the evaluation of sequences of neutralizing epitopes (Panshin *et al.*, 2002).

1.3 Justifications of the Study

- With the ongoing improvement programs on the local chicken as a promising poultry resource, a thorough knowledge of the defense status and production potentials of these fowls is necessary
- The prevalence of a rapid reduction in the total number of several local breeds and strains of fowls, some of which are at the verge of disappearance. Thereby, demanding for a resolution of improved management

- Development of early immune-competence and high disease resistance is key to modern intensive poultry production systems and high profitability

1.4 General objective:

To establish a better management approach on the Nigerian indigenous chickens so as to improve their survival rate against Newcastle Disease and equally enhance their production potentials

1.5 Specific objectives:

- To determine the antibody titre levels of *in ovo* and post-hatch vaccinates of three Nigerian Indigenous Chicken Genotypes (NICG)
- To evaluate the differential haematological and serological profiles among the NICG
- To evaluate growth performances in the NICG
- To evaluate the Hen-day production, internal and external egg qualities of the NICG

CHAPTER TWO

2.0 REVIEW OF LITERATURES

2.1 Haematology

Haematology is important in the movement of nutrients, metabolites and gaseous materials all round the body as a complete system (Zhou *et al.,* 1999). Additionally, the blood presents an opportunity for the assessment of the medical and dietary condition of animal species.

Haemato-biochemical examinations are frequently imbibed in dietary trials for fowls and some poultry species. The Full Blood Count (FBC) addresses in general the cellular aspects of the blood while bio-chemical assays address its chemical components (Zhou *et al.,* 1999).

Reports from blood tests can be utilized towards improving poultry birds (Ladokun *et al.,* 2008). Furthermore, blood indices assist in diagnosis of certain poultry diseases and may serve as fundamental knowledge for researches in defense mechanism and poultry pathology (Elagib and Ahmed, 2004).

Researches on Thai local fowls (Simaraks *et al.,* 2005), Kashmir's naked neck local fowls (Pampori and Iqbal, 2007) and laying fowls (Mohammed, *et al.,* 2005) indicated that haemato-biochemical conditions of fowls are correlated with so many factors such as sex, nutrition, raising/brooding temperature, population per unit area and other stress situations.

Other works showed that protein in the serum may be utilized as a form of non-direct measurement of nutritional quality of protein, while considerable drop in white and red corpuscles of blood suggests haemolytic-anaemia and this makes the fowls to be prone to deadly infection (Elagib and Ahmed, 2004).

Elagib and Ahmed, (2004) have reported differences in haemato-biochemical details in fowls of similar age and gender, and raised in similar environmental circumstances but examined at varied periods of the day, as a result of variations in daily physical activities and rates of metabolism (Islam *et al.* 2004), substitution of feed lots

(Adeyemo *et al.,* 2012), water pollution and breeds from different ecological zones (Elagib and Ahmed, 2004).

Recently, Ali *et al.,* (2011) established that serum cholesterol and serum lipids were notably decreased in post-hatch broiler birds.

Towards creating a fundamental data-base on haemato-biochemical details of breeds of fowl of Rajshahi, some important blood parameters of a native, 4 foreign and one crossbred fowl have been determined. Current results would give assistance in the assessment of diseases of poultry, denoting non-sick fowls, improvement on breeds that are desirable and formulating suitable breeding plans for the country's poultry population.

Haematological indices provide very important database on the conditions of immune systems of animals, and such database, in addition to management and diagnostic reasons would assist breeding schemes to improve the hereditary potentials of poultry birds (Elagib and Ahmed, 2004).

2.2 Defense Mechanisms of Birds

Vertebrates, including the native fowls, have two basic kinds of defense mechanisms against disease causing organisms. :

1. Inherent (non-specific) protection comprises a wide-range of protection methods: (a) physical and biochemical blockages planned to avoid attack by the disease causing and non- disease causing factors (antigens), and

(b) soluble and cellular apparatus which have the capacity of removing disease causing agents (antigens) which have effectively penetrated into the cells of the body (Davison *et al.,*1996).

The cellular apparatus of the inherent defense mechanisms are: monocytes/ macrophages, heterophils (neutrophils in animals that suckle their young ones), natural killer cells, eosinophils and basophils. In many cases, inherent defense mechanism is very efficient in destroying disease causing agents.

However, when inherent defense mechanism is not efficient, it becomes crucial to exclusively center immune action on the disease causing agents. At this time, the non - inherent defense mechanism is put into action.

2. The non-inherent (specific) defense mechanism comprises two categories of immunity, and these include:
(a) humoral protection, that is executed by antibodies, and
(b) cell-mediated protection, referring principally to actions of T-cell.
The cellular aspect of non-inherent defense mechanism is regarded to as Lymphocytes.

Lymphocytes are of diverse types and all the types are morphologically impossible to differentiate but vary in terms of location of maturity, tissue position, outward appearance of cell surface molecules, and functional capabilities (Davison *et al.,* 1996).
Bursa of fabricius is responsible for build-up of B cells that fowls' lymphocytes comprise while in the thymus, T-cells build up**.** The T and B cells convey foreign substances receivers on their surfaces.
Each T and each B cells have an identical array of antigen receptors which are definite for specific antigens. Jointly, the T and B cell section each possesses a stock of close to 10^9 various foreign substances-specifications, (Higgins, 1996).

At the initial meeting with a disease causing agent, for instance a pathogen "A", there are moderately a small number of B and T cells carrying receptors that are definite for such antigen.
Prior to disease causing agent "A" being removed by definite defense apparatus, B and T cells carrying disease causing agent "A" definite receptors will multiply and separate into effector cells (e.g. plasma cells generating antibody).
Even though, multiplication and delineation seize a lot of time, giving disease causing agent "A" the chance to trigger ailment (Rataliffe *et al.,* 1996).

In place of effector cells, cells identifying disease causing agent "A" can also delineate to form prolonged, fast-acting recall cells. Therefore during a B or T cell reaction to disease causing agent "A", the number of disease causing agent "A" definite cells improves, releasing huge figures of disease causing agent "A" definite reacting and recall cells.
The reacting cells take part in the removal of disease causing agent "A", while the recall cells are put sideways and ready to efficiently react to pathogen "A" upon a recurrence, prior to pathogen "A" causing infection. However, disease causing agent

"A" - definite recall cells will not offer protection to any victim from another disease causing agent (e.g. disease causing agent "B").

The conception of increasing the band of antigen-definite cells and the production of antigen-definite recall cells is frankly imbibed in immunization programs, wherein a non-pathogenic (a non-disease causing) form of a pathogen is administered into a victim's defense system.

The defense system will build up a reaction to definite apparatus of the disease causing agent as enumerated above. During a future contact with the disease-laiden state of disease causing agent, the defense mechanism is prepared to react and get rid of the disease causing agent prior to it causing infection (Rataliffe *et al.,* 1996).

2.3 Geometric Mean Antibody Titer (GMAT)

Apart from information on the defense mechanism in laying birds, definite information which addresses the defense mechanism and function in broilers and indigenous chickens is scarce.

Research findings on profitable egg-producing birds may have been supported, partly, by the comparatively long-term stay of laying birds, demanding special health care from a day old throughout the period of production of eggs.

Marketable broilers, in contrast, are often sold off prior to age of seven weeks. The little span of these marketable broilers has, to a great level, deprived interest in researching defense mechanism enlargement and its role in the egg-producing fowls.

Assessment of non-specific resistance in the 1957 Athens-Canadian broiler strain and the 1991 Arbor Acres broiler strain has been documented (Qureshi and Havenstein, 1994).

Assessment of macrophage role and natural killer cell action showed slight or no difference between the 1957 and 1991 stocks of fowls, indicating that choosing/picking on genetic basis for improved performance on growth characters did not significantly affect non-specific aspect of defense mechanism in broilers.

However, when the 1957 and 1991 fowls were introduced with the Sheep Red Blood Cells at age 2 weeks and evaluated for anti Sheep Red Blood Cells Geometric Mean Antibody Titer (GMAT) after day 7 , the 1957 stocks recorded a significantly elevated GMAT of anti Sheep Red Blood Cells (IgM and Ig) than 1991 stocks. After fourteen

days of SRBC administration, stock variations in anti-SRBC GMAT values were no more noticeable.

The decrease in response to a T-reliant foreign substance in 1991 population of broiler in comparison to 1957 population appeared to be a straight impact of choosing/picking on gene basis for improved performance on growth characteristics (Qureshi and Havenstein, 1994).

The drop in humoral defense role on live-ability of broilers is important, because two to three-weeks-old chicks are not completely protected and again are also not protected from maternal antibodies (Peleg *et al.,* 1985).

In contrary, Praharaj *et al.,* (1995) reiterated that strains of meat-type fowls that have been specifically chosen on the ground of improved body-weight gains for more than 36 generations when compared to the modern day broiler were identified to have a better ability to generate anti-SRBC GMAT.

The integration of immune competence as one of the criteria for selection within a breeding scheme for the enhancement of performance on growth characters consequently appeared to come up with effects that are beneficial.

In addition, the choosing of broiler fowls near the beginning of defense responsiveness to *Escherichia coli* appeared to give a general boost in near the beginning defense mechanism maturation, and these include: 1) superior humoral defense reactions to other T-reliant foreign substances (e.g. bovine serum albumin or SRBC), 2) superior T cell *in-vitro* propagation in reaction to *Escherichia coli* or mitogenic T cells, and 3) better phagocytic action (Heller *et al.,* 1992).

2.4 B cells and antibodies

The B-cell normally originates from the bursal of fabricius, the B cell foreign substances binder (B cell receptor or BCR) comprises foreign substances definite membrane-attached immune-globulin and BCR-associated signal proteins.

While a B cell encounters the disease-causing agent (antigen) with a definite BCR, it can get attached to the disease-causing agent via the BCR.

This attachment of the disease-causing agent to the BCR provides an initial indication for B-cell initiation. For the majority of B cell - causative agents interrelations, the B-cell needs a second indication from T-cells, like soluble materials or via express T cell-B cell link, prior to it being completely initiated.
Once initiated, the B cell replicates and undergoes differentiation into either recall cells or antibody-generating plasma cells. Any B-cell response that needs a subsequent indication from T cells is regarded as a T dependent response.

Certain antigens, or the so-called T-self-regulating antigens, are capable of activating B-cells that lack any supplementary support from T-cells (T- self-regulating response) (Davison *et al.,* 1996).

Antibodies, sooner than B cell in particular, are the real executors of the humoral protection reaction. As a result of their capacity to exclusively interrelate with antigen, antibodies can significantly improve the efficacy of non-specific defense components. For instance, antibodies enhance the capability of phagocytic cells to arrest and eradicate causative agents, set the balance mechanism in motion, and also stop antigens from getting attached to and infecting the cells.
In poultry birds, 3 categories of antibody factors (immunoglobulins; Ig) have been established. These are: Ig-M, Ig-G (Ig-Y), and Ig-A.

Various categories of antibodies prevail and they depend on the kind and phase of a humoral defense reaction (Davison *et al.,* 1996). The greater part of antibodies during a main defense reaction is of the class Ig-M. A switch from Ig-M to Ig-Y or Ig-A can be seen at the tail part of a main defense reaction, though, Ig class switch to Ig-Y or Ig-A is most obvious during a succeeding encounter to a similar disease causing agent.

In addition, diverse classes of antibodies demonstrate varied functional capabilities. Mentioning just a few, Ig-M possesses the capability of easy agglutination of large causative agents and causing the settling down of soluble causative agents, hence significantly improving the defense mechanism's capacity to get rid of causative agents via phagocytosis. Ig-A is instituted in discharges and acts at mucosal surfaces, and Ig-Y can be assigned through peripheral transportation mechanism of hens into the eggs in the form of maternal antibody, (Davison *et al.,* 1996).

2.5 T cell populations with functions

The T cell originates from the thymus, and in a similar form of action to that of the B cells, each T cell is a non heterogeneous array of causative agents binders (T Cell Receptor; TCR) which is so definite for a particular antigen.

It is noteworthy to state categorically that: without the influence of causative agents - definitiveness, a T-cell's TCR belongs to the following 3 categories.

These are: TCRI; a hetero-dimer comprising a delta (δ) and a gamma (y) protein chain, TCR2, a hetero-dimer comprising a beta (β) and an alpha (∝) protein chain determined partly by a VβI gene; and TCR3, with a cell hetero-dimer but its' β chain is encoded partly by a Vβ2 gene.

Expression of TCR is usually connected to signaling proteins group, jointly called the CD3 complex. The TCR are firstly expressed by T-cells during thymus T-cell proliferation. At this point, most T-cells begin CD4 and CD8 cell surface molecules expression. Selection processes regarding thymocytes with γδ TCR ($TCR1^+$) have been studied but are less understood but yet are existing (Erf *et al.,* 1997).

Even though certain exceptions do exist, the conveyance of some molecules on T-cells' surfaces is connected to T cell's purpose distinctiveness.

In poultry birds, as found in animals that suckle their young ones, matured $CD4^+$ $CD8^-$ T cells comprise T cells subsets which identify exogenous disease causing agents (phagocytosed disease causing agents) in conjunction with MHC class II molecules on disease causing agents projecting cells (e.g. macrophages).

Furthermore, $CD4^+$ $CD8^-$ T cells encompass the cytokine-producing regulatory subcomponent of T-cell (helper T-cell) which is needed for the disease causing agents definite launch of B-cells, other T cells, and non-definite defense cells (e.g. macrophages).

Matured poultry birds $CD4^-$ $CD8^+$ T-cells constitute T-cells subsets which identify endogenous antigens (disease causing agents or tumor antigens) in conjunction with MHC class I. Once they are produced, $CD4^-$ $CD8^+$ T cells turned cytotoxic, capable of the disease causing agents-specific destruction of the viruses-contaminated cells and tumor cells.

The proportion between $CD4^-$ $CD8^+$ and $CD4^+$ $CD8^-$ T-cells (CD4 : CD8 ratio) is being utilized as a final decision for the assessment of the stage of every animal's defense mechanisms. In poultry birds, the ratio of CD4 to CD8 has been established to

have positive correlation with the quantity of antibodies generated in reaction to SRBC, a T reliant disease causing agent (Parmentier *et al.,* 1995).

Mature T cells have been partitioned into subpopulations based on the category of TCR they put across. Among immature unsick egg-producing and broiler chickens (Erf *et al.,* 1997), $TCR3^+$, $TCR2^+$ and $TCRI^+$ T cells account for 10-20% , 30-40% and 15-35% of spleen's lymphocytes respectively.

TCR distinguished T cell populations vary in terms of tissue location, with $\propto\beta$T cells ($TCR3^+$ and $TCR2^+$) preferentially homing to the white pulp area in the spleen, while $TCRI^+$ T cells are mainly abundant within the red pulp area in the spleen.

Unlike $TCRI^+$ and $TCR2^+$ T cells, $TCR3^+$ T-cells are hardly found in gut's micro-environment. Finally, TCR distinguished T cell subpopulations appear to vary in their functional capabilities.

For instance, Cihak *et al.,* (1991) showed that selective *in vivo* depletion of $TCR2^+$ T cells also resulted in a serious Ig-A shortage. Therefore, $TCR2^+$ T cells appear to be required for the immunoglobulin isotype change from Ig-M to Ig-A and/or the expansion of Ig-A producing B cells.

Although, functional differences within the TCR conveyed T cell subsets are not fully explainable, T-cells conveying TCR3, TCR2 or TCR1 nonetheless comprise various T-cells' subsets, and may represent an investigative instrument for the assessment of immune system status.

Strains and genotypes antigenicity can equally be distinguished by cross HI tests, that show a relationship to variations in vaccine conservation as determined by shedding of virus after an outbreak (Gu *et al*., 2011).

Although, information as regards immune reaction in Avians to ND is so scanty, both immuno-globulins and cell-mediated immunity (CMI) play an important task in shielding and authorization of NDV after an outbreak (Reynolds and Maraqa, 2000).

2.6 Newcastle Disease Virus (NDV) Sero-conversion

Sero--conversion is the transition from the point of viral infection to when antibodies of the virus become present in the blood. Given the fact that many diagnostic tests use

the presence of antibodies to infer illness, understanding sero-conversion becomes a very important part of immunology and virology (Miller *et al*., 2009).

In immunology, sero-conversion is the time period during which a specific antibody develops and becomes detectable in the blood. After sero-conversion has occurred, the antibodies can be detected in blood tests for the diseases (Miller *et al*., 2009).

NDV sero-conversion: Sero-conversion is the period during which the body starts producing detectable levels of NDV antibodies. This normally occurs several days after initially contracting the virus. The bird's immune system responds by providing antibodies in response to the virus. This period is known as SEROCONVERSION. (Kapczynski and King, 2005).

The scientific study or diagnostic examination of blood serum, especially with regards to the response of the immune system to pathogens or introduced substances is called Serology whereas a serologically distinguishable strain of a microorganism is called a Serotype.

Discovery of antibodies against NDV roughly 6-10 days after an outbreak has been recorded, while disease causing agent-specific cytotoxic T-cells (CTLs) stimulation usually requires close to 7-10 days. (Miller *et al*., 2009).

For the fact that the average death time following an outbreak of vNDV is 2-6 days, the existence of preexisting protection before an outbreak happens to be the most vital factor for clinical diseases protection (Kapczynski and King, 2005). Immuno-globulins formed against the haemagglutinin (HN) and fusion (F) trans-membrane surface glycoprotein will neutralize NDV upon a successive outbreak (Boursnell *et al.*, 1990).

Since the pathology for leNDV is less than vNDV, the presence of pre-existing protection is not as that significant to prevent diseases, turning them outstanding vaccine alternatives.

Challenging fowls with vNDV leads to a quick passing away of immunologically immature fowls, and as a result of these, the involvement of cell-mediated protection is probably insignificant since this results into the death of most birds after 5-10 days post vaccination (Reynolds and Maraqa, 2000).

On the contrary, challenging chickens with leNDV types in immunologically immature fowls normally leads to a restricted local outbreak, whereby leading to the generation of both humoral immuno-globulins and disease-causing agent-definite T cells. Medical signs of outbreak may not be visible in restricted challenges with leNDV strain, while the existence of resultant disease causing agents and/or immune-suppression can aggravate medical infection.

Additionally, there is formation of mucosal immunoglobulin A (IgA) in fowls' intestinal and respiratory tracts (Al-Garib *et al*., 2003). There can also be detection of Immuno-globulin G (IgG) on mucosal surfaces and this was thought to have contributed to the general local defense also (Chimeno *et al*., 2008).

The stimulation of mucosal antibody aids in the lessening of viral load which additionally aids in the lessening of viral load following secondary exposure to NDV.

Fascinatingly, even low amount of antibodies can proffer defense to fowls against vNDV outbreak (Gough and Allan, 1973). The quantity of vNDV released to the surroundings by stocks that have been vaccinated has increased and this serves as an indication of vaccine effectiveness (Miller *et al*., 2009).

Other experiments on ND have indicated that with the utilization of vaccines manufactured with a NDV having the same genetic make-up with the vNDV challenge virus, for both genetic make-ups, there is a possibility of decreasing both the number of fowls releasing vNDV, and also the quantity of vNDV released from all fowls through the evaluation of oral-pharyngeal and cloaca swab substance (Miller *et al*., 2009).

Results indicated that the quantity of viral load from vaccinated fowls with vaccines that are heterologous to the genetic make-up of the infection virus was equally reduced, but at lesser quantities. There are some arguements that vaccination breakdown is primarily a result of insufficient administration (Dotmans *et al*., 2012).

Nonetheless, other researchers suggested that vaccines manufactured with genetic make-up similar to that of the infection virus which results in reduction of viral load should be an important aspect of disease management (Hu *et al*., 2011).

Information on whether the utilization of elevated doses of standard vaccines towards inducing elevated antibody status is unavailable, or whether this is sufficient in the prevention of ND caused by vNDV from viruses that are of genetic make-up which are more distant from vaccine strains, or which genetic make-ups have the possibility of leading to unsuccessful immunization with standard vaccines made from genetic make-ups I and II NDV types.

Additionally, it is important to know if stale vaccines can appreciably decrease viral load from infection with isolates that are new. Poultry birds were immunized with a live lasota vaccine and later infected with the heterologous vNDV (identified as a virus of a distinct of genetic make-up) at various days after-immunization to appraise the quantity of viral load from every group and to later establish how triumphant the quantity of virus that was passed down to other poultry fowls (Hu *et al*., 2011).

Also, with a more detailed examination on sero-conversion of poultry birds immunized with various genetic make-up of NDV and tested with homologous and heterologous genetic make-ups of vNDV to establish vaccine efficiency and humoral protection on viral load.

Observations revealed that immunization with NDV vaccines manufactured with homologous disease causing agents (antigen of identical genotypes) compared to heterologous disease causing agents, and that there is a relationship between antibody response after infection and ability to transmit to unprotected poultry populations.

2.7 Symptoms of Newcastle Disease Outbreaks

During outbreaks of Newcastle disease, symptoms usually comprise:

(1) Respiratory distress and rasping,

(2) Followed by 1 or 2 days of wings and legs paralysis and bowing down between legs or straight back over shoulders.

(3) Torticolis: A twisting of the neck (staggering) may also be witnessed among older birds,

(4) Loss of egg production potentials along with some respiratory sufferings and

(5) Total paralysis after 4 to 6 days may also be witnessed.

(6) Deterioration of both the internal and external egg quality parameters have also been reported.

2.8 Factors Contributing to ND Outbreaks

2.8.1 Virus: Bird - Host Factor

Strains of NDV differ in pathogenicity for local fowls, from velogenic to non-pathogenic strains. NDV strains observed in local fowls differ in their capability to cause outbreaks through various pathways, the less potent strains lying mostly on the enteric pathway (Alexander, 1988).

Furthermore, NDV strains are composed of different genetically and physicaIly dis-similar virus 'clones'. These clones that form a farmland strain of NDV possess varied infective capability, thermo-stability, shedding-capacity, replicability and pathogenicity (Hanson, 1988). Their collective incidence permits the strain survivability in various circumstances, and this allows them rapid adaptation to unfriendly ecological conditions.

There are differences in thermo-stability of strains and clones of NDV. These qualities of NDV render it difficult to generalize regarding the circumstances that favour epidemics of ND among local fowls, particularly when the conditions within the rural community may be made complex by the incidence of a number of strains of NDV.

In some rural communities, this is made more complex by the incidence of one or more vaccine strains.

During the outbreaks of NDV, it is most likely that birds and environmental factors are the most critical for the development of disease. Local fowls resistance to ND increases as they grow (Beard and Hanson, 1984). Velogenic strains will result into infection among healthy un-protected grown-ups, but some fowls will definitely survive.

2.8.2 Concurrent disease

Local fowls are equally attacked by a number of diseases: parasitic, viral and bacterial and some of them, predominantly parasites, are persistently there in the rural community environments. Feeding (nutrition) is often poor in local fowls which are dependent on scavenging for feed which is available in a limited quantity.

Post mortem findings may reflect ND as leading to death, some coexisting infections and incapacitating burdens of both ecto-parasites and endo-parasites may be present.

The coexisting infections increase local fowls susceptibility to ND. An all-embracing ND vaccination program was attached with parasite control in Burkina Faso, in their findings, the parasite control was observed to be of significant importance in the control of ND in that country (Verger, 1986).

2.8.3 Breed susceptibility

Positions vary on the degree of vulnerability of local stocks and exotic stocks. Cherdchai (1988) stated that in Thailand, local fowls are further opposed to ND than the exotic stocks, while Higgins and Shortridge (1988) said that no proof for variations in vulnerability among native fowls in Hong Kong small holdings, and exotic fowls.

It is possible to have variations in vulnerability within local fowls across the globe.

2.8.4 Environmental factor

ND occurs throughout the year among most native populations of poultry, though it is most widespread and more deadly most times during periods of climatic distress. Disease outbreaks are often connected with seasonal changes, especially at the commencement of the raining season (Thitisak *et al*., 1988). Cold conditions of the weather have been cited as one of the contributory factors during outbreaks of ND as it has equally being found in hot weather (Bell *et al*., 1990).

A higher density of native poultry population makes the environment more vulnerable to contamination and paves way for the spread of ND, when compared to a more dispersed population.

2.8.5 Hosts

Several species of fowls, both household and undomesticated birds are vulnerable, while geese and are the least vulnerable poultry species. Certain undomesticated species may be carriers.

2.8.6 Mode of Transmission

This could be through direct contact with body discharges: particularly faeces from infected fowls, contaminated feed, water, equipment and facilities, premises, human clothing and overalls, etc.

The percentage mortality and morbidity rates differ among species and with the strain of virus.

Incubation period ranges between 4 to 6 days.

2.8.7 Sources of Virus

These could be through respiratory discharges, faeces and dead carcasses. Virus is released in period of incubation and for a limited moment at recovery period.

Psittacoses have been identified to release the virus from time to time for over 1 year.

Symptoms/Diagnosis

Breathing and/or nervous signs: panting and coughing, droopiness of the wings, leg dragging , head and neck twisting (torticolis), rotating, dejection, total paralysis, incomplete or total termination of egg production.

Egg malformation: irregular-shaped shell, lean-shelled and eggs with watery albumen. Also, there have been reports of greenish watery diarrhoea, tissues around the eyes and the neck become swollen.

Mortality and morbidity depend on virulence of the virus, level of immunity derived from vaccination, conditions of the environment and of the stocks.

Preventive and Control measures for Newcastle Disease Virus

Currently, there is no treatment but available control measures for the disease include the following:

(1) Strict prevention of infections, destruction of all contaminated and unprotected fowls

(2) Meticulous cleaning-up and disinfection of surroundings

(3) Proper disposal of dead carcasses

(4) Adequate control of pest among flocks

(5) Avoidance of congestion

(6) Adequate control of human and animal trafficking, and

(7) Adequate and timely vaccination of poultry birds.

2.9 Immunity to Newcastle Disease Virus

Poultry birds that continue to exist after disease outbreak with a virulent Newcastle disease virus come up with a long lasting protection against a future encounter with Newcastle disease virus. The reasons for this protection are as follows:

- Revolving antibodies
- Secreted antibody resulting in mucosal protection.
- Cell mediated protection

Newcastle disease virus of minimal potency imposes comparable defense reactions without resulting into brutal outbreaks. This is the reason behind Immunization.

Live vaccines

Live vaccines are made up of viruses that are living and which are able to cause infection within the cells. Virus Strains of minimal potency are employed. Live vaccines normally imitate natural outbreaks and the 3 immune reactions.

Killed Vaccines

The viruses' capability to cause infection on the cells is shattered by treatment using either a chemical, irradiation or thermal treatment. Killed vaccines normally raise a revolving antibody reaction.

2.9.1 Strains of vaccine for Newcastle disease virus

Strains of Newcastle disease virus are largely categorized into 4 pathological types:

- Avirulent strain: This strain causes no disease
- Lentogenic strain: This strain is of little potency, mortality is minor and results into drop in production of eggs.
- Mesogenic strain: This strain is of reasonable potency, with mortality of up to 50% and equally results into loss of egg production

- Velogenic strain: This strain is of high virulence, resulting into a very severe disease condition and with a high mortality percentage. (Spadbrow, 1993).

Several strains of Newcastle disease virus other than velogenic strains are used in the manufacture of live vaccines. Some of these strains are listed below:

Table 1: Strains of Newcastle disease virus used in the manufacture of live vaccines:

Strains	Functional Description
F strain	Lentogenic in nature: This is usually used in young chickens but is equally appropriate for use among chickens of all ages.
B1 strain	Lentogenic in nature: This is slightly more virulent than the F strain and it's equally appropriate for use among chickens of all ages.
Lasota strain	Lentogenic in nature: This often causes post vaccination respiratory signs, and it's usually used as a booster vaccine among flocks that have been previously vaccinated with the F or B1vaccines.
V4 strain	Avirulent in nature: Appropriate for use among chickens of all ages.
V4-HR strain	Avirulent in nature: This is otherwise called Heat Resistant V4, it is thermo-stable and it is appropriate for use among chickens of all ages.
1-2 strain	Avirulent in nature: Thermo-stable, and it is appropriate for use among chickens of all ages.
Mukteswar strain	Mesogenic in nature: This is an invasive strain that is usually used as a booster vaccine. It causes adverse reactions (respiratory distress, emaciation, drop in production of eggs) and even death if used in a population of partially or not fully immuned chickens. Administration of this vaccine is usually by injection.
Komarov strain	Mesogenic in nature: This is a less pathogenic strain than Mukteswar, often used as a booster vaccine. Administration of this vaccine is also usually by injection.

(Spadbrow, 1993).

2.10 Infectious Bursal Disease

Infectious bursal disease, (IBD) (otherwise called Gumboro disease, Infectious bursitis and Infectious avian nephrosis) is known to be an extremely transmittable ailment of growing birds initiated by infectious bursal disease virus (IBDV) symbolized by immune-suppression and a high percentage of mortality generally between age 3 to 6 weeks (Thitisak *et al*., 1988).

Infectious Bursal Disease Virus (IBDV) was initially detected in Gumboro, Delaware in 1962. It is of economic importance to the poultry sector globally as a result of increased defenselessness to other secondary diseases and harmful interfering with efficient immunization.

Contamination is through the oral-fecal route, with the infected birds passing out soaring quantity of virus for roughly 2 weeks post contamination. Enlarged bursa of fabricius characterized by yellowish peri-bursal oedema is common during outbreaks.

IBDV is a dual stranded ribonucleic acid (RNA) virus with a genome bi-segmented and belonging to the genus: Avi-birna virus, family: Birna-viridae. Two different serotypes of the virus are in existence, but only serotype 1 viruses result into ailment in poultry birds populations (Thitisak *et al*., 1988).

Through the *in vitro* cross-neutralization assay, six different antigenic sub-types of IBDV serotype 1 are recognized. Any virus that belongs to one of the antigenic subtypes is regarded as a variant, which has been documented to crack into elevated degree of maternal antibodies in profit-making poultry birds, leading to mortality percentage of 60 to 100% among the birds.

Infection may emerge all of a sudden and morbidity usually attains 100%. In the non-chronic type of infection, birds show signs of prostration, debilitation and dehydration. The infection is also characterized by watery diarrhea and affected birds may have swollen feces-stained vents. Most of the affected birds are recumbent and with ruffled feathers.

Mortality percentages differ according to the level of potency of IBDV strain encountered, the incidence dose, initial protection, presence of secondary infection, and also the affected fowl's ability to build up a successful immune response. Immuno-

suppression of very young poultry birds of less than three weeks of age (3 weeks old), is possibly one of the most important symptoms and this may not be clinically visible (sub-clinical).

Additionally, outbreaks caused by a less potent strain will not present obvious medical signs, but poultry fowls that have bursal atrophy with fibrotic or cystic follicles and lympho-cytopenia before the age of six weeks may be vulnerable to secondary infections and this may result into death caused by agents that should not have caused outbreaks in immune competent poultry birds.

2.11 Fowl Pox

Fowl pox is a global infection of fowl and man caused by viruses of the family: *Pox-viridae* and the genus *Avipox virus*.

The causative viruses of fowl pox disease are different from one another but they are the same antigenically, the possible vectors for this disease include: chickens, turkeys, quail, canaries, pigeons, and several other poultry species of.

Two distinct forms of fowl pox disease exist:

(1). The first one is normally transmitted by the biting insects (particularly the mosquitoes) and wound contamination and resulting into lesions on the combs, wattles, and beaks. Poultry birds that are affected by this first form of the disease normally recover within just few weeks.

(2). The second form is normally transmitted by breathing - in of the virus and this causes a diphtheritic membrane to accumulate in the mouth, pharynx, larynx, and many times the trachea. The diagnosis for this form of fowl pox disease is very poor (Sumaya, 2005).

Fowl pox is a universal infection of local fowls which have not been previously immunized, usually affected fowls will survive outbreaks of this infection, even though very immature or feeble poultry birds may be nowhere to be found.

The lesions from this virus attack at the outset appear like a whitish wound and become visible on the comb, wattles and other skin areas.

Occasionally, lesions/wound can be seen on the body parts, legs region and even at times on the lower/softer parts of the beak.

The wounds transform into a dark coating/scab and this may take up to 3 - 4 weeks to finally heal-up and fall - off. Wounds/lesions resulting from Fowl pox, when found in the affected birds' mouths and throats can easily result into difficulties in breathing or even death in many occasions (Sumaya, 2005).

Scars may be formed and as a result of this, poultry farmers/ breeders who engage in poultry exhibitions choose to immunize against this disease and prevent its outbreaks at all cost. Extermination of mosquito population within the farm areas will assist in reducing outbreaks of fowl pox.

2.12 Coccidiosis Disease

Coccidiosis is a disease that is caused by different kinds of Eimeria species, an Apicomplexa parasite of the protozoan origin. It is a protozoan ailment in poultry, which normally results into alarming economic losses globally (Razmi *et al.,* 2000).

The disease occurs only after ingestion of sporulated oocysts in vulnerable poultry birds. Clinically infected and recovered poultry birds release oocysts in their fecal droppings, which eventually infect feed, water, litter, soil and the entire poultry premises.

Oocysts may also be transmitted by mechanically and these include: equipment, clothing, insect-pests, farm attendants and other workers, and various animals that are present on the farm).

Coccidiosis is found in the intestine around the epithelial cells and despite advances in poultry nutrition, chemo-therapy, management, and genetic improvements. *Eimeria tenella* and *Eimeria necatrix* are very serious pathogenic species resulting into bloody lesions, elevated morbidity, and mortality (Gari *et al.,* 2008).

The majority of *Eimeria species* affect poultry birds of between ages 3 to 18 weeks and resulting in very high mortality in young poultry birds. Some secondary infections are usually seen under field conditions during outbreaks of Coccidiosis (Gyorke *et al.,* 2013).

Coccidiosis disease in poultry birds is associated with dysentery, enteritis, emaciation, droopiness, and retarded growth. Also, feed and water intakes are depressed. Loss of weight, development of runts among the birds leading to so many culls, reduction of egg production potentials, amplified morbidity and higher mortality percentage usually go together with outbreaks (Sharma *et al.*, 2013).

Poor management practices, such as wet litters which promotes oocyst multiplication, contaminants such as poultry equipment and tools, poor ventilation and higher stocking densities can exacerbate these clinical signs.

Coccidiosis disease is still a bane to successful poultry production globally, and this is as a result of poor diagnosis. Diagnosis of clinical coccidiosis is only reliable if oocysts, merozoites, or schizonts are revealed under the microscopes and if lesions/wounds are serious (Soulsby, 1998).

Knowledge of the history of the endemicity of the farm and farm situations is necessary towards developing a suitable prevention program, and also the recognition of factors that may influence the possibility of outbreaks.

2.13 Salmonella

Salmonella belongs to the genus: bacillus, shape: rod-like bacteria (gram-negative), family: *Entero-bacteriaceae*. Two *species* of Salmonella exist and these are *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is that species which is sub-divided into 6 different subspecies that included above 2,500 serotypes (Su and Chium, 2007). *S. enterica* subspecies have been found throughout the Globe in all warm-blooded species.

S. bongori is restricted to cold-blooded species, predominantly the reptiles (Tortora, 2008). Strains of Salmonella result into fever diseases such as typhoid and paratyphoid and also food poisoning infections (Ryan and Ray, 2004).

Salmonellosis is one of various important bacterial infections which affect large number of host-birds internationally (Baumler *et al.*, 1998). Poultry birds are the important reservoirs of many pathogens of zoonotic importance, of which Salmonella is of leading significance (Berhravesh *et al.*, 2014).

Salmonellosis in poultry birds is important because it does not only affect the poultry industry but also affects humans through the eating of infected poultry meats and eggs. Salmonellosis has been described to be endemic in the poultry industry (Ramya *et al.*, 2012). A lot of researchers have identified variations in the prevalence of Salmonella infection among various poultry establishments. Different serovars of Salmonella have been reported from the poultry industries internationally.

Over 53 serovars have been documented in Nigeria and the number of serovars is on the increase. Management and biosecurity measures are part of the ways of preventing this infection in poultry farms.

2.14 *In Ovo* Vaccination

In ovo immunization is an unconventional method to Post-Hatch immunization in poultry birds principally in the meat-type birds (broilers), when chicks are vaccinated at the embryo stage.

Vaccination during embryonation at the 18^{th} day assists in closing the windows of vulnerability to diseases' outbreaks i.e. the interval connecting immunization and early introduction to causative factors when juxtaposed with vaccination at post-hatch (Ricks *et al.*, 1999).

In ovo vaccination implies the vaccination of the chicken embryos while they are still in the egg.

After making a small hole through the blunt end of the eggshell, the vaccine is injected slightly beneath the membranes at the floor of the air cell (Gildersleeve *et al*, 1993).

Vaccines are deposited into the extra-embryonic compartment mainly into the amniotic sac (Sharma *et al*, 1994) and are also injected directly into the embryonic tissues in the area of the neck, shoulder and breast.

The deposition of the vaccine into the embryonic tissues or into the extra-embryonic compartments depends on the duration of incubation of the eggs and on the size of the needle.

The embryo ingests the surrounding amnion fluid with the vaccine (Sharma *et al*, 1994). The antigen can stimulate the immune competent cells of gut-associated

lymphoid tissues. The role of cloacal sucking in the uptake of *in ovo*-injected substances has been also documented (Jochemsen and Jeurissen, 2002).

The presence of a choanal cleft that communicates with the nasal and oral cavities assists in the deposition of vaccines into the nasal cavity and into the respiratory tracts by aspiration (Sharma *et al*, 1994). The antigen might stimulate the nasal- and bronchus-associated lymphoid tissues.

Efforts to vaccinate at the embryonic stage in the hatchery as a means of vaccine administration was predicated on the fact that birds would have developed some immunologic functions prior to the period of hatch.

The defense mechanisms in poultry starts to build up in the early hours during embryogenesis and several defense system reactions with the benefit that because of this pre-natal vaccination, *in ovo* immunized fowls would have come up with a substantial level of defense by the time the chicks are coming out of the shell (hatch).

The impacts of maternal antibodies on vaccines to be utilized during *in ovo* immunization can be disallowed by coming up with vaccines that are not sensitive to maternal antibodies.

Administration of (BDA-IBDV) combined vaccine *in ovo*, has been distinguished to be safer, more effective than the usual IBDV vaccine alone basically because it delays the manifestation of bursal lesions, zero early mortality among the chicks, production of higher geometric mean antibody titres against IBDV sooner than the conventional vaccinates and equally generated protection against outbreaks of viral diseases. (Embrex, 2011).

The benefits of *in ovo* vaccination using the egg injection system (Embrex *In ovo*ject® Egg Injection System, Research Triangle Park, NC) compared to post-hatch vaccination include possible earlier immunity, no stress related to handling of chicks that can occur in post-hatch vaccination, accurate and uniform dosage, reduced labor costs and reduced contamination (Gildersleeve *et al*, 1993).

Advantages *in ovo* immunization are as follows:

i) Early protection against diseases with a negligible intervention from maternally-generated antibodies.

ii) Chicks have the best beginning when they hatch and a better protection from disease outbreaks right from their day one.

iii) To guarantee an active defense response, vaccine administration must occur either in the amnion or the embryo.

iv) A less laborious process with the minimal influence from human errors using bio-devices.

v) Minimizes birds' stress during handling and equally improves the health status of the chicks.

Critical Success Factors during *In ovo* Injection :

1. Egg location
2. Shell penetration
3. Site of injection
4. Vaccine delivery
5. Sanitation

Shell Penetration

1. Needle inside a punch
2. Punch is designed for egg shell penetration
3. Needle is designed for vaccine delivery to the embryo or amnion
4. Needle inside punch permits targeted, efficient sanitation of contact points of injection mechanism:

18 days + 0 hrs = 98.4% of injections were in amniotic fluid + embryo body

18 days + 12 hrs = 99.6% of injections were in amniotic fluid + embryo body

19 days + 0 hrs = 100.0% of injections were in amniotic fluid + embryo body

Vaccine delivery:

Vaccine Integrity – Safe delivery from bag to needle

Vaccine Efficacy – whole process, from bag to embryo, development of immunity defense against challenge.

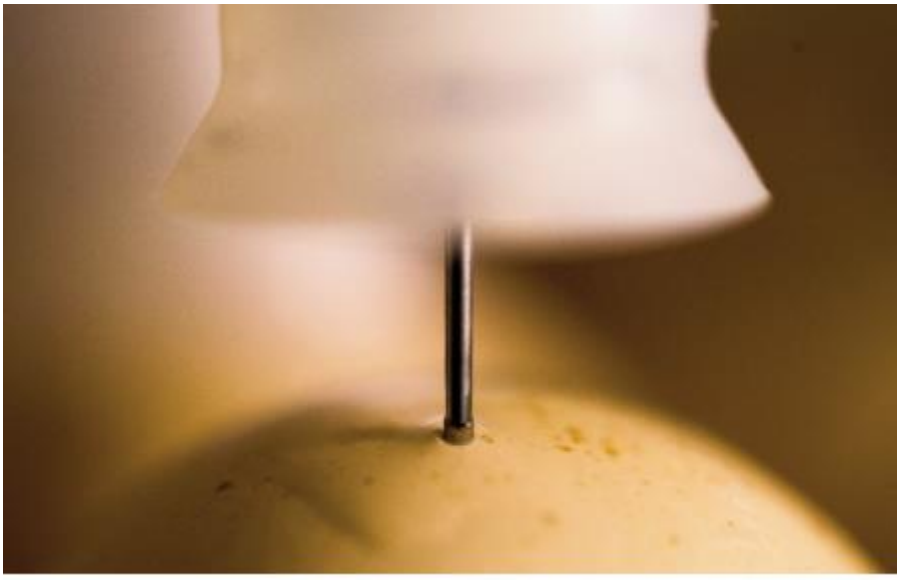

Figure1: Egg shell Punch

Williams, (2002).

Injectables

Licensed *In ovo* Vaccines MDV (3 serotypes)

IBDV (Gumboro)

Fowl Pox

AAC (IBDV, ND)

Eimeria app (coccidia)

Injectables

Other Vaccines used *In ovo* Reo virus (Tenosynovitis)

Vectored Fowl Pox (LT*, AI, AE, MG, ND)

HVT (IBDV*, ND* ILT*)

Adenovirus (AI)

Antimicrobials

*newly licensed by USDA for *in ovo* application

Lower Vaccine Costs

Precise, safe and accurate, flock inflammation system, offers decreased priming volume (EMBREX *IN OVO* TECHNOLOGY, 2011)

2.14.1 Sites of *in-ovo* vaccination

There are 5 basic compartments in an egg during the final stage of incubation:

Air Cell, Allantoic sac (waste), Amniotic sac, the Embryo itself and the Yolk sac.

- The air cell which is basically filled with gas.
- The allantoic sac which is filled with fluid containing embryo development by-products.
- Amniotic sac which is composed by amniotic fluid and the embryo body.
- The embryo itself which is located inside the amniotic sac.
- The yolk sac which is also inside the amniotic sac

Any one of these aforementioned compartments can be accessed by the needle either manually or by automation.

However, in order to achieve and maximize immune response by *in ovo* vaccination, it is essential to assure that the correct compartment inside the egg is accessed. Williams, (2002).

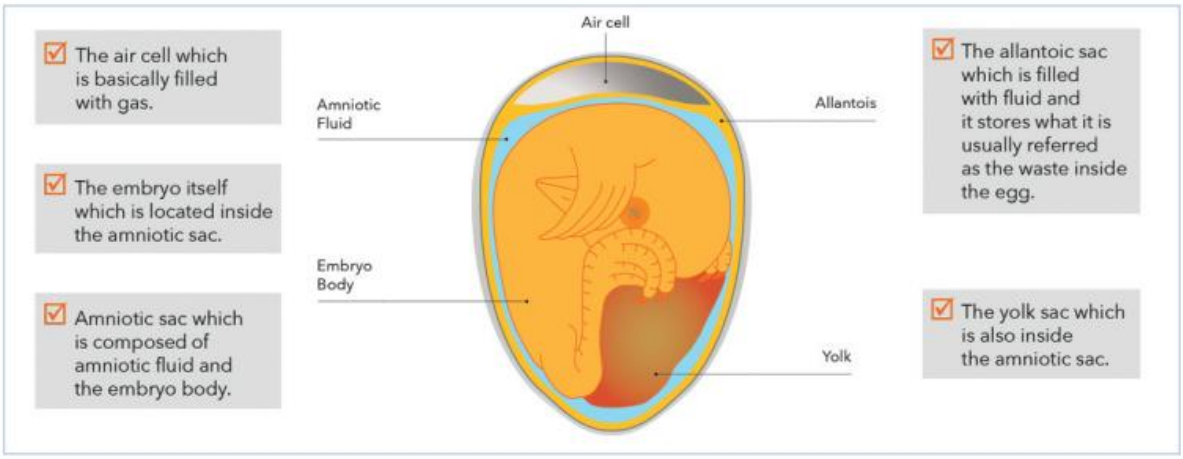

Figure 2: Sites of *in-ovo* vaccination

Williams, (2002).

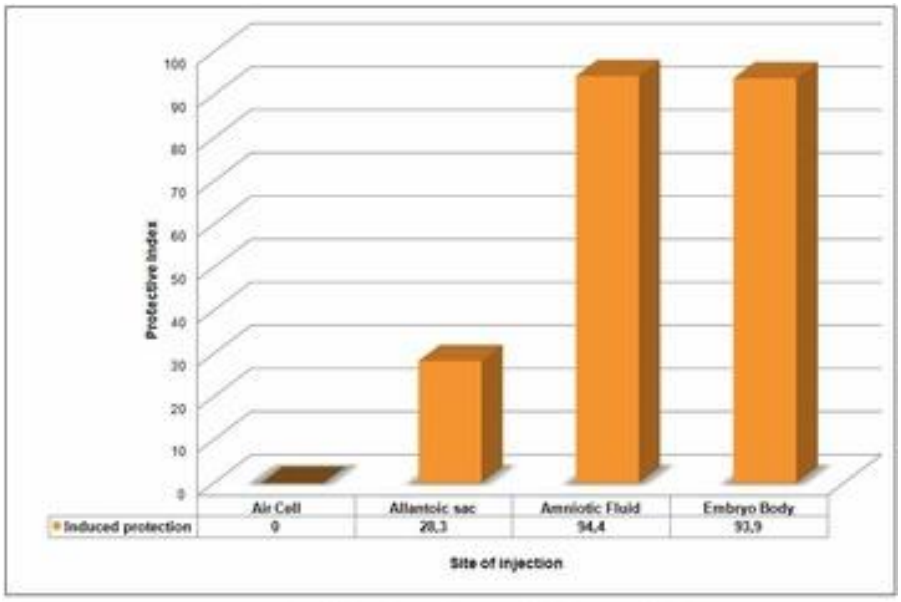

Figure 3: **Protection levels from different sites of *in-ovo* vaccination for Mareks Disease vaccine deposition**

Williams, (2002).

2.15 *In Ovo* Vaccination and Maternally Derived Immunity

During the development of vaccines for *in ovo* administration to embryos of poultry birds, two cogent points must be noted: firstly - the influence of maternally derived antibodies on the vaccines to be administered and secondly - the influence of the administered vaccines on the living embryos (Wakenell *et al.,* 2004).

Maternally derived antibodies have been noted to interfere with active vaccination. Vaccines from gentle and intermediary IBDV strains did not provoke defensive antibody status during their administration to broiler birds with maternal antibodies and these suggested that these vaccines have been consumed by the maternal antibodies.

Poultry birds with elevated degree of maternal antibodies do necessitate an extremely infectious IBDV vaccine strains to bring on vigorous protection (Wakenell *et al.,* 2004).

However, such strains are extremely infectious to poultry fowls with small levels of maternal antibodies. Poultry fowls from immunized parent stocks enclosed elevated degree of motherly generated antibodies (ranged between 285.51 to 289.43) at day one and later reduced steadily below protection level within 15-20 days post-hatch.

Saeed *et al.,* (1988) documented that motherly generated antibody reduced to almost zero at 25 days post-hatch. A higher degree of motherly generated antibody in one day-old-fowls was also documented (Wakenell *et al.,* 2004).

The rate at which the maternally derived antibody declines, was almost by 50% at every 5 days of haemaglutination inhibition test. The above findings agree with the findings of Gough and Allan, (1973) who projected that each two-fold decay in HI antibody titer that was maternally derived took about 4.5days.

Maternally derived antibody has been found to be protective and it is thus taken into contemplation as rendering the vaccine un-effective (Gough and Allan, 1973).

Highly immunogenic birds when compared to that of live vaccine vaccinated poultry birds were reboosted using live vaccine at day 60 and at day 120, antibody levels were measured and it was found out that the levels declined in both groups.

Birds from both groups were re-vaccinated at day 120 with an inactivated oil adjuvant vaccine and antibody titers were measured 30days later, higher antibody titer levels were found in both groups with variations that are significant ($P<0.05$). Antibody titer levels were increasing steadily and maintained production levels for a longer period of time.

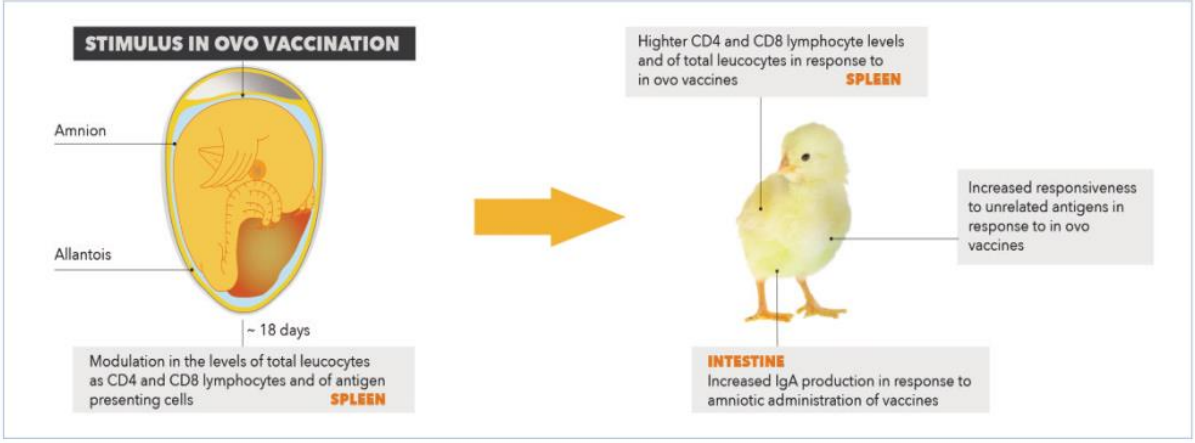

Figure 4: Sero-conversion during *in-ovo* vaccination

Williams, (2002).

2.17 Post-Hatch Vaccination

Post – hatch vaccination refers to the vaccination of chicks after they have hatched out of the shell so as to offer protection to them against viral diseases.

The post-hatch methods of vaccination against most of the viral diseases in poultry have been discussed above and it remains the most conventional methods of vaccination used against most of the known viral diseases throughout the world including Nigeria.

The conventional methods of post-hatch vaccinations include:

- Subcutaneous route of vaccination whereby prepared vaccines are administered under the skin of the chicks/poultry birds;
- Oral administration via drinking water;
- Aerosol spray over the chicks;
- Intra-nasal administration through the nostrils and
- Also Intra-ocular administration through the eyes etc.

2.18 Poultry Industry

The term poultry refers to all birds that benefit man economically which include guinea fowl, quail, pheasant, duck, pigeon, chickens and of recent ostrich all which belong to the class Aves zoologically.

Atteh,(2003) stated that poultry have reigned for more than One hundred and fifty million years, drawing back to the unique wild jungle fowl.

Poultry provided a lot of benefits to man and these include: egg and meat, medicine and research, improvement of soil fertility through the provision of manure, feathers from poultry birds provide human with aesthetic values.

Tremendous growth of interest in poultry industry and its products has been recorded in the past 20 years (Atteh, 2003).

Virtually all countries on the globe have recorded their involvement in poultry production. Japan domestic output for both poultry meat and eggs has continued to increase so as to meet their demands.

China, the Middle East and Africa at large are all areas where ever-increasing request for products from poultry has resulted into tremendous rise in the total figure of fowls that are reared for meat and eggs (FAO, 2010).

Nigeria's poultry industry is important as a chief supply of animal protein to the populace. For several years, the growth of poultry industry has followed a direction closely determined by the economic successes of many nations. USDA, (2013) estimated profit-oriented poultry production in Nigeria at about Eight hundred million dollars.

Around 25% of the domestic agricultural outputs of the Nigerian economy arouse from the poultry sector (FAO, 2010). USDA (2013) currently positioned Nigeria as the African leader with respect to the supply of eggs, but 4th in broilers supply and this is an indication that Nigeria needs improvement in the output of broiler meat-producing birds.

Production of poultry is becoming more popular in developing countries because of its position in bridging the protein undernourishment in their diets and empowerment of the poor dwellers in the rural communities economically.

Production of poultry is practiced at every level ranging from non- commercial to large scale profit-making operations.

Meat and eggs from poultry take the frontline in the consumption of animal proteins which are unlimited by any religion or cultural practices in Nigeria. FAO, 2010 reported that the production of poultry contributed almost 25% of Nigeria's Agricultural Gross Domestic Products.

Nigeria's present annual production has grown beyond 550,000 metric tons of poultry meat and 700,000 metric tons of eggs (FAO, 2010).

Notwithstanding, Nigeria has still not met her internal demand when compared with developed states that engaged in supply of poultry. FAO, (2010) documented that poultry growth was 3.2 percent as against global rise of 2.2 percent.

Nigeria's contribution had grown beyond her surrounding borders. Poultry sub-sector has a very huge capacity in Nigeria to be an employer of labour.

The opportunities in Nigeria are great and all these potentials can only be harnessed and gaps filled by farmers who are well-organized.

2.19 Origin of Domestic Chicken

There is a proper documentation by several authors. Going by the proofs from archaeologists, researchers like Carter (1971) and Crawford (1984) showed that fowls were firstly tamed in the Indus valley of South East Asia by 2000 BC.
The tamed fowls were thought to have emanated from the Indian undomesticated forest chicken and the Asian South-East Red forest chicken (Moiseyeva *et al.,* 2003).

This species is among the mainly familiar and persistent household species with over 24 billion population in 2003 and raised mainly for the supply of food from both their eggs and meat (Ganabadi *et al.,* 2009).

At large, Macdonald and Edward, (1993) stated that fowls had lived from 332 BC and that the South African's tamed species was most likely transported by traders travelling to India and European dwellers near the beginning of 15th and 16th centuries. Native fowls differ considerably in terms of morphological distinctiveness (Horst, 1991).

Native fowls referred to as *Gallus domesticus* occur all over the Globe and with reference to Manson (1984), these are the mainly consumed species and the mostly accepted fowl species reared under local environments.

2.20 Populations of Nigerian Indigenous Chickens (NI C)

Nigeria Indigenous Chickens (NIC) are the major poultry species reared in the villages and their documented populations differ from state to state.

Their distribution surpasses other live-stocks and the single species that are raised by the villagers in the non-urban areas because of their reliance on local feed resources and by- products from the farms.

Gueye, (2003) stated that in Africa, NIC account for close to 98% of the totality of poultry fowls reared. Nigeria contributes 80% of 120 million poultry fowls (Fayeye, 2005), Malawi, NIC contribute 83% (Gondwe, 2004).

In spite of the fame and prospects of these native fowls, Sonaiya and Olori, (1989) reiterated that village poultry practices were hardly given most important considerations in financial development policies. On the other hand, villagers often see these NIC as less important to other live-stocks and cropping systems**.**

Adebambo *et al.,* (2009) found that populations of indigenous poultry portrayed hereditary similarities as a result of intermixes of germ-plasm in Nigeria. Sonaiya and Olori, (1989) reported a frequency distribution of 75%, 15%, 6% and 4% for the Normal, Frizzled, Naked and Fray rural chickens in South-western Nigeria respectively.

Indigenous chickens are renowned to be somewhat independent, strong poultry birds that have the ability to survive cruel conditions of weather. Indigenous chickens although possess an appreciable level of immunity from endemic diseases, but still come down with Newcastle Disease (ND) particularly during the dry season (Horst, 1989).

Newcastle Disease has been established to be so potent to the extent that so many indigenous poultry birds die without presentation of any signs that are clinical. In many occasions, a mortality rate of approximately 100% does occur in indigenous poultry flocks that have not been initially vaccinated during outbreaks of ND (Ricks *et al.,* 1999).

2.21 Molecular Root of Nigerian Native Fowls

Nigeria as a country is gifted with diverse zones of ecology and has varied livestock genetic potentials that are of NIC strains. The native strains possess genes appropriate to adjustment to a specific location and a particular native improvement goal (Romanov *et al*., 1996).

The NIC in Nigeria are fitting gravely in danger of extinction due to a high level of genetic erosion caused by diseases' outbreaks, endo and ecto-parasites and predations. Serious steps must be taken so that the adaptation features of the native fowls will not be lost before characterization and conservation. A little research works have been conducted on the molecular categorization of NIC .

Adebambo *et al*., (2009) established that there were no considerable variations in the genetic gap of NIC in 3 different regions (South-west, North-west and North-east ecological zones) of the country.

The researchers arrived at the conclusion that these NIC displayed genetic similarities caused by inter-breeding of germ-plasms in Nigeria due to the trans-border movement of humans and animals.

Molecular markers have been employed in the characterization of variety that gives room for relatively quick and inexpensive evaluations when quality phenotypic measures are not available (Toro *et al*., 2006).

The use of geographical locations in the classifications of genetic resources requires the support of molecular data towards providing an impartial estimate of hereditary distinctiveness (Pimm and Lawton, 1998) for the intention of hereditary material conservation and exploitation.

Characterization refers to a comprehensible description of the heritable traits/ characteristics of a species of livestock, with an exclusive identification to the location to which live-stocks have been modified (FAO, 2010).

The hereditary uniqueness of a livestock is the starting point for differentiating varied livestock species and also for the assessment of the existing distinctiveness (FAO, 2010).

2.22 Production and Reproductive Performance of Native Fowls

The NIC possess small body size with a slow growth rate, these local birds attain the stage of maturity in advance than their imported counterparts.

The body-size of an animal is also resolute on its growth rate. Olawunmi *et al*. (2008) reported that NIC of North-West Ecological Type (ET) fowl was superior in body-size than the South-West ET, with 1.76 ± 0.40 and 0.79 ± 0.21 kg for North-West and South-West ET respectively.

The male NIC is superior in body-size than the female categories respectively with 1.50 ± 0.06 kg against 1.29 ± 0.04 kg.

Genes that are major were documented to reflect a marked outcome on the potentials of NIC in the tropical environments (Ibe, 1993).

Reports showed that the frizzle feathered and naked neck genes conferred a superior feed efficiency on these birds when compared to their normal feathered genotype (Gunn, 2008).

Table 2 below shows the rate of growth at different stages for undiluted NIC of different ecological types, crossbred groups with the imported stocks.

Existences of differences in morphology between the various ET have been established (Olawunmi *et al*., 2008). Crossbreeding of NIC with the imported ones also led to a great improvement in body weight at twelve weeks (Adebambo, 2005).

Table 2: Pattern of Growth of Undiluted NIC, Imported and Crossbred fowls

Genetic Sources resources	Day old	Age in weeks (g) 1	4	8	12	20	
Ind. A	27.45				484.72		Nwosu *et al*, (1980)
Ind. B	26.83				564.69		
Ind. C	29.66				557		
Exotic	42.28				728.18		
Ind.		33.13±0.9	85.18±6.1	286.93±19.4	545.05±36.9		Adebambo, (2005)
Exo.X Ind.		43.00±1.1	132.0±7.1	456.2±22.7	804.0±43.4		Adebambo, (2005)
Ind.X Exo.		38.00±0.9	119.8±6.0	409.0±19.1	742.5±36.9		
Fulani ecotypes	27.00±4	37.00±7	116.00±27	306.00±45			Fayeye *et al*, (2005)
	28.00±4	39.00±7	114.000±23	312.00±66			
Naked neck	36.17±0.75		142.90±8.46	348.61±4.21			
Frizzle	35.10±0.75		150.24±80.30	351.31±4.45			
White Leghorn	30.18±0.90		152.35±12.89	351.22±6.65			
Normal			135.1±6.00	314.0±12.56	511.3±13.33		Ibe, (1992)
Naked neck		118.3±14.96	290.8±31.34	496.7±33.25			
Frizzle			112.0±13.71	282.0±28.72	499.0±30.48		
Normal	29.45±0.29	53.89±1.1	168.45±3.8	330.51±8.6	520.13±11.5	986.12±21.32	
Frizzled	30.67±0.28	49.28±0.9	156.84±3.5	311.10±7.3	488.54±10.2	995.02±19.45	
Naked	30.22±0.3	52.10±1.4	158.52±4.7	341.87±10.9	572.56±17.3	1040.72±22.19	Gunn, (2008)

*Ind. – Indigenous, Exo. - Exotic

2.23 Egg Production of Nigeria Indigenous Chickens

The rate of growth and potentials of egg production in the villages are extremely poor, due to inadequate supply of feed, diseases outbreaks and social vices from the NIC (Ibe, 1992).

Egg production by these birds under free-range is about 40 eggs per annum (Ikeobi *et al*., 1996) while under better rearing circumstances, egg production potentials of these NIC may be in two-folds (Nwosu, 1979).

Sexual maturity is recorded at 133-169 days of age under free-range rearing condition and 189 days for battery cage system (Gunn, 2008).

Ibe (1992), indicated that the frizzle feathered and naked neck NIC in tropical environments attain maturity earlier than their normal feather counterparts. The variation that exists in the age of local pullets in attaining sexual development was majorly due to the system of managing the birds and their production traits (Gunn, 2008).

Even though egg-weight was significantly superior for the heavy ET chickens than the light ET chickens (Fayeye *et al*., (2005), number of eggs is inverse.

Adedokun and Sonaiya (2001) reported that NIC assume earlier sexual maturity and end laying of eggs during output cycle than the imported fowls. More feed was also recorded to have been utilized in the production of eggs by NIC than their hybrid counterparts.

Table 3: Egg Production of Nigerian Indigenous Chickens

Genetic Resource	Rearing System	Age at 1st egg (days)	Bodyweight at 1st egg (kg/ bird)	Egg Weight (g/ bird)	Egg Mass Egg (g)	Feed/doz (g/ bird)	Reference
Fulani ecotype	Deep litter						Fayeye *et al.,* (2005)
Localx white Leghorn	Cage	135.0±0.422	1.07±0.007	40.73±4.08		1.83	
Local xGirirj	Cage	165.20±0.668	1.47±0.011	41.14		2.02	
Local	Cage	136.36±0.45	0.95±0.007	37.15		2.14	
Ind. A	Scavenging	135		39.45			
Ind. B	Scavenging	139					
Ind. C	Scavenging	136					
Exotic	Cage	145					
Ind.	Scavenging	169.5±28		34.5±0.7			
Heavy ecotype	B. Cage			40.34±0.24	5740.85±21.42		
Light ecotype	B. Cage			37.32+0.23	5008.21±17.86		
White Leghorn (WL)	B. Cage	137.84	1.25	41.78		2.01	Adebambo, (2005)
Giriraj.	B. Cage	161.41	2.171	36.83		2.86	
Black Nera	B. Cage	178.88	1.356	47,2		2.56	
WLx Local	B. Cage	135.6	1,079	41.14		2.23	
Giri x Local	B. Cage	162.2	1.471	35.15		2.14	
Local nana	B. Cage	133.36	0.94	39.45		2.54	
Normal	B. Cage	168.68	1,134.76	42.24			Gunn, (2008)
Frizzled	B. Cage	189.02	1.129.33	40.76			
Naked neck	B. Cage	189.68	1.047.81	37.36			

*B. Cage = Battery cage

2.24 Egg Fertility and Hatchability of NIC

Fertility and hatch-ability of NIC in Nigeria can be compared in hatch-ability and fertility with local hens from other parts of the globe under village rearing conditions.

Fertility and Hatch-ability of eggs were 76.00 % and 48.00 % respectively for the Fulani ET hens which were close to 83.00 % - 92.70 % and 52.40 % - 87.00 % given for local normal feathered Bangladesh hens (Islam and Nishibori, 2009).

Even though, the percentage fertility of the normal feathered fowls (nana), naked neck (NaNa) and frizzle feathered (ff) hens were a little higher than average (Table 4), all the local strains have high percentage hatchability of between 72.00 - 93.10 % except the normal feathered hens with close to 45.00 % hatch-ability (Ajayi *et al*., 2008).

Table 4: Egg Fertility rate and hatch-ability of eggs of NIC

Traits	Heavy/ Fulani ecotypes	Light/Yoruba ecotypes Normal Feathered	Naked Neck	Frizzled Feathered	Reference
Fertility (%)	76				Fayeye *et al.,* (2005)
Live-germ(day18)	75				
Hatchability(%)	48				
Fertility (%)		55.0	52.0	58.0	Peters *et al.*,(2004)
Hatchability (%)		72.0	54.0	84.0	
Fertility (%)		92.3	78.4	80.5	
Hatchability (%)		45.0	93.1	81.8	Ajayi *et al.,* (2008)

Table 5: Body Weight Heritability in NIC

Trait	Heavy ecotype	Main Crossbred	Reciprocal crossbred
Body weight			
0	017±0.19	0.08± 0.10	0.19± 0.22
4	0.18±0.19	0.09±0.16	0.20±0.21
8	0.43±0.26	0.02±0.16	0.31±0.28
12	0.29±0.21	0.22±0.15	0.36±0.31
16	0.16±0.18	0.25±0.17	0.26±0.25
20	0.30±0.23	0.16±0.13	0.20±0.22

Omeje, (1985)

2.25 Estimation of Heritability of Growth Characteristics in NIC

The term heritability refers to the level of the superiority of the parents over their colleagues, which are usually passed on to the siblings of the next generation.

Towards the establishment of a breeding program, it is essential to rely on heritability estimates of those traits that are economically important and genetic relationship between all of them.

This is basically because the degree of heritability permits a breeder for the estimation of the amount of improvement through a selection criterion and also the genetic relationship that can state the selection method.

There is a paucity of information on heritability estimation of body weights of the NIC at different live stages.

At ages 8 and 4 weeks, heritability estimates of 0.32, 0.36 and 0.34, 0.36, 0.38 and 0.37, were recorded for father, mother and combined effects, respectively (Nwosu *et al*, 1984). Estimation of herita-bility are very important towards predicting reactions to an indirect or a direct selection criterion.

With three different mating groups: undiluted light and heavy ecological-type birds and a crossbred between light and heavy ecological-type birds.

Ndofor *et al*. (2006) reported that herita-bility estimates for light bird was 0.40 ± 0.44 at ages 4-20 weeks, whereas 0.29 ± 0.57 and 0.37 ± 0.09 were documented respectively for main and heavy crossbred birds.

The researchers finalized that a substantial trait enhancement would be realized if the undiluted parents could be picked separately at age 12 or 16 weeks.

In 2008, Momoh and Nwosu documented estimation of herita-bility for weight of the body-size as 0.30 and 0.43 respectively for heavy ET birds at weeks 20 and 8 for layers and broilers.

It was indicated that the heavy ET local birds may have double potentials to be chosen as an egg-type fowl or a meat-type, owning to the fact that weeks 20 and 8 are respectively layer and broiler ages.

2.26 Breed Development for Future and NIC

The NIC stand for an enormous pool of fowl genome. Their continuous utilization under rural community output practices provide a non expensive on the-farm conservation method (Olori, 2009).

The frizzle feathered and the naked neck genes are specifically demonstrated as adaptableness genes serving as marker for sex and resistance to disease factors/genes (Islam and Nishibori, 2009).

The NIC must be sustained towards the conservation of the large gene reservoir they stand for through the future and also future breed improvement and development.

The NIC possess the greatest worth particularly in this age of genomics findings and improved potentials towards developing newly upgraded breeds for the future. This can only be realized principally through an enhanced use of molecular genetics in poultry production research (Fulton, 2008).

Very large distinction exists across NIC and their imported counterparts raised under unfriendly circumstances of environment.

Crossbreeding NIC with the imported ones will assist tremendously towards the improvement of the performance of the NIC without an adverse effect on their adaptive features such that their advantageous genes are conserved (e.g. resistance to diseases).

This will result in better production performances of these local fowls and also assist towards planning a sustainable breeding program and goal for the future.

2.27 Characteristics of NIC

The NIC grow slowly when juxtaposed with the exotic fowls that are fast growers owing to genetic selection, proficient systems of management, better feeding, and access to standard animal care services.

Characteristically, NIC form an essential component of the farming systems that require low capital-inputs with outputs that are easily available to all house-hold categories (Kitalyi, 1999).

The scheme of output is still under-developed and experiences severe impediments such as deprived managemental practices, undernourishment, outbreaks of diseases and attack from predators.

Production performance is low due to the very low genetic potential of the local fowls, under-feeding and poor management, a very harsh condition of the environment.

The NIC have been categorized as double-purpose fowls owing to their capability to provide eggs and meat for man's utilization.

The NIC are broody, and hence are able to look after their chicks (Horst, 1989). When placed side by side with exotic fowls, the NIC lay fewer number of eggs that weighed about 43grams.

About 4 clutches per NIC per annum that comprised 10 eggs per clutch have been recorded (Horst, 1989).

Most local farmers use all eggs produced by their NIC for generation of chicks but with challenges of poor hatchability and very poor survivability of the chicks.

On the other hand, Horst, (1989) reported that other factors as attack by predators (dogs eating eggs and chicks) and high level ecto-parasite infestations which result into poor breeding were the foremost reasons for the low percentage hatch-ability percentage.

Poor housing facilities particularly among NIC growers, early exposure of the freshly hatched chicks to adverse environmental conditions and predators have also led to poor performance.

Many researchers have also given reports on the production potentials of native fowls to fall below the standard of commercial layers and broilers with their small body sizes (Ebangi and Ibe,1994).

Matured body weights of 1.20kg in males and 1.00kg in females at the age of 5 months were documented by Barua and Yoshinura (1997). Though, there are very scanty details on egg output from native fowls in Namibia.

2.28 Characteristics of Frizzle Feathered Chickens

Figure 5: Frizzled Chicken

The frizzle feathered (FF) is a breed of poultry bird with distinctive curl of frizzled plumage. Whereas the FF gene has been observed in several breeds, as Perkins and Polish, the FF is recognized as a different breed in so many countries in Europe (Sonaiya and Olori, 1989).

The designated gene for feather curling is incompletely dominant over the normal feathered; but it should be noted that the desired frizzling is not displayed by all components of the breed.

The FF manifest heterozygousity for the gene, when 2 individuals are mated together, their offsprings came up with the usual Mendelian 1:2:1 ratio: 25% have normal feathering , 50% are heterozygous and frizzled similar to the parents, and "over-frizzled" are 25%, with easily broken feathers resembling pipe-cleaners. The frizzled breed possesses single-comb and with clear legs.

The FF is a very superior layer of tinted eggs or white eggs, and often assume broodiness. The breed forages well and is very hardy, even though the curled feathers do not offer adequate protection to it from rain.

2.29 Characteristics of Naked neck Chickens

Figure 6: Naked neck Chicken

The nakedneck (NN) is a type of poultry bird that physically lacks feathers on its vent and neck region. The NN is otherwise named the Transylvanian and has been extensively developed in Germany (Mathur and Horst, 1990).

The term "Turken" came through the erroneous belief that the fowl was a crossbreed of the domestic turkey and chicken. The NN today in Europe are fairly common, but rare in North America.

The NN is controlled by a dominant gene and is restricted by only one gene which is reasonably easy to launch into other fowls, although these products are Crossbreds rather than true Nakednecks, which is a fowl-type that is acknowledged by the Poultry Association of America since 1965, the NN gene was accepted in the 1920s across Britain.

There are other strains of NN, which include: the French NN that is often confused with the Transylvanian, and the NN game bird (Horst, 1989).

The NN breed is not specifically known as a show/trade fair bird, but is a double function utility fowl.

They lay a reasonable quantity of light browned eggs, and have been recognized as a meat-type fowl owing to the fact that they require little feather removal with a meaty carcass.

They have a very good foraging habit and are immuned to several diseases. Notwithstanding its lack of feathers, NN is considerably resistant to colds**.** The NN bear a single comb, and the breed possesses just about half the feathers of other local chicken breeds, turning it more resistant to hot weather and easier plucking of the feathers (FAO, 2010). Recognized colour varieties are: buff, black, cuckoo, white, blue and red in the United States of America.

The NN gene which categorizes this fowl is governed by an incompletely dominant allele (Na) which is positioned close to the mid region of the chromosome.

In view of the fact that this allele is a dominant one, birds which are either heterozygous (Na/na+) or homozygous dominant (Na/Na) will show evidence of NN characteristics, although the heterozygous birds will display a decrease in feathering. Pure individuals of this breed must then be homozygous dominant and all individual in the accepted breed must also be members that are homozygous dominant.

Wild type feathered (na+/na+) or Homozygous recessive birds will not show evidence of reduction attributes of feather for the Nakednecks and baring mutation would not be able to pass down the trait to the next generation (Ibe, 1992).

Research studies have shown that naked-neck gene (Na) leads to improvement in breast-size and reduction in heat-stress in birds of non broiler origin which are equally identical for the feature.

Furthermore, the nakedneck feature (Na) when bred into broiler strains in the tropics was shown to assist in lowering temperature of the body, increased bodyweight gain, enhanced feed efficiency and improved carcass-traits when juxtaposed with the normalfeathered broilers (Ibe, 1992).

2.30 Characteristics of Normal Feather Chicken

Figure 7: Normal Feather Chicken

The Normal-feathering (NF) village chicken is by far the commonest kind of fowl in Nigeria, comprising over 97% of the total population (with an estimated 190 million domestic fowl in the country). It is therefore an invaluable resource for the country (Romanov *et al*, 1996).

The NF village chicken is also sub-divided into two types, the large or "Fulani" type which is produced in semi-arid North of the country (Adedokun and Sonaiya, 2001) and the small or "Yoruba" type which is the typical type in the southern states.

The Fulani chicken is one-third larger than the Yoruba chicken (Fayeye *et al*., 2005). So called native or "village" chickens are remarkable in that they can survive with minimal management (Momoh *et al.*, 2009).

The native African chicken breeds tend to be more flavorsome but have tougher meat than introduced breeds and the eggs have bright yolks (FAO, 2010).

2.31 The Nakedneck Gene (na)

The NN fowls resemble a crossing of a fowl and a turkey with their totally feather-less necks and faces. They are frequently known as turkeys, Transylvania Naked-necks, bare neck and are characterized by the NN trait, initiated by a single-autosomal incompletely dominant gene (Nthimo, 2004).

The NN trait is distinguished by feather-less skin on ventral part of the thigh, on the breast and on the neck. New researches in molecular genetics and genomics have given better approach to the evaluation of these native fowls.

Major Histo-compatibility-Complex (MHC)-linked markers, MCW-0371 and LEI-0258 identified 10 allelic pairs (198-207 base-pairs) and 46 allelic pairs (194-550 base-pairs) respectively for the native fowls of Kenya (Ngeno *et al*., 2014).

The nakedneck gene (Na) is not completely dominant and the heterozygote (Nana) poultry birds can be recognized by feather tuft above the crop on the ventral side. Homozygous dominant poultry fowls (NaNa) however, either lacks this tuft or minimized to just few pin-feathers.

Scott and Crawford, (1977) demonstrated that absence of tuft or its presence could be imbibed to recognize 2 different NaNa genotype precisely at the time of hatch. The

resultant bare-skin turns red, predominantly in male-fowls as they draw near sexual maturity (Some, 1990).

The Na is linked with notably reduced feather cover than poultry birds that are not bearing the gene (Nthimo, 2004). NaNa genotypes are immensely colorful – black, red, brown and white combinations of feather are common.

In addition to the responsibility for de-feathering the neck region, the autosomal incompletely dominant Na also regulates the feathered part of the body by close to 40% in homozygous (NaNa) fowls and 20 to 30% in heterozygous (Nana) due to the Na incomplete dominance (Islam and Nishibori, 2009).

2.32 Effect of the Na on Performance of Birds

The Na brought about better feed efficiency, improved growth rate, and superior percentage of dressing than the NF birds.

The feather distribution and structure genes are well modified to the unfriendly environments of the tropics; high survivability on poor calorie feeds, high resistance to endemic infections and also better than their foreign counterparts.

Significant improvement in body weight was noted after at 12 weeks of age after crossbreeding NIC with the exotic ones.

Merat, (1986) evaluated temperature's effect dissimilarity on egg production of 2 strains (NN and NF NIC). Their studies indicated that there was a different response of the NN and NF genotypes to high environmental temperature.

The Na has witnessed tremendous concentration in the previous years in broiler management due to its relationship with tolerance to heat (Merat 1986) which is the major significant limiting reason for production of poultry in the hot tropics (Horst, 1987).

In broiler chicken, a relatively higher rate of growth and yield of meat has resulted from Na than the NF NIC at temperature that is normal and the impact is noticed more at higher temperatures (Younis and Cahaner, 1999).

2.33 Carcass Characteristics of Na

Plumage reduction of (20-40%) gives 1.50 - 3.00% more yields of carcass to the NN NIC than the NF NIC not regarding the temperature. Owing to the superior quantity of carcass of NN NIC at the pectoral region, when their carcasses are dressed, nakedneck fowls give 1.80 - 7.10 % more meat than NF NIC (Merat, 1986).

Fathi *et al*., (2008) indicated that NN NIC gave superior comparative dressed carcass load, breast-muscles and drum-stick when compared to normallyfeathered stocks (nana) and percentage of fat in the abdomen reduced in both NN NIC when compared with the NF NIC.

2.34 Body Weight and Growth Rate of Na

At 20^0C, adult body weight was lower in NN hens, especially the homozygote, than in hens with complete plumage cover, but shows inverse relationship when the temperature increased above 30 ^{o}C Younis and Cahaner (1999).

The decrease in feather coverage gives room for comparative tolerance to heat and as such, during elevated temperatures, heterozygous NN NIC are superior to normallyfeathered individuals (Younis and Cahaner ,1999).

The NN NIC has been connected with improved rate of laying, eggsize and eggmass in hot environment.

Abdel-Rahman (2000) studied the impact of Na on the egg production potentials of Sharkasi chickens under subtropical situations and concluded that the NN hens showed significant enhancements in egg production, 90day hen-day production and eggmass by 9.0, 17.80 and 13.30% for Na/Na and 3.70, 7.30 and 7.30% for Na/Na respectively compared with the na/na fowls.

Garces *et al*., (2001) gathered that the NN NIC also reached sexual maturity significantly earlier than the NF NIC by about 5 days. The NN birds were also heavier at 24, 40 and 72 weeks than NF birds.

Average percent of mortality during the laying season was less in NN NIC than NF NIC (na/na); however, the variations were not significant. Garces *et al*., (2001) stated that the Na also reduced feed intake by 12.40 and 13.60% in Na/Na respectively.

The NN NIC had a significantly better feed efficiency than na/na genotypes. The Na led to a significant reduction in egg yolk and shell percentages.

Egg produced from NN NIC had a lower breaking strength and egg shell thickness compared with the na/na. Previous studies on productivity noted reduction on the impact of elevated environmental temperature on egg fertility, reduction in body weight loss during heat stress and greater amount of Heat-Shock Protein, HSP-70 (Hernandes *et al.*, 2002).

According to Yushimara *et al*., (1997) across the local chickens, the NN is noted to be ahead in terms of production of eggs, eggsize and liveweight in a hot humid climate. Other positive advantages associated with this Na on broiler are enhanced body weight and meat yield, reduced fat-content and enhanced efficiency of feed (Merat, 1986).

Barua *et al*. (1998) showed that across the native fowls of Bangladesh, the NN performed higher in terms of production of meat and egg, and were more resistant to diseases than NF NIC.

These feather tracts are also either not present or minimized in terms of coverage (Nthimo, 2004). Feather pterylae are not present on the neck and head but around the comb. Islam *et al*., (2004) indicated that the Na and its impacts on heat-loss significantly affect appetite owing to higher calorie requirements in cool climates, and owing to a rise in the upper limits of the critical body temperature in hot climate with resultant higher intake of feed, leading to improvement in bodyweight, egg sizes and livability.

The introduction of the Na in chicken breeds also leads to improvement in the resistance of these birds to heat stress (Islam and Nishibori, 2009). The incorporation of Na in commercial breeds was to contribute to the production of birds with a high genetic potential and better production performance at higher temperatures.

The relationship between the presence of Na and the resistance of the NN bird to heat stress might be due to the fact that the Na led to reduction in feathering by close to 40% in homozygous fowls and 30% in the heterozygous birds.

The NaNa is superior to Nana for bodyweight and efficiency of feed.

Feather reduction in NN fowls probably caused their greater ability in heat dissipation through the exposed areas compared to poultry birds not carrying the gene.

According to Singh *et al*. (2004) the NN and frizzle birds were not initially accepted by most Indians because of their unfamiliar look, however demand is on the increase due to the superiority of these genotypes in tropical adaptation and productivity.

2.35 The Frizzle Gene (F)

The frizzlefeathered (FF) gene was originally narrated in 1600 by Aldrovandi. According to Horst (1989) the frizzle state resulted from a single non completely dominant autosomal gene, with symbol F, that coordinates frizzling is located on locus 6 of the chromosome.

The F is infrequently constrained by a modifier autosomal recessive (mf). As described by Somes (1990), in homozygous unmodified frizzlefeathered native fowls, all feathers have their rachises exceptionally curved. The FF breaks easily and so the native fowls appear bare.

The modifier gene reduces the excessive components of the homozygote so they do not present much wool. The original heterozygous FF bear feathershafts and barbs of contourfeathers curved, to a greater reduced level than the homozygote.

The activities of the F were established to be situated in the featherfollicle and have not resulted from basis of metabolism (Somes, 1990). He further stated that the modifying gene modifies the heterozygote making them less different from the normally feathered ones.

2.36 Frizzle Gene on Poultry Birds' Performance

Merat (1990) indicated that F has the capacity to reduce the insulating properties of the feather coverage and hence facilitating heat radiation more proficiently from their body. Furthermore, F came up with an increased quantity of egg and egg mass, in addition to dropping the percentage death-toll in hot and humid environments.

Younis and Cahaner (1999) studied F/f and f/f progenies compared under two temperatures, (18-20^{0}C) and (32^{0}C), revealed that the native fowls exhibiting F gave 24 extra eggs over a period 364 day of laying in hot (32^{0}C) climate.

Conversely, the F native fowls laid only 3 eggs less on the average in the cooler (18^{0}C) environment. There was also an increase in egg weight, feed efficiency and viability in the hot climate for the FF birds.

According to Horst (1987) the F is connected with increased number of eggs, mass of egg and mortality reduction when the birds are raised under hot and humid conditions.

Haunshi *et al*. (2002) on the effect of Na and F on immune- competence in chickens and reported that serum haemolytic complement levels were higher statistically in the FF birds than NF siblings.

Younis and Cahaner (1999) suggested that when reared at ambient temperature (32^{0}C), birds with F perform better in terms of weight gain from 4-7 weeks than their counterparts which are NF.

And that the decrease in feather coverage by F provide relatively better tolerance to heat, and under hot climate the F/f broilers were superior to their NF poultry birds concluding that FF broilers be accorded preference in hot environments.

Adebambo *et al*.(2009) also observed that the birds with the F were superior to their siblings which were either NN or NF in body weights and the majority of the egg production traits evaluated, thus indicating that the F may be of special advantage in poultry production in the humid tropics.

2.37 Interaction between the Nakedneck (Na) and Frizzlefeathered (F) Genes

Adebambo *et al*. (2009) reinstated that some major genes like NN and F are utilized towards improvement of heat tolerance in birds and are usually incorporated in breeding programs with NIC to enhance optimal production performances in poultry.

Studies by Younis and Cahaner (1999) have shown that cross-breeding the Na allele-carrier fowl with another heat-tolerant gene-carrier such as F gave a favorable additive effect on various production parameters.

Mathur and Horst (1990) reported 3 genes: FF, NN and NF interaction that cross-breeding effect of 2 genes is minimized than the totality of their contributory gene effects.

Mahrous *et al*. (2008) indicated a positive additive influence on potentials when Dahlem Red NN were cross-bred with Dahlem White FF. In addition, NNFF cross-bred hens had appreciably higher egg performance, egg quality traits and attainment of sexual maturity earlier than the NF hens.

2.38 Meat Quality and Consumers' Preference for NIC

There is scarcity of information on meat-quality attributes of NIC. At 20 weeks of age, main genes have been documented to impose important effects on weight of organ and carcass.

The NN had a higher percentage breast weight than both FF and NF fowls (Gunn, 2008) though FF and NN strains gave higher weight of other primal-cuts than their NF counterparts.

Current research works on the integration of Na into broiler strains revealed the supremacy of the product over the NF fowls for growth-rate, efficiency of feed utilization, dress weight percentage and some economically significant broiler production attributes (Younis and Cahaner, 1999).

The NIC meat and eggs are favorites among the largest percentage of the local people majorly for leanness, taste, pigmentation, toughness and appropriateness for special dishes within villages (Islam *et al.,* 2004). Eggs and meat from native fowls command reasonable prices as against products from commercially raised exotic fowls (Horst, 1989; Gueye, 1998).

Gueye, (1998) stated that meat of native fowls was 27% and 13% higher in supermarkets and markets in comparison to processed meat from exotic fowls.

According to Souza *et al*. (2011), proximate analysis of meat was not affected by genetics in all the broiler strains, although sex influenced the Ash content, with the maximum amount recorded in the cocks.

Eva *et al*. (2011) reported that broilers and pheasant vary greatly in the chemical composition of thigh and breast muscles. On the other hand, Souza *et al*. (2011) did not observe such variations in their own experiment and this might be due to the fact that the original weight of the muscles rather than the dry matter content was used.

Eva *et al*. (2011) showed that pheasant and broiler meats differ considerably in their nutritional values which might be due to genetic variations and may also be influenced by the nutrition.

Eva *et al*. (2011) equally gave reports of major variations and that protein content in breast muscles was 5.16% higher in female pheasant and 7.95% in male pheasant than in broiler chicken.

2.39 Conservation strategies for Nigerian Indigenous Chickens

Due to a rapid reduction in the population of numerous local breeds and strains of NIC, some of which are already running into a danger of extinction. Hence, there is a need to conserve these endangered species.

The NIC have been favoured by local people, basically because of their special adaptation abilities to environments that are not favourable and superior immune-competence.

Lateness into sexual-maturity, reduced output of eggs, slow growth rate, broody nature, small egg number, small size of the body and non-availability of detailed conservation efforts are part of the reasons for this reduction in population.

To mitigate the aforementioned problems, breeders have imbibed several steps to intensify selection and integration of excellent performing imported genes (Besbes *et al.,* 2007) thus leading to dilution or replacement of NIC.

Tixier-Boichard *et al.,* (2009) reiterated that close to 50% of the 938 stocks of Avian of the 5 species (Muscovy duck , turkey , duck, goose and chicken) are being identified to be at the verge of extinction.

Other factors that contributed to this danger of extinction include: Most commercial farms do not make public the capacity and operational condition and wherewithal for

personal classification towards keeping confidential reports (Tixier-Boichard *et al.*, 2009).

Also, the non all-encompassing closed-door managemental steps at the educational, commercial and regional levels and all these have exposed most native hereditary materials to be at the verge of extinction.

Different genetic principles imbibed in long-standing conservation and managing of genetic resources and heterozygosity of the populace has been noted to be the most vital. By incorporating molecular and population genetics, conservation can evaluate differences among stocks.

Maintenance of allelic diversity across the species is of paramount importance. Conservation strategies should be tailored towards all farmers, breed groups, native societies and commercial hatcheries. Conservation has 4 major stages: **inventory**, **evaluation, choice** and **conservation.**

Assessment of distinctiveness utilizing microsatellite markers was done in some African countries including Nigeria. Single Nucleotide Polymorphisms (SNPs) is a molecular marker system, new and proffers a lot of chances for assessment of the genetic diversification in several species of animals.

The SNPs are at present in use to evaluate gene diversification in Kenyan native fowls. Several methods of conservation prevail, the ideal conservation could be in situ or on-farm conservation in the villages.

These adaptation traits (NN and FF) that suit the environ are genes that are noted to reduce coverage of feather and affect their structure which facilitate loss of heat by convection.

The NN and FF native fowls have improved rate of growth and body weights, enhanced feed efficiency and production of eggs and tolerance to diseases in the tropics with temperatures beyond 25^{o}C (Mahrous *et al.*, 2008).

Within cold and mountain surroundings, feathered-shank and bearded stocks have good adaptations and with enhanced bodyweight and mass of egg (Fayeye *et al.,* 2005) and far better meat and egg productions in environments that are cold.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Sites

The studies were conducted at the Poultry Breeding Section, Federal College of Animal Health and Production Technology (FCAH&PT), Ibadan with the approval from Animal Science Department, University of Ibadan.

The FCAH&PT is sited in Ibadan South-West Local Government, Oyo State, Nigeria.

Ibadan is the largest city in West Africa and the second largest in Africa, with land size covering an area of 240km^2. The city is located on geographic grid reference longitude 3^{o}5E, latitude 7^{o}20N.

The average total annual rainfall for Ibadan is 1420.06mm, falling for roughly 109 days.

There are 2 extremes in the rainfall season: June and September. The average temperature is 26.46 ^{o}C, minimum 21.42 ^{o}C and a relative humidity of 74.55%. (Filani,1994).

3.2 Experimental animals and management

One-day old chicks of indigenous breeds (n=720) were generated from their initially stocked parent stocks. At first week, brooding was done at a relative humidity of 64.05% and temperature of 32 ^{o}C, while subsequent weeks witnessed a decrease of 2.4 ^{o}C.

The chicks were weighed and bled at the inception of the studies and afterwards weekly basis. Routine management practices were strictly adhered to.

3.3 Experimental design

One-day NIC breeds (n=720) generated from their initially stocked parent stocks were allotted to four of six replicates and ten chicks per replicate employing RCBD in a 2x2x3 factorial arrangement.

3.4 Experimental Diet

Both growers mash and layers mash containing 2500kcal/kg/M.E with 15% CP and 2600kcal/kg/M.E with 17% CP respectively were used across all the genotypes.

TABLE 6: GROSS COMPOSITION OF GROWERS MASH

GROWERS MASH (1000KG)	
Corn	436
Soybean meal	100
Wheat offal	300
Groundnut Cake	120
Oystershell	10.0
Bone Meal	25.5
Methionine	1.50
Lysine	1.00
Grower premix	3.00
Salt	3.00
Total	1000kg
CRUDE PROTEIN	15% C.P
METABOLISABLE ENERGY	2500kgcal/kg M.E

TABLE 7: GROSS COMPOSITION OF LAYERS MASH

LAYERS MASH (1000KG)	
Maize	500
Soya bean meal	160
Wheat offal	180
Groundnut Cake	30.0
Oyster shell	80.0
Bone Meal	40.0
Methionine	1.50
Lysine	2.50
Layer premix	2.50
Salt	3.50
Total	1000kg
CRUDE PROTEIN	17% C.P
METABOLISABLE ENERGY	2600kgcal/kg M.E

TABLE 8: Experimental Layout (For Each NICG)

***In ovo* group**		**Post-hatch group**	
Control $T1_1$	Vaccinated $T1_2$	Control $T2_1$	Vaccinated $T2_2$
0.2mls injection water	0.2mls NDV (BILS)	0.2mls injection water	0.2mls NDV (BILS)
$T1_1R_1$	$T1_2R_1$	$T2_1R_1$	$T2_2R_1$
$T1_1R_2$	$T1_2R_2$	$T2_1R_2$	$T2_2R_2$
$T1_1R_3$	$T1_2R_3$	$T2_1R_3$	$T2_2R_3$
$T1_1R_4$	$T1_2R_4$	$T2_1R_4$	$T2_2R_4$
$T1_1R_5$	$T1_2R_5$	$T2_1R_5$	$T2_2R_5$
$T1_1R_6$	$T1_2R_6$	$T2_1R_6$	$T2_2R_6$

STUDY ONE

3.5 Immunological Assay/Antibody Titre Determination

3.5.1 Experimental animals and management

As stated in section 3.2 of page 60

3.5.2 Experimental design

As stated in section 3.3 of page 60

3.5.3 Hatching

Candling and in-*ovo* (INO) vaccination of half of the 50% of fertilized eggs (n=180) was carried out at 18th day using 0.2mLs B1 Lentogenic strain (B1LS-INO) of Newcastle Disease Vaccine (NDV) and the remaining half of the 50% of fertilized eggs (n=180) was administered 0.2mLs injection water (IW-INO) (control) by careful drilling and intervention into the amniotic fluid and the created holes were sealed using candle wax.

Thereafter, the *in-ovo* group embryos were returned into the hatcher for hatching into chicks.

The one-day old chicks (n=180) at day one post-hatch were administered 0.2mLs B1 LS NDV-P, while another set of one-day old chicks (n=180) were administered 0.2mLs IW-P (control). Routine management practices were strictly adhered to across all treatments.

3.5.4 Haemagglutination Inhibition (H.I) Test

Haemaglutination inhibition (H.I) test using quantitative Enzyme Linked Immunosorbent Assay (ELISA) (Allan and Gough, 1974) at week: 1,3,6,9 and 12 respectively was conducted at the Avian Medicine Unit, Veterinary Medicine Faculty, University of Ibadan, via blood collection into sample bottles through the jugular veins.

3.5.4.1 Details of Haemagglutination Inhibition Test

The nucleic acids of various viruses encode surface proteins (e.g. haemaagglutination (HA) of Newcastle disease virus) that agglutinate red blood cells (RBC) of a variety of species. (Microbe-online, 2015).

The reaction of viral hemagglutinins with red blood cells results in a lattice of agglutinated cells that settle irregularly in a tube or microtiter wells. Unagglutinated cells settle in a compact bottom. This process is called hemagglutination.

Hemagglutination occurs when Newcastle disease viruses and red blood cells are mixed together. But if the serum of a bird infected with Newcastle disease virus is mixed with RBC and Newcastle disease virus, there won't be any agglutination of RBC, this phenomenon is called Haemagglutination inhibition (Microbe-online, 2015).

This arises because antibodies present in the serum of that sick bird reacts with the Newcastle disease viruses and neutralize them (POSITIVE RESULT).

If the sick bird's serum does not contain antibodies against surface proteins of test virus, there will be presence of hemagglutination as surface molecules are free to haemagglutinate RBCs (NEGATIVE RESULTS) (Microbe-online, 2015).

The basis of HAI assay is that antibodies to that particular virus (e.g. Newcastle disease virus) will prevent attachment of the virus to RBC. Therefore, hemagglutination is inhibited when antibodies are present (Microbe-online, 2015).

HAI Titer: The highest dilution of serum (Ab) that prevents hemagglutination is called HAI titer of the serum.

3.5.4.2 PROCEDURES FOR HAEMAGGLUTINATION INHIBITION TEST

1. Blood samples from each genotype (breed) collected into sample bottles.
2. Diluents (e.g. Bovine albumin veronal buffer) added at suitable level of pH
3. Formation of solution to get rid of non-specific haemagglutinins from the serum.
4. Infection of cultural solution or typical antigen (e.g. grounding of ND virus) for serum analysis.
5. A two-fold dilution of each genotype/test serum was prepared to be evaluated e.g. from 1:8 to 1:512.
6. A predetermined quantity of viruses was added to all 96-wells plate, corresponding to 4 Haemagglutination Assay units (but this varies in relation to the disease/virus type), apart from the control wells for the serum.
7. Those plates were later permitted to stand at room temperature for 60 minutes (but time varies according to definite necessities).
8. Red blood cells were added and later incubated at $40^{O}C$ for 30minutes.
9. The wells reading were taken (Microbe-online, 2015).

3.5.5 Statistical Analysis

Data were analyzed using SAS package by RCBD employing two-way Analysis of Variance (ANOVA) at $p<0.05$ level and a repeated measure test. Means were partitioned employing new Duncan Multiple Range Test (DMRT), while impacts of interaction were partitioned using Least Square Means (LSM), (Steel and Torrie, 2004).

STUDY TWO

3.6 Comparative growth performance of the Nigerian Indigenous Chicken Genotypes (NICG)

The following performance parameters were examined for the NICG as follows:
Initial bodyweight, Mean weekly feed intake, Mean weekly weight-gain, Mortality percentage, Final bodyweight, FCR (Feed conversion ratio) using standard procedures.
The following data were collected;

Performance parameters:

Records of daily feed intake, weight gain per week were taken, Feed conversion ratio were calculated at the end of the experiment. These values were calculated thus;

Weight gain (g)

The initial body weight of the chick was taken on a replicate basis while subsequent body weight was recorded on weekly basis.

Body Weight Gain (BWG) = final weight gain – initial weight gain

Feed intake (g)
A known weight of feed given to the chicks measured while the leftover of feed was weighed to determine daily feed intake and consequently weekly feed intake. The feed intake was calculated as follows;

Feed intake per bird (g) = feed consumed - feed leftover

Feed conversion ratio (FCR)
The feed conversion ratio of each of the group of chicks were determined by

calculating ratio of feed intake to weight gain and thus calculated as;

$$\textbf{FCR} = \frac{\textbf{Feed consumed}}{\textbf{BWG}}$$

3.6.1 Statistical Analysis

As stated in section 3.6.5 of page 67

STUDY THREE

3.7 Differential haematological and serological profiles among the Nigerian Indigenous Chicken Genotypes (NICG).

3.7.1 Blood Collection and Evaluation

At the growers phase (week 16), there was collection of blood samples from the jugular veins of the NIC from each genotype into a set of **EDTA** fortified glass tubes for haematological studies and also into a set of plain glass tubes for serum biochemistry.

While laboratory analysis was executed at the Animal Physiology unit, Animal Science Department, University of Ibadan.

3.7.2 Statistical Analysis

As stated in section 3.6.5 of page 67

STUDY FOUR

3.8 Evaluation of Internal and External egg-quality Indices of the NICG

The following external and internal egg quality indices of the NIC were examined using standard procedures:

- Haugh-unit
- Yolk index
- Albumen index
- Yolkcolour
- Shape-index
- Shellthickness
- Phases 1,2 and 3 Hen-day production

3.8.1 Yolk-Index (YKI): This was calculated as below (Doyon *et al*, 1986):

$$YKI = \frac{YKH}{YKW} \times 100$$

Note, YKI = Yolkindex

YKH = Yolkheight

YKW = Yolkwidth

3.8.2 ShapeIndex:

$$SPI = \frac{EW}{EL} \times 100$$

Note, EW = EggWidth

EL = EggLength

3.8.3 Albumen-Index (ABI):

$$ABI = \frac{(ABH)}{(ABL + ABW)/2} \times 100$$

ABI = Albumenindex

ABH = AlbumenHeight

ABL = AlbumenLength

ABW = AlbumenWidth

Measurement of albumenheight was done using 0.01mm accuracy tripod micrometer in a flat dish

3.8.4 Eggshell thickness; Measurement of thickness was done after removing the internal membrane of eggshell.

- ❖ Shell measurements were taken at 3 regions of the shell and means were determined. Shell measurements were achieved using precision micrometer to the nearest 0.01mm (Thickness Gage of Mitutoyo Dial).

3.8.5 Haugh Units: Each HaughUnit (HU) score was determined using eggweight and albumenheight (Haugh, 1937).

- ❖ The HaughUnit values were determined for each egg as below:
  - HGU = $100 \log_{10} (AOH + 7.5 – 1.7EW^{0.37})$
  - AOH = Albumen's observedheight (mm)
  - EW = EggWeight (grams)

3.8.6 Eggyolk colour: Determined by Roche yolk-colour fan.

3.8.7 Hen-day egg production per day (HDEP):

- ❖ HDEP = $\frac{\text{Total no. of eggs prod. per day}}{\text{Total no. of hens present per day}} \times 100\%$
- ❖ Eggweight was determined by weighing of eggs on a digital weighing scale on a daily basis

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Stages of Vaccination in Nigerian Indigenous Chickens at Various Ages.

The main effects of stages of vaccination in the NIC at various ages are shown in Table 8. The geometric mean antibody titres in the *in-ovo* stage were significantly higher at weeks 1 and 9 than that of their post – hatch counterparts.

Whereas, the geometric mean antibody titres for both the *in-ovo* and post-hatch vaccinators at week 12 were not significantly different and equally reflected a decline in the geometric mean antibody titres of both vaccination stages.

Table 8: Main effect of stages of vaccination at different ages (GMAT)

Age (weeks)	*In-ovo*	Post hatch	±SEM
1	3.58[a]	2.25[b]	0.11
3	4.67	4.46	0.14
6	3.88	3.96	0.07
9	4.67[a]	3.79[b]	0.19
12	2.83	2.88	0.06

[a b] means in row having different superscripts are significant ($p < 0.05$).

SEM = Standard Error of Mean

4.2 Effect of Stages of Vaccination Groups at different ages of GMAT across the Nigerian Indigenous Chickens.

Effects of Stages of Vaccination Groups at different ages across the NIC are shown in Table 9. Observed results indicated that the GMAT levels were significantly higher ($p < 0.05$) in *in-ovo* vaccinates ($T1_2$) than those of *in-ovo* control ($T1_1$) , post – hatch control ($T2_1$) and post-hatch vaccinates ($T2_2$) with GMAT of 4.00, 3.17, 2.00 and 2.50 respectively at week 1, GMAT of 5.50, 3.83, 3.75 and 5.17 respectively at week 3, GMAT of 4.17, 3.58, 3.58 and 4.33 respectively at week 6, GMAT of 6.00, 3.33, 3.08 and 4.50 respectively at week 9.

Table 9: Main effect of stages of vaccination groups at different ages of GMAT in NIC

	In ovo		Post Hatch		
Age (weeks)	**$T1_1$**	**$T1_2$**	**$T2_1$**	**$T2_2$**	**±SEM**
1	3.17^{b}	4.00^{a}	2.00^{d}	2.50^{c}	0.11
3	3.83^{b}	5.50^{a}	3.75^{b}	5.17^{a}	0.14
6	3.58^{b}	4.17^{a}	3.58^{b}	4.33^{a}	0.07
9	3.33^{c}	6.00^{a}	3.08^{c}	4.50^{b}	0.19
12	2.83	2.83	2.83	2.91	0.06

$^{a\ b\ c\ d}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1|}$- *In-ovo* control, $T1_2$ – *In-ovo* vaccinated, $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated

4.3 Main effect of Vaccination across Breeds

The main effect of vaccination across all the evaluated NIC is shown in Table 10. The effect of immunization against ND using the B1LS across all the breeds was similar respectively ($p > 0.05$) at weeks 1, 3 and 6. The FF had a significantly higher ($p < 0.05$) GMAT (4.75 and 3.19) than NF (4.38 and 3.00) and NN (3.56 and 2.38) at weeks 9 and 12 respectively.

Table 10: Main effect of vaccination across breeds (antibody titre)

Age (weeks)	FF	NF	NN	±SEM
1	2.88	2.88	3.00	0.11
3	4.75	4.19	4.75	0.14
6	3.81	3.81	4.13	0.07
9	4.75[a]	4.38[ab]	3.56[b]	0.19
12	3.19[a]	3.00[a]	2.38[b]	0.06

[a b] means in row having different superscripts are significant ($p < 0.05$).

SEM = Standard Error of Mean

FF = Frizzle feather, NF= Normal feather, NN=Naked neck

4.4 Interaction Effect of Stages of Vaccination and Breeds at different ages.

Interaction effect of stages of vaccination and strains at various ages shown in Table 11. The GMAT as a result of interaction between the stage of vaccination and breed were statistically higher ($p < 0.05$) at the *in-ovo* stage of vaccination than the post-hatch stage of vaccination.

The GMAT of 4.00, 3.50 and 4.00 for FF, NF and NN were recorded respectively at week 1 of *in-ovo* stage of vaccination whole, the GMAT of 2.50, 2.00 and 2.50 for FF, NF, and NN were recorded respectively at week 1of post-hatch stage of vaccination.

Also, the GMAT of 7.00, 3.25 and 4.00 for FF, NF and NN were recorded respectively at week 9 of *in – ovo* stage of vaccination, while the GMAT of 3.00. 5.75 and 3.25 for FF, NF and NN were recorded respectively at week 9 stage of vaccination.

Table 11: Interaction effect of stages of vaccination groups at different ages of GMAT in NIC

		In-ovo						Post hatch						
		Control			Vaccinated			Control			Vaccinated			
Age(week)	FF	NF	NN	FF	NF	NN	FF	NF	NN	FF	NF	NN	FF	SEM
1	3.00^{c}	4.00^{a}	3.00^{c}	4.00^{a}	3.50^{b}	4.00^{a}	2.00^{e}	2.50^{d}	2.00^{e}	2.50^{d}	2.00^{e}	2.50^{d}	0.11
3	3.50^{ef}	6.75$^{a`}$	3.75^{e}	3.75^{e}	4.25^{d}	6.00^{b}	3.25^{f}	5.50^{c}	3.75^{e}	5.50^{c}	4.25^{d}	4.50^{d}	0.07
6	3.25^{c}	4.25^{a}	3.50^{bc}	3.75^{b}	4.00^{ab}	4.50^{a}	3.25^{c}	4.50^{a}	3.50^{bc}	4.50^{a}	4.00^{ab}	4.00^{ab}	0.06
9	3.25^{e}	7.00^{a}	3.50^{de}	7.00^{a}	3.25^{e}	4.00^{c}	3.00^{e}	5.75^{b}	3.25^{e}	3.75^{de}	3.00^{e}	4.00^{c}	0.19
12	3.00^{b}	3.50^{a}	3.25^{ab}	3.25^{ab}	3.25^{ab}	2.00^{d}	3.00^{b}	3.25^{ab}	3.00^{b}	3.00^{b}	3.00^{b}	2.50^{c}	0.06

$^{a\ b\ c\ d\ e\ f}$means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

4.5 Correlation Between GMAT and Weight Gains across Breeds at *In-ovo* Stage of Vaccination

The correlation between GMAT and weight gains across all NIC examined at the *in – ovo* stage of vaccination is shown in Table 12.

Correlation results revealed that the association between the bodyweight gains and GMAT was significant and negative across NIC breeds.

However, the coefficients of correlation ranged between - 0.53 to - 0.77 for NN and NF while FF NIC recorded – 0.72 respectively.

Table 12: Correlation Between GMAT and Weight Gains (*In-ovo* Groups)

	FFT	**NFT**	**NNT**	**FFW**	**NFW**	**NNW**
FFT	0.00	0.64**	0.85**	-0.72**	-0.78**	0.70**
NFT	0.64**	0.00	0.61**	-0.73**	0.77**	0.68**
NNT	0.85**	0.61**	0.00	-0.56**	-0.69**	-0.53**
FFW	-0.72**	-0.73**	-0.56**	0.00	0.98**	0.95**
NFW	-0.78**	-0.77**	-0.69**	-0.98**	0.00	0.93**
NNW	-0.70**	-0.68**	-0.53**	0.95**	0.93**	0.00

** P<0.0001

FFW – Frizzle feather weight, NFW – Normal feather weight, NNW – naked neck weight

FFT = Frizzle feather titre, NFT = Normal feather titre, NNT = Naked neck titre

4.6 Correlation between GMAT and Weight Gains Across Breeds at Post-Hatch Stage of Vaccination.

The correlation between GMAT and weight gains across all NIC examined at the post-hatch stage of vaccination is shown in Table 13.

The correlation results showed that the relationship between GMAT and weight gains was significant and negative across NIC breeds.

However, correlation coefficients ranged between – 0.43 to – 0.72 for FF and NN while NF NIC recorded – 0.46 respectively.

Table 13: Correlation between GMAT and Weight Gains Across Genotypes (Post-hatch).

	FFT	NFT	NNT	FFW	NFW	NNW
FFT	0.00	0.94**	0.52**	-0.43**	-0.46**	-0.46**
NFT	0.94**	0.00	0.43**	-0.43**	-0.46**	0.46**
NNT	0.52**	0.43**	0.00	-0.71**	-0.71**	-0.72**
FFW	-0.43**	-0.43**	-0.71**	0.00	0.99**	0.99**
NFW	-0.46**	-0.46**	-0.71**	0.99**	0.00	0.99**
NNW	-0.46**	-0.46**	-0.72**	0.99**	0.99**	0.00

** P<0.0001

NNW = Naked neck weight, NFW = Normal feather weight, FFW = Frizzle feather weight

FFT = Frizzle feather titre, NFT = Normal feather titre, NNT = Naked neck titre

4.7 Main effect of Stages of Vaccination and Stages on the Growth Performance of the NIC

The average effects of routes of vaccination and breeds on the growth of NIC are shown in Table 14. Observed results showed that the effect of stages of vaccination (*In ovo* or post - hatch*)* was significant ($p < 0.05$) on the initial bodyweight, FCR and % mortality.

While, the effect of breeds of the indigenous chicken was important ($p < 0.05$) on the first bodyweight, final bodyweight, bodyweight gain, Feed intake, FCR and the % mortality initial bodyweight of chicks ranged between 29.31g/bird to 29.51g/bird for *in – ovo* post – hatch stages of vaccination respectively.

Also, FCR ranged between 6.90 to 6.93 for post – hatch and stages of vaccination respectively, while the % mortalities for both stages of vaccination were similar. Control groups for both *in – ovo* stage and post – hatch stage recorded 2.28% mortality while the vaccinated groups for both *in – ovo* stage and post stage recorded 1.78% mortality.

Table 14: Main effect of Stages of Vaccination and Strains on the growth performance of the NIC (week 18)

	Vaccination Stages					Breeds			
Parameters	**$T1_1$**	**$T1_2$**	**$T2_1$**	**$T2_2$**	**±SEM**	**FF**	**NF**	**NN**	**±SEM**
Initial wt (g/b)	29.39^{ab}	29.31^{b}	29.49^{a}	29.51^{a}	0.05	29.38	29.43	29.48	0.05
Final wt (g/b)	844.32	843.95	844.18	844.36	0.14	839.42^{b}	805.10^{c}	888.08^{a}	0.12
BWG (g/b)	814.92	814.63	814.69	814.85	1.16	810.05^{b}	775.67^{c}	858.61^{a}	0.14
FI (g/b)	5633.87	5633.55	5633.65	5606.88	8.19	5590.44^{b}	5752.06^{a}	5538.46^{c}	7.09
FCR	6.93^{a}	6.93^{a}	6.93^{a}	6.90^{b}	0.01	6.90^{b}	7.42^{a}	6.45^{c}	0.01
Mortality (%)	2.28^{a}	1.78^{b}	2.28^{c}	1.78^{b}	0.00	1.92^{b}	2.25^{a}	1.91^{c}	0.01

$^{a\ b\ c}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1|-}$ *In-ovo* control, $T1_{2\ -}$ *In-ovo* vaccinated , $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated, Feed-Intake (FI), BWG (Bodyweight gain), FCR (Feed conversion ratio).

4.8 Interaction Effect of Stages of Vaccination and Strains on the growth of the NIC

The interaction effect of stages of vaccination and breeds on the growth of the NIC are shown in Table 15. Observed results indicated that the interaction effect of stages of vaccination with breed was significant on all the growth parameters: Initial body weight, final bodyweight, bodyweight gains, feed-intake, FCR and % mortality.

Table 15: Interaction effect of Stages of Vaccination and strains on the growth performance of the NIC

	In-ovo						Post hatch						
	Control			Vaccinated			Control			Vaccinated			
Parameters	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**±SEM**
Initial wgt (g/b)	29.40^{bc}	29.34^{bc}	29.36^{bc}	29.38^{bc}	29.42^{bc}	29.22^{c}	29.40^{bc}	29.36^{bc}	29.35^{bc}	29.62^{ab}	29.72^{a}	29.54^{ab}	0.03
Final wgt (g/b)	839.55^{bc}	838.98^{c}	805.56^{d}	805.02^{d}	888.24^{a}	887.84^{a}	839.26^{bc}	839.90^{b}	805.18^{d}	805.04^{d}	888.10^{a}	888.14^{a}	4.43
BWG (g/b)	810.15^{bc}	809.64^{c}	775.80^{d}	775.64^{d}	858.82^{a}	858.62^{a}	809.86^{bc}	810.54^{b}	775.83^{d}	775.42^{d}	858.38^{a}	858.60^{a}	4.43
FI (g/b)	5591.04^{c}	5590.08^{c}	5771.71^{a}	5772.04^{a}	5538.84^{d}	5538.52^{d}	5589.96^{c}	5590.68^{c}	5772.54^{a}	5691.92^{b}	5538.44^{d}	5538.04^{d}	12.68
FCR	6.90^{c}	6.90^{c}	7.44^{a}	7.44^{a}	6.45^{d}	6.45^{d}	6.90^{c}	6.90^{c}	7.44^{a}	7.34^{b}	6.45^{d}	6.45^{d}	0.05
Mortality (%)	2.17^{b}	1.67^{e}	2.50^{a}	2.60^{d}	2.16^{c}	1.66^{f}	2.17^{b}	1.67^{e}	2.50^{a}	2.00^{d}	2.16^{c}	1.66^{f}	0.04,

$^{a\ b\ c\ d}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

Feed conversion ratio (FCR), Feed Intake (FI), Bodyweight gain (BWG).

4.9 Main Effects of Stages of Vaccination and strains on the mean live-weight of sexes of NIC

The main effects of Stages of vaccination and breeds on the mean live-weight of sexes of NIC are shown in Table 16.

Observed results indicated that the mean live weight of *in – ovo* vaccinated cocks and post – hatch vaccinated cocks was similar ($p > 0.05$) 1147.73 g, while the average live-weights of *in – ovo* vaccinated hens and post – hatch vaccinated hens were different ($p < 0.05$) with 1034.71 and 1034.86 g respectively.

Also, observed results indicated that the NN cocks are significantly heavier ($p < 0.05$) than the cocks of FF and NF with the average live-weight of 1224.44g, 1178.34g and 1041.43g respectively.

It was also observed that the NN hens are statistically heavier ($p < 0.05$) than the hens of FF and NF with the average live-weights of 1065.12g, 1064.34g and 975.08g respectively.

Table 16: Main effects of Stages of Vaccination and Breeds on the average live-weight of sexes of NIC (32 weeks)

	Vaccination Stages					Breeds			
Sex	**$T1_1$**	**$T1_2$**	**$T2_1$**	**$T2_2$**	**±SEM**	**FF**	**NF**	**NN**	**±SEM**
Male	1148.41^{a}	1147.73^{b}	1148.41^{a}	1147.73^{b}	0.04	1178.34^{b}	1041.43^{c}	1224.44^{a}	0.04
Female	1034.91^{a}	1034.71^{b}	1034.91^{a}	1034.86^{ab}	0.05	1064.34^{b}	975.08^{c}	1065.12^{a}	0.05

$^{a\,b\,c}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1|}$- *In-ovo* control, $T1_2$ – *In-ovo* vaccinated, $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated. FF = Frizzlefeather, NF = Normalfeather, NN = Nakedneck

4.10 Interaction Effect of Stages of Vaccination and Breeds on the average live-weight of Sexes of NIC (32 weeks).

Interaction effect of Stages of vaccination and breeds on the average liveweight of sexes of indigenous fowls are shown in Table 17.

Observed results indicated that the interaction effects of stages of vaccination with breed on the average live-weights of both the males and females of the indigenous chickens were considerable ($p < 0.05$).

Table 17: Interaction Effect of Stages of Immunization and Breeds on the average liveweight of Sexes of (NICG) (32 weeks)

	In-ovo						Post hatch						
	Control			Vaccinated			Control			Vaccinated			
Sex	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**±SEM**
Male	1178.78^{c}	1177.90^{d}	1041.66^{e}	1041.20^{f}	1224.77^{a}	1224.10^{b}	1178.78^{c}	1177.90^{d}	1041.66^{c}	1041.20^{f}	1204.78^{a}	1224.10^{b}	10.12
Female	1064.30^{c}	1064.30^{c}	975.20^{d}	974.92^{d}	1065.22^{a}	1064.92^{b}	1064.30^{c}	1064.40^{c}	975.20^{d}	975.00^{d}	1065.22^{a}	1065.12^{ab}	5.50

$^{a\,b\,c\,d\,e\,f}$means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

FF = Frizzlefeather, NF = Normalfeather, NN = Nakedneck

4.11 Main Effect of Stages of Vaccination and NIC Breeds on haematological Parameters

The main effects of stages of vaccination and breeds on haematological parameters are shown in Table 18.

Observed results indicated that the effect of stages of vaccination breeds on the haematological parameters was significant ($p < 0.05$) with 30.35 PCV%, values were 9.10, 6.66 and 9.87 for Hb (g/dl), values were 3.11, 3.94 and 3.28 for RBC (X $10^3/mm^3$), values were 18.40, 17.65 and 18.35 for WBC (x $10^3/mm^3$), values were 17.12, 17.73 and 13.59 for Platelets (%),values were 63.18, 66.21 and 62.58 for Lymphocytes (%),values were 30.00, 26.96 and 31.03 for Heterophyl (%) respectively for each of FF, NF and NN indigenous chickens.

Table 18: Main effect of Stages of Vaccination and Breeds on haematological parameters

Parameters	Vaccination Stages				SEM±	Breeds			±SEM	Normal range
	$T1_1$	$T1_2$	$T2_1$	$T2_2$		FF	NF	NN		
PCV (%)	36.88^{b}	37.43^{a}	36.88^{b}	37.43^{a}	0.12	50.65^{a}	30.47^{b}	30.35^{b}	0.10	23.0-55.0
Hb (g/dl)	8.48^{b}	8.56^{ab}	8.48^{b}	8.65^{a}	0.05	9.10^{b}	6.66^{c}	9.87^{a}	0.04	7.00-18.6
RBC ($x10^3/mm^3$)	3.41^{b}	3.47^{a}	3.41^{b}	3.47^{a}	0.02	3.11^{c}	3.94^{a}	3.28^{b}	0.02	1.52-4.50
WBC ($x10^3/mm^3$)	18.03^{b}	18.27^{a}	18.02^{b}	18.20^{a}	0.06	18.40^{a}	17.65^{b}	18.35^{a}	0.05	9.00-32.0
PLAT (%)	161.33^{b}	161.57^{ab}	161.41^{ab}	161.61^{a}	0.07	171.27^{b}	177.30^{a}	135.91^{c}	0.63	13.0-70.0
Lymphocyte (%)	63.89^{b}	64.06^{a}	63.90^{b}	64.11^{a}	0.06	63.18^{b}	66.21^{a}	62.58^{c}	0.05	29.0 - 84.0
Heterophyl (%)	29.19^{b}	29.48^{a}	29.20^{b}	29.44^{a}	0.08	30.00^{b}	26.96^{c}	31.03^{a}	0.07	15.1 - 50.0
Monocyte (%)	3.31^{ab}	3.41^{a}	3.28^{b}	3.36^{ab}	0.04	3.10^{c}	3.62^{a}	3.31^{b}	0.03	0.05 - 7.00
Eosinophil (%)	3.38^{b}	3.48^{a}	3.41^{b}	3.45^{ab}	0.03	3.83^{a}	2.90^{c}	3.56^{b}	0.02	0.00 - 16.0
Basophil (%)	0.20^{b}	0.24^{a}	0.20^{b}	0.24^{a}	0.00	0.34^{a}	0.06^{c}	0.26^{b}	0.00	0.00 - 8.00

$^{a\ b\ c}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1}$- *In-ovo* control, $T1_{2}$ – *In-ovo* vaccinated , $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated, Normal range: (Mitruka and Rawnsley, 1979).

4.12 Interaction Effect of Stages of Vaccination and Breeds of Haematological Parameters

The interaction effect of stages of vaccination and breeds of haematological parameters are showed that the interaction effects of stages of vaccination with breeds on all the haematological parameters considered were significant ($p < 0.05$).

Table 19: Interaction effect of Stages of Vaccination and Breeds of Haematological Parameters

	In-ovo						Post hatch							
	Control			Vaccinated			Control			Vaccinated				
Parameters	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**±SEM**	**Normal range**
PCV (%)	50.38^{b}	50.97^{a}	30.11^{d}	30.82^{c}	30.20^{cd}	30.49^{cd}	50.33^{b}	50.97^{a}	30.11^{d}	30.82^{c}	30.20^{cd}	30.49^{cd}	1.24	23.0 - 55.00
Hb (g/dl)	9.02^{b}	9.07^{b}	6.64^{c}	6.70^{c}	9.78^{a}	9.81^{a}	9.02^{b}	9.27^{b}	6.64^{c}	6.64^{c}	9.78^{a}	10.03^{a}	5.50	7.00 - 18.60
RBC ($x10^3/mm^3$)	3.09^{d}	3.13^{d}	3.92^{a}	3.95^{a}	3.23^{c}	3.34^{b}	3.09^{d}	3.13^{d}	3.92^{a}	3.95^{a}	3.23^{c}	3.34^{b}	0.05	1.52 - 4.50
WBC ($x10^3/mm^3$)	18.29^{a}	18.53^{a}	17.55^{b}	17.80^{b}	18.26^{a}	18.49^{a}	18.29^{a}	18.49^{a}	17.53^{b}	17.71^{b}	18.26^{a}	18.39^{a}	0.05	9.00 - 32.00
PLAT (%)	179.08^{b}	171.39^{b}	177.22^{a}	177.33^{a}	135.83^{c}	135.98^{c}	171.19^{b}	171.41^{b}	177.22^{a}	177.43^{a}	135.83^{c}	136.00^{c}	2.37	13.0 - 70.00
Lymphiepte (%)	63.07^{b}	63.26^{b}	66.11^{a}	66.31^{a}	65.50^{c}	66.62^{c}	63.09^{b}	63.30^{b}	66.11^{a}	66.33^{a}	62.50^{c}	62.70^{c}	0.21	29.0 - 84.00
Heterophyl (%)	29.77^{c}	30.09^{bc}	26.91^{d}	27.07^{d}	30.90^{a}	31.27^{a}	29.82^{c}	30.31^{b}	26.91^{d}	26.93^{d}	30.87^{a}	31.07^{a}	0.23	15.1 - 50.00
Monocyte (%)	3.08^{de}	3.20^{cde}	3.63^{a}	3.66^{a}	3.23^{cde}	3.38^{bc}	3.04^{e}	3.06^{de}	3.55^{ab}	3.63^{a}	3.26^{cd}	3.37^{bc}	0.03	0.05 - 7.00
Eosimophil (%)	3.76^{ab}	3.85^{a}	2.89^{e}	2.93^{e}	3.48^{d}	3.67^{bc}	3.82^{a}	3.89^{a}	2.87^{e}	2.90^{e}	3.52^{d}	3.55^{de}	0.05	0.00 - 16.00
Basophil (%)	0.33^{a}	0.35^{a}	0.01^{e}	0.10^{d}	0.25^{bc}	0.26^{bc}	0.33^{a}	0.34^{a}	0.01^{e}	0.10^{d}	0.25^{bc}	0.27^{b}	0.02	0.00 - 8.00

$^{a\,b\,c\,d\,e}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

T11 - *In-ovo* control, T12 - *In-ovo* vaccinated , T21 - Post hatch control, T22 - Post hatch vaccinated, Platelets (PLAT), Normal range: (Mitruka and Rawnsley, 1979).

4.13 Main Effect of Stages of Vaccination and Breeds on Serum Biochemistry of NIC.

The main effect of stages of vaccination and breeds on serum biochemical parameters of the NIC are displayed in Table 20.

The effects of stage of vaccination on Albumin, Globulin, Glucose, Urea and ALT were similar ($p > 0.05$) while the effects of stage of vaccination on total protein, Triglyceride and cholesterol were significant. ($p < 0.05$).

Total protein (g/dl) values ranged between 4.16 to 4.24, Triglyceride ranged between 86.07 to 86.33 and cholesterol ranged between 96.86 to 96.96 for *in – ovo* post – hatch stages respectively.

Table 20: Main effect of Stages of Vaccination and Breeds on Serum Biochemistry of the NICG

	Stages of Vaccination					Breeds				
Parameters	**T11**	**T12**	**T21**	**R_2 V_2**	**±SEM**	**FF**	**NF**	**NN**	**±SEM**	***Normal**
TP (g/dl)	4.18	4.24	4.16	4.24	0.03	4.27^{a}	4.07^{b}	4.27^{a}	0.03	5.00 - 7.00
Alb (g/dl)	2.19	2.21	2.17	2.19	0.02	2.29^{a}	2.12^{b}	2.16^{b}	0.02	1.80 - 3.50
Glob (g/dl))	1.99	2.03	1.97	2.05	0.03	1.97^{b}	1.94^{b}	2.12^{a}	0.03	2.00 - 4.00
Glucose (mg/dl)	57.08	57.23	56.97	57.19	0.09	62.21^{a}	60.86^{b}	48.28^{c}	0.08	200.0 - 500.0
T.G. (mg/dl)	86.19^{a}	86.33^{a}	86.07^{b}	86.17^{ab}	0.07	89.57^{b}	95.38^{a}	73.55^{c}	0.06	35.0 - 135.0
CHOL (mg/dl)	96.89^{ab}	96.94^{ab}	96.86^{b}	96.96^{a}	0.03	96.18^{b}	138.93^{a}	75.64^{c}	0.03	52.0 - 148.0
Urea (mg/dl)	7.47	7.49	7.45	7.47	0.02	8.03^{a}	7.71^{b}	6.67^{c}	0.02	2.47 - 8.08
ALT (i.u/l)	31.72	31.67	31.73	31.65	0.04	37.48^{a}	29.18^{b}	28.42^{c}	0.03	10.0 - 37.0

$^{a\ b\ c}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1|-}$ *In-ovo* control, $T1_{2\ -}$ *In-ovo* vaccinated , $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated, TP (Total protein), Alb (Albumin), Glob (Globulin), Triglyceride (T.G), Cholesterol (CHOL), Alanine amino transferase (ALT), *(Mitruka and Rawnsley, 1979).

4.14 Interaction Effect of Stages of Vaccination and Breeds on the Serum Biochemistry of Indigenous Chicken.

The interaction effects of stages of vaccination and breeds on the serum biochemistry of NIC are shown in Table 21.

Observed results indicated that the interaction effect of stages of vaccination with breeds on the serum biochemical parameters was significant ($p < 0.05$).

Table 21: Interaction Effect of Stages of Immunization and Breeds on the Serum Biochemistry of the NIC

	In-ovo						Post hatch							
	Control			Vaccinated			Control			Vaccinated				
Parameter	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**±SEM**	***Normal range**
TP (g/dl)	4.24^{abc}	4.31^{a}	4.02^{d}	4.13^{bcd}	4.26^{abc}	4.29^{ab}	4.23^{abc}	4.29^{ab}	4.01^{d}	4.11^{cd}	4.23^{abc}	4.31^{a}	0.02	5.00 - 7.00
Alb (g/dl)	2.30^{a}	2.35^{a}	2.11^{b}	2.13^{b}	2.15^{b}	2.16^{b}	2.26^{a}	2.27^{a}	2.11^{b}	2.13^{b}	2.14^{b}	2.17^{b}	0.01	1.80 - 3.50
Glob (g/dl)	1.94^{bc}	1.96^{abc}	1.91^{c}	1.99^{abc}	2.11^{ab}	2.13^{a}	1.97^{abc}	2.02^{abc}	1.90^{c}	1.98^{abc}	2.09^{ab}	2.14^{a}	0.02	2.00 - 4.00
Glucose (mg/dl)	62.22^{a}	62.42^{a}	60.77^{a}	60.93^{b}	48.24^{c}	48.34^{c}	60.01^{a}	62.22^{a}	68.77^{b}	60.92^{b}	48.14^{c}	48.34^{c}	0.82	200.0 - 500.0
TG (mg/dl)	89.51^{c}	89.87^{b}	95.33^{a}	95.50^{a}	73.48^{d}	73.61^{d}	89.39^{c}	89.51^{c}	95.33^{a}	95.36^{a}	73.48^{d}	73.63^{d}	1.20	35.0 - 135.0
CHOL (mg/dl)	96.14^{b}	6.21^{b}	138.89^{a}	138.93^{a}	75.63^{c}	75.68^{c}	76.14^{b}	76.21^{b}	138.89^{a}	138.99^{a}	75.56^{c}	75.68^{c}	3.87	52.0 - 148.0
Urea (mg/dl)	8.03^{a}	8.04^{a}	7.69^{b}	7.73^{b}	6.68^{c}	6.70^{c}	8.02^{a}	8.03^{a}	7.69^{b}	7.73^{b}	6.63^{c}	6.66^{c}	0.08	2.47 - 8.08
ALT (i.u/l)	37.45^{a}	37.47	29.21^{b}	29.17^{b}	28.50^{c}	28.37^{c}	37.55^{a}	37.44^{a}	29.21^{b}	29.14^{b}	28.44^{c}	28.37^{c}	0.53	10.0 - 37.0

$^{a\ b\ c\ d}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

TP (Total protein), Alb (Albumin), Globulin (Glob), Triglyceride (T.G), Cholesterol (CHOL), Alanine amino transferase (ALT), *(Mitruka and Rawnsley, 1979).

4.15 Main Effect of Stages of Vaccination and Breeds on the HDEP of the NIC.

The main effects of routes of vaccination and breeds on the hen-day production of the indigenous chickens are shown in Table 22.

Results indicated that both the stages of vaccination and breeds significantly enhanced ($p < 0.05$) the percentage hen – day production (HD %) at phases 1, 2 and 3.

In the present study, 53.63 and 54.62, 68.56 and 69.63, 50.48 and 51.43 (HD %) were recorded for phases 1, 2 and 3 respectively for control and vaccinated groups.

Results also showed that the HD % for FF hens were significantly higher in % hen – day production at phases 1, 2 and 3 than their NF and NN breeds.

Table 22: Main effect of Stages of Vaccination and Breeds on the Hen-day Production of the NIC

	Vaccination Stages					Breeds			
Parameter (%)	**$T1_1$**	**$T1_2$**	**$T2_1$**	**$T2_2$**	**±SEM**	**FF**	**NF**	**NN**	**±SEM**
Hen-day Phase 1	53.63^b	54.62^a	53.63^b	54.62^a	0.00	58.50^a	51.17^c	52.1^b	0.00
Hen-day Phase 2	68.56^b	69.63^a	68.56^b	69.63^a	0.00	73.19^a	65.45^c	68.66^b	0.00
Hen-day Phase 3	50.48^b	51.43^a	50.48^b	51.43^a	0.00	54.19^a	48.56^c	50.14^b	0.00

$^{a\ b\ c}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1|}$- *In-ovo* control, $T1_2$ – *In-ovo* vaccinated , $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated. FF = Frizzlefeather, NF = Normalfeather, NN = Nakedneck

4.16 Interaction effect of Stages of Vaccination and Breeds on the Hen-day Production of Indigenous Chickens.

The interaction effects of stages of vaccination and breeds on the HD % of the hens of the indigenous fowls are displayed in Table 23.

Results showed that the interaction effects of stages of vaccination with breeds of hens were significant ($p < 0.05$) at phases 1, 2 and 3 of HD % production.

Table 23: Interaction effect of Routes of Vaccination and Breeds on the Hen-day (HD %) Production of the NIC

	Inovo						**Post hatch**						
	Control			**Vaccinated**			**Control**			**Vaccinated**			
Parameter (%)	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**±SEM**
HD Phase 1	58.00^{b}	59.00^{a}	50.67^{f}	51.67^{e}	52.22^{d}	53.20^{c}	58.00^{b}	59.00^{a}	50.67^{f}	51.67^{e}	52.22^{d}	53.20^{c}	0.42
HD Phase 2	62.67^{b}	73.70^{a}	64.90^{f}	66.00^{e}	68.11^{d}	69.20^{c}	72.67^{b}	73.70^{a}	69.90^{f}	66.00^{e}	68.11^{d}	69.20^{c}	0.42
HD Phase 3	53.67^{b}	54.70^{a}	48.11^{f}	49.00^{e}	49.67^{d}	50.60^{c}	53.67^{b}	54.70^{a}	48.11^{f}	49.00^{e}	49.67^{d}	50.60^{c}	0.31

$^{a\,b\,c\,d\,e}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

FF = Frizzlefeather, NF = Normalfeather, NN = Nakedneck

4.17 Main impact of Stages of Vaccination and Breeds on EggQuality Parameters of NIC

The main impact of stages of vaccination and breeds on eggquality parameters of the hens of NIC are shown in Table 24.

Observed results showed that the effect of stages of vaccination on all the egg-quality parameters considered were identical ($p > 0.05$), while the effects of strains on the eggquality parameters were only significant ($p < 0.05$) with shellthickness (mm) and shellweight (g).

Table 24: Main effect of Stages of Vaccination and Strains on Eggquality parameters of the NICG

	Stages of Vaccination					Breeds			
Parameters	**$T1_1$**	**$T1_2$**	**$T2_1$**	**$T2_2$**	**±SEM**	**FF**	**NF**	**NN**	**±SEM**
Egg wt (g)	39.27	38.27	39.27	38.27	0.82	38.75	38.75	38.00	0.71
Shellthickness (mm)	0.43	0.43	0.43	0.43	0.01	0.45^a	0.45^a	0.40^b	0.01
Shellweight (g)	3.53	3.40	3.53	3.40	0.12	3.60^a	3.60^a	3.20^b	0.10
Egg width (mm)	37.95	36.96	37.95	36.96	0.53	37.02	37.02	38.34	0.46
Yolk weight (g)	20.93	21.00	20.93	21.00	0.80	20.45	20.45	22.00	0.69
Yolkheight (cm)	1.09	1.16	1.09	1.16	0.08	1.18	1.18	1.01	0.07
Yolk width (mm)	40.55	39.04	40.55	39.04	1.63	40.21	40.21	38.96	1.41
Yolk colour	1.53	1.00	1.53	1.00	0.38	1.40	1.40	1.00	0.33
Albumin hgt (cm)	0.05	0.06	0.05	0.06	0.00	0.05	0.05	0.06	0.00
Yolkindex	0.03	0.03	0.03	0.03	0.00	0.03	0.03	0.03	0.00
Yolk (%)	53.54	55.00	53.54	55.00	2.09	52.94	52.94	56.93	1.81
Haugh unit	18.04	19.71	18.04	19.71	2.32	18.37	18.37	19.89	2.01
Alb. wgt (g)	14.87	14.20	14.87	14.20	0.95	15.00	15.00	13.60	0.82
Alb. wgt (%)	46.46	45.00	46.46	45.00	2.09	47.06	47.06	43.07	1.81
Shapeindex	0.74	0.73	0.74	0.73	0.01	0.73	0.73	0.74	0.01
Shell wgt (%)	10.21	8.83	10.21	8.83	0.84	9.26	9.26	10.04	0.73
Shell surf. area (mm)	52.55	51.82	52.55	51.82	0.73	52.19	52.19	52.19	0.64
Egg length (mm)	50.54	50.68	50.59	50.68	0.39	50.30	50.30	51.30	0.34

$^{a\ b}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

$T1_{1|-}$ *In-ovo* control, $T1_2$ – *In-ovo* vaccinated , $T2_1$ - Post hatch control, $T2_2$ - Post hatch vaccinated, Albumin weight (Alb wgt), Albumin height (Alb), Shell weight (Shell wgt), Shell surface area (Shell surf area).

4.18 Interaction effect of Stages of Vaccination and Breeds on EggQuality Parameters

The interaction effect of stages of vaccination and breeds on egg-quality parameters of the hens of NIC are displayed in Table 25.

Results showed that the interaction effects of stages of vaccination with breeds on the egg-quality were similar for most of the parameters considered except for shellthickness (mm), shellweight (g), eggwidth (mm) and yolk-index.

Table 25: Interaction effect of Stages of Vaccination and Breeds on Eggquality parameters

	In-ovo						Post hatch						
	Control			Vaccinated			Control			Vaccinated			
Parameter	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**FF**	**NF**	**NN**	**±SEM**
Egg wgt (g)	40.40	35.80	39.00	39.80	38.40	39.20	39.00	39.80	40.40	35.80	38.40	39.20	0.42
Shell thickness (mm)	0.42^{b}	0.45^{ab}	0.48^{a}	0.45^{ab}	0.40^{b}	0.41^{b}	0.48^{a}	0.45^{ab}	0.42^{b}	0.45^{ab}	0.39^{b}	0.41^{b}	0.01
Shell wgt (g)	3.40^{ab}	3.40^{ab}	3.80^{a}	3.80^{a}	3.80^{a}	3.40^{ab}	3.80^{a}	3.80^{a}	3.40^{ab}	3.40^{ab}	3.40^{ab}	3.00^{b}	0.06
Egg width (mm)	37.55^{ab}	36.11^{b}	36.87^{ab}	37.53^{ab}	39.43^{a}	37.25^{ab}	36.87^{ab}	37.53^{ab}	37.55^{ab}	36.11^{b}	39.43^{a}	37.25^{ab}	0.27
Yolk weight (g)	20.20	19.80	20.20	21.60	22.40	21.60	20.20	21.60	20.20	19.80	21.40	21.60	0.38
Yolk height (cm)	1.00	1.10	1.22	1.40	1.04	0.98	1.22	1.40	1.00	1.10	1.04	0.98	0.54
Yolk width (mm)	49.17	39.03	42.17	37.68	37.51	40.41	42.17	37.68	41.17	39.03	37.51	40.41	0.77
Yolk colour	1.00	1.00	2.60	1.00	1.00	1.00	2.60	1.00	1.00	1.00	1.00	1.00	0.19
Alb. hgt (cm)	0.05	0.05	0.05	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.06	0.06	0.00
Yolk index	0.02^{b}	0.03^{ab}	0.04^{a}	0.04^{a}	0.03^{ab}	0.03^{ab}	0.04^{a}	0.04^{a}	0.02^{b}	0.03^{ab}	0.03^{ab}	0.03^{ab}	0.00
Yolk (%)	50.27	55.56	51.85	54.06	58.49	55.37	51.85	54.06	50.27	55.56	58.49	55.37	1.00
Haugh unit	20.65	21.47	15.80	15.57	17.68	22.09	15.80	15.57	20.65	21.47	17.68	20.09	1.10
Alb. weight (g)	17.00	12.60	15.00	15.40	12.60	14.60	15.00	15.40	17.00	12.60	12.60	14.60	0.47
Alb wgt (%)	49.73	44.44	48.15	45.94	41.51	44.63	48.15	45.94	47.73	41.44	41.51	44.63	1.00
Shape index	0.72	0.72	0.74	0.73	0.75	0.72	0.74	0.73	0.72	0.72	0.75	0.72	0.00
Shell wgt (g)	8.52	9.51	9.74	9.26	12.37	7.71	9.74	9.26	8.52	9.51	12.37	7.71	0.42
Shell surf. Area (mm)	53.43	49.50	52.39	53.42	51.83	52.54	52.38	53.42	53.43	49.50	51.83	52.54	0.37
Egg length (mm)	51.26	49.94	49.49	50.51	51.00	51.60	49.49	50.51	51.26	49.95	51.00	51.60	0.20

$^{a\,b}$ means in row having different superscripts are significant ($p< 0.05$). SEM = Standard Error of Mean

CHAPTER FIVE

5.0 Discussion

5.1 Geometric Mean Antibody Titres (GMAT)

Results in Tables 12 and 13 showed a negative correlation between bodyweight gains and geometric mean antibody titres across the indigenous chickens evaluated, these findings corroborate one of the conclusions of Parmentier. *et al.* (1996) who indicated a negative correlation between bodyweight gains and GMAT for Newcastle disease virus in the White Leghorn cockerels.

Correlation results (Tables 12) for *in-ovo* vaccinates revealed that the association between the bodyweight gains and GMAT was significant and negative across NIC breeds.

The coefficients of correlation ranged between - 0.53 to - 0.77 for NN and NF while FF NIC recorded – 0.72 respectively.

Also, correlation results (Tables 13) for post-hatch vaccinates showed that the relationship between GMAT and weight gains was significant and negative across NIC breeds.

However, correlation coefficients ranged between – 0.43 to – 0.72 for FF and NN while NF NIC recorded – 0.46 respectively.

Also, results from tables 9, 10 and 12 concur with the findings of Williams, (2002) that *in-ovo* vaccinates would have developed an appreciable degree of protection against a particular viral disease by the time of hatch.

At days 21, 42 and 84, GMAT of birds vaccinated *in ovo* ($T1_2$) and post-hatch ($T2_2$) and their controls ($T1_1$ and $T2_1$, respectively) were similar. At days 1 and 63, GMAT differed significantly across all treatments and were 3.2±0.1 and 3.3±0.2 ($T1_1$), 4.0±0.1 and 6.0±0.2 ($T1_2$), 2.0±0.1 and 3.1±0.2 ($T2_1$), 2.5±0.1 and 4.5±0.2 ($T2_2$), respectively.

At day 63, GMAT was significantly higher in FF (4.8±0.2) than NF (4.4±0.2) and NN (3.6±0.2), while at day 84, GMAT in FF (3.2±0.1) was similar to NF (3.0±0.1) but significantly higher than NN (2.4±0.1).

5.2 Growth Performance

Results from Table 14 showed that the B1-LS had no significant effect on Body Weight Gain (BWG) across all the NIC evaluated.

Results from Tables 14, 15, 1 and 17 showed that FF and NN NIC were superior to their NF counterpart in terms of growth performance.

These results affirm the conclusions of Adebambo *et al*. (2009) and several other authors that the frizzle (FF) and naked (Na) genes confer a better FCR on these chickens which in turn leads to better growth performances.

Results (Table 14) showed that NN NIC had the highest final weight (g/b) (888.08), followed by FF (839.42), while the NF NIC had the lowest value of 805.10 respectively.

Also, results (Table 14) showed that NN NIC had the highest body weight gains (g/b) (858.61), followed by FF (810.05), while the NF NIC had the lowest value of 775.67 respectively .

Results of Feed intake (g/b) and Feed conversion ratio (Table 14) showed significant strain differences: NN NIC had 5538.46 and 6.45. FF had 5590.44 and 6.90, while NF NIC had 5752.06 and 7.42 respectively.

Results (Table 14) showed that NN NIC had the lowest mortality (%) (1.91), followed by FF (1.92), while the NF NIC had the highest value of 2.25 respectively during the course of these experiments.

5.3 Haematological and Serological Profiles

Results of PCV in Table 18, the three strains fell within the normal range of 23.00-55.00 proposed by Mitruka and Rawnsly, (1979). FF NIC had the highest average value (50.65%), followed by NN and NF with matching values of 30.35% and 30.47% respectively.

Observations gathered in this research work partly alighned with that given by Udoh, *et al*. (2012) who gave the superiority of the frizzle genes in PCV in comparison with that of the fullyfeathered and the naked neck. This could enhance the growth and other production potentials of both the normal feathered and the naked neck fowls.

Results (Table 20) revealed that Hb level of fowls in the 3 strains be within the normal range of 7.0-18.6g/dl for NF (Mitruka and Rawnsley, 1979) and 4.0-14.0g/dl by Lewis (1998). The NF recorded the lowest Hb level (6.66g/dl), seconded by FF (9.10g/dl) whereas the NN had the uppermost concentration (9.87g/dl).

The Hb level generated for FF fell within the normal range of 11.4±2.75g/dl for normal FF native fowls Udoh *et al*. (2012). The levels generated in this research work slightly vary from those gathered by Udoh *et al*. (2012). They gave mean Hb levels of 9.24, 8.91 and 8.05 g/dl respectively for NF, NN and FF.

Ologhobo *et al*. (1986) indicated that a rise in WBC count beyond normal range is suggestive of the existence of waste materials and strange agents in the circulatory system of livestocks. In this research work (Table 19), such abnormality in the levels of WBC was not recorded.

The WBC values gathered fell within the normal range of 9.0 - $32\times10^3/mm^3$ opined by Lokhande *et al.* (2008). Findings indicated that FF recorded the maximum count 18.40 ($\times10^3/mm^3$), followed by NN 18.35 ($\times10^3/mm^3$) and NF 17.65 ($\times10^3/mm^3$) respectively.

Lymphocytes are of special importance in creating obstructions against local infections and are equally engaged in the development of antibody (Frandson, 1981).

In this research work (Table 19), NF had the highest lymphocytes value (66.21%) while the NN had the lowest value (62.58%) and values fell within the range levels (29 – 84%) for healthy birds (Mitruka and Rawnsley, 1979) which is suggestive of strong defense system among the native fowls.

Lymphocytes in *in ovo* vaccinated ($T1_2$) (64.1±0.1%) and post-hatch vaccinated ($T2_2$) (64.2±0.1%) were significantly higher than in *in ovo* control ($T1_1$) (63.2±0.1%) and post-hatch control ($T2_1$) (63.1±0.1%, respectively)

Monocytes which strongly look like neutrophils because of their active motility and phagocytic activities, allowing the blood stream to gulp foreign agents and certain micro-organisms that penetrated the tissues.

The reduced levels gathered for monocytes might be due to seasonal differences or effect of strain of native fowls.

Monocytes in *in ovo* vaccinated ($T1_2$) (3.41±0.03%) and post-hatch vaccinated ($T2_2$) (3.36±0.03%) were significantly higher than in *in ovo* control ($T1_1$) (3.31±0.03%) and post-hatch control ($T2_1$) (3.28±0.03%) respectively.

These findings suggested supreme capacity of NF NIC in combating infections, in comparison to other strains. This might be one of the reasons for their adaptability and greatest population across the 3 major strains in Nigeria.

Elagib and Ahmed, (2004) established large variations in lymphocytes principally owing to nutritional status and age of livestocks.

Findings gathered from this work disagreed with these documentations since all the fowls utilized for this research work were of similar age and were subjected to identical feed. The variation in lymphocytes count in this work might be as a result of strain effects.

Heterophil is the major granulocyte recognized in Avians. The normal range of heterophils (Table 19) ranges between 15.1 - 50% (Mitruka and Rawnsley, 1979). Its variations in uniqueness and number do occur with recourse to the fowl's health status since litle

troubles such as mild inflammation, low grade infection and stress do appear (Adeyemo, 2012).

The values of heterophils gathered in this research work were within the reference levels of healthy fowls and agreed with the reports of Adeyemo (2012) for laying hens.

Similarly, heterophil and white blood cell count ($x10^3/mm^3$) in $T1_2$ (29.6±0.1%, 3.47±0.02) and $T2_2$ (29.5±0.1%, 3.47±0.02) were significantly higher than in $T1_1$ (29.2±0.1%, 3.41±0.02) and $T2_1$ (29.1±0.1%, 3.42±0.02, respectively).

Variations in RBC depend on whether the fowls are adults or juvenile and the strain of the fowls for examination, findings gathered in this research work were in the range of levels for healthy fowls (Mitruka and Rawnsley, 1979).

The RBC in *in ovo* vaccinated ($T1_2$) ($3.47±0.02×10^3/mm^3$) and post-hatch vaccinated ($T2_2$) ($3.47±0.02×10^3/mm^3$) were significantly higher than in *in ovo* control ($T1_1$) ($3.41±0.02×10^3/mm^3$) and post-hatch control ($T2_1$) ($3.41±0.02×10^3/mm^3$), respectively.

Serum indices are very crucial for proper osmotic-pressure maintenance within the transporting fluid and cellular spaces' fluid for the proper transfer of materials within the cells and the blood.

The biochemical components of the serum equally add to the viscosity and upholding of normal pressure and pH of blood. The higher globulin levels in the NF enhances better cell-mediated immune response.

Results of the serum biochemistry indicated that all the parameters varied significantly across genotypes ($p < 0.05$)

All the values were within the normal reference range of chicken for serum ALT which was reported to be 10 - 37 iu/L by Mitruka and Rawnsley, (1979). As ALT is considered one of the more reliable indicators of Liver disease in birds Mitruka and Rawnsley, (1979).

However, the values for serum ALT gathered across the evaluated NIC did not suggest a state of illness among the birds.

Total protein (g/dl) in the plasma are mainly created by hepatocytes (Mitruka and Rawnsley, 1979), with assumption that the lower values: FF (4.27), NF (4.07) and NN (4.27) recorded across the NIC was a direct effect of breed differences as against the normal reference values of 5.00 – 7.00 (Mitruka and Rawnsley, 1979).

The glucose concentration (mg/dl) in the serum across all the NIC breeds varied significantly: FF (62.21), NF (60.86) and NN (48.28) and values were far lower than the reference values of 200.00 – 500.00 for chicken (Mitruka and Rawnsley, 1979).

These results of serum's glucose concentration suggested a lower rate of gluconeogenesis among the NIC which may be one of the reasons attributable for small body sizes among the NIC breeds.

5.4 Hen-day Production (%)

The average values for hen-day production (%) presented in Tables 22 and 23 suggested statistical variations ($p<0.05$) across the genotypes in phases 1, 2 and 3 of the laying cycle.

Hen-day production was significantly improved by vaccination and ranged from 53.63±0.00% *in ovo* control ($T1_1$) to 54.62±0.00% post-hatch vaccinated ($T2_2$), 68.56±0.00% *in ovo* control ($T1_1$) to 69.63±0.00% post-hatch vaccinated ($T2_2$), and 50.48±0.00% *in ovo* control ($T1_1$) to 51.43±0.00% post-hatch vaccinated ($T2_2$) for early, mid and late laying phases, respectively.

The FF genotype recorded the highest performance with 58.50, 73.19 and 54.19 percent at phases 1, 2 and 3 of the laying cycle respectively. Also, the NN genotype recorded a significantly higher performance than their normal feathered counterpart with 52.10, 68.66 and 50.14 percent at phases 1, 2 and 3 of the laying cycle respectively.

These results confirm the conclusions of Adebambo *et al*. (2009) that both FF and NN genotypes because of their special affinity for heat loss by convection and consequently a better FCR and a better egg production potentials when compared to their normal feathered counterpart.

5.4.1 Effects of Newcastle Disease on Egg Production in laying Hens

Newcastle disease presents a fairly definite pattern in its effect on egg production. Flocks in high production may suddenly drop to zero production within 4 to 5 days. Many eggs will be laid on the floor during the course of this drop and many eggs will be found with soft shell and with no shells at all.

Production will be interrupted temporarily for a period of approximately two weeks and it then takes the birds another three to four weeks to return to normal production. While returning to normal production, brown eggs turning to white shelled eggs is common (Williams, 2002).

Many of the eggs will have rough shells and the interior quality of the eggs will be quite poor. In some instances, the birds may go into molt. When this occurs of course, egg production will be interrupted for an even longer period of time. If feeding and management are such as to maintain a high feed intake, the molt may be prevented.

Where flocks have had some protection by previous vaccination against Newcastle disease at the time of an outbreak, the drop in egg production is not quite as severe and it is difficult to distinguish the disease in vaccinated birds (Williams, 2002).

Outbreaks of infections bronchitis usually has a more lasting effect on the quality of eggs from pullets than when attacked by Newcastle disease, while birds that are just coming into production will return into normal production much more quickly than older birds, usually in a matter of two to four weeks.

Frequently the egg production will be down at the low point only for a very few days.

5.4.2 Internal EggQuality Parameters

The mean values for internal egg-quality traits presented in Tables 24 and 25 suggested that no significant variations ($p>0.05$) were recorded among the strains in eggweight, yolkweight, yolkheight, yolkwidth, yolk-colour, albumenheight, yolk-percentage, Haugh-unit, albumen-weight, albumen-weight percentages. However, significant variations were gathered across the three genotypes in yolk-index. The egg weight as observed in the result above were within the range of egg-weight documented by Eghi *et al.* (2013): that eggweight in the genetic groups ranges from 32.29±0.27 to 43.15±0.23, but not in accordance with the result of his experiment which concluded that frizzle and nakedneck native fowls laid heavier eggs than their normal feathered counterpart, because the result of this experiment showed otherwise. Nevertheless the average eggweights in the current research work are in accordance with 38.00, 38.50 and 39.90 reported for Sudanese native fowls. The result of this experiment showed that were no significant variations in albumen-heights and weights across the genotypes which does not corroborate with the report from Eghi *et al.* (2013) that breeds have significant effect in albumen-height and weight. It was further stated in that experiment that frizzle and nakedneck genetic group had better albumen quality than their counterpart the normalfeathered. Furthermore, all yolk characteristics which are yolkweight, yolkheight, yolkwidth, yolkcolour and percentage had no statistical difference ($p>0.05$) across the genotypes except for yolkindex. This observation contradicts the report of Eghi *et al*. (2013) that there were significant variations in the characteristics looked out for across the genotypes when comparing two or the three genotypes in study. Moreover, the results concurred with the finding of Rajkumar *et al*. (2009) who compared normalfeathered and nakedneck chickens but had significant variations in yolkindex, yolk-colour and height. Yolkindex and Haugh-unit have been reported to be the best assessors of internal egg quality.

5.4.3 External Egg Quality Parameters

The shellthickness of eggs produced by the normalfeathered and frizzlefeathered was thicker significantly than those of nakedneck native fowls. Average thickness of shell were 0.45mm, 0.45mm and 0.40mm respectively.

Fraga *et al.* (1989) gathered that eggshell quality of nakedneck could be linked to a elevated Cholecalcipherol synthesis from deposition of 7-dehydrocholesterol on nakedneck fowls in body parts that lack feather, thus turning the receiver of the non-direct radiation from the sun. Nonetheless, eggs with thick shellwall are able to resist externally inflicted pressure and as such preventing egg breakage and this is a good monetary attribute for profit-making poultry farmers, consumers and hatchery operators. The values generated in this work are greater than those reported by Momoh *et al*. (2010). They gave exertions that nakedneck produced heavier shell weight than the other native fowls in Nigeria. Results gathered in this research are comparable to that of light ecological types (Momoh *et al*., 2010) but values to some extent lesser than the ones given by heavy ecological types.

Significant positive correlation between egglength and eggweight, eggwidth and eggweight, shellweight and eggweight, shellweight and eggwidth, eggindex and eggwidth, in the strains compared favourably with the reports of Omeje, (1985). In this research work, eggwidth was identified to be a good estimator of eggshape index. Eggshape index could be utilized as one of the criteria for determining stiffness of eggshell (Omeje, 1985).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

In-ovo groups are better protected than the post-hatch groups, i*n-ovo* vaccination will be more appropriate in the meat-type chicken that will be slaughtered at an early age of between 8 to 12 weeks.

Bodyweight gains and antibody titres were correlated negatively for both vaccination groups and route of vaccination is genotype specific because as BWG increases, GMAT decreases.

Also, routine vaccinations against Newcastle disease must be carried out at not more than every three months in the NICG.

Haematological and serological profiles of the examined NICG are not the same, FF and NN genotypes were superior to NF in terms of live bodyweight gains.

The internal egg quality parameters of the examined NICG were not the same.

Therefore, FF and NN genotypes should be incorporated into our livestock improvement programmes because of their exceptional production performances conferred by the major genes they possess.

6.2 Conclusion and Recommendations

Vaccination at *in-ovo* and post hatch stages offered protection to the NICG against ND, with a better protection from *in-ovo* vaccination. Vaccination against ND significantly improved % HDP of the NICG hens and other production parameters.

Also, it is recommended that routine vaccination programmes be incorporated into our rural poultry production so as to offer better protection to them against viral diseases and equally enhance their production potentials.

CONTRIBUTIONS TO KNOWLEDGE

- *In ovo* vaccination offered better protection to the NIC against ND, but stage of vaccination is genotype specific

- *In ovo* vaccination will be more appropriate in the meat-type chicken that will be slaughtered at an early age

- Body weight gains and antibody titres are negatively correlated for both stages of vaccination

- Routine vaccinations against Newcastle disease must be carried out at less than or exactly 12 weeks in the indigenous chickens.

- Internal and external egg quality parameters of the indigenous chickens were similar except for shell thickness and shell weight.

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