

**Studies on the oxidative balance in rats
maintained on ripe *Musa paradisiaca* peel**

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 ($33.43g \pm 4.41g$) 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 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 parasidiaca; peel; superoxide dismutase; catalase; malondialdehyde; cornstarch

1. INTRODUCTION

The potential of *Musa parasidiaca* 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 parasidiaca* 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 parasidiaca* 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

38 loss of vital physiological functions, later in life. Reactive oxygen species can attack vital cell
39 components like polyunsaturated fatty acids, proteins, and nucleic acids. To a lesser extent,
40 carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane
41 properties like fluidity, ion transport, loss of enzyme activity, protein synthesis, DNA damage;
42 ultimately resulting in cell death [7]. This research was therefore, designed to provide
43 scientific information on the oxidative balance in rats maintained on processed ripe *Musa*
44 *paradisiaca* peel.

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46 **2. MATERIALS AND METHODS**

47 **2.1 MATERIALS**

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

53

54 **2.2 SAMPLE PREPARATION**

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

59

60 **2.3 Feed formulation**

61 The pulverized peels were mixed with other ingredients to formulate four experimental diets
62 to replace corn starch at 0%, 50% and 100%. Diet C (Control) contained 100% inclusion
63 level of corn starch. Diets P100, P70 and P50 respectively contained 100%, 70% and 50%
64 inclusion levels of ripe *Musa parasidiaca* peel. The food items were thoroughly mixed

65 together and manually made into pellets to feed albino rats. The proximate composition of
66 the formulated diet was determined in accordance with the methods of AOAC [9]

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Table 1: Components of the formulated diets (gkg⁻¹)

	C	MP100	MP70	MP50
C	413	-	123.9	206.5
MP	-	413	-	-
MP	-	-	289.1	-
MP	-	-	-	206.5
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

Percentage proximate composition of the formulated diets

Protein	22.88±0.22 ^a	22.85±0.10 ^a	22.99±0.05 ^a	22.93±0.07 ^a
Lipid	6.12±0.11 ^a	6.07±0.04 ^a	5.99±0.07 ^a	6.09±0.13 ^a
Fibre	7.07±0.07 ^a	8.48±0.01 ^b	8.31±0.02 ^{b^c}	8.15±0.04 ^c
Ash	7.38±0.04 ^a	7.40±0.08 ^a	7.47±0.07 ^a	7.40±0.03 ^a
Carbohydrate	56.54±0.27 ^a	55.16±0.16 ^b	55.23±0.15 ^b	55.43±0.17 ^b

69 Results are means of 3 determinations ± SEM .Values along the same row with the same
70 superscript are not significantly different (P >0 .05), and are significantly different (p<0.05) if
71 the superscripts are different. C: (control): diet based on 100% inclusion level of cornstarch,
72 MP100: diet based on 100% inclusion level of processed ripe *Musa parasidiaca* peel, MP70:
73 diet based on 70% inclusion level of processed ripe *Musa parasidiaca* peel, MP50: diet
74 based on 50% inclusion level of processed ripe *Musa parasidiaca* peel.
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76 **2.4 Assigning of experimental animals**

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

81

82 **2.5 Growth response**

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

88 **2.6 Animal sacrifice and preparation of tissue homogenate**

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

95 **2.7 Determination of superoxide dismutase (SOD) activity**

96 The superoxide dismutase activity was determined by the method described by Misra and
97 Fridovich [12]. In this method, the inhibition of auto-oxidation of adrenaline to adrenochrome
98 is determined by measuring the absorbance of adrenochrome formed at 480 nm. The assay
99 mixture was constituted by mixing 0.2 ml of enzyme source (tissue homogenate), 2.5 ml of
100 0.05M phosphate buffer and 0.3 ml of 0.3mM adrenalin in a test-tube. A blank was
101 constituted by replacing enzyme source with 0.2 ml of distilled water. The constituents in
102 each tube were mixed well and the absorbance was read at 480nm.

103 **2.8 Determination of catalase activity**

104 Catalase activity was determined by the method described by Beers and Sizer [13]. In this
105 method, the disappearance of peroxide is followed spectrophotometrically at 240 nm.
106 Distilled water (1.9 ml) was mixed with 1.0 ml of hydrogen peroxide in a test-tube. Tissue
107 homogenate (0.1 ml) was added to the resulting solution after 5 minutes and the decrease in
108 absorbance was monitored at 240 nm for 2 minutes.

109 **2.9 Determination of malondialdehyde**

110 Malondialdehyde was determined by the method described by Satoh [14]. A chromogenic
111 reagent (2-thiobarbituric acid) reacts with MDA at 25°C to yield a chromophore (MDA-TBA₂)
112 with absorbance maximum at 532 nm. The intensity of the absorbance is proportional to the
113 concentration of malondialdehyde present in the biological sample.

114 **2.10 Statistical Analysis**

115 Results were expressed as means ± SEM. One-way analysis of variance (ANOVA)
116 computed from statistical package program (SPSS 16.0 version) was used to determine
117 statistical significance; p value less than 0.05 was considered significant.

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120 **3. RESULTS AND DISCUSSION**

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122 **3.1 Growth response of rats maintained on ripe *Musa paradisiaca* peel**

123 Feed intake, weight gain and feed conversion ratio in rats maintained on different inclusion
124 levels of ripe *Musa paradisiaca* peel did not differ significantly ($p>0.05$) from the response of
125 the rats to the control diet (Table 2). The non-significant difference in the feed intake
126 indicates that the test diet may have the adequate taste and texture required of animal
127 feeds. Feed intake has been reported to be influenced by taste [15], habitual consumption
128 [16] and fulfilment of consumers' expectations of sensory quality [17]. Agbabiaka *et al* [2]
129 reported similar consumption pattern of *Musa paradisiaca* peel in African catfish fingerlings.
130 If processed, the peel has the potential of producing adequate growth. This is supported by

131 the weight gain and feed conversion ratio of rats maintained on the test diets; they did not
132 differ significantly from weight gain and feed conversion ratio of rats maintained on the
133 control diet. In a similar research, Idoko *et al* [1] reported normal growth and organ
134 development in rats fed on the peels as source of protein fraction.

135 **3.2 SOD activities in selected tissues of rats maintained on ripe *Musa*** 136 ***parasidiaca* peel**

137 The activities of SOD in the liver, kidney and heart of rats fed 50% and 70% inclusion levels
138 of ripe *Musa parasidiaca* peel were not significantly different ($p>0.05$) from the activities in
139 rats maintained on the control diet. However, rats maintained on 100% inclusion level of ripe
140 *Musa parasidiaca* peel had significantly lower ($p<0.05$) activities of SOD in the studied
141 organs when compared with the control (Table 3). Superoxide is the primary reactive oxygen
142 species produced from a variety of sources and its dismutation by SOD to the less reactive
143 species (H_2O_2) is of primary importance for each cell. The result of the activities of SOD
144 shows that use of processed *Musa parasidiaca* peel up to 70% replacement for corn starch
145 in animal feed does not cause the overproduction of superoxides nor do the diets lack the
146 nutrients needed for the synthesis of SOD. The activity of SOD has been reported to be
147 affected by nutritional status. For instance, a dietary deficiency of protein not only impairs the
148 synthesis of antioxidant enzymes but also reduces tissue concentrations of antioxidants,
149 thereby resulting in a compromised antioxidant status [18]. It could be inferred from the
150 result that long term consumption of diet based on replacement of corn starch by processed
151 ripe *Musa parasidiaca* peel may have no adverse effect if the replacement does not exceed
152 70%.

153 **3.3 Catalase activities in selected tissues of rats maintained on ripe *Musa*** 154 ***parasidiaca* peel**

155 There was no significant difference ($p>0.05$) between the catalase activities in the organs of
156 rats maintained on 50% inclusion level of ripe *Musa parasidiaca* peel and activities in the
157 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 parasidiaca* 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 parasidiaca* could be due to increased production of H_2O_2 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_2O_2 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 parasidiaca* peel

The difference between the concentration of malondialdehyde in the studied organs of rats maintained on 50% inclusion level of *Musa parasidiaca* peel and control diet was not significant ($p>0.05$). Rats maintained on 100% and 70% inclusion levels of *Musa parasidiaca* 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 parasidiaca* 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 parasidiaca* 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 parasidiaca* peel, the results of catalase activity and malondialdehyde concentration shows that only up to 50% replacement of corn starch by processed ripe *Musa parasidiaca* peel may be safe for long term feeding of the animals. in other words, only 50% inclusion level of processed ripe *Musa parasidiaca* 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 2: Growth response of rats maintained on ripe *Musa parasidiaca* peel

	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 parasidiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa parasidiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa parasidiaca* peel. FCR: feed conversion ratio

Table 3: SOD activities (unit/mgprotein) in selected tissues of rats maintained on ripe *Musa parasidiaca* peel

ORGAN	C	P100	P70	P50
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Liver	58.49±0.33 ^a	25.78±0.17 ^b	57.76±0.16 ^a	58.39±0.33 ^a
Kidney	38.57±0.20 ^a	18.35±0.06 ^b	38.30±0.08 ^a	38.69±0.09 ^a
Heart	21.67±0.11 ^a	13.29±0.06 ^b	21.04±0.23 ^a	21.49±0.01 ^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 parasidiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa parasidiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa parasidiaca* peel

Table 4: Catalase activities (unit/mgprotein) in selected tissues of rats maintained on ripe *Musa parasidiaca* peel

ORGAN	C	P100	P70	P50
Liver	97.33±0.00 ^a	49.80±0.26 ^b	67.88±0.05 ^c	97.19±0.05 ^a
Kidney	59.16±0.22 ^a	27.69±0.12 ^b	33.81±0.23 ^c	58.60±0.10 ^a
Heart	13.22±0.01 ^a	6.97±0.09 ^b	10.24±0.02 ^c	13.15±0.02 ^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 parasidiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa parasidiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa parasidiaca* peel

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

Organ	C	P100	P70	P50
Liver	54.98±0.16 ^a	106.91±0.48 ^b	79.71±0.33 ^c	54.48±0.28 ^a

Kidney	11.10±0.30 ^a	25.23±0.06 ^b	21.60±0.12 ^c	11.42±0.31 ^a
Heart	3.65±0.26 ^a	9.49±0.27 ^b	6.25±0.02 ^c	3.73±0.29 ^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 parasidiaca* peel, P70: rats maintained on diet based on 70% inclusion level of processed ripe *Musa parasidiaca* peel, P50: rats maintained on diet based on 50% inclusion level of processed ripe *Musa parasidiaca* peel

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 parasidiaca* peel is safe for long term feeding of the animals and is as such recommended.

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