Animal Nutrition 3 (2017) 186–190

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

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

ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form
25 March 2017
Accepted 3 April 2017
Available online 13 April 2017

Keywords:
ADIN
Protein degradability
Soluble protein
Streptomyces griseus protease

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.

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2017.04.003

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 by-products (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