European Journal of Science, Innovation and Technology

ISSN 2786-4936

Vol. 2 No. 1 (2022)

Liver and Kidney Health Biomarkers of Broiler Chickens Fed Vitamins A, C and E

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Abstract. The effects of vitamins A, C and E based-diets were investigated on the biomarkers of liver [alanine amino transferase (ALT), aspartic amino transferase (AST), alkaline phosphatase (ALP)] and kidney biomarkers [blood urea nitrogen (BUN) and creatinine] to determine liver and kidney health status of broiler chickens. Ninety day-old chicks were used in the investigation. The chicks on arrival at the venue of the study were brooded and similarly reared for 4 weeks to fully adjust them to their new environment. At the end of the adjustment period, the animals were randomly assigned to 3 dietary treatments with 30 birds/treatment and 3 replicates of 10 birds/replicate as: T_0 (control diet, contained the vitamins at basal levels of 30mg/kg of diet), T_1 (diet 2, contained vitamins A and E at 100mg of vitamin A + 100mg of vitamin E/kg of diet) and T_2 (diet 3, contained vitamins C and E at 100mg of vitamin C + 100mg of vitamin E/kg of diet). The animals ingested their dietary treatments for 4 weeks. At the end of trial, 9 birds from each treatment group composed of 3 birds from each replicate were bled and their blood collected into treated ethylene diamine tetra-acetic acid (EDTA) tubes and immediately snap frozen for liver and kidney biomarkers analyses. ALT serum levels of T_0 and T_1 animal treatment groups were significantly ($P < 0.05$) higher compared with those of T_2 animals. ALP levels of T_1 and T_2 treatment groups were similar ($P > 0.05$) but significantly ($P < 0.05$) lower compared with the T_0 group. There were no differences ($P > 0.05$) in the levels of AST, BUN and creatinine amongst all the treatment groups. It was concluded that the vitamins-based diets had effect on ALT and ALP but had no effects on AST, BUN and creatinine.

Key words: Vitamins A, C, and E, Liver and Kidney Biomarkers, Health Status and Broiler Chicken

Introduction

Vitamins A, C and E are anti-oxidant vitamins. Therefore, it is touted that they can aid in maintaining good health of animals that ingest them during their growth process, especially when they are fed in combinations (Johnson *et al.*, 2019). Liver and the kidney are essential organs in the body of the animal, including chickens. When the animal is challenged or experienced oxidative stress, the functions of the liver and those of the kidney can be compromised; this is usually judged by their biomarkers. When this occurs quality of life and the well-being of the animal is often negatively impacted, particularly in the absence of anti-oxidant vitamins that regulate the defense system of the animal, such as the glutathione system (Ekhato *et al.*, 2014). The major means of detecting damages to these organs are the serum levels of alanine amino transferase (ALT), aspartic amino transferase (AST) and alkaline phosphatase (ALP) for the liver/bile duct and serum level of creatinine and blood urea nitrogen (BUN) for the kidney, respectively as they signal malfunctioning of the liver and kidney when their levels are above normal serum levels (Ognik & Wertelecki, 2012). Such conditions become worse as earlier alluded to in the absence of anti-oxidant vitamins that up-regulate the defense system of the animal, especially the glutathione defense system that deactivates reactive oxygen species (ROS) that have been implicated for cells, tissues and organ damages during oxidative stress (Aslan & Meral, 2007; Michael & Navdeep, 2014).

To this extent, ALT is mostly abundant in the liver of animals (Lalita *et al.*, 2016). High level of ALT in blood serum has been shown to be a dependable biomarker of oxidative damage in the cells of the liver. Again, Kim *et al*. (2008) and Shokrzadeh *et al.* (2012) have consequently demonstrated that the activity of ALT in the liver is 3,000 times its activity in the blood serum and thus any damage in the liver cells will lead to the releasing of high amount of ALT into the blood of the animal. Furthermore, Shokrzedah *et al.* (2012) showed that anti-oxidant vitamins reversed damage done to the liver thereby conferring protective effect on the liver in the presence of oxidative stress as evidenced by normal levels of ALT in Wister rats exposed to organophosphate pesticides when compared with the control in that study.

In order words, when the functions of the liver and kidney are impeded the efficiency of animal production is usually compromised as the health of the animal nose dives thereby making the animal uncomfortable and susceptible to pathogenic diseases (Hungu *et al.*, 2013). Usually, ROS and hydroxyl radicals produced during oxidative stress leads to cells damages that eventually result in programmed cell death and more so if the ROS are not eliminated from the cells of the animal (Michael & Navdeep, 2014). ALT, AST and ALP are liver enzymes although ALP is also indicative of bone development. Thus liver/bile duct damage leads to liver leakage leading to high serum levels of these enzymes and are therefore collectively refer to as biomarkers of liver functions (Lalita *et al.*, 2016).

Blood creatinine concentration is a usual practice used to determine the level of oxidative damage to the kidney of animals. To this point, Fulya *et al.* (2012) demonstrated that blood creatinine level increased when animals were treated with cisplatin that led to injury of the kidney resulting in the increased level of blood creatinine suggesting that creatinine is a good biomarker of oxidative stress in determining the health status of the kidney. Thus high concentration levels of blood creatinine are usually an indication of kidney malfunctioning. Similarly, Lalita *et al.* (2016) demonstrated that high levels of BUN are related to damage or malfunctioning of the kidney suggesting that BUN test is one of the well-established practices in evaluating damage in the kidney. Animal production environments are not 100% pathogen-free and are thus often exposed to oxidative stress. Therefore, the objectives of this investigation are to determine the effects of dietary vitamins A, C and E on the health statuses of the liver and kidney in broiler chickens using their biomarkers.

Materials and Methods

Animals

Ninety (90) *Agrited* day-old chicks were acquired and used in this study. When the animals arrived at the Rivers State University Teaching and Research Farm, they were similarly managed for 4 weeks to ensure that they were fully adapted to their new environment. After the four weeks of adjustment, the animals were randomly allotted into three treatment groups of 30 birds/treatment group with 3 replications of 10 birds/replicate. To ensure that the animals were comfortable and also "pathogen-free" their pens were properly cleaned and disinfected before the birds' arrival. Their feeders and drinkers were also kept under high sanitary conditions. During the first 4 weeks' period of rearing the birds' management protocols in terms of the birds' safety, 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* to all the animals throughout the study period.

Experimental Diets and Design

Commercial Top Feed^{TM} grower mash was used in the study. Therefore, diets fed to the animals during the last four weeks of the experimental period were isocaloric and isonitrogenous except that diet of group 2 animals (T_1) contained additional vitamins A and E while diet of group 3 animals (T_2) contained additional vitamins C and E, respectively. The control (T_0) diet contained these vitamins A, C and E at the basal levels only. Treatment T_1 contained vitamins A and E at: (vitamin A 100mg + vitamin E 100mg/kg of diet) and T_2 diet contained vitamins C and E at: (vitamin C 100mg + vitamin E 100mg/kg of diet). The animals were fed these vitamin-based diets for 4weeks (28days). The study was designed and carried out as a completely randomized design (CRD).

Blood Sample Collection

At the end of feeding trial period, nine (9) birds were bled (slaughtered) for blood collection. Three birds were randomly collected from each replicate of the three treatment groups. The blood was collected from each bird into treated tubes with ethylene diamine tetra-acetic acid (EDTA) and immediately snap frozen for later analyses for ALT, AST, ALP, BUN and creatinine.

Blood Sample Analyses for Liver and Kidney Biomarkers

ALT and AST were analyzed for by the method of Reitman and Frankel (1957). ALP was analyzed according to the method of Bodansky (1930). Urea concentration was measured according to the method of Machado and Horizonte (1958) whereas creatinine was determined according to the method of Max (2011).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS. Treatment means were compared using Bonferoni's test. Therefore, 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} treatment and E_{ij} = the error term. An *α-level* of *0.05* was used for all statistical comparisons to represent significance.

Results

The results of liver biomarkers of the three treatment groups are shown in Table 1.

Table 1. Serum Concentrations of Liver Biomarkers: ALT, AST and ALP of Broiler Chickens Fed Vitamins A, C and E

Parameter	Treatments			SEM	*P-value*
	T_0	T_1	T_2		
ALT (iu/l)	100^a	90^a	76^b	3.89	0.04
AST (iu/l)	99	95	93	4.13	0.13
ALP (iu/l)	28.96^a	23.98^b	23.43^b	1.04	0.05

Note: Means with different superscripts within the same row are significantly ($P < 0.05$) different.

Although ALT serum concentrations were all within normal ranges for all dietary treatment groups, T_0 and T_1 animal treatment groups had similar ($P > 0.05$) levels of ALT which were significantly ($P < 0.05$) higher compared with the T_2 animal treatment group. There were no differences ($P > 0.05$) in AST for all the three treatment groups. Animals in the T_0 treatment group had significantly ($P < 0.05$) higher serum concentrations of ALP compared with the T_1 and T_2 animals' treatment groups that had similar ($P > 0.05$) serum concentrations. The results of kidney biomarkers of the three treatment groups are shown in Table 2.

Table 2. Serum Concentrations of Kidney Biomarkers: BUN and Creatinine of Broiler Chickens Fed Vitamins A, C and E

Parameter	Treatments			SEM	*P-value*
	T_0	T_1	T_2		
BUN (mmol/l)	4.45	4.42	4.35	0.11	0.14
Creatinine (mmol/l)	0.92	0.90	0.90	0.23	0.19

Note: There were no significant ($P > 0.05$) differences for kidney biomarkers, namely BUN and creatinine amongst the three dietary treatment groups.

Discussion

ALT is mainly a hepatocyte enzyme as it is abundantly found in the liver cells compared to other organs in the body (NRC, 2012). ALT plays major role in the conversion of amino groups to pyruvate and glutamate indicating that the enzyme is involved in protein metabolism (Ekhato *et al.*, 2014). Nevertheless, abnormal high levels of serum ALT is mostly an indication of liver leakage resulting in the excess release of the enzyme into the bloodstream. This fact therefore, confers on ALT as an indicator enzyme of liver damage or malfunctioning. In this current study, although the serum concentrations of ALT were within normal ranges, those of T_0 and T_1 animals tended towards the upper border line indicating that the liver of the animals in these treatment groups possibly would have experienced some mild forms of oxidative stress compared with the T_2 animals. This can be partially explained on the ground that dietary combination of vitamins C and E is a better strategy in combating oxidative stress in broiler chickens. This observation is substantiated to in this study via the findings of Alcala *et al.* (2017). In Alcala *et al.* (2017) in the presence of oxidative stress induced by obesity vitamin E reduced serum levels of ALT and in fact brought it down to normal level in the obese mice. Furthermore, it is a confirmation that there was a better synergy between vitamins C and E compared with A and E in activating the animal defense system to protect the animal. This finding is also in agreement with those of Johnson *et al.* (2019) and Johnson and Popoola (2020).

Another important serum enzyme usually used in the assessment of liver health status is AST. Although AST is not as specific as ALT in liver testing principally as a result of AST presence in both the heart and muscle, it is a common diagnostic practice to often check for ALT and AST together as it aids in proper liver health status diagnosis. As is with ALT during liver leakage AST is released into the blood stream leading to high levels of serum AST. In this study however, there were no significant effects of the vitamins on AST as all dietary treatment groups had similar ($P > 0.05$) levels of AST. This of course sharply differed with the findings of Johnson and Diri (2020) as well as that of Okejim *et al.* (2020).

Alkaline phosphatase is found in bones, bile ducts and the liver. To this point therefore, high serum levels of ALP may indicate liver inflammation, blockage of the bile ducts, a bone disease or bone malfunctioning. In this study, animals of the T_0 treatment group had significantly ($P < 0.05$) higher serum levels of ALP compared with animals of the T_1 and T_2 treatment groups. This is an indication that animals of the T_1 and T_2 treatment groups had better stabilized internal body environment during the trial period compared with those of T_0 animals' treatment groups as evidenced by the lowered ALP serum levels in the animals of the T_1 and T_2 treatment groups. This finding agrees with those of Ognik and Wertelecki (2012) and Lalita *et al.* (2016). Ingested vitamins had no effects on BUN and serum creatinine. One major deduction that could be drawn at least in part to explain some of the findings of this study would be that the animals and by extension their livers and kidneys were not environmentally challenged as a result of the high sanitary and hygienic conditions maintained from the beginning of the trial to the end.

Conclusion

From the findings of this study, it was concluded that dietary ingestion of vitamins A and E as well as vitamins C and E aid in liver health status, especially that of vitamins C and E as evidenced by the serum levels of liver biomarkers, namely ALT and ALP but had no effects on serum BUN and creatinine.

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