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Haemato-biochemistry of Albino Rats Fed African Kudzu (*Pueraria phaseoloides* Roxb. Benth) Seed Diet

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Target audience: Nutritional biochemists, Animal breeders, Feed formulators

Abstract

The effects of cooked and acid-extracted kudzu seed meals on some nutritional and blood and liver biochemical parameters were investigated. Rats fed cooked kudzu meal had significantly ($p<0.01$) slower rates of growth than the casein control. Cooking enhanced feed efficiency (FE) but the acid-extracted meal could not support growth resulting in negative protein efficiency ratio (PER). Serum and liver protein in kudzu-fed rats were non-significantly lower than the control ($P<0.05$). The kudzu meals exerted significant ($P<0.05$) hyperglycemic effect relative to casein. Serum and liver total lipids and cholesterol were non-significantly elevated relative to the controls. Alterations in the haematocrit were not significant, but the significantly ($p<0.01$) lower white blood cell (WBC) in kudzu-fed rats was inexplicable. The possible reasons and nutritional implications of these observations were briefly highlighted. It was concluded that cooked kudzu meal, with adequate amino acid supplementation, could possibly be well tolerated as protein supplement in food and animal feeding.

Key words: African kudzu, nutritional and biochemical evaluation, blood and liver parameters, albino rats

Description of Problem

Faced with protein malnutrition caused by acute food shortages, researchers are subjecting some wild underexploited crops to chemical analysis and indications are that quite a number of them are highly nutritious (1,2). It may well be that such sources of protein will provide large quantities of animal feed in the future. This could both reduce the cost of livestock products for human consumption and spare for human use protein feed that could otherwise have been consumed by livestock. However, many plants are capable of synthesizing a wide variety of chemical substances, which, under practical circumstances, can impair some aspects of animal metabolism when ingested by man or animals (3). Therefore, prior to the utilization of such novel sources, either in human food or feed, thorough toxicological evaluation of possible biochemical, haematological or epidemiological response to their ingestion is necessary (4).

Studies have shown that malnutrition in the form of specific nutrient deficiency results in general stunting and reduced organ size and marked changes in some biochemical parameters. Also ingestion of certain toxic factors or chemicals

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have manifested in distorted haematology-biochemical parameters. However, Dong *et al.* (5) reported that lower weight gain in rats fed poor sources of protein was mainly due to essential amino acid deficiency and/or lower *in vivo* apparent protein digestibility and that subsequent supplementation of protein with limiting amino acids restores normal growth response in the animals. Kudzu (*P. phaseoloides*) is a common wild forage legume widely used as green manure and in soil conservation (6). It is a good source of leaf protein concentrate (7) and the tubers give starches (8). Our previous study (9) indicated a crude protein content of 28.10 g/100g and a fair complement of essential amino acids (EAAs) marginally limiting in lysine. However, while the raw kudzu meal proved toxic, resulting in the death of experimental animals, animals fed the cooked and acid-extracted meals recorded severe retardation in weight gain. But whether the body biochemical composition was altered was an unanswered question. Therefore, it was thought to be of interest to further investigate the effects of ingestion of kudzu meal on the nutritional and biochemical parameters in rats.

Material and Methods

The collection, processing, formulation of diets and feeding protocol were carried out as previously described (9). At the end of the 21 days experimental period, the rats were weighed, anaesthetized with chloroform and dissected. Blood was collected from the heart by cardiac puncture using a syringe and needle and deposited in heparinised tubes. Hematocrit was determined immediately by standard methods (10). The whole blood were kept in the refrigerator and later centrifuged and separated at 3,000g for 10 minutes to obtain a clear serum. The livers were excised, weighed and homogenized with 5ml. phosphate buffer (pH 7.5) using a hand homogenizer. Serum and liver proteins, lipids, cholesterol and total sugars were determined according to the methods outlined by Lynch *et al.* (10)

All analysis was done in duplicate. Treatment means were compared using the LSD test (11).

Results and Discussion

Nutritional indices, presented in Table 1, followed the same trend as obtained in the previous study (9). However, rats on the cooked meal recorded relatively better indices in this study. This may probably be due to the use of younger growing rats in this study. The HCl-extracted meal recorded significantly reduced weight gain ($P<0.05$). Kawatara *et al.* (12) have reported similar low growth in rats fed acid-extracted meals. Cooking enhanced feed efficiency (FE) better than acid extraction. Palatability and feed consumption seems to be affected negatively by acid treatment.

Serum and liver biochemical parameters are shown in Table 2. Rats placed on the casein control diet recorded non-significantly higher total serum and liver proteins relative to those on the acid-extracted and cooked meals ($P<0.05$). The acid-extracted diets recorded the lowest serum protein compared to both the cooked and the control diets. Liver protein values showed same trend as serum proteins, being related physiologically. However, lower serum total protein as observed for the test diets confirms the inferior quality of kudzu protein relative to casein. Amino acid profile of kudzu showed lysine as the only limiting amino acid (9). Reduction in plasma total protein is regarded as the most important biochemical change in protein malnutrition in man (13) and rats (14) and thus a good index of the quality of dietary proteins. On the other hand, it is possible adequate protein is taken in the test diet, but absorption from the alimentary tract may be defective. Loss of protein in the urine, into alimentary tract and increased catabolism of proteins may also be responsible for low serum and liver proteins (15). Blood sugar levels were elevated significantly ($p<0.05$) in rats fed both test meals relative to casein. Elevated blood sugar may be due to inhibition of glycolysis by the presence of glycoproteins and saponins which may have some adverse effects on regulation of insulin from pancreatic B-cells and on blood sugar, as suggested by Mandal *et al.* (16).

Serum and liver total lipids were elevated but not significantly ($p<0.01$) in rats fed the kudzu meals compared to casein (Table 2). Serum lipids were highest in rats on acid-extracted meal and

Table 1 Nutritional Indices of Kudzu-fed Rats

	Casein	Cooked	HCl-extracted
Weight gain, g/day	0.84±0.08[a]	0.34±0.13[b]	-0.22±0.07[c]
Food consumption, g/day	3.41±0.22[a]	2.96±0.25[b]	2.76±0.02[b]
Feed Efficiency	2.46±0.3[a]	1.12±0.4[b]	-0.14±0.10[c]
Protein Efficiency Ratio	2.47±0.08[a]	1.12±0.40[b]	-0.81 ±0.48[c]

Mean±SD (Standard Deviation)
abc (Means not followed by the same superscript on the same row are significantly different (P<0.05)

Table 2. Effect of Kudzu Seed Meal on Biochemical Parameters of the Liver and Blood

	Blood (mg/100ml)				Liver (mg/g)		
Diet	Sugar	Protein	Lipid	Cholesterol	Protein	Lipids	Cholesterol
Casein	**37.58±3.95[a]**	**4.20 ±0.38[a]**	**374.48±61.17[a]**	**55.23±2.7[a]**	**41.23±10.33[a]**	**268.10±22.64[a]**	**6.10±5.13[a]**
Cooked	51.51±13.31[a]	3.42±0.78[a]	391.54±13.60[a]	105.12±17.52[b]	35.78±5.40[a]	282.87±46.36[a]	7.40±1.18[b]
HCl-extracted	48.96±8.85[a]	2.95±0.69[a]	396.28±17.47[a]	125.95±9.23[b]	32.37±3.13[a]	292.00±75.8[a]	6.34±2.02[c]

Mean±SD (Standard Deviation)
abc (Means not followed by the same superscript on the same column are significantly different (P<0.05))

Table 3. Effect of Kudzu Seed Meal on Haematological Parameters

Hb g/100ml	RBC x $10^6 mm^{-3}$	WBC x$10^3 mm^{-3}$	PCV %	
Casein	14.95±0.94[a]	5.97±0.64[a]	10332±883.95[a]	40.0±6.38[a]
Cooked	12.6±3.00[a]	6.41±1.71[a]	5037.5±577.89[b]	33.5±3.70[b]
HCl-extracted	11.48±2.28[b]	5.76±1.00[a]	4612.5±593.54[b]	28.25±10.28[c]

Mean±SD (Standard Deviation)
abc: Means not followed by the same superscript on the same column are significantly different (P<0.05)
Hb: Haemoglobin, RBC: Red blood cell, WBC: White blood cell, PCV: Packed cell volume

least in those on the casein diet. However, the serum and liver cholesterol of rats on the kudzu meals were elevated, but only significantly ($p<0.01$) for blood cholesterol, compared to controls and this may have some physiological significance (17). This observation, however, is in conflict with the reported hypocholesterolaemic effect of plant protein compared to casein diet (18). Plants have been reported to have lipid-lowering action while animal proteins have some lipid elevating action (19). But findings by Leelamma *et al.* (20) have shown that the effect of dietary protein on lipid levels depends upon the nature of particular protein rather than its source. This may be related to the amino acid composition and digestibility of different proteins and also the experimental animal used (21). Since undigested protein is able to bind bile acids reabsorption of bile acids in the intestine is reduced (22). Recycling of bile acid is thus diminished and consequently synthesis of bile acids from cholesterol is stimulated which results in lower levels of serum cholesterol (23). Also studies in experimental animals have demonstrated that presence of certain plant fibers in the diet is accompanied by significant lowering of serum and tissue cholesterol levels (24). Dietary fiber adsorbs bile salts and thus is excreted (25, 26). Recycling of bile acid is diminished resulting again in the lowering of serum cholesterol levels.

On the other hand the results of this study seem to agree with Kuyvenhoven *et al* (27), who reported that protein digestibility may not be an important determinant of serum cholesterol. While Balogun *et al.* (28) reported significantly higher levels of serum lipids in rats fed plant proteins as compared with rats fed similar diets containing casein as the only protein source, other workers observed no consistent differences in serum lipids in rats fed diets containing either plant or animal proteins (5, 19, 29). Based on the inferior true digestibility (TD) values and fiber content of the test meals earlier reported (9), hypocholesterolemic effect should be expected. Similarly, Yadav *et al.* (30) observed that, some plant proteins exhibited hypoglycemic and hypocholesterolemic effects, while some others had the opposite effects.

Effects of kudzu diets on haematological parameters are presented in Table 3. There is no consistent pattern in haematocrit trend, though non-significant decreases in packed cell volume (PCV) and haemoglobin (Hb) were exerted by the test diets ($P<0.05$). The protein quality of the test diets probably exerts direct influence on both enzyme activities and concentration of factors and precursors regulating these blood variables. However, the significant decrease in white blood cell (WBC) ($p<0.05$) for the test diets is inexplicable and possibly not a physiological response to toxicants (31).

Neither the control nor the treated animals showed any sign of behavioral abnormality, side effect or any toxic reaction throughout the experimental period. It seems therefore that the cooked kudzu meals exerted little or no negative effect on the dynamic equilibrium of the blood and liver metabolites. The negative effect on weight gain may therefore be due to amino acid imbalance and not a result of any toxic assault. Normal growth rate would probably be restored in younger growing rats by amino acid supplementation (5). Further investigation to ascertain this possibility is continuing.

Conclusions and Applications

1. Cooked kudzu meal is well tolerated by experimental rats and thus could be fed to livestock.
2. In view of the high protein kudzu meal could possibly be combined with cereals to yield products of good nutritional value. Kudzu can thus be utilized as a protein supplement in food and animal feeding.
3. Further research on the possible improvement of kudzu meal by amino acid supplementation is continuing.

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