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Hemogram and antibody profiles of local and broiler chickens under different vaccination programs

T. I. OJIEZEH[1*], V. O MORKA[2], and P. A OKIKI[3]

[1]*Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria*
[2]*Department of Medical Laboratory Science, Achievers University, Owo, Ondo State, Nigeria*
[3]*Department of Biological Science, College of Sciences, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria*

Abstract

The assessment of maternal immunity of poultry birds transferred to young chicks was carried out using both local breed and broiler chickens, with an objective to determine the antibody level pattern and hematological parameters of chickens in the first thirty one (31) days of life. One hundred and twenty birds were used for the study. Ninety (90) broilers and thirty (30) local breed chickens were grouped into four of 30 birds per group. Group 1 was local breed chickens only (LC), group 2 was vaccinated with "Primer" - Newcastle disease virus (SIM), group 3 was vaccinated with Primer and booster (Lasota) dose of Newcastle disease virus (DIM) and group 4 was non vaccinated broilers (NIM). Chickens were fed with standard feeds and adequate water ad libitum. Venous blood samples were collected from the groups at every 72 hour interval using standard methods. The leukocyte count was higher among the groups, packed cell volume was unstable, but increased gradually with age, heterophil / lymphocyte (H/L) ratio was decreasing with age among groups. Unlike NIM and SIM, Immunoglobulin Y was raised following vaccination on day 21 in DIM group; it was gradually increased in LC with age. Immunoglobulin M was not significant between the groups. The maternal antibody in LC and DIM were statistically significant but there was no significant difference in hematological parameters of the birds used in this study irrespective of the species. NDV vaccination had no effect on packed cell volume and hemogram. H/L ratio and antibody level decreased with age, given that the birds were not immunologically challenged.

Keywords: Broilers, Hemogram, Local breed, Maternal immunity, Newcastle disease virus

*Correspondence author: Tel: +2348027120080
E-mail address: pastortksm@gmail.com

Introduction

Newcastle disease (ND) is caused by virulent strains of avian paramyxovirus type 1(APMV-1) serotype of the genus Avulavirus belongs to the subfamily Paramyxovirinae, family Paramyxoviridae. The paramyxoviruses isolated from avian species have been classified by serological testing and phylogenetic analysis into ten subtypes designated APMV-1 to APMV-10 (Miller et al., 2010a); ND virus (NDV) has been designated APMV-1 (Alexander and Senne, 2008b). One of the most characteristic properties of different strains of NDV has been their great variation in pathogenicity for chickens. Strains of NDV have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens (Alexander and Senne, 2008b).

Immunity is the ability to resist the entry, manipulation and colonization of harmful microorganisms in a host system. In other words, it is the capability of the body to resist harmful microbes from infecting it (Janeway et al., 2001).

The immune system defence against foreign cell, this may be in the form of an invading organism or an abnormal body cell. There are two major types of responses in the bird's immune system; the specific, consisting of the humoral and cell mediated response, and the nonspecific responses. The humoral and cell mediated responses need a processed antigen to stimulate a response, and their response is to create a specific antibody for each particular antigen (Alberts et al., 2007). The nonspecific immune system responds to all antigens through the surface and layered barrier mechanisms of the body. Passive immunity is acquired through transfer of antibodies or activated T-cells from an immune host, and is short lived -usually lasting only a few weeks or months (Soares, 2008). Active immunity is induced in the host itself by antigen, and lasts much longer, sometimes lifelong (Janeway et al., 2001). Animal immune systems continually adapt to changing pathogen challenges and several studies suggest that immune function plays a major part in the life-history (Sheldon and Verhulst, 1996). The acquired (passive) immunity protects the offspring against pathogens, which the hens have encountered in their environment (Klasing and Leshchinsky, 1998). Immunization is often required shortly following birth to prevent diseases such as tuberculosis, infectious bronchitis and infectious laryngotracheitis. However, maternal antibodies can inhibit the induction of protective vaccine responses throughout the first year of life. This effect is usually overcome by secondary responses to booster immunization (Lambert et al., 2005). Birds hatch with an incomplete immune system. Maternal immunity is passed on in the amniotic fluid and the yolk of the egg (Wakenell, 1999).

There are three principal classes of immunoglobulins in birds: Immunoglobulin M, Immunoglobulin Y (the ancestor of mammalian IgG and IgE) and Immunoglobulin A. These classes of immunoglobulins are produced by B-lymphocytes that develop in the Bursa of Fabricius. In the chicken, bursal rudiments appear between embryonic day 3 and 5 as a dorsal evagination of the cloacal wall and epithelial folds appear on day 10 as documented by Oláh et al. (1986) and other researchers (Glick, 1995; Paramithiotis and Ratcliffe, 1996a cited by Kirk and Leshchinsky,1998). The bursa becomes seeded with precursors between embryonic

day 7 and 15. Within the bursa, B cells undergo gene conversion to diversify the variable region of their immunoglobulin genes and then begin to proliferate. The half-life of IgY in hatching chickens and in Blue and Gold Macaws is about 4 days, and maternal IgY is virtually undetectable by 4 weeks of age. Maternal immunoglobulin Y has been demonstrated to be effective in protecting the chicken against a variety of different bacterial, viral, and parasitic challenges during the first week or two post-hatch (Shawky et al., 1993; Shawky et al., 1994; Smith et al., 1994; Sugita-konishi et al., 1996; Ling et al., 1998), but its protective capacity is in question in Psittaciformes (Ritchie et al., 1992; Phalen et al., 1995). These antibodies give the newly hatched chick's immunity a start, while their own system is developing, thereby protects them from infection. Therefore, our objective was to compare the maternal antibody level and hematological profile of poultry birds in the first thirty one (31) days of life between the local chickens and the broilers, using immunoglobulins Y and M as classes of antibody that may be transferred from mother hens to young chicks and assessment of peripheral leucocyte count, H/L ratio and PCV parameter to ascertain the hemogram.

Materials and Method

Source of experimental birds

A total of one hundred and twenty (120) chickens was used for this experiment, Ninety (90) were day old broiler chickens from Obasanjo farm, Oluyole estate, Ibadan and Thirty (30) were day-old chicks from local hens of free range mothers in Owo, Ondo State.

Grouping and treatment of experimental birds

The chickens were divided into four groups of thirty (30) chickens per group after 3 days of acclimatization. Group 1 was the local breed only (LC); Group 2 was vaccinated with Newcastle disease virus "Primer" only on day 1 (SIM), Group 3 was vaccinated with Primer on day 1 and Lasota (booster dose) on day 21 with Newcastle disease virus (DIM) and group 4 was non vaccinated broilers (NIM).

The chickens were housed in battery cages of size 0.304 m^2/bird as recommended by Mustafa et al. (2010), maintenance and treatment of birds were in accordance with the principle of the "Guide for the care and use of laboratory animals in research and Teaching" prepared by the National Academy of Science and published by the National Institute of Health (National Research Council, 1985). The ethical approval was obtained from ethical and research committee, Achievers University, Owo. The chickens were reared under standard management practices with wood shaving and were administered oral antibiotic containing Tylosine –tartrate (Tyloretplus; Globeret, England) and vitamin A and C. During the entire study, the broilers were fed with feeds compounded carefully to meet 23% crude protein (CP) and 3200 Kcal metabolizable energy (ME) for broiler starter and 20% CP and 3000 Kcal for broiler finisher (table 1). It was ensured that the levels of mycotoxins in feed were maintained relatively low throughout the experiment, the quality and quantities of groundnut cake (GNC), soya bean and rice bran included in the feeds were the same for both starter and

finisher mash. The percentage CP in feed and ingredients were determined by the burette method (Ranjna, 1991), while the ME was determined by the Bomb calorimeter method (AOAC, 1990).

Blood Sample Collection and hematological Analysis

Venous blood samples from the wings were collected from day 4 and at every 72 hour interval into EDTA bottle and plain bottles for the assessment of hematological parameters and immunoglobulin level respectively. The samples in EDTA bottles were processed for white blood cell count (WBC) and differential counts. Total leucocyte and differential counts were obtained by the technique described by Fudge (2000), following the staining procedure described by Lewis et al. (2000), and packed cell volume (PCV) was determined by the method described by Jain (1986).

Immunological analysis

The blood samples collected in plain bottles were allowed to cloth at room temperature of 28 ± 10 ºC for 2 hours and centrifuged at 2000 rpm for 10 min using Gallen kamp bench centrifuge and the sera thus formed was then separated into sterile plain bottles and frozen at 40 ºC until required for analysis. Serum of each bird was separated and analyzed for levels of antibody titre using HA-HI technique (not reported in this paper) and immunoglobulins (IgY and IgM) using Immunological analyzer (ELISA). The parameters were measured as a batch from each serum using immunoassay methods as described by Clarke and Dufour (2006).

Statistical analysis

The results presented are means ± SEM of five independent determinations. Results obtained from this study were statistically analyzed using one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using SPSS 17.0. Significant differences between the treatment means were determined at 95% confidence level.

Table 1: Gross composition of experimental diets for broiler chicken

Ingredient	Starter diet (%)	Finisher diet (%)
Maize	55.00	55.00
Groundnut cake (GNC)	30.10	30.00
Soya bean meal	9.10	5.00
Fish meal (72%)	2.00	1.50
Bone meal	3.00	3.00
Common salt	0.25	0.25
Lysine	0.20	0.20
Methionine	0.20	0.20
*Premix	0.25	0.25
Wheat offal	-	4.60
Crude protein (%)	23	20
Metabolizable energy (KJ/Kg)	3200	3000

Good quality GNC was used to prepare feed for the birds.

*Premix - Vitamin/mineral premix provided the following vitamin and minerals per kg of diet: A, 10,000 I.U.; D3, 300 I.U.; E. 8.0 I.U.; K, 2.0mg; B1, 2.0mg; B6, 1.2mg; B12, 0.12mg; Nianin 1.0mg; Panthothenic acid, 7.0mg; Folic acid, 0.6mg; Cholic, 500mg; C, 10.0mg; Fe, 60mg; Mn, 80mg; Mg, 100mg; Cu, 8.0mg; Zn, 50mg; Co, 0.45mg; I, 2.0mg and Se, 0.1mg.ANUPCO, Anglican Nutrition Products Company, England

Results

The white blood cell count (10^3/µl) of broilers and local chickens are shown in Table 2. The values were analogous in the first 4 to 10 days of life, the count was within 19–23 and it gradually increased with age. The values were unstable in all the groups for the 31 days of study.

The lymphocyte count (%) of broilers and local chickens are shown in Figure 1. The count was observed to increase with age in all the groups. The chickens recorded higher lymphocyte values at day 31, LC (69.0 ± 1.0), DIM (60.6 ± 0.1) and NIM (45.03 ± 0.3) respectively. There was a statistically significant difference in lymphocyte count of LC at *P* f< 0.05 when compared with the broilers. Lower values were recorded on day 10 for DIM and SIM (30.6 ± 0.0) and NIM (32.0 ± 6.0).

Table 2. Total white blood cell count (103/µl) of broilers and local chickens in the first 31 days of life

	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22	Day 25	Day 28	Day 31
Local breeds	35.0	41	41.0	48.0	58	51.0	49.0	49.0	58.0	69.0
Single Immunized	43.3	40	30.6	42.0	48	56.6	46.0	55.3	50.6	52.0
Double immunized	43.3	40	30.6	42.0	48	56.6	50.0	52.0	54.0	60.6
Non immunized	42.0	36	32.0	35.3	34	40.0	38.6	40.3	41.3	45.3

All values are means ± SEM of 5 values per group. Values in the same row carrying different superscripts are significantly different at $P < 0.05$.

Table 3. Immunoglobulin Y (mg/ dl) of local chickens and broilers

Treatment groups	Local chick	Non Immunization	Single Immunization	Double Immunization
Day 4	1.02 ± 0.01	0.99 ± 0.01	1.02 ± 0.02	1.01 ± 0.02
Day 7	1.04± 0.01	1.02 ± 0.02	1.06 ± 0.00	1.08 ± 0.00
Day 10	1.00 ± 0.10	0.89 ± 0.01	1.21 ± 0.01	1.21 ± 0.01
Day 13	1.36 ± 0.01	1.00 ± 0.00	1.24 ± 0.40	1.24 ± 0.40
Day 16	1.71 ± 0.01	1.01 ± 0.01	1.29 ± 0.01	1.30 ± 0.01
Day 19	1.38 ± 1.01	1.14 ± 0.01	1.32 ± 0.01	1.34 ± 0.01
Day 22	1.54^{a} ± 0.02	1.07 ± 0.01	1.14^{c} ± 0.11	1.30^{b} ± 0.30
Day 25	1.60^{a} ± 0.01	1.07 ± 0.01	1.11^{c} ± 0.11	1.42b ± 0.02
Day 28	1.63^{b} ± 0.03	1.19 ± 0.01	1.30^{c} ± 0.01	1.66^{a} ± 0.01
Day 31	2.42^{a} ± 0.02	0.33 ± 0.03	1.29^{c} ± 0.02	1.60^{b} ± 0.33

All values are means ± SEM of 5 values per group. Values in the same row carrying different superscripts are significantly different ($P < 0.05$)

Table 4. Immunoglobulin M (mg/dl) of Local chickens and broilers

Treatment groups	Local chick	Non Immunized	Single Immunized	Double Immunized
Day 4	0.42 ± 0.02	0.31 ± 0.01	05.37 ± 0.01	0.37 ± 0.01
Day 7	0.58 ± 0.01	0.46 ± 0.01	0.38 ± 0.01	0.38 ± 0.01
Day 10	0.35 ± 2.01	0.42 ± 0.02	0.38 ± 0.01	0.38 ± 0.01
Day 13	0.35 ± 0.02	0.36 ± 0.01	0.52 ± 1.02	0.52 ± 1.02
Day 16	0.46 ± 0.01	0.35 ± 0.02	0.33 ± 0.01	0.33 ± 0.01
Day 19	0.51 ± 1.00	0.47 ± 0.01	0.49 ± 0.01	0.49 ± 0.01
Day 22	0.45 ± 0.01	0.38 ± 1.01	0.31± 0.01	0.31 ± 0.01
Day 25	0.35 ± 0.02	0.38 ± 0.02	0.31 ± 0.01	0.40 ± 0.01
Day 28	0.41 ± 0.01	0.38 ± 0.01	0.31± 0.01	0.40 ± 0.01
Day 31	0.43 ± 0.01	0.35 ± 0.02	0.35 ± 0.01	0.32 ± 0.02

All values are means ± SEM of 5 values per group.

The H/L ratio of LC and broilers are shown in Figure 2. There was a reduction of the H/L ratio with age in all the groups, except NIM that was relatively stable. Although, the values recorded within the groups was statistically insignificant at $P < 0.05$. The first 4 to 13 days of life showed higher values. At day 10, DIM recorded (1.95), SIM (1.96), NIM (1.76) and on day 4, LC was 1.41.

The PCV (%) was unstable in the entire group in the first 4 -13 days of life but gradually increased with age. The values were analogous with the control group (NIM). The chicks recorded higher values at day 31, DIM (32.0 ± 1.0), SIM (30 ± 1.0), NIM (31.0 ± 0.5) and LC (31.5 ± 1.5) as shown in figure 3.

The values of IgY in the test birds were generally low in all the groups. The gradient tends to increase with age in LC and in DIM following booster dose at day 21. There was a statistically significant difference in the values of IgY in LC and DIM ($P < 0.05$) and between DIM and SIM ($P < 0.05$), as shown in table 3 below. The IgM of the LC and broiler chickens for the first 31 days of life is shown in table 4. The values of IgM observed in the studied birds (all the groups) were statistical insignificant ($P > 0.05$). The values of the LC, DIM and SIM and were analogous to the control group (NIM).

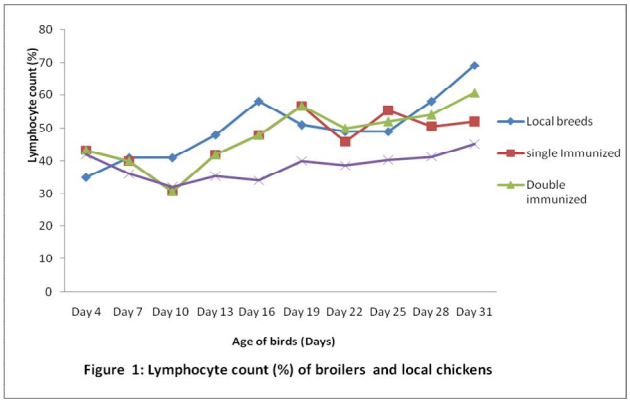

Figure 1: Lymphocyte count (%) of broilers and local chickens

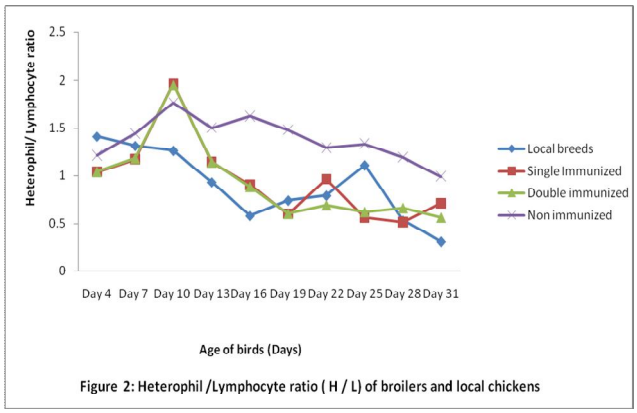

Figure 2: Heterophil /Lymphocyte ratio (H / L) of broilers and local chickens

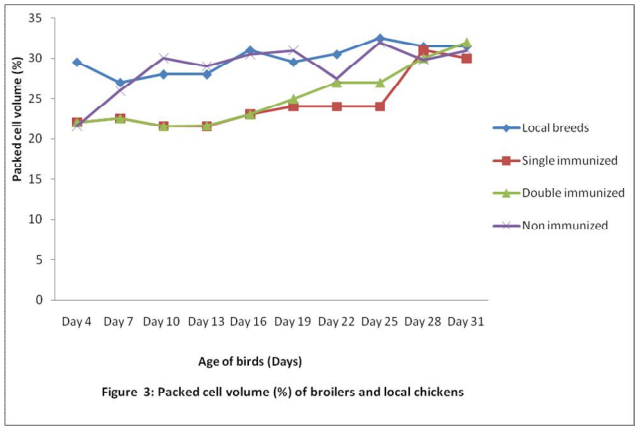

Figure 3: Packed cell volume (%) of broilers and local chickens

Discussion

An attempt was made to determine the hemogram and Newcastle Disease antibody in broiler chickens and locally breed chickens. The hen antibody transferred to young chick was studied using classes of immunoglobulins (Y and M), and the total white blood cell count and differential profiles; H/L ratio, was used to determine the cell mediated immunity transferred; Packed cell volume was used to determine the functionality of the young chick hematopoetic system.

The significant increase in leukocyte count of the birds indicates that the birds were responding to immunologically sensitization following immunization. Since the chicks were young in age, it may also be a physiological adjustment against environmental factor. The total white blood cells count recorded in the present study from the local chicks and broilers were slightly higher than the values reported by Alabi et al. (2008) for broiler finishers fed control diets. It could be that the supplements impacted the hematopoetic function of the birds, and the variation may be related to physiological adjustment since they were all young chick. Our finding is in agreement with the work of Ogbuewu (2008) and Emenalom et al. (2009), who in their reports documented that diets affect blood profile of animal, and the haemogram of birds varied slightly with treatment.

Differential leucocytes were used as indicators of stress response and sensitive biomarkers crucial to immune functions (Graczyk et al., 2003). It has however been reported that bacterial and viral diseases affect the number of white corpuscles and the ratio between the different types of white corpuscles and the percentages of the various types in healthy animals vary slightly, but are greatly modified in sick animals (Jean, 1993; Uchegbu et al., 2010). The WBC count in the first 4 to 7 days of life in all the groups may be due to the underdeveloped hematopoitic system. Though, the count was akin with the control group (NIM) and with that of the normal ranges mentioned by Bell and Sturkie (1965), who stated that the normal values of hematological parameters are as follows: PCV = 30.6 %, WBC = 20-30 thousands/mm^3.

Vaccination had no effect on the PCV and hemogram profile; there was an increase in PCV and lymphocyte count with age, thereby giving reduction of H/L ratio with age. These values were analogous

with the values of NIM and LC. Meanwhile, the values of WBC count were analogous within the groups, but there was a statistically significant difference in lymphocyte count ($P < 0.05$) in local chicks when compared with the broilers. These may not be related to the incomplete development of the hematopoitic system of the broilers. An increasing practice among ornithologists is the use of blood smears to assess innate immune function in birds by counting the numbers and proportions of white blood cells (leukocytes) on blood smears, a leukocyte profile (or differential) can be obtained for the individual, giving insight into its immune function at the time of sampling (Davis et al., 1994). The ratio of two leukocyte types, heterophils/lymphocytes (H/L ratio), has been increasingly used by ornithologists to monitor immune function, as it appears to increase with disease (Davis et al., 1994), injury (Vleck et al., 2000) and urbanization (Ruiz et al., 2002). The study showed a reduction in H/L ratio with age in the entire groups. The secondary dosage (LaSota) following the primer was to boost cell mediated and non-cellular responses of the birds, which in turn enhanced antibody production in DIM group.

Immunoglobulin Y generally confers immunity to a patient so far as that particular disease is concerned. The results revealed that the serum IgM of broilers (NIM, SIM and DIM) groups were significant low when compared with that of local breed. This finding further corroborate the report of Hamal et al. (2006) who stated that the level of antibody transferred from hen to progeny is between 27 and 40% and it is directly related to titres in the hen. It is very likely that IgM will be raised in concurrent infections. Serum IgY concentration in vaccinated groups increased with booster dose at day 21, which is an indication of reinforcement of immune system of the birds against possible NDV infection. This observation is not at variance with the reports of other researchers (Chinnah et al., 1992; Pugh 2001; Im et al., 2005; Boudreau and Beland, 2006). The vaccine may have stimulated humoral responses in the broiler chickens. The increase in IgY in the DIM group could be explained by the principle of active immunization against infectious diseases in the management and control of viral infections in poultry birds. Maternal antibody in local chickens was consistently high despite the fact that they were not vaccinated against NDV at all. It is very likely that the adaptive immune responses have been well developed in local chickens following maternal antibody passed on to the young chickens from the breeders, who might have built up enough antibody against NDV as a result of exposure to environmental factors, as infection and immunity are inseparable. This finding is in agreement with the report of Apanius (1998), who stated that maternal antibodies are transferred across the follicular epithelium into the yolk during oogenesis. The enhanced antibody in DIM chickens following day 21 implies that the booster dose vaccine impacted the broilers. It is therefore suggested that further studies be carried out to monitor closely environmental factors, and dietary constituents of feed and ruminants picked-up by the free range birds, perhaps, the study may be able to identify some of those constituent that contain antiviral properties capable of enhancing the immune responses of the local birds.

Conclusion

The anti NDV antibody derived from the hen provides protect for the young chicks but for how long. It is expected to have a protective effect on the defence less young species at least for a period of time. The level of IgY local breed may be due to the adaptive immunity following maternal antibody passed – on to the chicks. The IgY pattern in SIM and DIM groups were related to vaccine regimen (primer and booster dosages). NDV vaccination had no effect on PCV and hemogram, and in this circumstance, H/L ratio was decreasing with age, given that the birds were not infected with NDV irrespective of species of the birds.

Conflict of Interests

The authors do not have a direct financial relationship with the commercial identity mentioned in this paper.

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