BLOOD BIOCHEMICAL PROFILE AND HISTOPATHOLOGY OF VITAL ORGANS IN RABBITS FED ON PROCESSED NEEM (*Azadirachta indica*) KERNEL MEAL INCORPORATED DIETS

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Summary

Blood biochemical and histopathological changes in vital organs of rabbits were studied after 18 wk of feeding composite diets (75 concentrate : 25 roughage) incorporating either urea (2%, wt/wt) ammoniated or alkali (1.5%, wt/wt) treated neem kernel meal (NKM) replacing peanut meal protein of control diet by either 50 or 100%. The blood biochemical constituents (Haemoglobin, Alanine amino transferase, Aspartate amino transferase, Total protein, Blood urea nitrogen & Cholesterol) in rabbits fed on processed NKM diet at either levels, were comparable to the values of those on control diet except a lowered ($p<0.05$) blood glucose concentration in processed NKM fed rabbits as compared to that in control diet fed ones. Histological examination revealed increased goblet cell activity, stunting of jejunal villi, mild tubular degeneration in kidney and hepatic fibro-cellular reaction in rabbits fed on urea ammoniated and alkali treated NKM diets with less marked changes in the latter. Testicular changes with variable degree of disorganization and vacuolation of spermatogonial cells were noticed in rabbits fed higher levels of urea-ammoniated and alkali treated NKM. Thus, alkali treatment and urea ammoniation were effective in detoxification of meal, but the processing technology is to be further perfected to prevent cumulative effect of residual neem bitters in long term feeding.

(**Key Words** : Blood, Histopathology, Rabbits, Neem)

Introduction

Neem (*Azadirachta indica*) kernel meal (NKM), a by-product of oil industry is available to the extent of 0.7 million tonnes per year in India. Inspite of a rich protein (CP: 34-37%) source, its use in livestock feeding is restricted due to the presence of toxic and bitter triterpenoids (Devkumar and Sukhdev, 1993). Water washing the meal (Nath et al., 1983) was found promising in detoxifying, except for 22% DM loss. Alternate methods like alkali treatment and urea-ammoniation (Katiyar et al., 1992) without water washing were of considerable significance in the feeding of large (Reddy, 1992) and small ruminants (Musalia, 1994; Anandan, 1994) as well as in broiler poultry (Nagalakshmi, 1993) without inducing any adverse effects on blood biochemical constituents and histopathological changes in vital organs. However, the efficacy of these processing methods are to be tested in the biological system through long term feeding. Thus, an attempt has been made to investigate the blood biochemical profile and histopathological changes in target organs due to long term (18 wk) feeding of NKM detoxified by urea-ammoniation and alkali treatment in New Zealand white (NZW) and Angora rabbits.

Materials and Methods

Processing of neem kernel meal

Urea-ammoniated NKM (UANKM) was prepared by ensiling the meal in water (1:1.2, wt/vol) dissolved with fertilizer grade urea (2%, wt/wt) in an air tight steel silo for five days. Alkali treatment (ATNKM) was done by soaking the meal in water (1:1.2, wt/vol) containing NaOH (1.5%, wt/wt) overnight in a cement trough. These processed meals were sundried and ground before their incorporation into the composite rabbit diets.

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Animals and feeding

Thirty Angora and 20 NZW male rabbits of six wk age were randomly alloted equally to five composite diets (D1 to D5) prepared as per NRC (1977) requirements for growing rabbits (table 1). The deoiled peanut meal (DPNM) of control diet (D1) was replaced by either UANKM (D2 & D3) or ATNKM (D4 & D5) @50 and 100%. The rabbits were individually fed *ad lib.* up to 24 wk of age by housing them in self contained metallic cages (0.6 × 0.6 × 0.5 m^3) located in well ventilated barn. They were dewormed (0.1% mebendazole) and protected against coccidiosis (0.05% sulfaquinoxaline) regularly. Daily fresh water was made available through out the experimental period.

TABLE 1. PERCENT INGREDIENT COMPOSITION OF DIETS*

Ingredient	Diet				
	D1	D2	D3	D4	D5
Oat hay (ground)	12.5	12.5	12.5	12.5	12.5
Jowar hay (ground)	12.5	12.5	12.5	12.5	12.5
Maize	38.0	38.5	38.5	38.0	38.0
DPNM	14.0	7.0	–	7.0	–
UANKM	–	7.5	15.0	–	–
ATNKM	–	–	–	9.25	18.5
Fish meal	1.5	1.5	1.5	1.5	1.5
Wheat bran	12.5	12.0	11.5	10.25	9.0
Cane molasses	6.0	5.5	5.5	6.0	5.0
Mineral mix.	2	2	2	2	2
Salt	1	1	1	1	1
Calculated (%) CP	16.11	16.03	16.00	15.90	15.80
TDN	64.74	65.15	64.60	64.63	65.95
CF	10.10	10.15	11.50	10.70	11.70
CP from Veg. meal (%)	39.20	39.30	39.37	39.40	40.60

* Liquid vitamin supplement: Vitamin A- 12,000 IU; D_3- 6,000 IU; E- 48 mg; B_{12}- 20 mcg; 5 ml/100 ml of drinking water, once a wk.

Tissue and blood examination

The rabbits under all the five dietary regimes were sacrificed at the end of 18 wk of feeding to collect blood (EDTA @2 mg and sodium fluoride @5 mg/ml, separately) and target vital organs (intestine, kidney, liver, spleen, testes, lung and heart), which were preserved in 10 % formal saline. The representative tissues from each organ were fixed in paraffin wax, sectioned and stained with Haematoxylene & Eosin (Culling, 1963) for detailed microscopic examination. The blood or its plasma was subjected to the estimation of haemoglobin (Hb%: Oser, 1965) and glucose (Cooper and McDaniel, 1970), activities of alanine & aspartate amino transferases (ALT & AST: Reitman and Frankel, 1957), total protein (Biuret, 1949), urea-nitrogen (Rahmatullah and Boyde, 1980) and cholesterol (Wybenga et al., 1970). The data pertaining to blood analysis were tested for statistical significance (Snedecor and Cochran, 1967).

Results and Discussion

Blood biochemical profile

The results presented in table 2 show that the observed blood Hb concentration among rabbits on different dietary treatments did not differ significantly and were within the range (8 to 12 g%) reported in rabbits (Loeb and Quimbly, 1989), unlike depressed levels noticed due to feeding of raw neem seed meal in poultry (Chand, 1987) indicating that the factors responsible for decreasing Hb levels were dissociated during processing of meal with both alkali and urea. But the level of alkali as well as urea and the length of processing period appears to be insufficient to suppress the anti-hyperglycaemic factors usually reported with feeding of neem products in rabbits (Pillai and Santakumari, 1981) and guinea pigs (Murthy et al., 1978) as evidenced by significantly ($p < 0.05$) low glucose levels in rabbits on either of the processed meals even at low level of dietary incorporation in the present study against the normal range (90-110 mg%) seen in rabbits. The activities of ALT & AST were though comparable between treatments, they varied widely among rabbits in a given dietary treatment as indicated by higher standard error of each mean. The activity of transaminases is sensitive to myocardial infarction, hepato-cellular diseases and nephrosis (Oser, 1971) with toxic feed supplements (Camp et al., 1967). This further showed that residual neem bitters left after processing are not sufficient enough to vary the activity of these blood enzymes. The plasma total protein levels observed among rabbits on different diets were within the normal range (7-8 g%) which indicated that protein of neem meal after processing was wholesome to support optimum protein metabolism without producing any kidney or liver disorder normally seen with protein-energy malnutrition (Kaneko, 1989). This view was supported by the presence of normal blood urea nitrogen (BUN) levels among rabbits on different diets showing renal sufficiency (Lameire et al., 1977). Plasma cholesterol concentration, which is normally affected by physiological status of thyroid, was comparable among rabbits on all diets. Debitterization and detoxification of neem kernel meal by

either NaOH treatment or urea-ammoniation were thus evident by normal biochemical functioning of the important vital target organs in the rabbit biological system.

TABLE 2. BLOOD BIOCHEMICAL PROFILE

Attribute	Treatment mean ± SE				
	D1	D2	D3	D4	D5
Haemoglobin (g%)	9.8	10.4	10.1	10.3	10.5
	0.31	0.43	0.21	0.30	0.30
Glucose (mg%)	94.9^{a}	84.2^{ab}	78.5^{b}	77.4^{b}	76.9^{b}
	5.22	3.90	2.16	1.30	0.91
ALT (U/ml)	33.7	30.0	18.0	20.3	19.3
	4.87	6.63	5.94	4.21	3.33
AST (U/ml)	34.5	60.0	52.8	38.8	42.7
	1.65	10.70	10.61	1.25	13.13
Total protein (g%)	8.7	9.0	9.1	8.7	8.9
	0.20	0.38	0.15	0.33	0.20
Urea-N (mg%)	8.7	8.5	7.6	9.7	6.7
	0.80	0.98	0.81	1.00	1.20
Cholesterol (mg%)	63.9	61.8	52.2	67.4	80.8
	8.61	2.95	4.82	8.32	15.41

^{a,b} Means with different superscripts in a row differ significantly : $p < 0.05$.

Histopathological studies

Gastro-intestinal tract (GIT) :

Feeding of urea-ammoniated NKM at both 50 (D2) & 100 (D3) % levels of DPNM replacement led to slight mucosal irritation resulting in enhanced goblet cell activity (figure 1) with stunting of villi and appreciable mucosal thinning along with submucosal oedema in duodenum. Except for jejunal villi stunting at 50% replacement level (D4), the above changes were not associated with alkali treated NKM feeding. However, these pathological changes were not sufficient enough to induce any adverse effect on normal feed intake, nutrient digestibility and growth of the experimental rabbits in the present investigation. Since the dissociation of urea to constituent ammonia is a slow process providing mild alkaline medium, the selected level of urea (2%, wt/wt) and length of processing in the present study, as compared to NaOH treatment, appears to be insufficient for complete detoxification of NKM.

Figure 1. Intestine (D3) : Increased goblet cell activity (arrow) with stunting of villi. H & E × 230.

Kidney :

. Mild tubular degeneration with slight fibrocellular reaction were less pronounced at lower levels of processed NKM incorporation. However, even these nephrotic changes appear to be very mild to manifest nephritis associated with higher BUN levels, which was not noticed on these processed NKM incorporated diets. Moreover, the noticed mild effects would usually be compensated by tremendous reserve capacity of nephrons (Lameire et al., 1977).

Liver :

Cellular architecture of liver remained normal in rabbits on processed NKM incorporated diets and was found comparable to that on control diet, except for moderate fibrocellular reaction in portal areas with slight epithelial proliferation in bile duct. These changes due to residual neem bitters or their dissociated factors that remained after processing of meal can be viewed as mild, since birds fed on 30% raw neem seed meal containing diets exhibited paler visceral organs with fatty changes in liver and kidney (Christopher et al., 1976). Such changes were, however, could not be noticed in birds fed neem seed meal incorporated diets after alcohol extraction (Chand, 1987) similar to the finding of Reddy (1992) and Khan (1994) with UANKM (3.5% urea, wt/wt) and ATNKM (2.5% NaOH, wt/wt) feeding at 30 & 18% level of incorporation in buffaloes and rabbits, respectively. This shows that the reduction of urea & alkali respectively to 2 & 1.5% for processing the meal in the present study appeared to be inadequate for complete detoxification and/ debitterization. However, in the case of ruminants, the detoxification of residual neem bitters left after processing just like gossypol (Hungate, 1966) and mimosine (Kumar et al., 1987), cannot be ruled out.

Testis :

The testicular changes were well pronounced in comparison to control (figure 2) with increase in the level of incorporation of processed meals. Homogenous fluid material in the interstitium with mild disorganization of spermatogonial cells (figure 3) was noticed in rabbits fed diets of 50% UANKM (D2) & ATNKM (D4). Whereas, testis of those fed 100% UANKM (D3) diet exhibited variable degree of spermatogonial cell degeneration and disruption of their orderly arrangement. Some of the semeniferous tubules assumed irregular shape with reduced number of spermatozoa (figure 4). These changes were more pronounced in the testis of rabbits fed 100% ATNKM (D5) diet which varied from vacuolation and clumping of cells to formation of a sheet like pattern in the lumen (figure 5). Vijjan and Parihar (1983) also observed testicular degeneration in rats fed 10% raw neem seed cake. Feeding raw, water washed and solvent extracted NKM at 14% level in the diet to male rats for 50 days also resulted in sluggish spermatogenic activity (Garg et al., 1992). In light of the above observations, the work carried out in further perfecting the processing technology revealed no such reproductive degenerations in both sexes of sheep and goat fed 2.5% urea treated NKM from three months of age till lambing/kidding (Mahanta et al., 1995).

Lungs, heart and spleen :

The changes in lungs, heart and spleen were inappreciable as the cellular pattern of tissues being

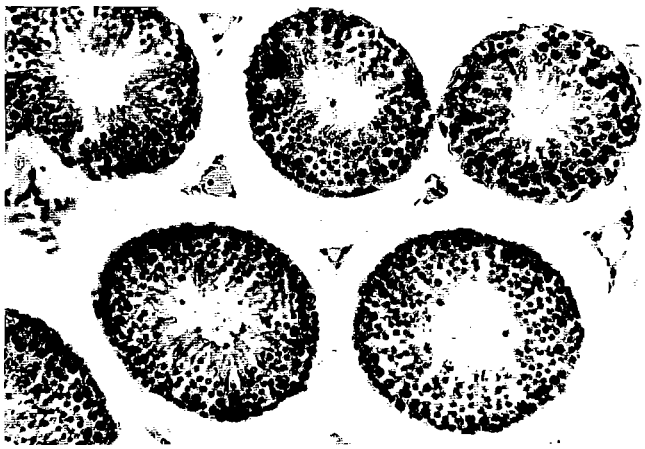

Figure 2. Testis (D1) : Orderly arrangement of cells with normal spermatogenic activity. H & E × 300.

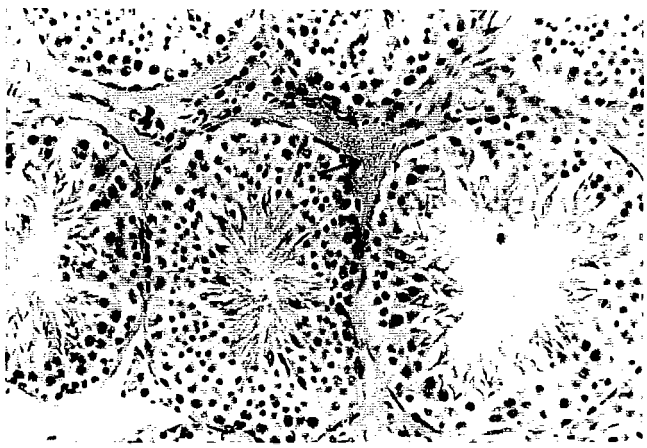

Figure 3. Testis (D2) : Homogenous fluid material (arrow) in the in terstitium with mild disorganization of spermatogonial cells. H & E × 300.

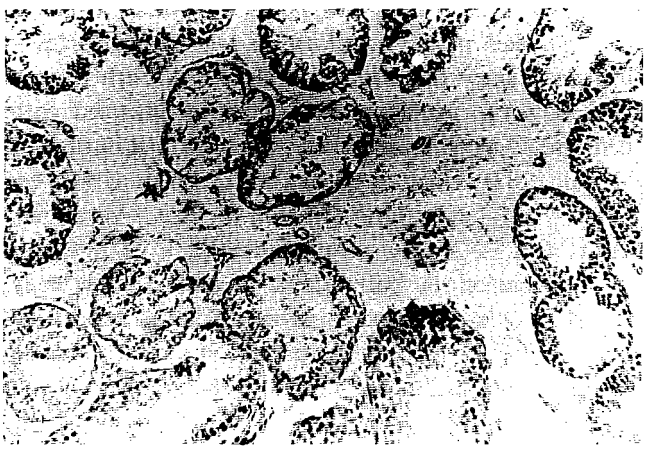

Figure 4. Testis (D3) : Disruption of orderly arrangement of spermatogonial cells with degeneration. H & E × 110.

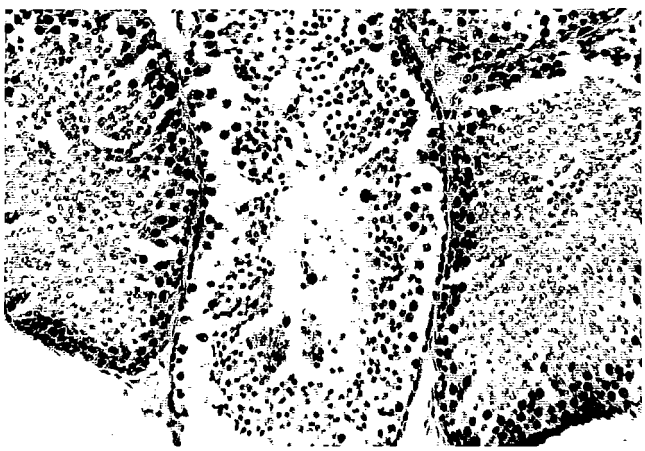

Figure 5. Testis (D5) : Vacuolation and clumping of spermatogonial cells. H & E × 300.

comparable between control and experimental diets fed rabbits.

Conclusions

The normal blood profile, except for hypoglycaemic effect, and mild histopathological changes in some of the vital organs due to long term feeding of treated NKM indicated the need for perfecting the processing technology with respect to level of urea and alkali to avoid any cumulative effect of residual neem bitters.

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Literature Cited

Anandan, S. 1994. Urea- ammoniated neem (*Azadirachta indica*) kernel cake as a protein supplement for growing kids. Ph. D. Thesis, IVRI., Izatnagar, India.

Biuret. 1949. (c.f. G.E. Delory, 1966). In: Photoelectric Colorimetry in Clinical Biochemistry; Higgler and Watts Ltd., London.

Camp, B. J., E. Steel and J. W. Dollahite. 1967. Certain biochemical changes in blood and liver of rabbits fed oak tannins. Am. J. Vet. Res. 28:290-292.

Chand, S. 1987. Nutrient evaluation of neem seed meal in chicks. Ph. D. Thesis, Rohilkhand University, Bareilly, India.

Christopher, J., N. Ahmed and G. A. Sastry. 1976. Effect of feeding deoiled neem seed cake to chickens (c.f. Ketker, 1976; Utilization of neem and its by-products. Final technical report. Khadi and village industries commission, Pune, India).

Cooper, G. R. and V. McDaniel. 1970. Standard Methods of Clinical Chemistry, (ed.) Slinger, Academic Press. p. 159.

Culling, C. F. A. 1963. Handbook of histopathological techniques, 2nd ed. Butterworths pub.

Devkumar, C. and Sukhdev. 1993. Chemistry. In: Neem research and development, Eds. Randhawa, N. S. & Parnar, B. S. Publication No. 3. society of pesticide science, India, pp. 63-96.

Garg, A. K., D. K. Agrawal, S. D. Singh and K. Nath. 1992. Growth and spermatogenesis in rats fed solvent extracted water washed and untreated neem (*Azadirachta indica*) seed kernel cake. Int. J. Anim. Sci. 7:223-225.

Hungate, R. E. 1966. The rumen and its microbes. Academic press, New York: p. 439.

Kaneko, J. J. 1989. Clinical biochemistry of domestic animals. 4th ed. Academic press, Inc., San Diego, California.

Katiyar, R. C., V. R. B. Sastry and D. K. Agrawal. 1992. Annual report, Animal nutrition Division, IVRI, Izatnagar, India.

Khan, M. H. 1994. Performance of growing rabbits on different conventional and unconventional oil seed cakes incorporated diets. Ph. D. Thesis, IVRI, Izatnagar, India.

Kumar, S., A. Singh, V. K. Saxena, G. S. Mann and S. Singh. 1987. Degradation of mimosine by rumen fluid and its fractions from buffalo. Indian J. Anim. Sci. 57:1128-1131.

Lameire, N. H., M. D. Lifschitz and J. H. Stin. 1977. Heterogenicity of nephron function. Ann. Rev. Phy. 39:159-184.

Loeb, W. F. and F. W. Quimbly. 1989. The Clinical Chemistry of Laboratory Animals, pergamon press, p. 50.

Mahanta, S. K., R. C. Katityar, V. R. B. Sastry and D. K. Agrawal. 1995. Effect of urea-ammoniated neem kernel cake on reproduction performance and wool growth in crossbred sheep. Abst. No. 136, Animal Nutrition Workers Conf, Bombay, India.

Murthy, K. S., D. N. Rao, D. K. Rao and L. B. G. Murthy. 1978. A preliminary study on hypoglycaemic and antihyperglycaemic effects of *Azadirachta indica*. Indian J. Pharmacol. 10:247-250.

Musalia, L. M. 1994. Performance of growing lambs fed urea ammoniated neem (*Azadirachta indica*) seed kernel cake. Ph. D. Thesis, IVRI, Izatnagar, India.

Nagalakshmi, D. 1993. Performance of broiler chicks fed processed neem (*Azadirachta indica*) seed kernel cake as protein supplement. M. V. Sc. Thesis, IVRI, Izatnagar, India.

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

N. R. C. 1977. Nutrient requirements of rabbits. (2nd ed.), Washington. D. C.

Oser, B. L. 1965. In : Hawk's Physiological Chemistry, (12th ed.), Tata McGraw Hill Pub. Co., New Delhi.

Oser, B. L. 1971. In : Hawk's Physiological Chemistry (14th ed.). Tata McGraw Hill pub. Co. Ltd., New Delhi.

Pillai, N. R. and G. Santakumari. 1981. Hypoglycaemic activity of *Melia azadirachta*. Indian J. Pharamacol. 13:91.

Rahmatullah, M. and T. R. C. Boyde. 1980. Improvement

in the determination of urea using diacetylmonoxime method with or without deproteinisation. Clinical Chemica Acta. 107:3-8.

Reddy, K. C. V. 1992. Performance of buffalo calves fed alkali treated and urea-ammoniated neem (*Azadirachta indica*) seed kernel cake incorporated rations. M. V. Sc. Thesis, IVRI, Izatnagar, India.

Reitman, S. and S. Frankel. 1957. A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28:56-63.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods (6th ed.). Oxford and IBH Pub. Co., New Delhi.

Vijjan, V. K. and N. S. Parihar. 1983. Toxic effects of neem (*Azadirachta indica*) seed cake feeding in rats. J. Environ. Biol. 4:39-41.

Wybenga, D. R., V. J. Pilegi, P. H. Dirstine and J. DiGiorgio. 1970. Direct manual determination of serum total cholesterol with a single stable reagent. Clinical Chemistry. 16:980-982.