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Effect of Solvent Extraction on Crude Protein, *in vitro* Protein Digestibility and Amino Acid Profile of Neem Seed Cake

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Abstract: Neem seed cake resulting after solvent extraction with water and different concentrations (35, 55, 75% and absolute) of methanol, ethanol and propanol were analyzed for crude protein, *in vitro* protein digestibility and amino acid composition Processed neem seed cake obtained from 55% methanol, 35% propanol and 100% propanol was significantly ($p<0.05$) lower in crude protein content compared to that obtained through water extraction while 75% ethanolic treated neem seed was significant ($p<0.05$) higher. There was a significant ($p<0.05$) increase *in vitro* protein digestibility of neem seed cake processed with 75% methanol over that with water. Neem seed cake obtained by extraction with 75% methanol, 75% ethanol and 55% propanol compared favorably with the amino acid profile to soya bean protein. The total essential amino acid content of neem seed cake obtained from 75% methanol was higher than that obtained for soya bean protein. The nutritional potential of neem seed cake, on the basis of these results, is discussed.

Key words: Neem seed cake, crude protein, *in vitro* protein digestibility, amino acid compositions, solvent extraction, Nigeria

INTRODUCTION

Recently attention has been focused on the non-convectional nutritive feed sources for animals, especially the possibility of using neem seed in feeds. Neem cake the solid residue left after the oil is removed from the kernel is useful when broadcast over farm field because it provides organic matter importance for fertility to the soil (NRC, 1992), but beyond this, neem cake is one product showing great potentials for livestock (Katiyar *et al*., 1991; Musalia *et al*., 2000; Rao *et al*., 2003). It is proteineous and relatively balanced in its amino acid profile (Tewari, 1992).

The chemical composition of the neem cake varies considerably depending on types of processing such as solvents or expeller extraction of undecorticated or decorticated seed (Gowda and Sastry, 2000). The cake is toxic due to triterprenoids like Azadirachtin, nimbin, salanin and others, which restrict its safe inclusion in livestock diet (Devakumar and Sukhdev, 1993).

NRC (1992) reported that these toxic compounds are slightly soluble in water and are freely soluble in organic solvents such as hydrocarbons, alcohols and ketones. However increase nutritive value and palatability of the cake has been achieved through various methods including water washing (Agrawal *et al*., 1987; Rao *et al*., 2003), alkali treatment (Katiyar *et al*., 1991), urea treated (Musalia *et al*., 2000) and solvent treated (Chand, 1987), the problem of putting the seed cake to use still remains this is because neem tree scattered around the world are genetically distinct and its nutritional potential are affected by climatic condition, method of processing and to a lesser extent the genetic make up of the animals (NRC, 1992). Secondly result of solvent processing on chemical composition has not taken into consideration effect of solvent polarity on the chemical composition.

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This study was done to find out the effect of different concentration of alcohols (methanol, ethanol and propanol) and water on the crude protein content, *in vitro* protein digestibility and amino acid profile of processed neem cake, so as to ascertain the effect of solvent polarity.

MATERIALS AND METHODS

Matured ripped neem (yellowish) fruits, collected from Zaria and its environment, Kaduna State Nigeria in the month of August, were depulp, dried in a hot air circulating oven at about 45°C for 48 h and decorticated to obtain seed kernels. 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. Petroleum Spirit (40-60°C) about 350 mL was poured into a previously weighed 500 mL round bottom flask, containing boiling chips and the soxhlet extractor was then fitted into the 500 mL bottom flask and the extraction carried out for 6 h. The cake was dried at an oven temperature of 45°C for 48 h and kept for further extraction.

Extraction of the Residual Bitter Component (Solvent Extracted Kernel)

Five grams of powdered neem seed cake was transferred into a conical flask and a 100 mL portion of the different concentrations % v/v (35, 55, 75% and absolute) of methanol, ethanol and propanol were added. These were set inside the mechanical shaker at 120 rpm at 60°C for 6 h following modified method of Mitra (1963) to remove the residual bitter component.

Chemical Composition

Analyses for the protein content were carried out using micro-kjedahl method (AOAC, 1980). while *in vitro* protein digestibility was carried out using the procedure of Mertz *et al*. (1984). Amino acid composition was determined by column chromatography with the automatic amino acid analyzer Technicon TSM-1, USA according to Trevor *et al*. (1981).

Statstical Analyses

Stastical analyses were by analysis of variance (ANOVA). Turkey test was used to identify means that differed significantly ($p<0.05$).

RESULTS AND DISCUSSION

From the result it is clear that 75% methanol, 75% ethanol and 100% ethanol treated neem seed cake significant ($p>0.05$) increased the protein contents of neem seed cake compared to water (control) processing method Table 1. Generally, processing affect the crude protein content of the neem cake as reported by Bedi *et al*. (1975), James *et al*. (2000) who reported 12.35 17.85 and 26.66%, respectively for neem seed. This increase is due to removal of Triterprenoids and other substances present in the cake. Chand (1987) has reported 40.35% crude protein content for alcohol extracted neem meal which is still lower than most of the extraction processing in this study.

The digestibility of a food is most accurately defined as the proportion of the ingested food which is not excreted in the faeces and which is therefore assume to be absorbed by the animal (Mc Donald *et al*., 1995). Digestibility determined *in vitro* is generally slightly lower than that determine *in vivo*. There was no significant ($p>0.05$) change of propanol concentrations of neem seed cake on *in vitro* protein digestibility. Ethanol (35%) gave the lowest significant ($p<0.05$) value while

methanol (75%) gave the highest value, this value is significantly ($p<0.05$) higher than other alcoholic treated neem seed cake and that of water (control). The differences in protein digestibility are brought about by the susceptibility of a protein to enzymatic hydrolysis in the digestive system and this is directly related to primary, secondary and tertiary structure of the protein (Barbara, 1994; Mc Donald *et al*., 1995). Processing and storage conditions alter the protein structure thus improving or lessening its susceptibility to enzymes. The high *in vitro* protein digestibility level observed for 75% methanol compared to water may be attributed to effect of this solvent in improving susceptibility of the cake to enzyme hydrolysis.

Table 2a and b showed the total essential amino acids and non-essential amino acids. The change in amino acid profile is as a result of the effect of different processing method on neem cake. All the essential amino acid except for leucine were significantly ($p<0.05$) higher in the cake processed with

Table 1: Crude protein and *in vitro* digestibility of alcohol- processed neem seed cake

Extracts	*In vitro* digestibility	Crude protein
35% Methanol	7.04^{f}	47.06^{cd}
55% Methanol	17.15^{f}	26.31^{e}
75% Methanol	83.61^{a}	53.37^{ab}
100% Methanol	69.48^{b}	48.13^{b-d}
35% Ethanol	13.00^{f}	50.31^{bc}
55% Ethanol	60.00^{bc}	43.75^{cd}
75% Ethanol	69.23^{b}	56.88^{a}
100% Ethanol	62.50^{bc}	52.5^{ab}
35% Propanol	42.84^{de}	30.62^{e}
55% Propanol	39.39^{e}	43.15^{d}
75% Propanol	52.27^{c-e}	45.93^{cd}
100% Propanol	52.20^{c-e}	27.50^{e}
100 mL of distilled water	53.08^{cd}	46.63^{cd}
Pooled SEM	3.47	1.54

Means with different superscripts in column differ significantly $p<0.05$, Values are triplicate determinations

Table 2a: Effect of alcoholic processing of neem seed cake on essential amino acid composition (g/100 g protein)

	Methanol				Ethanol			
Amino acids	C-1	C-2	C-3	C-4	C-1	C-2	C-3	C-4
Lysine	3.38^{de}	2.81^{e}	7.36^{a}	3.99^{de}	3.92^{de}	3.69^{de}	6.55^{ab}	5.59^{bc}
Histidine	1.89^{c}	2.02^{c}	2.69^{a}	1.45^{d}	2.00^{c}	1.94^{c}	1.88^{c}	1.39^{d}
Arginine	4.71^{d}	4.41^{d}	9.06^{a}	4.19^{d}	5.01^{d}	5.08^{d}	7.84^{ab}	5.62^{cd}
Threonine	3.32^{b}	2.97^{bc}	5.70^{a}	1.65^{d}	2.17^{cd}	2.23^{cd}	2.75^{bc}	2.20^{cd}
Valine	3.19^{b}	3.02^{bc}	4.12^{a}	2.57^{c-f}	2.75^{b-e}	2.17^{f}	2.92^{b-d}	2.97^{bc}
Methionine	1.10^{a}	0.91^{d}	1.52^{a}	0.79^{f}	0.82^{ef}	0.71^{f}	1.06^{b-e}	1.09^{b-d}
Isoleucine	2.53^{fg}	3.00^{e}	4.09^{a}	2.80^{d-f}	2.75^{ef}	3.02^{e}	3.70^{ab}	3.52^{abc}
Leucine	7.00^{ab}	6.45^{a-c}	4.12^{d}	5.40^{c}	6.10^{b}	5.10^{c}	6.68^{ab}	3.10^{d}
Phenyl-alanine	4.02^{c}	3.75^{c}	5.57^{a}	3.09^{d}	3.32^{cd}	3.06^{e}	3.96^{c}	4.95^{ab}
Total EAAs*	31.14^{c-e}	29.34^{de}	44.23^{a}	25.93^{ef}	28.84^{ef}	27.00^{ef}	37.34^{bc}	30.43^{c-f}

	Propanol							
Amino Acids	C-1	C-2	C-3	C-4	Water	SEM	Soya** Protein	Requirement for growing rat ***
Lysine	4.50^{cd}	6.07^{ab}	3.56^{de}	4.40^{cd}	3.07^{de}	0.39	5.90	5.30
Histidine	2.39^{ab+}	2.46^{a}	2.36^{a}	1.92^{c}	2.04^{bc}	0.10	2.50	3.50
Arginine	5.50^{cd}	6.20^{cd}	4.80^{d}	6.89^{bc}	4.08^{d}	0.41	8.20	1.80
Threonine	2.49^{b-d}	2.78^{bc}	2.50^{b-d}	3.02^{bc}	2.58^{b-d}	0.27	4.00	4.30
Valine	2.43^{df}	2.49^{df}	2.38^{ef}	2.36^{ef}	2.72^{b-d}	0.14	4.30	5.30
Methionine	1.01^{cd}	1.19^{bc}	0.92^{d}	1.28^{ab}	0.71^{f}	0.07	1.40	4.20
Isoleucine	3.33^{b-d}	3.39^{b-d}	3.13^{cd}	4.12^{a}	2.12^{g}	0.16	4.20	5.30
Leucine	6.84^{ab}	7.24^{ab}	6.53^{a-c}	7.46a	5.00^{e}	0.36	7.70	6.40
Phenyl-alanine	3.88^{cd}	4.29^{bc}	3.94^{c}	4.54^{bc}	3.06^{e}	0.21	5.00	6.90
Total EAAs*	32.37^{c-e}	36.11^{c}	30.12^{c-f}	35.99^{cd}	25.38^{f}	1.63	43.00^{ab}	43.00^{ab}

** Chandrasiri *et al*. 1987*** Smith *et al*. 1975, *Essential amino acidsmean with different superscripts in a row differ significantly, C-1 = 35%, C-2 = 55%, C-3 = 75% and C-4 = Absolute

Table 2b: Effect of alcoholic processing of neem seed cake on non-essential amino acid composition (g/100 g protein)

	Methanol				Ethanol			
Amino acids	C-1	C-2	C-3	C-4	C-1	C-2	C-3	C-4
Aspartic acid	5.61^{d}	5.00^{d}	12.92^{a}	6.82^{cd}	6.01^{c}	5.03^{d}	6.14^{c}	8.04^{bc}
Serine	2.88^{de}	2.54^{e}	5.70^{a}	3.30^{cd}	3.55^{c}	3.43^{cd}	4.27^{bc}	3.78^{b-d}
Glutamic acid	8.90^{d}	9.50^{d}	11.95^{b}	11.73^{b}	9.87^{d}	10.02^{cd}	12.02^{b}	13.52^{a}
Proline	2.53^{b-d}	2.19^{c-e}	4.15^{a}	1.78^{de}	1.80^{de}	2.12^{de}	3.32^{b}	2.84^{bc}
Glycine	2.70^{de}	2.55^{e}	3.58^{ab}	2.79^{c-e}	3.53^{ab}	3.39^{ab}	3.34^{ab}	3.25^{a-c}
Alanine	3.86^{b}	3.42^{bc}	5.52^{a}	2.40^{d}	4.13^{b}	3.88^{b}	4.11^{b}	3.50^{bc}
Cystine	1.91^{b-f}	1.65^{d-f}	2.82^{a}	1.58^{f}	2.19^{b}	1.85^{cde}	2.21^{b}	2.05^{b-d}
Tyrosine	2.43^{c-e}	2.57^{cd}	3.32^{ab}	1.79^{f}	1.93^{ef}	2.23^{def}	3.45^{ab}	3.11^{ab}
Total NEAAs*	30.79^{de}	29.42^{e}	49.96^{a}	32.19^{c-e}	33.01^{cde}	31.95^{de}	38.86^{b-d}	40.09^{bc}
TotalAAs**	61.93^{c-e}	58.76^{de}	94.19^{a}	58.12^{de}	61.84^{de}	58.95^{de}	76.20^{bc}	70.52^{b-d}

	Propanol							
Amino acids	C-1	C-2	C-3	C-4	Water	SEM	Soya protein**	Requirement for growing RAT***
Aspartic acid	5.83^{c}	6.01^{c}	4.78^{d}	9.57^{b}	4.97^{d}	0.64	10.60	-
Serine	3.92^{bc}	4.00^{bc}	4.00^{bc}	4.59^{b}	2.30^{e}	0.25	4.90	-
Glutamic acid	11.23^{bc}	11.22^{bc}	10.91^{bd}	12.16^{b}	9.84^{d}	0.36	17.30	-
Proline	1.88^{de}	2.00^{de}	1.80^{de}	3.11^{b}	1.63^{e}	0.21	5.50	-
Glycine	3.13^{b-d}	3.22^{ab}	3.00^{c-e}	3.65^{a}	2.01^{f}	0.13	4.00	-
Alanine	4.00^{b}	3.85^{b}	3.76^{bc}	3.88^{b}	3.08^{cd}	0.20	4.10	-
Cystine	2.06^{b-d}	2.11^{bc}	1.93^{b-e}	1.78^{c-e}	1.76^{d-f}	0.098	1.60	-
Tyrosine	2.34^{d}	2.47^{c-e}	2.35^{cd}	2.87^{bc}	2.23^{d-f}	0.14	3.70	-
Total NEAAs*	34.39^{b-e}	34.88^{b-e}	32.23^{c-e}	41.61^{b}	27.82^{e}	1.95	51.70^{a}	-
TotalAAs**	66.77^{b-e}	70.99^{b-d}	62.35^{c-e}	77.60^{b}	53.20^{e}	3.45	94.90^{a}	-

***Smith *et al.* (1975*) **Chandrasiri *et al.* (1987); *Non-Essential amino acids, Total amino acids, mean with different superscripts in a row differ significantly. C-1 = 35%, C-2 = 55%, C-3 = 75% and C-4 = Absolute

75% methanol. Propanol processing significantly ($p<0.05$) increased methionine, isoleucine and phenyalanine. However ethanol significantly ($p<0.05$) decreased the level of histidine, threonine and valine (55, 75 and 100%) over that of water, alcoholic processing neem seed cake quantitatively increased the total essential and non-essential amino acid compared to water (control) processing, this change may be attributed to less-polar nature of alcohols, which reduced the effect of chemical reactions (especially Maillard browning) which is responsible for significant reduction of essential amino acid, thus affecting the level of amino acid in processed neem seed cake (Mc Donald *et al*., 1995). The amino acid composition of Soya bean protein as reported by Chandrasir *et al*. (1987) and amino acid requirement for growing rat (Smith *et al*., 1975) were also presented in Table 2a provide comparable nutritional data.

CONCLUSIONS

The results of this analysis showed that 75% methanol processed neem cake showed significantly improved nutritive value when compared to other alcoholic processing method hence the possibility of its inclusion in animal feed as non convectional food source

REFERENCES

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

Agrawal, D.K., A.K. Garg and Nath, 1987. The use of water washed neem (*Azadirachta indica*) kernel cake in the feeding of buffalo calves. J. Agric. Sci. (Camb)., 108: 497-499.

Barbara, R.A., 1994. Protein Quality Tests. In: Chemical Analysis of Foods. Susainne, N. (Ed.), Publ. Chapman and Hall.

Bedi, P.S., V.K. Vijjian and S.K. Ranjihan, 1975. Utilization of neem (*Azadirachta indica*) seed cake and its influence on nutrients digestibility. Ind. J. Dairy Sci., 28: 104-107.

Chand, S., 1987. Nutritional evaluation of neem seed meal in chicks. Ph.D Thesis, Rohilkhand Univ. Bareilly, India, pp: 66-106.

Chandrasiri, V., H.M. Bau, C. Villaume, F. Giannangeli, D. Lorient and L. Mejean, 1987. Effect of germination of soyalbean on chemical composition: Sci. Aliments, 7: 139-150.

Devakumar, C. and Sukh Dev. 1993. Chemistry. In: Neem Research and Development. Randhava, N.S. and B.S. Plannar (Eds.), Publ. No. 3 Society of Pesticide Science India, pp: 63-96.

Gowda, S.K. and V.R.B. Sastry, 2000. Neem (*Azadirachta indica*) seed cake in animal feeding scope and limitations. Rev. Asian-Aus. J. Anim. Sci., pp: 13: 5.

James, D.B., D.A. Ameh and A.S. Agbaji, 2000. Proximate composition of seed of *Azadirachta indica* (Dogonyaro) and the extraction of its protein Nigeria. J. Bioc. Mol. Biol., 15: 75-78.

Katiyar, R.C., V.V.B. Sastry and D.K. Agrawal, 1991. Urea and alkali treated neem seed kernelcake as livestock feed in. Ann. Rep. Anim. Nutr. Div. IVRI. Izatnagar, India.

National Research Council, 1992. Neem. A Tree for Solving Global Problems. National Academy Press Washington. D.C.

Mertz, E.T., M.M. Hassen, C. Cairns-Whittern, A.W. Igleis, L. Tu and J.D. Axtel, 1984. Pepsin digestibility of protein in sorghum and other major cereals. Proc. Nath Acad. Sci. (USA), 81: 1-6.

Mc Donald, P., R.A. Edward, J.F.D. Greenhalgh and C.A. Morgan, 1995. Animal Nutrition Longman Publish, pp: 221-297.

MITRA, C.R., 1963. India Central Oil Seed Committee Hyderabad. India.

Musalia, L.M., S. Anandan, V.R.B. Sastry and D.K. Agrawal, 2000. Urea-treated neem (*Azadirachta indica*) seed kernel cake as a protein supplement for lambs. Small Ruminant Res., 35: 107-116.

Smith, R.H., R. Palmer and A.E. Reade, 1975. Chemical and biological assessment of A. oryzae and other filamentous fungi as protein sources for stomached animals. J. Sci. Food Agric., 26: 785-795.

Rao, V.K., B.N. Kowale and A.K. Verma, 2003. Effect of feeding water washed neem (*Azadirachta indica*) seed kernel cake on the quality, lipid profile and fatty acid composition of goat meat 47: 213-219.

Tewari, D.M., 1992. In: Monograph of Neem Inernational Book Distributor, Dehradun, India. pp: 105-135.

Trevor, W.B., J.O. Nicholas and T.W. Jeffrey, 1981. Composition and nutritional evaluation of *Aspergillus oryzae* biomass grown on palm oil processing effluent. J. Sci. Food Agric., 32: 1014-1020.