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Lipid Profiles and Antibody Responses of Broiler Chickens Fed Graded Levels of Vitamin C.

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ABSTRACT

This study was primarily carried out to investigate the effects of graded levels of vitamin C on lipid profiles and antibody responses in the broiler chicken. One hundred and twenty Agrited day-old chicks were used in the study. The chicks were initially brooded for four weeks after which they were randomly assigned to their experimental diets. There were 30 birds/treatment consisting of 3 replicates of 10 birds/replicate for each treatment; as: treatment 1 (T1 was the negative control, contained no added vitamin C), treatment 2 (T2, contained 200 mg of vitamin C), treatment 3 (T3, contained 300 mg of vitamin C) and treatment 4 (T4, contained 400 mg of vitamin C)/ kg of diet, respectively. Birds received their respective experimental diets for 4 weeks. At the end of the study, 3 birds from each replicate for all treatments were slaughtered by humanely severing their necks for blood collection. Blood were collected into non-ethylene diamine tetra-acetic acid (EDTA) tubes for analyses for total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) as well as immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM). The results showed that birds of T1 had significantly ($P < 0.05$) the highest levels of TC, TG and LDL levels compared with birds of T2, T3 and T4 groups; HDL levels were significantly ($P < 0.05$) lower in the T1 group compared with the T2, T3 and T4 groups. There were no significant ($P > 0.05$) differences amongst all treatment groups in IgG and IgM levels. Serum levels of IgA were very low for all treatment groups for analyzes. It was concluded that vitamin C reduced TC, TG and LDL contents and simultaneously increased HDL contents in the broiler chicken.

INTRODUCTION:

Broiler chickens are known fast growing species reaching market weights of about 3 kg of body weight within 8 weeks of age. However, one of the environmental stressors that impede the fast growing process of the birds is nutrition. Specifically, when the diet of the birds is deficient in nutrients or under-supplied the birds become runts leading to huge losses on the part of the broiler farmer (NRC, 1993). In the past, antibiotics were used in poultry diets at sub-therapeutic levels to overcome this as their uses were seen to improve growth and even reproductive performance. Currently, there are bans on the use of certain antibiotics in poultry diets because of the development of strains of pathogenic organisms that are resistant to some antibiotics (NRC, 2012; Bekenev *et al.,* 2015). To this extent, animals' producers, including poultry producers and nutritionists are in search for alternatives to the use of antibiotics. One of the means of achieving this is via the fortification of poultry diets with micro-nutrients, particularly vitamins which have been touted to improving growth rates of livestock, including poultry primarily as a result of their protective effects (Bekenev *et al.,* 2015).

Based on the above stated implications of vitamins in enhancing animal growth rates, Herbert *et al*. (2005) and Stahly *et al*. (2007), respectively demonstrated that fortifying animal diet with vitamins is a dependable nutritional means of enhancing animal growth and performance characteristics as they act as antioxidants thereby protecting animals from oxidative stress. Some of the vitamins are vitamins A, B, C and E. These vitamins have also been shown to synergistically work together in improving growth rate and meat quality (Stahly *et al*., 2007; Johnson *et al*., 2019).

With reference to vitamin C, it is required as an essential nutrient for various biochemical and physiological functions in the animal's body. It is a well-known fact that the animal cannot synthesize enough of vitamin C to meet all its biochemical and physiological requirements, hence the need for vitamin C supplementation (NRC, 2012). Supplementation levels needed in respect to broiler requirements regarding reduction of lipid depositions, particularly the 'bad' cholesterol as well as up-regulating antibodies in maintaining sound health of broiler chickens is not well established. The paucity of information on these data therefore warranted this current study. Therefore, the objectives of this study are: to investigate the effects of feeding graded levels of vitamin C on total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) in broiler chickens as well as to investigate broiler chickens' antibodies responses to graded levels of vitamin C: namely, immunoglobulin (Ig) G, immunoglobulin (Ig) A and immunoglobulin (Ig) M.

MATERIALS AND METHODS

***Experimental site*:** This study was carried out at the poultry unit of the Teaching and Research Farm, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt. The farm is situated at latitude 4^0 48'N and longitude 6^0 48'E at the Rivers State University campus.

Animals: One hundred and twenty (120) *Agrited* day-old chicks were acquired from a reputable commercial poultry dealer in Port-Harcourt, Rivers State. The animals on arrival at the Rivers State University Teaching and Research Farm were brooded to proper pre-condition them to their new environment. The animals by the fourth week were observed to have properly adapted to their environment and thus were randomly assigned to four dietary treatment groups of 30 birds/treatment group with 3 replications of 10 birds/replicate. The pens were properly cleaned and disinfected before the birds' arrival. Feeders and drinkers were also properly cleaned to also ensure that the animals' environment were "pathogen-free". During the brooding period all protocols, including the necessary medications were provided. Animals were fed similar diets from day one through the end of the 4^{th} week. Water was provided *ad libitum*. The experiment lasted for 8 weeks and thus animals received their respective experimental diets for 4 weeks.

Experimental Diets: Hybrid feed™ grower mash were used in the study. In other words, the diets fed to the animals during the last four weeks of the experimental period were similar in all dietary nutrients except their dietary vitamin C levels as: negative control or treatment 1 (T_1, contained only basal level of vitamin C of 30mg/kg of diet); whereas the positive treatments, viz: treatment 2 (T_2, contained 200mg of vitamin C), treatment 3 (T_3, contained 300mg of vitamin C) and treatment 4 (T_4, contained 400mg of vitamin C)/kg of diet, respectively. The animals were fed these graded levels of vitamin C-based diets for 4 weeks.

Experimental Procedure and Data Collection: At the end of the study period, birds were killed for blood collection. Three birds were randomly collected from each replicate of the four treatment groups. The blood was collected from each bird into non-treated ethylene diamine tetra-acetic acid (EDTA) tubes and immediately snap frozen in ice for later lipids and antibody analyses. Lipids were analyzed according to the method of Nauck *et al*. (2002) and antibodies were analyzed according to the method of Rey *et al.* (2017).

Experimental Design and Statistical Analysis: The study was carried out as a completely randomized design (CRD). Data were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS. Treatment means were compared using Tukey's test. The model was: $Y_{ij} = \mu + X_i + E_{ij}$, where Y_{ij}= individual observation of the treatment, μ = population mean, X_i = effect of the i^{th} dietary treatment (i^{th} diet = 1, 2, 3, 4) and E_{ij}= the error term. An *α-level* of *0.05* was used for all statistical comparisons to represent significance.

RESULTS

Animals in all the four dietary treatment groups readily consumed their respective diets and grew throughout the experimental period as evidenced by their sizes and weights at the end of the study period. Vitamin C ingestion was observed to have profound effects on the lipid profiles of the birds in a dietary vitamin C ingestion levels dependent fashions as depicted in Table 1.

Table 1. **Lipid profiles of broiler chickens fed graded levels of vitamin C.**

	TREATMENTS					
Parameter	1	2	3	4	SEM	*P-value*
Total cholesterol (mmo/l)	4.75^a	3.75^b	3.00^c	3.00^c	0.18	0.002
Triglyceride (mmo/l)	2.00^a	2.00^a	1.00^b	1.00^b	0.00	0.00
High density lipoprotein (mmo/l)	1.00^b	2.00^a	2.00^a	2.00^a	0.00	0.00
Low density lipoprotein (mmo/l)	4.00^a	3.00^b	2.25^c	2.00^c	0.13	0.00
Very low density lipoprotein (mmo/l)	1.00	1.00	1.00	1.00	0.00	0.18

^{a,b,c} Means within the same row with different superscripts are significantly different (*P < 0.05*)

Birds on diet 1 had significantly *(P < 0.05*) the highest levels of TC followed by birds on diet 2. Birds on diets 3 and 4 significantly (*P < 0.05*) had similar and lowest concentrations of serum TC compared with animals on diets 1 and 2. For TG, animals on diets 1 and 2 had significantly *(P < 0.05*) higher values compared with the levels of TG found in animals on diets 3 and 4 that had similar (*P > 0.05*) TG values that were significantly *(P < 0.05*) lower compared to those of animals on diets 1 and 2. For HDL, animals on diet 1 significantly (*P < 0.05*) had the lowest levels compared to HDL levels of animals on diets 2, 3 and 4 that had similar (*P > 0.05*) HDL concentrations that were significantly *(P < 0.05*) higher than those of diet 1. Conversely, animals on diet 1 significantly (*P < 0.05*) had the highest levels of serum LDL followed by animals on diet 2. Animals on diets 3 and 4 had similar levels of LDL that were significantly (*P < 0.05*) lower compared to those of animals on diets 1 and 2. There were no differences (*P > 0.05*) in the serum levels of VLDL as birds in the four treatment groups had similar (*P > 0.05*) serum concentrations. The antibody responses of broiler chickens fed graded levels of vitamin C are shown in Table 2.

Table 2. **Antibody responses of broiler chickens fed graded levels of vitamin C**

	Treatments					
Parameter	1	2	3	4	SEM	P-value
IgG (mg/l)	5.00	6.00	6.00	6.00	0.00	0.08
IgM (mg/l)	1.00	2.00	2.00	2.00	0.00	0.08

As shown in Table 2, there were no significant (*P > 0.05*) differences amongst all treatment groups in terms of the serum levels of IgG and IgM immunoglobulins. However, there were tendencies for higher levels of IgG and IgM in the sera of animals on diets 2, 3 and 4. Serum levels of IgA were very low for all treatment groups, hence could not be statistically analyzed.

DISCUSSION

Fast growing animal species, such as broiler chickens required their diets to be well-fortified with dietary nutrients, especially micro-nutrients such as minerals and antioxidant vitamins to elicit properties that better support their fast growth rates without incidence of sudden deaths (NRC, 2012; Johnson *et al*., 2019). This is more so now that the use of antibiotics had been banned in non-ruminant diets, including poultry (Bekenev *et al.,* 2015).Environmental stressors, such as unbalanced nutrition of the animals can lead to imbalance between oxygen reactive substances production and the animals' anti-oxidant defense system resulting in cellular damage and incidence of diseases in the presence of lowered immune response. This condition also results in high economic losses on the part of the farmer (NRC, 2012).

Many dietary additives have been studied in order to mitigate against stress, including oxidative stress. Antioxidant vitamins, such as vitamin C acts as a catalyst in oxide-reduction reactions and as a reducing agent that neutralizes free radicals in aqueous compartments (Frei *et al.* 1989).Therefore, supplementing broiler diets with vitamin C may be to the rescue by increasing antibody concentration, lower LDL and thus improve performance in addition to good quality meat (Holmannova *et al*., 2012).In this study, one of the major objectives was to investigate the effect of feeding graded levels of vitamin C on the lipid profiles of broiler chickens. As shown in Table 1, the ingestion of dietary vitamin C reduced the total amount of cholesterol in the animals that were fed the vitamin C-enriched diets. Furthermore, dietary ingestion of vitamin C reduced body triglycerides. More importantly dietary vitamin C reduced LDL levels, the "bad cholesterol" and increased the HDL levels, the "good cholesterol." These findings are in agreement with the findings of Das *et al.* (1997) and Frikke-Schmidt *et al.* (2011). From nutrition standpoint, these findings in this current study point to the fact that vitamin C could be used for value capture of broiler chickens as consumers prefer to pay higher premiums for broiler meats of good quality in respect to reduced fat contents of the broiler meat. This finding in this current study again is in agreement with the data of Holmannova *et al*. (2012).

It is common knowledge that LDL is one of the common causes of sudden deaths syndrome in fast growing animal species, such as broilers and the pig, including humans. LDL normally forms plaques such that when the plaques accumulate usually lead to the blockage or narrowing of blood vessels, such as the arteries thereby impeding the normal process of blood circulation. These characteristics of LDL when not well managed is very fundamental to cardiac arrest or heart attack of the animal resulting in atherosclerosis (Das *et al.,* 1997). Therefore, it is not a gainsaying that cardiovascular disease is highly correlated with the amount of lipids, particularly LDL in the blood. Since dietary vitamin C reduced the levels of circulating LDL as found in this study, it can be inferred that vitamin C can be used to protect the animal against cardiovascular disease. This is more so as vitamin C reduced the LDL and concomitantly increased the "good cholesterol." These findings are in tandem with those of Marc and McRae (2008) and Shariat *et al.* (2013).

The second object of this current study was also to investigate the effects of feeding graded levels of vitamin C on the antibodies status in broiler chickens. Many dietary additives have been investigated as it relates to mitigation of stress in the animal. To this point, many vitamins have been considered for this role. One of such vitamins is vitamin C as it acts as a catalyst in oxide-reduction reactions and as a reducing agent that neutralizes free radicals in aqueous compartments as evidenced by the findings of Hamilton *et al.* (2000). Vitamin C supplementation has been shown to up-regulate immunoglobulin concentrations and thus improve performance characteristics in piglets (Bekenev *et al.,* 2015). As also shown in Table 2, dietary ingestion of the vitamin C-enriched diets had no remarkable effects on the status of immunoglobulins compared to the control in this study. However, there were trends of improved IgG and IgM immunoglobulins by the vitamin C enriched diets. This suggests that vitamin C could be used to improve immunoglobulin status of broiler chickens to reduce stress in the chicken and thus improve performance parameters. Furthermore, vitamin C enriched diets had no effects on IgA immunoglobulin in this study.

Conclusions: Vitamin C can be used to reduce the total cholesterol, triglycerides and low density lipoprotein contents of the broiler chicken. On the other hand, vitamin C can be used to increase the high density lipoprotein contents in the broiler chicken. The overall benefits of these are that vitamin C can be employed in the production of broiler chickens with less fat contents for better value capture as vitamin C can improve the HDL contents of the chicken that consumers cherish.

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