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Effect of Open Fire Roasting on Reducing Tannin Content in Soybean (*Glycine max*), Cowpea (*Vigna unguiculata*) and Pigeon Pea (*Cajanus cajan*)

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Abstract:

The effect of roasting in reducing anti-nutritional factors (ANF), in legume grains (pigeon pea (PP), cowpea (CoP) and soybean (SB) as source of proteins for growing rabbits was evaluated. Flemish Giant (FG), California Black (CB) and New Zealand White (NZW)) were fed soybean (Glycine max), pigeon pea (Cajanus cajan) and cowpea (Vigna unguiculata) as protein sources in the ration. Eighteen rabbits weaned at six weeks of age were used for each breed (a total of 54 rabbits) out of which six of them were assigned to Ration 1 (containing 31.6 % soybean), six rabbits to ration 2 (containing 72 % cowpea) and the last six to Ration 3 (containing 70.18 % pigeon pea). The rabbits were randomly assigned to fifty four cages in a 3 x 3 factorial experiment. The three legume grains were roasted to a uniform light brown colour using ordinary open fire, before rations were compounded. Raw Legume Grains (RaLG), Roasted Legume Grains(RoLG) and the three Rations (1, 2 and 3) were analyzed for tannin content (TC). TC was significantly ($p<0.05$) higher in raw and roasted CoP and Ration 3. Rabbits fed SB based ration had significantly ($p<0.05$) higher growth rate than rabbits fed either CoP or PP based rations. Mortality was higher for rabbits fed on CoP based ration. Rabbits offered PP based ration demonstrated a significantly

($p<0.05$) higher growth rate than rabbits consuming the CoP based ration. The study revealed that the concentration of tannins per unit mass increases when roasted because of loss of moisture. The study also revealed that open fire roasting used in this study was less effective in reducing tannin content in CoP.

Key Words: Rabbit; Legume grains; Tannin, Roasting, Trypsin inhibitor, Feed Intake

Introduction

Grain legumes (except for soybean) are potential sources of energy and amino acids for rabbits, but their use is still limited because of uncertainty about the amount and effect of any anti-nutritional factors (ANF) that they may contain. The most commonly found ANFs in legumes are protease (trypsin and chymotrypsin) inhibitors, tannins, lectins, amylase inhibitors, glycosides, phytate and alkaloids. An increasing human demand for protein in developing countries and a relatively high cost of imported ingredients has turned the attention of animal nutritionists to the exploitation of non-conventional ingredients and by-products which these regions have in abundance. Other grain legumes are potential substitutes for soybean meal because of the similarity of their amino acid profiles. Other legume grains could totally replace soybean meal without adversely affecting weight gain provided suitable processing methods are established. The ingredients included will vary between countries and between districts within countries depending on the potential availability of the ingredients for particular livestock species. Traditionally, maize and soybean are used as primary ingredients in most countries. However, the potential of other legume grains in rabbit rations has not been investigated and little is known about their effectiveness relative to soybean (Cheeke 1987). McNitt (2000) reported that many problems still remain unsolved in rabbit meat production and less information is available on optimal feeding, breeding, disease prevention and management systems. Concentrates especially of leguminous origin are high in crude protein and gross energy when compared to non-

legume forages such as *Bidens pilosa* and *Pennisetum purpureum* which are commonly fed to rabbits (Fielding 1991).

The purpose of the study was to evaluate the effectiveness of open fire roasting of soybean, pigeon pea and cowpea as an alternative method of reducing anti-nutritional factors. The study was specifically designed to:

1. To evaluate tannin levels before and after roasting in pigeon pea, cowpea and soybean as single sources of protein for growing rabbits.

Materials and Methods

Study site

The study was conducted at Bunda College of Agriculture in Malawi from February 2001 to February 2002. The study area was located between 14 º 11‘ S latitude and 33 º 46‘ E longitude. The area lay 1100 m above sea level.

Animals and diets

Three pure bred rabbits, *Oryctolagus cunniculus* (Flemish Giant, California Black and New Zealand White) were used in the study. Twenty four does and six bucks were estimated to breed the 54 weaners (18 of each breed) required for the experiment. The kits were weaned at 6 weeks of age. The 54 weaners with an average weight of 0.761 kg selected for fattening were identified using ear tattoos. The difference in age between the youngest and the oldest kits was 2 weeks. The average litter size was four kits per doe. The 54 weaners (18 of each breed) were offered the test rations namely: soybean, pigeon pea and cowpea. The three test rations of soybean, pigeon peas and cowpeas were identified with numbers 1, 2 and 3 respectively. Six weaners of each breed were randomly allocated to each of the three rations by picking lots. Soybean (*Glycine max*), Cowpea (*Vigna unguiculata*), Pigeon pea (*Cajanus cajan*), Maize (*Zea mays*) and Madea (*Zea mays*) were used as the major feed ingredients for this experiment. Ocepara, V418 and Royes were the varieties of Soybean, Cowpea and Pigeon pea used in the study, respectively. The major protein sources in the rations were roasted to a

consistent brown colour using open fire prior to grinding and mixing to reduce trypsin inhibitors. The tannin content in raw and roasted Soybean, Cowpea and Pigeon pea as well as in rations containing SB, PP and CoP was determined by the laboratory procedure developed by Kalade et al. (1974). The rations were formulated to meet the basic energy and nutrient requirements of growing rabbits (NRC, 1996). The rations were formulated to contain 17 % CP and 2500 kcal/kg using the Linear Programme with Bounds (BLP88) computer package (1987).

Feeding Trial

Rabbits were weighed at the start of the feeding trial. The average weight for the 54 kits was 0.761 kg. The weaners were fed on the test feeds for 7 days so that they could adapt to the new feeds before data collection started. Each rabbit was weighed at the end of the adaptation period and this was taken as the initial weight for the feeding period of 84 days. The three grower rations were replicated twice in the experiment. The rabbits were offered 100g of feed once daily during morning hours (07.00 hours) over a 24-hour period and the rejected feed was weighed the following morning (07.00 hours). The difference was taken as the feed consumed by each rabbit per day. Rabbits were weighed individually weekly (every Friday) to obtain weekly body weight from which average daily weight gain and weekly body weight gain were calculated. Weekly body weights were recorded until the rabbits reached the targeted average weight of 2.5 kg. Clean water and feed were provided everyday using drinkers and feeders mounted in each cage. Each rabbit was offered 7 g of *Leucaena* leaf meal (LLM) as a source of fiber twice a week.

Statistical Analysis

Data were analysed using the General Linear Model (GLM) procedures of Statistical Analysis System (SAS 1989). Treatment means within and between breeds were tested for differences using Least Significant Means (Montgomery 1984).

Results

Average Daily Weight gains for rabbits fed rations containing soybean or pigeon pea were significantly higher ($p<0.05$) than the rabbits fed a ration containing cowpea. The average daily weight gains for rabbits fed rations containing soybean, pigeon pea and cowpea over the 12 week experimental period were; 15.59 g/day (S.E=1.02), 14.33 g/day (S.E=1.01) and 10.69 g/day (S.E=0.98), respectively (Table 1). Across rations, average body weight gain for Flemish Giant was significantly ($p<0.05$) higher than the average body weight gain of either the New Zealand White or the California Black (Table 1). The Flemish Giant, New Zealand White and California Black had average daily weight gains of 14.99 g/day (S.E=1.01), 12.98 g/day (S.E=1.02) and 12.71 g/day (S.E=1.03), respectively. Across breeds, average body weight gain of rabbits fed a ration containing 31.2 % soybean was not significantly different from that of rabbits fed a ration containing 70.18 % pigeon pea but both had significantly ($p<0.05$) higher average daily weight gain than rabbits fed a ration containing 72.00 % cowpea (Table 1).

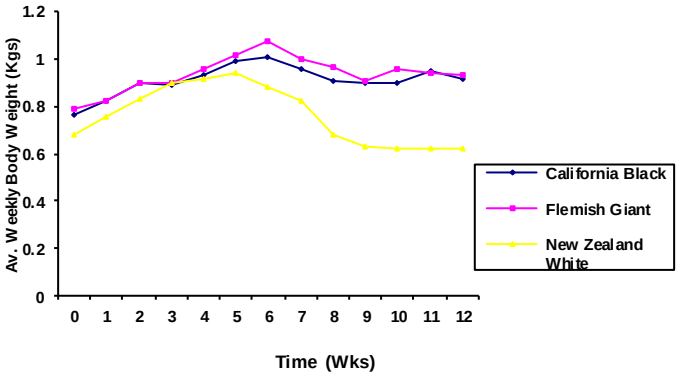

Figure 1: Response of three rabbit breeds to Cowpea ration

Tannin Levels in Legume Grains

The results of tannin content of raw, roasted legume grains and rations of the three legume grains (soybean, cowpea and pigeon pea) are shown in Table 1. Cowpea had significantly ($p<0.05$) higher tannin content in all the three samples (raw, roasted and rations). The analysis revealed that tannin content was higher in roasted samples than in raw samples for all the three legume grains.

Table 1. Means for Tannin Content (%) in Soya bean, Pigeon pea, Cowpea and Rations 1, 2, 3

Source of Variation	n	LSmeans	Stderr
Raw			
Soybean	2	.327[c]	.005
Pigeon pea	2	.408[b]	.003
Cowpea	2	1.129[a]	.001
Roasted			
Soybean	2	.719[b]	.008
Pigeon pea	2	.779[b]	.005
Cowpea	2	1.794[a]	.002
Ration			
Soybean 1[d]	2	0.134[b]	.003
Pigeon pea 2[e]	2	0.157[b]	.003
Cowpea 3[f]	2	0.573[a]	.001

[a b c]Means with different superscripts are significantly different ($p<0.05$)
1[d] contains 31.2% soybean n=number of replications
2[e] contains 70.18% pigeon peas CV=1.13 %
3[f] contains 72.00% cowpeas

The highest post-weaning mortality was observed in rabbits fed cowpea-based ration. Rabbits fed cowpea-based ration were found to have pale, pathologically enlarged kidneys during post-mortem and carcass analysis (Plates 1, 2). Rabbits fed cowpea-based ration were also found with haemorrhagic intestines and pale livers during post-mortem.

Plate 1. Pathologically Enlarged Kidneys (k) from a Rabbit Fed Cowpea

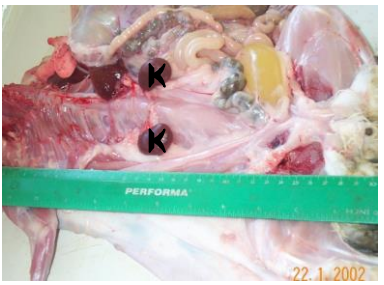

Plate 2. Normal Kidneys (k) from a Rabbit Fed Soybean/Pigeon pea

Discussion

Growth in rabbits was more pronounced during the first 70 days (ranging from 0.30 to 2.5 kg body weight) post-weaning (Fortun-Lamothe and Gidenne, 2000). In general weight gain was significantly higher ($p<0.05$) for rabbits that were fed soybean. This trend is indicative of the influence of feed type on growth of rabbits. Average daily weight gains for the breeds observed in the present study (11-16 g/day) are in agreement with results reported by Fortun-Lamothe and Gidenne (2000) who indicated that average daily weight gains ranged from 13.2 g/day to 38.9 g/day for growing rabbits (26 to 70 days of age) depending on source of protein. In the present study, when evaluated across breeds, rabbits fed, soybean had a significantly ($p<0.05$) higher growth rate (15.59 g/day; S.E=1.02) than rabbits fed either cowpea or pigeon pea. Rabbits offered the pigeon pea ration demonstrated a significantly higher average daily weight gain (14.33 g/day; S.E=1.01) ($p<0.05$) than rabbits consuming the cowpea ration (10.69 g/day; S.E=0.98) across breeds. These differences in the effectiveness of the three types of legume grains on weight gain, is a result of differences in trypsin inhibitor activity, available lysine and tannin content. Trypsin inhibitor activity and tannin content were lower in soybean, making protease enzymes more effective. Available lysine, the most limiting amino acid was higher in soybean than in either cowpea or pigeon pea.

Tannins are naturally occurring plant polyphenols that can have a large influence on the nutritive value of legume grains and forage legumes. Tannins are defined as water-soluble polymeric phenolics that precipitate proteins. Tannins exist in mixtures with many other classes of plant phenolic compounds such as lectins (Reed, 1995). Tannins may reduce intake by decreasing palatability or by negatively affecting digestion. Tannins were reported by Waghorn et al. (1994) to lower protein degradation. In the present study the negative effect of tannins on growth rate was caused by a combination of reduced feed intake and low digestibility of proteins.

Feed efficiency was highly influenced by feed type. When evaluated across breeds, rabbits fed soybean ration were observed to have the lowest feed to gain ratio (highest growth rate). Rabbits fed cowpea ration had the highest feed to gain ratio (lowest growth rate) while those fed pigeon pea ration had an intermediate feed to gain ratio.

Barry and McNabb (2001) reported that the quantity of tannins (60-150 g/kg DM) found in most legume grains is too high for rabbit nutrition. The rabbit is the most sensitive domestic animal to toxicity. While chickens can tolerate up to 2.6 g/kg tannin content, young rabbits can tolerate toxic levels only up to 0.3 mg/kg body weight, one of the lowest toxic levels of any species studied (Clark et al. 1992). The concentration of tannins detected in the ration containing cowpea (0.55 mg/kg or 0.55 %) was high enough to cause pathophysiological changes observed in this study. Results observed in this study support results reported by Filippich, Zhu and Alsalmi, (1991) who reported that the major lesions associated with tannin poisoning were; haemorrhagic gastroenteritis, necrosis of the liver and kidney damage with proximal tubular necrosis resulting in death. The poor weight gains observed in rabbits offered cowpea ration can be explained in terms of anti-nutritional factors (ANFs). The difference in effectiveness among the three rations was manifested in the levels of trypsin inhibitor and tannins in the respective legume grains used as sources of proteins. Heat treatment was least effective in reducing trypsin inhibitor in cowpea and most effective in soybean, while it was satisfactorily effective in pigeon pea. Roasted soybean had the lowest trypsin inhibitor (5.87 %),

roasted pigeon pea had an intermediate value (20.40 %). Cowpea had the highest trypsin inhibitor (34.56 %). This implies that soybean had the highest (94.13 %) trypsin activity, pigeon pea had intermediate (79.60 %) trypsin activity and cowpea had the lowest (65.44 %) trypsin activity. Consequently, rabbits fed soybean had the highest weight gain because the proteins were easily degradable. Makinde et al. (1998) reported that cowpea feeding in pigs resulted in pathophysiological changes in gut morphology leading to impaired absorption of nutrients. These researchers further reported that ANFs in cowpeas could account for the observed changes in gut morphology and faecal parameters. The decrease in growth may have been due to reduced digestive and absorptive capacities as a result of intestinal mucosal changes. It is postulated that some diseases such as enteritis in rabbits are initiated by dietary components, such as lectins, tannins or allergens, which induce diarrhoea and consequently create the favourable environment in the caecum for secondary opportunistic bacterial involvement (McLeod and Katz 1996). Nutritional factors can affect animals indirectly in a number of ways by, for instance creating conditions which favour commensalistic organisms e.g. Clostridium perfringens type D, the cause of pulpy kidney in sheep. Nutritional stress can also induce pathological lesions of the gastro-intestinal tract (GIT) which are ideal sites for bacterial infiltration and colonization (McLeod and Katz 1996).

Rabbits fed cowpea ration were found with pathophysiological changes in the gastrointestinal tract (GIT), pale and enlarged kidneys (hypertrophy of kidneys), haemorrhagic intestines and pale livers. The average weight of normal kidneys from rabbits fed soybean and pigeon pea was 5.24 g, while pathologically enlarged kidneys from rabbits fed cowpeas had an average weight of 20.61 g. The average length and width of normal kidneys from rabbits fed soybean and pigeon pea were 3.10 cm and 1.00 cm, respectively. Pathologically enlarged kidneys measured 6.5cm in length and 2.00 cm in width. Three of the five rabbits from cowpea ration were diagnosed with *coccidia* and *pasteurella* protozoa in the GIT and liver. All the five rabbits from cowpea ration which later died gradually deteriorated in health especially from week

six of the feeding trial. Observations showed reduced feed intake and lost body weight before death. Hypertrophy of kidneys and pale livers were evident in all rabbits fed cowpea during carcass analysis.

Conclusion

The results observed in this study indicate that the open fire roasting of legume grains used in this study was unable to reduce tannin levels in cowpea grains. The study further revealed that cowpea contains more tannins than either soybean or pigeon pea. The study also indicated superiority of soybean over pigeon pea and cowpea as sole sources of protein in rabbit rations under similar heat treatment. The study further demonstrated that pigeon pea was superior to cowpea as a sole source of protein in rabbit rations. The study has also indicated that even though soybean has been observed to be more effective than pigeon pea and cowpea as a protein supplement, pigeon pea can replace soybean without adversely affecting the performance of rabbits. The results of this study suggest that the variety of cowpea used in these experiments is not suitable as a sole source of protein in rabbit rations.

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