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Antioxidants and Oxidants Status of Broiler Chicken Fed Garlic-and Ginger-Based Diets

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Abstract
Effects of ingesting garlic-and ginger-based diets were investigated on antioxidants and oxidants in broiler chickens. 120 day old chicks were used in the study. The chicks were brooded and similarly reared for four weeks to fully adjust them to their new environment. At the end of the first four weeks, the birds were randomly assigned to four dietary treatments with 30 birds/treatment and 3 replicates of 10 birds/replicate as: T_1(control diet, contained neither garlic nor ginger), T_2 (ginger diet, contained 10g of ginger/kg of diet), T_3 (garlic diet, contained 10g of garlic/kg of diet) and T_4 (garlic and ginger diet, contained 5g of garlic + 5g of ginger/kg of diet). The birds received their respective experimental diets for 4 weeks (28 days). At the end of study, 9 birds from each treatment group composed of 3 birds from each replicate were slaughtered and their blood collected into non-ethylene diamine tetra-acetic acid (EDTA) tubes for analyses for antioxidants and oxidants. Results showed that the T_2, T_3 and T_4 animal groups had significantly ($P<0.05$) higher values of glutathione (GSH) and superoxide dismutase (SOD) compared with T_1 animal group. However, the T_1 animal group had significantly ($P<0.05$) higher values of glutathione peroxidase (GSH-Px) and catalase (CAT) compared with the T_2, T_3 and T_4 animals' treatment groups. Furthermore, the T_1 animal group had significantly ($P<0.05$) the highest levels of the oxidant malondialdehyde (MDA). It was concluded that animals of the T_1 group experienced more of oxidative stress compared with the T_2, T_3 and T_4 animal groups as MDA is an indicator of oxidative stress. Therefore, it was concluded that ginger and garlic use should be employed, particularly ginger at 10g of ginger/kg of diet for broiler chickens to optimize their productivity via protection of the bird against oxidative stress.

Keywords: Oxidative Stress, Phytochemical, GSH, CAT and SOD

Introduction

Commercial poultry production according to different independent studies is highly associated with oxidative stress that is further triggered by environmental, other internal/biological and nutritional factors (Peter and Surai, 2020) [16]. To date, despite the efforts imputed into commercial poultry production, stress in poultry production is still inevitable. Therefore, there is the need to reduce stress levels in poultry birds, especially oxidative stress as to improve their productivity. To this point therefore, it should be noted that it is the excess production of free radicals that is majorly responsible for the oxidative stress the birds undergo during production and is known to contribute to the 'sudden death syndrome' in commercial poultry production (Peter and Surai, 2020) [16]. This has led to strategizing for inclusion of antioxidants in poultry rations with the aim of reducing oxidative stress.

Antioxidants in respect to their origins can be classified into synthetic or natural. Synthetic antioxidants are widely used in food preservation as preservatives and as a result of their relatively low costs as well as their effectiveness. Some examples of synthetic antioxidants are butylated hydroxyanisole, butylated hydroxytohene and ethoxyquin. They are antioxidants of phenolic structure except ethoxyquin (Alayash *et al.*, 2001) [4]. Antioxidants stop or slow down the damage caused to cells by free radicals. It is important to note that antioxidant could be produced by or into the animal. Therefore, there are two types of antioxidants. These are endogenous and exogenous antioxidants. Those produced by the animal are the endogenous whereas those introduced into the animal are known as exogenous antioxidants, respectively. To this extent therefore, independent studies have shown that medicinal values of plants are inherent in their bioactive phytochemical components withhigh potential positive effects in respect to the health of the animal (Chinedu *et al.*, 2019) [6].

Ginger (*Zingiber officinale*) is a spice with great medicinal value. According to the data of Chinedu *et al.* (2019) [6] ginger exhibits a powerful antioxidant activity due to its oil that possesses protective effect on DNA. It also has preventive effects on lipid peroxidation by inhibiting it or breaking its chain. With these properties of ginger, it is possible that when added to poultry feed it is capable of improving their performance via improvement of their immune status. Similarly, garlic (*Allium sativum*) has a bioactive component known as *allicin*. It is also thought that garlic exhibits antioxidant effect and therefore it can enhance antioxidant productions to protect the animal against lipid peroxidation leading to improved performance. It is also capable of eliminating pathogenic microbes and thus improve the health of the animal for better performance. Oleforuh-Okoleh *et al.* (2014) [13] showed that ginger and garlic when added to poultry feed improved weight gain, feed efficiency and survivability of the birds. These observations might not be unrelated to anti-inflammatory, antioxidant and anti-microbial properties of ginger and garlic (Irivboje *et al.*, 2020) [8]. Therefore, the objectives of this study are: To investigate the effects of ginger- and garlic-based diets on the antioxidants (GSH, SOD, GSH-Px and CAT) and oxidant (MDA) status of broiler chickens as to gain some insights on their protective potentials of broiler chickens during production.

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.

Experimental animals: One hundred and twenty (120) *CHI*day-old chicks used in the study 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 properly condition them to their new environment for 4 weeks. The animals by the fourth week were observed to have properly adapted to their environment and thus were randomly assigned into four dietary 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. Feeders and drinkers were also kept under high sanitary conditions. During the 4 weeks of brooding and 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*. The experiment lasted for 8 weeks in total as the animals received their respective experimental diets for 4 weeks.

Experimental diets and animal feeding: Top feed™ mash was used in the study. The diets fed to the animals during the last four weeks of the experimental period were similar in all nutrients except the test ingredients as: Control or treatment 1 (T_1, contained neither garlic nor ginger), treatment 2 (T_2, contained 10g of ginger), treatment 3 (T_3, contained 10g of garlic) and treatment 4 (T_4, contained 5g of garlic + 5g of ginger)/kg of diet, respectively. The animals were fed these levels of the garlic- and ginger-based diets for 4weeks.

Blood Sample Collection: At the end of the study period, nine (9) birds from each treatment group were bled for blood collection. Three (3) birds were randomly collected from each replicate of the four treatment groups. The blood was collected from each of the bird into non-treated ethylene diamine tetra-acetic acid (EDTA) tubesfor later antioxidants and oxidant analyses.

Antioxidant and oxidant Analyses: Antioxidant molecules analyzed for were GSH, GSH-Px, CAT and SOD whereas the oxidant analyzed for was MDA. GSH and GSH-Px were measured according to the method of Agerganrd and Jensen (1982) [2]. CAT was analyzed for according to the method of Aebi *et al.* (1974) [1]. SOD was analyzed for according to the method of Misra and Fridorich (1972) [10], whereas MDA was analyzed for according to the method of Varsney and Kale (1990) [19].

Experimental design and Statistical analyzes: The study was designed and carried out as a completely randomized design (CRD). Data obtained 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} treatment and E_{ij} = the error term. An *α-level* of *0.05* was used for all statistical comparisons to represent significance.

Results

The result of the antioxidants analyzes is shown in Table 1.

Table 1: Antioxidant status of broiler chicken fed garlic- and ginger-based diets

Parameter	Treatments				SEM	*P-value*
	T_1 (n = 9)	T_2 (n = 9)	T_3 (n = 9)	T_4 (n = 9)		
GSH (iu/mg)	1.2^c	1.5^a	1.41^b	1.48^a	0.02	0.001
GSH-Px (iu/mg)	0.07^a	0.04^c	0.05^b	0.05^b	0.06	0.001
CAT (iu/mg)	5.61^a	4.60^b	4.24^c	4.15^c	0.00	0.000
SOD (iu/mg)	0.22^d	0.50^a	0.32^c	0.47^b	0.01	0.04

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

There were significant (*P < 0.05*) differences in the GSH levels of the birds amongst all treatment groups. Animals of the T_2 and T_4 treatment groups had the highest serum levels of GSH compared with T_3 and T_1 with the T_1 treatment group had significantly (*P < 0.05*) the lowest GSH serum levels.Similarly, there were significant (*P < 0.05*) differences in the serum levels of GSH-Px among treatment groups. T_1 had the highest level followed by the T_3 and T_4 groups that had similar (*P > 0.05*) levels whereas the T_2 treatment group had the lowest level. For CAT, T_1 treatment group had significantly (*P < 0.05*) the highest level followed by the T_2 treatment group whereas the T_3 and T_4 groups had significantly *(P < 0.05*) the lowest levels. For SOD, T_2 had significantly (*P < 0.05*) the highest level followed by T_4 and T_3 while the T_1 group had significantly (*P < 0.05*) the lowest level.

The result of the oxidant analyzes is shown in Table 2.

Table 2: Oxidant status of broiler chickens fed garlic- and ginger-based diets

Treatments						
Parameter	**T_1 (n = 9)**	**T_2 (n = 9)**	**T_3 (n = 9)**	**T_4 (n = 9)**	**SEM**	***P-value***
MDA (umol/l)	0.56^a	0.27^d	0.49^b	0.31^c	0.01	0.001

^{a,b,c,d}Means within each row with different superscripts differ significantly ($P < 0.05$).

There were significant ($P < 0.05$) differences in the MDA serum levels of the birds amongst all treatment groups. T_2 animal group had significantly ($P < 0.05$) the lowest level followed by T_4 and T_3 groups whereas T_1 group had significantly ($P < 0.05$) the highest level.

Discussion

With the current ban of antibiotics from use in animals' feeds all stakeholders, including the nutritionists have been strategizing for alternatives to antibiotics. Natural antioxidants, such as garlic and ginger have been touted to possess the potentials that can confer on them attributes that can enable them to be adopted as alternatives to antibiotics (Khan *et al.*, 2012) [9]. This was one of the major aims of this current study; the test of garlic and ginger on their potentials of regulating reactive oxygen species (ROS) by removing or converting ROS to less harmful products, such as water. To this point therefore, they can significantly contribute immensely to the health of the animal and thus improve growth and overall animal productivity.

In a study of this nature therefore, antioxidants that would normally be evaluated usually include GSH, GSH-Px, CAT and SOD whereas the oxidant MDA which is used to assess the degree of oxidative stress in the animal (NRC, 2012) [12] would be the molecules to be investigated as is an indicator of the degree of oxidative stress. Antioxidants protect cells, tissues and organs of the animal by reacting with ROS and converts them to less harmful products as previously stated. Dietary natural antioxidants instigate and trigger the production of antioxidant molecules endogenously for them to elicit their effects in the animal. From nutrition stand point, SOD is believed to be the first level of defense by endogenous antioxidants (Surai, 2002) [17]. Thus, the finding in this study that the positive control diets had higher values of SOD compared with the negative control diet therefore is not surprising. SOD is the antioxidant molecule that catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen (Bourne *et al.*, 2000) [5]. To this point, animals in these positive control treatments' groups grew faster and also had a better average daily weight gains compared with the T_1 animals as also observed in this current study, though not reported here. This finding in this study is in agreement with the data of Oleforuh-Okeleh *et al.* (2014) [13] that observed improved growth performance, carcass quality and economics of productivity in broiler chickens.

GSH is the body's major antioxidant molecule that aids to preserve all other antioxidants and therefore often refer to as the 'mother of all antioxidants' master detoxifier and master of the immune system. Therefore, its functions can be summed as antioxidant defense, nutrient metabolism and regulation of pathways essential for whole body homeostasis (Wu *et al.*, 2004; Oyewopo *et al.*, 2018) [20, 15]. These functions of GSH would have also been responsible for the results obtained with the positive control diets in this study, including better weight gains that was one of the data collected in this study but not reported here as previously mentioned.According to NRC (2012) [12] GSH-Px principally functions by converting oxidized GSH (GSSG) back to its reduced and biologically bioactive glutathione form (GSH). Thus, GSH-Px increased levels in the negative control group suggests that there would have been more of GSSG levels in birds of this group (NRC, 2012) [12]. CAT levels were highest in the negative control group of birds.CAT converts or degrades hydrogen peroxides to water and oxygen (Peter and Surai, 2020) [16]. CAT is located in the cytosol of living cells. In this way, CAT effects are found more in the cell. Thus its increased levels in the negative control group is not surprising as the productit catalyzes would have not been much in the cells of the birds of the group. This finding is in agreement with other previous workers (Noor *et al.*, 2011) [11].

In the overall, we found that garlic and ginger up-regulated GSH and SOD and thus proved to be effective and therefore demonstrated the potentials in protecting the birds as judged by the levels of MDA for the positive control treatment groups (Surai *et al.*, 2017) [18]. To this point, we found in this study that the negative control group birds had the highest levels of MDA. This was an indicator that birds in this group was most stressed from physiological standpoint. This further bring to the fore that probably the bioactive compounds in garlic and ginger would have aided the birds of the positive control groups to reduce the activities of ROS in those groups of birds (Ahmed *et al.*, 2000; Estevez, 2015) [3, 7]. Furthermore, garlic and ginger possess antibacterial, antiviral as well as antifungal properties. These characteristics of garlic and gingeralso have the potentials to biologically impact the findings of this study as they are also yardsticks for assessing health status of animals (Onu, 2010) [14].

Conclusions

Garlic and ginger up-regulated GSH and SOD but had no influence on GSH-Px and CAT. However, garlic and ginger significantly reduced oxidative stress as evidenced by the significant reduction levels of MDA in the positive control treatment groups. Furthermore, further studies are warranted involving the assessment of GSSG levels of the animals to gain a better insight of the impacts of garlic and ginger on oxidative stress in broiler chickens.

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