Effect of Replacement of Groundnut Cake with Urea-treated Neem (*Azadirachta indica* A. juss) Seed Kernel Cake on Nutrient Utilisation in Lambs**

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ABSTRACT : The effect of urea treatment on chemical composition of neem seed kernel cake (NSKC) was investigated by soaking the cake in 2.1% urea solution (1.2 l kg^{-1} NSKC) for five days. The effect on utilisation of nutrients by replacing groundnut cake (GNC) (30%) with urea-treated neem seed kernel cake (UTNSKC) (33%) in a concentrate mixture fed to meet 70% of the protein requirements of lambs (8 males and 8 females), was monitored in a digestibility study. Following urea treatment of NSKC only 9.5% of urea was hydrolysed and the crude protein content of the cake was increased by 6.65%. The tannin content in depulped neem seeds was 37% catechin equivalent. Whereas feeding UTNSKC had no effect on intake of dry matter (72.5 vs 66.3 g/kg $BW^{0.75}$ day^{-1}) and digestibility of crude fibre (41.3 vs 43.4%), the cake depressed ($p<0.01$) the percent digestibility of dry matter (63.7 vs 70.2), crude protein (63.2 vs 70.2), nitrogen free extract (73.8 vs 80.5) and gross energy (64.3 vs 69.1). Digestibility of ether extract (75.8 vs 70.9%) was higher ($p<0.05$) in animals offered UTNSKC. The nutritive value of the composite ration consumed by lambs offered UTNSKC was lower ($p<0.01$) in terms of total digestible nutrients (64.7 vs 70.2%) and digestible energy (2.8 vs 3.0 Kcal/g DM). Intake of digestible energy (199.8 vs 194.1 Kcal/kg $BW^{0.75}$ day^{-1}) and retention of nitrogen (7.53 vs 8.23 g day^{-1}) and calcium (2.12 vs 1.84 g day^{-1}) were comparable on the 2 rations. Animals fed UTNSKC retained less ($p<0.01$) phosphorus (0.37 vs 1.05 g day^{-1}). The results indicate that urea treatment increased the protein level of NSKC whereas feeding the treated cake as a replacement of GNC, lowered the digestibility of nutrients and retention of phosphorus. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 9 : 1273-1277)***

Key Words : Neem Seed Kernel Cake, Urea Treatment, Tannins, Digestibility, Utilisation of Nutrients

INTRODUCTION

The rapid increase in human population has been accompanied by a deficit in animal feeds especially the concentrates. This has resulted in the use of unconventional feed resources like neem (*Azadirachta indica* A. juss) seed cake (NSC), a by-product of neem oil industry. The utilisation of the cake in livestock ration has been limited by growth depression effects. Low growth rate was reported in crossbred cow calves fed 25% NSC in the ration (Bedi et al., 1975), lambs on ration with 30% NSC (Vijjan et al., 1982) and growing cow calves offered concentrate mixture containing 45% NSC (Garg, 1989). Gupta and Bhaid (1981) observed a gradual decline in growth rate of rams with higher levels of NSC in concentrate ration. Considering the growth depressant factors in NSC, the cake has been treated by various methods, including alcohol extraction (Nath and Vijjan, 1976), water washing (Nath et al., 1983; Agrawal et al., 1987) and alkali treatment (Katiyar et al., 1991), to improve its utilisation in livestock rations. Though alkali treatment followed by water washing gave good results, it was accompanied by a dry matter loss of 22%. To reduce the dry matter loss and avoid any side effects that may accompany the process of alkali treatment it was postulated that alkali conditions could be achieved by ensiling the cake with urea for five days (Reddy, 1992). Urea-treated neem seed kernel cake (UTNSKC) was found to be a good substitute of conventional cakes in concentrate mixtures when fed to growing buffalo calves (Reddy, 1992), broilers (Nagalakshmi, 1993), goats (Anandan et al., 1996) and lambs (Musalia et al., 2000). In the later study the rumen enzymes activities and digestibility of nutrients were depressed. The present study was designed to monitor the method of urea treatment and investigate the effects on feed intake, digestibility and utilisation of nutrients by growing lambs fed concentrate mixture containing UTNSKC.

** The Indian Council for Cultural Relations is highly acknowledged for providing funds to carry out this study. The Director of Indian Veterinary Research Institute is also acknowledged for granting permission to carry out the study in the Institute.

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Received May 28, 2001; Accepted April 30, 2002

MATERIALS AND METHODS

Urea-treated neem seed kernel cake was prepared by soaking neem seed kernel cake (NSKC) in 2.1% urea solution (1.2 l kg^{-1} NSKC) in an air-tight steel drum. The contents were mixed daily and sundried after 5 days. The method of urea treatment was investigated in glass-stoppered bottles in the laboratory to monitor the pH (day 0, 2, 4 and 5 of treatment) and residual urea using groundnut cake (GNC) as a control.

A control concentrate mixture containing GNC was formulated, as shown in Table 1. The experimental concentrate mixture was prepared by replacing 100% of GNC with UTNSKC on protein equivalent basis. Sixteen Mandya× Merino crossbred lambs (8 males and 8 females) aged one year were allocated randomly to four groups of four animals each; control males, control females, experimental males and experimental females in a randomised block design. The control and experimental concentrate mixtures were assigned to the lambs in the respective groups. The lambs were offered allotted concentrate mixture at 7.00 a.m. to meet 70% of the animals' protein requirements for daily gains of 100 g (NRC, 1985) followed by jowar (*Sorgum vulgare*), *ad libitum* as from 10.00 a.m.

The lambs were fed for an adaptation period of seven days in metabolic cages, followed by total faecal and urine collection for six days. Urine was collected in buckets containing 30 ml of dilute sulphuric acid (1:4). Duplicate aliquots of 1/100 of the total daily urine output for each animal were pooled in kjeldhal flasks containing 50 ml of concentrated sulphuric acid for 6 days for estimation of nitrogen after digestion. An aliquot of 1/100 of daily urine output for each animal was pooled into plastic containers for 6 days and preserved by deep freezing for analysis of minerals and energy. One twentieth of faeces voided daily was weighed and mixed with 10 ml of dilute sulphuric acid (1:4) and pooled in a previously weighed airtight glass stoppered bottles for 6 days. After mixing the pooled samples ten grams was taken for digestion and estimation of faecal nitrogen. Samples of feeds offered as well as feed residues and faecal output were taken daily for dry matter determination and bulked for eventual chemical analyses.

Proximate composition of feeds, feed residues and faecal samples were determined by methods described in AOAC (1984). Urea content in feeds was estimated by the methods described by Hogg (1967). The gross energy (GE) of feeds, faeces and urine were determined by an adiabatic oxygen bomb calorimeter. Methane production was estimated using the equation suggested by Swift et al. (1948), as quoted by Maynard and Loosli (1979). Tannin level in depulped neem seeds was determined by the procedure of Burns (1963), as modified by Price et al. (1978).

Data were subjected to a two-way analysis of variance with the main effects of source of protein (GNC vs UTNSKC) and sex (male vs female) (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Neem seed kernel cake was acidic and did not show major changes in pH when treated with urea for five days (Table 2). The drop in pH on the second day of treating GNC with urea might be due to production of acids by the fermentation of water-soluble carbohydrates. The rise in pH after the second day was probably due to depletion of water-soluble carbohydrates followed by the production of ammonia either from protein breakdown or urea hydrolysis. Low levels of residual urea (0.29% DM) after five days of treating GNC is indicative of urea hydrolysis. After 5 days of treatment, the residual urea was 2.49% DM in UTNSKC indicating that only 9.5% of urea was hydrolysed after treating the cake with 2.5% urea as compared to 89.6% after similarly treating GNC. Consequently, the crude protein (CP) content of UTNSKC

Table 1. Ingredient and chemical composition (%) of concentrate mixtures, jowar (Sorghum vulgare), neem seed kernel cake (NSKC), urea-treated neem seed kernel cake (UTNSKC) and groundnut cake (GNC)

	Concentrate mixture containing		Jowar	NSKC	UTNSKC	GNC
	GNC	UTNSKC				
Ingredient composition						
Maize	59	57				
GNC	30	0				
UTNSKC	0	33				
Wheat bran	8	7				
Mineral mixture*	2	2				
Salt	1	1				
Chemical composition						
Organic matter	92.38	90.00	89.06	84.34	83.50	92.09
Crude protein	19.42	20.20	7.84	33.68	40.33	43.48
Ether extract	2.52	4.85	1.25	8.60	8.46	1.23
Crude fibre	5.82	7.00	38.01	13.64	15.38	12.13
Nitrogen free extract	64.62	57.90	41.96	28.42	19.33	32.55
Calcium	0.93	1.07	0.36	-	0.68	-
Phosphorus	0.54	0.56	0.30	-	0.67	-
Ash	7.62	10.00	10.95	15.66	16.50	7.91
Urea	-	0.83	-	-	2.49	-

* Mineral mixture per 3 kg (in kg): dicalcium phosphate, 1.65; sodium chloride, 0.9; chalk, 0.3312; magnesium carbonate, 0.09; ferrous sulphate, 0.015; copper sulphate, 0.0021; manganese dioxide, 0.0021; cobalt chloride, 0.0015; potassium iodide, 0.0003; sodium fluoride, 0.0003 and zinc sulphate, 0.0075.

Table 2. Changes in pH and residual urea during urea-treatment of neem seed kernel cake (NSKC) and groundnut cake (GNC)

	Period (days)			
	0	2	4	5
	pH			
NSKC*	5.57	5.37	5.42	5.49
Urea-treated NSKC	5.57	5.57	5.57	5.34
Urea-treated GNC	7.15	5.52	6.96	7.17
	Residual urea (%DM)			
Urea-treated NSKC	2.73	-	-	2.49
Urea-treated GNC	1.04	-	-	0.29
	Crude protein (%DM)			
NSKC*	33.41	-	-	34.57
Urea-treated NSKC	40.02	-	-	39.99

* Soaked in water.

was increased by 6.65% DM. However, Reddy (1992) reported an increase in CP content of only 1.73%, despite treating NSKC with a higher level of urea (3.5%). The antiurease activity observed in this study is in agreement with the findings of Sahrawat (1982). Depressed activities of urease enzyme in the rumen liquor was also reported in animals fed UTNSKC (Musalia et al., 2000). The low pH during treatment in addition to the low level of urea hydrolysis, indicated that there was no ammoniation or alkali condition during treatment.

The increase in CP content after treating the cake had a diluting effect on the concentration of other nutrients, especially nitrogen free extract, which dropped by 32% (Table 1). The probable reason for high ash content in NSKC and UTNSKC is the unorganised method of collecting the seeds from the ground. The composite ration consumed was lower ($p<0.001$) in OM and NFE content but contained higher levels of EE for the ration based on UTNSKC (Table 3). These differences in chemical composition of dry matter intake were reflected in the composition of GNC and UTNSKC (Table 1). The composite ration consumed by the female lambs contained more CP ($p<0.05$) and NFE ($p<0.001$) which is attributed to low intake of jowar as compared to the male lambs.

Replacement of GNC with UTNSKC had no effect on palatability as is evident from similar intake of concentrate offered (Table 4). This is in agreement with earlier studies where NSKC (Rajagopal and Nath, 1981), water washed neem seed kernel cake (Agrawal et al., 1987; Nath et al., 1989) and UTNSKC (Reddy, 1992; Anandan et al., 1996; Musalia et al., 2000) totally replaced GNC in concentrate mixtures. Although lambs on the two rations were offered the same amount of concentrate, those on UTNSKC consumed more ($p<0.05$) jowar (30.48 vs 25.08 g/kg $BW^{0.75}$) leading to a nonsignificantly higher intake of dry matter (72.46 vs 66.26 g/kg $BW^{0.75}$). Gupta and Bhaid (1981) also reported higher intake of dry matter on feeding neem seed cake. Similarly the concentrate offered to males was comparable to the amount given to female lambs but females consumed less ($p<0.001$) jowar (22.13 vs 33.42 g/kg $BW^{0.75}$) compared to male lambs (Table 4) resulting in lower ($p<0.05$) intake of dry matter (64.56 vs 74.16 g/kg $BW^{0.75}$).

Apparent digestibilities of dry matter (63.7 vs 70.2%), crude protein (63.2 vs 70.2%), nitrogen free extract (73.8 vs 80.5%) and gross energy (64.3 vs 69.1%) were depressed ($p<0.01$) on feeding UTNSKC. In contrast, feeding UTNSKC increased ($p<0.01$) the digestibility of ether extract (70.9 vs

Table 3. The chemical composition, apparent digestibility and nutritive value of composite ration consumed by lambs offered concentrate mixtures containing either urea-treated neem seed kernel cake (UTNSKC) or groundnut cake (GNC)

	Protein source (P)		Sex (S)		Significance		
	GNC	UTNSKC	Male	Female	P	S	P×S
Chemical composition (%)							
Organic matter	91.6[a]	90.2[b]	90.9	90.9	***		
Crude protein	15.0	14.7	14.1[a]	15.5[b]		*	
Ether extract	2.5[a]	3.5[b]	3.1	3.2	***		
Crude fibre	15.0	16.3	16.9	14.3			
Nitrogen free extract	57.8[a]	56.8[b]	55.5[a]	59.2[b]	***	*	
Apparent digestibility (%)							
Dry matter	70.2[a]	63.7[b]	65.7	68.2	**		
Organic matter	72.1[a]	66.0[b]	67.7	70.4	**		
Crude protein	70.2[a]	63.2[b]	65.7	67.6	**		
Ether extract	70.9[a]	75.8[b]	70.5[a]	76.2[b]	*	**	
Crude fibre	43.4	41.3	43.7	41.1			
Nitrogen free extract	80.5[a]	73.8[b]	76.0	78.3	**		
Energy	69.1[a]	64.3[b]	65.4	68.1	**		
Nutritive value (% or Kcal/g DM)							
Digestible crude protein	10.5	9.3	9.3	10.5			
Total digestible nutrients	70.2[a]	64.7[b]	66.0	68.9	**		
Digestible energy (Kcal/g DM)	3.0[a]	2.8[b]	2.8	2.9	**		
Metabolizable energy (Kcal/g DM)	2.5[a]	2.3[b]	2.4	2.4	*		

[a,b] Means with different superscripts in a row between protein sources or sexes, differ significantly (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$).

Table 4. Utilisation of nutrients and plane of nutrition in lambs fed rations containing either urea-treated neem seed kernel cake (UTNSKC) or groundnut cake (GNC)

	Protein source (P)		Sex (S)		Significance		
	GNC	UTNSKC	Male	Female	P	S	P×S
Nitrogen (g/day)							
Intake	15.46	16.27	16.87	14.86			
Faecal loss	4.62	6.05	5.84	4.84			
Urinary loss	2.60	2.68	2.89	2.40			
Retained	8.23	7.53	8.14	7.62			
Energy (Kcal/day):							
Intake	2,801	3,032	3,244	2,589			
Faecal loss	872	1,092	1,136	829			
Methane loss[1]	248	242	257	234			
Urinary loss	48	53	56	46			
MEI[2]	1,632	1,645	1,796	1,481			
Daily intake (g or Kcal/kg $BW^{0.75}$) of:							
Concentrate	41.18	41.98	40.73	42.42			
Jowar	25.08[a]	30.48[b]	33.43[a]	22.13[b]	*	***	
Dry matter	66.26	72.46	74.16[a]	64.56[b]		*	
Gross energy	282.5	311.4	317.5	276.4			
Digestible energy	194.1	199.75	206.91	186.94			**
Metabolizable energy	163.68	168.88	175.66	156.9			
Digestible crude protein	7.72	7.48	7.55	7.65			

[1] Estimated from equation of Swift et al. (1948) as quoted by Maynard et al. (1979).
[2] Metabolizable energy intake.
[ab] Means with different superscripts in a row between protein sources or sexes, differ significantly (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$).

75.8%). Reddy (1992) and Anandan et al. (1996) did not observe any depression in apparent digestibility of dry matter by offering concentrate mixtures containing 30% and 22.5%, respectively, of UTNSKC. In the study of Anandan et al. (1996) the results may be attributed to the low intake of the cake (0.38% of live body weight) as compared to this study (0.78% of live body weight). Whereas high dry matter intake may be implicated in the variations of the digestibility of dry matter between diets, this may not be the case since the females which consumed less ($p<0.05$) dry matter had comparable digestibility to that of male lambs. Low digestibility of dry matter (Nelson et al., 1975) and depressed activities of enzymes (Griffiths and Mosley, 1980) have been attributed to high levels of tannin in the diet. Although the tannin levels were not determined in the rations fed to the animals in this study, analysis of depulped neem seeds revealed levels of 37% catechin equivalent. It is possible that the antinutritional factor in neem seed cake is due to high tannin content. Although it was speculated that ensiling NSKC with urea could give rise to alkali condition leading to the treatment of the cake (Reddy, 1992), this was not true in this study. Thus the antinutritional factors observed in this study and that of Musalia et al. (2000) may be due to residual tannin contents in UTNSKC. This needs further investigation.

Depression in digestibility of the ration containing UTNSKC resulted in low nutritive value of the composite ration consumed in terms of TDN (64.7 vs 70.2%) and DE (2.8 vs 3.0 Kcal/g DM). There was no difference between sexes in nutritive value of the ration consumed, which had similar digestibility. All animals in the study were in positive nitrogen, calcium, phosphorus and energy balance (Tables 4 and 5). Feeding UTNSKC lowered ($p<0.05$) the retention of phosphorus which agrees with the findings of Elangovan et al. (2000) on feeding neem kernel meal to Japanese quails (*Coturnix cotrnix japonica*). The intake of metabolisable energy and retention of nitrogen were not affected by differences in sex or diet. Animals in this study were on the same plane of nutrition in terms of daily intake of DCP and DE (Table 4). Since the ration containing UTNSKC was of low nutritive value (Table 3), it is possible that the animals on UTNSKC consumed more jowar to ensure comparable intake of DE with the lambs offered GNC which may be attributed to low supply of DE from concentrate mixture containing UTNSKC. The higher intake of jowar and retention of calcium by males may be attributed to their inherent higher growth rates as compared to females. Similarly the feeding regime was based on body weight to meet 70% of the protein requirement of animals. Since the protein requirements reduce with the body size of animal (NRC, 1985) it is possible that the smaller females received relatively more concentrate that met most of their energy requirements and in turn consumed less jowar as compared to the males.

IMPLICATIONS

Urea treatment of NSKC increased the crude protein content by 6.65% because of residual urea. Replacement of GNC with UTNSKC in the concentrate mixture depressed the

Table 5. Calcium and phosphorus balance in lambs fed rations containing either urea-treated neem seed kernel cake (UTNSKC) or groundnut cake (GNC)

	Protein source (P)		Sex (S)		Significance		
	GNC	UTNSKC	Male	Female	P	S	P×S
Calcium (g/day)							
Intake	4.80	5.71	5.68	4.82			
Faecal loss	2.91	3.52	3.40	3.03			
Urinary loss	0.046	0.058	0.040	0.064			
Retained	1.84	2.12	2.24^{a}	1.73^{b}		*	
Phosphorus (g/day)							
Intake	3.12	3.29	3.47	2.95			
Faecal loss	1.90^{a}	2.66^{b}	2.52	2.04	*		
Urinary loss	0.181^{a}	0.278^{b}	0.258	0.201	*		
Retained	1.05^{a}	0.37^{b}	0.70	0.71	**		

^{a,b} Means with different superscripts in a row between protein sources or sexes, differ significantly (* p<0.05 and ** p<0.01).

digestibility of nutrients and retention of phosphorus. The possibility of tannins being the antinutritional factor in NSKC needs further investigation.

REFERENCES

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

Anandan, S., V. R. B. Sastry, L. M. Musalia and D. K. Agrawal. 1996. Growth rate and nutrient efficiency of growing goats fed urea ammoniated neem (*Azadirachta indica*) seed kernel meal as protein supplement. Small Rumin. Res. 22:205-212.

AOAC. 1984. Official methods of analysis of the Association of Official Analytical Chemists. 13th ed. Association of Official Analytical Chemists, Washington, D.C. pp. 125-131.

Bedi, S. P. S., V. K. Vijjan and S. K. Ranjhan. 1975. Effect of neem (*Azadirachta indica*) seed cake on growth and digestibility of nutrients in crossbred calves. Indian J. Anim. Sci. 45:618.

Burns, R. E. 1963. Methods of tannin analysis for forage crop evaluation. Georgia Agric. Exp. Tech. Bull. No. 32:1-14.

Elangovan, A. V., S. V. S. Verma, V. R. B. Sastry and S. D. Singh. 2000. Effect of feeding neem (*Azadirachta indica*) kernel meal on growth, nutrient utilisation and physiology of Japanese quails (*Coturnix cotrnix japonica*). Asian-Aus. J. Anim. Sci. 13:1272-1277.

Garg, A. K. 1989. Studies on deoiled neem (*Azadirachta indica*) seed cake as cattle feed. Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, India.

Griffiths, D. W. and G. Mosley. 1980. Effect of diet containing field beans of high and low polyphenolic content on the activity of digestive enzymes in the intestine of rats. J. Sci. Food Agric. 31:255-259.

Gupta, R. S. and M. V. Bhaid. 1981. Studies on agro-industrial by-products: Deoiled neem fruit cake in sheep feed composition. Indian Vet. J. 58:311-315.

Hogg, M. L. 1967. Methods for the estimation of urea in foods. In: Urea as a Protein Supplement (Ed. M. H. Briggs). Pergamon press, London, pp. 455-461.

Katiyar, R. C., V. R. B. Sastry and D. K. Agrawal. 1991. Urea and alkali treated neem seed kernel cake as livestock feed. Annual report. Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, India.

Maynard, L. A., J. K. Loosli, H. F. Hintz and R. G. Warner. 1979. Animal Nutrition. 7th ed. McGraw-Hill Book Company, New York. p. 199.

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 Rumin. Res. 35:107-116.

Nagalakshmi, D. N. 1993. Performance of broiler chicks fed processed neem (*Azadirachta indica*) as a protein supplement. M.V.Sc. Thesis, Indian Veterinary Research Institute, Izatnagar, India, pp. 126-130.

Nath, K., D. K. Agrawal, Q. Z. Hassan and S. J. Daniel. 1989. Water washed neem (*Azadirachta indica* A. juss) seed kernel cake in the feeding of milch cows. Anim. Prod. 48:497-502.

Nath, K., S. Rajagopal and A. K. Garg. 1983. Water washed neem (*Azadirachta indica*) seed kernel cake as a cattle feed. J. Agric. Sci. Camb. 101:323-326.

Nath, K. and K. Vijjan. 1976. Palatability of alcohol treated neem seed cake. In: Utilisation of Neem (*Azadirachta indica* A. juss) and its by-products (Ed. C. M. Ketkar). Final Technical Report. Khadi and Village Industries Commission . Pune, India

Nelson, T. S., E. L. Stephenson, A. Burgos, J. Floyd and J. O. York. 1975. Effect of tannin content and dry matter digestion on energy utilisation and average amino acid availability of hybrid sorghum grains. Poult. Sci. 54:1620-1623.

NRC. 1985. Nutrient requirements of sheep. National Academy Press, Washington DC.

Price, M. L., S. Van Scoyoc and L. G. Butler. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26:1214-1218.

Rajagopal, S. and K. Nath. 1981. Note on the nutritive value of cake of neem seed kernel. Indian J. Anim. Sci. 51:661-663.

Reddy, K. V. C. 1992. Performance of buffalo (*Bubalus bubalis*) calves fed alkali treated and urea-ammoniated neem (*Azadirachta indica*.A.juss) seed kernel cake incorporated rations. M.V.Sc thesis, Indian Veterinary Research Institute, Izatnagar, India.

Sahrawat, K. L. 1982. Comparative evaluation of Karanjin and extracts of Karanja and neem seeds for retardation of nitrification of urea in soil. J. Indian Soc. Soil Sci. 30:156-159.

Snedecor, G. W. and W. G. Cochran. 1989. In: Statistical methods, 6th ed. Oxford and IBH Publishing company, New Delhi.

Swift, R. W., E. J. Thacker, A. Black, J. W. Bratzler and W. H. James. 1947. Digestibility of rations for ruminants as affected by proportion of nutrients. J. Anim. Sci. 6:432.

Vijjan, V. K., H. C. Tripathi and N. S. Parihar. 1982. A note on the toxicity of neem (*Azadirachta indica*) seed cake in sheep. J. Environ. Biol. 3:47.