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Potash Pretreatment Enhances Carbohydrate Biodegradability and Feed Potential of Groundnut (*Arachis hypogea*) Shell Meal

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Abstract: The rising cost of commercial non-ruminant feeds in Ghana has necessitated a search for local alternatives such as modified crop residues. The present study was carried out to investigate the extent to which maize stalk potash treatment might improve groundnut shell biodegradability and whether or not a low dietary intake of the treated groundnut shell would support the growth of rats. Groundnut Shell (GS) meal was immersed in 1% (w/v) potash, pH 11.2, for 24 h at room temperature (solid to liquid ratio of 1:4). The enzymatic degradation of the cellulose fraction of groundnut shell *in vitro* increased 18 fold following the potash treatment. Three dietary treatments were evaluated on five randomly selected rats over a period of four weeks: normal broiler mash diet, broiler mash partially substituted with either 10% potash-treated GS or 10% untreated GS. The mean values for daily feed intake (g rat^{-1}) were 14.2±1.4, 15.8±1.3 and 13.2±0.8 while the specific growth rates were 2.03±0.06, 2.34±0.20 and 2.31±0.15 for normal diet, untreated GS and potash-treated GS, respectively. Although the differences were not statistically significant, the growth performance of rats fed on the diet partially substituted with potash-treated GS appeared to be better than the other two groups. The feed conversion ratio of 4.05±0.48 for this dietary treatment was the lowest. Taken together, the results suggest that a low dietary concentration of alkali-treated GS should support the growth of rats and other monogastrics without supplementation with costly feed additives.

Key words: Groundnut shell, non-ruminant, cellulose, biodegradability, animal feed

INTRODUCTION

The rising cost of commercial feeds for non-ruminants in Ghana has necessitated a search for affordable alternative feed ingredients. The conversion of the country's abundant crop residues into nutritive feed ingredients would be highly desirable from several standpoints-maximization of bioresource utilization, sustainable animal production and appropriate waste disposal (Metzler and Mosenthin, 2008; Oduguwa *et al*., 2008; Bratte *et al*., 2010; Adamafio *et al*., 2011; Ozung *et al*., 2011). Numerous studies have demonstrated the presence cellulolytic bacteria in the distal part of the small intestine and in the caecum of monogastrics (Yang *et al*., 1969; Montgomery and Macy, 1982; Teunissen *et al*., 1991; Varel *et al*., 1995; Fuller and Reeds, 1998; Wenk, 2001; Grieshop *et al*., 2001; Montagne *et al*., 2003). Therefore, there is convincing, but largely overlooked, evidence in support of the ability of monogastrics to utilize cellulose, a major constituent of crop residues. The use of crop residues as non-ruminant feed ingredients would require prior chemical or biological treatment to disrupt the strong association between structural polysaccharides and lignin which restricts the enzymatic degradation of cellulose (Silverstein *et al*., 2007; Vancov and McIntosh, 2011).

Simple but effective local resource-based processes need to be developed in order to promote the utilization of crop residues in Ghana. In this direction, immersion in potash obtained from maize stalks has been found to be highly effective in improving the degradability of the cellulose fraction of cocoa pod husk *in vitro* (Adamafio *et al*., 2004). The present study was aimed at investigating the effectiveness of that procedure on the enzymatic degradation of groundnut shell, as well as the influence of potash-treated groundnut shell on non-ruminant growth. The selection of groundnut shell as a possible non-ruminant feed ingredient was predicated on two factors: (1) Human consumption of whole boiled groundnuts (nut and shell) in a number of communities in southern Ghana has not been linked to any deleterious effects and (2) Huge quantities of the shell

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are generated in Ghana annually as waste from the processing of half a million metric tonnes of groundnuts.

MATERIALS AND METHODS

Groundnut shells were obtained from the Western Region of Ghana brushed free of soil particles, sun-dried for three weeks, milled and stored at 4°C. Dried post-harvest maize stalks were obtained locally. Normal male Sprague-Dawley albino rats (28 days old) were purchased from the Animal Experimentation Unit of the Noguchi Memorial Institute of Medical Research, University of Ghana. Cellulase (45 mU mg^{-1}) was purchased from Fluka AG, Switzerland.

Treatment of milled groundnut shells with potash: Samples of milled groundnut shells were then immersed in 1% (w/v) potash solution or distilled water for 24 h at room temperature (solid to liquid ratio of 1:4). The potash was prepared from maize stalk ash as previously described and the concentration used in the present study had been validated in a previous study (Adamafio *et al*., 2004). Potash treatment was terminated by neutralizing samples with an equal volume of 0.5 M HCl. Samples were then filtered through cheese cloth, rinsed thoroughly with distilled water and sun-dried to constant weight, after which the moisture content was determined by the air-oven method. The detergent system of analysis was employed in determining the fibre composition of untreated and potash-treated samples (Van Soest and Robertson, 1980). Nitrogen was estimated by the Kjeldahl method (AOAC, 1970), while the water soluble non-structural carbohydrate (WS-NSC) content was measured as previously described (Adamafio *et al*., 2009).

***In vitro* biodegradability:** The enzymatic hydrolysis of the cellulose component of untreated and alkali-treated groundnut shell samples was assessed *in vitro* as previously described (Adamafio *et al*., 2009). Acid-neutralized potash-treated controls were also included.

Growth studies: Normal male Sprague-Dawley albino rats (28 days old) were housed under constant temperature (23°C) on a 12 h light/darkness cycle. Control rats were fed *ad libitum* on their normal diet, commercial broiler mash (energy-2520 Kcal and expressed as %, protein: 14, fibre: 12, Ca: 1.5, P: 1.0, Lys: 0.6, Met and Cys: 0.5, Try: 0.2). The test diets offered *ad libitum* to the rats comprised commercial broiler mash to potash-treated or untreated groundnut shell (9:1, w/w). No feed additives were included. Each feed was evaluated on five randomly selected rats housed in a single cage. Serial body weight measurements were recorded over a four week period. The daily feed and water intake per group were also measured. The growth performance of rats was measured in terms of body weight gain, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR). Specific growth rate was calculated as dm/dt/m where dm is change in mass; dt is the trial period and m the mass the animal at the beginning of the study. Feed conversion ratio was calculated as the ratio of average daily feed intake to average daily weight gain. The FCR calculation was based on the assumption that the feed provided in each cage was consumed in equal parts by all occupants.

Haematological characteristics and urine biochemistry: Rats were tail bled and 0.5 mL blood samples collected into Eppendorf tubes containing 3.8% sodium citrate. Haematological indices were then determined in an automated analyser (Hema Screen 13, Hospitex). Urine samples were collected from each group on a weekly basis and pooled. Urine biochemistry parameters were subsequently estimated using a urine analyzer (URIAN Sean UA 1001).

Statistical analysis: Statistical analysis was performed using the analysis of variance (ANOVA) test. Differences resulting in p-values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The pre-treatment of groundnut shell with potash had no observable effects on the fibre composition of groundnut shell but increased the water-soluble non-structural carbohydrate sugar content by 193.1% (Table 1). As depicted in Fig. 1, pre-treatment with potash also resulted in a massive increase (18 fold) in the rate of enzymatic hydrolysis of cellulose *in vitro* which was statistically significant ($p<0.05$). Clearly, potash was effective in improving the enzymatic hydrolysis of groundnut shell samples *in vitro*, susceptibility to cellulase increased by several orders of magnitude. These findings are consistent with the improved the biodegradability of lignocellulosic materials following pretreatment with various alkali sources (Silverstein *et al*., 2007; Hamed and Elimam, 2009; Vancov and McIntosh, 2011). Presumably, the mechanism by which

Table 1: Effect of potash on selected constituents of groundnut shell

Constituent (%)	Untreated	Potash-treated
Cellulose	47.50	47.40
Hemicellulose	11.30	10.90
Lignin	34.20	34.70
N	0.78^a	1.01^b
WS-NSC	1.02^a	2.99^b

WS-NSC: Water soluble non-structural carbohydrates, Values in the same row with different superscripts are significantly different from each other at $p<0.05$

Table 2: Growth and haematological parameters of rats

	Dietary treatments		
Parameter	Normal diet	Untreated-GS	Potash-GS
Av. daily feed intake (g rat^{-1})	14.20 ± 1.4^{a}	15.80 ± 1.3^{ab}	13.20 ± 0.8^{ab}
Av. daily water intake (mL rat^{-1})	14.80 ± 1.8^{c}	17.50 ± 1.4^{c}	16.80 ± 1.3^{c}
Av. initial wt (g rat^{-1})	44.00 ± 4.3^{d}	42.00 ± 6.1^{d}	40.00 ± 3.0^{d}
Av. daily wt gain (g rat^{-1})	3.28 ± 0.25^{d}	3.59 ± 0.49^{d}	3.44 ± 0.37^{d}
Specific growth rate	2.03 ± 0.06^{e}	2.34 ± 0.20^{e}	2.31 ± 0.15^{e}
Feed conversion ratio	4.41 ± 0.31^{f}	4.78 ± 0.74^{f}	4.05 ± 0.48^{f}
RBC (10^{6} mm^{-3})	4.90 ± 0.9^{g}	5.10 ± 0.9^{g}	4.60 ± 0.6^{g}
WBC (10^{3} dL^{-1})	14.00 ± 3.3^{h}	16.50 ± 2.2^{h}	13.00 ± 1.5^{h}
Haemoglobin (g dL^{-1})	10.50 ± 1.1^{j}	10.30 ± 0.5^{j}	10.10 ± 1.8^{j}

Results are expressed as mean values±SE of five determinations, Values in the same row with similar superscripts are significantly different from each other at $p<0.05$

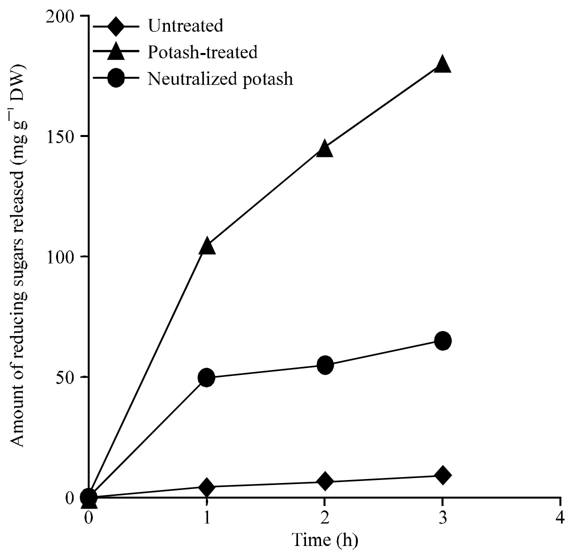

Fig. 1: Effect of potash pretreatment of groundnut shell on rate of cellulose degradation *in vitro*

potash improved groundnut shell digestibility involved the disruption of chemical bonds between lignin and cellulose thereby improving enzyme accessibility. Alterations in the crystallinity of cellulose during the potash treatment cannot be ruled out and might have contributed to the change in susceptibility to biodegradation. Crystallinity has been identified as an important parameter for the rate of cellulose degradation (Szijarto *et al*., 2008; Park *et al*., 2010).

At the end of the trial period, the average weight gain of the rats fed on the untreated groundnut shell substituted diet was higher than that of the other two groups. However, the values obtained for the specific growth rates which takes into account the initial weight of the animal, were remarkably similar for all of the three dietary treatments (Table 2). The highest average daily feed consumption recorded was by rats on the untreated groundnut shell substituted diet and this was significantly different ($p<0.05$) from that of animals fed on the alkali-treated groundnut shell substituted diet

Table 3: Urinary biochemical parameters of rats

	Dietary treatments		
Parameter	Normal diet	Untreated-GS	Potash-GS
pH	8	8	8
Nitrite	+	+	+
Bilirubin	+	+	+
Urobilinogen	+	+	+
Glucose	-	-	-
Ketones	-	-	-
Protein (g L^{-1})	10	10	10
Leucocyte	++	++	+++

+: Trace, ++: Low, +++: Moderate, -: Absence of the parameter

(Table 2). Ingestion of the alkali-treated groundnut shell diet resulted in the lowest feed conversion ratio, while the untreated groundnut shell group had the highest feed conversion ratio.

Considering the fact that the average daily feed intake of the rats fed on potash-treated groundnut shell was 7.0% lower than feed consumption by the normal diet group, probably because of differences in organoleptic properties, the striking similarity between the mean specific growth rates of the two groups is quite remarkable. Obviously, the rats fed on the alkali-treated groundnut shell succeeded in deriving adequate amounts of metabolisable biomolecules from their diet to sustain a normal growth rate, despite the reduction in feed intake. It is reasonable to presume that energy derived from the groundnut shell fraction of the feed contributed to the attainment of normal growth. This would not be entirely surprising since the *in vitro* studies provide evidence that pretreatment with potash increased the reducing sugar content of the groundnut shell samples and dramatically improved susceptibility to enzymatic depolymerization. A critical analysis of the feed conversion ratio data suggests that partial substitution of the diet with untreated groundnut shell might not have been beneficial. This is because, unlike the rats fed on alkali-treated feed, the normal growth rate attained by rats in this group required an 11.3% increase in feed intake. Given that the level of substitution with untreated groundnut shell was 10%, it is highly probable that the increase in feed consumption was a compensatory mechanism to ensure adequate energy. It is well established that when animals are unable to degrade fibre, it only serves as a dietary diluent whose content is inversely related to the amount of metabolisable energy available per gram of feed.

The present findings are consistent with the reported ability of non-ruminants to utilize fibre, albeit to a limited extent, owing to the presence of cellulolytic, hemicellulolytic and pectinolytic organisms in the caecum and distal part of the small intestine (Varel *et al*., 1995; Fuller and Reeds, 1998; Wenk, 2001; Grieshop *et al*., 2001; Montagne *et al*., 2003). The urinary biochemical and haematological parameters of the three dietary treatment groups were similar (Table 3).

The haematological indices which mirror the nutritional status of an animal (Ahamefule *et al*., 2006; Ozung *et al*., 2011), provide no evidence of malnourishment as a result of the inclusion of groundnut shell in the diet of rats. Although the differences in daily water intake by rats were not statistically significant, water intake by rats fed alkali-treated groundnut shell substituted diets appeared to be somewhat higher than that of rats on the normal diet. This was also true of rats fed on untreated groundnut shell substituted diets (Table 2). Thus, potash treatment did not cause an increase in urine alkalinity or excessive thirst.

Because rats are far less proficient than pigs and poultry in the utilization and conversion of cellulose (Yang *et al*., 1969; Gargallo and Zimmerman, 1981), it is tempting to predict that the growth performance of pigs and poultry fed on the potash-treated groundnut shell is likely to exceed that observed for rats in the present study. In pigs, dietary fiber is the main substrate for bacteria in the gastrointestinal tract (Grieshop *et al*., 2001; Hedemann *et al*., 2006). These include *Ruminococcus* sp., *Bacteroides* sp. and *Clostridium* sp. with cellulolytic, pectinolytic and hemicellulolytic activities such (Metzler and Mosenthin, 2008). The inclusion of potash-treated groundnut shell in small ruminant feed is also likely to lead to improved growth performance as has been observed with alkali-treatment of other lignocellulosic materials (Abiola *et al*., 2002; Genin *et al*., 2002; Al-Bowait and Al-Sultan, 2006; Masoudi *et al*., 2011; Takahashi *et al*., 2011).

CONCLUSION

Taken together, the findings of the present study provide ample evidence that, if properly treated to substantially improve biodegradability, groundnut shell could serve as a suitable feed ingredient for non-ruminants. If adopted by farmers and feed producers, this would reduce the high animal production costs in Ghana.

REFERENCES

AOAC, 1970. Official Methods of Analysis. 11th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Abiola, S.S., A.C. Amalime and K.C. Akadiri, 2002. The utilization of alkali-treated melon husk by broilers. Bioresour. Technol., 84: 247-450.

Adamafio, N.A., D.A. Annan, V. Amarh, G.O. Nkansah and M. Obodai, 2011. An *in vitro* evaluation of *Pleurotus ostreatus* EM-1-modified maize (*Zea mays*) cob as a non-conventional energy source for livestock in Ghana. J. Biol. Sci., 11: 442-447.

Adamafio, N.A., E. Cooper-Aggrey, F.O. Quaye, J.K. Laary and J. Quaye, 2004. Effectiveness of corn stalk ash in reducing condensed tannin levels and improving *in vitro* enzymatic degradation of polysaccharides in crop residues. Ghana J. Sci., 44: 87-92.

Adamafio, N.A., M. Obodai and B.B. Brimpong, 2009. Solid state fermentation of maize (*Zea mays*) cob by *Pleurotus ostreatus* strain EM-1: Biopolymer profiles and cellulose degradability. Int. J. Biol. Chem. Sci., 3: 1459-1466.

Ahamefule, F.O., G.O. Eduok, A. Usman, K.U. Amaefule, B.E. Obua and S.A. Oguike, 2006. Blood biochemistry and haematology of weaner rabbits fed sundried, ensiled and fermented cassava peel based diets. Pak. J. Nutr., 5: 248-253.

Al-Bowait, M. and S.I. Al-Sultan, 2006. Aspects of the serum biochemistry, carcass quality and organoleptic characteristics of broilers fed alkali-treated date pits. Int. J. Poult. Sci., 5: 284-288.

Bratte, L., F.U.C. Mmereole, O.J. Akpodiete and S.I. Omeje, 2010. The nutrient composition of seeds of the African Pear (*Dacreodes edulis*) and its implications for non-ruminant nutrition. Pak. J. Nutr., 9: 255-258.

Fuller, M.F. and P.J. Reeds, 1998. Nitrogen cycling in the gut. Annu. Rev. Nutr., 18:: 385-411.

Gargallo, J. and D.R. Zimmerman, 1981. Effects of dietary cellulose levels on intact and cecectomized pigs. J. Anim. Sci., 53: 395-402.

Genin, D., P. Abasto, S. Choque and J. Magne, 2002. Dung ash treatment of a native forage to improve livestock feeding in low-input Andean pastoral systems. Livestock Res. Rural Dev., Vol. 14.

Grieshop, C.M., D.E. Reese and G.C. Fahey Jr., 2001. Nonstarch Polysaccharides and Oligosaccharides in Swine Nutrition. In: Swine Nutrition, Lewis, A.J. and L.L. Southern (Eds.). CRC Press, Boca Raton, FL., USA., pp: 107-130.

Hamed, A.H.M. and E.M. Elimam, 2009. Effects of rabaa ash alkali treatment of sesame straw on chemical composition and degradation in the rumen of Nubian goats. Pak. J. Nutr., 8: 1344-1348.

Hedemann, M.S., M. Eskildsen, H.N. Laerke, C. Pedersen, J.E. Lindberg, P. Laurinen and K.E. Knudsen, 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. J. Anim. Sci., 84: 1375-1386.

Masoudi, A., M. Chaji, M. Bojarpour and K. Mirzadeh, 2011. Effects of different levels of date pits on performance, carcass characteristics and blood parameters of broiler chickens. J. Applied Anim. Res., 39: 399-405.

Metzler, B.U. and R. Mosenthin, 2008. A review of interactions between dietary fiber and the gastrointestinal microbiota and their consequences on intestinal phosphorus metabolism in growing pigs. Asian-Aust. J. Anim. Sci., 21: 603-615.

Montagne, L., J.R. Pluske and D.J. Hampson, 2003. A review of interactions between dietary fibre and the intestinal mucosa and their consequences on digestive health in young non-ruminant animals. Anim. Feed. Sci. Technol., 108: 95-117.

Montgomery, L. and J.M. Macy, 1982. Characterization of rat cecum cellulolytic bacteria. Applied Environ. Microbiol., 44: 1435-1443.

Oduguwa, O.O., M.O. Edema and A.O. Ayeni, 2008. Physico-chemical and microbiological analyses of fermented corn cob, rice bran and cowpea husk for use in composite rabbit feed. Bioresource Technol., 99: 1816-1820.

Ozung, P.O., I.I. Bitto and S.A. Ikurior, 2011. Carcass yield, gut morphology, reproductive tract morphometry and some biochemical characteristics of serum in female rabbits fed cassava peel meal based diets. Cont. J. Anim. Vet. Res., 3: 22-32.

Park, S., J.O. Baker, M.E. Himmel, P.A. Parilla and D.K. Johnson, 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol. Biofuels, Vol. 3.

Silverstein, R.A., Y. Chen, R.R. Sharma-Shivappa, M.D. Boyette and J. Osborne, 2007. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresour. Technol., 98: 3000-3011.

Szijarto, N., M. Siika-Aho, M. Tenkanen, M. Alapuranen, J. Vehmaanpera, K. Reczeya and L. Viikari, 2008. Hydrolysis of amorphous and crystalline cellulose by heterologously produced cellulases of *Melanocarpus albomyces*. J. Biotechnol., 136: 140-147.

Takahashi, T., K. Horiguchi, N. Yoshida and Y. Cao, 2011. Effect of cellulase and urea addition on *in situ* degradability and preference for pruned persimmon branch chips in cattle. Grassland Sci., 57: 179-183.

Teunissen, M.J., H.J. Op den Camp, C.G. Orpin, J.H.H. Veld and G.D. Vogels, 1991. Comparison of growth characteristics of anaerobic fungi isolated from ruminant and non-ruminant herbivores during cultivation in a defined medium. J. Gen. Microbiol., 137: 1401-1408.

Van Soest, P.J. and J.B. Robertson, 1980. Systems of Analysis for Evaluating Fibrous Feeds. In: Standardisation of Analytical Methodology for Feeds, Pigden, W.J., C.C. Bolch and N. Graham (Eds.). International Development Research Center, Ottawa, Canada, ISBN-10:100889362173, pp: 46-60.

Vancov, T. and S. McIntosh, 2011. Alkali pretreatment of cereal crop residues for second-generation biofuels. Energy Fuels, 25: 2754-2763.

Varel, V.H., R.S. Tanner and C.R. Woese, 1995. *Clostridium herbivorans* sp. nov., a cellulolytic anaerobe from the pig intestine. Int. J. Syst. Bacteriol., 45: 490-494.

Wenk, C., 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Technol., 90: 21-33.

Yang, M.G., K. Manoharan and A.K. Young, 1969. Cellulose in cecum of rats. J. Nutr., 97: 260-264.