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ORIGINAL ARTICLE

Effect of Newcastle Disease Virus (Kudu 113) Infection on Calcium Metabolism in Response to Endocrinological Changes in Commercial Layers

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SUMMARY
The effect of experimental Newcastle disease virus (NDV) infection on some aspects relating to endocrinology of calcium metabolism, which affects eggshell quality and production, was studied. Twenty 22-week-old ISA brown layers vaccinated against Newcastle disease (ND) were allocated to infected and uninfected groups of 10 chickens each. The chickens in the infected group were inoculated intranasally with 0.1 ml of NDV (Kudu 113 strain). Blood samples were collected every other day for the first week, weekly for 5 weeks and analysed for haematology and serum chemistry. Clinical signs such as anorexia, ruffled feathers, greenish diarrhoea, misshapen, small-sized and white-shelled eggs were observed in the infected chickens from day 3 post infection (pi). The eggshell abnormalities were observed in the 2nd and 3rd week pi, which subsequently normalized. The mean packed cell volume (PCV) in the infected chickens on day 6 pi was significantly lower ($P<0.05$) than that of the uninfected. There was a significant difference in plasma calcium levels between both groups. There were significant decreases in the concentrations of calcium and phosphorus from onset to day 18 and then an increase in phosphorus on day 25. Plasma oestrogen activity showed a significant increase ($P<0.05$) from onset to day 18 and then decreased on day 25 pi. The activity of parathormone also increased progressively until day 32 pi. From this study, ND induced decline in plasma calcium and phosphorus levels which triggered an increase in oestrogen and parathormone activity with a consequent rise in plasma calcium and phosphorus levels.
Key words: Newcastle Disease, Calcium, Oestrogen, Parathormone

INTRODUCTION

The Nigeria poultry industry contributes the largest to the national economy through revenue next to the oil industry (Nnadi and George, 2010). This industry has become increasingly organized, specialized and integrated into an industry of major national and international importance but faced with myriads of challenges including diseases such as Infectious Bursal Disease

(Gumboro), fowl cholera, coccidiosis, salmonellosis, infectious laryngotracheitis, fowl pox, chronic respiratory disease, Marek's Disease, egg drop syndrome, infectious bronchitis, avian influenza, Newcastle disease and so on (Oluwayelu *et al*., 2005; Adene and Oguntade, 2006).

Newcastle disease (ND), a highly contagious viral disease, affecting wild and domestic avian species of all ages, caused by Newcastle disease virus (NDV) belongs to the genus Avularvirus, subfamily Paramyxovirinae, family Paramyxoviridae (Gogoi *et al*., 2017). Varying lesions including eggshell and skeletal abnormalities are major findings in ND (Bwala *et al*., 2011 and Anonymous, 2014). This associated deformity could be related to calcium concentrations in the blood; to the best of my knowledge, there is a dearth of information on the effects of the disease on aspect relating to calcium metabolism.

Calcium is an important macro-element that is absorbed and released by the intestine, bone and kidney with its homeostasis maintained by parathyroid hormone (PTH), calcitonin, oestrogen, thyroxin and vitamin D (Veum, 2010). Oestrogen promotes the formation of vitellogenins in the liver which are lipoproteins that are incorporated into the egg yolk. They bind calcium and their production is followed by a rise in serum calcium levels (Wistedt *et al*., 2012). PTH is a protein hormone released by the parathyroid gland and controls blood calcium and phosphate levels (Veum, 2010). However, the calcium required for eggshell production and normal skeletal system development is mainly obtained from increased intestinal absorption and reservoir found in the medullary bone, and the homeostatic control of this process involves oestrogen, parathormone and calcitonin activities (Webster *et al*., 2004; Jonchere *et al*., 2012). Suggestive, therefore, that egg and bone lesions associated with ND disease could be linked to calcium metabolism. Hence, this study is aimed at understanding the effect of infection with NDV on some endocrine functions as it relates to calcium metabolism in vaccinated commercial layers.

MATERIALS AND METHODS

Chicken

Twenty 18-week old ISA brown layers used for this study were obtained from a commercial farm in Zaria. They were fed commercial grower mash for four weeks after which they were fed layer mash till the end of the research at 6 weeks and water was provided *ad libitum*.

Challenge Virus

NDV (Kudu 113), the velogenic viscerotropic type was obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria.

Experimental Design

At 22 weeks old, the chickens were allocated at random into two groups (infected and uninfected) of 10 birds each. Each of the chickens in the infected group was intra-nasally inoculated with 0.1 ml of the NDV inoculum (virus titre of $10^{8.0}$ EID_{50} per ml). At 2days pi, 2ml of blood was collected through the brachial vein every other day from every five birds selected at random from both groups in the first week and then weekly in the next five weeks. About 0.5 ml of blood from the heparinized tube was used for determination of PCV while the remaining 1.5 ml was centrifuged to obtain plasma.

Biochemical Analysis

The plasma obtained was used to evaluate concentrations of calcium and phosphorus using SPACE analyzer (Randox) following atomic absorption spectrophotometric technique while oestrogen and parathormone activities were measured using CAYMAN ELISA kit (catalogue no 58225) and

ABNOVA ELISA kit (catalogue no KA0924) respectively.

Statistical Analysis

The data generated were analyzed using Graph Pad Prism Version 4.00 for Windows, Graph Pad Software, San Diego California USA. Mean ± SE of the mean for each variable was calculated. Pearson's coefficient of correlation was used to compare variables in the infected group while Student T-test was used to compare variables between the infected and uninfected groups. Values of $P<0.05$ were considered significant.

RESULTS

Haematology

The mean PCV for the two groups are shown in Figure 1. The mean PCV for both groups remained relatively unchanged until day 6 pi when it significantly decreased in the infected group from 44.00 ± 0.7 % to 41.20 ± 0.7 %. The mean PCV in the infected group then rose and fluctuated between 41.2 and 41.4 %, a consistently lower range ($P>0.05$) than that in the uninfected group.

Biochemical Parameters

The mean plasma calcium concentrations for both groups are shown in Figure 2. The mean plasma concentration of calcium in the infected group was higher (2.55 ± 0.04 mmol/L) and statistically significant than that in the uninfected group which was 2.40 ± 0.01 mmol/L from day 2 pi. Thereafter, it decreased gradually from 2.55 ± 0.04 mmol/L on day 2 pi to reach a statistically significant low level of 2.37 ± 0.02 mmol/L on day 18 pi.

The mean plasma activities of oestrogen for both groups are shown in Figure 3. The activity of oestrogen was observed to increase in the infected group from 429.2 ± 19.68 pg/ml on day 2 pi to 515.0 ± 43.52 pg/ml on day 18 pi. Thereafter, there was a significant decrease on day 25 pi to 395.8 ± 20.22 pg/ml.

The mean plasma parathyroid hormone activities for both groups are shown in Figure 4. The mean parathyroid hormone activity increased ($P>0.05$) from day 2 pi value of 26.24 ± 2.18 to 30.28 ± 1.75 pg/ml on day 4 pi in the infected group. It stabilized on days 6, 11 pi and there was a statistically significant difference between both groups on day 18 pi. An increase in the infected group was observed from 25.96 ± 1.25 pg/ml on day 18 pi to 27.74 ± 2.02 pg/ml on day 25 which then subsequently decreased to 23.08 ± 0.79 pg/ml on day 32 pi.

The Pearson's coefficient of correlation for calcium and oestrogen in the infected group is shown on Table I. A negative correlation was observed on day 18 pi, which corresponded with a statistically significant low value of calcium (2.37 ± 0.02 mmol/L) associated with an increase in oestrogen from 465.4 ± 36.00 pg/ml on day 11 to 515 ± 43.52 pg/ml on day 18 pi. The activity of oestrogen also decreased significantly when the concentration of calcium increased on day 25 pi thus showing a negative correlation.

Pearson's coefficient of correlation for calcium and parathormone for the infected group is presented in Table II. On day 25 pi, there was a negative correlation between calcium and parathormone which resulted in an increase on day 25 pi in calcium concentration. Also, a negative correlation was observed on day 32 pi when the value for parathormone decreased following the increase in calcium concentration.

Table I: Pearson's coefficient of correlation of Calcium (Ca) and Oestrogen (E2) in ND infected hens

Days	2	4	6	11	18	25	32	39
Ca (mmol/L)	2.55±0.04	2.47±0.05	2.45±0.03	2.44±0.02	2.37±0.02	2.41±0.02	2.42±0.01	2.43±0.01
E2(pg/ml)	429.2± 19.68	477.4± 37.61	472.0± 15.72	465.4± 36.00	515.0± 43.52a	395± 20.22a	555.6± 40.07	461.2± 41.17
R- value	0.2430	0.6020	0.0244	0.0038	-0.2011	-0.6407	0.5433	-0.6728
P-value	0.69	0.28	0.96	0.99	0.74	0.24	0.34	0.21

a- negative correlation on days 18 and 25.

Table II: Pearson's coefficient of correlation of Calcium (Ca) and Parathormone (PTH) in NDV infected hens

Days	2	4	6	11	18	25	32	39
Ca (mmol/L)	2.55±0.04	2.47±0.05	2.45±0.03	2.44±0.02	2.37±0.02	2.41±0.02	2.42±0.01	2.43±0.01
PTH (pg/ml)	26.24±2.18	30.28±1.77	25.66±1.47	25.24±0.13	25.96±1.25	27.74±2.0a	23.08±0.79	25.08±0.01
R- value	-0.6367	-0.7757	-0.4276	0.6301	0.8775	0.8420	-0.7825	-0.4385
P-value	0.25	0.12	0.47	0.25	0.05	0.07	0.11	0.46

a- Negative correlation on day 25.

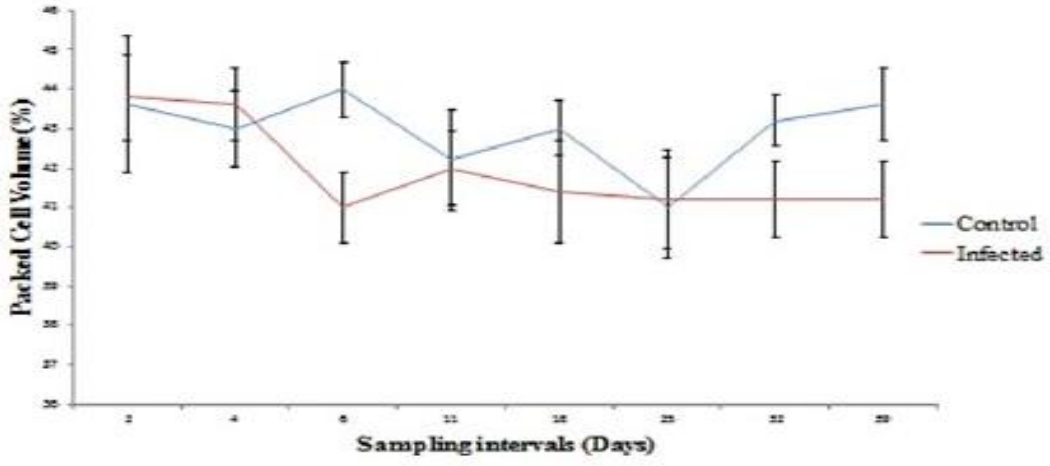

Figure 1: Packed Cell Volume of NDV Infected and Control Groups.

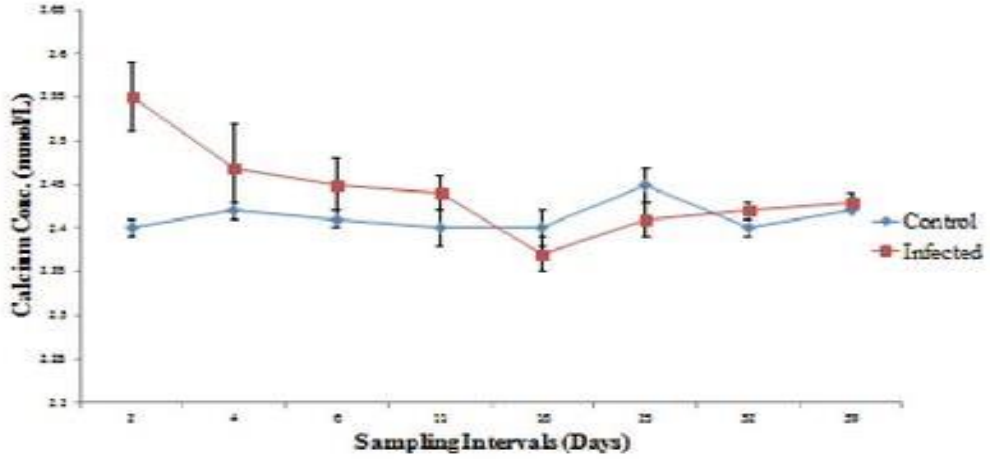

Figure 2: Calcium concentrations of NDV infected and control groups

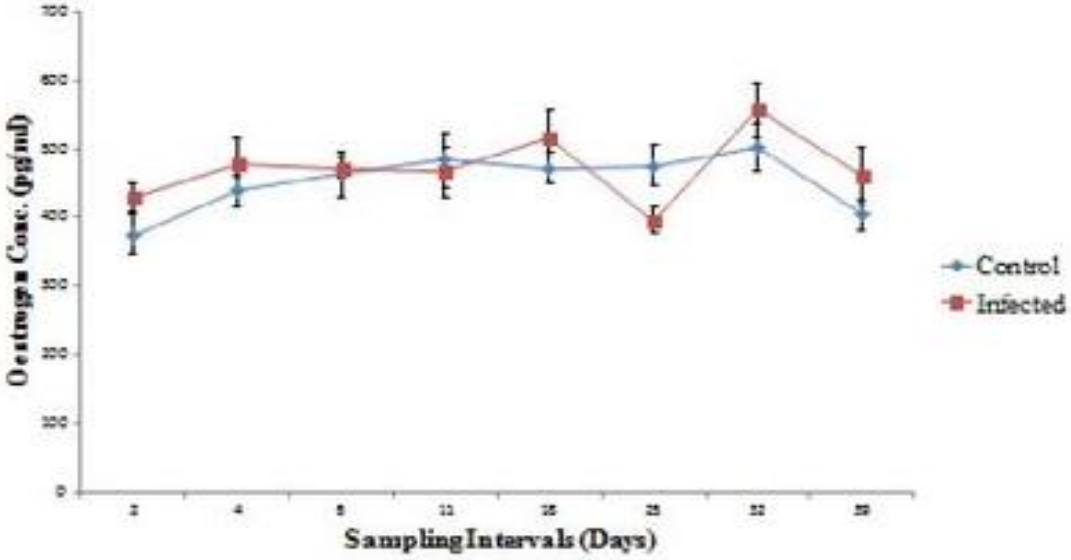

Figure 3: Oestrogen concentration of NDV infected and control groups

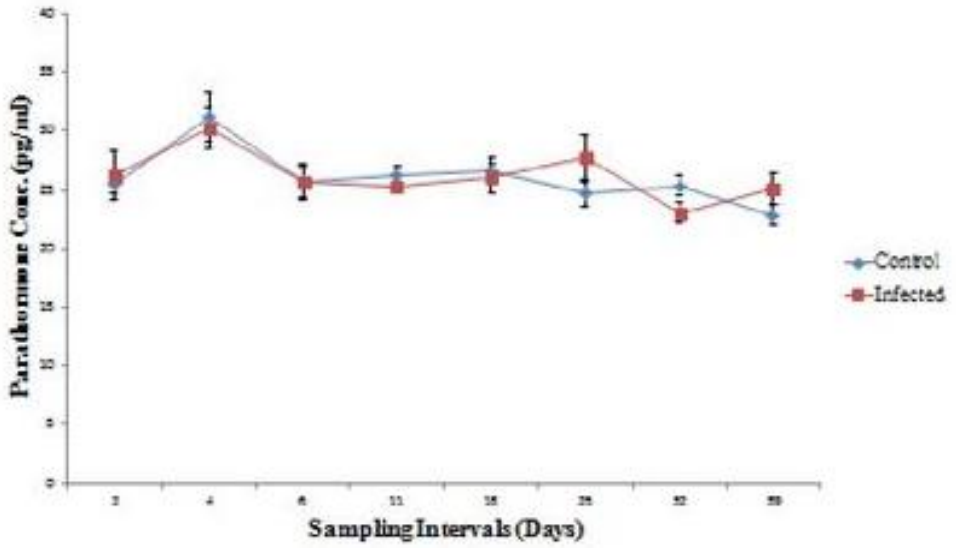

Figure 4: Parathormone concentration of NDV Infected and control groups

DISCUSSION

The incubation period of three days recorded in this study based on clinical signs had previously been reported for ND by other authors (Bwala *et al*., 2011; Badau *et al*., 2015). However, in this study, mortality was not recorded in the chickens challenged throughout the experiment and this could be attributed to the fact that the birds might have developed immunity following vaccines previously administered at the farm of purchase. A significant decrease in the mean PCV in the infected chickens on day 6 pi could be attributed to the onset of anaemia in the chickens, indicating the presence of the disease resulting from the effect of the virus. This finding is similar to that of Ruwaan *et al*. (2009) and (Oladele, 2004) who reported that the sialidase of the virus, desialidated the red blood cell surface sialic acid and resulted in decreased PCV, hence, anaemia in the infected chicken. Also, molecular studies had demonstrated that oestrogen inhibited erythroid gene expression, delayed progenitor cell maturation, and induced apoptosis in erythroid cell lineages *in vitro* (Perry *et al*., 2000). The mean plasma values for oestrogen in this study were consistently higher in the infected group from day 2 up to day 6 pi than in the uninfected group; but lower on day 11 pi, which probably resulted in the decrease observed in calcium levels on day 18 pi. But the increase in the value of plasma calcium on day 25 pi could be the result of an increase in the value of plasma oestrogen on day 18 pi. This agrees with the report by Shore and Shemesh (2003) that oestrogen is a steroid hormone produced by the developing follicles in the ovaries and plays an important role in egg formation and also increases calcium levels. The value for parathyroid hormone was consistently low in the infected group from day 4 up to day 18 as compared to the uninfected chickens. But on day 25 pi, the value was observed to increase as a compensatory response to the low level of calcium obtained previously on day 18 pi. This is similar to the report by Wittow (2000) who reported that the major physiological stimulus for parathyroid hormone secretion from the chief cells was a fall in plasma calcium concentration, while a rise in calcium suppressed it. The mean plasma calcium concentration in the infected group decreased gradually from day 2 Pi until day 18 pi. This observed decrease coincided with the period of egg

abnormalities; watery albumin, misshapen egg, cracked/soft-shelled egg and whitish coloured shell (Plate 1) seen in the eggs laid by the infected chickens. The decrease could be attributed to the neuroendocrine response to disease and stress induced by the viral challenge (Eiler, 2004) as it leads to the

Plate 1. Eggs from NDV infected hens with white shell (A) compared to uninfected hens with brown shell (B).

activation of the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal system with the subsequent peripheral secretion of cortisol and corticosterone that affects the metabolism and availability of calcium ions required for use in egg calcification. But on day 25 pi, the plasma calcium concentration in the infected group was increased to 2.41 ± 0.02 mmol/L and this could be attributed to the increase in plasma parathyroid hormone on day 25 pi, coupled with previously increased oestrogen level on day 18 pi.

From this study, the clinical signs, especially, the egg abnormalities observed in the infected vaccinated layers during the second and third week were mild compared to what is usually observed in unvaccinated chickens with the eventual cessation of these egg abnormalities. It is also evident that the presence of immunity against ND did not prevent the challenge virus from infecting and replicating in the host (Kapczynski and King, 2005; Miller *et al*., 2007).

CONCLUSION

Infection with NDV (Kudu 113 strain) in vaccinated layers caused mild form of the disease which included varying eggshell abnormalities, a decline in plasma calcium level with accompanying changes in eggshell qualities which possibly triggered a significant surge first in oestrogen and later in parathormone plasma activities with eventual increase in calcium concentration and phosphorus needed for proper eggshell quality.

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