

DIGESTIVE AND ABSORPTIVE ENZYMES IN RATS FED PHYTIC ACID EXTRACT FROM SWEET POTATO (*IPOMOEA BATATAS*)

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*Key words: phytate, sweet potato, intestine, disaccharidase,
ATPase*

SUMMARY

*Phytic acid was extracted from sweet potato (*Ipomoea batatas*) and fed to wistar rats with or without zinc for three weeks. Animals were then sacrificed and blood glucose, bone zinc and calcium levels were assessed. Intestinal disaccharidases and Na^+/K^+ ATPase activities were determined. All groups fed phytic acid extract from sweet potato displayed low blood glucose levels compared to their controls. However, it was more pronounced in the groups fed zinc supplement along with phytic acid. There were no significant changes in the activities of intestinal disaccharidases in study groups. The supplemented diets significantly reduced Na^+/K^+ ATPase activity in lower intestine compared to controls. The consumption of supplemented diets did not show any adverse effects on bone minerals in rats. In conclusion, this study showed the rate of absorption to be stepped down as demonstrated by the observed decrease in the activity of the transport enzyme, Na^+/K^+ ATPase leading to a blood glucose decrease. The consumption of Jamaica sweet potato with a high phytic acid to zinc ratio may not adversely affect bone minerals in rats.*

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INTRODUCTION

Phytic acid is a storage form of phosphorus which is found in plant seeds and in many roots and tubers (1). Phytic acid has long been recognized as an antinutrient for its ability to bind to, precipitate and decrease the availability of di- and trivalent cationic minerals (2). The reduced availability of minerals will prove disadvantageous, as minerals play a major role in many enzyme systems and metabolic pathways in animals. Apart from binding to minerals, phytic acid is also thought to regulate the process of digestion by binding to some digestion products, thereby delaying the onset of diabetes and hyperlipidemia (3). Phytic acid may also prove beneficial as it is thought to impair starch digestion and thus help in blood glucose control (4). It has also been suggested that phytic acid may directly bind to some metabolic by-products and even some enzymes, thereby directly affecting the activities of those enzymes. Both phytic acid and polyphenols have been shown to play a role in reducing the *in vitro* rate of starch digestibility and possibly blood glucose response (5). Studies carried out by Yoon *et al.* (6) also show that phytic acid may affect starch digestibility through interaction with amylase, protein and/or binding with salivary minerals such as calcium.

Disaccharides (sucrose, lactose and maltose) are important sources of metabolic energy. The first step in the catabolism of disaccharides is hydrolysis to their

constituent monosaccharides. The enzymes responsible for the hydrolysis of sucrose, lactose and maltose are sucrase, lactase and maltase, respectively. These enzymes are located in the epithelial cells of the small intestine. The end-products resulting from the activities of these enzymes are actively translocated from the intestine to the blood by ATPases.

Sweet potato (*Ipomoea batatas*) is an important staple food crop in the Caribbean. In addition to the presence of many beneficial nutrients, which may be obtained by the consumption of sweet potato, preliminary studies in our laboratory have shown that sweet potato has a high phytic acid to zinc ratio of 18:1 even after cooking, compared to other tuber crops grown in Jamaica. The use of diets high in phytic acid to zinc ratio has been reported to induce mineral deficiencies in mammals (7). There are concerns about the probable effects of consuming Jamaica sweet potato with a high phytic acid to zinc ratio. To test the implications of this in the Caribbean, the effects of consumption of sweet potato phytic acid extract was evaluated to assess intestinal responses and bone minerals using animal models. Commercial phytic acid at the same level was used for comparison.

MATERIALS AND METHODS

Fresh mature sweet potato (*Ipomoea batatas*) tubers were harvested from a local farm in the Parish of Manchester, Jamaica. Tuber samples were washed with distilled water, peeled, diced, oven dried at 65 °C to constant weight, and ground to fine powder.

Extraction and determination of phytic acid

Phytic acid was extracted by a modification of the method of Samotus and Schwimmer (8). A known amount of sweet potato was blended for 5 min with 10% trichloroacetic acid in a Warring blender. The

slurry was filtered by suction in a sintered glass funnel and the residue washed successively with known volumes of 5% trichloroacetic acid. Filtrates were combined, neutralized with 5M NaOH, and freeze-dried for use as phytic acid extract. Phytic acid level in the extract was determined by the method of Holt (9) as described by Davies and Reid (10).

Experimental animals were 36 adult Wistar rats (18 male and 18 female). They were assigned by weight into 6 groups of 6 rats each, mean body weight 236.4 g. Groups 1 and 2 were designed as controls. The composition of test diets (groups 3 and 4) was designed to simulate the high phytic acid to zinc ratio of 18:1 as observed in cooked sweet potato commonly consumed in the Caribbean. Groups 5 and 6 were included in the study to assess the effects of phytic acid (at the same level as above) on low dietary zinc intake. Diets were prepared according to standard methods (AIN-76A purified rodent diet, AIN-76A vitamin mix, and AIN-76A mineral mix (11,12). Commercial phytic acid was purchased from Sigma-Aldrich, St. Louis, MO, USA. The feeding groups were as follows: normal rats fed formulated diet without zinc supplementation (group 1); normal rats fed formulated diet supplemented with zinc (group 2); normal rats fed formulated diet plus zinc supplement along with phytic acid extract from sweet potato (group 3); normal rats fed formulated diet plus zinc supplement along with commercial phytic acid (group 4); normal rats fed formulated diet supplemented with phytic acid extract from sweet potato (group 5); and normal rats fed formulated diet supplemented with commercial phytic acid (group 6). The levels of phytic acid and zinc in the extract were 9.46 mg/g and 0.002%, respectively (Table 1).

Zinc was added as part of the mineral mix to the diets of group 2, 3 and 4 animals, in the form of zinc carbonate at a concentration of 1.60 mg/kg. The zinc level in the diets fed to groups 1, 5 and 6 was negligible as determined by Atomic Absorption Spectrophotometry.

Table 1. Levels of zinc, phytic acid and phytate to zinc ratio in selected tuber crops

Tuber crops	Uncooked samples			Cooked samples		
	Phytate (mmole/g)	Zinc (μmole/g)	Phytate:Zinc	Phytate mmole/g	Zinc (μmole/g)	Phytate:Zinc
Yellow yam	6.85x10 ⁻³	1.88x10 ⁻¹	38:1	1.136x10 ⁻³	1.55x10 ⁻¹	8:1
Cocoyam	5.52x10 ⁻³	1.66x10 ⁻¹	38:1	2.27x10 ⁻³	1.92x10 ⁻¹	13:1
Sweet potato	4.98x10 ⁻³	1.18x10 ⁻¹	67:1	2.238x10 ⁻³	1.24x10 ⁻¹	18:1

metry. Ingredients of the standard formulated diets are shown in Table 2.

Table 2. **Diet composition**

Ingredient	Grams/kilogram
Casein	200.00
DL-methionine	3.00
Corn starch	150.00
Sucrose	500.00
Cellulose	50.00
Corn oil	50.00
Salt mix	35.00
Vitamin mix	10.00
Choline bitartate	2.00

Spectrophotometry

Commercial phytic acid (dodecasodium salt) of 92% purity and other components of the commercial diet preparation were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Phytic acid (extract or the commercial variety) was added to the diets of animals in groups 4-6 in quantities that would simulate a phytate to zinc ratio of 18:1.

Rats were housed in stainless steel cages in a room kept on a 12-h light-dark cycle, and were allowed access to their respective diets and water freely. The cages were cleaned daily. Prior to the start of the experiment, all animals were fed with formulated diet plus zinc (group 2 diet) for one week to allow for acclimatization. The rats were then fed their respective diets for three weeks. Body weight change and total food intake were recorded. At the end of the experiment, the rats were sacrificed by a blow to the head. Approval for the study was obtained by the protocol presentation to the Department Board.

Blood glucose and intestinal enzyme assay

Fasting blood samples were obtained in oxalate/fluoride containers for glucose determination as described by Trinder (13). The intestine of each rat was divided into two portions: the proximal segment (duodenum) representing upper intestine, and the mid and distal segment (jejunum and ileum) representing lower intestine. Rat intestine, which was free of food materials, was excised and the lumen flushed out

several times with 0.9% NaCl. The mucosal washing and the scraped mucosa were pooled, homogenized, centrifuged (5,000 g), and the supernatant was frozen until required for enzymatic assays. The activities of intestinal disaccharidases were determined by measuring the amount of glucose released from their various substrates (14). Disaccharidases break disaccharides down to glucose and monosaccharide. The glucose formed is acted upon by glucose oxidase in the presence of oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide produced is hydrolyzed by peroxide to produce water and oxygen. The amount of glucose formed *per* time unit as determined by brown color at 450 nm is a measure of disaccharidase activity.

ATPase activities were measured by the amount of inorganic phosphate liberated following incubation with disodium ATP and cations for the appropriate ATPase using the method of Bonting *et al.* (15), as modified by Bonting (16) and Takeo *et al.* (17). The method of Fiske and Subarrow (18) was used to quantify the amount of inorganic phosphate released. Protein determination was carried out by the method of Bradford (19).

Bone mineral assay

Femurs from all animals were cleaned of soft tissue using stainless steel scissors and stainless steel forceps. They were dried in an oven at 100 °C for 3 hours. Dry weights were taken and femurs were ashed in silica glass crucibles (20). The samples were placed in a cold furnace and temperature was gradually raised to 450 °C and kept so for 24 hours. One milliliter of concentrated high purity nitric acid was then added. After the bones had dissolved, the solutions were taken to dryness on a hotplate, then fired for 30 to 60 seconds over a Bunsen burner. The resulting white ashes were dissolved in 5 ml of 20% HCl, the crucible was covered and digested at low heat for about 15 minutes. This was allowed to cool and made up to 25 ml in a volumetric flask. Solutions were further diluted with 1M HCl as required, after which zinc and calcium determinations were made using atomic absorption spectrophotometry.

Table 3. **Body weight changes and food intake in rats fed supplements of sweet potato phytic acid extract or commercial phytic acid or formulated normal diets**

Rat group		Initial body weight (g)	Final body weight (g)	Daily food intake/rat (g)
Formulated diet	(1)	236.8±16.5 ^a	256.3±23.9 ^a	12.1±1.1 ^a
Formulated diet +Zn	(2)	236.5±14.5 ^a	260.9±19.3 ^a	11.9±0.8 ^a
Formulated diet +Zn +phytate extract	(3)	236.3±14.9 ^a	225.6±9.33 ^{a,b}	11.2±1.9 ^a
Formulated diet +Zn +commercial phytate	(4)	236.5±21.2 ^a	250.0±21.1 ^a	11.4±0.8 ^a
Formulated diet +phytate extract	(5)	236.0±15.7 ^a	202.5±8.9 ^b	10.1±0.4 ^a
Formulated diet +commercial phytate	(6)	236.7±16.5 ^a	241.4±17.1 ^{a,b}	13.4±0.8 ^a

Values expressed as mean ±SEM; figures in columns with different superscripts were significantly different ($p < 0.05$).

Table 4. **Blood glucose levels in rats fed supplements of sweet potato phytic acid extract or commercial phytic acid or formulated normal diets**

Group	Blood glucose (mM)
1 Formulated diet	7.12±0.89 ^a
2 Formulated diet + Zn	7.62±1.32 ^a
3 Formulated diet + Zn + phytic acid extract	5.43±1.66 ^a
4 Formulated diet + Zn + commercial phytic acid	5.17±0.78 ^a
5 Formulated diet + phytic acid extract	5.94±0.28 ^a
6 Formulated diet + commercial phytic acid	6.78±1.04 ^a

Values are expressed as mean ±SEM; figures in rows with different superscripts were significantly different ($p < 0.05$).

Statistical analysis

Results were expressed as means ± SEM. Analysis of variance (ANOVA) was used to test for between group differences. Duncan's Multiple Range Test was used to test for significant difference among the means and $p < 0.05$ was considered significant (21).

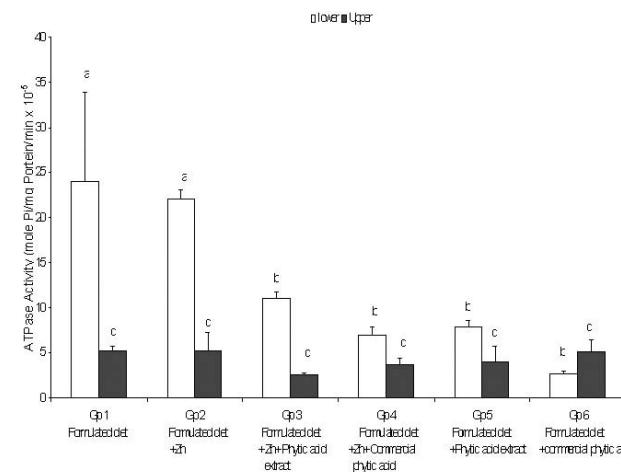
RESULTS

Table 3 shows body weight changes and food intake in rats fed sweet potato phytic acid extract. Final body weight was significantly reduced in rats fed phytic acid extract compared to groups fed control diets (groups 1 and 2) or commercial phytic acid plus zinc even though daily food intake was not significantly different among the groups. Table 4 shows blood glucose levels in rats which had their diets supplemented with commercial phytic acid or crude phytic acid extract from sweet potato. There was no significant difference among the groups.

Table 5 shows intestinal disaccharidase activities in rats fed phytic acid supplemented diets. Generally, there were no significant differences in disaccharidase activities among the groups. However, sucrase activity was lowest in the groups fed phytic acid diets supplemented with zinc (groups 3 and 4).

Figure 1 shows the activity of intestinal lower and upper segment Na^+/K^+ ATPase in rats fed phytic acid supplemented diets. The activity of Na^+/K^+ ATPase in the lower intestine of the four groups (groups 3-6) was significantly lower than that recorded in the control groups (1 and 2).

Figure 1. **Lower and upper intestinal Na^+/K^+ ATPase activity in rats fed supplement sweet potato phytic acid extract or commercial phytic acid or formulated normal diets.**



Means ±SEM; figures that share different superscripts were significantly different ($p < 0.05$).

There was no significant change in the bone calcium level. However, there was a significant decrease in the bone zinc level in the group fed commercial phytic acid

Table 5. Effect of supplements of sweet potato phytic acid extract or commercial phytic acid or formulated normal diets on the intestinal digestive enzyme activities

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Enzyme activity (μ mole enzyme / mg protein/min) $\times 10^{-3}$	Formulated diet	Formulated diet +Zn	Formulated diet +Zn + phytic acid extract	Formulated diet + Zn+ commercial phytic acid	Formulated diet +phytic acid extract	Formulated diet + commercial phytic acid
<i>Maltase</i>						
Upper	2.02 \pm 0.16 ^a	1.59 \pm 0.14 ^a	0.95 \pm 0.01 ^a	2.03 \pm 0.14 ^a	1.83 \pm 0.37 ^a	1.65 \pm 0.23 ^a
Lower	1.80 \pm 0.99 ^a	2.17 \pm 0.16 ^a	1.98 \pm 0.47 ^a	2.21 \pm 0.22 ^a	2.44 \pm 0.15 ^a	1.97 \pm 0.13 ^a
<i>Sucrase</i>						
Upper	2.09 \pm 0.03 ^a	1.29 \pm 0.34 ^a	0.45 \pm 0.28 ^a	1.12 \pm 0.22 ^a	1.9 \pm 0.35 ^a	1.36 \pm 0.22 ^a
Lower	1.27 \pm 0.18 ^a	0.79 \pm 0.24 ^a	0.77 \pm 0.08 ^a	0.90 \pm 0.25 ^a	1.18 \pm 0.31 ^a	1.43 \pm 0.68 ^a
<i>Lactase</i>						
Upper	0.19 \pm 0.03 ^a	0.15 \pm 0.04 ^a	0.13 \pm 0.004 ^a	0.25 \pm 0.04 ^a	0.17 \pm 0.004 ^a	0.24 \pm 0.02 ^a
Lower	0.17 \pm 0.03 ^a	0.21 \pm 0.02 ^a	0.14 \pm 0.001 ^a	0.25 \pm 0.006 ^a	0.15 \pm 0.04 ^a	0.19 \pm 0.04 ^a

Values are expressed as mean \pm SEM; figures in columns with different superscripts were significantly different ($p > 0.05$)

Table 6. Bone minerals in rats fed supplements of sweet potato phytic acid extract or commercial phytic acid or formulated normal diets

	Calcium (mole/g)	Zinc (mole/kg)
Group 1 Formulated diet	2.98175 \pm 1.134 ^a	6.106 \pm 0.224 ^a
Group 2 Formulated diet + Zn	5.3285 \pm 0.383 ^a	6.077 \pm 0.1679 ^a
Group 3 Formulated diet + Zn + phytic acid extract	2.53175 \pm 1.478 ^a	5.828 \pm 0.2967 ^a
Group 4 Formulated diet + Zn + commercial phytic acid	5.0895 \pm 1.012 ^a	6.428 \pm 0.144 ^{ac}
Group 5 Formulated diet + phytic acid extract	5.2625 \pm 0.172 ^a	5.718 \pm 0.025 ^a
Group 6 Formulated diet + commercial phytic acid	4.72125 \pm 0.775 ^a	5.055 \pm 0.744 ^{ab}

Values are expressed as mean \pm SEM; figures in columns with different superscripts were significantly different ($p < 0.05$).

plus zinc compared to the group fed commercial phytic acid without zinc added to the diet (Table 6).

DISCUSSION

Although there were no significant between-group differences in the levels of blood glucose, the study clearly demonstrated that the groups on phytic acid supplemented diets showed lower blood glucose levels compared to groups 1 and 2 that were not fed phytic acid. An interesting observation was that out of four phytic acid fed groups (groups 3-6), blood glucose levels were lowest in the two groups that had zinc supplementation (groups 3 and 4). This implies that adequate supplementation of the diet with zinc is necessary for phytic acid to be truly effective in lowering blood glucose. However, commercial exploitation of sweet potato phytic acid with zinc

supplementation as a potential blood glucose-lowering agent for diabetics needs additional long-term investigations.

Intestinal ATPases are membrane bound enzymes that function primarily in the active transport of minerals across mucosal membrane (22). Increased Na^+/K^+ ATPase activity has been shown to increase glucose translocation across the cell membrane by creating an inward sodium ion gradient (23). The observed significant lowering of ATPase activity could largely explain the decreased blood glucose in the groups fed test diets, which may prove beneficial for diabetics.

The significant decrease in the level of bone zinc in the group fed commercial phytic acid plus zinc compared to the group fed commercial phytic acid without zinc added to the diet shows that the consumption of a diet high in phytic acid and low in

zinc may adversely affect the bone zinc level. Of note, however, is that there was no significant difference in the level of bone zinc among the groups fed phytic acid extract from sweet potato. This shows that the consumption of phytic acid from sweet potato may not adversely affect the bone zinc level, and this may be due to its interaction with other antinutritive factors contained in the crude extract. The absence of such interactions in the commercial phytic acid [(dodecasodium salt) 92% purity] may account for the observed adverse effect of this supplement.

Overall, although phytic acid has been reported to lower mineral absorption, data from this rat model study did not show any adverse effect of phytic acid

extract consumption from sweet potato on bone minerals. This study also showed that the rate of absorption was stepped down, as demonstrated by the observed decrease in the activity of the transport enzyme, Na^+/K^+ ATPase, leading to a blood glucose decrease. However, due to anatomical and physiological differences between the species, this study could only provide an indirect evaluation of the extracts for human use.

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