Studies on the Oxidative Balance in Rats Maintained on Ripe *Musa paradisiaca* Peel

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author ASI designed the experiment, performed the statistical analysis and interpreted the results. Author NL administered the diets and typed the manuscript. Author LEI was in charge of the literature search and writing of the first draft. All authors jointly performed the biochemical analysis, read and approved the final manuscript.

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**ABSTRACT**

The use of ripe *Musa paradisiaca* peel as a replacement for corn starch is promising as the search for alternative inexpensive feed sources are sought to reduce the cost of live stock production. However, the possible long term effects of the consumption remained unclear. This research was designed to investigate the oxidative balance in rats maintained on the ripe *Musa paradisiaca* peel. The peel was mixed with other ingredients to formulate four diets replacing cornstarch at 0%, 50%, 70% and 100%. Twenty-four weanling albino rats (33.43 g±4.41 g) were divided into four groups of equal average weight. The groups were then assigned to the four diets. Diet C (Control) contained 100% inclusion level of cornstarch. Diets P100, P70 and P50 respectively contained 100%, 70% and 50% inclusion levels of the peel. The rats were fed on their respective diets and water *ad libitum*. Growth response of the rats to the diets was computed. Superoxide dismutase activity, catalase activity and concentration of malondialdehyde in some organs of the rats were also evaluated using standard methods. The results revealed that feed intake, feed conversion ratio and average weekly weight gain were not significantly (p>0.05) affected by the test diets. Diet based on 100% inclusion level caused significant (p<0.05) decrease in superoxide dismutase and catalase activity.
activities, and significant (p<0.05) increase in malondialdehyde concentration. Diet based on 70% inclusion level did not significantly (p>0.05) affect the superoxide dismutase activity, but caused significant (p<0.05) decrease in catalase activity and increase in malondialdehyde concentration. All the studied biochemical parameters were not significantly (p>0.05) affected by 50% inclusion level of the peel. It is our conclusion from the findings that up to 50% replacement of corn starch by the peel is safe for long term feeding of the animals and is as such recommended.

Keywords: Musa paradisiaca; peel; superoxide dismutase; catalase; malondialdehyde; cornstarch.

1. INTRODUCTION

The potential of Musa paradisiaca peel as a replacement for corn starch in animal feed has been investigated by many researchers. While Idoko et al. [1] reported that normal growth and organ development are maintained in rats fed with the peels as source of protein fraction, Agbabiaka et al. [2] found that Musa paradisiaca peel diets are well tolerated by African catfish and can replace maize in fish feed. The focus on the peel becomes necessary as the search for alternative inexpensive feed sources are sought to reduce the cost of live stock production. The high cost of feed ingredients and the competition existing between humans and animals over the limited supply of grains such as maize remain a major challenge facing the poultry industry in Nigeria [3]. Commonly called plantain, Musa paradisiaca is a tropical plant with Nigeria being one of the largest producing countries in the world [4]. But the possible long term effects of the consumption remained unclear. Biochemical biomarkers are used in assessment and identification of such possible effects; they identify effects at sub-cellular level before they manifest at higher levels of biological organization [5]. The biomarkers include activities of antioxidative enzymes and rate of malondialdehyde generation. Body cells and tissues are threatened continuously by damage caused by toxic reactive oxygen species (ROS) which are produced during normal oxygen metabolism. Because ROS production is a naturally occurring process, a variety of enzymatic and non-enzymatic mechanisms have evolved to protect cells against ROS [6] by balancing between the pro-oxidant and anti-oxidant systems in intact cells. Enzymes involved in the elimination of ROS include superoxide dismutases (SODs) and catalase. Oxidative damage causes a net stress on the normal body functions, leading to a gradual loss of vital physiological functions, later in life. Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein synthesis, DNA damage; ultimately resulting in cell death [7]. This research was therefore, designed to provide scientific information on the oxidative balance in rats maintained on processed ripe Musa paradisiaca peel.

2. MATERIALS AND METHODS

2.1 Materials

Ripe peel of Musa paradisiaca fruits, corn, soybeans, soybean oil and sucrose were bought from Azare central market, while rice bran was collected from a rice milling factory in Azare, Bauchi State, Nigeria. Twenty-four (24) weanling albino rats (33.43 g±4.41 g) were used for the research. All the chemicals and reagents used in this study were of analytical grade and were kept under ideal laboratory condition.

2.2 Sample Preparation

The sample was prepared in accordance with the method of Idoko and Oladiji [8]. Here, the peels were washed with water, cut into pieces and then oven dried at 60°C for 72 hours to constant weight. The pieces were then pulverized using an electric blender and thereafter kept in polythene bag.

2.3 Feed Formulation

The pulverized peels were mixed with other ingredients to formulate four experimental diets to replace corn starch at 0%, 50% and 100%. Diet C (Control) contained 100% inclusion level of corn starch. Diets P100, P70 and P50 respectively contained 100%, 70% and 50% inclusion levels of ripe Musa paradisiaca peel. The food items were thoroughly mixed together and manually made into pellets to feed albino rats. The proximate composition of the formulated diet was determined in accordance with the methods of AOAC [9].
2.4 Assigning of Experimental Animals

The twenty-four weanling albino rats were assigned into four groups. The six rats in each group were housed in standard plastic laboratory cages with stainless steel covers. The groups were then maintained on their respective experimental diets and water *ad libitum* for six weeks. The rats were kept in accordance with the recommendation of ARRP [10].

2.5 Growth Response

The feed intake was determined as the difference between the quantity of feed served and the quantity of feed left over. The weekly change in weight was computed by weighing the rats at the commencement of the feeding trial and thereafter on a weekly basis until termination of the experiment. The weekly feed conversion ratio was calculated by dividing the total feed consumed by total weight gained.

2.6 Animal Sacrifice and Preparation of Tissue Homogenate

After 42 days of feeding trial, the animals were weighed and sacrificed by anaesthetizing them in a jar containing cotton wool soaked in diethylether. The sacrificed rats were dissected to remove the liver, kidney and heart. The isolated tissues were weighed and a portion cut out, cut into very small pieces and then homogenized in ice-cold 0.25M sucrose solution [11]. The tissue homogenates were appropriately diluted (x5) and kept frozen while being used.

2.7 Determination of Superoxide Dismutase (SOD) Activity

The superoxide dismutase activity was determined by the method described by Misra and Fridovich [12]. In this method, the inhibition of auto-oxidation of adrenaline to adrenochrome is determined by measuring the absorbance of adrenochrome formed at 480 nm. The assay mixture was constituted by mixing 0.2 ml of enzyme source (tissue homogenate), 2.5 ml of 0.05M phosphate buffer and 0.3 ml of 0.3mM adrenalin in a test-tube. A blank was constituted by replacing enzyme source with 0.2 ml of distilled water. The constituents in each tube were mixed well and the absorbance was read at 480 nm.

2.8 Determination of Catalase Activity

Catalase activity was determined by the method described by Beers and Sizer [13]. In this method, the disappearance of peroxide is

| Table 1. Components of the formulated diets (gkg⁻¹) |
|-------------------|------------------|------------------|------------------|
|                  | C           | MP100          | MP70             | MP50             |
| Soy meal         | 353         | 353            | 353              | 353              |
| Soybean oil      | 40          | 40             | 40               | 40               |
| Rice bran        | 40          | 40             | 40               | 40               |
| D-L methionine   | 04          | 04             | 04               | 04               |
| Sucrose          | 100         | 100            | 100              | 100              |
| Vit/min mix      | 50          | 50             | 50               | 50               |

Results are means of 3 determinations±SEM. Values along the same row with the same superscript are not significantly different (P > 0.05), and are significantly different (p<0.05) if the superscripts are different. C: (control): diet based on 100% inclusion level of cornstarch, MP100: diet based on 100% inclusion level of processed ripe Musa paradisiaca peel, MP70: diet based on 70% inclusion level of processed ripe Musa paradisiaca peel, MP50: diet based on 50% inclusion level of processed ripe Musa paradisiaca peel.
followed spectrophotometrically at 240 nm. Distilled water (1.9 ml) was mixed with 1.0 ml of hydrogen peroxide in a test-tube. Tissue homogenate (0.1 ml) was added to the resulting solution after 5 minutes and the decrease in absorbance was monitored at 240 nm for 2 minutes.

### 2.9 Determination of Malondialdehyde

Malondialdehyde was determined by the method described by Satoh [14]. A chromogenic reagent (2-thiobarbituric acid) reacts with MDA at 25°C to yield a chromophore (MDA-TBA$_2$) with absorbance maximum at 532 nm. The intensity of the absorbance is proportional to the concentration of malondialdehyde present in the biological sample.

### 3. RESULTS AND DISCUSSION

#### 3.1 Growth Response of Rats Maintained on ripe *Musa paradisiaca* Peel

Feed intake, weight gain and feed conversion ratio in rats maintained on different inclusion levels of ripe *Musa paradisiaca* peel did not differ significantly (p>0.05) from the response of the rats to the control diet (Table 2). The non-significant difference in the feed intake indicates that the test diet may have the adequate taste and texture required of animal feeds. Feed intake has been reported to be influenced by taste [15], habitual consumption [16] and fulfilment of consumers’ expectations of sensory quality [17]. Agbabiaka et al. [2] reported similar consumption pattern of *Musa paradisiaca* peel in African catfish fingerlings. If processed, the peel has the potential of producing adequate growth. This is supported by the weight gain and feed conversion ratio of rats maintained on the test diets; they did not differ significantly from weight gain and feed conversion ratio of rats maintained on the control diet. In a similar research, Idoko et al. [1] reported normal growth and organ development in rats fed on the peels as source of protein fraction.

#### 3.2 SOD Activities in Selected Tissues of Rats Maintained on Ripe *Musa paradisiaca* Peel

The activities of SOD in the liver, kidney and heart of rats fed 50% and 70% inclusion levels of ripe *Musa paradisiaca* peel were not significantly different (p>0.05) from the activities in rats maintained on the control diet. However, rats maintained on 100% inclusion level of ripe *Musa paradisiaca* peel had significantly lower (p<0.05) activities of SOD in the studied organs when compared with the control (Table 3). Superoxide is the primary reactive oxygen species produced from a variety of sources and its dismutation by SOD to the less reactive species (H$_2$O$_2$) is of primary importance for each cell. The result of the activities of SOD shows that use of processed *Musa paradisiaca* peel up to 70% replacement for corn starch in animal feed does not cause the overproduction of superoxides nor do the diets lack the nutrients needed for the synthesis of SOD. The activity of SOD has been reported to be affected by nutritional status. For instance, a dietary deficiency of protein not only impairs the synthesis of antioxidant enzymes but also reduces tissue concentrations of antioxidants, thereby resulting in a compromised antioxidant status [18]. It could be inferred from the result that long term consumption of diet based on replacement of corn starch by processed ripe *Musa paradisiaca* peel may have no adverse effect if the replacement does not exceed 70%.

|          | C              | P100           | P70            | P50            |
|----------|----------------|----------------|----------------|----------------|
| Feed intake (g) | 64.87±1.48$^a$ | 66.56±1.36$^a$ | 66.50±1.15$^a$ | 66.27±1.35$^a$ |
| Weight gain (g)  | 5.95±0.09$^a$  | 5.53±0.17$^a$  | 5.74±0.16$^a$  | 5.89±0.12$^a$  |
| FCR            | 10.90±0.19$^a$ | 12.09±0.52$^a$ | 11.65±0.47$^a$ | 11.28±0.40$^a$ |

Results are means of 3 determinations ± SEM. Values along the same row with the same superscript are not significantly different (P > 0.05), and are significantly different (p<0.05) if the superscripts are different. C: (control): rats fed on diet based on 100% inclusion level of cornstarch, P100: rats maintained on diet based on 100% inclusion level of processed ripe *Musa paradisiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa paradisiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa paradisiaca* peel. FCR: feed conversion ratio.
Table 3. SOD activities (unit/mg protein) in selected tissues of rats maintained on ripe *Musa paradisiaca* peel

| Organ   | C      | P100     | P70      | P50      |
|---------|--------|----------|----------|----------|
| Liver   | 58.49±0.33<sup>a</sup> | 25.78±0.17<sup>a</sup> | 57.76±0.16<sup>a</sup> | 58.39±0.33<sup>a</sup> |
| Kidney  | 38.57±0.20<sup>a</sup> | 18.35±0.06<sup>b</sup> | 38.30±0.08<sup>a</sup> | 38.69±0.09<sup>a</sup> |
| Heart   | 21.67±0.11<sup>a</sup> | 13.29±0.06<sup>a</sup> | 21.04±0.23<sup>a</sup> | 21.49±0.01<sup>a</sup> |

Results are means of 3 determinations ± SEM. Values along the same row with the same superscript are not significantly different (P >0.05), and are significantly different (p<0.05) if the superscripts are different.

C: (control): rats fed on diet based on 100% inclusion level of cornstarch, P100: rats maintained on diet based on 100% inclusion level of processed ripe *Musa paradisiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa paradisiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa paradisiaca* peel.

### 3.3 Catalase Activities in Selected Tissues of Rats Maintained on Ripe *Musa paradisiaca* Peel

There was no significant difference (p>0.05) between the catalase activities in the organs of rats maintained on 50% inclusion level of ripe *Musa paradisiaca* peel and activities in the organs of rats maintained on the control diet. Compared with the control, the catalase activities were significantly lowered (p<0.05) in rats maintained on 100% and 70% inclusion levels of *Musa paradisiaca* peel in a dose dependent manner (Table 4). The decrease in the activities of catalase in rats maintained on 100% and 70% inclusion levels of *Musa paradisiaca* could be due to increased production of H<sub>2</sub>O<sub>2</sub> or decreased synthesis of the enzyme. Whichever way, the decrease is potentially dangerous because catalase is the enzyme responsible for the degradation of hydrogen peroxide generated either from the reactions of SOD or from other sources. The danger of H<sub>2</sub>O<sub>2</sub> largely relates to its ease of conversion to the indiscriminately reactive hydroxyl radical by interaction with transition metal ions, of which the most important in *vivo* is probably iron [19], leading to hydroxyl radical -mediated oxidative DNA damage.

### 3.4 Malondialdehyde (nmol/ml) Levels in Selected Tissues of Rats Maintained on Ripe *Musa paradisiaca* Peel

The difference between the concentration of malondialdehyde in the studied organs of rats maintained on 50% inclusion level of *Musa paradisiaca* peel and control diet was not significant (p>0.05). Rats maintained on 100% and 70% inclusion levels of *Musa paradisiaca* peel had higher malondialdehyde than the control (Table 5). The non significant difference between the concentration of malondialdehyde in the studied organs of rats maintained on 50% inclusion level of *Musa paradisiaca* peel and control diet is consistent with the activities of the antioxidant enzymes (SOD and catalase). Thus, rise in the levels of ROS may be properly checked in the organs of rats fed this diet thereby preventing the development of oxidative stress. The increased concentration of malondialdehyde in rats maintained on 100% and 70% inclusion levels of *Musa paradisiaca* peel is also consistent with the decreased activity of catalase in rats maintained on these diets. This increase could become so damaging to the tissues because it could lead to the development of oxidized lipids and fatty acids that give rise to free radicals. Malondialdehyde react with proteins and nucleic acids leading to decrease in membrane fluidity, an increase in the leakiness of the membrane to substances that do not normally cross it except through specific channels. Malondialdehyde may damage membrane protein, inactivate receptors, enzymes and ion channels [20]. Oxidative stress has been associated with increased production of ROS, leading to increase in concentration of malondialdehyde, and a decrease in the effectiveness of anti-oxidant defence such as catalase [21]. Despite the normal activities of SOD in rats maintained on 70% inclusion level of processed ripe *Musa paradisiaca* peel, the results of catalase activity and malondialdehyde concentration shows that only up to 50% replacement of corn starch by processed ripe *Musa paradisiaca* peel may be safe for long term feeding of the animals. in other words, only 50% inclusion level of processed ripe *Musa paradisiaca* peel could supply the nutrients needed for adequate synthesis of antioxidative enzyme (catalase and SOD) thereby preventing the oxidation of lipid and fatty acids and generation of free radicals.
Table 4. Catalase activities (unit/mg protein) in selected tissues of rats maintained on ripe *Musa paradisiaca* peel

| Organ  | C           | P100         | P70           | P50           |
|--------|-------------|--------------|---------------|---------------|
| Liver  | 97.33±0.00<sup>a</sup> | 49.80±0.26<sup>b</sup> | 67.88±0.05<sup>c</sup> | 97.19±0.05<sup>a</sup> |
| Kidney | 59.16±0.22<sup>a</sup> | 27.69±0.12<sup>b</sup> | 33.81±0.23<sup>c</sup> | 58.60±0.10<sup>a</sup> |
| Heart  | 13.22±0.01<sup>a</sup> | 6.97±0.09<sup>b</sup>  | 10.24±0.02<sup>c</sup> | 13.15±0.02<sup>a</sup> |

Results are means of 3 determinations ± SEM. Values along the same row with the same superscript are not significantly different (P >0.05), and are significantly different (p<0.05) if the superscripts are different.

C: (control): rats fed on diet based on 100% inclusion level of cornstarch, P100: rats maintained on diet based on 100% inclusion level of processed ripe *Musa paradisiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa paradisiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa paradisiaca* peel.

Table 5. Malondialdehyde (nmol/ml) levels in selected tissues of rats maintained on ripe *Musa paradisiaca* peel

| Organ  | C           | P100         | P70           | P50           |
|--------|-------------|--------------|---------------|---------------|
| Liver  | 54.98±0.16<sup>a</sup> | 106.91±0.48<sup>b</sup> | 79.71±0.33<sup>c</sup> | 54.48±0.28<sup>a</sup> |
| Kidney | 11.10±0.30<sup>a</sup> | 25.23±0.06<sup>b</sup>  | 21.60±0.12<sup>c</sup> | 11.42±0.31<sup>a</sup> |
| Heart  | 3.65±0.26<sup>a</sup> | 9.49±0.27<sup>b</sup>  | 6.25±0.02<sup>c</sup>  | 3.73±0.29<sup>a</sup>  |

Results are means of 3 determinations ± SEM. Values along the same row with the same superscript are not significantly different (P >0.05), and are significantly different (p<0.05) if the superscripts are different.

C: (control): rats fed on diet based on 100% inclusion level of cornstarch, P100: rats maintained on diet based on 100% inclusion level of processed ripe *Musa paradisiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa paradisiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa paradisiaca* peel.

4. CONCLUSION

Diets based on replacement of corn starch by processed ripe *Musa paradisiaca* peel have no obvious adverse effect on the growth response of rats. However, only up to 50% replacement of corn starch by processed ripe *Musa paradisiaca* peel is safe for long term feeding of the animals and is as such recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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