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The Effect of Feed Quality on the Development and Function of Immune System in Chicken

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Abstract: A total of 72 day old cockerel Harco chicks purchased from a local hatchery were used to investigate the effect of feed quality on the development and function of immune system in chicken. Upon procurement, they were divided into 3 equal groups (n = 24) and brooded separately in three mini deep litter brooder pens with adequate brooding requirement. Three diets with crude protein contents of 26, 22 and 17% were prepared and from day zero of hatch till day 26, chicks in group A were fed 26 while groups B and C were fed 22 and 17% respectively. During and after brooding, signs of ill health such as diarrhoea, anorexia, huddling, drooping of wings, coughing and mortality were carefully monitored. Post brooding, each group retained its pen but all the groups were maintained on one diet, the one on which they were fed till the end of the study. On day 21 Post Hatch (PH), 8 chicks per group were randomly selected, weighed individually and their group means and standard deviation calculated. They were subsequently sacrificed and through jugular venipuncture, 2 ml of blood was collected into an anticoagulant bottle for general haematology while 5 ml was collected into a non anticoagulant containing bottle for serum biochemistry and serology. This was followed by necropsy of all the chicks after which some lymphoid organs and the liver were harvested and their weights determined followed by that of their relative weights. The remaining birds in each group were vaccinated with ND vaccine lasota^R. Five days later, all the operations of day 21 were repeated. The remaining chicks in the group were hereafter maintained on 17% CP till day 42 PH when they were vaccinated with ND Komarov^R vaccine. Five days later, all the operations of day 21 were repeated. We observed the loss of 17% of the flock population in group A during brooding. On days 21, 26 and 47 PH groups A and B had comparable mean live weights which were significantly higher than the mean weights of group C (p<0.05). Also, all the groups had comparable mean relative organ weights throughout the period of study. Moreover, on days 21 and 26 PH, all the groups had comparable PCV while on day 47, groups A and B had mean values significantly higher than C (p<0.05). Generally, we observed that dietary protein appears to modulate the rate of growth and function of some lymphoid organs in growing chicks.

Key words: Feed quality, body and lymphoid organ weights, serum proteins, antibody titre

INTRODUCTION

Various interactions exist within the bio-environment of the flock which ultimately determine the overall success of the enterprise and these are; management, host genetic make up and the rate of exposure and virulence of infectious agents (Klasing, 1998; Amir *et al.*, 2001). As flocks are continually confronted by these pathogens, the severity of clinical signs, duration of the disease, morbidity and mortality are influenced among other factors by the immune status of the birds, their management including dietary characteristics. Research findings have shown that severe and chronic deficiencies of most nutrients impair the development and function of immune system thereby increasing susceptibility to infectious diseases (Dietert *et al.*, 1993; Latshaw, 1990; Cook, 1991). They attributed these to the roles of nutrients as sources of substrates for the development, maturation and function of the immune system. The development of some lymphoid organs like

the thymus, bursa of Fabricius and spleen have earlier been demonstrated to be influenced by the nutrition of the bird (Dibner *et al.*, 1998; Gross and Siegel, 1997; Panda and Reddy, 2007). Nutrients are required to provide the building blocks for the immune cells and tissues including the non specific immune mechanisms such as skin which present a physical barrier to pathogens as well as cells such as T and B lymphocytes, macrophages and natural killer cells (Korver and Klasing, 1995). Ontogenically, the development of the immune system in poultry is a dynamic process initiated during embryogenesis but not completed until weeks or months after hatch. Therefore, maternal nutrition as well as early nutrition is expected to play some important roles in the development and function of the immune system.

Poultry production is a world wide agricultural activity with variations among producers on the capability to provide the right dietary components that constitute the

right feed for the various developmental and productive utilities for the various classes of poultry. Feed scarcity and cases of poorly formulated feed is a common condition among producers in poor nations. Moreover, inefficiency in feed compounding and delivery occasioned by nutrient scarcity and interruption in feed delivery mechanism is also common in the industry.

For adequate productivity including resistance against many endemic diseases, provision of adequate feed resource both in quality and quantity is mandatory. These are in the light of the increasing evidence that immune function is compromised by limiting access to quality nutritional resources especially energy and protein and some micronutrients (Glick *et al.*, 1981; Glick *et al.*, 1983; Lockmiller *et al.*, 1993; Siano *et al.*, 1997). These are observed in the role of nutrients in the development of the immune system, substrates supply, effect on hormonal milieu reduction of immunopathology among others (Klasing, 1998). Furthermore, in the event of a disease challenge the host's first reaction called acute phase response is characterized by synthesis of acute phase proteins, fever, accelerated whole body protein turnover and high rates of gluconeogenesis by the liver utilizing both endogenous and nutritional substrate sources (Grimble, 1996; Moldawer and Copeland, 1997). Generally, chronically severe deficiencies of micronutrients are more debilitating to the immune system than macronutrients and include vitamin A, iron, selenium and several of the B vitamins. Moreover, in spite of the apparent good knowledge of the dietary requirements of most species for adequate productivity, it is not yet known whether the requirement values that maximizes productivity in healthy unchallenged birds are also optimal for immunocompetence and disease resistance (Klasing, 1998; Amir *et al.*, 2001).

Hitherto, most of the studies on the effect of nutrient on the development of function of the immune system have focused on broiler chicks (Gross and Siegel, 1997; Saki, 2005; Deif *et al.*, 2007). Their findings indicated that nutrition is essential for general growth of the chick as well as development and function of its immune system. Cockerel chicks are males co-hatched with pullets and therefore of the same genetic constitution with them. In advanced societies, its rearing to maturity is not popular due to long maturity period and low dressing percentage relative to broiler as source of meat. However, its rearing is still popular in less developed societies as source of poultry meat. Unfortunately, these same societies suffer from the problems of both recurring feed scarcity and common cases of poor quality feed in their poultry industry. Moreover, as cockerel chickens shares the same genetic make up with the more popular pullets research results obtained using one can be extrapolated to the other. It was therefore the aim of this study to

investigate the effect of dietary protein on the development and function of immune system in cockerel chicks.

MATERIALS AND METHODS

Chicks and general management: A total of 72 day old Harco cockerel chicks purchased from a local hatchery in Nnewi, Anambra State Nigeria, were used for the study. The study was carried out in the Poultry Unit of the Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The birds were brought in within 12 h of hatch and on arrival were divided into three equal groups, A, B and C (n = 24) and brooded separately in three mini brooder pens A, B and C. Adequate brooding provisions like heat, lighting, and humidity were maintained in all the pens within the brooding period.

Three diets, A, B and C with 26, 22 and 17.6% crude protein levels respectively and designated High (H) Medium (M) and Low (L) protein were formulated prior to the arrival of the chick. Chicks in pens A, B and C were started on diets H, M and L respectively from the day of hatch till day 28. Water containing mineral and multivitamin preparation (Vitalyte[®]) by Anglia Nutritional Company UK was also provided ad libitum to all the birds throughout the period of investigation.

Brooding in all the pens was by deep litter and lasted for four weeks during which signs of ill health such as anorexia, diarrhoea; huddling, drooping of wings, coughing and mortality were carefully monitored. After brooding, the chicks were all retained in their respective pens but their diet was changed to the L protein diet till day 47 when the study was terminated.

Vaccinations, live-weight and relative organ weight

determination: As the birds had the history of hatchery vaccination with Newcastle Disease (ND) vaccine Intra-ocular (I/O) no further vaccinations were carried out till the chicks were three weeks old. At exactly 21 days of age, 8 chicks were randomly selected from each group. These were weighed individually using a weighing balance and the various group means determined. Thereafter, the chicks were sacrificed and 2 mls of blood immediately collected into an anticoagulant containing bottle for the general haematology while another 5 mls was collected into a bottle without an anticoagulant for serum harvest to be used for the determination of serum biochemistry and serology.

The chicks were subsequently necropsied and some lymphoid organs; bursa, spleen, caecal tonsils and liver harvested and weighed using a weighing balance. The relative weights of these organs were calculated as percentages of the live weights. The sum of the organ weights for each group was calculated and the mean and standard deviation determined. Moreover, samples of each of these organs were fixed in 10% formal saline

and prepared for histopathology. The remaining eighteen birds in each group were subsequently vaccinated against ND using Lasota^R vaccine according to the manufacturer's specification, Five days later, eight birds were again randomly selected from each of the groups. These were treated in a similar manner with those selected on day 21 post hatch.

The remaining eight birds in each group were thereafter maintained on low protein diet and on day 42 of age vaccinated with ND Kamorov^R vaccine according to manufacturer's instruction. They were again sacrificed five days later after which all the operations of day 21 were again carried out.

Haematology serum biochemistry and serology: The PCV was determined using the haematocrit method according to the method of Dacie and Lewis (1995). The total and differential leukocyte counts were carried out using the method of Coles (1986). Furthermore, total serum protein was determined in each sample by the biuret method (Weichselbaum, 1946) 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 histopathology/histology: Determination of antibody titre in the serum samples on the sampling dates were carried out using the ND Lasota^R vaccine as the antigen. Thereafter, Haemagglutination Inhibition (HI) test was carried out as described by Beard (1989). Determination of the Geometric Mean Titre (GMT) was as described by Villegas and Purchase (1989). The samples of the organs were processed embedded in paraffin wax, sections 5 µm thick were cut, stained with haematoxyline and eosin and studied under the light microscope.

Statistics: Data on each parameter were arranged in groups and the means calculated. 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

General clinical observations: Feeding was uniformly uninterrupted among members of the various groups within the first two days of hatch. However, on day three post hatch three chicks representing 17% of the members of group A started exhibiting droopy wings, signs of anorexia and listlessness. Upon close examination, these were observed to have swollen yolk sacs which have failed to regress with time. These

eventually died on days five and six post hatch. All other members of the group including members of groups B and C showed no signs of illness throughout the remaining period of study.

Live-weight, relative liver and lymphoid organ weights:

On day 21 post hatch, groups A and B had comparable mean live-weights which were however significantly higher than the mean live-weight of group C ($p < 0.05$). However, on day 21 only group A was significant relative to C ($p < 0.05$) while on day 49 group B had higher value relative to both A and C ($p < 0.05$) while A was higher than C ($p < 0.05$) (Table 1).

Analysis of the data on the basis of the relative weights of these organs to the live-weights of the chicks showed lack of variations between groups throughout the study period.

Table 1: Mean live-weights of the various dietary groups

Days of activity	Groups		
	A	B	C
21	190±28.5	202.5±45.4	115±13.7
26	275±17.7	260±18.22	188±27.9
47	445±33.4	550±25.2	350±21.3

Table 2: The percentage packed cell volume of the various dietary groups

Days post hatch	Dietary groups		
	High (%)	Medium (%)	Low (%)
21	31.6±0.89	30.6±2.11	27.2±2.68
27	35.4±2.6	30.4±2.51	31.47±4.34
47	22.6±1.22	23.8±0.23	23.6±1.3

Haematology, serum biochemistry: The analysis of the PCV of the various groups showed that on day 21 groups A and B had comparable values which were significantly higher than the mean value for group C ($p < 0.05$). The same situation was still observed on day 26 while on day 47 all the groups have similar values as shown in (Table 3).

Similarly, comparisons between groups on the total leukocyte count on day 21 showed that except for group B with significantly higher value than C ($p < 0.05$), the value for group A was comparable to B and C. On day 26 however, groups A and B had comparable values both of which were significantly higher than C ($p < 0.05$). On day 47, all the groups had comparable total leukocyte count. Analysis of the data on the lymphocyte count showed that on day 21 all the groups had comparable values while on day 26, group A had significantly higher count relative to C ($p < 0.05$) while groups A and B had similar values. On day 47, group B had significant value relative C ($p < 0.05$) while the values for A and C were comparable as well as A and B.

There were some interactions between dietary types and the level of serum protein composition. Hence, on day 21, group A had mean total protein level significantly

Table 3: Mean serum protein, albumin and globulin of the various dietary groups

	Total protein			Serum albumin			Serum globulin		
	A	B	C	A	B	C	A	B	C
21	3.72±0.28	4.22±0.11	3.92±0.38	1.94±0.12	1.97±0.24	1.91±0.14	1.78±0.22	2.25±0.20	2.01±0.45
26	3.18±0.49	3.64±0.35	2.78±0.51	2.67±0.13	2.33	2.75±0.12	0.97±0.3	1.13±.26	0.70±0.3
47	2.8±0.32	3.06±0.26	2.84±0.29	1.19±0.11	2.17±0.17	1.8±0.17	1.01±0.3	0.89±0.28	1.04±0.26

higher than the value for group B and C ($p < 0.05$). The mean value of group B was also higher than that of group C though this was not significant ($p > 0.05$). On day 26, groups A and B had statistically comparable total protein values while only group B was higher than C ($p < 0.05$). However, on day 47 all the groups had comparable values. There were minimal interactions between dietary types, vaccinations and changes in the serum albumin levels. Thus, all the groups had similar values on day 21 post hatch while on day 26 groups A and B had comparable values which again were significantly higher than the value for C ($p < 0.05$). Analyses of serum globulin indicated that on days 21 and 26 group A had mean value superior to group B whose value was also superior to C ($p < 0.05$). However, on day 47 all the groups had similar values.

Serology and organ histology: Analyses of the data from the serology indicate that on day 21, group B had a significantly higher GMT ($p < 0.05$) relative to groups A and C whose values were comparable. However, five days post vaccination with ND lasota^R vaccine group A had antibody GMT significantly superior to the other two groups ($p < 0.05$). Group C still maintained a significantly lower GMT relative to B. On day 47, the GMT of group B had again risen to level significantly higher than those of groups A and C though C was still significantly inferior to B. A noteworthy observation is the sequential fall in GMT among members of group C over time and in response to vaccination. Table 4 shows the geometric mean antibody titres for the various groups on the various days of study.

The histopathology examination showed no lesions in all the groups.

DISCUSSION

The results of this study indicated some possible complication of high proteinaceous diet on the function of yolk sac. The is element of fact in the earlier believe that young chicks should be deprived of feed until they have fully resorbed their yoke and in order to speed up its resorption feeding should be commenced thereafter (Knight and Dibner, 1998; Keereman and Vermeersch, 1994). Excessively high dietary protein was shown to suppress the rate of utilization of the yolk and may predispose the wet and juvenile organ to infection. Panda and Reddy (2007) had reported that the absorption of essential nutrients and maternal antibodies from the yolk sac are critical for the survival

during the early stages of life post hatch. Moreover, it has been established that deficiency of excess of dietary protein changes immune responses (Glick *et al.*, 1981; 1983; Payne *et al.*, 1990). We speculate that under this condition, the chicks will have higher preference for the excess protein in the diet over that of the yolk protein thereby reducing the amount of absorbed and circulating maternal antibodies. Dibner *et al.* (1998) have reported that fasted/fed restricted chicks early in life did not have any effect on the rate of yolk utilization to compensate for the absence of extraneous feed. Though we did not investigate this relationship, Nnadi *et al.* (2010) confirmed their observation in early fed and fasted cockerel chicks. The slow rate of yolk or lack of resorption may have resulted in its infection by many opportunistic pathogens that abound in the poultry house resulting in the observed clinical signs and mortality. Groups maintained on diets with adequate or even marginal protein however did not show any overt signs of disease.

The linear relationship between dietary quality and rate of body weight gain is attributable to the readily available substrate for body development among members of groups A and B but not those on marginal diet. This is in agreement with the findings of Rama-Rao *et al.* (1999) but contrasts the report by Deif *et al.* (2007) at 21 days of age. Interestingly, the latter study was carried on using broiler chicks while the former was on pullets with growth peculiarities of cockerels. That group B had superior body weight at day 47, three weeks after diet normalization indicates that poultry, if not other species have a dietary protein range within which it does best under various stressful conditions. Thus, for cockerels the 22% crude protein diet when fed early in life appears to fulfill this requirement.

On the profile of the total serum protein, our result is in agreement with those of Tewe (1985) Eggum (1989) and Deif *et al.* (2007). These workers have established a consistent correlation between total serum protein, albumen and globulin with the protein quality and quantity of the diet. However, this disagrees with the report by Agbede and Aletor (2003) who reported that total serum protein, albumen and globulin syntheses were not affected by the sources of dietary protein (quality of protein) Our results showed that groups A and B on high and moderate protein levels consistently had significantly higher total serum protein levels relative to group C on marginal protein.

Table 4: Geometric mean antibody titres for the various dietary groups

Groups	Days post hatch		
	21	26	47
A	128	337.8	64
B	831.7	97	84.4
C	128	64	16

Albumin fraction of the serum proteins serves as the reservoir of protein, is involved in colloidal osmotic pressure, acid-base balance and also acts as transport carriers for small molecules like vitamins, minerals and even hormones (Margaret, 2001). Our result is consistent with those of Deif *et al.* (2007) with groups A and B having higher albumen values relative to C especially post vaccination with ND lasota^R vaccine.

The globulin, the third fraction of the serum proteins is composed of three fraction; alpha, beta and gamma which are entirely synthesized in the liver. Their elevation in the serum is usually associated with nephritis, hepatitis, acute inflammation and malnutrition (Margaret, 2001). The gamma globulin fraction contains most of the immuno-proteins and these are usually elevated in serum during antigenic stimulation or by infectious agents. (Margaret, 2001).

The profile of the globulin fraction of the serum proteins also showed that groups on high protein diet proved to be more capable immunological wisely than the group on marginal protein diet. Group A showed superiority over B and C and B over C on days 21 and 27 indicating either superior response to vaccination or an earlier pre vaccination stress. The mechanism responsible for the latter is not clear though it might be connected with metabolic stress due to unduly high protein diet which again may be related to the observed mortality in this group.

Nutritional studies in animal rarely report on consequences of over-nutrition, an aspect of malnutrition because of economic implications arising from the cost of protein source. However, producers who may be tempted to blindly increase the protein content of the diet because of possible result regarding the known function of protein should be careful. This is especially so in slow growing breeds/strains during the neonatal period.

The result of the serology showed a perfect correlation with an earlier indication of depressed resistance to infections among members of group A with antibody titre similar to group C on day 21. The group however, reacted adequately to vaccination using lasota^R, vaccine but again proved inferior to group A to ND Kamorov^R vaccination. These observations indicate the importance of caution in the quality and source of dietary protein to be used in formulating feed for young chicks especially

producers in poor countries with no poultry feed formulation standard. Furthermore, the similarity in the histology of the sectioned tissues between groups may be due to lack of undue severity and duration of the nutritional insult.

In summary, protein nutrition appears to modulate the growth and some immune parameters in developing chicks but with little or no effect on the relative weights of most lymphoid organs and the liver.

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