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Full Length Research Paper

Effect of dietary substitution with solvent extracted neem seed cake on growth and nitrogen metabolism of albino rats (wistar strain)

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A study was conducted with 24 albino rats (wistar strain) ages 3 weeks to determine the effect of substitution of diets with processed neem seed cake on growth, performance and nitrogen metabolism of rats. Group 1 and those of groups II and III were fed experimental diets containing water, 75% methanol and 75% ethanol processed neem seed cake, respectively. Two groups were given standard and commercial diet (Pfizer Nigeria Limited). The diets were iso-nitrogenous and iso-caloric. The feeding experiment was conducted for two weeks (7days preliminary and 7days collection). Except for methanol neem seed cake (MNSC diet), there was significant ($p<0.05$) decrease in daily weight gain, protein efficiency ratio and feed efficiency ratio for all animals fed with processed neem seed cakes compared to standard protein. Nitrogen intake and urinary nitrogen excretion of processed neem seed cakes was significantly ($p<0.05$) lower than that of standard, but there was no difference in faeceal nitrogen excretion. Nutritional indices (true nitrogen digestibility, biological value and protein utilization) for all the processed neem seed cakes were comparable with standard diet.

Key words: Neem seed, processed cake, substitution, solvent extraction, nitrogen metabolism, albino rats.

INTRODUCTION

Animal protein in most developing world including Nigeria is very low. The increased cost of feed ingredients, which constitute about 70% of animal production cost, has led to increase in the cost of animal protein. This has reduced protein intake especially among the vulnerable groups of the developing countries (FAO, 1996; Igoeli, 2000) hence reduction in investment by incorporating hitherto wasted and under utilized agro-industry unconventional stuffs need to be examined.

Neem (*Azadirachta indica*) kernel meal, a protein rich source (CP: 34-38%) is an important oil industry by product in India, feeding of which in raw form to livestock is generally discouraged due to presence of bitter triterpenoids (Gowda and Sastry, 2000). Consequently, the cake has been treated by various methods, including water washing (Rao et al., 2002), urea treatment (Musalia et al., 2000) and solvent treatment (Chad, 1987; Reddy and Rao, 1988; James et al., 2007) to avoid the growth depression.

We earlier reported that processing with different concentrations of methanol, ethanol and propanol (different solvent polarity) affect the chemical composition of neem seed cake (James et al., 2007). Previous study of solvent processing on chemical composition has not taken into consideration polarity of these solvents. However there is no information on the effect of these treated neem seed cake on the growth and nitrogen metabolism of animals; and secondly, nutritional evaluations are affected by chemical composition. The present study was therefore designed to investigate the effect of total replacement of soya meal by solvent treated neem seed cake, as a major source of protein on nitrogen metabolism in growing rats.

MATERIALS AND METHODS

Ripe neem (yellowish) fruits were collected from the tree in Zaria

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Table 1. Dietary component (w/w) of the diet containing processed neem seed cake.

Ingredient	WNSC I	MNSC II	ENSC III	SFD IV	CFD V	PFD VI
Yellow maize[1]	46.62	51.10	52.19	44.41	-	76.80
Soya bean meal	-	-	-	32.38	-	-
Processed Neem cake	30.18	25.69	24.60	-	-	-
Corn Pomace	10	10	10	10	-	10
Limestone	0.50	0.50	0.50	0.50	-	0.50
Salt	0.50	0.50	0.50	0.50	-	0.50
Vitamin Premix[2]	0.20	0.20	0.20	0.20	-	0.20
Corn Oil	10	10	10	10	-	10
Bone Meal	2.00	2.00	2.00	2.00	-	2.00
TOTAL	100	100	100	100	-	100
Crude Protein[3]	19.65	19.93	19.75	19.50	15.00	-
Crude Fibre [3]	6.62	4.22	6.60	5.02	7.00	0.50

WNSC = Water processed neem seed cake, MNSC = Methanol processed neem seed cake, ENSC = Ethanol processed neem seed cake, RFD = Reference diet containing soya bean meal, CFD = Commercial feed diet PFD = Protein free diet.

[1]Maize starch was used in place of yellow Maize as energy source for animals on protein free diet

[2] The vitamin-premix used in this study is a product of Pfizer Nig.Ltd. Each contains Vit.A (I.U), 10,000; Vit.D (I.U); 2,000; Vit..E (I.U); 2.5; Vit.K (mg); 20 Choline (mg), 300; Riboflavin (mg),4.2; Folic acid (mg), 0.5; Methionine (mg), 0.225; Mn (mg), 56.0; I (mg), 1.0; Fe (mg), 20.0; Cu (mg),10.0; Zn (mg), 1.25; and Co (mg), 1,25. [3]Determined.

environ in the month of August, they were depulped, dried in a hot air circulating oven at about 45°C for 48 h, and then decorticated to obtain seed kernel. These kernels were then stored in screw-capped bottles until required for processing and subsequent analyses.

Oil extraction

The well ground sample (10 g) was accurately weighed into a pre-weighed fat-free thimble. About 350 cm^3 petroleum ether (40 – 60°C) was poured into a previously weighed 500 cm^3 round bottom flask, containing boiling chips. Soxhlet extractor was then fitted and the extraction carried out for 6 h. The neem cake was in an oven temperature of 45°C for 48 h, and kept in an air tight container for further extraction

Extraction of the residual bitter component (neem seed cake alcoholic extract)

Five grams of ground neem seed cake were weighed into three conical flasks and 100 cm 3 portions of 75% methanol, 75% ethanol (%v/v) and distilled water were added separately into the flasks. These were set inside the mechanical shaker at 120 r.p.m at 60°C for 6 h as described by modified method of Mitra (1963) to remove the residual bitter component, the extracts were collected through filtered paper and the debitterised neem cake was dried at 45°C for 2 h. It was then stored in a screwcapped bottle until needed.

Animal housing and management

Twenty- four albino rats (wistar strain) of both sexes ages 3 weeks were used for the study. The rats were obtained from Animal unit of Pharmacology Department, Ahmadu Bello University, Zaria, weaned and kept in Biochemistry Department Ahmadu Bello University, Zaria. Three days were allowed for the animals to adapt to the control diet and experimental condition in metallic cages fitted with feeders and waterers, located in well ventilated animal house. Clean drinking water was provided *ad libitum*. At the end of conditioning periods, animals weighing between 70 to 103 g were randomly distributed into 6 groups (I, II, III, IV, V and VI) of 4-rats each of both sexes and were housed individually in well ventilated metabolic cages for nitrogen metabolism experiments.

Feeding schedule

Rats in group I, II and III were offered neem seed cakes processed with 75% methanol, 75% ethanol and water, group IV were given diet containing soya meal as a protein source, group V was fed commercial diet (Pfizer Nigeria Limited) while group VI were animals on protein free diet fore estimation of endogenous urinary and feacal nitrogen. The different proportions of the feed ingredients are shown in Table 1. Nitrogen balance study lasted for 14 days (7 days preliminary and 7 days collection). Food and water were given *ad libitum* and pre-measured quantity of food calculated to provide at least 4.0 Kcal of gross energy (Rahman et al., 1996) with 150 mg N and 10 g dry matter per rat per day were given to each rat for N-balance test as described by Eggum (1973).

The procedure for net protein utilization (NPU) determination, true nitrogen digestibility (TDN) and biological value (BV) were as described by Armstrong and Mitchell (1955). Body weights were recorded using a sensitive top loading balance. Urinary output was monitored as change in urine weight while faecal output was recorded as excess pellet weight per animal per cage according to Igile et al. (1995).

Daily feed intake was measured, as decrease in the quantity of weighed feed served daily. Urine out put was measured and the weight taken for nitrogen determination, while faeces were dried in an open air and stored for faecal nitrogen determination. Faecal and urinary nitrogen were determined by Kjeldahl method (AOAC, 1980).

Statistical analysis

The data were subjected to two-way analysis of variance and diff-

Table 2. Effect of processed neem seed cake on growth performance of rats.

Diet	Average Initial Weight (g/rat)	Average Final Weight (g/rat)	Average Weight Gain (g/rat)	Daily Weight Gain (g/d/rat)	Daily feed Intake (g/d/rat)	Protein Intake (g/d/rat)	Protein Efficiency Ratio	Feed Efficiency Ratio
WNSC	103.22	110.45	7.23^{b}	0.52^{b}	6.69^{c}	1.31^{c}	0.39^{b}	0.08^{a}
MNSC	101.55	114.44	12.89^{a}	0.92^{a}	10.00^{b}	1.99^{b}	0.46^{a}	0.09^{a}
ENSC	94.01	101.79	7.78^{b}	0.56^{b}	7.97^{bc}	1.57^{c}	0.35^{b}	0.07^{b}
SFD	117.66	133.60	15.95^{a}	1.14^{a}	12..35^{a}	2.41^{a}	0.47^{a}	0.09^{a}
CFD	84.70	97.98	13.28^{a}	0.95^{a}	9.75^{b}	1.90^{b}	0.50^{a}	0.10^{a}
Pooled SEM			0.97	0.07	0.56	0.11	0.02	0.003

WNSC = Water Processed Neem seed cake, MNSC = Methanol processed neem seed cake, ENSC = Ethanol Processed neem seed cake, SFD = Standard protein diet, CFD = Commercial feed diet, SEM = Standard error of the mean.
Values are means of four determinations.
Means with different superscripts in a column differ significantly (P<0.05).

Table 3. Effect of processed neem seed cake on dry matter digestibility and faecal output.

Diet	Average Water Intake (ml/d)	Faecal Wet Weight (g/d)	Faecal Dry Weight (g/d)	Dry Matter Digestibility (%w/w)
WNSC	9.71^{b}	1.31^{b}	1.12^{c}	18.06^{b}
MNSC	10.79^{b}	1.45^{b}	1.07^{c}	47.16^{a}
ENSC	11.60^{b}	1.45^{b}	1.15^{c}	22.86^{b}
SFD	16.97^{a}	2.63^{b}	2.07^{b}	46.80^{a}
CFD	15.25^{a}	7.30^{a}	3.15^{a}	30.53^{b}
Pooled SEM	0.79	0.66	0.23	3.47

WNSC = Water processed neem seed cake, MNSC = Methanol processed neem seed cake, ENSC = Ethanol processed neem seed cake, SFD = Standard protein feed diet, CFD = Commercial feed diet, SEM=Standard error of the means
Values are means of four determinations.
Means with different values in a column differ significantly (P<0.05).

erences between the means of diets were tested (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Significant (P<0.05) reduction in daily feed intake and daily protein intake compared with standard protein diet, were observed for rats fed with processed neem seed cakes, this observation may be as a result of decrease palatability of the diets (Table 2). Daily weight gain, protein efficiency ratio and feed efficiency ratio show no significant (P<0.05) difference between rats fed methanol treated neem and that of the standard diet. Generally a protein efficiency ratio (PER) describes a protein quality. PER of animal protein is higher than plant protein, the higher the value the higher the quality of a protein (Friedman, 1996). In this study rats fed methanol processed neem seed cake compared favorably with soybean protein diet.

There was also correlation (R=0.97) between feed or protein intake and the growth indices of rats in this study. The study shows that the diet was of good quality and adequate nutrients, since growth of the animals was not actually limited by the quality of the feed consumed, but a function of the bioavailability of the total nutrient consumed (Rahman et al., 1996; Friedman, 1996).

There was no significant (P>0.05) difference between the dry matter digestibility of rats fed with methanol processed neem seed cake meal (MNSC) and the dry matter digestibility of the standard protein diet. Other neem cake processing method resulted in a decrease (P<0.05) in dry matter digestibility (Table 3).

There was correlation (R=0.61) between the water intake and faecal wet weight of rats used in this study. The water intake of rats fed with processed neem cakes are significantly (p<0.05) lower than those on the standard diet (Table 3). Increase in bulkiness of food or feed intake is responsible for increase in water intake (Aremu, 2000), whereas, the decrease in the water intake of rats fed processed neem seed cake may be as a result of decrease in the feed intake.

Significant (P<0.05) decreases in nitrogen intake and urinary nitrogen excretion were found in all the rats fed

Table 4. Nitrogen balance studies in rats fed with processed neem seed cake.

Parameter	WNSC	MNSC	ENSC	SFD	CDF	Pooled SEM	PFD
Nitrogen Intake (mg/d)	209.60^{c}	318.72^{b}	251.72^{c}	386.38^{a}	271.05^{b}	17.50	27.56
Faecal Nitrogen Excretion (mg/d)	41.94^{b}	28.97^{b}	24.60^{b}	35.10^{b}	72.42^{a}	4.89	10.70
Urinary Nitrogen Excretion (mg/d)	34.16^{c}	33.60^{c}	34.40^{c}	76.72^{a}	52.55^{b}	4.86	22
Biological Value	0.93^{a}	0.96^{a}	0.95^{a}	0.85^{b}	0.85^{b}	0.01	-
Digestibility	0.85^{a}	0.94^{a}	0.94^{a}	0.94^{a}	0.65^{b}	0.03	-
Net Protein Utilization	0.79^{a}	0.91^{a}	0.90^{a}	0.80^{a}	0.56^{b}	0.04	-
Nitrogen Balance	133.50^{b}	256.15^{a}	192.72^{b}	274.56^{a}	146.08^{b}	16.38	-5.14

WNSC = Water Processed Neem seed cake, MNSC = Methanol Processed Neem seed cake, PFD = Protein free diet, ENSC = Ethanol Processed Neem seed cake, CDF = Commercial feed diet, SFD= Standard protein diet, SEM = Standard error of the means.
Values are means of four determinations.
Means with different superscripts in a row differ significantly (P< 0.05).

with processed neem cake diets compared with standard protein diet, this is as a result of decrease in feed intake of rats fed with processed neem seed cakes shown in Table 2. However there was no significant difference in faecal nitrogen excretion for the rats fed with processed neem seed cakes and the standard protein diet. This suggests that there is no significant difference in the dietary fibre in their diet (Table 4). Increase dietary fibre has been reported to decrease bowel transit time, increase faecal bulk and number of defecation as well as faecal excretion of nitrogen (Glore et al., 1994).

The biological value (BV), is significantly ($p<0.05$) higher in all the rats fed with processed neem seed cakes than that of the standard protein diet, while there was no significant ($p>0.05$) difference between rats fed with standard and processed neem seed cakes for the true digestibility and net protein utilization (NPU).

For BV and NPU, 100% denotes the highest quality protein, though high protein digestibility does not always mean high protein quality. Digestibility is a measure of protein hydrolysis, whereas, protein quality is a measure of the balance of the amino acids that are absorbed and utilized for growth and other purposes (Friedman and Cuq, 1988). The NPU values for all the rats fed with processed neem seed cakes diet were observed to be within the values (>0.70) recommended for good food protein and dietary mixture (FNB, 1974) this suggests that even though growth depression occurred with rats fed water processed neem seed cake diet, the processed neem seed cake are still adequate and can meet the basic physiological needs of the animals (Istifan et al., 1983; Hannaning et al., 1992; Hoffiman, 1993).

Conclusions

The result shows that weight gain, protein efficiency ratio and feed efficiency ratio of methanol processed neem seed cake diet were significantly ($p<0.05$) higher than other processing method employed on neem seed cake. The result of nitrogen balance study also indicates that methanol processed neem seed cake compared well with standard diet; hence it can be included in animal feeds. Improved utilization can be obtained by carrying out nutritional evaluation of different graded level of 75% methanolic processed neem seed cake in the diet of rat so as to establish the maximum level that could be tolerated especially in monogastric animals.

REFERENCES

AOAC (1980). Official methods of Analysis. Horwitz (Ed.), 13th Edn Assoc. Office Anal. Chem., Washington D.C. p. 211.

Aremu A (2000). Effect of source and varying levels of sawdust and maize cobs on nutrient digestibilities of Layers. Nig. J. Biotechnol. 11(1): 72-76.

Armstrong DG, Mitchell HH (1955). Biological values of protein in various foods for maintenance and growth for the growing pigs. J. Anim. 14: 53-56.

Chad S (1987). Nutritionalevaluation of neem seed meal in chicks.PhD Thesis, Rohilkhand Univ. Bareilly, Indian, pp. 66-106.

Eggum BO (1973). Factors influencing protein utilization in rats and pigs. Forlag No.406

FAO (1996). Food agriculture organization population reports on winning the food race serias No.13.

FNB (1974). Recommended daily allowances. National Research Council Publication NO. 1694. Washington D.C National Academy of Science.

Friedman M (1996). Nutritional values of protein from different food sources. J. Agric. Food Chem. 44: 6-29.

Friedman M, Cuq JL (1988). Chemistry, analysis, nutritional value and toxicology of tryptophan. J. Agric. Food Chem. 36: 1079-1093.

Glore SR, Van Treek D, Knehans AW, Guild M (1994). Soluble fiber and serum lipids: a literature review. J. Am. Diet. Assoc. 94: 425-436.

Gowda SK, Sastry VRB (2000). Neem (*Azadirachta indica*) seed cake in animal feeding scope and limitation. Rev. Asian-Aus. J. Anim. Sci. 13: p. 5.

Hannaning RM, Paes B, Atkinson SA (1992). Protein metabolism and growth of term infants in response to reduced-protein, 40:60 whey: casein formula with added tryptophan. Am. J. Clin. Nutr. 56: 1004-1011.

Hoffiman K (1993). Nutritional values of proteins and protein requirements of people with special reference to meat proteins Mitteilungbl. Bundesanst. Fleisch Forsch. 32: 422-429.

Igile GO, Oleszek W, Burda S, Jurzysta M (1995). Nutritional assessment of *vernonia amygdalina* leaves in growing mice. J. Agric. food Chem. 43: 2162-2166.

Igoeli G (2000). Animal production and agriculture in the new millennium, in proceedings of 25th Annual Conference of Nig. Soc

Anim. Prod. Edits Ukanchukwu SN, Ibeamuchi JA and Ibe SN, pp. 1-3.
Istifan N, Murray E, Janghorbani M, Young MR (1983). An evaluation of the nutritional value of soy protein concentrate in young adult using short term N-balance method. J. Nutr. 113: 2516-2523.
James DB, Ameh DA, Agbaji AS (2007). Proximate composition of seed of *Azadirachta indica* (Dogonyaro) and the extraction of its protein. Nig. J. Bioc. Mol. Biol. 5: 75-78.
Mitra CR (1963). Indian central oil seed committee Hyderabad. I edn. Examiner Press, Bombay, Indian, p. 190.
Musalia LM, Anandan S, Sastry VRB, Agrawal DK (2000). Urea-treated neem (*Azadirachta indica*) Seed kernel cake as a protein supplement for lambs. Small Ruminant Res. 35: 107-116.
Rahman MH, Hossain I, Moslehuddin M (1996). Nutritional evaluation of sweet lupin (*Lupinus angustifolius*): net protein utilization (NPU), nitrogen balance and fraction studies. Brit. J. Nutr. 77: 443-4457.
Rao VK, Kowale BN, Verma AK (2002). Effect of feeding water washed neem (*Azadirachta indica)* seed kernel cake in the quality, lipid profile and fatty acid composition of goat meat. Small Ruminant Res. 47: 213-219.
Reddy VR, Rao PV (1988). Utilization of chemically treated neem cake in broilers. Indian J. Anim. Sci. 58: 958-963.
Snedecor GW, Cockhran WG (1980). Stastistical methods, 6th edn. Oxford and IBH Publishing Company, New Delhi, pp. 258-298.