The protective role of zinc in palm kernel cake (PKC) toxicity in sheep

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ABSTRACT

Male Malin x Polled Dorset crossbred sheep were stall-fed with grass (10%) and PKC (90%) and supplemented with either zinc at 500 ug/g, as zinc sulfate (PKC+Zn group) or zinc (113 ug/*g)* and ammonium molybdate (500 ug/g) (PKC+Zn+Mo group) or unsupplemented diet (PKC group) for 20 weeks. Another group which acts as a control was fed with a diet consisting of corn and fish meal (2 0%) and grass (80%). The animals were monitored daily and the body weights were recorded at a period of two weeks intervals throughout the trial. Blood samples were also collected for mineral analysis. At the end of the trial the animals were slaughtered. The carcasses were examined for gross lesions, whilst the right liver lobes and renal cortex were isolated for histopathological evaluation and mineral analysis. All animals in the PKC group died before the end of the trial with the main clinical signs of generalised jaundice and haemoglobinuria. The kidneys were firm, enlarged and reddened or darkened. Histologically, the hepatocytes were swollen, vacuolated and necrotized, particularly at the periacinar zone. Hepatic fibrosis was observed at the periportal zone. Cellular swelling, vacuolation and necrosis were found in the tubular epithelial cells of the renal cortex. Neither clinical signs nor gross or remarkable histological lesions were observed in the other groups of animals. The hepatic, renal and blood copper levels In the PKC group were elevated when compared to the control. Addition of zinc either with or without ammonium molybdate in PKC diet inhibit the copper content in the organs, however the zinc contents were increased. The average daily gain of the PKC group was remained consistent to those of the other groups, except it was reduced starting at about 1 to 2 weeks prior to death. It was concluded that feeding PKC In excess in sheep can cause chronic copper toxicity. However, this effect can be prevented by dietary zinc supplementation either with or without ammonium molybdate.

INTRODUCTION

Palm kernel cake (PKC) is an important by-product of the oil palm industry and is obtained after the extraction of oil from the kernel of the oil palm fruit. It has a high nutritive value and is widely used as feed ingredients in ruminants, although its usage in excess in sheep can cause chronic toxicity (Abdul Rahman *et al.,* 1989: Wan Mohamed *et al.,* 1989). Death is mainly due to hepatic necrosis, whilst jaundice and haemoglobinuria were the most remarkable clinical signs demonstrated. These changes are quite consistent to those of chronic copper toxicity (lshmael *et* al, 1971; Underwood, 1977; Soli, 1980). The level of copper in PKC was found to be relatively high which was about 11 to 55 ug/g dry weight (Abdul Rahman *et al.,* 1989; Jalaludin *et al.,* 1991) and supplementation with either sodium molybdate or ferous sulphate was reported to reduce the incidence of the disease (Abdul Rahman *et al.,* 1989; Wan Mohamed *et al.,* 1989). However, knowledge of the disease, including the biochemical and morphological changes of the liver, blood and kidney to PKC excess are little understood.

Copper is absorbed from the gastrointestinal tract, bound to albumin In the portal circulation and is mainly accumulated in the liver with excess excreted in the bile (Evans, 1973; Cousins, 1985). A small amount of copper also passes directly from the plasma into the urine and Intestinal epithelium, whilst a negligble amount is lost in the sweat (Underwood, 1977). Disturbances in copper homeostasis may lead to an excessive accumulation of this element in body tissues, especially the liver and kidneys and can cause serious tissue injury and cell death.

Copper toxicosis is a complex problem, and it can be primerly due to excess intake of dietary copper (Soli, 1980) or secondary to liver diseases (Haywood *et al.,* 1988). Copper toxicosis occurs as a familial copper storage disorder with liver and kidney injury as in Wilson's disease in man (Scheinberg & Sternlieb, 1976) and in Bedlington terriers (Twedt *et al,* 1979). Alternatively, copper poisoning may be acquired as in sheep (Soli, 1980) and less commonly reported in cattle (Marschang *et al.,* 1980) and pigs (Higgins, 1981). Sheep as a species are more prone to the disease with some breeds more susceptible than others (Underwood, 1977).

The existence of the antagonistic interaction between copper and zinc suggest that zinc which is less toxic than copper might be useful in the treatment of the disease (Hair-Bejo *et al.,* 1991), and indeed, zinc has been used in the treatment of or to confer protection against copper toxicity in Wilson's disease patients (Brewer *et al.,* 1983), but its usage is still far from universal and mode of action is little understood. Thus, the aim of the study was to assess the effects of dietary zinc supplementation in sheep fed with excess PKC diet.

MATERIALS AND METHODS

Animals

Twelve male Malin x Polled Dorset crossbred sheep, weighing 19.4 ± 1.5 kg., were divided into 4 groups of 3 animals each. They were stall-fed with a basal diet of grass (10%) and PKC (90%) and supplemented with either zinc at 500 ug/g, as zinc sulfate ($ZnSO_4$. $7H_20$) (PKC+Zn group) or zinc (113 ug/g) and ammonium molybdate (500 ug/g) (PKC+Zn+Mo group) or unsupplemented diet (PKC group) for 20 weeks. The control group was fed a diet consisting of corn and fish meal supplernentations (20%) and grass (80%). Animals were monitored daily and the body weights were recorded at a period of two weeks intervals. Blood samples were also collected every fortnight from the jugular vein and stored at -20^0C until required for analysis. At the end of the trial the animals were slaughtered. The carcasses were examined for gross lesions, whilst the right liver lobes and renal cortex were isolated for histopathological evaluation and mineral analysis.

Histopathology

The right liver lobes and the renal cortex were fixed in freshly prepared 10% formalin for at least 48 hours. The blocks were sub-sequently dehydrated in alcohol, cleared with xylene and embedded in paraffin wax, sectioned at about 5 um and mounted on glass slides. Sections were stained with haematoxylin and eosin (Lillie, 1965).

Copper and zinc analysis

Triplicate samples of liver and kidney from each animals, and PKC were oven dried in plastic containers at 700C until they reached a constant weight. The dry weight of the samples were recorded prior to acid digestion. Duplicate whole blood samples of 2.0 ml each from each animals were used for the analysis. All samples were digested in a pyrex glass tube (150 mm X 18 mm) with 70% aristar grade nitric acid (BDH Chemicals Ltd) and 60% spectrosol grade perchloric acid (BDH Chemical Ltd.) in 2 to 1 *(v/v)* respectively. Fifty ul (1mg/ml) of spectrosol grade cupric nitrate and zinc nitrate solutions (BDH Chemicals Ltd.) were added into each recovery tube. All tubes were covered with glass marbels and left overnight. They were then heated in a heating block (Thermolyne Dri Bath Incubator Type 28100) at 140^0C until all the samples were completely digested and changed from dark brown to colourless.

The digested samples were diluted in distilled water to 10 ml in volumetric flasks and further diluted if required. Copper and zinc contents were analysed in an Atomic Absorption Spectrophotometer (Varian Spectra 400) at wavelengths of 324.7 nm and 213.9 nm respectively. The spectro-photometer was standardized with a solution containing 2.00, 4.00, 6.00, 8.00 and 10.00 ug/ml of copper and 0.20, 0.40, 0.60, 0.80 and 1.00 ug/ml of zinc prepared from spectrosol grade cupric nitrate and zinc nitrate (1mg/ml) respectively, in 0.1M nitric acid. The performance of the spectro-photometer was frequently

monitored and restandardised if required. Copper and zinc concentrations were expressed as the mean of the three or two samples reading and the mean ± standard error of mean of the groups in p-g/g dry weight or ug/ml. Statistical analysis was performed using Student's t-test (Rafferty *et al.*, 1985).

RESULTS

Clinical signs

All animals in PKC group died before the end of the trial. Jaundice was first observed on day 53 in one of the sheep and this was followed by haemoglobinuria at day 56. The animal was weak, depressed, anorexic and on sternal recumbency and was slaughtered. The other animals in the group died on day 95 and 129 of the trial with similar clinical signs. Neither the PKC+Zn group nor the PKC+Zn+Mo and the control groups showed any clinical abnormalities throughout the trial. The average daily gain in the PKC group (54.8g) was quite consistent ($p>0.05$) to those of the control (59.2g), PKC+Zn (62.2g) and PKC+Zn+Mo (41.8g) groups, but started to decrease at about 1 to 2 weeks prior to death.

Gross lesions

The carcasses of animal died of PKC toxicity had moderate to severe generalised jaundice. The liver was mild to moderately yellowish and presence of multifocal pale areas of necrosis. The kidneys were firm, slightly enlarged and reddened or dark black in appearance involving both the cortex and the medulla. The urine was also reddened and darkened. No significant gross lesions were observed in the other groups of animals.

Histological lesions

Moderate to severe swelling and vacuolation of the hepatocytes, particularly at the periacinar zone (central vein) were observed in animals died of PKC toxicity. Single cell necrosis (apoptosis) was also frequently observed in the region, whilst mild to moderate fibrosis observed in the periportal zone in two of the animals. Cellular swelling, vacuolation and necrosis were found in the tubular epithelial cells of the renal cortex. No remarkable histological lesions were observed in the other groups of animals.

Copper concentration

The hepatic and renal copper contents in PKC group were increased ($p<0.05$) when compared to the control (Table 1). However, supplementing zinc either with or without ammonium molybdate to the diet appeared to inhibit the accumulation of copper in the organs. The blood copper content in P1(C group remained low during the first 5 weeks of the trial (1.08±0.05 ug/ml). It was slightiy elevated thereafter (1.40±0.11 ug/ml), but was markedly increased ($p<O.O5$) during or at about 1 to 2 days prior to the appearance of haemoglobinuria (6.47±0.09 ug/ml). The blood copper content in the PKC+Zn (1.05±0.04 ug/ml) and PKC+Zn+Mo (1.19±0.18 ug/ml) groups were remained low ($p<O.O5$) when compared to those of the control (1.07±0.07 ug/ml). Copper content in the PKC was 21.6±0.2 ug/g,

Table 1. Copper and zinc concentrations in the right liver and renal cortex (ug/g dry weight)*

Treatments (Groups)	Copper Concentration		Zinc Concentration	
	Right Liver	Renal Cortex	Right Liver	Renal Cortex
PKC	1026.3 ± 43.5^{a}	425.7 ± 29.8^{a}	143.3 ± 40.9^{a}	136.5 ± 0.9^{a}
PKC + Zn	$713.7 \pm 191.1^{a,b}$	39.4 ± 7.1^{b}	208.2 ± 27.4^{b}	202.3 ± 43.1^{b}
PKC + Zn + Mo	$767.3 \pm 178.4^{a,b}$	190.7 ± 36.9^{c}	$183.3 \pm 37.6^{a,b}$	$153.1 \pm 15.8^{a,b}$
Control	466.3 ± 52.1^{b}	$196. \pm 1.3^{b}$	123.9 ± 7.2^{a}	116.5 ± 14.1^{a}

* All values are express as mean ± standard error of mean.[a,b,c] Means with same superscript in the same column shows non significant differences ($p > 0.05$).

whilst the estimated recovery for the copper concentration during acid digestion was 97.9 ± 0.7 **%.**

Zinc concentration

The hepatic and renal zinc concentrations in PKC+Zn group were increased ($P<0.05$) when compared to those of the control (Table 1). However, despite some elevation of zinc content in the organs of PKC+Zn+Mo group they were not significantly different ($p>0.05$). The hepatic and renal zinc contents in PKC group remained unchanged. The zinc concentration in the blood of PKC+Zn (5.96±0. 16 ug/ml) and PKC+Zn+Mo (5.21±0.16 ug/ml) groups were increased ($p<0.05$) at week 5 and thereafter when compared to the control (3.92±0.07 ug/ml). However, the zinc content in the blood of the PKC group (4.09±0.08 ug/ml) remained low throughout the trial. Zinc content in the PKC was 43.8±0.1 ug/g, whilst the estimated recovery for the zinc concentration during acid digestion was 98.4 ± 1.0 **%.**

DISCUSSION

This study demonstrated that feeding PKC in excess (90%) in sheep can cause chronic copper toxicity and support the preliminary observation as reported previously (Abdul Rahman *et al.,* 1989; Wan Mohamed *et al,* 1989). The clinical manifestations, gross and histopathological changes as well as the elevation of hepatic, renal and blood copper contents of PKC toxicity in sheep are consistent to those of acquired chronic copper poisoning (Ishmael *et al.,* 1971; Soli, 1980). Furthermore, the copper content of the PKC in the present study was relatively high (21.6 ug/g). The dietary requirement of copper for growing sheep is about 4-6 ug/g and chronic copper poisoning was reported in sheep fed with diet containing copper at 10-20 ug/g (Soli, 1980). The primary source of copper in PKC is little known, although preliminary studies showed that the copper content in the kernel from the fresh fruits was high (17.0±0.3 ug/g dry weight) (Hair-Bejo & Alimon, unpublished data). High copper content in PKC could also be associated with copper contamination during processing of the by-products.

The ability of zinc either with or without ammonium molybdate to inhibit hepatic, renal and blood

copper contents and clinical manifestations of toxicity as well as hepatic and renal damages is an interesting phenomenon and suggest that zinc and ammonium molybdate can be useful in the therapy for PKC toxicity in sheep. It was suggested that the antagonistic lnteracoon between copper and zinc takes place at the luminal level of the Intestinal tract (Hair-Bejo *et al.,* 1991), whilst molybdate may react with sulphide in the rumen to form thiomolybdate which subsequently combines with dietary and tissue copper to form complexes in which copper is unavailable (Mill, 1980; Goo-nerathne *et al,* 1981).

The elevation of hepatic, renal and blood zinc concentrations in zinc supplemented animals in the present study Is another Interesting finding and It might further explain on the protective effect of zinc In copper toxicity. Zinc is less toxic than copper. Furthermore, the element is a better inducer of metarothionein (MT), a low molecular weight metal binding protein, when compared to copper (Oestrelcher & Cousins, 1985). However, MT has a higher ability to bind copper than zinc (Breamer, 1980). Thus, the elevation of zinc content In the organs in the present study could be associated with the induction of MT in the organ. This metal binding protein sequester copper and make it non toxic to the tissue (Mehra & Breamer, 1984).

In conclusion, this study showed that feeding PKC in excess in sheep can cause chronic copper toxicity. This effect can be prevented by zinc supplementation either with or without anunonium molybdate.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Dr Mahyuddin Dahan, Dr. Hallimatun Yackob, Mr lbrahim Mohsin and Mr Abdullah Misron for their kind assistance in this project.

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