Indian J. Anim. Nutr. 2009. 26(2) : 165-168

Indian Journal of Animal Nutrition

Near-infrared Spectroscopy Method to Evaluate UDP Content of Protein Supplements

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ABSTRACT

Protein meals (1-mm particle size) were used to determine the rumen undegradable protein (UDP) by in vitro ammonia release (IVAR) and by near-infrared spectroscopy (FOSS NIR System, 1100 – 2500 nm) methods. UDP values in various formaldehyde treated and untreated protein meals, estimated by NIR were compared with that of traditional IVAR method and was found to be highly correlated ($r=0.67$ to $r=92$; $p<0.01$). Partial least square analysis (PLS) model was used to develop the calibration equations with F values of >10, indicating acceptance of these calibration equations for predicting the UDP values. Regression coefficients were used to develop the mathematical relationship for UDP values, between the NIR spectra and IVAR methods. Based on the values obtained by IVAR and NIR methods, various regression equations have been developed, for various treated and untreated protein meals.

Key words : Protein meals, Rumen undegradable protein, Near Infrared spectroscopy, In vitro ammonia release method.

Credit for the discovery of the need of escape protein in the diet of producing ruminants, is difficult to assign, as it slowly evolved from basic observations when ruminant nutrition was in its infancy. Protein meals, particularly rumen escape proteins, play a very important role as being excellent protein supplement in livestock feeding. NIRS methods are becoming popular in animal science to predict the chemical composition of forages and other feeds, feed digestibility and responses to feeds including feed intake and growth (Coates, 2000; Stuth and Tolleson, 2000). Since, traditional method (In vitro ammonia release) for determining UDP in feed supplements requires use of rumen liquor for which rumen cannulated animals are to be maintained, the mathematical equations developed on NIRS could be used for predicting UDP values. So this study was undertaken, to develop regression equations can be used by the feed industry as well as livestock owners.

The rapeseed, sunflower, guar, cottonseed, soyabean and groundnut meal were treated with appropriate quantity of formaldehyde (37-40 % w/v) and incubated for 10 days in sealed vials (Ashes et al., 1984). Treated and untreated protein meals were ground to 1-mm particle size by cyclotec instrument. Moisture and crude protein were estimated as per AOAC (1995). The protein meals were tested for degree of protection using IVAR method (Ashes et al., 1995; Gulati et al., 1999). Rumen fluid was collected at 0800 hour (prior to feeding) from four ruminants (Two HF x Jersey bulls and two Mehsani buffalo bulls of 4 years of age) and pooled in a pre-warmed thermos flask, strained through a muslin cloth (80-100 microns). Animals were fed basal

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diet, comprising of green maize fodder, paddy straw and concentrate mixture according to their requirement. Known quantities of test samples of protein meals were incubated for 24 hours with strained rumen liquor (SRL), anaerobically at 39°C. Rumen protein degradation was measured by analyzing ammonia nitrogen level in SRL, at the end of incubation period (Scott and Ashes, 1993; Gulati et al., 2005) and UDP values were quantified.

UDP values obtained by IVAR method were entered in WinISI II software for calibration of NIRS. This software programme was used to process the data and to develop models for determination of UDP contents in various protein meals. Spectra were taken in the wavelength range of 1100-2500 nm and were recorded in the linked computer as absorbance. The calibration was performed using PLS regression method (Shenk and Westerhaus 1991). The amount of radiation reflected from the sample was quantified as the reflectance (R) of the sample. The value was expressed as log (1/R), which gave higher values at higher levels of absorbance. There was an almost linear relationship between log (1/R) and the concentration of an absorbing component (Norris et al., 1976 and Hruschka, 1987). PLS model was used to predict the moisture, crude protein and UDP values in protein meals. The results were compared with the respective reference values. The validation errors were combined into a standard error of cross validation (SECV), which was accepted as a measure of the accuracy of determination. One of several different multivariate calibration methods was used to relate the spectral data from a sufficiently large and representative sample set to the primary IVAR data (Blanco et al., 1997). Finally, calibrations were subjected to validation procedures with an independent set of samples. Data generated for UDP by IVAR method and NIRS were used to develop regression equations for different protein meals using statistical model (Snedecor and Cochran, 1968).

Moisture content in protein meals (Table 1) ranged from 5.6 to 8.9 % (AOAC) and 5.7 to 8.6 % (NIR). The protection of proteins amongst various untreated protein meals ranged from 31.8 to 53.8 % (IVAR) and 31.5 to 54.2 % (NIR); whereas, protection of proteins amongst various treated protein meals ranged from 72.1 to 73.0 % (IVAR) and 72.2 to 72.9 % (NIR). Spectral calibration and validation statistics for UDP in various protein meals revealed that lowest SECV was observed in groundnut meal (0.1) and highest in treated cottonseed meal (2.2). In all protein meals, the F values (10.6 to 142.0) were > 10, indicating acceptance of calibration

Table 1. Comparison of moisture, crude protein and UDP content (%) in protein meals

Protein meal	Moisture		Pooled	Crude protein		Pooled	UDP		Pooled
	AOAC	NIR	SE	AOAC	NIR	SE	IVAR	NIR	SE
RSM	5.9	6.0	0.07	37.8	38.0	0.11	37.8	37.9	0.26
FTRSM	7.3	6.9	0.17	37.2	37.3	0.13	72.5	72.3	0.42
SFM	5.9	6.0	0.10	28.4	28.7	0.27	32.1	31.9	0.30
FTSFM	7.0	7.0	0.13	27.8	27.9	0.16	73.0	72.3	0.45
GM	6.0	6.0	0.09	47.7	47.6	0.30	40.1	39.7	0.24
FTGM	7.0	7.0	0.09	46.6	46.5	0.47	72.8	72.9	0.33
CSM	5.6	5.7	0.11	37.5	37.5	0.33	53.8	54.2	0.23
FTCSM	7.9	7.7	0.10	39.0	38.8	0.17	72.1	72.2	0.36
SBM	6.0	5.9	0.43	45.3	45.4	0.60	37.5	37.7	0.30
FTSBM	8.9	8.6	0.13	45.9	46.2	0.31	72.4	72.1	0.33
GNM	6.3	6.3	0.16	37.7	37.9	0.80	31.8	31.5	0.24
FTGNM	7.5	7.5	0.16	40.5	39.9	0.36	72.7	72.6	0.43

Rapeseed meal – RSM; Sunflower meal – SFM; Guar meal – GM; Cottonseed meal –CSM; Soybean meal – SBM; Groundnut meal – GNM; Formaldehyde treated - FT

equations. R values ranged from 0.660 (treated sunflower meal) to 1.094 (treated guar meal) indicating significant relationship between two methods of analysis. Regression equations, developed for various protein meals are mentioned below :

Rapeseed meal (YIVAR = 1.54 XNIR – 20.542; r=0.85; p<0.01; n=15),

Treated rapeseed meal (YIVAR = 0.978XNIR + 1.756; r=0.85; p<0.01; n=15)

Sunflower meal (YIVAR = 1.10 XNIR – 3.197; r=0.70; p<0.05; n=15),

Treated sunflower meal (YIVAR = 0.660XNIR + 25.197; r=0.69; p<0.01; n=15)

Guar meal (YIVAR = 0.813 XNIR + 7.801; r=0.77; p<0.01; n=15),

Treated guar meal (YIVAR = 1.094XNIR – 7.063; r=0.83; p<0.01; n=15)

Cottonseed meal (YIVAR = 1.242 XNIR – 13.434; r=0.81; p<0.01; n=15),

Treated cottonseed meal (YIVAR = 1.010XNIR – 0.832; r=0.67; p<0.01; n=15)

Soybean meal (YIVAR = 1.03 XNIR – 1.31; r=0.96; p<0.01; n=15),

Treated soybean meal (YIVAR = 0.792XNIR + 15.197; r=0.85; p<0.01; n=15)

Groundnut meal (YIVAR = 0.967 XNIR + 1.35; r=0.91; p<0.01; n=15) and

Treated groundnut meal (YIVAR =0.929XNIR + 5.198; r=0.92; p<0.01; n=15).

Where,

XNIR = UDP by near-infrared spectroscopy method.

YIVAR = UDP by In vitro ammonia release method.

n = Number of observations.

Equations were developed from a calibration data set, which have been generated by IVAR method. The optimum size for calibration data sets has not been developed; however, at least 15 samples taken for each different constant and for each parameter in the regression equation (Hruschka, 1987). Regression coefficients were used to develop the mathematical relationship for UDP values, between NIR spectra and IVAR methods.

IVAR and NIRS methods were highly correlated as measure of escape protein. Correlations were setimated using IVAR, as these were considered to be more accurate estimates.

It was concluded that NIR spectroscopy was an adequate method for determination of UDP in various protein meals. UDP values estimated by IVAR and NIR methods were highly correlated. Partial least square analysis (PLS) method for mathematical treatment was found best for predicting the UDP values in various treated and untreated protein meals.

ACKNOWLEDGEMENT

Financial assistance and necessary facilities provided by the management of National Dairy Development Board, Anand, for undertaking this study, are gratefully acknowledged.

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