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Effects of *Aspergillus niger*-fermented *Terminalia catappa* seed meal-based diet on selected enzymes of some tissues of broiler chicks

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ABSTRACT

Effects of *Aspergillus niger*-fermented *Terminalia catappa* seed meal-based diet on the activities of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamate transferase (γ-GT) in the crop, small intestine, gizzard, heart, liver and serum of broiler chicks were investigated. Milled *T. catappa* seed was inoculated with spores of *A. niger* (2.21×10^4 spores per ml) for 3 weeks. Forty-five day-old broiler chicks weighing between 27.62 and 36.21 g, were divided into three groups. The first group was fed soybean-based (control) diet; the second on raw *T. catappa* seed meal-based diet; and the third on *A. niger*-fermented *T. catappa* seed meal-based diet for 7 weeks. The results revealed a significantly increased ($p < 0.05$) activity of ALP in the tissues. Contrarily, there were significant reductions ($p < 0.05$) in the activities of ALP, ALT, AST and γ-GT in the liver and heart of the broilers fed the raw *T. catappa* seed meal-based diet while there were significant increase ($p < 0.05$) in the activities of these enzymes in the serum of the broilers in this group. The data obtained showed that *A. niger*-fermented *T. catappa* seed meal reduced the toxic effects of the raw seed meal on the tissues of broiler chicks.

1. Introduction

Consumption of certain ingredients in foods and feedstuffs could be deleterious to the cells of the body (Muhammad et al., 2004; Muhammad and Oloyede, 2004). This is because foods and feedstuffs contain in addition to the useful nutrients, some antinutrients (Osagie, 1998). Antinutrients are known to elicit their deleterious effects on the tissues and cells of the body (Butler, 1989, 1992; Osagie, 1998). Tannins had been implicated as impairing the intestinal absorption of iron, reduce the digestibility of starch and proteins (Reed, 1995; Giner-Chavez, 1996). In fact, the presence of tannins in food sources for monogastric animals, is generally viewed adversely. Even in ruminants, levels of tannins exceeding 6% of the diet resulted in negatively affecting the growth rates and milk yield of lactating animals (Reed, 1995; Giner-Chavez, 1996). Tannin–protein complexes can cause inactivation of digestive enzymes and reduce protein digestibility by interaction of protein substrate with ionizable iron (Salunkhe et al., 1990). It has also been reported that phytate chelates, virtually all divalent ions, reducing their bioavailability in the living system (Reddy et al., 1989). Some of these ions are known to be important cofactors for the activity of some enzymes (Muhammad and Oloyede, 2001; Oloyede and Muhammad, 2001; Muhammad et al., 2006). *Terminalia catappa* seed has been reported to contain certain antinutrients like phytate, oxalate, tannins and hydrocyanic acid (Jeremiah, 1992; Muhammad and Oloyede, 2004; Muhammad, 2007). These antinutrients are known to affect the digestibility and bioavailability of starch, protein and minerals (Reed, 1995; Giner-Chavez, 1996), and consequently the growth of animals (Muhammad et al., 2004; Muhammad and Oloyede, 2004, 2010). That raw *T. catappa* seed meal could damage the tissues of animals was first hinted by Muhammad and Oloyede (2004) and Muhammad et al. (2006).

The effects of antinutrients on the proper synthesis and functioning of enzymes cannot be overemphasized. Enzymes are known to give the signal of damage to the cells long before histological studies could reveal such (Malomo, 2000). Antinutritional factors have been suggested to decrease protein digestibility by partly complexing with trypsin and pepsin (Reddy and Pierson, 1994). Trypsin inhibitors have been known to interfere with the physiological process of digestion through interference with the normal functioning of the pancreatic proteolytic enzymes in non-ruminants, leading to severe growth depression (White et al., 1989).

However, processing methods like cooking, autoclaving, heating, germination and microbial fermentation have been used, successfully, to either eliminate or reduce these antinutrients in foods (Aderibigbe et al., 1997; Bedford, 2000; Owoyele et al., 2003;

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Muhammad, 2007; Muhammad and Oloyede, 2010). Microbial fermentation using *Aspergillus. niger*, for example, has been reported to reduce the phytate, tannins and hydrocyanic acid of *T. catappa* seed meal (Muhammad, 2007). The present work was therefore aimed at evaluating the effect of *A. niger*-fermented *T. catappa* seed meal-based diet on the activities of some enzymes in selected tissues of broiler chicks.

2. Materials and methods

2.1. Sources of materials

Ripe fruits of *T. catappa* (authenticated at FRIN, Ibadan, Nigeria, with a voucher number of FHI 107767) were picked from the premises of the main campus of the University of Ilorin, Ilorin, Nigeria. The fruits were cracked using a 125 mm Bench vice, FUKUNG Brand and milled using the magic blender SHB-515 model. Stock of *Aspergillus niger* was obtained from the Plant Health Management Department of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The broiler chicks were obtained from Rofat Feed Limited, Ilorin, Nigeria. The paranitrophenyl phosphate used was a product of Sigma–Aldrich Chemie GmbH, Germany; the AST and ALT kits used were products of Dialab GmbH, Austria; while the GGT kit was produced by Cypress Diagnostics, Belgium.

2.2. Methods

The ripe *T. catappa* fruits were oven-dried at 60 °C and cracked to remove the seeds, using 125 mm Bench vice and the seeds were milled using magic blender. The milled sample of *T. catappa* seed was inoculated with spores of *A. niger* (2.21×10^4 spores per ml) and fermented for 3 weeks as described by Muhammad, (2007). The fermented substrate was oven-dried at 60 °C for 48 h to terminate the growth of *A. niger* and the dried substrate was used as a source of protein in the formulating diet as shown in Table 1. Proximate analysis of the formulated feed was carried out as described in AOAC (1990). The metabolisable energy was obtained as described by Pike and Brown (1975).

Forty-five-day-old broiler chicks of both sexes, weights ranging from 27.62 to 36.21 g, were randomly assigned into three dietary treatment groups. Each treatment had three replicates with five birds per replicate. The broilers were kept in an environment that was warm (40–45 °C) and disinfected. The animals were allowed to acclimatize to the laboratory environment, on commercial feed, for 1 week and weighed prior to the commencement of the feeding experiment and, thereafter on weekly basis for 7 weeks. Group feeding was carried out and the animals were supplied their different feeds and water *ad libitum*. Appropriate medications and vaccinations were administered at the 1st, 2nd and 3rd weeks of the feeding trial.

2.3. Collection of blood for serum preparation

Blood was collected from the broilers by simply incising the neck and evacuating the blood into sample bottles without anticoagulant for serum separation. Blood samples for serum were allowed to stand at room temperature for 30 min to form clot after which it was centrifuged at 1000*g* for 10 min. After centrifugation, the solid blood settled and the supernatant which was the serum was obtained using a Pasteur pipette. The sera obtained were appropriately labeled and stored in the freezer, at −5 °C, and used for analysis within 24 h (Ogbu and Okechukwu, 2001).

Table 1
Percentage composition of the diets (g/100 g).

Ingredients	A	B	C
Maize	47.00	32.00	49.00
Soybean meal	35.00	–	–
Raw *T. catappa* seed meal	–	50.00	–
Fermented *T. catappa* seed meal	–	–	33.00
Maize bran	6.00	6.00	6.00
Wheat offal	8.00	8.00	8.00
Bone meal	2.54	2.54	2.54
Oyster shell	1.00	1.00	1.00
NaCl	0.20	0.20	0.20
Vit/Min premix[a]	0.25	0.25	0.25
Lysine	0.01	0.01	0.01
Methionine	0.01	0.01	0.01
Total	100	100	100

A = Soybean meal-based diet (Control).
B = Raw *Terminalia catappa* seed meal-based diet.
C = *Aspergillus niger* treated *Terminalia catappa* seed meal-based diet.
[a] Vit. A, 4,000,000 IU; Vit. D_3, 800,000 IU; tocopherols, 4000 IU; Vit. K_3, 800 mg; folacin, 200 mg; thiamine, 600 mg; riboflavin, 1800 mg; niacin, 6000 mg; calcium panthothenate, 2000 mg; pyridoxine, 600 mg; cyanocobalamin, 4 mg; biotin, 8 mg; manganese, 30,000 mg; zinc, 20,000 mg; iron, 8000 mg; choline chloride, 80,000 mg; copper, 2000 mg; iodine, 480 mg; cobalt, 80 mg; selenium, 40 mg; BHT, 25,000; anticaking agent, 6000 mg.

2.4. Preparation of tissue homogenates

The animals were quickly dissected, the tissues excised and immersed in ice-cold 0.25 M sucrose solution (to maintain the integrity of the tissues). Homogenates were prepared for the liver, heart, crop, gizzard and small intestine. This was done by cutting a known weight of the tissue finely with a clean scissors. The tissues were thereafter homogenized in ice-cold, 0.25 M sucrose solution (1:5 w/v) using pestle and mortar. Triton x-100 was added to a final concentration of 1% (Ngaha et al., 1989; Muhammad et al., 2006). All operations were carried out at between 0 and 4 °C. The homogenates were stored in the freezer (each in a labeled specimen bottle) and used for analysis within 24 h (Ogbu and Okechukwu, 2001).

2.5. Determination of enzyme activities in the tissues studied

Alkaline phosphatase (ALP) was assayed using the method described by Bassey et al. (1946) and modified by Wright et al. (1972), which employs the use of p-nitrophenyl phosphate as substrate. In this method the amount of phosphate ester that is split within a given period of time is a measure of the phosphatase enzyme activity. ρ-Nitrophenyl phosphate (ρNPP) is hydrolysed to ρ-nitrophenol and phosphoric acid at a pH 10.1. The ρ-nitrophenol confers a yellowish colour on the reaction mixture and its intensity, measured spectrophotometrically at 400 nm gives the measure of the enzyme activity.

The activity of aspartate transaminase (AST) in the serum and tissue homogenates of the broilers was determined following the method reported by Reitman and Frankel (1957) as modified by Schmidt and Schmidt (1963). AST catalyses the formation of oxaloacetate from L-aspartate and α-ketoglutarate. The oxaloacetate generated is unstable and it spontaneously decarboxylated to form pyruvate. The absorbance of the red coloured complex formed from the reaction of pyruvate with p-nitrophenyl hydrazine was then read on a spectrophotometer at 546 nm.

The activity of glutamate–pyruvate transaminase (ALT) in the serum and tissue homogenates of the broilers was determined following the method reported by Reitman and Frankel (1957) as modified by Schmidt and Schmidt (1963). ALT catalyses the reaction between L-alanine and α-ketoglutarate to produce L-glutamate and pyruvate. The method measures spectrophotometrically the absorbance of the red coloured complex formed from the reaction between pyruvate and 2,4-dinitrophenylhydrazine.

The method of Szasz (1969) was used to assay for gamma-glutamyl transferase. Gamma-glutamyl transferase catalyses the transfer of the glutamyl from a peptide to an acceptor molecule, glycylglycine. The change in absorbance at 405 nm due to the p-nitroaniline formed in the reaction measured spectrophotometrically gives the activity of the enzyme.

2.6. Statistical analysis

The data obtained were subjected to analysis of variance and the means were compared using the Duncan Multiple range test (Steel and Torrie, 1980).

3. Results

The proximate compositions of the diets are shown in Table 2. There were no significant differences ($p > 0.05$) in the quantity of nutrients of the formulated diets. That is, the diets were isocaloric and isonitrogenous. The alkaline phosphatase activity in the tissues of the broiler chicks fed the control, raw *T. catappa* seed meal-based diet and *A. niger* treated *T. catappa* seed meal-based diet over a period of 7 weeks is shown in Table 3. The ALP activity in the liver and heart of the broilers fed the raw *T. catappa* seed meal-based diet was significantly low ($p < 0.05$) compared with those on the control and *A. niger*-fermented *T. catappa* seed meal-based diets. Whereas, the activity of the ALP in the crop, gizzard and small intestine of the raw *T. catappa* seed meal-based diet was significantly higher ($p < 0.05$) than those of the animals on the control and *A. niger*-fermented *T. catappa* seed meal-based diets. Furthermore, the serum ALP activity of the broilers on raw *T. catappa* seed meal-based diet was significantly higher ($p < 0.05$) than those on the control or *A. niger*-fermented *T. catappa* seed meal-based diet.

The AST activity of the tissues of the broilers fed the control, raw *T. catappa* seed meal-based and *A. niger*-fermented *T. catappa* seed meal-based diets for 7 weeks are shown in Table 4. The AST