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The Effect of Phytase and Zinc Supplementation on Palm Kernel Cake Toxicity in Sheep

H.D. Akpan[1], E.O. Udosen[2], A.A. Udofia[3], E.J. Akpan[1] and A.A. Joshua[1]
[1]Department of Biochemistry, Faculty of Basic Medical Sciences,
University of Uyo, Uyo, Akwa Ibom State, Nigeria
[2]Department of Biochemistry, College of Medical Sciences,
University of Calabar, Calabar, Cross River State, Nigeria
[3]Department of Chemical Pathology, University of Calabar Teaching Hospital,
Calabar, Cross River State, Nigeria
E-mail: henrydan4u@yahoo.com

Abstract: The toxicity effect of excess palm kernel cake and the effect of phytase and zinc supplementation on palm kernel cake (PKC) toxicity in sheep were investigated. Three experimental diets A, B, and C were prepared using grass and PKC at 10% and 90% respectively. Diet A contained grass and PKC only (PKC group). Diet B was same as A but in addition was supplemented with Ronozyme™ P at 750 FYT/Kg (PKC + Phytase (P) group). Diet C was also as A, but in addition, was supplemented with zinc sulphate at 500µg/g (PKC + Zn group). Another diet D, containing corn and fish meal at 20% and grass at 80% served as control. These diets were fed to twelve male West African Dwarf sheep (average weight 20.0±0.8kg) divided into 3 groups and the control. Clinical signs and body weights were monitored for 20 weeks. The animals were slaughtered and the right lobes and renal cortex were isolated for copper and Zinc estimation and histopathological evaluation. The results obtained showed that animals in the PKC + phytase group, PKC + Zinc group and the control performed alike in terms of clinical signs, gross and histopathological lesions. In terms of body weight gain, animals in PKC + phytase group performed better than animals in PKC + Zinc group and the control, although the differences were not statistically significant (p>0.05). Animals in the PKC group all died of PKC toxicity. Supplementation with zinc and phytase prevented chronic PKC toxicity and can be useful in the therapy for PKC toxicity in sheep. Supplementation with zinc however, led to significant elevation in zinc concentration in the liver and kidney. It is interesting to discover that the use of phytase did not elevate the concentration of zinc in these organs.

Key words: Phytase, zinc, supplementation, palm kernel cake, toxicity, sheep

Introduction

Palm kernel cake (PKC) is a by-product obtained after extraction of palm kernel of the fruits of the oil palm tree *Elaeis guineensis*. Palm kernel cake is produced by grinding the palm kernel followed by pressing which could be done with or without an intermediary flaking and cooking stages (Tang and Toeh, 1985). Raw palm kernel oil is obtained during the pressing and is diverted for classification while the residual cake (Palm kernel cake) is cooled and stored for use (Tang and Toeh, 1985).

The most important usage of PKC is as ingredient for the formulation of animal feeds (Collingwood, 1958). It has a high nutritive value and is considered to be excellent for ruminants but also suitable for use in feed formulations for swine, poultry and horses (Hutagulung *et al.*, 1982; Yeong *et al.*, 1983; Collingwood, 1958). It usage in excess in sheep are known to cause chronic toxicity and death due to hepatic necrosis (Abdul Rahman *et al.*, 1989; Wan Mohammed *et al.*, 1989). Jaundice and haemoglobinuria are the most remarkable

clinical signs demonstrated (Hair-Bejo and Alimon, 1995).

Changes occurring after ingestion of excess PKC in sheep are consistent to those of chronic copper toxicity (Sandstead *et al.*, 1970; McCall *et al.*, 1971; Ishmeal *et al.*, 1971; Underwood, 1977; Soli, 1980). Supplementation of diets with sodium molybdate and ferrous sulphate reduces the incidence of the disease (Abdul Rahman *et al.*, 1989; Wan Mohammed *et al.*, 1989). The toxicity can be prevented by dietary zinc supplementation either with or without ammonium molybdate (Hair-Bejo and Alimon, 1995) and is possible due to antagonistic interaction between copper and zinc, which make zinc to be preferentially absorbed instead of copper (Hair-Bejo and Alimon, 1995). However, the use of supplementary zinc in the prevention of PKC toxicity in sheep led to an elevated blood, hepatic and renal zinc concentration (Hair-Bejo and Alimon, 1995). The long-term effect of this alteration is not known. Furthermore, little information is available on the use of supplementary zinc in the prevention of PKC toxicity in

sheep (Hair-Bejo and Alimon, 1995). Few approaches are made to prevent PKC toxicity in sheep (Abdul Rahman *et al.*, 1989; Wan Mohammed *et al.*, 1989; Hair-Bejo and Alimon, 1995). In view of the abundance of PKC in many West African Countries, the potential of PKC as cheap material for feeding sheep and other animals and livestock and the economic importance of sheep product such as cheese, wool, and meat etc, it is desirable to investigate widely on PKC toxicity and ways of prevention in sheep.

It is known that copper bioavailability depends on the relative proportion of zinc. The zinc and copper contents of PKC have been reported to fall between the ranges of 43.8-77.0mg/kg and 11-28.5mg/kg respectively (Yeong *et al.*, 1983; Abdul Rahma *et al.*, 1989; Jalaludin *et al.*, 1991; Mustaffa *et al.*, 1991; Hair-Bejo and Alimon, 1995). It is obtained naturally in the ratio of 1:4 of copper to zinc. It is suggested that if most of the natural zinc in PKC were bioavailable, it may make zinc to be preferentially absorbed instead of copper and reduce the burden of having to source for inorganic zinc.

Factors that aggravate zinc deficiency include high copper, phosphorus, calcium and phytate (Maga, 1982; Davis and Olpin, 1979; Bingham, 1978; Davis and Reid, 1979; Sandberg *et al.*, 1982). Siew (1989) gave the composition of zinc, copper, phosphorus and calcium in PKC (out of the seventy eight samples from 23 palm kernel crushers analyzed) to be 77.0, 28.5, 47.4 and 17.4 mg/kg respectively. Therefore, calculated molar proportion of zinc to copper; zinc to phosphorus and zinc to calcium is 3:1; 2:1 and 4:1 respectively. At this ratio, copper, phosphorus and calcium appear not to exhibit significant effect to interfere with zinc absorption in PKC. Only phytate may play significant role, as phytate level as high as 1309±20.8mg/kg was obtained for Nigerian PKC (Akpan and Joshua, 2003, unpublished). Davis and Olpin (1979) demonstrated zinc deficiency symptoms in rat fed a diet with phytate: zinc ratio of 10:1.

The objective of the experiment was to investigate the effect of supplementary phytase and zinc on palm kernel cake toxicity in sheep and to assess the toxicity effect of excess palm kernel cake in sheep.

Ronozyme[TM] P was used to represent the universe of phytase and is a highly active phytase derived from *Peniophora lycil*. It is formulated as a multi coated granulate (CT) with superior heat stability and function well above pelleting temperature of 80°C. With proven efficacy in pigs and poultry, Ronozyme [TM]P makes better use of phosphorus and other nutrients such as calcium, zinc, magnesium, and phytate-bound proteins, which are present naturally in the diet, thus reducing the need for inorganic supplements and saving unnecessary cost. It is recommended at 750 FYT/kg diet.

Materials and Methods

Three experimental diets A, B, and C similar to the experimental feed of Hair-Bejo and Alimon, (1995), were prepared using grass and PKC at the level of 10 and 90% respectively. The PKC used in the study was purchased from Basu Palm Kernel crushing industry located at Itam in Itu Local Government Area in the South-Southern part of Nigeria. Grass was only elephant grass. Diet A contained grass and PKC only (PKC group). Diet B was same as A but in addition was supplemented with Ronozymes[TM] P (phytase) at 750FYT/Kg (PKC + Phytase (P) group). Diet C was also as A, but in addition was supplemented with Zinc sulfate ($ZnSO_4.7H_2O$) at 500µg/g (PKC + zinc (Zn) group). Another diet D containing corn and fishmeal at 20% and grass at 80% was prepared to serve as control.

Twelve male West African Dwarf sheep weighing 20.0 ± 0.8kg were divided into 4 groups of 3 animals each and were randomized to the three experimental diets and the control. They were stall-fed for 20 weeks. All animals were monitored for clinical signs. Body weights were recorded at two weeks interval. Blood samples were also collected every two weeks from the jugular vein and analyzed same day for copper and zinc content or stored at 0 - 4°C until required for analysis. At the end of the 20 weeks, the animals were slaughtered and carcasses were analyzed for gross lesions. The right liver lobes and renal cortex were isolated for histopathological evaluation and estimation of copper and zinc content.

Histopathological studies: 10% formalin was freshly prepared and both the right lobe and the renal cortex were fixed in the formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylene and embedded in paraffin wax. Sections of the lobe and cortex at about 5µm were mounted on glass slides and stained with haematoxylin and eosin (Lillie, 1965).

Analysis of zinc and copper: Copper and zinc were estimated in blood, liver lobe, renal cortex and the PKC used for this study. The liver and kidney from each of the animals in each group were prepared for copper and zinc analysis by oven drying at 70°C until they reached a constant weight. The dry weights were recorded and the material ground to powder, sieved with 0.5 mesh and samples of the sieved were digested. The PKC used for this study was also oven dried to constant weight, ground to powder and sieved with 0.5 mesh and samples of the sieved were also digested. Also, 2.0ml of the blood from each animal collected fortnightly over the 20 weeks were also digested.

Digestion was carried out in a pyrex glass tube (150mm x 18mm) using 70% aristar grade Nitric acid (BDH Chemicals Ltd) and 60% spectrosol grade perchloric acid (BDH Chemical Ltd) in 2 to 1 (v/v) respectively. Added into each recovery tube was fifty µl (1mg/ml) of spectrosol grade cupric nitrate and zinc nitrate solutions (BDH Chemical Ltd). The tubes were then covered with

Table 1: Effect of different dietary treatments of PKC on the average daily weight gain in sheep (0-20 weeks)

Treatment	Average weight gain (g)
PKC	40.1 ± 0.05[b]
PKC + Zn	50.3 ± 0.10[a]
PKC + P	55.3 ± 0.02[a]
Control	54.0 ± 0.11

[a]Mean with different superscript are significantly different (p>0.05)

Table 2: Effect of different dietary treatments of PKC in sheep on blood, hepatic and renal copper concentration

Treatment	Blood (µg/ml)	Right liver (µg/g)	Renal cortex (µg/g)
PKC	3.02±0.82[b]	1058.3±0.20[b]	430.5±0.50[b]
PKC+Zn	1.25±0.11[a]	458.8±20.20[a]	40.7±10.50[a]
PKC+P	1.05±0.20[a]	430.3±30.20[a]	34.2±5.80[a]
Control	1.05±0.11[a]	430.0±0.50[a]	30.5±7.00

[a]Mean with different superscript are significantly different (p>0.05)

glass marbles, left overnight and on the next morning heated until they were completely digested and turned from dark brown to colourless.

The digest were further diluted in distilled water to 10ml in volumetric flask and analyzed for copper and zinc using Atomic Absorption Spectrophotometer (Virian Spectra 400) at wavelengths of 324.7nm and 213.9nm respectively. The spectrophotometer was standardized using a solution containing 2.00, 4.00, 6.00, 8.00 and 10.00µg/ml of copper and 0.20, 0.40. 0.60, 0.08 and 0.10µg/ml of zinc prepared from spectrosol grade cupric nitrate and zinc nitrate (1mg/ml) respectively, in 0.1M Nitric acid. The spectrophotometer was closely monitored and restandardised if necessary to ensure better performance. Triplicate determinations were carried out per sample and the result of copper and zinc concentration were expressed as the mean of the three determination ± standard error of mean of the groups in P-g/g dry weight or µg/ml. Student's t-test was employed to test the significance of difference between treatment means.

Results

There was no mortality except in the PKC group. All animals died in the PKC group. The clinical signs were jaundice observed in the first animal on day 54, second and third on day 56 and followed by haemoglobinuria at day 57 for that first animal and at day 58 by the other two animals. The animals severely flake off their hairs at day 58 and were generally weak, depressed and anorexic and all died on day 130, the first one in the morning followed by the other two in the evening. The animals in the PKC + Zn group, PKC + phytase group and the control group did not die and did not show any clinical abnormalities throughout the trial.

The average daily gain of the animals in the various groups is shown in Table 1. The average daily weight gain was similar in all groups over the 20 weeks except at two weeks prior to death where it began to reduce in the PKC group and at one week to death was significantly lowered (P < 0.05) The numerical values of the daily gain were 35.6g for the PKC group, 50.5g for the PKC + Zn group, 55.3g for the PKC + P group and 54.0g for the control. The daily gain was numerically higher in the PKC + P group than the PKC + Zn group and the control, though the difference was not statistically significant (P>0.05).

Analysis of carcasses for gross lesions revealed that animals that died in the course of the experiment died of copper toxicity of PKC. All animals that died had severe generalized jaundice. All animals in the PKC group had liver that was moderately yellowish with multi focal pale area of necrosis. Kidneys were firmed, enlarged and dark black in appearance at both the cortex and medulla. Carcasses of animals of the other groups analyzed did not show significant gross lesions.

The hepatocytes of animals that died in PKC group were highly swollen, vacuolated and necrotized at the periacinal zone. Moderate fibrosis occurred at the periportal zone. Tubular epithelial cells of the liver lobes and renal cortex of animals in the other groups did not show remarkable histological lesions.

The copper concentration in the PKC used in this study was 20.5 ± 0.09µg/g. The copper concentration in the blood, renal cortex and liver for each treatment is shown in Table 2. The blood copper concentration in all the groups remained alike for the first 4 weeks of the trial (PKC group, PKC + Zn, PKC + P and the control had blood copper concentration of 1.85 ± 0.02µg/ml, 1.02 ± 0.20µg/ml and 1.00 ± 0.35µg/ml respectively). It was slightly elevated in the PKC group from 4th to 8th week ranging from 2.45 ± 0.20µg/ml but highly increased to 7.50 ± 0.10µg/ml at the 10th week and thereafter (which was about 2 weeks after the appearance of haemoglobinuria). The blood copper concentration in the PKC + Zn group, PKC + P group and the control increased only slightly to 1.30 ± 0.20µg/ml, 1.08 ± 0.02µg/ml and 1.2 ± 0.85µg/ml respectively, throughout the duration of the experiment. The mean blood copper concentration of the PKC (3.02 ± 0.82µg/ml) was significantly higher (P>0.05) than those of the PKC + Zn group, PKC + P group and the control. The blood copper concentration of PKC + Zn, PKC + P and the control were similar.

The copper concentration in the right liver lobe and renal cortex in the PKC group (1058.3 ± 0.20 and 430.5 ± 0.5µg/g respectively) were significantly higher (P > 0.05) when compared with those of the control (460.3 ± 0.50 and 32.5 ± 7.0µg/g respectively) and those of the PKC + Zn group (458 ± 0.20 and 40.7 ± 10.5µg/g respectively) and those of PKC + P group (430.0 ± 30.2 and 34.2 ± 5.8

Table 3: Effect of different dietary treatments of PKC in sheep on blood, hepatic and renal Zinc concentration

Treatment	Blood (μg/ml)	Right liver (μg/g)	Renal cortex (μg/g)
PKC	3.50±0.10[c]	90.50±15.20[c]	102.30±12.50[c]
PKC+Zn	6.09±1.80[a]	219.20±30.20[a]	212.50±35.30[a]
PKC+P	4.90±0.50[b]	110.00±8.20[b]	118.20±8.50[b]
Control	4.90±1.50[b]	108.50±18.20[b]	108.20±12.10

[b]Mean with different superscript are significantly different (p>0.05)

μg/g respectively). But those of PKC + Zn group (430.0 ± 30.2 and 34.2 ± 58μg/g respectively) and the PKC + P group (430.0 ± 30.20 and 34.2 ± 38μg/g respectively) were not significantly different when compared with those of the control.

The Zinc content in the PKC used in this study was 75.0 ± 0.2μg/g. The result of the Zinc concentration of the blood, renal cortex and the right liver lobe for each treatment is shown in Table 3. The blood Zinc concentration in all the groups remained alike for the first 4 weeks of the trial (PKC group 3.2 ± 0.21μg/ml, PKC + Zn group 3.91 ± 0.5μg/ml, PKC + P group 3.5 ± 0.5μg/ml and the control 3.4 ± 1.5μg/ml). It was slightly elevated in the PKC + Zn group from 4th to 8th week ranging from 3.9 ± 0.8μg/ml but highly increased to 7.02 ± 0.10μg/ml at the 10 week and thereafter (which was 2 weeks after haemaglobinuria). The blood Zinc concentration in the PKC group, PKC + P group and the control increased only slightly throughout the duration of the trial. The mean blood Zinc concentration in the PKC + Zn group (6.09 ± 1.80μg/ml) was significantly higher (P >0.05) than those of the other groups. The mean blood Zinc concentration of the PKC + P group (4.90 ± 0.50μg/ml) was significantly higher (P>0.05) than those of the PKC group (3.50 ± 0.10μg/ml) but was consistent with the control (4.90 ± 1.5μg/ml).

The mean Zinc concentration in the right liver lobe and renal cortex in the PKC + Zn group (219.2 ± 30.2μg/g and 212.5 ± 35.3μg/g respectively) were significantly higher (P > 0.05) when compared with those of the PKC group (90.50 ± 15.2 and 102.3 ± 12.5μg/g respectively), PKC + P group (110.0 ± 8.2 and 118.2 ± 8.5μg/g respectively) and the control group (108.5 ± 18.2 and 108.2 ± 12.1μg/g respectively). The mean Zinc concentration in the right liver lobe and renal cortex in the PKC + P group, and the control were similar.

Discussion

The objective of the study was to assess the effect of phytase and zinc supplementation on palm kernel cake toxicity in sheep. It was to further assess the toxicity effect of excess palm kernel cake in sheep. The results obtained show that animals with phytase supplementation (PKC + P group) performed as those of the zinc supplementation (PKC + Zn group) and the control in terms of clinical signs, gross and histological

lesions. In terms of body weight gain, animals with phytase supplementation (PKC + P group) even performed better than animals with the zinc supplementation (PKC + Zn group) and the control, though the differences were not statistically significant (P>0.05).

Animals in the PKC group all died of PKC toxicity. Supplementation with zinc and phytase prevented chronic PKC toxicity and can be useful in the therapy for PKC toxicity in sheep. Supplementation with zinc led to a significant elevation of zinc concentration in the blood, right liver lobe and renal cortex. These findings on the elevated zinc concentration in the blood and these organs support previous findings by Hair-Bejo and Alimon (1995). We obtained a significant elevation in our study. Hair-Bejo and Alimon (1995), in their study did not obtain a significant elevation. The variation in the results might be due to the differences in the composition of PKC used which might be caused by differences in soil type, species of palm tree and processing instruments etc. Our PKC material contained 75.0 ± 0.2μg/g of zinc and 20.5 ± 0.09μg/g of copper while those of Hair-Bejo and Alimon (1995) contained 43.8 ± 0.1μg/g of zinc and 21.6 ± 0.2μg/g of copper. It was interesting to observe that the use of supplementary phytase did not at all elevate the concentration of zinc in those organs.

Phytases are enzymes specialized in breaking down phytates (myo-inositol hexakisphosphates). In addition to phosphorus, phytate also binds tightly to other nutrients such as proteins and minerals and render them unavailable. Phytase breaks down phytate and release bound nutrients, thus improves the digestibility of those nutrients. In our work, we observed that the addition of phytase increased blood zinc concentration significantly (P>0.05) in the PKC + P group compared with other groups. The mean blood zinc concentration was 4.9 ± 0.5μg/ml in the PKC + P group and was significantly higher (P>.0.05) than the mean blood zinc concentration in the PKC group 3.50 ± 0.10μg/ml. This result suggests that the added phytase increased the availability of natural zinc of the PKC. Contrarily, we observed that while the blood zinc concentration was increased with the added phytase, the blood copper concentration was decreased. The enzyme appears to improve the utilization of zinc of PKC, which then make zinc to be preferentially absorbed instead of copper. Similar observations of the role of phytase in copper and zinc utilization have been reported by a number of researchers. Aoyagi and Baker (1995) reported decreased utilization of copper in soya bean meal by 50% when microbial phytase was added. There is also report showing increased calcium availability and decreased copper and sulphur availability in barley grains when phytate level was reduced. In growing and finishing pigs, Murray (1998) reported increased availability of zinc, magnesium, phosphorus and iron,

but decreased availability of calcium and copper utilization when a low level of phosphorus corn-soya bean meal was fed with phytase enzyme. Perhaps the increased zinc utilisation in PKC when phytase was added overcame the copper toxicity in the PKC + P group.

But why should sheep as a ruminant requires an extrinsic phytase to utilize phytate when ruminant readily utilize phytate because of the phytase produced by the rumen microorganism? More recent findings shows that the efficacy of phytase depend not only on the present of the enzyme but also on the type of feed (phytate), the dose and the source of phytase (Dekker *et al.*, 1992; Eeckhout and De Paepe, 1992; Dungelhoef *et al.*, 1994). The toxicity effect of excess PKC in sheep without an added phytase may be caused by the inability of the endogenous phytase to handle the increased phytate level in the PKC. The supplemental phytase may have had an additive effect on the ruminal phytase activity. An increased phytase activity was demonstrated with an increased phytase level (Simons *et al.*, 1990).

This study agrees with previous findings that feeding PKC in excess (90%) in sheep caused chronic copper toxicity (Hair-Bejo and Alimon, 1995; Abdul Rahman *et al.*, 1989; Wan Mohammed *et al.*, 1989). The clinical signs, gross lesion and histopathological changes were consistent with those reported by Hair-Bejo and Alimon (1995) except that in our study, the kidney, were completely darkened. Our work also agrees with the work of Hair-Bejo and Alimon (1995), who also observed an elevated hepatic, renal and blood copper in the PKC group. Our work, supports other works that the PKC toxicity in sheep is consistent to those of acquired chronic copper poisoning (Ishmael *et al.*, 1971; Soli 1980). Our works also agree with Hair-Bejo and Alimon (1995) that dietary zinc supplementation can prevent chronic copper toxicity of excess PKC in sheep.

It is concluded that feeding PKC in excess in sheep can cause chronic copper toxicity in sheep but supplementing the diet with zinc and phytase can prevent this effect. Supplementation with phytase does not elevate zinc concentration in the liver and kidney, as it is the case with zinc supplementation.

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