THE EFFECTS OF BOILED *CROTALARIA OCHROLEUCA* (MAREJEA) SEEDS IN CHICKENS

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SUMMARY

The effects of boiled seeds of *Crotalaria ochroleuca* (Marejea) were studied in 2.5 month-old cockerels. The cockerels were randomly divided into four groups of seven each. Group A individuals were fed Broiler Mash (BM) only while group B, C, and D were fed broiler mash mixed with 10%, 30% and 50% boiled marejea seed meal (BMSM) respectively. The experiment lasted for five weeks. The clinical findings were related to the level of BMSM supplementation; group D individuals were the most severely affected, group B was least severely affected while group A individuals were not affected. The clinical signs observed were emaciation, ruffled feathers, anorexia, anaemia, weakness and diarrhoea at terminal stages. Gross findings were clear fluid effusions in body cavities, enlarged mottled liver, enlarged lungs and generalized congestion and petechial hemorrhages. Others were rudimentary wattles, combs, testes, kidneys and spleen. The hearts were enlarged and roundish; microscopically there were generalized congestion, hemorrhages, oedema and megalocytosis of the heart. Others were interstitial pneumonia, necrosis of the hepatocytes and tubular epithelium of the kidneys and generalized endothelial damage. These results did not differ significantly from the ones observed on feeding Marejea Seed Meal (MSM) before treatment as earlier thought. Therefore it was established that boiling marejea seeds did not affect the toxicity of the seeds. It is therefore recommended that in areas where marejea is grown as fodder, free-range chicken should be confined when potential exposure to marejea seeds is imminent.

INTRODUCTION

Crotalaria is a widespread tropical and subtropical plant with about 600 species. Native species occur in nearly every tropical country, but the greatest number are found in Africa. They are erect annuals or short-lived perennial shrubs with coarse profusely branched stems. The leaves are uni-or-trifoliate and leaflets linear to broad ovate. The plants are generally leafy, with showy, yellow flowers and set seed freely. The seed colour varies from yellow to brown and black, this genus is self fertilizing. The crotalarias are grown throughout the tropics, especially in India, mainly for green manure and soil cover (White, *et al.*, 1953). In 1950s, *Crotalaria ochroleuca* (Marejea) was introduced in Tanzania, Ruvuma region by Peramiho

missionaries and has since then spread almost all over the country. However several unidentified *Crotalaria spp* have been noted growing wild in the Southern highlands of Tanzania. The plant is grown for its fodder values in ruminants and its ability to add nitrogen in the soil (Sarwatt, 1990). It was later on discovered that the plant had some properties of killing weeds (Narayanan and Dabadghao, 1972). Farmers in Tabora, intercrop marejea with maize for the purpose of killing a popular weed known locally as "Nkansimba" (Mugittu, *et al.*, 1996). Marejea also kills pest in the storage house and nematodes in the field (Hughes, *et al.*, 1962). Farmers in Kibondo district, Kigoma, plant marejea after harvest for soil cover, green manure and fodder (Nditiye, D. personal communication, 1996).

Despite actual beneficial values of marejea, farmers around the Peramiho Mission centre accepted the plant with difficulties since they believed that the marejea seeds caused most of the chicken mortalities. *Crotalaria spp* have also been incriminated for chicken mortalities in Australia (Norton and Rowrke, 1979). However through practical demonstration it became easier for the farmers and peasants to accept the fact that marejea was actually beneficial to them despite the toxic effects to the chickens. From then on, the plant gained popularity and spread all over the country.

The widespread use of marejea in improving soil fertility, killing weeds and use as fodder run parallel with the increase in the number of chickens at intoxication risk. Most of the farmers in the rural areas do not restrict their chickens from eating marejea seeds, since they are freely ranged. In most cases, chickens will suffer from a chronic toxicity due to daily ingestion of small amounts of the marejea seeds.

Marejea plant is known to have pyrrolizidine alkaloids (PAS) as the principle factor of toxicity of *Crotalaria* genus (Kellerman, *et al.*, 1988). The genus is found to affect different species of animals including rats, pigs, horses, sheep (Hooper, 1978). Pyrrolizidine alkaloid causes toxic effects in the liver, lungs, kidney and gastrointestinal tract (Hooper, 1978). Also other researchers and investigators have shown the toxic effects of marejea in different animal species (Jago, *et al.*, 1970 cited by Hooper, 1978; Dickinson and Robert, 1986; Peterson, 1972 and Hsu, *et al.*, 1973 cited by Hooper, 1978).

However it had been suggested that soaking in water or heating seeds of marejea reduces toxicity, (White, *et al.*, 1953 and Purseglove, 1972). Therefore the purpose of this project was to investigate the effect of feeding different levels of boiled marejea seed meal to chickens and subsequently recommend on possibility of exploitation of its nutritive potentials in chickens and other animals.

MATERIALS AND METHODS

Marejea seeds (30 kg) were boiled for three hours, sun-dried and ground into flour, which was mixed with broiler mash at different levels to give 10%, 30% and 50% Boiled Marejea Seed Meal (BMSM).

Twenty-eight cockerels were randomly divided into 4 groups, A, B, C and D each with 7 birds (Table 1). In each group, individual birds were assigned numbers; e.g. in group A numbering was A_1, A_2, A_3, A_4, A_5, A_6 and A_7. Corresponding arrangement was applied in other groups.

The groups received similar treatment except on feeding quality. Group A received (BMSM)-free broiler mash, while groups B, C and D received broiler mash mixed with 10%, 30% and 50% (BMSM) respectively.

Table 1: Allocation of chickens in groups and the feeding regime.

Groups	Number of chickens	Feeding regime.
A	7	Broiler mash (BM)
B	7	10% BMSM and 90% BM
C	7	30% BMSM and 70% BM
D	7	50% BMSM and 50% BM

Following random grouping of the cockerels all groups continued feeding on broiler mash for three days, in order to get used to the new environment. Only cockerels were used since they were cheap and easily available considering the limited financial resources.

On the fourth day all cockerels were weighed and started feeding on their respective experimental feed.

The cockerels were weighed after every 7 days and immediately after death during experimental feeding. The cockerels were examined clinically for any abnormal signs or behavioral change. Dead birds were immediately examined grossly and the following organs were taken for histopathology examination: Proventriculus, small and large intestines, spleen, lung, kidney, heart, liver and testes.

At the end of 5 weeks of experimental feeding the cockerels, which were still alive, were sacrificed and examined grossly and microscopically.

RESULTS

Clinical signs

Clinical signs and body weight were monitored and recorded after every seven days. The average body weight of individuals in group A increased progressively. In group B there was average body weight increase in the first week followed by decreasing body weight. Group C individuals had decreasing average body weight and so had group D.

Death intervened in group D individuals from the second week and all birds were dead at the end of the third week of experimental feeding. Group C individuals started dying on the third week and only one individual survived to the end of experiment.

The most important clinical signs were anorexia, emaciation, whitish diarrhoea and pale mucous membranes of combs and wattles. The severity of these clinical signs was dose-dependent. Group A did not manifest the above clinical signs. Group D had severe signs followed by group C and Group B had least severe clinical signs.

Pathological findings

The pathological findings were dose related. The most severely affected group was group D while group A which was fed broiler mash only had no pathological lesions.

Group B (10% BMSM and 90% broiler mash)

The carcasses were emaciated with small, rudimentary and pale combs and wattles. The vents were soiled with whitish feces. The peritoneal cavity had colourless fluids varying in volume from 70 to 110 ml. Livers were slightly enlarged with roundish margin and mottled to grey in colour with dark red spots of hemorrhages. Microscopically the livers were congested and had distended blood vessel with thickened walls. There was hepatocyte enlargement and slight proliferation of bile ducts.

The thoracic cavity had straw coloured fluids. The lungs were edematous and congested and there were dark-blue spots, which were concentrated more in the caudal lobes. Microscopically the lungs exhibited septal oedema, epithelialization, mononuclear cell infiltration, endothelial swelling and thickened arterials. Also there were yellowish to green particles in the parenchyma.

The hearts were roundish, slightly enlarged, flabby with clear pericardial fluid ranging from 1 to 3 ml. Microscopically there were mild congestion and hemorrhages.

The gastrointestinal tracts were full of ingesta. The mucosae were congested with hemorrhagic spots. Microscopically there were mild congestion, hemorrhages and villous atrophy.

The kidneys, spleen and testes were reduced in size and showed mild congestion and hemorrhages.

Group C (30% BMSM and 70% Broiler mash)

The carcasses were emaciated and anaemic with very small dull and pale wattles and combs. The peritoneum and pericardium had variable amounts of fluid ranging from 10-70 ml and 2-10 ml respectively.

The livers were enlarged and mottled with brownish yellow discoloration. The gall bladders were distended with thick yellowish green fluid, which ranged from 2 to 3 ml. Microscopically the liver exhibited severe congestion, hemorrhages, necrosis of hepatocytes, enlarged blood vessels and proliferation of bile ducts.

The lungs were edematous and abnormally firm in consistency. Microscopically there were thickening of blood vessel walls, perivascular hemorrhages and interstitial pneumonia.

The intestines had thick and dirty mucoid contents and congested mucosa. Microscopically there were mucosal hemorrhages, congestion, endothelial swelling, necrosis of enterocytes and glandular epithelium. There were also marked villous atrophy.

The kidney, testes and spleen were

very small in size (rudimentary) and on microscopic examination they exhibited congestion, hemorrhages and vascular damage which was also observed in the hearts. The renal tubular cells showed a degree of necrosis.

Group D (50% BMSM and 50% Broiler mash)

The carcasses were severely emaciated with enlarged, dark-brown livers. The peritoneal fluid, which was frothy, measured from 5 to 10 ml. The pericardial fluid was clear and measured up to 2 ml. The gastrointestinal tracts were completely empty. The microscopic lesions were similar to but more severe than those in group C.

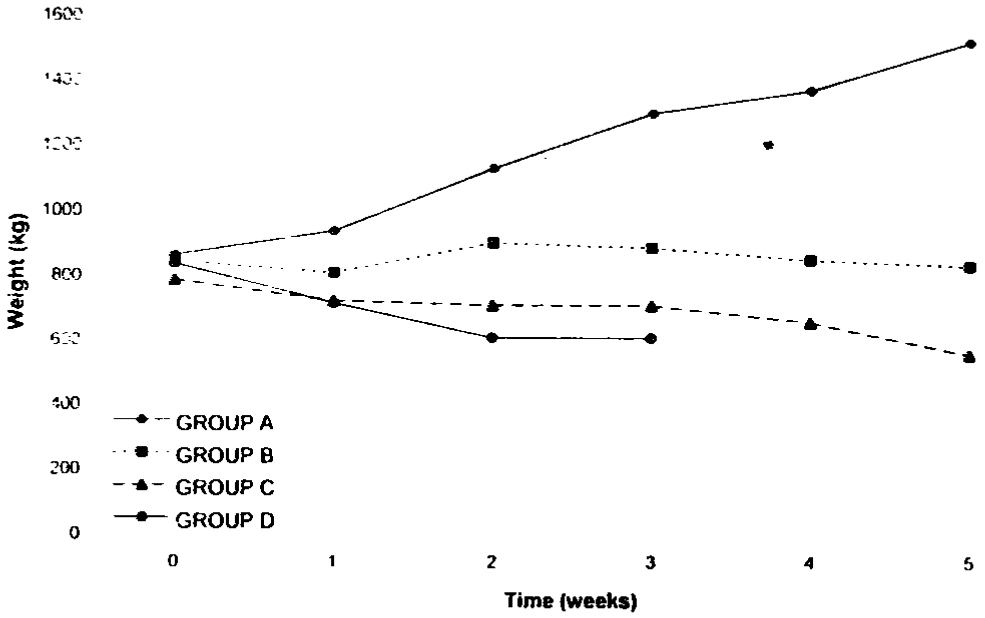

Fig. 1: Body weight changes during experimental feeding

DISCUSSION

Group A (100% broiler mash)

This was a control group, it did not manifest any significant abnormal clinical signs or pathological lesions. In this group there was progressive increase in body weight to the end of experimental feeding, Fig., 1.

Group B (10% BMSM)

In this group individuals exhibited increase in body weight in the first week followed by progressive decrease of body weight up to the fifth week, Fig 1. They had an average death weight of 786 grammes. Initial increase in the body weight could be due to that the BMSM supplement level of 10% was small such that the palatability of feed did not

change much, so the birds could feed normally with effects of poisoning being minimal. The loss of body weight observed at the end of the second week onwards could be due to the effect of marejea poisoning and decreased appetite. The chickens continued eating up to the fifth week.

Group C (30% BMSM)

This group exhibited clinical signs and pathological findings similar to but slightly less severe than in group D. The average loss of body weight was high in the first week, then it progressively decreased in the second week and then increased again in the third week when death intervened; the average death weight was 557 grammes. The initial high rate of weight loss was possibly due to loss of feed interest while at the terminal stage the high rate was probably due to marejea poisoning, Fig. 1. Feather ruffling started at the end of the first week. In the second week palor mucous membrane, wattles and combs became marked and the cockerels became dull and inactive.

The combs and wattles were pale with reduced size, the mucous membranes were also pale. The rudimentary combs and wattles were a result of starvation. The ruffling of feathers was evident on the fourth week and during this time whitish diarrhoea set in.

At postmortem the birds were emaciated and anaemic possibly due to poisoning. There was diarrhoea and anorexia at terminal stages. The gastroinstestinal tracts were full of ingesta and mucus. The intestines were congested with some petechial hemorrhages. On microscopy there were mild congestion and hemorrhages with mild enterocytes swelling and villous atrophy. These were caused by the damage effect of PAS and its metabolites and also due to enteroblasts mitotic inhibition. Bull, *et al.*, 1968 (cited by Hooper, 1978), also observed these findings.

The livers were slightly enlarged because of congestion and megalocytosis, the architecture of the liver was intact. The main findings included congestion and hemorrhages that were characteristic of initial changes caused by endothelial damage.

The group exhibited large amounts of peritoneal fluids. Also there was fluid in the air sac and the pericardium because the chickens survived longer for the lesion to advance. These findings were also observed in rats fed on *Crotalaria ochroleuca* by Mkiwa, (1990).

The hearts were roundish possibly because of anaemia and exhibited mild congestion and hemorrhages. The kidneys, spleen and testes were reduced in size and showed mild congestion and hemorrhages due to endothelial damage. Peckham, (1974) also observed these changes in *Crotalaria spp* poisoning in pigs, horses and sheep. Apart from one individual, which died at the fourth week others, persisted to the end of the experiment.

Group D (50% BMSM)

This group received 50% BMSM and it exhibited rapid loss of body weight of 100 grammes average in the first week and 90 grammes in the second week, followed by death during the third week at the average weight of 479 grammes. Rapid loss of body weight was due to marejea poisoning and partly due to starvation because the birds had

marked anorexia from the first week, probably because of high levels of BMSM which caused acute toxicity. Other features included ruffled feathers and emaciation. Similar findings have been reported by Mkiwa, *et al.*, (1994) in rats poisoned by *Crotalaria*.

The mucous membranes, were severely pale while the wattles and combs were equally pale and reduced in size. This indicated severe anaemia which could be due to starvation and/or damage to reticuloendothelial organs, failure of incorporation of iron in red blood cells (Swick, *et al*, 1982) and severe hemorrhages caused by endothelial damage. Thomas, 1934 (cited by Hooper, 1978), observed anaemia in *Crotalari spectabilis* poisoning in chickens. There was also whitish diarrhoea at terminal stages, which was probably caused by observed intestinal mucosal damage and villous atrophy. Cockburn, 1955 (cited by Clarke and Clarke 1975) observed anorexia, depression, emaciation and rough hair coat in *Senecio spp* poisoning due to PAS.

Grossly the birds were emaciated and anaemic. There was fluid effusion in the peritoneum, air sac and pericardium. This was a result of endothelial damage observed in the livers, intestines, lungs, mycocardia and pericardia. The amount of the fluid was small compared to that in groups C and B. This was due to acute poisoning such that the birds did not live longer for the accumulation of fluid in body cavities. These effusions were also observed by Mkiwa, (1990) in rats fed on *Crotalaria ochroleuca*. Diffuse hepatocyte necrosis, endothelial swelling and hermorrahages was also observed. Necrosis was so severe that normal architecture of the liver could not be appreciated; these findings were also reported by Bull, *et al.*, 1968 (cited by Hooper 1978) when reporting that PAS had higher predilection for pulmonary blood vessel endothelium. Mkiwa, *et al.*, (1994) found endothelial swelling in lungs and also interstitial pneumonia in rats fed on *Crotalaria ochroleuca*.

Intestines were empty because of anorexia and there was congestion and haemorrages possibly due to endothelial damage. On microscopic examination, there was marked villous atrophy caused by PAS and its metabolites. Bull, *et al.*, 1968, (cited by Hooper, 1978) demonstrated severe ulceration and mitotic inhibition of enteroblasts in rats and sheep given purified alkaloids.

Kidneys were reduced in size and microscopically exhibited congestion, haemorrahage and tubular necrosis. Peckham, *et al.*, (1974), described megalocytosis in epithelial cells of proximal tubules and thick Loops of Henle in *Crotalaria retusa* poisoning in pigs and horses, and by *Senecio jacobeae* poisoning in sheep and mice.

The hearts were roundish and the spleens reduced due to anaemia. Both organs exhibited congestion and hemorrhages. Reduced size of spleen could also be as a result of starvation. Mkiwa, *et al.*, (1994), reported endothelial swelling in the lungs, liver, intestines and myocardium together with interstitial pneumonia.

Testes were small in size compared to those in-group A (control) and other groups because of high supplementation with BMSM. This

reduced size could be due to starvation and PAS poisoning since these compounds have been found to be antimitotic by Peterson, *et al.*, (1972).

In conclusion it is observed that recent studies on *Crotalaria ochroleuca* poisoning in chickens (Mugittu, *et al.*, 1996), showed that the levels of 10% of marejea seed meal, were detrimental such that the birds could not survive beyond 7 weeks on that supplementation level.

This study, using boiled marejea seed meal showed no significant difference in clinical signs, pathological findings, onset of clinical signs and deaths. This shows that there was no significant reduction of toxicity level in the seeds by boiling.

To-date there is no effective method of detoxifying PAS in *Crotalaria spp.* Therefore in order to utilize the valuable potentials of the plant in agriculture and at the same time maintaining chicken industry especially in rural areas, there must be confinement of chickens prior to the ripening of pods and throughout the harvesting, sowing and germination season while at the same time efforts of treating marejea seeds are being researched on, for example, through chemical treatment and breeding strains of *Crotalaria spp* which are less toxic.

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