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Influence of Delayed Feeding on the Performance, Development and Response of Immune System to Newcastle Disease Vaccination in Chickens

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Abstract: The influence of delayed feeding on the performance, development and response of immune system to Newcastle disease vaccination were investigated. 72 day old cockerel chicks purchased from a local hatchery were used. These were at the onset divided into two groups A and B of equal numbers (n = 36) and housed separately. Group A was provided with a diet containing 25% crude protein and 2800Kcal of metabolizable energy from the day of hatch while group B was deprived of feed for the first 72 h post hatch (ph) after which they were introduced to feed and thereafter maintained on same diet as group A. Once feeding is commenced in each of the groups, same regimen was maintained till the end of the study. Other brooding arrangements were common to the groups. 21 days ph 12 chicks were randomly selected per group, weighed, sacrificed after which two blood samples were collected for general haematology and serum biochemistry respectively. They were then necropsied and lymphoid organs as bursa of fabricius, spleen and caecal tonsil as well as liver harvested weighed and their relative weights calculated. The remaining 24 chicks per group were then vaccinated with Newcastle Disease (ND) vaccine lasota[®]. Five days later, 12 chicks were again randomly elected per group and same operations as on day 21 carried out on the chicks. On day 42 ph, all the remaining chicks in each of the groups were vaccinated against ND using ND vaccine komarov[®] and 5 days later, they were sacrificed and again all the activities of day 21 repeated. Our results showed that delaying feeding for up to 72 h ph does not significantly affect the growth, development and function of some organs of the immune system. Moreover, it also produced no effect on Packed Cell Volume early in life as significant variation between the two groups (p<0.05) was only observed on day 47 ph. However, our finding showed that early feeding significantly improved early response to vaccinations (p<0.05) shown by higher geometric mean antibody titre. There were no differences in organ morphology and histopathology between the two groups. The study demonstrated that early feeding could be beneficial in the response of chicks to early vaccinations but does not confer other productive advantage.

Key words: Delayed feeding, body and lymphoid organ weights, immune response

INTRODUCTION

Optimum productivity in poultry as in livestock establishments is governed by the genetics of the flock and general management including nutrition. According to Gross and Siegel (1988), a good husbandry should be a combination of genetics and environment that maximizes productivity and disease resistance at minimum resource cost. Attainment of this paradigm is however contradicted by the known inverse relationship that exists between such genetic traits as fast growth rate, improved feed efficiency and egg production and disease resistance (Parmentier *et al.*, 1996; Gross and Siegel, 1997). This has resulted in suboptimal immune response in such strains/breeds leading to an increase in the incidence of metabolic disorders, reduced resistance to infectious diseases and higher mortality. This low immune responsiveness of chicks breed for fast growth and egg production has been attributed to nutritional resource allocation that are usually prioritized

leading to increased susceptibility to diseases (Gross and Siegel, 1988; 1997; Dunnington and Siegel, 1996; Coop and Kyriazakis, 1999).

As in mammals, immunity in birds develops through the lymphoid system composed of bursa of Fabricius and thymus as primary organs which are relatively near to maturity at hatch. Also, secondary immune organs including spleen, caecal tonsils and the non lymphoid but important immune organ, the liver are also incomplete at hatch. The development of the avian immune system is among the factors believed to be influenced by the age the hatchlings are introduced to oral feeding. In the newly hatched chicks, the yolk provides the immediate post hatch energy and protein for growth and maintenance. The yolk is believed to be used up within the first four days of life. Under the traditional chick management it is also believed that young chicks should be deprived of feed until they have fully resorbed their yolk sac and to hasten its resorption

and that exogenous feed can only be given after its resorption (Beekbergen, 1994). It is also believed by the same author that such fasted chick usually show a compensatory growth that enable them to surpass those fed on the day of hatch. This according to some opinions involves a lot of mortalities and reduction in production efficiency (Knight and Dibner, 1998; Panda and Reddy, 2008). According to Uni and Ferket (2004) the system is also responsible for 2-5% mortalities associated with post hatch adjustments and loss of production efficiency. They also claimed that under this management system, many of the survivors showed stunted growth, inefficient feed utilization and reduced disease resistance. Moreover, Dibbner *et al.* (1998) have argued that while the survival of the hatchlings may depend on its use of the residual yolk as a nutrient source in the absence of other feed that this practice does not make for the optimum use of the residual yolk. It has been demonstrated that the development of the immune system in particular appears to respond to early feeding on the basis its provision of limiting substrates, effect of dietary nutrients on the endogenous levels of hormones and the stimulatory effect of early feed usually recognised as an antigen by the immune system (Schaffner *et al.*, 1974; Ekino *et al.*, 1980). Achieving the maximum potential of poult in the brood period accounts for as much as 70% of the final turkey performance.

However, some producers would withhold feed and water from their hatchlings until 12-24 h to allow them to mature and to initiate a vaccine response while the birds are under low immunological challenge from other antigens (Dibbner *et al.*, 1998). Panda and Reddy (2008) had reported that residual yolk was used up faster in chickens that had access to feed immediately after hatch than those fasted for 48 h.

Many investigators have examined the effect of feed restriction alone on various components of the immune system such as haematological values (Maxwell *et al.*, 1990; De Jong *et al.*, 2002) macrophage function (Afzal *et al.*, 1999; Stapleton *et al.*, 2001) antibody responses (Christadoss *et al.*, 1984; Klasing, 1988; Dong *et al.*, 2000; Khajavi *et al.*, 2003; El Hadri *et al.*, 2004).

In our tropical environment with limited hatcheries and scarce means of transportation, it is not unusual for the newly hatched chicks to stay between 48-72 h post hatch without feed. Here the effect of feed restriction on the performance, development and function of the immune system of the hatchlings are better imagined than assessed. This study was therefore designed to investigate the effect of delayed feeding on the productivity and development of immune system in chicken.

MATERIALS AND METHODS

Birds and their management: This study was carried out in the Poultry Unit of the Faculty Demonstration Farm,

Faculty of Veterinary Medicine, University of Nigeria, Nsukka. 72 day old cockerel chicks purchased from a local hatchery were used for the study. They were on reception divided into two equal groups A and B (n = 36) and housed separately in pens A and B. Due to their uniform body weights, allocations to groups was random.

Group A was provided with a diet containing 25% crude protein and 28000kcal/kg of metabolizable energy, a feeding regimen that was maintained till the end of the study. Water was also provided *ad libitum* during the period. Group B however, was deprived of feed for the first 72 h post hatch though given access to water, after which it was also maintained on the same diet as was started with group A. All the chicks were brooded and reared under the same environment and management conditions for the 47 day duration of the study. All the chicks were vaccinated against ND in the hatchery using Newcastle disease vaccine intra-ocular manufactured by Nigerian Veterinary Research Institute (NVRI).

Vaccination, live-weight and organ measurements: On day 21 post hatch, 12 chicks were randomly selected from each group after which they were weighed using a weighing balance to determine their live-weights in grams. Group mean live-weights and standard deviation were subsequently calculated. These were sacrificed after collecting two blood samples per chick from the jugular vein. A sample in an anticoagulant bottle was used for the determination of Packed Cell Volume (PCV) and leucocyte counts while the other sample in non anticoagulant containing bottle was allowed to clot and clear serum collected for the determination of serum biochemistry and serology. After blood collection, each chick was necropsied and lymphoid organs such as the bursa, spleen, caecal tonsils and liver were harvested and their weights also in grams determined. Thereafter, the relative weights of these organs calculated as a percentage of the live weights were determined followed by the calculation of their group means and standard deviation. Samples of these organs were preserved in Phosphate Buffered Saline (PBS) for histopathological processing and examination. The remaining twenty four chicks per group were further given the second dose of ND vaccine Lasota^R by NVRI according to the manufacturer's instruction. Five days post Lasota^R vaccination, 12 chicks were again randomly selected from each of the two groups and similarly treated as was done on day 21. All the remaining 12 chicks per group were reared under the same management regimen till day 42, when they were all given ND vaccine Komarov^R manufactured by NVRI and administered deep intra muscularly. Five days later, all the remaining birds were sacrificed as before. Blood samples, organ recovery and weighing as well as relative weights to live-weights were repeated as on days 21 and 27 post hatch. Similarly, serum samples were collected.

Haematology and serum biochemistry: The PCV was determined using the haematocrit method (Dacie and Lewis, 1995). The total and differential leukocyte counts were carried out using an improved Neubaur haemocytometer the method (Campbell, 1995). Furthermore, total serum protein was determined in each sample using the biuret method (Lumeiji, 1987) using the standard Randox diagnostic kit (Randox Laboratories, LED UK). Serum albumin concentration was determined using the bromocresol green method according to the method of Doumas (1971) using the standard Randox diagnostic kit. The serum globulin fraction was calculated by subtracting the value of the albumin fraction from the total serum protein (Nnadi *et al.*, 2007).

Serology and histology: Determination of antibody titre on the serum samples on the sampling dates was carried out using Haemagglutination Inhibition (HI) test as described by Beard (1989). Geometric Mean Titre (GMT) was determined as described by Villegas and Purchase (1989).

The organs collected on each day of investigation were fixed in 10% formal saline for a minimum of 24 h. They were processed, embedded in paraffin wax, sectioned, stained with haematoxyline and eosin and studied under the light microscope.

Statistics: Data on each parameter were arranged in groups and the means calculate. These were subjected to one way analysis of variance with time of feeding as the main effect using the General Linear Model (GLM) procedure of SAS users guide (2001).

RESULTS

Live weights and relative organ weights: The results of this study generally demonstrate that feed provision to the newly hatched chicks within the functional period of the residual yolk sac post hatch (3-4 days) does not significantly affect the early growth, development and function of some of the organs of the immune system.

Table 1: Mean live weight of the fasted and early fed chicks at the various days of investigation (gm)

Days of activity post hatch	Groups	
	A	B
21	202±4.5	194±1.9
26	260±7.6	255±8.0
47	350±5.7 ^a	321±9.8

This is shown by the fasted and early fed chicks having comparable values in most of the parameters that were assessed throughout the period of investigation. Table 1 below shows the mean live-weights of the two groups at days 21, 26 and 47 of study.

It was only on day 47 post hatch that group A had a significantly higher mean live weight relative to group B ($p < 0.05$).

For the liver and the lymphoid organs, our analyses showed that the values of their relative weights to the body weights were comparable for the two groups throughout the period of study. These are as shown in Table 2 below.

Haematology and serum biochemistry: The study also showed that the two groups had comparable PCV values on days 21 and 27. However, on day 47, the mean PCV for group A was significantly higher than the mean value for group B ($p < 0.05$).

Also, the values for the total leukocyte count, lymphocytes, monocytes and heterophils showed comparable values for the two groups throughout the period of study ($p > 0.05$).

Furthermore, analyses of serum proteins indicated that except on day 21 post hatch when group A had higher total serum protein value relative to B ($p < 0.05$), the two groups had comparable values on days 26 and 47. The two groups also had comparable serum albumin values within the three investigative days. On the day 21 post hatch, group A had a higher globulin value relative to B, a situation that was reversed on day 27 ($p < 0.05$) while on day 47 their values were comparable.

Table 2: Mean relative weights of some lymphoid organs and the liver from the fasted and early fed chicks (%)

Days of activity post hatch	Liver		Bursa		Spleen		Caecal tonsil	
	-----		-----		-----		-----	
	A	B	A	B	A	B	A	B
	3.05±0.25	3.08±0.24	0.51±0.14	0.48±0.16	0.19±0.11	0.08±0.04	0.15±0.09	0.16±0.05
	2.81±0.39	2.76±0.32	0.54±0.11	0.41±0.20	0.18±0.09	0.23±0.06	0.29±0.07	0.29±0.08
	4.43±0.5	4.44±0.65	0.53±0.21	0.48±0.23	0.13±0.10	0.12±0.08	0.31±0.12	0.32±0.13

Table 3: Mean PCV, total leukocyte count and lymphocyte count for the two dietary groups

Days of activity post hatch	PCV (%)		Total leukocyte count cm ³ /ml		Lymphocyte count cm ³ /ml	
	-----		-----		-----	
	A	B	A	B	A	B
21	31	30	16820	13440	15306	11827
26	30	31	6380	6940	5614	5552
47	24	21	4348	4648	3565	3718

Table 4: Mean values of the components of the serum biochemistry (mg/ml)

Days of activities post hatch	Total serum protein		Serum albumin	
	A	B	A	B
21	3.64±0.4	3.84±0.3	1.97±0.21	1.98±0.11
26	3.06±0.3	3.08±0.23	2.33±0.09	2.33±0.07
47	3.24±0.24	2.98±0.41	2.17±0.11	2.03±0.12

Serology and lesions: The result of our serological analyses showed that early feeding was beneficial both in preparing the birds for possible antigenic challenge and also their response to vaccination. Thus on day 21 and 27, their mean antibody GMT were 831 and 84 and 97 and 49 respectively. However, on day 49, this advantage has been lost as a result of feed normalization for three weeks with mean GMT values of 84: 81 for groups A and B respectively. Also, examination of tissue samples did not show any difference in the gross and microscopic morphology of the two groups.

DISCUSSION

Upholding the earlier observed benefits of early and adequate nutrition in the development of the young chick especially during the first few weeks of hatch, specific reports on different species, breeds and strains have resulted in the modification in the early post hatch feeding management in poultry. Thus according to Beekbergen (1994), the nutrition of the young chick has to be seriously reduced especially during the first few days post hatch. This practice is believed to result in among other things compensatory growth among the hatchlings when they receive feed in the subsequent stage of management. It is also believed that the young chicks should be deprived of feed until they have resorbed their yolk sac in order to speed up its resorption and that extraneous feed can only be provided thereafter. This system of feeding has in recent past received a series of criticisms (Gross and Siegel, 1997; Dibbner *et al.*, 1998; Saki, 2005). These authors argue that early nutrition gives the birds an early start that can not be made up later by their fasted counterparts, Also it is argued that early fed birds develop immunological competence earlier and of higher quality and faster than fasted individuals.

Time of commencement of feeding within the design of this study between the two groups had no effect on the rate of weight gain. Earlier reports on the effect of early feeding on growth performance have used broiler chicks instead of cockerels or pullet chicks with slower rate of weight gain. According to Hangalapura *et al.* (2005) the outcome of feed restriction in newly hatched poultry correlates highly with the severity of and duration of the insult. Thus, in this study either the yolk sac was providing the necessary survival requirements within the period of fasting or the duration was short to have provoked a noticeable pathology. We postulate that in

broiler chicks engineered genetically for faster rate of weight gain, the implication may involve faster utilization of yolk sac and earlier need of extraneous nutrient source to actualize its special trait. This phenomenon may not be the case with slower growing poultry strains/breeds as was the case in this study. Our results also did not demonstrate any compensatory growth as earlier reported (Beekbergen, 1994; Dibbner *et al.*, 1998) in broiler chicks among the cockerel chicks that experienced delayed access to feed in this study. Moreover, neither mortality nor any undue clinical manifestation was observed among the fasted individuals to indicate compromised viability. This contrasts with the reported incidence of undesired mortality or poor performance that ultimately results in loss of production efficiency in birds that are not fed immediately after hatch (Panda and Reddy, 2008). Furthermore throughout the period of investigation, both groups had comparable relative organ weights especially with respect to liver, spleen, bursa and caecal tonsils. The assessment of the development of these organs was in the recognition of their special roles in general homeostasis and defense against microbial insults all of which will manifest in lowered productivity. These findings contrast with the reports by Fanguy *et al.* (1980), Knight and Dibner (1998), Saki (2005) and Deif *et al.* (2007) who demonstrated that early feeding improved early development of the internal organs including those of the immune system leading to improved disease resistance. These authors however, used broiler chicks which are fast growing in their studies.

Among genetically engineered birds, more resources are allocated to growth and egg production than to immune function. This has been demonstrated in the pathology of lymphoid organs from atrophy of the thymus to loss of lymphoid cells in the spleen and depletion of lymphoid cells in the lymph nodes under nutritional deficiency (Chandra and Kumari, 1994).

According to Korver and Klasing (1995) much of the development of immune system in birds occurs late in incubation and early part of life. As a result, the nutrition of the hen reflected on the egg quality as well as early feeding of chicks are supposed to positively impact on the juvenile immune system of the chicks. Its development is initiated during embryogenesis and is not completed till weeks to months post hatch (Klasing, 1988; Dibbner *et al.*, 1998). It is believed that fasting or delayed access to feed early in life delays its development, This was not demonstrated in this study as most of the organs had comparable weights between fasted and early fed. It appeared that withholding feed for three day was not critical for the envisaged changes to manifest as observed in this study. The argument that early feeding was associated with large bursa or that it prepares the chicks for better immune response

following antigenic challenge/vaccination was not observed in this study. Ferket and Kellems (2007) have reported that under the influence of juvenile under-nutrition that most lymphoid organs; thymus, bursa, spleen and lymph nodes will become atrophied with reduced circulating lymphocytes. This also does not agree with our observation. However, the underlying point is how long and of what severity will be the insult (Hangalapura *et al.*, 2005) This is because in nature animal have a programmed adaptation range within which environmental insults can be absorbed. Moreover, Deif *et al.* (2007) earlier showed that splenic size was not affected by feed restriction and that reduction in crude protein levels between 20 to 17% did not have any effect on the secondary immune tissue.

Our findings with respect to antibody response did not support those of earlier workers (Khajavi *et al.*, 20003) in which feed restriction caused an enhanced antibody response to Sheep Red Blood Cells (SRBC). The early fed chicks in this study appeared to be better prepared immunological wise than their fasted counterparts. The explanation for this may be the availability of substrates with which immune molecules are synthesized. Hangalapura *et al.* (2005) also reported that antibody responses to either keyhole limpet haemocyanin or mycobacterium butyricum immunizations were not affected by level of feed restriction. However, this finding is in agreement with those of Christadoss *et al.* (1984); El Hadri *et al.* (2004) who demonstrated suppressed antibody response in feed restricted mice and poult to T cell dependent antigens. The reasons for these discrepancies are not clear but may be related to the type of immune response measured (Hangalapura *et al.*, 2005) or the quality of ingredients used in feed formulation. The choice of five days post vaccination for the assessment of reaction to the vaccine was based on the report of Gross and Siegel (1988) as the time for peak response by chicks to sheep red blood cells.

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