Chemical Composition and Biological Evaluation of Debitterized and Defatted Neem (*Azadirachta indica*) Seed Kernel Cake

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Two samples of debitterized and defatted neem (*Azadirachta indica*) seed kernel cakes were analyzed for their nutrient composition, and the cake protein quality was evaluated in weanling rats. The cake is a good source of protein (45-50%), phosphorus, calcium and iron. Trypsin inhibitory activity was detected in the cake, but it was low (15-17 TIU/mg protein). Lysine content of the cake protein was higher than most of the cereal proteins but lower than that of legume proteins. Sulphur-containing amino acids were the limiting amino acids of the cake protein. When the cake contained a low amount of fat and was devoid of neem smell, its PER and NPU were comparable to those of any other oilseed cake. Animals receiving 84% neem cake diet did not exhibit any toxic symptoms. Therefore, neem seed kernel cake may be included in animal feeds.

Neem (*Azadirachta indica*) is the national tree of India. Neem oil is used as an antiseptic and in treating boils, ulcers resulting from chronic syphilis, and ringworm (1). The oil and cake are bitter in taste and have a disagreeable odor. Their bitter taste is due to sulphur-containing compounds like nimbin, nimbidin and nimbosterol. These compounds have insecticidal properties. Traditionally neem cake is used as a fertilizer and could not be used as animal feed due to its bitter taste and characteristic neem smell. Animals fed on diets containing neem cake lost body weight (2).

Recently, a simple process for extracting compounds having insecticidal properties from neem seed kernels has been developed at the Regional Research Laboratory, Hyderabad. In this process ground neem kernels were extracted initially with ethanol followed by hexane (3). The resultant cake was devoid of bitter taste and the usual neem smell. Therefore, the suitability of the debitterized and defatted neem seed kernel cake as animal feed was investigated. In this study the nutrient composition of neem seed kernel cake and its protein quality were evaluated.

MATERIALS AND METHODS

Materials. Two samples of neem cake (5 kg each) were received from the division of oils and fats, RRL, Hyderabad (3). The first sample was dark brown in color and had a characteristic neem odor, resembling that of ghani expelled cake, and contained 9% ether extractives. The second cake was light in weight, buff colored and devoid of neem smell.

Chemical composition. Chemical composition of both the cakes was determined. Protein (Nx 6.25), fat (ether extractives), ash, crude fiber, phosphorus, calcium and iron were estimated according to AOAC methods (4). Trace elements were determined after ashing the cake in an atomic absorption spectrophotometer, varion techtron model AAS 1000. Trypsin inhibitory activity was determined by the method of Kakade et al. (5) as modified by Roy and Rao (6).

For amino acid analysis of neem cake protein, 20 mg of finely powdered cake sample was hydrolyzed in 6N hydrochloric acid at 110 C for 20 hr in evacuated sealed

TABLE 1

Percent Composition of Experimental Diets

Group	Diet	Vitamin mixture[a]	Choline chloride[b]	Salt mixture[c]	Oil	Casein	Neem cake	Starch
			Experiment No. 1					
I	Protein free	1	1	4	10	–	–	84
II	13% casein	1	1	4	10	13	–	71
III	22% neem cake	1	1	4	10	–	22	62
IV	22% neem cake	1	1	4	10	–	22	62
V	84% neem cake	1	1	4	10	–	84	–
			Experiment No. 2					
I	Protein free	1	1	4	10	–	–	84
II	13% casein	1	1	4	10	13	–	71
III	20% neem cake	1	1	4	10	–	20	64
IV	17.5% neem cake 2% casein	1	1	4	10	2	17.5	64.5
V	84% neem cake	1	1	4	10	–	84	–

[a]Vitamin mixture according to Campbell (9).
[b]50:50 choline chloride starch.
[c]Salt mixture according to USP XVII (4).

tubes. Excess acid was removed by flash evaporation under reduced pressure. The amino acid analysis was carried out in an automatic amino acid analyzer (7). Tryptophan content of the cake protein was determined in an alkaline hydrolysate by the microbiological method of Barton-Wright (8).

Biological evaluation. Biological evaluation of the cake protein was carried out in 21-day-old male rats weighing 37 to 44 g. Rats were randomly distributed into five groups of 6 animals each, and the experimental diets were given as follows: For Experiments 1 and 2, rats in Group I received a protein-free diet and those in Group II received a 10% casein protein diet. Group III received a 10% neem cake protein diet in both experiments; Group IV received a 10% neem cake protein cooked diet in Experiment 1 and an 8.5% neem cake protein/1.5% casein protein diet in Experiment 2, and Group V received an 84% neem cake diet in both experiments.

Table 1 gives the composition of experimental diets. All the diets, except diet No. 3 in Experiment 1, were mixed with double the amount of water and cooked by steaming in an autoclave for 15 min. Food and water were given ad lib. Daily food intake and weekly body weights of individual rats were maintained during the four weeks of experiment for calculating protein efficiency ratio (PER) (9). During the last three days of the experiment fecal samples of individual animals were collected, and their nitrogen content was estimated by the Kjeldahl method. From these data, protein and dry matter digestibilities were calculated. At the end of the experiment, all animals were killed under ether anesthesia. The eviscerated carcasses were hydrolyzed by autoclaving with 6N hydrochloric acid at 15 lb pressure for 2 hr, and the nitrogen content of the hydrolysate was determined by the Kjeldahl method. The net protein utilization (NPU) was calculated from the nitrogen content of carcasses (10). The data were tested by analysis of variance for statistical significance.

RESULTS

The data on chemical composition of debitterized and defatted neem seed kernel cakes are presented in Table 2. The cake is a good source of protein, phosphorus, calcium and iron. Its protein content is comparable to that of groundnut cake. Phosphorus and calcium contents of neem cake were significantly higher than those observed in groundnut cake. Trypsin inhibitory activity was detected in the cake, but the activity was low when compared to *Lathyrus sativus* (6). The first sample of neem cake had higher amounts of fat and slightly lower protein content than the second cake.

Lysine content of the cake was higher than any of the cereal proteins (sorghum, maize and wheat) but lower than most of the legumes like pigeon pea, chickpea, blackgram and greengram (11). As in legumes, the cake protein was deficient in sulphur-containing amino acids and they constituted its limiting amino acids (Table 3). Chemical score of the neem cake protein was 33, compared to 55 for groundnut cake and 100 for whole egg protein.

Table 4 gives the biological data on neem seed kernel cake protein.

Experiment No. 1. All the parameters studied except dry matter digestibility were significantly lower in 10% neem cake protein diets, raw and cooked, than in casein. Food intake of animals on the cake diet was 50% that of the animals on casein diet. Their body weight gain ranged between 0 and 10 g, compared to 73-103 g in animals receiving a casein diet over a period of four weeks. Group No. V was included in the study to find out the toxicity of neem cake if any. Animals in this group consumed more food and gained more weight than the animals receiving 10% neem cake protein diets,

TABLE 2

Nutrient Composition of Debitterized and Defatted Neem Seed Kernel Cake

		Groundnut Cake	Sample I	Sample II
Moisture	g%	7.2	9.0	8.7
Protein	g%	40.9	45.0	49.4
Fat	g%	7.4	9.1	3.6
Ash	g%	2.5	7.6	9.5
Crude fiber	g%	3.2	5.5	8.6
Phosphorus	mg/100 g%	548	879.0	938.0
Calcium	g%	213	399.0	415.0
Magnesium	g%		190.0	193.5
Iron	g%		8.7	10.5
Zinc	g%		8.0	7.1
Copper	g%		2.4	2.6
Manganese	g%		2.2	2.1
Chromium	g%		0.3	0.2
Trypsin inhibitory activity TUI/mg protein	g%		17	15

TABLE 3

Amino Acid Composition of Debitterized and Defatted Neem Seed Kernel Cake—2[a]

	Neem cake	Groundnut[b] cake
Lysine	3.3	5.1
Histidine	1.9	2.4
Arginine	8.5	6.3
Aspartic acid	10.6	12.8
Threonine	3.2	3.9
Serine	4.9	4.0
Glutamic acid	24.2	9.6
Proline	3.9	11.0
Glycine	4.3	4.8
Alanine	3.7	5.0
Cystine	0.3	1.3
Methionine	1.1	1.4
Valine	3.6	5.0
Isoleucine	2.6	4.0
Beucine	6.5	7.0
Tyrosine	2.1	3.7
Phenylalanine	3.8	5.2
Tryptophan	1.2	0.7

[a]Values given are g/16 g N.
[b]Amino acid composition of foods and biological data on proteins (11).

TABLE 4

Protein Quality of Debitterized and Defatted Neem Seed Kernel Cake[a,b]

Group	Diet	Food intake (g/4 weeks)	Gain in body weight (g/4 weeks)	PER	Dry matter digestibility (%)	Protein digestibility (%)	Net Protein Utilization
			Experiment No. 1				
I	10% casein protein	320 ± 8.8[a]	95 ± 3.8[a]	3.03 ± 0.051[a]	93 ± 0.4[a]	88 ± 0.1[a]	69 ± 2.6[a]
III	10% neem protein (Raw diet)	165 ± 4.2[b]	4 ± 0.6[b]	0.26 ± 0.33[b]	91 ± 0.4[a]	75 ± 1.2[b]	20 ± 0.6[b]
IV	10% neem protein (cooked diet)	158 ± 6.1[b]	5 ± 1.6[b]	0.29 ± 0.90[b]	89 ± 0.6[a]	71 ± 1.3[b]	21 ± 1.0[b]
V	84% neem cake	194 ± 6.0[c]	43 ± 1.7[c]	0.223 ± 0.0034[c]	77 ± 2.1b	81 ± 1.8c	–
			Experiment No. 2				
II	10% casein protein	295 ± 6.4[a]	92 ± 4.1[a]	3.08 ± 0.782[a]	94 ± 1.0[a]	86 ± 1.6[a]	63 ± 2.0[a]
III	10% neem protein	228 ± 4.1[b]	42 ± 2.4[b]	1.91 ± 0.100[b]	90 ± 0.6[a]	75 ± 0.8[b]	38 ± 1.9[b]
IV	8.5% neem protein + 1.5% casein protein	279 ± 6.3[a]	63 ± 2.8[c]	2.22 ± 0.061[b]	92 ± 0.7[a]	83 ± 0.8[a]	43 ± 0.8[b]
V	84% neem cake	227 ± 9.3[b]	73 ± 5.2[c]	0.319 ± 0.0100[c]	81 ± 3.5[b]	85 ± 2.0[a]	–

[a]Six animals in each group; values given are mean ± SE.
[b]Figures having the same superscript are not statistically different.
[c]Feed efficiency ratio.

but ate and gained significantly less than those on casein diet.

Experiment No. 2. Even in this experiment food consumption, gain in body wieght, PER, protein digestibility and NPU of animals receiving a 10% neem cake protein diet were significantly lower than of those on a casein diet. In comparison to the animals of Experiment 1 (Group IV) receiving a similar diet, animals of this group consumed 40% more food and gained an eightfold higher body weight. These animals also registered sixfold increases in PER and about a twofold increase in NPU when compared to the animals in Experiment 1 (Group IV). The presence of 9% fat and characteristic neem odor of the cake used in Experiment 1 may be responsible for the lower intake of food in this group, due to nonpalatability of the diet. The eightfold increase in body eight gain by animals in Experiment 2 may be due to removal of growth inhibitory substances along with fat during defatting of the second sample of neem cake.

This conclusion is further strengthened by the observation on the animals fed 84% neem cake diets in the two experiments. Animals receiving the 84% neem cake diet in Experiment 2 consumed 17% more food, but their weight gain was 70% more and feed efficiency ratio 45% higher than the animals receiving 84% neem cake diet in Experiment 1. These results seem to indicate that neem seed kernel cake may not be toxic to rats.

In the first experiment, gain in weight by animals receiving 10% neem cake protein was very low (0-10 g). To find out whether supplementation of this diet with 2% casein can improve its quality animals of Group V in Experiment 2 were fed an 8.5% neem cake protein- and 1.5% casein protein-containing diet. Supplementation of neem cake diet with 2% casein brought about a significant increase in food intake, body weight gain and protein digestibility.

TABLE 5

Protein Quality Parameters of Debitterized and Defatted Neem Seed Kernel Cake

	Groundnut cake[a]	Neem cake
B.V.	54.5	–
Digestibility	86.6	75
NPU	42.7	38
PER	1.65	1.91
Chemical score	55	33

[a]Amino acid composition of foods and biological data on proteins (11).

The results of the second experiment demonstrated that the PER and NPU of neem seed cake are comparable to those of groundnut cake (Table 5). Hence, neem seed cake can be used in animal feeds in place of groundnut cake because the latter often contains aflatoxin, a potential carcinogen. However, long term toxicity studies have to be carried out before the use of neem seed cake in animal rations can be avocated.

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