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Incriminating factors in neem seed kernels and *in vitro* evaluation of graded levels of raw and detoxified neem seed cake based concentrate mixtures

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Raw neem seed cake as a residue after oil extraction is high in crude protein (36–38%) and is estimated to be available in sizable quantities of around 0.9 million tonnes/ annum in India alone (Radhakrishna 2003). In the present study, neem seeds/kernels obtained from different parts of Karnataka were analysed for chemical composition, incriminating factors like azadirachtin, salannin and trypsin inhibitor activity (TIA). Detoxification of neem seed cake was done successfully by removing the oil (Saxena *et al*. 2010).They developed a process to remove the oil and further detoxify the cake. The cake was evaluated for *in vitro* dry matter digestibility (IVDMD) and gas production kinetics for assessing its use as protein supplement. Defatted cake (RNC) and detoxified neem cake (DNC) was incorporated in concentrate mixtures replacing soybean meal and evaluated under *in vitro* conditions for its suitability as a replacement protein supplement.

Samples (16) of neem seeds collected from different regions of Karnataka with the help of Karnataka forest department officials. The kernels were separated from seeds and chemical composition in terms of organic matter, crude protein, crude fat (AOAC 2000) and acid detergent fibre (Van Soest *et al.* 1991) were determined. Azadirachtin, salannin contents (%) in neem kernels were estimated by HPTLC (Saxena *et al.* 2010). Trypsin inhibitory activity (TIU/g) was determined (Chitra and Sadasivam 1986).The cakes (both defatted raw and detoxified) required for *in vitro* experiment were procured commercially. To find out the optimum level of inclusion of RNC and DNC, iso-nitrogenous and iso-caloric (20% CP and 70% TDN) concentrate mixtures were prepared using conventional feed ingredients like maize grain, soybean meal, wheat bran, mineral mixture and salt. RNC was included at 3 replacement ratios (25%, 50%, 75%) whereas DNC was included at 4 replacement ratios (25%, 50%, 75%, 100%).

To find out the rate of degradation and gas production parameters, the concentrate samples were subjected to *in vitro* gas production test (IVGPT) for 96 h to determine their rate of degradation and $t^{-1/2}$. The gas volumes were recorded at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h. The data were subjected to non-linear regression in a graph pad prism and Y min (minimum gas production), Y max (maximum potential to produce gas), k (rate of degradation) and $t^{1/2}$ were calculated (Menke and Steingass 1988).

Tilley and Terry 1-stage *in vitro* assay (Goering and Vansoest 1970) was conducted on all concentrate mixtures for finding out *in vitro* dry matter digestibility. At the end of incubation period of 24 h the contents of the flask were filtered through sintered crucibles (grade I) and the filtrate was collected. The residue was dried in the hot air oven at 100° C and IVDMD was calculated. The ammonia nitrogen in the filtrate was estimated by Conway micro diffusion analysis using 2% boric acid as an indicator (Conway 1957). The TVFA was measured using Markham's apparatus (Barnett and Reid 1957).

Data obtained on various parameters were subjected to one-way analysis of variance (Snedecor and Cochran 1994). The statistical software SPSS was used for analysis of data and analysis variance assuming for independent constant variance structure with post-hoc Turkey to find the pair wise significance between treatments. A P-value of less than or equal to 0.05 was accepted to indicate statistical significance.

Compared to neem seeds, neem kernels had more CP, crude fat and less ADF (Table 1). The average content of azadirachtin (%), salannin (%) and TIA (IU/g) in neem kernels was 0.09±0.01, 0.22±0.01 and 1894±87, respectively. Large deviations in CP (11.22 to 26.07%) and crude fat (oil) (42.33 - 48.36%) in neem seeds/kernels were observed. Variations were also observed in azadirachtin, salannin and

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Table 1. Chemical composition and anti-nutritional factors of neem seeds and kernels (on dry matter basis) collected from different places of Karnataka

Parameter	Neem seeds (n= 16)	Neem kernels (n=13)
Chemical composition (% on dry matter basis)		
Organic matter	95.82 (94.38–96.96)	95.93 (95.43–95.93)
Crude protein	16.14 (8.55–26.09)	21.71 (11.22–26.07)
Crude fat	23.76 (15.00–26.29)	44.99 (42.33–48.36)
Acid detergent fiber	36.81 (22.85–46.37)	11.38 (5.83–15.93)
Anti-nutritional factors		
Azadirachtin (%)	-	0.09 (0.04–0.135)
Salannin (%)	-	0.22 (0.13–0.29)
Trypsin inhibitor activity (TIA/IU)	-	1894 (1776–2082)

TIA concentrations. In a multicentric study, it was observed that azadirachtin content (ppm) varied widely in neem seed kernels collected across different locations of India from 200 to 16,000 ppm and neem trees from southern India possessed more azadirachtin (Kaushik *et al*. 2007). Similarly, TIA contents in different cultivars of soybean revealed that there exists a genotype × cultivar interaction (Vineet *et al.* 2003, Manjaya*et al.* 2007).

The CP content of various concentrate mixtures was found to be 19.62 to 20.40 (Table 2a). The calculated energy (TDN) value of the diets was also same (69.99 to 70.05%) indicating that concentrate mixtures are iso-nitrogenous and iso-caloric. The minimum volume of gas production that can be produced was ranging from 1.6 to 5.2 ml and the maximum potential for gas production was ranging from 84.19 to 88.77ml in neem defatted and detoxified cakes at different levels of replacement (Table 2b). The rate of degradation (mg/h) was same in different rations (0.058 to 0.061). The half time ($t^{1/2}$) in hours was ranging from 11.39 to 11.87 in different rations. Compared to DNC, RNC containing concentrate mixtures had low IVDMD indicating detoxification of neem seed kernels is proper. However, other parameters related to gas production, rate of degradation and $t^{1/2}$ were same in all

Table 2.Ingredient composition of concentrate mixtures and gas production parameters and *in vitro* rumen fermentation

Ingredient	Proportions							
	Control	RNC (25%)	RNC (50%)	RNC (75%)	DNC (25.0%)	DNC (50.0%)	DNC (75%)	DNC (100%)
a. Ingredient composition of concentrate mixtures								
Maize grain	26.0	29.25	31.3	33.8	29	32.5	34.6	38.5
Soybean meal	26.5	20	13.2	6.6	20	13.3	6.6	0
Wheat bran	44.5	41	39	36.4	42	39.4	38	34
Neem seed cake*	-	6.75	13.5	20.2	6	11.8	17.8	24.5
Mineral mixture	2	2	2	2	2	2	2	2
Salt	1	1	1	1	1	1	1	1
CP	20.00	19.62	19.84	20.09	19.77	19.84	20.09	20.40
TDN**	70.12	70.05	70.01	70.01	70.01	70.02	70.01	70.01

Treatment	Y Min (ml)	Y Max (ml)	K	$t^{1/2}$ (h)	IVDMD (%)	NH_3 – N (mg/100 ml)	TVFA (meq/100 ml)
b. Gas production and fermentation parameters							
Control	5.0	85.56	0.061	11.32	89.86^{a}	16.54^{c}	7.34^{d}
RNC 25.0	3.5	87.95	0.061	11.42	66.59^{b}	11.67^{ab}	6.54^{cd}
RNC 50.0	3.7	88.77	0.058	11.87	60.60^{b}	9.80^{ab}	5.50^{a}
RNC 75.0	3.1	84.19	0.061	11.39	58.83^{b}	9.80^{ab}	5.96^{abc}
DNC 25.0	5.2	87.83	0.060	11.63	82.05^{a}	12.37^{b}	6.58^{cd}
DNC 50.0	3.8	87.93	0.060	11.49	83.83^{a}	8.17^{a}	5.66^{ab}
DNC 75.0	2.6	88.76	0.060	11.47	83.65^{a}	11.67^{ab}	6.59^{cd}
DNC100.0	1.6	88.25	0.060	11.52	83.08^{a}	12.83^{bc}	6.66^{cd}
SEM	0.4	0.58	0.00	0.06	2.29	0.55	0.13

*raw cake (RNC); detoxified cake (DNC) **calculated values.

Y Min- Minimum volume of gas that can be produced; Y Max, maximum potential for gas production; K, rate of degradation (mg/h); $t^{1/2}$ – time in hours required; IVDMD-*In vitro* dry matter digestibility; NH_3 – N, ammonia nitrogen concentration; TVFA, total volatile fatty acid concentration; [abcd] values bearing different superscripts in a column differ significantly (P<0.05).

concentrate mixtures. It was observed that there was a poor correlation between IVDMD and gas production of 12 ruminant feedstuffs (Getachew *et al.* 2004). IVDMD (%) was comparable to control in neem detoxified cake replaced rations. Ammonia-N (mg/100 ml) in incubation medium was more in control (16.54) compared to rest of treatment combinations. TVFA concentration (meq/100 ml incubation medium) ranged from 5.50 to 6.58 compared to 7.60 in control. Significant ($P<0.05$) differences observed in ammonia-N and TVFA concentrations are not following any trend and were in normal ranges across different concentrate mixtures indicating optimum fermentation across all the treatments (Dineshkumar *et al.* 2011).

SUMMARY

Variations were observed in chemical composition and incriminating factors of neem seeds/kernels collected from different parts of Karnataka. Graded levels of neem seed cake (defatted- raw (RNC) and detoxified (DNC) containing concentrate mixtures were further evaluated under *in vitro* conditions based on *in vitro* dry matter digestibility and gas production kinetics. RNC at all levels of incorporation reduced IVDMD without affecting gas production and rate of degradation whereas incorporation of DNC at all replacement levels did not affect IVDMD and rate of degradation, thus, can be recommended for *in vivo* evaluation.

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REFERENCES

AOAC. 2000. *Official Methods of Analysis*. Association of Official Analytical Chemists. 16th edn. Washington DC, USA.

Barnett A J G and Reid R L. 1957. Studies on volatile fatty acids from grass by rumen liquor in artificial rumen. *Journal of Agriculture Science* **48**: 315.

Chitra R and Sadasivam S. 1986. A study of the trypsin inhibitor of blackgram (*Vignamungo*). *Food Chemistry* **21**: 315–20.

Conway E J. 1957. *Micro diffusion Analysis and Volumetric Error*. 4th edn. Crossby Lockwood and sons, London.

Dineshkumar D, Rao S B N, Jash S, Elangovan A V and Hemalatha S. 2011. Chemical composition and anti-nutritional factors in karanja (*Pongamia pinnata*) seed kernels and its *in vitro* evaluation, *Indian Journal of Animal Sciences* **81**: 478–83.

Getachew G, Robinson P H, DePeters E J and Taylor S J. 2004.Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds, *Animal Feed Science and Technology* **111**: 57–71.

Goering H K and Vansoest P J. 1970. *Forage fiber analyses* (Apparatus, reagents, procedures and some applications). *Agriculture Handbook No. 379*. Agriculutre Research Service, United State Department of Agriculture (USDA).

Kaushik N, Gurudev S B, Tomar U K, Naik S N, Satya V, Bisla S S, Sharma K K, Banerjee S and Thakkar P. 2007. Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica*, *A. Jusieu*). *Current Science* **92**: 1400–06.

Manjaya J G, Gopalakrishna T, Pawar SE and Bapat V A. 2007.Genetic variability for trypsin inhibitor content in soybean [*Glycine max* (L.) Merrill.] and its correlation with oil and protein. *Indian Journal of Genetics* **67**: 51–55.

Menke K H and Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**: 7–55.

Radhakrishna P. 2003. *Tree borne oil seeds as a source of energy for decentralized planning*. Government of India, Ministry of Non-Conventional Energy Sources, New Delhi, India,

Saxena M, Ravikanth K, Kumar A, Gupta A, Singh B and Sharma A. 2010. Purification of *Azadirachtaindica*seed cake and Its Impact on Nutritional and Anti nutritional Factors, *Journal of Agricultural Food Agricultural Food Chemistry* **58**: 4939–44.

Snedecor G W and Cochran W G 1994. *Statistical Methods.* 8th edn. Oxford and IBH Publications Company, Kolkata.

Van Soest P J, Robertson J B and Lewis B S. 1991. Methods for dietary fibre, neutral detergent fibre and non-starchpolysacharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.

Vineet K, Anita R, Chanda T and Mukti J. 2003. Lipoxygenaseisozymes and trypsin inhibitor activities in soybean as influenced by growing location. *Food Chemistry* **83**: 79–83.