Fermentation Pattern and Enzymatic Activity in Caecum of Rabbits Fed Processed Neem (*Azadirachta indica*) Kernel Meal Incorporated Diets

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ABSTRACT: A caecal fermentation study was conducted in 30 Angora rabbits equally placed under five whole diets (75 concentrate : 25 roughage) supplemented with processed Neem (*Azadirachta indica*) kernel meal (NKM ; 2% urea or 1.5% NaOH, W/W), replacing isonitrogenously either at 50 or 100% level of deoiled peanut (*Arachis hypogea*) meal, for 18 wk period. The total volatile fatty acids level was depressed ($p < 0.05$) in all the experimental groups (0.02 to 0.04mEq/g) and ammonia-nitrogen was lowest ($p < 0.05$; 24.9 μmol/g) in rabbits fed NaOH treated NKM supplemented diet. Enzyme activity (unit/g) of carboxy methyl-cellulase, α-amylase, protease and urease exhibited much variation and did not differ significantly. Hence, the results could not confirm a possible adverse effect of feeding NKM on caecal fermentation.

(**Key Words**: Caecal Fermentation, Rabbits, Neem Kernel Meal)

INTRODUCTION

The caecal microbial fermentation plays a significant role in nutrient utilization by the rabbit (Cheeke, 1987). Volatile fatty acids produced in the caecum contribute 12 to 40% of energy requirement for maintenace in adult rabbits (Marty and Vernay, 1984). But ammonia of dietary origin is the principle source for caecal microbial protein synthesis. Neem (*Azadirachta indica*) kernel meal (NKM) can serve as an alternate vegetable protein supplement (crude protein (CP): 34-37%) in India. However, its use in raw form for livestock feeding is discouraged due to the presence of deleterious and bitter triterpenoids (Azadirachtin, Nimbin) (Singh, 1993). Feeding of NKM to crossbred calves (Mondal et al., 1995) and rats (Garg et al., 1984) resulted in adverse effect on rumen fermentation and growth, respectively. However, urea-ammoniation and alkali soaking of NKM was found to improve its palatability and utilization (Nagalakshmi, 1993). Similarly processed NKM was included in rabbit diet in the present experiment for caecal fermentation study to ascertain the effect of feeding NKM.

MATERIALS AND METHODS

Proximate composition

The proximate composition of NKM was estimated as per AOAC (1980).

Feed processing

Urea-ammoniation of NKM (UANKM) was done by ensiling the meal in water (1 : 1.2, W/V) dissolving 2% fertilizer grade urea (W/W) for six days in an air tight silo before it was sundried and ground. While alkali treatment of NKM (ATNKM) was done by soaking in water (1 : 1.2, W/V) dissolved with 1.5% NaOH (W/W) for 24 hr, followed by sundrying and grinding.

Experimental design

Thirty Angora rabbits of six wk age were randomly alloted in equal numbers to five whole diets (75 concentrate : 25 roughage) computed to contain 16% CP, 10.46 MJ/kg digestible energy (DE), 10 to 12% crude fibre (CF) and 15 to 20% acid detergent fibre (ADF) as per the standards of National Research Council (1977) for growing rabbits (table 1). The nitrogen moeity of deoiled peanut meal (DPNM, control: D_1) was respectively replaced by 50 or 100% with either UANKM (D_2, D_3) or ATNKM (D_4, D_5). After 18 wk of experimental feeding, rabbits were sacrificed and caecal content were collected individually in ice cold condition.

Processing of caecal content

After recording pH of homogenously mixed caecal content, 3 g were diluted with 9 ml of distilled water, mixed thoroughly before centrifuging at 3,000 rpm for 10 min to obtain a caecal liquor (CL). Suitable aliquots of

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CL for the estimation of total volatile fatty acids (TVFA) and ammonia-nitrogen (NH_3-N) were stored after respectively adding two drops of $HgCl_2$ and 1 : 4 H_2SO_4 in plastic vials at −20℃. Another 3 g of caecal content were diluted with 9 ml of phosphate buffer (0.05 M, pH 7), ultrasonicated at 8 microns for 10 min at ice cool temperature and centrifuged at 10,000 rpm for 15 min at 4℃. The supernatent was utilized for different enzyme analysis in duplicate.

Table 1. Ingredient composition of diets (g/kg as fed basis)

Ingredient	Diet				
	D_1	D_2	D_3	D_4	D_5
Oat hay	125	125	125	125	125
Jowar hay	125	125	125	125	125
Maize (crushed)	380	385	385	380	380
DPNM	140	70	–	70	–
UANKM	–	75	150	–	–
ATNKM	–	–	–	92.5	185
Fish meal	15	15	15	15	15
Wheat bran	125	120	115	102.5	90
Cane molasses	60	55	55	60	50
Mineral mix[1].	20	20	20	20	20
Salt	10	10	10	10	10
Calculated (g or M J/kg)					
CP	161.1	160.3	160.0	159.0	158.0
DE	10.36	10.41	10.38	10.30	10.45
CF	101.0	101.5	115.0	107.0	117.0
ADF	201.0	172.5	151.8	149.0	164.0

DPNM : Deoiled peanut meal, UANKM : Urea-ammoniated neem kernel meal, ATNKM : Alkali treated NKM.
[1] Contained Ca – 28%, P – 12%, Fe – 0.5%, I – 0.026% Cu – 0.077%, Mn – 0.01%, Co – 0.013%, Zn – 0.18%. Liquid vitamin supplement (Vitamin A – 12,000 IU ; D_3 – 6,000 IU ; E – 48 mg; B_{12} – 20 μg per ml) – 5 ml per 100 ml of drinking water of each rabbit, once a week.

Analytical methodology

The TVFA (mEq/g) and NH_3-N (μmol/g) of caecal content were analysed as per Barnet and Reid (1956) and Pearson and Smith (1943), respectively. Activity (μmol/min/g) of Carboxy methyl (CM) – cellulase and α-amylase were estimated as per Miller (1959) respectively, with CM-cellulose and 1% starch as substrates. The protease (mg/hr/g) was estimated as the activity required to solubilise 1 mg of casein in 1 hr under standard assay condition and thus obtained trichloroacetic acid soluble protein was estimated (Lowry et al., 1951). Urease (μmol/min/g) was estimated as the enzyme activity required for liberating 1 μmol of NH_3 from urea under standard assay condition (Bergmeyer, 1974).

Statistical analysis

The data were subjected to the analysis of variance in one way classification for completely randomised design as per Snedecor and Cochran (1967). The test of significance among the treatment differences was analysed by Duncan multiple range test in a micro 32 computer.

RESULTS

Proximate composition of NKM

Laboratory analysis of NKM revealed 36% CP, 15.5% CF, 4.8% ether extract and 15.8% total ash. Alkali and urea-ammoniation did not change the chemical composition of NKM except the later increased its CP value to 40%.

Caecal pH, TVFA and NH_3-N

The TVFA and NH_3-N concentrations were significantly ($p < 0.05$) lowered in NKM fed group of rabbits with no significant change in pH value. Activities of CM-cellulase, α-amylase, protease and urease enzymes among the different dietary groups were statistically comparable (table 2).

Table 2. Caecal fermentation pattern and enzymatic activity

Attribute	Dietary treatement (Mean)					SEM	Significance
	D_1	D_2	D_3	D_4	D_5		P
pH	6.2	6.2	6.1	6.1	6.2	0.03	N.S.
TVFA (mEq/g)	0.05^{a}	0.04^{b}	0.04^{b}	0.02^{c}	0.03^{bc}	0.002	< 0.05
NH_3-N (μmol/min/g)	53.1^{a}	43.1^{ab}	48.6^{ab}	30.6^{bc}	24.9^{c}	3.31	< 0.05
CM− Cellulase (μmol/min/g)	1.43	1.03	1.40	1.23	1.09	0.11	N.S.
α −Amylase (μmol/min/g)	4.57	4.33	4.18	5.38	4.78	0.48	N.S.
Protease (mg/hr/g)	8.49	7.18	6.69	7.72	7.70	0.81	N.S.
Urease (μmol/min/g)	9.50	9.37	14.03	13.54	14.10	0.97	N.S.

SEM : Standard error of means ; N. S. : Non-significant.

DISCUSSION

Caecal pH, TVFA and NH_3-N

The pH of rabbit caecal content under normal conditions was found close to 6.5 (Vernay et al., 1984) and is influenced by the concentration of TVFA and NH_3-N, which will inturn be dictated by type of diet, time of feeding and rate of caecotrophy (Santoma et al., 1989). The caecal pH in rabbits was not affected by dietary variation despite significantly ($p < 0.05$) higher TVFA in rabbits on DPNM incorporated diet (D_1) and lower NH_3-N on ATNKM containing diets (D_4 & D_5), with comparable values between DPNM and UANKM included diets. The NKM as such or after ammoniation by urea also depressed the TVFA concentration in large (cow calves) and small (lambs) ruminants (Garg, 1989; Musalia, 1994). The availability of more soluble and easily digestible carbohydrates in DPNM than in NKM might explain for higher TVFA concentration in caecum of those on DPNM diet. However, the values were within the normal range (0.02 to 0.05 mEq/g) and comparable to that in rabbits on diets of varying levels of protein and energy (Deshmukh, 1989). On the other hand, reduced ($p < 0.05$) NH_3-N in caecal content of rabbits on ATNKM diets in spite of comparable pH and urease activity could not be attributed to dietary variation. Hence, further detailed investigations are required before arriving at a reasonable conclusion as caecal fermentation is influenced by type of diet, rate of passage, caecal retention of digesta besides caecotrophy (Cheeke, 1987).

Caecal enzymes

The higher standard error of means (SEM) on some of the diets for CM-cellulase, α-amylase, protease and urease due to individual variation nullified the dietary effect. However, the anti-urease action observed in NKM (Reddy and Prasad, 1975) and depressing effect on cellulase, protease and urease activities even after processing by urea-ammoniation (Musalia, 1994) could not be observed in the present study. In addition, the experimental feed was equally palatable as indicated by comparable feed intake (66-68 g/day), with no adverse effect on growth performance. Hence, this study could not confirm a possible adverse effect on caecal fermentation due to NKM feeding in rabbits.

REFERENCES

A. O. A. C. 1980. Official methods of analysis (12 th ed.). Association of Official Analytical Chemists. Washington, D. C., U. S. A.

Barnet, J. C. A. and R. L. Reid. 1956. Studies on the production of volatile fatty acids from the grass by rumen liquor in an artificial rumen. J. Agric. Sci (Camb.). 48:315-321.

Bergmeyer, H. V. 1974. In : Methods of Enzymatic Analysis. Vol. 2. Academic press, Inc., U. S. A, pp. 320-321.

Cheeke, 1987. Rabbit Feeding and Nutrition. Orlando, F. L. Academic press. pp. 82-83.

Deshmukh, S. V. 1989. Growth performance and carcass characteristics in New Zealand White rabbits as influenced by different planes of nutrition. Ph. D. Thesis, IVRI, Izatnagar, India. p. 255.

Garg, A. K., S. D. Singh, D. K. Agrawal and K. Nath. 1984. Effect of feeding processed neem kernel cake on growth and reproductive development a male rats. In: Society of Biological Chemists India. p. 150.

Garg. A. K. 1989. Studies on deoiled neem (*Azadirachtaindica*) seed cake as cattle feed. Ph. D. Thesis. IVRI, Izatnagar, India. p. 173.

Lang, J. 1981. The nutrition of commercial rabbit. Part-I. Physiology, digestibility and nutrient requirements. Nutr. Abst. Rev. (B). 51:179-225.

Lowry, O. H., N. J. Rosabrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193:265-275.

Marty, J. and M. Vernay. 1984. Absorption and matabolism of the volatile fatty acids in the hind gut of rabbit. Br. J. Nutr. 51:265-277.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Ann. Chem. 31:426-428.

Mondal, D. P., A. K. Garg, B. C. Mondal and N. N. Pathak. 1995. Effect of feeding neem kernel cake on ruminal protozoal assay. Abst. No. 137, 7th Animal Nutrition Workers Conf. Bombay, India.

Musalia, L. M. 1994. Performance of growing lambs fed urea-ammoniated neem (*Azadirachta indica*) seed kernel cake. Ph. D. Thesis. IVRI, Izatnagar, India. p. 74.

Nagalakshmi, D. N. 1993. Performance of broiler chicks fed processed neem kernel cake as protein supplement. M. V. Sc. Thesis, IVRI, Izatnagar, India. pp. 50-60.

National Research Council. 1977. Nutrient Requirements of Rabbits. 2nd ed., National Academy of Science. Washington, D. C.

Pearson, R. M. and J. A. B, Smith. 1943. The utilisation of urea in bovine rumen. Biochem. J. 37:148-152.

Reddy, R. N. S. and R. Prasad. 1975. Studies on the mineralisation of urea and nitrification inhibitor treated urea in soil. J. Soil Sci. 26:304-312.

Santoma, G., J. C. De Blas, R. Carabano and M. J. Fraga. 1989. Nutrition of rabbits. In : Recent Advances in Animal Nutrition. Eds. Haresign, W. and Cole, D. J. A. Butterworths, pub.

Singh, K. 1993. Livestock Production and Health. In : Neem Research and Development, Eds. Randhawa, N. S. and Parmar, B. S. pub No. 3. Society of pesticide science, India, pp. 187-198.

Snedecor, G. W. and W. G. Cochran. 1967. In : Statistical Methods. 6 th ed., Oxford and I. B. H. pub., New Delhi, pp. 258-265.

Vernay M., J. Marty and J. Moatil. 1984. Absorption of electrolytes and volatile fatty acids in hind gut of the rabbit, circadian rhythm of hind gut, electrolytes and plasma aldosterone. Br. J. Nutr. 52:419-428.