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# Plantain Ash Enhances Dietary Mineral Elements Absorption in Pullets

**Research Article**

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## ABSTRACT

Generally, only a fraction of the mineral ingested by an animal is effectively absorbed, while most are bound to other components such as fibre and then excreted. In this study, faecal mineral composition differentials were used as indicators of mineral uptake by pullets supplemented varying levels of plantain ash in their daily rations for nine weeks. Plantain stalk and root base samples were collected, sun dried and ashed to produce plantain stalk ash (PSA) and root base ash (PRA), respectively. Ninety six (96) day old Isa Brown pullets were reared to 15 weeks of age and thereafter divided into 2 major groups of 48 birds each. Each group was divided into 4 subgroups of 12 birds each and each subgroup divided into three replicates giving four birds per replicate in a completely randomized design. The PSA and PRA were fed to the pullets as mineral supplements to commercial grower from the 15th to 18th week (4 wk) and layer mashes from the 19th to 24th week (6 wk) at the rate 0, 1, 2 and 3 g per kg body weight so that one group received PSA and the other PRA. In each case, the zero supplementation served as control. At the 9th week of feeding, faecal samples were collected from the groups, dried in the sun and analyzed for their mineral compositions by atomic absorption spectrophotometry. Among the macro minerals, both PSA and PRA supplementation resulted in reductions in the faecal concentrations of K and Mg, with increasing supplementation level, resulting in further reduction of faecal content of these minerals. Mg showed highly significant ($P<0.05$, $P<0.01$) regression effects for PSA and PRA, respectively. Reductions in faecal Ni and Fe concentrations were high (47.62 and 79.19%) across 1 g/kg body weight (BW) PSA and PRA supplementations, while it was 83.33% for Mn at the same 1 g/kg BW PRA supplementation. Ni (PSA) and Cr (PSA and PRA) values were significant ($P<0.10$) for regression effects, implying that the faecal values of these minerals could be predicted from any given quantity of plantain ash using the regression equations. PRA supplementations resulted in increasing faecal content of the two heavy metals, that is, lead and cadmium, indicating selective poor intestinal absorption of these. Plantain ash could serve as potential sources of absorbable mineral supplements and also could improve mineral uptake from commercial diets offered to pullets.

KEY WORDS faeces, heavy metals, mineral absorption, plantain ash, pullets.

## INTRODUCTION

Mineral elements are important nutrients in animal diets because they participate in metabolic, enzymatic and biochemical reactions needed for sustenance, feed efficiency, growth and development of animals. According to Underwood and Suttle (1999), 26 elements are considered essential for animal wellbeing. Eleven of these are classed as macro elements and include carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, phosphorous, potassium, sodium, chlorine and magnesium. The remaining 15 are classed as micro elements and include iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium and arsenic (Underwood and Suttle, 1999). Macro mineral elements are those required in relatively large amounts by the animal body, while micro (trace) mineral elements are those required in very small or minute amounts (Esonu, 2006).

All natural feedstuffs contain some minerals but in intensive animal production, especially poultry, supplemental minerals are provided in various forms including salt, trace mineralized salt, oyster shell, limestone, bone meal and a wide variety of other forms (Damron, 2009; Power and Horgan, 2007; Oso *et al.* 2011). Majority of the minerals used in supplementing intensively farmed animals come from rock and often are thought of as inert, inorganic substances. These naturally occurring minerals are usually mined in their most unstable states and thereafter processed or used directly in animal feed manufacture (Rodrigues, 2010). Other mineral containing binders include activated charcoal, which is prepared from wood, vegetables or other organic materials. Such charcoal and plant ash are the residue of burnt plant parts like the bark, wood, saw dust, leaves, woody debris, pulp, husk, fronds and other plant debris (Ndhlovu, 2007; Rodrigues, 2010).

Most inorganic mineral elements are usually not readily bioavailable to the animal mainly due to antagonism amongst themselves, feed ingredients and other nutrients resulting in reduced absorption (Soetan *et al.* 2010). Feed producers have tended to overcome this problem by increasing the quantity of the mineral element sources included in the feed, with resultant further reduction in bioavailability, increased fecal residues and attendant environmental problems (Power and Horgan, 2007). Generally, only a fraction of the minerals ingested by an animal is effectively absorbed, while most are bound to other components such as fiber and excreted (Underwood and Suttle, 1999). Different terms are used to assess and express the nutritive value of minerals for animals, for example, digestibility, absorbability, bioavailability or even bio-efficacy (Patridge, 1980). Digestibility and absorbability pertain to the gastrointestinal tract (feed minus feces). ARC (1981) defined bioavailability as the fraction that is retained in the body [feed - (feces+urine)]. Mineral bioavailability is also defined as the proportion of the element consumed that is utilized for a biochemical or physiologic function (Ammerman *et al.* 1995; Fairweather Tait, 1996; O'Dell, 1997), indicating that it involves both the absorption and the ultimate metabolic utilization of a mineral within the cell. Thus, for a mineral to have high bioavailability, it must be readily absorbed and easily assimilated by the body.

Bioavailability is influenced by the chemical form of the mineral, the amount in the diet, the amount stored in the body, the concentration of other minerals in the diet, and the health, age, and physiological state of the animal to which it is fed (Hazel, 1985; Whitehead *et al.* 1996; Cao *et al.* 2000). Since numerous intrinsic and extrinsic factors have been shown to affect the bioavailability of dietary inorganic trace elements, continuous efforts have been made over the years to improve their utilization by humans and animals. For example, the use of metal chelates of $Cu^{2+}$, $Zn^{2+}$ and $Mn^{2+}$ with amino acids and peptides has been shown to enhance their bioavailability and has led to the development of many such products in the animal feed industry (Power and Horgan, 2007; Scheideler, 2008; Ashmead, 2012). These products have been termed organic metals because the metal elements needed to be made more bioavailable are complexed with organic molecules (Close, 1999).

Internationally, various types of organic trace metal products used in livestock production have been recognized (AAFCO, 1998) and include essentially, metal amino acid complex, metal amino acid chelate, metal polysaccharide complex and metal proteinate (Power and Horgan, 2007; Ashmead, 2012). The chemical structures differ between chelate, complex or proteinate compounds and have direct effects on the nutritional and overall industrial values of these products. According to Hynes and Kelly (1995), in metal amino acids chelates, the amino acids act as 'bidentate' ligands, which bond to metal ions via an oxygen of the carboxylic acid group and the nitrogen of the amino group. Chelation therefore occurs where such ligands bind to a metal ion via two or more donor atoms to form a complex containing one or more heterocyclic rings bearing the metal atom. While enormous progress has been made in research and development of products for more nutrient bioavailability, there is still room for progress (Ashmead, 2012), especially in experimentations with simpler processes amenable to small scale rural farming and feeding systems in developing countries (Ebere, 2012; Iwu, 2012; Nwogu, 2013). Wood or plant ash has been used in a variety of agricultural applications such as excellent source of potassium, lime and other plant nutrients (Campbell, 1990; Etiegni, 1990), source of alkaline material, which has been used successfully in treatment of wheat straw to improve its digestibility (Nolte *et al.* 1987; Ramirez *et al.* 1992) and treatment of high tannin sorghum for poultry feeding (Kyarisiima *et al.* 2004). Several reports from Africa and many other cultures have also shown that treatment with high alkaline plant ash improves the nutritional value of many foods (Kyarisiima *et* *al.* 2004). Ochetim (1988) suggested the use of wood ash as a source of minerals for poultry. However, Oso *et al.* (2011) reported occurrence of lameness and poor gaits in broilers fed diet containing wood ash as Ca source and therefore concluded that wood ash inclusion in broiler diets should be discouraged since it results in low Ca availability to the birds. Recent studies at our station have however shown that supplementation with coconut shell ash at the level of 1 g/kg body weight was optimal for growing rabbits fed normal commercial diet, while higher levels of supplementation resulted in some mildly detrimental serum biochemical responses (Iwu, 2012; Ebere, 2012). Wood ash is produced when plant materials are subjected to high temperature treatment to burn off all the organic parts leaving behind only the inorganic part as plant minerals. Analysis of the ash samples obtained at different combustion temperatures indicated that Ca, K, Mg, Si and P were the major components (Ndhlovu, 2007). Wood ash is substantially different from coal ash, which has a lower alkalinity but higher silicon, aluminium, iron and heavy-metal content (Campbell, 1990). The mineral content of wood ash is however highly influenced by the combustion system and handling of the wood prior to incineration as well as wood type. The alkalinity of wood ash depends on its carbonate, bicarbonate and hydroxide content. Carbonates and bi-carbonates predominate below 500 ˚C, whereas oxides become more prevalent above 1000 ˚C where most of the industrial wood fired boilers operate (Naylor and Schmidt, 1986). Ash composition will also change during storage and under varying environmental conditions as carbon dioxide ($CO_2$) and moisture react with ash to form carbonates, bicarbonates and hydroxides (Etiegni and Campbell, 1991). In making wood ash, the burnt organic components of the plant including phytates, certain sugars, fibre sources and polyphenols, which ordinarily interact with mineral molecules to reduce their absorption via specific mucosal receptors (Johnson, 1989; Power and Horgan, 2007; Scheideler, 2008), are completely eliminated. It is therefore expected that plant ash may be a valuable mineral source to animals and may successfully replace intact plant and rock mineral sources in human and animal diets if properly processed (Ndhlovu, 2007).

Such ash minerals in their free oxide states (Etiegni and Campbell, 1990) might bind and form complexes or chelates with available ligands in the feed such as synthetic amino acids or with the numerous endogenous ligands in the gastrointestinal tract to prevent their hydroxy-polymerisation and improve their delivery, subsequently absorption and assimilation by enterocytes (Ashmead *et al.* 1985; Hynes and Kelly, 1995; Powell *et al.* 1999). In this study, the absorption of mineral in pullets fed commercial rations supplemented with varying levels of plantain ash were determined. Faecal minerals composition differentials were used as indicators of mineral absorption.

## MATERIALS AND METHODS

### Collection and preparation of test material

The stalk and root base of mature plantain plants (*Musa paradisiacal*) from which the fruits had already been harvested were collected, cut into smaller pieces and sun dried for about five days and thereafter stored in labeled sample envelopes. The sun dried plantain stalk and root base samples were ashed according to the method described by Nwogu (2013). Briefly, weighed sun dried plantain stalk and root samples were placed in local clay pots and ashed for nine hours over a fire provided by a kerosene stove. At the end of the ashing process, the ash obtained was weighed again to determine the ash yield of each sample type.

### Mineral analysis

The ashes were analyzed for their mineral contents using the atomic absorption spectrophotometer (Bulk Scientific, 205). A measured quantity (2 g) of the ash samples was transferred into a kjeldahl flask; 20 mL of concentrated nitric acid ($HNO_3$) was added and the sample pre-digested by heating gently for 20 minutes. More acid was thereafter added and the digestion was continued for 30-40 minutes and was stopped when a clear digest was obtained. The flask was cooled and the contents filtered into a 50 mL volumetric flask through a Whatman No.42, 150 mm diameter filter paper and made to mark with distilled water. The resulting solutions were analyzed for Nickel (232 nm), Chromium (357. 9 nm), Lead (283.2 nm), Iron (248.3 nm), Copper (324.7 nm), Zinc (213. 9 nm), Cobalt (240. 7 nm), Calcium (424.7 nm), Magnesium (285.2 nm), Sodium (589 nm), Manganese (279.5 nm), Potassium (766.5 nm) and cadmium (228.9 nm).

### Experimental birds and diets

One hundred and twenty (120) day old Isa Brown pullets (body weight range of 350-360 g) procured from a local commercial hatchery in Owerri was used for the experiment. The birds were brooded under proper feeding, medication and vaccination management as practiced at the Teaching and Research farm of Federal University of Technology Owerri (FUTO), Nigeria. At eight weeks of age, they were transferred from the brooding house to the rearing pen, where they were raised until needed for the experiments. During the brooding, rearing and laying periods, the bird were fed commercial chick, grower and layer rations. The nutrient compositions of the commercial rations as declared by the manufacturer are presented in Table 1. The mineral composition of these test diets were also determined using AAS.

**Table 1** Nutrient compositions of the commercial feed used as shown on the product label

| Parameter | Chick mash | Grower mash | Layer mash |
|---|---|---|---|
| Crude protein (%) | 21.00 | 16.00 | 16.50 |
| Fats / oil (%) | 6.00 | 5.00 | 5.00 |
| Crude fiber (%) | 5.00 | 7.00 | 6.00 |
| Calcium (%) | 1.00 | 1.00 | 3.80 |
| Available phosphorus (%) | 0.45 | 0.45 | 0.45 |
| Lysine (%) | 1.00 | 0.75 | 0.80 |
| Methionine (%) | 0.50 | 0.36 | 0.34 |
| Salt (% min) | 0.30 | 0.30 | 0.30 |
| Minimum metabolizable energy (kcal/kg) | 2800 | 2450 | 2500 |

At 13 weeks of age, 96 of these birds were selected on the bases of viability and transferred to battery cages. For the purposes of this study, the birds were placed two per cage in order to eliminate stress arising from feeding and watering space competition (Nwogu, 2013). The 96 birds were randomly divided into two groups of 48 birds each. Each of these groups was subjected to either PSA or PRA treatments. Each of these ash treatment groups were further divided into four groups of 12 birds each and replicated three times with four birds per replicate. These were assigned to the treatment diets in a completely randomized design (CRD). In each case, the group receiving the zero supplemented diet served as the control.

At 15 weeks of age, the experimental diets were introduced to the birds. These were again the commercial grower and layer rations supplemented with plantain stalk and root base ashes (PSA and PRA respectively) at the rate of 0, 1, 2 and 3 grams per kilogram body weight of the birds to correspond to $T_1$, $T_2$, $T_3$ and $T_4$ or $R_1$, $R_2$, $R_3$ and $R_4$ treatments for PSA and PRA respectively. During the grower and laying stages of the feeding experiment, $T_1$ and $R_1$ with zero ash supplementations served as controls.

The grower mash supplemented with the test materials was given to the birds until point of lay, which in this study was determined at 19 weeks of age, when the first egg was laid by one of the experimental birds. Thereafter, the ration was changed to layer mash supplemented with the test materials and given to the birds till the 24th week of age. The birds were fed on a divided dose regime, with the first batch of feeds offered in the morning containing the PSA and PRA thoroughly mixed with the commercial ration, while the afternoon batch was offered straight. Water was offered *ad libitum*.

### Data collection

At the ninth week of the experimental period, faecal samples were collected from each treatment group and prepared for mineral content analysis. The samples were oven dried at 60 ˚C over night or sun dried until they become crispy to touch. Thereafter, they were subjected to AAS analysis as earlier described to determine their concentrations of calcium, sodium, potassium, magnesium, iron, chromium, manganese, zinc, lead, nickel, cobalt, arsenic, copper and cadmium (AOAC, 1995).

### Data analysis

Data collected were subjected to analysis of variance (Steel and Torrie, 1980). The treatments mean values were tested for significant difference by Duncan's new multiple range test (SAS, 2000).

Furthermore, percentages deviations of mineral values in the faecal samples from the control group values were calculated. Simple regression equations relating mineral concentrations in faecal samples to dietary inclusion levels of plantain ash were also developed according to SAS (2000).

## RESULTS AND DISCUSSION

### Mineral composition of experimental grower and layer diet

Table 2 shows the mineral composition of the commercial grower and layer rations used in the study and their comparisons with the standard organization of Nigeria recommended values (SON, 2003). In all cases, the mineral concentrations of the commercials feeds were quite below the values recommended by SON (2003).

**Table 2** Mineral concentration in experimental commercial diets (mg/100 g)

| Minerals | Grower mash | Layer mash | SON recommended grower | SON recommended layer |
|---|---|---|---|---|
| Ca | 192 | 244 | 1000.000 | 3500.000 |
| Mg | 20 | 52 | 47.500 | 52.500 |
| K | 0.47 | 0.44 | 375.000 | 375.000 |
| Na | 1.0 | 1.10 | 225.000 | 225.000 |
| P | 0.05 | 0.047 | 400.000 | 450.000 |
| Fe | 0.04 | 0.07 | 9.250 | 5.500 |
| Mn | 1.41 | 1.13 | 3.500 | 5.500 |
| Zn | 1.40 | 1.70 | 4.500 | 3.500 |
| Cu | 0.26 | 0.14 | 0.950 | 0.950 |
| Se | - | - | 0.015 | 0.015 |
| I | - | - | 0.035 | 0.0525 |
| Pb | 0.0 | 0.73 | - | - |
| Cd | 0.002 | 0.002 | - | - |

### Concentrations of macro minerals in the faeces of experimental birds

The faecal macro mineral contents of the PSA and PRA supplemented birds are shown in Table 3. The concentration of Ca was 236 mg/100 g in the control faecal sample and was followed by Mg (7.10 mg/100 g), while the lowest value was recorded for sodium (0.002 mg/100 g). Concentrations of K and Mg in the control faecal samples were significantly ($P<0.05$) higher than the values recorded in the faeces of ash supplemented groups. Thus, both PSA and PSA supplementation of the control diet, resulted in reductions in the faecal concentrations of K and Mg with increasing supplementation level resulting in further reduction of faecal content of these minerals. PRA supplementation gave a similar result for faecal Ca concentration.

Computations of the percentage deviations of this faecal macro mineral contents from the control values as presented in Table 4 showed that PSA supplementation at 1 g/Kg BW resulted in a 200% increase in faecal Na, while the same level of PRA supplementation resulted in 50% reduction in faecal content of the same mineral. At 2 g/kg BW PSA supplementation the situation reversed with faecal Na level being reduced to 50% of the control, while for PRA group, it took up to 3 g/kg BW supplementation to achieve 50% increase above faecal control values for Na. The results recorded for potassium and magnesium are particularly interesting since plantain ash supplementation generally resulted in the reduction in the faecal content of these minerals in pullets at the early laying stage.

Table 5 shows the macro minerals Ca, Na, K and Mg which were generated from two ash sources, plantain stock ash (PSA) and plantain root ash (PRA) and their relationship with dietary inclusion levels of plantain ash in their diets. Their prediction equations had both positive and negative coefficients, but only positive correlation coefficients (r) ranging from 0.153 to 0.99, which is low to high. The coefficient of determination ($R^2$) ranged from 0.023 to 0.980, translating to the range of 2.3 to 98% in terms of percentage of $R^2$. There was no significant ($P>0.10$) regression effect for Ca, Na and K, while Mg showed highly significant ($P<0.05$, $P<0.01$) regression effects for PSA and PRA respectively. The positive values of r imply a direct relationship between the faecal macro-minerals and the levels of inclusion of plantain ash. With the range of $R^2$ being 2.3 to 98%, it shows that there is a low to very high strength of determination of the various macro minerals using the regression equation. Hence the faecal Na, K and Mg values of PRA supplemented birds and Mg only in PSA supplemented birds had very strong coefficient of determination, while others were weak. However, the non significant ($P>0.10$) regression effects on Ca, Na and K implies that their values cannot be predicted using the prediction equation for any given quantity of plantain ash. But Mg can be predicted given a quantity of plantain ash supplied in the diet of the experimental birds at 25 weeks of age.

### Faecal micro minerals concentrations of the experimental birds

Micro mineral contents (mg/100 g) of faecal samples collected form layers fed plantain ash were presented in Table 6. The manganese concentration in the control faecal was highest (2.40 mg/100 g) and was followed by the 2.10 mg/100 g recorded for iron, thus making these values significantly ($P<0.05$) higher than the values recorded in the faeces of the plantain ash supplemented groups. Increasing supplementation generally resulted in the reduction of the faecal contents of Ni and Fe, while only PSA supplementation could be used to achieve this for cobalt.

Table 7 showed the computations of percentage deviations of faecal micro minerals concentration from the control values. At 1 g/Kg BW ash supplementation, the faecal copper (PSA), from the birds rose by 428.57% but reduced with higher supplementation levels to 35.71% above and 35.71% below control values at 2 and 3 g/kg BW supplementations respectively. PRA supplementation resulted in initial 85.71% reduction in faecal level, followed by a rise of 114.29% at 2 g/kg BW supplementation and an amelioration of the situation (7.74% increase) with higher PRA supplementation. Similarly, reductions in faecal Ni and Fe concentrations were high (47.62-79.19%) across 1 g/Kg BW PSA and PRA supplementations, while it was 83.33% reduction in the faecal concentration of Mn for the 1 g/Kg BW PRA supplementation.

Again, while increase in supplementation levels of PSA resulted in continued reduction in faecal value of Co, 3 g/kg BW supplementation of PRA resulted in an increase of 12.50% above control value. PSA supplementation however resulted in a consistent higher yield of faecal chromium above the control values (23.53, 217.65, 741.17% faecal increase for 1, 2 and 3 g/kg BW supplementation respectively) indicating an inability of the birds to absorb this mineral from the PSA supplemented diet. PRA supplementation however resulted in reduction of faecal chromium content, especially at the higher supplementation levels.

Table 8 showed the simple linear regression equations relating micro-mineral uptake from the GIT (Y) to dietary inclusion levels of plantain ash (X). The r values were all positive, with the values ranging from medium to high. The $R^2$ values ranged from 0.018 to 0.840, which is 1.8 to 84% in percentages of $R^2$. Ni (PSA) and Cr (PSA and PRA) values were significant ($P<0.10$) for regression effects which implies that only Ni and Cr could be predicted from any given quantity of plantain ash using the regression equations. Furthermore, the faecal Ni, Fe, Zn, Mn and Cr concentrations of PSA supplemented birds and Fe and Cr only in PRA supplemented birds had very strong coefficient of determination, while others were weak. However, the non significant ($P>0.10$) regression effects on Fe, Zn and Mn implied that their values could not be predicted using the prediction equation for any given quantity of plantain ash-sup-plemented in the diet of the experimental birds at 25 weeks of age.

**Table 3** Macro mineral concentration (mg/100 g) in faecal samples from layers fed plantain ash

| Mineral | Ash type | Control | 1 g/kg body weight | 2 g/kg body weight | 3 g/kg body weight | SEM |
|---|---|---|---|---|---|---|
| Calcium | PSA | $236^{a}$ | $236^{a}$ | $199^{b}$ | $262^{a}$ | 13.00 |
| | PRA | $236^{a}$ | $148^{b}$ | $208^{ab}$ | $232^{a}$ | 20.30 |
| Sodium | PSA | $0.002^{b}$ | $0.006^{a}$ | $0.001^{b}$ | $0.002^{b}$ | 0.0011 |
| | PRA | $0.002^{ab}$ | $0.001^{b}$ | $0.002^{ab}$ | $0.003^{a}$ | 0.0004 |
| Potassium | PSA | $0.72^{a}$ | $0.67^{bc}$ | $0.65^{c}$ | $0.71^{ab}$ | 0.0165 |
| | PRA | $0.72^{a}$ | $0.52^{bc}$ | $0.65^{ab}$ | $0.46^{c}$ | 0.0594 |
| Magnesium | PSA | $7.10^{a}$ | $6.50^{b}$ | $6.70^{ab}$ | $6.50^{b}$ | 0.141 |
| | PRA | $7.10^{a}$ | $6.90^{ab}$ | $6.70^{b}$ | $6.60^{b}$ | 0.111 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
PSA: plantain stock ash; PRA: plantain root ash and SEM: standard error means.

**Table 4** Percentage deviation of faecal macro minerals concentrations from the control values

| Mineral | Ash type | 1 g/kg body weight | 2 g/kg body weight | 3 g/kg body weight |
|---|---|---|---|---|
| Calcium | PSA | 0.00 | -11.86 | +11.02 |
| | PRA | -37.29 | -11.86 | -1.70 |
| Sodium | PSA | +200.00 | -50.00 | 0.00 |
| | PRA | -50.00 | 0.00 | +50.00 |
| Potassium | PSA | -13.89 | -9.72 | -1.39 |
| | PRA | -27.78 | -9.72 | -26.00 |
| Magnesium | PSA | -8.45 | -5.63 | -8.45 |
| | PRA | -2.82 | -5.63 | -7.04 |

-: percentage reduction from the control values and +: percentage increase from the control values.
PSA: plantain stock ash and PRA: plantain root ash.

**Table 5** Simple linear regression equations relating macro mineral absorption from the GIT of experimental birds at 25 weeks of age (Y) to dietary inclusion levels of plantain ash (X)

| Dependent variable (Y) | Ash type | Prediction equation | r | $R^2$ | % of $R^2$ | Significance | |
|---|---|---|---|---|---|---|---|
| Calcium | PSA | Y= 0.127+0.00102X | 0.204 | 0.042 | 4.20 | 0.796 | ns |
| | PRA | Y= 1.50+0.000485 | 0.153 | 0.023 | 2.30 | 0.847 | ns |
| Sodium | PSA | Y= 2.966-169.492X | 0.291 | 0.085 | 8.50 | 0.709 | ns |
| | PRA | Y= 0.50+1000.00X | 0.632 | 0.400 | 40.00 | 0.368 | ns |
| Potassium | PSA | Y= 07.748-7.634X | 0.195 | 0.038 | 3.80 | 0.805 | ns |
| | PRA | Y= 7.017-7.688X | 0.707 | 0.500 | 50.00 | 0.293 | ns |
| Magnesium | PSA | Y= 24.833-3.33X | 0.730 | 0.533 | 53.30 | 0.016 | * |
| | PRA | Y= 41.831-5.763X | 0.990 | 0.980 | 98.00 | 0.01 | ** |

r: correlation coefficient; $R^2$: coefficient of determination and % of $R^2$: percentage of $R^2$.
* (P<0.05) and ** (P<0.01).
NS: not significant.

**Table 6** Micro mineral concentration (mg/100 g) in faecal samples from layers fed plantain ash

| Mineral | Ash type | Control | 1 g/kg body weight | 2 g/kg body weight | 3 g/kg body weight | SEM |
|---|---|---|---|---|---|---|
| Cu | PSA | $0.014^{b}$ | $0.074^{a}$ | $0.019^{b}$ | $0.009^{b}$ | 0.0151 |
| | PRA | $0.014^{ab}$ | $0.002^{b}$ | $0.030^{a}$ | $0.015^{ab}$ | 0.0057 |
| Ni | PSA | $0.198^{a}$ | $0.062^{b}$ | $0.098^{b}$ | $0.079^{b}$ | 0.0305 |
| | PRA | $0.198^{a}$ | $0.090^{b}$ | $0.136^{ab}$ | $0.156^{ab}$ | 0.0224 |
| Fe | PSA | $2.10^{a}$ | $1.10^{b}$ | $0.80^{b}$ | $0.90^{b}$ | 0.298 |
| | PRA | $2.10^{a}$ | $0.50^{b}$ | $0.80^{b}$ | $0.70^{b}$ | 0.364 |
| Zn | PSA | $0.10^{ab}$ | $0.11^{a}$ | $0.10^{ab}$ | $0.09^{b}$ | 0.0041 |
| | PRA | $0.10^{a}$ | $0.08^{b}$ | $0.11^{a}$ | $0.10^{a}$ | 0.0063 |
| Mn | PSA | $2.40^{b}$ | $2.30^{b}$ | $3.10^{ab}$ | $3.40^{a}$ | 0.268 |
| | PRA | $2.40^{ab}$ | $0.40^{c}$ | $3.60^{a}$ | $1.80^{abc}$ | 0.665 |
| Co | PSA | $0.08^{a}$ | $0.07^{a}$ | $0.05^{b}$ | $0.07^{a}$ | 0.0063 |
| | PRA | $0.08^{ab}$ | $0.05^{b}$ | $0.07^{ab}$ | $0.09^{a}$ | 0.0132 |
| Cr | PSA | $0.17^{b}$ | $0.21^{b}$ | $0.54^{b}$ | $1.43^{a}$ | 0.293 |
| | PRA | $0.17^{ab}$ | $0.19^{a}$ | $0.07^{bc}$ | $0.02^{c}$ | 0.0405 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
PSA: plantain stock ash; PRA: plantain root ash and SEM: standard error means.

## Faecal heavy metals concentrations of the experimental birds

Tables 9 and 10 showed the heavy metals (lead and cadmium) contents of the faecal samples and their percentage deviations from the control values respectively. Generally, increasing PRA supplementations resulted in increasing faecal content of the two minerals. For example, at 3 g/kg BW supplementation of PRA the faecal lead and cadmium content had increased to 100 and 112.50 mg/100 g respectively, indicating their very poor absorption from this source. However, at 3 g/kg BW PSA supplementation an undesirable improvement in the uptake of these metals (15.39 and 12.50% below control values) was observed and may be detrimental to the health of the animals since these elements are known to be toxic to animals.

Table 11 shows the simple linear regression equation relating heavy metals uptake from the GIT to dietary inclusion levels of plantain ash (X). The coefficients of X in the regression equations were both positive and negative, and r values lay between 0.047-0.939, all being positive. The $R^2$ values were between 0.002 and 0.883, which is 0.20 to 88.30% for the percentage of $R^2$. Pb (PRA) was significant ($P<0.10$) for regression effect, while Cd (PSA and PRA) and Pb (PSA) were not. This implies that only Pb could be predicted from any given quantity of plantain ash using the regression equation.

The mineral composition results of the commercial feeds were in agreement with earlier results reported at our station (Okoli *et al.* 2012) and highlight the low mineral concentration of commercial poultry feeds produced in Nigeria. The supplementation of extra mineral elements when feeding these commercial rations is therefore recommended. Thus, it is probable that plantain ashes would not only provide readily bioavailable minerals in their pure oxide forms to the birds, but also might improve the intestinal uptake of earth mineral sources from the rations as recently observed in rabbits we fed coconut shell ash in our station (Ebere, 2012; Iwu, 2012).

The present study shows a general trend of increasing uptake of minerals from the intestine in the presence of the plantain ashes. Specifically, the study shows significant relationships between ash supplementation and faecal content of the macro mineral, Mg, two micro minerals Ni and Cr, and the heavy metal, Pb, all exhibiting high r and $R^2$ values. This implies that the quantities absorbed or passed out with faeces from the GIT of the chicken could be predicted using the prediction equation for any given quantity of plantain ash supplied in the diet. Similarly, relationships between ash supplementation and faecal content of other minerals such as Na, K, Fe, Zn and Mn also recorded very strong coefficient of determination, while others were weak.

Before minerals could be absorbed from the gastrointestinal tract, they must become available in the ionic form which is the only form suitable for their uptake and transport into the enterocytes. Mineral transport into the mucosal cells of the GIT could be either by passive para-cellular transport which depends on chemical and electrical gradients existing across the mucosal cell wall or by an active trans-cellular transport, which is usually regulated (Nys and Mongin, 1980; Schroder *et al.* 1996; Jongloed and Mroz, 1997).

The highly soluble monovalent minerals such as sodium, potassium and chlorine could be transported passively. However, for other minerals, solubility is dependent on the presence of other compounds in the GIT, since these minerals can easily precipitate or form non-absorbable complexes at the normal pH of the GIT (Ashmead, 2012).

It is therefore possible that the improved uptake of Na and K observed in this study is as a result of improvements in their passive absorption influenced by ashing of the plantain materials. Ashing has been reported to convert most plant minerals to their oxide states. It has also been reported that the degree of solubility of most metal oxides is $M^{3+} > M^{2+} > M^{+}$, indicating that the plantain ash may contain more of the $M^{3+}$ and $M^{2+}$.

However, the ability of the birds to absorb more of the passively transported metals as the supplementation levels increased showed that the threshold for further absorption has not been attained, since such passive absorption is gradient dependent.

According to Power and Horgan (2007) and Ashmead (2012), there are two categories of ingested minerals; those soluble throughout the potential pH range of the gastrointestinal lumen such as Na, Mg, Ca etc and those susceptible to hydroxy-polymerisation such as Cu, Fe, Mn, Zn etc. The latter group has been referred to as hydrolytic metals and includes potentially toxic heavy metals. These are usually soluble at low pH.

However, as the pH is raised, they readily hydroxy-polymerise to form insoluble precipitates (Powers and Horgan, 2007). Hynes and Kelly (1995) also reported that different metal ions have different stability constants and thus, the percentage of a metal present as a particular species will depend not only on the pH of the solution but also on the stability constant of the complex. The use of metal chelates of $Cu^{2+}$, $Zn^{2+}$ and $Mn^{2+}$ with amino acids and peptides has been shown to enhance the absorption of these minerals (Power and Horgan, 2007; Scheideler, 2008; Ashmead, 2012). These products have been termed organic metals because of the fact that the metal elements needed to be made more bioavailable are complexed with organic molecules or ligands. Available literature (Whitehead *et al.* 1996; Power and Horgan, 2007; Ashmead, 2012) show that metal ions require both endogenous soluble ligands and mucosally associated ligands present in the gut for their uptake. The endogenous soluble ligands prevent hydroxy-polymerisation of cations, while the mucosally associated ones allow selective absorption between toxic and essential metals (Whitehead *et al.* 1996). Crowther and Marriott (1984) and Conrad *et al.* (1991), Power and Horgan (2007) stated the predominant endogenous mucosally associated ligand is probably the large glycoprotein mucin, which is secreted throughout the GIT.

**Table 7** Percentage deviation of faecal micro minerals concentrations from the control values

| Mineral | Ash type | 1 g/kg body weight | 2 g/kg body weight | 3 g/kg body weight |
|---|---|---|---|---|
| Copper | PSA | +428.57 | +35.71 | -35.71 |
| | PRA | -85.71 | +114.29 | +7.74 |
| Nickel | PSA | -68.68 | -20.71 | -60.10 |
| | PRA | -54.56 | -31.31 | -21.21 |
| Iron | PSA | -47.62 | -61.91 | -57.14 |
| | PRA | -79.19 | -61.91 | -66.00 |
| Zinc | PSA | +10.00 | 0.00 | -10.00 |
| | PRA | -20.00 | 10.00 | 0.00 |
| Manganese | PSA | -4.17 | 29.17 | 41.67 |
| | PRA | -83.33 | +50.00 | -60.00 |
| Cobalt | PSA | -12.50 | -37.50 | -12.50 |
| | PRA | -37.50 | -12.50 | +12.50 |
| Chromium | PSA | +23.53 | +217.65 | +741.17 |
| | PRA | +11.77 | -7.00 | -15.00 |

-: percentage reduction from the control values and +: percentage increase from the control values.
PSA: plantain stock ash and PRA: plantain root ash.

**Table 8** Simple linear regression equations relating micro mineral uptake from the GIT of experimental birds at 25 weeks of age (Y) to dietary inclusion levels of plantain ash (X)

| Dependent variable (Y) | Ash type | Prediction equation | r | $R^2$ | % of $R^2$ | Significance | |
|---|---|---|---|---|---|---|---|
| Copper | PSA | Y= 2.87–12.73X | 0.298 | 0.089 | 8.90 | 0.702 | ns |
| | PRA | Y= 1.901+39.265X | 0.349 | 0.122 | 12.20 | 0.651 | ns |
| Nickel | PSA | Y= 4.073–14.394X | 0.68 | 0.462 | 46.20 | 0.090 | * |
| | PRA | Y= 3.461–6.627X | 0.230 | 0.053 | 5.50 | 0.770 | ns |
| Iron | PSA | Y= 4.738–1.827X | 0.844 | 0.712 | 71.20 | 0.156 | ns |
| | PRA | Y= 3.759–1.228X | 0.692 | 0.479 | 47.90 | 0.308 | ns |
| Zinc | PSA | Y= 12.50–100-00X | 0.632 | 0.400 | 40.00 | 0.368 | ns |
| | PRA | Y= 0.579+31.579X | 0.308 | 0.095 | 9.50 | 0.692 | ns |
| Manganese | PSA | Y= -3.686+2.209X | 0.916 | 0.840 | 84.00 | 0.953 | ns |
| | PRA | Y= 2.230+0.132X | 0.136 | 0.018 | 1.80 | 0.864 | ns |
| Cobalt | PSA | Y= 6.053–52.63X | 0.513 | 0.263 | 26.30 | 0.487 | ns |
| | PRA | Y= 0.429+28-57X | 0.378 | 0.143 | 14.30 | 0.662 | ns |
| Chromium | PSA | Y= 1.327+1.997X | 0.906 | 0.821 | 82.10 | 0.094 | * |
| | PRA | Y= 4.130–14.485X | 0.909 | 0.826 | 82.60 | 0.091 | * |

r: correlation coefficient; $R^2$: coefficient of determination and % of $R^2$: percentage of $R^2$.
* (P<0.05) and ** (P<0.01).
NS: not significant.

**Table 9** Heavy metals concentrations (mg/100 g) in faecal samples from layers fed plantain ash

| Mineral | Ash type | Control | 1 g/kg body weight | 2 g/kg body weight | 3 g/kg body weight | SEM |
|---|---|---|---|---|---|---|
| Pb | PSA | $0.013^{b}$ | $0.019^{a}$ | $0.014^{ab}$ | $0.011^{b}$ | 0.0017 |
| | PRA | $0.013^{b}$ | $0.014^{b}$ | $0.018^{ab}$ | $0.026^{a}$ | 0.0030 |
| Cd | PSA | $0.008^{a}$ | $0.002^{b}$ | $0.004^{ab}$ | $0.007^{a}$ | 0.0014 |
| | PRA | $0.008^{b}$ | $0.004^{b}$ | $0.009^{ab}$ | $0.017^{a}$ | 0.0027 |
| As | PSA | BDL | BDL | BDL | BDL | |
| | PRA | BDL | BDL | BDL | BDL | |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
PSA: plantain stock ash; PRA: plantain root ash; BDL: below detection level and SEM: standard error means.

**Table 10** Percentage deviation faecal heavy metals contents from the control values

| Mineral | Ash type | 1 g/kg body weight | 2 g/kg body weight | 3 g/kg body weight |
|---|---|---|---|---|
| Lead | PSA | +46.15 | +7.69 | -15.39 |
| | PRA | +7.69 | +38.46 | +100.00 |
| Cadmium | PSA | -75.00 | -50.00 | -12.50 |
| | PRA | -50.00 | +12.50 | +112.50 |

-: percentage reduction from the control values and +: percentage increase from the control values.
PSA: plantain stock ash and PRA: plantain root ash.

**Table 11** Simple linear regression equations relating heavy metals uptake from the GIT of experimental birds at 25 weeks of age (Y) to dietary inclusion levels of plantain ash (X)

| Dependent variable (Y) | Ash type | Prediction equation | r | $R^2$ | % of $R^2$ | Significance | |
|---|---|---|---|---|---|---|---|
| Lead | PSA | Y= 4.755-158.273X | 0.417 | 0.174 | 17.40 | 0.368 | ns |
| | PRA | Y= -1.143+205.251X | 0.939 | 0.883 | 88.30 | 0.061 | * |
| Cadmium | PSA | Y= 2.615-21.978X | 0.047 | 0.002 | 0.20 | 0.953 | ns |
| | PRA | Y= 0.792+179.78X | 0.758 | 0.575 | 57.50 | 0.42 | ns |

r: correlation coefficient; $R^2$: coefficient of determination and % of $R^2$: percentage of $R^2$.
* (P<0.05) and ** (P<0.01).
NS: not significant.

The affinity of mucin for metals has been shown to follow the pattern, $M^{3+} > M^{2+} > M^{+}$ with binding occurring at more than one binding site on mucin since the molecule contains sulphated groups and carboxylate groups while for absorption the pattern is $M^{+} > M^{2+} > M^{3+}$ (Whitehead *et al.* 1996). Indeed, efficient absorption of Cu, Fe, Mn and Zn depends on a number of factor including the extent of prevention of luminal hydroxy-polymerisation, the rates of metalligand exchange and the rate of passage across the mucosally-adherent mucus layer (Powell *et al.* 1999). We therefore postulate from the present results that different metal forms in the ashes, especially the $M^{+}$ and $M^{2+}$ forms may have combined with available synthetic amino acids in the commercial feeds to form more inert and bioavailable organic molecules. Similarly, other metal ions in the ashes may also have strong affinity for mucin and may bind at one or more of the binding site on the mucin molecule (Whitehead *et al.* 1996). These complexed metal molecules are protected from subsequent polymerization as the pH of intestine increases and are therefore transported more efficiently across the cell walls into enterocytes. These results need to be validated. The improved uptake of minerals as a result of plantain ash supplementation is therefore facilitated by the improved solubility of some macro minerals in their oxide forms, formation of organic complexes and possibly chelates between some of the micro minerals and free amino acids in the commercial feeds as well as with endogenous mucin in the GIT of the birds. These complexes are also believed to be protected from negative interactions with dietary phytate and fibers which would normally bind cations, making them unavailable for absorption (Fairweather Tait, 1996). Again, it is possible that the highly alkaline plant ashes of pH 12.0 (Nwogu *et al.* 2012) may have exerted a leaching effect on the rock mineral sources incorporated in the commercial feeds. This effect may be early enough in the digestion process, possibly in the crop, thereby releasing the metal components for binding with mucin or free amino acids. This probably explains the reduction of faecal Cu concentration from +428.57 to -35.71 and Na from +200.00 to 0.0 at 1 g/Kg BW and 3 g/Kg BW ash supplementation respectively. It seems from the present study that PRA is a better source of absorbable minerals than PSA.

This is in agreement with Nwogu *et al.* (2012) who had earlier reported that for example, the chromium content of PSA and PRA were 0.09 and 0.27 mg/100 g respectively, suggesting that the poor absorption of the mineral from PSA could be as a result of differences in the chemical nature of the mineral in two ash types. Similar disparities are also observable with other minerals in this study with the PRA causing better absorption in most cases. Mineral supplements which are used to correct deficiencies in feeds may also contain metals other than those of primary interest. The amount of other elements depends on the native material from which the mineral supplement is obtained and the type of processing that it undergoes (NRC, 1984). Poor quality mineral supplements can therefore contain excess levels of Fe, Pb, As, Al and Cl which may cause toxicoses in supplemented animals.

The present results showed that birds were able to selectively exclude Cr from absorption, especially when supplemented with PSA, while Pb and Cd were excluded from absorption from the PRA. The mucosally associated ligand has been reported to be responsible for this observed selective absorption between toxic and essential metals (Whitehead *et al.* 1996).

## CONCLUSION

This study has shown the potential value of plantain ash as good sources of absorbable mineral supplements in the diets of pullets. Supplementation of plantain ash could improve mineral uptake from commercial diets offered to the birds. It seems from the present study that PRA is a better source of absorbable mineral than PSA.

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