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Short Communication

Nitrogen fractionation of certain conventional- and lesser-known by-products for ruminants

M.S. Mahesh*, Sudarshan S. Thakur, Rohit Kumar [1], Tariq A. Malik, Rajkumar Gami [2]

Animal Nutrition Division, ICAR – National Dairy Research Institute (Deemed University), Karnal, Haryana 132001, India

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ABSTRACT

Dietary proteins for ruminants are fractionated according to solubility, degradability and digestibility. In the present experiment, 11 vegetable protein meals and cakes used in ruminant nutrition were included with a main focus on determining various nitrogen (N) fractions *in vitro*. Total N (N × 6.25) content varied from 22.98% (mahua cake) to 65.16% (maize gluten meal), respectively. Guar meal *korma* contained the lowest and rice gluten meal had the highest acid detergent insoluble nitrogen (ADIN; N × 6.25). Borate-phosphate insoluble N (BIN, N × 6.25) and *Streptomyces griseus* protease insoluble N (PIN; N × 6.25) were higher ($P < 0.01$) in maize gluten meal than in other feeds, whereas groundnut cake and sunflower cake had lower ($P < 0.01$) BIN, and PIN, respectively. Available N, calculated with the assumption that ADIN is indigestible, was maximum in guar meal *korma* and minimum in rice gluten meal. Furthermore, rapid and slowly degradable N (N × 6.25) was found to be higher ($P < 0.01$) in groundnut cake and coconut cake, respectively. Intestinal digestion of rumen undegradable protein, expressed as percent of PIN, was maximum in guar meal *korma* and minimum in rice gluten meal. It was concluded that vegetable protein meals differed considerably in N fractions, and therefore, a selective inclusion of particular ingredient is needed to achieve desired level of N fractions to aid precision N rationing for an improved production performance of ruminants.

1. Introduction

Recent advances in the quantitative understanding of nitrogen (N) requirements have witnessed a paradigm shift in protein evaluation systems for ruminants. While the ration balanced for total crude protein (CP; N × 6.25) still serves as the basis in most parts of the developing world, it cannot sufficiently define the actual requirements, e.g., of high yielding cows, which need an additional rumen protected proteins and/or amino acids. Therefore, many of the improved systems of protein evaluation like rumen degradable and undegradable protein (NRC, 2001; ICAR, 2013), absorbable protein (NRC, 1989), metabolisable protein (AFRC, 1993; NRC, 2001; ICAR, 2013), Nordic AAT/PBV system (Madsen 1985; Hvelplund and Madsen, 1993), protein digested in the intestine (Jarrige, 1989), German utilisable crude protein (Lebzien and Voigt, 1999), Dutch DVE/OEB system (Tamminga et al., 1994), Australian CSIRO (2007) and Cornell Net Carbohydrate and Protein System (Van Amburgh et al., 2015) have been developed. Essentially, all these systems consider N requirements of rumen microbes in the form of rumen degradable protein (RDP) and host tissue requirements in the form of undegradable protein/amino acids to be available for absorption at the intestines.

Although *in sacco* nylon bag technique (Mehrez and Ørskov, 1977) served as the reference method for estimating the degradability, several inherent errors have been associated with the technique making the results poorly reproducible among laboratories. Besides, the technique needs surgically prepared animals and has implications for animal welfare and costs of

* Corresponding author.
E-mail address: drmaheshmsvet@gmail.com (M.S. Mahesh).
[1] Current address: Division of Animal Nutrition, Sher-e-Kashmir University of Agricultural Science and Technology - Jammu, Jammu and Kashmir 180009, India.
[2] Current address: NDDB – Ration Balancing Program Office, Jaipur, Rajasthan 302017, India.

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maintenance (Mohamed and Chaudhry, 2008). Alternative *in vitro* method simulating ruminal proteolysis has been proposed (Krishnamoorthy et al., 1995; Licitra et al., 1998), which involves treating feedstuffs with protease from *Streptomyces griseus* and the fraction that is insoluble upon enzyme treatment is considered as rumen undegradable protein (RUP).

Knowledge on various N fractions of feedstuffs including nature and extent of degradability is necessary in order to apply new protein systems in practical ration formulation. However, only a limited number of studies (Sampath, 1990; Krishnamoorthy et al., 1995; Ramachandra and Nagabhushana, 2006; Das et al., 2014) on N fractions of few feedstuffs are reported employing different methods. Concurrently, a meta-analysis by Suresh et al. (2011) revealed a requirement of 571 g of RUP for Indian cows yielding up to 10 kg of milk. On the other hand, Chandrasekharaiah et al. (2011) observed rumen degradable nitrogen (RDN) deficiency under crop residue-based feeding system and hence, recommended 12 g of RDN/kg digestible organic matter intake in sheep. These findings emphasise that studying N fractions, at least degradability, becomes imperative in formulating nutritionally balanced rations for ruminants.

As there exists a wide variation in the solubility of dietary protein among diverse feedstuffs, and lack of appropriate scientific database, the present experiment was designed to bridge the knowledge gap in various N fractions of common as well as some new and/or lesser-known feed resources like rice gluten meal, guar meal *korma*, niger-seed cake and mahua cake for ruminant feeding under Indian context.

2. Materials and methods

2.1. Sample collection and processing

Eleven samples of vegetable protein feed ingredients ($n = 5$ per feed) available across India with a broad range in protein content were procured from local market. These comprised of 6 oilseed cakes/meals obtained after oil extraction (groundnut cake, soyabean meal, mustard cake, cottonseed cake, niger-seed cake and sunflower cake), 2 wet milling co-products of cereal grains (rice gluten meal and maize gluten meal) and 3 agro-industrial byproducts (coconut cake, guar meal *korma* and mahua cake). Samples were dried in hot-air oven at 65 °C for 48 h, ground in laboratory Wiley mill, passed through 1-mm screen, and stored in zip lock bags to avoid moisture gain until analysis.

2.2. Protocols to fractionate dietary N

The estimation of total N, acid-detergent insoluble N (ADIN; Licitra et al., 1996), buffer-insoluble N (BIN; Licitra et al., 1996) and protease insoluble N (PIN; Krishnamoorthy et al., 1995; Licitra et al., 1998) were done by Kjeldahl method (# 984.13) according to AOAC (2005). The PIN was regarded as rumen undegraded N, which was estimated using commercial broad-spectrum protease of *S. griseus* (type XIV, Sigma P-5147, St Louis, MO, USA). Briefly, 0.5 g of feed sample was incubated in 40 mL of borate-phosphate buffer (pH 7.8 to 8.0) for one hour in 125 mL of Erlenmeyer flask followed by treatment with 10 mL of *S. griseus* protease solution containing 330×10^{-3} units/mL for 18 h with intermittent shaking. Afterwards, the contents were filtered through Whatman No. 54 filter paper and the residue along with filter paper was transferred to Kjeldahl digestion tube for N estimation, which was assumed to be rumen undegradable protein (Licitra et al., 1998). The various other fractions viz. available N, rapid and slowly degradable N as well as intestinally available N were calculated as detailed in Table 1. All the analyses were completed at least in triplicate.

2.3. Statistical analysis

The results obtained in this experiment were tabulated as means and standard error of means (SEM) for all fractions. Data were subjected to one-way analysis of variance (ANOVA) using SAS 9.3 software package. Studentised Range Test was applied to make post-hoc comparison among means to distinguish significant differences at $P \leq 0.05$.

3. Results and discussion

The various N fractions of feeds are presented in Table 2. The total N (N × 6.25) content of the studied feed ingredients ranged from 22.98% to 65.16% in mahua cake and maize gluten meal, respectively. The ADIN (N × 6.25) content (%) was the lowest ($P < 0.01$) in guar meal *korma* followed by groundnut cake, and very high in rice gluten meal followed by sunflower cake and mahua cake. Furthermore, BIN (%) value was noted to be significantly ($P < 0.01$) higher in maize gluten meal followed by mahua cake, coconut cake and rice gluten meal, and it was the lowest ($P < 0.01$) in groundnut cake. Fraction of feed protein resistant to proteolysis by *S. griseus* (PIN) was significantly ($P < 0.01$) higher in maize gluten meal followed by rice gluten meal and was least in sunflower cake. On the contrary, reverse trend was true for RDN (N × 6.25) content. Rapidly rumen soluble N was higher ($P < 0.01$) in groundnut cake, and coconut cake contained higher slowly rumen soluble N. Intestinally available N or available rumen escape N (as % of PIN) was higher ($P < 0.01$) in guar meal *korma* and groundnut cake, and it was lowest ($P < 0.01$) in rice gluten meal.

Significance of various N fractions has been widely recognised in ruminant nutrition (AFRC, 1993; NRC, 2001; Van Amburgh et al., 2015). Although ration that is balanced to be optimum in CP could suffice the needs of low producing tropical cows (<10 kg/d), the cows with high dairy merit may not perform to their fullest potential if protein requirements are met only on CP basis as they need a considerable proportion of RUP. Therefore, it is important to generate an accurate database on N fractions of feeds that could be used in ration formulation.

In the present experiment, all the studied feeds differed widely with respect to various N fractions. The total N contents are in close range with the reported literature values (Krishnamoorthy et al., 1995; Stern and Bach, 1996; Ramachandra and Nagabhushana, 2006; Habib et al., 2013; Das et al., 2014; Kumar et al., 2016). Protein soluble in borate-phosphate buffer, commonly referred to as soluble protein, is generally assumed to be rapidly degradable in the rumen (Licitra et al., 1996), which comprises mostly of non-protein N compounds like ammonia, urea, nitrates, amino acids as well as some small peptides and true protein. However, neither all soluble proteins are degradable nor all insoluble proteins resist ruminal proteolysis (Ramachandra and Nagabhushana, 2006; Mohamed and Chaudhry, 2008). The PIN observed in this study is in line with previous reports of Krishnamoorthy et al. (1995) and Ramachandra and Nagabhushana (2006), who also estimated RUP content by PIN method, except for feeds like rice gluten meal, guar meal *korma*, niger-seed cake and mahua cake, for which we did not find literature values to compare our results. Of specific interest is maize gluten meal and rice gluten meal, which contained substantial proportion of RUP. This could be attributed to the presence of high concentration of resistant cereal storage proteins (glutamines and prolamins), which result from wet milling procedure used for starch extraction (Wadhwa et al., 2012; Kumar et al., 2016). In addition, Sehgal and Makkar (1994) also recorded a high RUP value of 77% in sorghum gluten meal (48.9% CP) having only 10% of soluble protein. Overall, the present findings on majority of feeds agree with the general assumption of high

Table 1
Methods used to determine various nitrogen (N) fractions of feedstuffs.

N fraction	Method of determination	Nutritional property	Reference
CP	N × 6.25 (Kjeldahl method)	True protein and non-protein N	AOAC (2005)
ADIN	N estimation in ADF	Undegradable in the rumen and unavailable at intestine (heat damaged Maillard products, N bound to lignin and tannins)	Licitra et al. (1996)
Available N	N−ADIN	N free from ADIN is considered digestible and utilisable by the animal	Licitra et al. (1996)
BIN	Insoluble N upon treatment with borate-phosphate buffer (pH = 6.7) for 3 h	Slowly rumen degraded, rumen undegraded and indigestible N	Licitra et al. (1996)
PIN	Insoluble N upon treatment with commercial protease (*Streptomyces griseus*)	Rumen undegraded N	Krishnamoorthy et al. (1995); Licitra et al. (1998)
RDN	N−PIN	Total rumen degraded N	Krishnamoorthy et al. (1995); Licitra et al. (1998)
Rapidly rumen soluble N	N−BIN	Fraction of RDN that is rapidly hydrolysed in the rumen	Krishnamoorthy et al. (1995)
Slow rumen soluble N	BIN−PIN	Fraction of RDN that is slowly hydrolysed in the rumen	Krishnamoorthy et al. (1995)
Intestinally available N	PIN−ADIN	Rumen undegraded N that is assumed to be digested and absorbed at intestine	AFRC (1993); Krishnamoorthy et al. (1995)

CP = crude protein; ADIN = acid detergent insoluble nitrogen; BIN = borate-phosphate insoluble nitrogen; PIN = protease insoluble nitrogen; RDN = rumen degradable nitrogen.

Table 2
Nitrogen[1] (N) fractions and estimates of N availability in the rumen and intestine for various protein feedstuffs.

Ingredient	Total N	N fraction, % of total N				Available N				
		ADIN	BIN	PIN	RDN	Total	Rumen, % of RDN		Intestine	
							Rapid	Slow	% of total N	% of PIN
Groundnut cake (*Arachis hypogaea*)	43.12^{d}	2.74^{g}	39.55^{k}	24.97^{i}	75.03^{a}	97.26^{a}	80.57a	19.43^{g}	22.23^{e}	89.01^{b}
Soyabean meal (*Glycine max*)	44.40^{d}	4.58^{f}	55.48^{h}	31.73^{gf}	68.27^{cd}	95.42^{b}	65.22c	34.78^{e}	27.15^{d}	85.51^{c}
Mustard cake (*Brassica juncea*)	38.12^{e}	5.65^{e}	45.27^{j}	28.95^{h}	71.05^{b}	94.35^{c}	77.03ab	22.97^{gf}	23.30^{e}	80.48^{d}
Cottonseed cake (*Gossypium hirsutum*)	26.30^{g}	8.14^{d}	73.23^{e}	51.70^{d}	48.30^{f}	91.86^{d}	55.42d	44.58^{d}	43.56^{c}	84.25^{c}
Niger-seed cake (*Guizotia abyssinica*)	33.34^{f}	5.26^{ef}	51.03^{i}	33.42^{f}	66.58^{d}	94.74^{bc}	73.56b	26.44^{f}	28.16^{d}	84.25^{c}
Sunflower meal (*Helianthus annus*)	31.99^{f}	20.39^{c}	65.75^{g}	24.03^{i}	75.97^{a}	94.36^{c}	45.09e	54.91^{c}	18.39^{f}	76.51^{e}
Mahua cake (*Madhuca longifolia*)	22.98^{h}	15.62^{b}	86.61^{b}	58.54^{c}	41.46^{g}	84.38^{f}	32.30f	67.70^{b}	42.92^{c}	73.32^{f}
Rice gluten meal (*Oryza sativa*)	47.50^{c}	22.04^{a}	82.83^{d}	69.44^{b}	30.56^{h}	77.96^{g}	56.26d	43.74^{d}	47.40^{b}	68.26^{g}
Maize gluten meal (*Zea mays*)	65.16^{a}	12.09^{c}	90.14^{a}	79.30^{a}	20.70^{i}	87.91^{e}	47.72e	52.28^{c}	67.20^{a}	84.74^{c}
Coconut cake (*Cocos nucifera*)	23.09^{h}	5.17^{ef}	84.94^{c}	46.04^{e}	53.96^{e}	94.83^{bc}	27.91g	72.09^{a}	40.87^{c}	88.78^{b}
Guar meal *korma* (*Cyamopsis tetragonoloba*)	51.41^{b}	2.06^{g}	68.23^{f}	30.87^{gh}	69.13^{bc}	97.94^{a}	45.97e	54.03^{c}	28.81^{d}	93.31^{a}
SEM	0.48	0.22	0.34	0.67	0.67	0.22	1.03	1.03	0.77	0.75

ADIN = acid detergent insoluble nitrogen; BIN = borate-phosphate insoluble nitrogen; N = nitrogen; PIN = protease insoluble nitrogen; RDN = rumen degradable nitrogen; RUP = rumen undegradable protein.

$^{a-h}$Means bearing different superscripts within a column differ significantly at $P < 0.01$.

[1] Expressed as N × 6.25 (% dry matter basis).

undegradability when protein is insoluble, and moderately corroborate with previous reports (Krishnamoorthy et al., 1995; Ramachandra and Nagabhushana, 2006).

Extensive degradation of dietary protein in the rumen is one of the main constraints for its efficient utilisation by high yielding cows. On the other hand, N compounds that are released during protein degradation are crucial for microbial protein synthesis in the rumen (reviewed by Bach et al., 2005). Therefore, there is a need to balance the ratio of RDP to RUP (60:40 as per NRC, 2001) for optimum rumen function and to fulfil N needs. In addition, higher protein degradability leading to higher ammonia production than that could be captured by rumen microbial cells, leads to higher blood urea N and eventually higher urinary N excretion, which is a potential source of environmental pollution associated with dairy farming. Moreover, higher N use efficiency (ratio of milk N to total ingested feed N) has also been obtained with the lowest N and RDP levels (Bach et al., 2005).

Feedstuffs differ in their rates of degradation in rumen, which is influenced by factors like hydrophobicity of peptides, molecular weight, degree of secondary and tertiary structures as well as disulfide bonds (reviewed by Wallace et al., 1999). Slowly degradable N has been reported to utilise completely for ruminal microbial protein synthesis (Thirumalesh and Krishnamoorthy, 2013) compared to rapidly degradable N that is utilised with only 80% efficiency (AFRC, 1993). Furthermore, there is a general agreement that most of the high yielding cows respond positively to increasing dietary RUP during early lactation (NRC, 2001). In this regard, feeds like maize gluten meal, rice gluten meal and cottonseed cake could be ranked as sources of high RUP. Data obtained in the present study on PIN closely resembles with that of literature values (Table 3); nonetheless, there is a difference between degradability estimates of protease and *in sacco* method.

In true sense, the response to supplemental RUP depends on their intestinal digestion, which varies due to factors like extent of heat processing and ADIN content, among others. A wide range of 25% to 95% of intestinal digestibility coefficient has been stated for various feeds by French PDI (protein digested in intestines) system (Jarrige, 1989). In regards to ADIN, which is generally supposed to be completely indigestible (AFRC, 1993; Wang et al., 2015), comprises of heat damaged proteins (Schiff's bases) and proteins bound with tannin and/or lignin. However, much of the compelling evidences has demonstrated a substantial digestibility (up to 60%) of

Table 3
Literature values of actually estimated rumen undegradable protein (RUP) and intestinal digestibility for various protein feedstuffs.

Ingredient	RUP, % of total N				Intestinal digestibility, %	
	Streptomyces griseus protease method	Reference	*In sacco* nylon bag method	Reference	Three-step *in sacco–in vitro* method	Reference
Groundnut cake	10.80	Krishnamoorthy et al. (1995)	15.40	Wadhwa et al. (2007)	94.1	Stern and Bach (1996)[1]
	12.97	Prabhu et al. (1996)	26.33	Das et al. (2014)	76.9	Chatterjee and Walli (2002)[2]
Soyabean meal	50	Roe et al. (1990)	28.17	Lamba et al. (2014)	82.1	Hippenstiel et al. (2015)[3]
	25.58	Prabhu et al. (1996)	46.0	Peng et al. (2014)	88.9	Peng et al. (2014)[4a]
	48.30	Licitra et al. (1998)	33.50	Habib et al. (2013)	96.4	Stern and Bach (1996) [1]
Mustard cake	24.60	Krishnamoorthy et al. (1995)	28.48	Mondal et al. (2008)	70.9	Stern and Bach (1996)[1]
	44.34	Prabhu et al. (1996)	24.39	Das et al. (2014)	75.2	Chatterjee and Walli (2002)[2]
Maize gluten meal	95.20	Licitra et al. (1998)	86.40	Mondal et al. (2008)	96.2	Stern and Bach (1996) [1]
	94.10	Roe et al. (1990)	61.79	Lamba et al. (2014)	94.2	Piepenbrink and Schingoethe (1998)[4b]
	84.80	Susmel et al. (1993)	83	Stern et al. (1997)	96.8	Harstad and Prestløkken (2000)[5]
Cottonseed cake	28.60	Krishnamoorthy et al. (1995)	36.70	Das et al. (2014)	92.9	Stern and Bach (1996)[1]
	30.12	Prabhu et al. (1996)	17.09	Lamba et al. (2014)	77.4	Promkot et al. (2007)[2]
	19.59	Ramachandra and Nagabhushana (2006)	61.0	Gao et al. (2015)	77.8	Chatterjee and Walli (2002)[2]
Coconut cake	16.70	Krishnamoorthy et al. (1995)	63	Hvelplund and Madsen (1993)	89.6	Stern and Bach (1996)[1]
	9.80	Prabhu et al. (1996)	–	–	–	–
Niger-seed cake	21.58	Prabhu et al. (1996)	29.40	Negi et al. (1990)	–	–
	–	–	19	Sampath (1990)	–	–
Guar meal *korma*	–	–	42.54	Mondal et al. (2008)	–	–
Sunflower meal	28.91	Ramachandra and Nagabhushana (2006)	21.60	Habib et al. (2013)	87.8	Stern and Bach (1996)[1]
	19.24	Prabhu et al. (1996)	31.70	Gao et al. (2015)	88.5	Gao et al. (2015)[4b]
	17.80	Ramachandra and Nagabhushana (2006)	43.73	Mondal et al. (2008)	–	–
Mahua cake	–	–	51.40	Negi et al. (1990)	–	–
			75.0	Sampath (1990)	–	–

[1] Estimated by *in situ* mobile bag technique using duodenally cannulated cattle.
[2] Estimated by subjecting *in sacco* nylon bag (Mehrez and Ørskov, 1977) residues to *in vitro* enzymatic method (Antoniewicz et al., 1992).
[3] Estimated by sequential treatment of *Streptomyces griseus* protease followed by pepsin–pancreatin digestion of residue.
[4] Estimated by 3-step method (Calsamiglia and Stern, 1995) and expressed as % of CP[4a] and RUP[4b].
[5] Estimated by *in situ* mobile bag technique (Hvelplund et al., 1992) after 16 h of rumen incubation.

ADIN fraction in feeds like xylose (12.8%) treated maize gluten meal (induced Maillard protein, Nakamura et al., 1994), dried distillers grain plus solubles (Klopfenstein, 1996) and others (Cabrita et al., 2011). In the present study, higher ADIN content decreased the total available N as well as other available fractions including intestinal digestion, as we assumed ADIN to be completely indigestible and thus unavailable. In particular, three of the studied feed ingredients i.e., rice gluten meal, sunflower cake and mahua cake were found to have intestinal digestibility of RUP lower than the average value of 80% to 85% considered by AFRC (1993). It is also plausible that intestinal digestibility was under-estimated when compared with literature values (Table 3) that could probably be due, in part, to ADIN content that was assigned zero digestibility in the present study. Therefore, for determining intestinal digestibility, it would be desirable to go for realistic estimate using pepsin–pancreatin digestion, thereby imitating *in vivo* digestive process for ADIN rich feedstuffs like rice gluten meal, sunflower cake, mahua cake, maize gluten meal etc. Furthermore, most recently, Wang et al. (2015) also concluded that use of ADIN in estimating digestibility of RUP is not applicable to the majority of feeds.

4. Conclusions

This study presented novel database on N fractions of certain feeds like rice gluten meal, guar meal *korma*, niger-seed cake and mahua cake. Selection of ideal combination of feed ingredients with desired level of particular N fraction would be expected to aid precision diet formulation to improve production performance and minimise N pollution to environment. Further studies are warranted to determine actual intestinal digestion to ascertain quality of RUP contained in various tropical by-product feedstuffs fed to ruminants.

Conflict of interest

M.S. Mahesh, on behalf of all the authors, states that they have no financial or any other conflict of interest that could have an inappropriate influence on the results of this study.

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