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Dietary zinc treatment for chronic copper intoxication in palm kernel cake (PKC) fed sheep

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ABSTRACT

Thirty, 4 month-old male Maim x Polled Dorset crossbred sheep were allocated into 6 groups of 5 animals each. Four groups of animals were stall-fed with basal diet of 90% palm kernel cake (PKC) and 10% grass (G) for 16 weeks. One group of the animal was slaughtered at the end of the 16 weeks feeding trial (PKC group), whilst the other three groups were further fed with either the same diet (PKC+PKC group) or fed with a new diet consisting of 30% corn and 10% fish meals (CF) and grass (60%) either with (PKC+CF+Zn group) or without (PKC+CF group) zinc supplementation (500 μg/g Zn as zinc sulfate) for another 16 weeks and were slaughtered at the end of the feeding trial, The other two groups which act as controls were fed with corn (30%) and fish meals (10%) and grass (60%), and were slaughtered at weeks 16 (CF group) and 32 (CF+CF group) of the trial. The blood, right and left liver, renal cortex and medulla, pancreas, bile and urine of all animals were analysed for copper and zinc contents using an atomic absorption spectrophotometer. The liver and kidney were also fixed in 10% buffered formalin for histopathological examination. The study showed that neither clinical signs nor gross lesions of copper or zinc toxicity were observed throughout the trial. However, the copper concentration in both the right and left liver of PKC fed sheep at weeks 16 and 32 rose to about 3 times that of the controls and remained high in both the PKC+CF and PKC+CF+Zn groups. A similar pattern of copper concentration was observed in the blood. The copper and zinc contents in the renal cortex and medulla, pancreas, bile and urine remained low in all groups. The zinc content in the liver of PKC+CF+Zn group was significantly increased. Histologically, moderate hepatic lesions were observed in the PKC fed sheep at week 32. The lesions were milder in the other groups especially in the PKC+CF+Zn group. No significant renal lesions was recorded in all groups. It was concluded that the usage of dietary zinc supplementation (500 μg/g) in the treatment of PKC toxicity in sheep was unsatisfactory. The ability of Malin x Polled Dorset crossbred sheep to tolerate the high copper content in PKC at least during the first 16 weeks of the feeding trial may provide more avenue in the utilization of PKC as a major feed ingredient in sheep.

INTRODUCTION

Copper and zinc are essential trace elements in animals. Copper is absorbed from the gastrointestinal tract and is stored in the liver, any excess being excreted through the bile and urine (Evans, 1973). An accumulation of excess copper in the liver can cause severe hepatic damage and death. This can be of genetic origin as in copper storage disorder in Wilson's disease in man (Sternlieb, 1980) and copper toxicosis Bedlington terriers (Johnson *et al.,* 1980) or acquired as in sheep (Soli, 1980). Sheep is a species known to be prone to chronic copper

poisoning (Soli, 1980), in contrast to the Dominican toad and Mute swan which are apparently resistant to excess copper (Stemlieb, 1980). Rats have been shown to adapt to copper overload and became tolerant (Haywood, 1985).

The drug of choice in the treatment of chronic copper toxicity is limited and their mode of action is uncertain. Penicillamine or dimethyl cysteine is used for treatment of copper excess such as in Wilson's disease patients (Walshe, 1975) and Bedlington terriers (Ludwing et al., 1980), although it is relatively toxic (Brewer et al., 1983; Hardy, 1983) and too expensive for routine use for animals under field condition (Soil et al., 1978). Alternatively, an organic compound containing molybdenum and sulphur, and a tetramine compound (triethylene tetramine dihydrochloride) have been reported to be effective for the treatment of chronic copper poisoning in sheep (Humphries *et al.,* 1988) and Bedlington terriers (Twedt *et al.,* 1988), respectively. The existence of antagonistic effect between copper and zinc suggest that zinc, which is less toxic than copper (Bises *et al.,* 1989) can be useful as an alternative therapy for copper toxicity and indeed, zinc has been made use of in the treatment of Wilson's disease in man, although the success of the treatment was unpredictable (Walshe, 1984; Brewer *et al.,* 1983; Hill *et al.,* 1987). Supplementation of zinc in palm kernel cake (PKC), an important by product of the oil palm industry with high copper content, can inhibit hepatic copper accumulation and prevent the incidence of chronic copper toxicity in sheep fed with the diet (Hair-Bejo & Alimon, 1995). However, the role of zinc in the treatment of the disease is unknown. The aim of this study is to determine the role of zinc in the treatment of chronic copper intoxication in PKC fed sheep.

MATERIALS AND METHODS

Animals

Thirty, 4-month-old male Malin x Polled Dorset crossbred sheep, weighing 16.8+0.2kg were allocated into 6 groups of 5 animals each. Four groups of animals were stall-fed with basal diet of 90% palm kernel cake (PKC) and 10% grass (G) for 16 weeks. One group of the animals was slaughtered at the end of the 16 weeks feeding trial (PKC group), whilst the other three groups were further fed with either the same diet (PKC+PKC group) or fed with a new diet consisting of 30% corn and 10% fish meals (CF), and grass (60%) either with (PKC+CF+Zn group) or without (PKC+CF group) zinc supplementation (500 μg/g Zn as zinc sulfate ($ZnSO_4$ $7H_20$) for another 16 weeks and were slaughtered at the end of the feeding trial. The other two groups which act as the controls were fed with corn (30%), fish meals (10%) and grass (60%), and were slaughtered at weeks 16 and 32 of the trial. The animals were monitored daily for any clinical abormalities. The body weight and heparinised blood samples from the jagular vein were collected prior to slaughter. The carcasses were examined for gross lesions, whilst the right liver and kidney were taken for histopathological examination. Samples of right and left liver, renal cortex and medulla, pancreas, blood, bile and urine were isolated and stored at -200C until required for copper and zinc determination.

Histopathology

The right liver and kidney were fixed in freshly prepared 10% buffered formalin for at least 48

hours. The blocks were subsequently dehydrated in series of alcohol, cleared with xylene and embedded in paraffin wax. The tissues were sectioned at about 4 pm and mounted on glass slides. Sections were stained with haematoxylin and eosin (Lillie, 1965).

Copper and zinc analysis

Triplicate samples of the right and left liver, renal cortex and medulla, and pancreas from each animals, and samples of PKC, corn, fish meal and grass were oven dried in plastic containers at 70ºC until they reached a constant weight. The dry weight of the samples (about 0.2g) were recorded prior to acid digestion. Duplicate samples of whole blood, urine and bile of 1.0 or 2.0 ml each from each animal were used for the analysis. All samples were digested in a pyrex glass tube (150 mm x 18 mm) with 70% nitric acid (Aristar grade, BDH Chemicals Ltd) (Hair-Bejo *et al.*, 1995). Fifty µ1 (1mg/ml) of cupric nitrate and zinc nitrate solutions (Spectrosol grade, BDH Chemicals Ltd.) were added into each recovery tube. All tubes were covered with glass marbles and left overnight. They were then heated in a heating block (Thermolyne Dri Bath incubator Type 28100) at 140ºC 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 spectrophotometer was standardized with a solution containing 2.00, 4.00, 6.00, 8.00 and 10.00 µg/ml of copper and 0.20, 0.40, 0.60, 0.80 and 1.00 µg/ml of zinc prepared from cupric nitrate and zinc nitrate (1mg/ml), respectively (Spectrosol grade, BDH Chemicals Ltd.), in 0.1M nitric acid (Analar grade, BDH Chemicals Ltd.). The performance of the spectrophotometer was frequently monitored and restandardised if required. Copper and zinc concentrations were expressed as the mean of the three or two samples readings and the mean ± standard error of means of the groups in µg/g dry weight or µg/ml. Statistical analysis was performed using Student's t-test in Cricket software (version 1.1) for Macintosh (Rafferty *et al.*, 1985).

All glasswares were washed and rinsed throughly with running water and later soaked in 0.1N nitric acid (Analar grade, BDH Chemicals Ltd.) for at least 48 hours. This was followed by 2 washes of 24 hours each in distilled water, rinsed and dried in oven at 70ºC.

RESULTS

Clinical signs and gross lesions

Neither the clinical signs nor the gross lesions of chronic copper or zinc toxicity were observed in any groups of animals throughout the trial. The average daily gain (ADG) of the PKC fed animals (PKC (41g), PKC+PKC (47g), PKC+CF (50g), PKC+CF+Zn (42g)) during the first 16 weeks of the trial was much higher ($p<0.05$) than the control group (25g). The ADG in all group remained low ($p>0.05$) in the last 16 week of the trial (PKC+PKC(-4g), PKC+CF(0g), PKC+CF+Zn (7g) and CF(5g)).

Histological lesions

Most of the liver in the PKC+PKC group showed moderate swelling, vacuolation and necrosis of the hepatocytes, especially at the periacinar zones (central vein). Fatty degeneration were observed in some sheep. Mild mononuclear inflammatory cell infiltration and bile duct proliferation were present in the periportal zone. Moderate fibrosis which bridged portal zones was observed in one animal.

The histological changes in the livers of PKC, PKC+CF and PKC+CF+Zn groups were less severe than those seen in the PKC+PKC group. Mild to moderate lesions were observed in the hepatocytes, whilst fatty degeneration, bile duct proliferations, fibrosis and infiltration of mononuclear inflammatory cells were only observed in a few sheep in both the PKC and PKC+CF groups. Similar lesions were observed in the PKC+CF+Zn group, except that three of the animals in the group showed much milder lesions in the hepatocytes. No remarkable histological changes were observed in the control groups.

The overall histological changes in the kidney were unchanged, although mild swelling, vacuolation and necrosis of the tubular epithelial cells were occasionally recorded in a few animals.

Copper concentration

The copper content in the right liver of sheep fed PKC at weeks 16 (1716.92±303.17μg/g) and 32 (1813.10±271.32 μg/g) rose to about 3 times ($p<0.05$) than the controls (487.47±32.87 μg/g and 715.58±87.55 μg/g) and remained high in both the PKC+CF (1560.47 ± 299.82 μg/g) or PKC+CF+Zn (1573.20±303.91 μg/g) groups. The copper content in the left liver were remain unchanged ($p>0.05$) when compared to those of the right liver (Table 1).

The copper concentration in the renal cortex of sheep fed PKC at week 32 (82.39±40.45 μg/g) was about two times higher than that at week 16 (41.73±15.39 μg/g), although it was statistically insignificant ($p>0.05$). Copper level in the PKC+CF (35.44±10.82 μg/g) was slightly higher ($p>0.05$) than the PKC+CF+Zn (19.73±2.48 μg/g) and the control groups (22.94±3.84 μg/g) and 19.11±2.55 μg/g). Copper content in the renal medulla remained low in all group of animals (Table 1).

The blood copper content in PKC fed animals were elevated at week 16 (0.91±0.06 μg/ml) and remained high thereafter at week 32 (1.18±0.15 μg/ml), and in both the PKC+CF (1.23±0.14 μg/ml) and PKC+CF+Zn (1.30±0.07 μg/ml) groups when compared ($p<0.05$) to the controls (0.62±0.01 μg/ml and 0.89±0.06 μg/ml) (Table 1).

The copper content in the pancreas of PKC fed animals (8.47 + 0.66 μg/g) was significantly elevated ($p<0.05$) at week 32 than the control group (4.95±0.26 μg/g). However, despite some increased in copper content in the PKC+CF (6.43±0.59 μg/g) and PKC+CF+Zn (5.04±0.22 μg/g) groups they were statistically insignificant ($p>0.05$) when compared to the control. The bile and urine copper concentrations remained low in all groups of animals (Table 1).

Copper concentration in the PKC, corn, fish meal and grass were 23.42±0.84 μg/g, 6.94±0.36 μg/g, 9.67±0.12 μg/g and 3.08±0.58 μg/g, respectively. The estimated recovery for copper during acid digestion was 101.83±0.73%.

Zinc concentration

The zinc content in the PKC+CF+Zn group (255.36±25.64 μg/g) increased signficantly ($p<0.05$) when compared to those of PKC+PKC (158.85±21.33 μg/g), PKC+CF (180.10±17.05 μg/g) and the control groups (190.38±11.72 and 166.62±17.68 μg/g). The zinc concentration in both the right and left liver remained unchange ($p>0.05$) in all groups (Table 2). Zinc concentration in the renal cortex (161.99±10.27 μg/g) of the PKC+CF+Zn group was slightly elevated when compared to the other groups although they were statistically insignificant ($p>0.05$) (Table 2).

The blood, pancreas, bile and urine zinc contents were remained low ($p>0.05$) in all group of animals (Table 2). The zinc concentration in the PKC, corn, fish meal and grass were 50.80±0.38 μg/g, 38.47±1.51 μg/g, 76.00±2.14 μg/g and 29.07±1.36 μg/g, respectively. The estimated recovery for zinc during acid digestion was 105.20±1.49%.

Table 1. Copper concentration in the liver, kidney, blood, bile, urine and pancreas (μg/g dry weight or μg/ml)* of sheep treated with various dietary regimen

Treatments / 0-16	Weeks 17 - 32	Right	Left	Cortex	Medulla	Blood	Bile	Urine	Pancreas
1. PKC		1716.92 ±303.17	1580.12 ±306.62	41.73 ±15.39	16.49 ±2.45	0.91 ±0.06	-	-	-
2. PKC	PKC	1813.10 ±271.32	1844.78 ±320.48	82.39 ±40.45	23.21 ±6.44	1.18 ±0.15	0.67 ±0.09	0.23 ±0.15	8.47 ±0.66
3. PKC	CF	1560.47 ±299.82	1643.69 ±313.25	35.44 ±10.82	14.73 ±2.11	1.23 ±0.14	0.50 ±0.07	0.16 ±0.02	6.43 ±0.59
4. PKC	CF + Zn	1573.20 ±303.91	1481.84 ±311.4	19.73 ±2.48	11.30 ±0.72	1.30 ±0.07	0.94 ±0.40	0.16 ±0.12	5.04 ±0.22
5. CF		487.47 ±32.87	483.73 ±27.62	22.94 ±3.84	13.32 ±2.14	0.62 ±0.01	-	-	-
6. CF	CF	715.58 ±87.55	711.93 ±91.37	19.11 ±2.55	12.17 ±1.04	0.89 ±0.06	0.58 ±0.16	0.16 ±0.02	4.95 ±0.26

* All value are expressed as mean ± standard error of mean of 5 sheep in each group. PKC = palm kernel cake, CF = corn and fish meals, Zn = zinc, and (-) = not done.

Table 2. Zinc concentration in the liver, kidney, blood, bile, urine and pancreas (μg/g dry weight or μg/ml)* of sheep treated with various dietary regimen

Treatments / 0-16	Weeks 17 - 32	Right	Left	Cortex	Medulla	Blood	Bile	Urine	Pancreas
1. PKC		169.39 ±5.73	186.39 ±9.06	140.46 ±2.25	93.49 ±9.32	3.07 ±0.21	-	-	-
2. PKC	PKC	158.85 ±21.33	120.71 ±20.86	138.00 ±11.30	129.98 ±9.77	3.73 ±0.45	2.58 ±0.22	1.37 ±0.69	102.81 ±7.63
3. PKC	CF	180.10 ±17.05	232.48 ±46.21	143.17 ±7.91	146.80 ±7.90	2.65 ±0.15	3.35 ±0.65	1.51 ±0.61	78.86 ±4.05
4. PKC	CF + Zn	255.36 ±25.64	251.67 ±15.04	161.99 ±10.27	174.43 ±11.48	3.20 ±0.09	3.94 ±0.58	2.05 ±0.58	92.04 ±6.39
5. CF		190.38 ±11.72	191.63 ±13.39	143.24 ±5.99	101.44 ±2.04	2.52 ±0.06	-	-	-
6. CF	CF	166.62 ±17.68	176.82 ±16.92	138.82 ±2.48	158.05 ±8.54	2.96 ±0.16	4.15 ±0.78	1.59 ±0.66	80.75 ±1.47

* All value are expressed as mean ± standard error of mean of 5 sheep in each group. PKC = palm kernel cake, CF = corn and fish meals, Zn = zinc, and (-) = not done.

DISCUSSION

The study showed that feeding PKC in sheep up to 16 weeks significantly increased the copper concentration in the liver. However, despite the withdrawal of the diet up to 16 weeks the high copper content in the organ failed to be removed. Dietary zinc treatment did not reduce the hepatic copper content which had been accumulated previously. This might explain the inconsistent effect of zinc in the treatment of Wilson's disease in man (Walshe, 1984) and further support the previous finding which showed that the antagonistic interaction between copper and zinc occurs at the luminal level of the intestinal tract during the process of absorption (Hair-Bejo *et al.*, 1991). Excess hepatic copper accumulation and death can be prevented in sheep fed with high copper content when zinc was supplemented in the diet prior to feeding (Hair-Bejo *et al.*, 1993; Hair-Bejo & Alimon, 1995). The absence of any increase in the copper content in the renal cortex and medulla, bile, and urine in the zinc treated animals in the present study suggest that zinc does not facilitate copper removal through these routes. Excretion of copper through the bile and urine play an important role in reducing excess copper in copper loaded rats (Haywood, 1985).

The elevation of hepatic zinc concentration in the zinc treated animals may be associated with an increased in the induction of metallothionein (MT), a low molecular weight metal binding protein in the organ. Furthermore, the histological changes in the liver of the group was much milder than the other groups. Zinc is a better inducer of MT than copper. However, this protein has a higher affinity for copper than zinc and thus reduces its toxicity to tissue (Mehra &

Bremner, 1984). The ability of sheep to tolerate the potentially toxic effect of copper in PKC (23.42±0.84 μg/g) particularly during the first 16 weeks of the trial is an interesting phenomenon and this is in contrast to previous study, as chronic copper toxicity were exhibited as early as 8 weeks of the feeding trial (Hair-Bejo & Aliomon, 1995). Dietary copper content as low as 10 to 20 μg/g dry matter can cause chronic copper poisoning in sheep (Soli, 1980). However, despite the absence of marked clinical signs and gross lesions of chronic copper toxicity, except for reduction in body weight, moderate hepatic lesions were observed in most sheep fed with PKC up to 32 weeks. This indicate that chronic copper toxicity may occur in the animals if the feeding trial was prolonged.

Chronic copper toxicity in sheep is considered to have three different phases: prehaemolytic, heaemolytic and post-haemolytic phases (Ishmael *et al.,* 1971). During the pre-haemolytic phase copper is continously accumulated in the liver, the target organ of the disease, over a period of weeks or months with absence of clinical signs. However, as the hepatic copper concentration reached levels up to 1000 to 4000 μg/g dry weight (Ishmael *et al.,* 1971; Hair-Bejo *et al,* 1993; Hair-Bejo & Alimon, 1995) severe hepatic necrosis occured. The renal and whole blood copper concentrations increased up to ten fold followed by onset of a haemolytic crisis and death (Ishmael *et al.,* 1972; Hair-Bejo *et al.,* 1993: Hair-Bejo & Alimon, 1995). The copper content in the right liver and renal cortex were much higher than the left liver and medulla respectively (Hair-Bejo *et al.,* 1993; Hair-Bejo & Alimon, 1995). The equal distribution of copper in the right and left liver in the PKC fed animals in the present study may delay the development of hepatic lesions, although the slight elevation of copper content in the blood and renal cortex can be an early indication for the haemolytic crisis.

Variation in the susceptibility to copper intoxication in different breeds of sheep was reported previously. Merino or pure breed of sheep appears to be more resistant to copper than sheep of mixed bred (Marston & Lee, 1948; Wiener & Field, 1970), the Ronaldsay breed, by contrast is extremely copper sensitive (Mac Lachlan & Johnston, 1982). The ability of Malin x Polled Dorset crossbred sheep to tolerate to high copper content in PKC at least during the first 16 weeks of the feeding trial may provide more avenue in the utilization of PKC as a major feed ingredient in the species.

It was concluded that the usage of dietary zinc supplementation (500 μg/g) in the treatment of PKC toxicity in sheep is unsatisfactory. Malin x Polled Dorset crossbred sheep were able to tolerate the potential toxic effect of copper in PKC at least up to 16 weeks of the feeding trial.

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