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Studies on Glycogen storage and activities of Liver Enzymes in Broiler Chickens fed Diets supplemented with a mixture of Ginger, Garlic and Cinnamon

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Abstract

Glycogen storage and liver enzymes` activities in broiler chickens fed diets supplemented with a mixture of ginger, garlic and cinnamon were studied. Dried and pulverized cinnamon, garlic and ginger were mixed in ratio 4:2:1 respectively. The blended spices were combined with starter and finisher feeds to formulate four diets; 1 and 2 were 0% mixed-spices supplemented starter feed and 2% mixed-spices supplemented starter feed respectively. Diets 3 and 4 were respectively 0% mixed-spices supplemented finisher feed and 2% mixed-spices supplemented finisher feed. During starter phase, 150 broiler chicks were divided into groups A and B and were randomly fed diets 1 and 2 respectively for 3 weeks. In the finisher phase, group A was sub-divided into groups C and D while group B was subdivided into groups E and F and fed for additional 3 weeks. The breast and thigh ultimate pH values were found to be significantly decreased ($p<0.5$) in the groups maintained on mixed spices supplemented diets. Compared with the control, test groups had significantly lower serum activities of gamma – glutamyl transferase and alkaline phosphatase but higher transaminases ($p<0.05$). Although the blended spices may improve shelf life quality of broiler meat, it`s use is not encouraged until the cause of the increased serum transaminases is ascertained.

Keywords: cinnamon, garlic, ginger, glycogen, liver enzyme, spices

Introduction

Poultry meat such as broiler chicken meat contains vital and balanced nutrients which make it very important in human nutrition. The nutritive quality however depends on many factors such as diet and carcass part (Mustafa and Orkide, 2017). After slaughter, glycogen remains the main metabolic fuel for the anaerobic glycolysis due to lack of oxygen supply to the muscles (Vonick*et al*., 2011). The amount of stored glycogen is an important index of physical meat qualities and is inversely proportional to pH of the meat. Decrease in pH is largely due to post- slaughter glycolysis, with glycogen being converted into lactic acid (Scheffler *et al*., 2011).

Birds are predisposed to fat deposition in the liver and various infectious agents and toxins (Cherian *et al*., 2002). The disorders of liver which include hepatosis, necrosis, fatty liver, hepatitis and cholangitis (Faegheh *et al*., 2019) have far reaching consequences on the performance and health of birds. Some serum biochemical constituents reflect conditions such as the health and nutrition to which the animals are subjected (Minafra, 2010) and can therefore be used to assess the productive performance and health status of birds (Rotava *et al*., 2008).Therefore, any candidate for fortification of chicken diet should be proven to have no deleterious effects on the liver. Alanine-aminotransferase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphotase (ASP) are usually referred to as liver enzymes and their concentrations in the serum are usually used to assess the integrity of the liver.

The imperative of animal health and considerations for carcass and meat quality make studies on alternative ways of making broiler feed more functional a continuing one. The present study was conducted to investigate glycogen storage and activities of liver enzymes in broiler chickens fed diets supplemented with a mixture of ginger, garlic and cinnamon.

Materials and Methods

One hundred and fifty (150) day-old broiler chickens were purchased from Chi Farms Limited Ibadan in Nigeria. Commercial starter and finisher feeds were purchased from Central Market, Dustinma of Katsina State in Nigeria. The dried garlic and ginger were separately pulverised in a mortar with pestle to pass through 0.5mm mesh sieve. Pure cinnamon powder was bought from Al-Hilal Islamic Chemist Store, Kastina city, Katsina State.

Feed formulation

The spices; cinnamon, garlic and ginger were thoroughly mixed in ratio of 4:2:1 respectively. The blended spices and starter were mixed in a ratio of 2:98 receptively to formulate 2% mixed-spices supplemented starter. Also, 2% mixed-spices supplemented finisher was formulated using the same method (Idoko *et al*., 2019; Idoko *et al*., 2020_a). The following feeds were then formulated for the experiment;

1. 0% mixed-spices supplemented starter feed
2.2% mixed-spices supplemented starter feed
3.0% mixed-spices supplemented finisher feed
4.2% mixed-spices supplemented finisher feed

Experimental design and animal management

Starter phase: In this phase of the experiment, the 150 chicks were divided into groups A and B of 75 birds each and randomly assigned to diets 1 and 2 respectively.

Finisher phase: In the second (finisher) phase of the experiment, group A in the first phase was sub-divided into groups C and D while group B in the first phase was sub-divided into groups E and F(Idoko *et al*., 2020_b). Group C was maintained on 0% mixed spices - supplemented sarter feed and then 0% mixed-spices -supplemented finisher feed (Control). Group D was maintained on 0% mixed spices-supplemented starter feed and then 2% mixed spices-supplemented finisher feed. Group E was maintained on 2% mixed spices-supplemented starter feed and then 2% mixed spices-supplemented finisher feed. Group F was maintained on 2% mixed spices -supplemented starter feed and 0% mixed spices-supplemented finisher feed.

The broilers were kept in accordance with the method of Idoko *et al*., (2019). In this method, the birds were put in a warm (35°C-38°C), appropriately ventilated and disinfected environment. After seven days of acclimatization to the experimental environment, the birds were maintained *ad libitum* on their respective experimental starter feeds for three (3) weeks and Finisher feeds for another three (3) weeks. The birds received gumboro disease vaccines on the $10^{th}$and 24^{th}days. Newcastle disease vaccine (Lasota strain) was administered on the $17^{th}$and 31^{st}days.

Animal sacrifice and preparation of specimen

At the end of the feeding trial, 5 birds with the least standard deviation from the mean among them were selected from each group and starved for 12 hours, weighed after the fasting and sacrificed by severing the jugular vein. Blood was collected into untreated sample tubes. The sacrificed birds were scalded and plucked.

Determination of ultimate pH

Ultimate pH of the breast and thigh meat was measured at 24 h with pH meter after homogenization of 10gmeat in 100 ml distilled water (Wardlaw *et al*., 1973).

Determination of liver enzyme activities

Serum was separated from the blood in plain tubes by allowing the blood to clot for 3 hours. The clotted blood was spun in a bench top centrifuge at 1500 rpm for 15 minutes to obtain serum. The serum samples were thereafter separated into another set of plain sample tubes and kept in the refrigerator. The serum activities of transaminases (ALT and AST) were determined using ALT and AST Randox Kit based on the method of Reitman and Frankel (1957) modified by Schmidt and Schmidt (1963), ALP was determined according to the Recommendations of the Deutsche Gesellschaft für Klinische Chemie (1972) while GGT was determined using Randox assay Kits based on the method of Szasz (1974).

Statistical analysis

Results were expressed as means ± SEM. The data generated were subjected to one-way ANOVA using SPSS (16.0 version) statistical package program at 0.05 degree of freedom.

Result and Discussion

Broiler chickens fed on diets supplemented with the blended spices caused significant decrease ($p<0.05$) of pH in the breast and thigh meat (Table 1). The amount of muscle glycogen in chicken determines to a larger extent the alteration in the pH of meat at death and other physical properties such as water-holding capacity, colour and firmness (Berri *et al*., 2005). The lower pH values in the meat of birds fed blended spices supplemented diet points to a possible higher stored glycogen and this could lead to improved meat quality. Higher stored glycogen would cause decrease in pH which is largely due to post-slaughter glycolysis, with glycogen being converted into lactic acid (Scheffler *et al*., 2011). A low pH retard bacterial growth and so slows spoilage by promoting the diffusion of short chain fatty acids such as formic acid and acetic acid across the membrane. Such diffusions dissipate proton motive force across the membrane thereby inhibiting microbial growth (Jin and Kirk, 2018). In addition, high level of post- slaughter glycogen prevents total conversion of glycogen to lactic acid. The residual glycogen allows for improved keeping qualities. This is so because microbial population would utilise glycogen as a fuel and not protein. It should be noted that utilisation of protein by the microbes results in the production of ammonia which gives the characteristic offensive odour of spoilt meat. Also, the hydrophilic nature of glycogen molecule facilitates maintenance of the moisture content of meat. In a research by Džinić *et al*. (2013), 2% of garlic powder in feed led to excellent meat quality regarding the juiciness and tenderness of breast meat. Deterioration in meat quality is also caused by lipid peroxidation (Ana *et al*., 2018) and this could be prevented by antioxidants in the spices.

Table 1: Ultimate meat pH of broiler chickens fed diets supplemented with a mixture of ginger, garlic and cinnamon

Group	BpH	TpH
C	5.56 ± 0.05^{a}	5.74 ± 0.04^{a}
D	5.36 ± 0.02^{b}	5.34 ± 0.09^{b}
E	5.36 ± 0.04^{b}	5.28 ± 0.06^{b}
F	5.26 ± 0.07^{b}	5.26 ± 0.07^{b}

BpH- Breast pH TpH- Thigh pH

Values along the column with the same superscript are not significantly different at $P>0.05$, but are significantly different if the superscripts are different at $P>0.05$.

Group C was maintained on 0% mixed spices - supplemented Starter and then 0% mixed-spices -supplemented Finisher feeds (Control).

Group D was maintained on 0% mixed spices-supplemented Starter and then 2% mixed spices-supplemented Finisher feeds.

Group E was maintained on 2% mixed spices-supplemented Starter and then 2% mixed spices-supplemented Finisher feeds.

Group F was maintained on 2% mixed spices -supplemented Starter and 0% mixed spices-supplemented Finisher feeds.

Compared with the control, all test groups had significantly lower serum activities of GGT but higher AST. Serum activities of ALP were significantly ($p<0.05$) decreased in Groups D and F while serum ALT activities were raised (Table 2). The blended spices could protect the birds against cholestasis as shown by the significantly lower serum GGT. GGT is widely used as a biomarker for liver diseases (Woo *et al*., 2015) with increased serum level being associated with cholestasis which could lead to hepatocellular injury and bile duct obstruction. Lower level of serum ALP is also reported to correlate well with decreased risk of cholestasis (Siddique and Kris, 2012). The two enzymes are found in the plasma membrane of hepatocytes. As the concentration of bile acids increase in the liver, they act as detergents and mediate the release of the enzymes from the plasma membrane of hepatocytes (Graham *et al*., 2013). Furthermore, GGT is one of the markers of oxidative stress because it is important in the extracellular catabolism of glutathione (GSH), the representative intracellular antioxidant (Jiang *et al*., 2013) and can influence coronary atherosclerotic plaques and endothelial dysfunction (Ruttmann *et al*., 2005). Therefore, the blended spices may not only give protection in broiler against cholestasis but could also prevent GGT-mediated oxidative

stress. However, the use of the blended spices calls for caution since there were significant elevations of serum AST and ALT in the test groups. Idoko *et al*. (2020_b) reported diets supplemented with a mixture of pomegranate, ginger and garlic to have hepato-protective effect in broiler chickens. From this study, it is observed that replacement of pomegranate with cinnamon could cause hepatocellular injury. ALT and AST are found within the cytoplasm of hepatocytes and are released when the cell is injured. They are useful markers of hepatocellular liver damage.ALT is found in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body.

Table 2: Serum activities of liver enzymes in broiler chickens fed diets supplemented with a mixture of ginger, garlic and cinnamon

Group	GGT(u/l)	ALP(u/l)	ALT(u/l)	AST(u/l)
C	150.33 ±19.22c	478.00±3.55a	26.00±2.31a	184.33±8.33b
D	89.67 ±3.28a	410.33±5.78b	47.00±4.36b	207.00±3.51a
E	131.33 ±11.35b	509.33±2.17d	25.67±0.67a	224.33±1.86a
F	99.00 ±1.53a	450.67±2.00c	55.66±5.78b	204.67±8.81a

GGT-Gamma-glutamyltransferase**; ALP-** Alkaline phosphatise**; ALT-** Alanine-aminotransferase**;**
AST- Aspartate transaminase
Group C was maintained on 0% mixed spices - supplemented Starter and then 0% mixed-spices -supplemented Finisher feeds (Control).
Group D was maintained on 0% mixed spices-supplemented Starter and then 2% mixed spices-supplemented Finisher feeds.
Group E was maintained on 2% mixed spices-supplemented Starter and then 2% mixed spices-supplemented Finisher feeds.
Group F was maintained on 2% mixed spices -supplemented Starter and 0% mixed spices-supplemented Finisher feeds.

Although, concentration of ALT is greater in the liver, it is also found in the kidney, kidney, heart and muscle, while heart has higher concentration of AST in comparison with other tissues of the body including liver, kidney and skeletal muscle (Edoardo *et al*., 2005).It is therefore premature to conclude that the blended spices cause liver injury in the broiler chickens.

Conclusion

Although the blended spices may increase glycogen storage and guard against cholestasis in broiler chickens and thus improve the quality of their meat, its use is not encouraged until the cause of the increased serum ALT and AST is ascertained.

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