IN SITU PROTEIN DEGRADABILITY, *IN VITRO* AMMONIA RELEASE AND N SOLUBILITY OF SOME FEED INGREDIENTS

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(Received on September 14, 1998)

ABSTRACT

Nine feed ingredients, viz, rapeseed cake, groundnut cake, sunflower seed cake, cotton seed cake, soybean meal, wheat bran, rice polish, maize gluten and tamarind seed cake were subjected to *in situ* protein degradability, *in vitro* ammonia release and N solubility determination. The effective protein degradability was lowest for maize gluten (28.2%) followed by tamarind seed cake (55.1%) and highest for GN cake (85.8%). The values for soybean meal, cotton seed cake and rapeseed cake were 60.1, 61.9 and 82.5 per cent, respectively. Accordingly, the undegraded dietary protein (UDP) value of feeds was highest in maize gluten (35.5%) followed by soybean meal (19.84%), cotton seed cake (14. 5%), sunflower cake (8.9%), tamarind seed cake (8.72%), G.N. Cake (6.86%), rapeseed cake (6.39%), wheat bran (6.11%) and rice polish (4.0%), on DM basis. However, N solubility in phosphate buffer was highest in rice polish (54.28%) and lowest in tamarind seed cake (12.5%). *In vitro* ammonia release as mg/100 ml SRL was highest on cotton seed cake (17.0 mg) and lowest on tamarind seed cake (3.62 mg). The correlation coefficient between *in vitro* N solubility and effective protein degradability by *in situ* technique was found to be poor ($r = 0.41$, $r^2 = 0.16$). Similarly, the correlation between *in vitro* ammonia release and *in situ* protein degradability was also not high ($r=0.56$, $r^2=0.31$).

Key words : *In situ* protein degradability, *In vitro* ammonia release, N solubility, RDP and UDP value.

The concept of evaluating protein quality for ruminant feeds has undergone a lot of change in the recent times and the traditional DCP system is being slowly replaced by more realistic approach, based on the RDP (rumen degradable protein) and UDP (undegraded dietary protein) value of feeds[2,3]. Measurement of protein degradability is the essential part of this system. *In situ* technique is considered more accurate and has been widely used[4,5,6,7]. However, it is somewhat time consuming and laborious. Efforts are going on to replace it by some simpler laboratory technique[8,9]. The present investigation was therefore taken up to estimate protein degradability

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and RDP and UDP values of some feed ingredients and to compare the protein degradability values with *in vitro* ammonia release and N solubility values, estimated for the same feeds.

MATERIALS AND METHODS

There rumen fistulated crossbred calves (200-250 kg B.wt.) were used to estimate *in situ* protein degradabilities of nine feed ingredients, viz. rapeseed cake, groundnut cake, sunflower seed cake, cotton seed cake, soybean meal, wheat bran, rice polish, maize gluten and tamarind seed cake. The experimental animals were fed a diet of straw and concentrate (65:35) along with 5 kg green fodder, to meet the nutrient requirement of animals as per NRC[3]. After 3 weeks of feeding, preweighed nylon bags (9x13 cm., 40 mesh) containing 5g samples (2.5 mm) were incubated in the rumen of 3 fistulated animals at 4, 8, 12, 16, 24, 36 and 40 h[10] The bags after removal from the rumen, were washed throughly under running water for 15 minutes and dried in an oven to a constant weight.

The residue was subjected to CP analysis[11]. The constants 'a', 'b' and 'c' were obtained from the degradability data, using the equation p= a+bc/c+k[12], where p is the effective degradability at 'K' value (fractional outflow rate from rumen), the constant 'a' is the intercept on Y-axis, 'b' is the potentially degradable faction and 'c' is the rate contant or degradation rate.

The N solubility of the above ingredients was determind by the extraction of 1 g sample in 50 ml phosphate buffer in triplicate, kept for 2 h at 39°C in a water bath, and estimating the soluble N in the supernatant after filteration through Whatman filter paper No 40[13]. For the *in vitro* ammonia release, 0.5 g sample was incubated with 40 ml of strained rumen liquor (SRL) for 6 h under anaerobic conditions, using 0 h sample as control for subtraction[14]. However, the quantity of ammonia estimated in the vial at the end of incubation was not the total ammonia generated, but the residual ammonia left, as part of the ammonia released must have been taken up by microbes for protein synthesis during incubation. Just to minimise that effect, the short time incubation was conducted.

Table 1 Degradation characteristics of some feed ingradients with respect to protein degradabilty

Ingredient	'a' intercept on Y axis (%)	'b' potentially degradable fraction (%)	'c' degradation rate	RSD (Residual Standard Deviation)
Tamarind seed cake	20.01	70.29	0.0334	2.79
Rapeseed cake	49.15	46.08	0.1277	0.48
GN cake	62.01	34.29	0.0698	1.52
Sunflower cake	42.58	53.42	0.0486	3.31
Cotton seed cake	23.09	64.21	0.0480	3.29
Soybean meal	10.60	80.49	0.0302	1.97
Wheat bran	34.74	51.86	0.0455	3.00
Rice polish	44.06	39.28	0.2277	1.34
Maize gluten	2.16	77.24	0.0160	0.69

RESULTS AND DISCUSSIONS

Protein degradation charachteristics of feeds are presented in Table 1. While the potentially degradable faction (b value) was highest for soybean meal (80.49%) followed by maize gluten (77.24%), it was lowest for GN cake (34.29%) followed by rice polish (39.28%). However, the degradation rate was highest for rice polish (0.2277) and lowest for maize gluten (0.0160). The degradation rate was also lower for soybean meal (0.0302) and tamarind seed cake (0.0334), while it was quite high for rapeseed cake (0.1277) and medium for GN cake (0.0698) and cotton seed cake (0.0480). Very low degradation rate for maize gluten and lower degradation rate for cotton seed cake has also been reported earlier[6]. While the potentially degradable fractions is related to potential degradability (a+b) value, the degradation rate has a bearing on effective degradability of feeds.

Effective CP degradability at fractional flow rate of $0.05h^{-1}$ (Table 2) was maximum for GN cake (85.8%) followed by rapeseed cake (82.5%) and rice polish (76.3%) and was minimum for maize gluten (28.2%). Cotton seed cake (61.9%) and soybean meal (60.0%) had medium values for protein degradability. Values for effective CP degradability with respect to cotton seed cake, GN cake and sunflower cake are in close agreement with the earlier studies[15], but were different from other studies[6,16]. After making correction for bacterial N contamination, the effective protein degradability value for GN cake, cotton seed cake and soybean meal were reported to be 78.49, 50.70 and 38.40 percent, respectively[17]

Table 2 Effective CP degradability at the fractionl flow of 0.05% h^{-1} and RDP and UDP value of feeds

Ingredient	% effective CP degradabilty	% CP	%RDP	%UDP
Tamarind seed cake	55.1	19.42	10.70	8.72
Rapeseed cake	82.5	36.54	30.15	6.39
GN cake	85.8	47.00	40.14	6.86
Sunflower cake	70.9	30.60	21.70	8.90
Cotton seed cake	61.9	38.06	23.56	14.50
Soybean meal	60.0	49.60	29.76	19.84
Wheat bran	59.5	15.07	8.96	6.11
Rice polish	76.3	16.80	12.80	4.00
Maize gluten	28.2	50.40	14.50	35.50

Factors affecting the degradability of feeds in rumen, such as size and pore size of the bag, particle size of the feed samples and the method of washing[11], were controlled to a large extent, however, the microbial contamination of the residue left in the nylon bag[17] was not taken into account in the present experiment.

The differences which exist in the protein degradability of various feed ingradients in rumen, could be attributed to feed characheristics, including protein structure and amino acid sequence[18], type of binding between protein and other components, mainly acid detergent

insoluble N (ADIN) content of feeds[19]; and the animal factors, like rumen environment (type of microbes, ruminal pH), retention time and flow rate[20]. In the present experiment, the rumen environment was somewhat similar in all the three animals, which were maintained on a similar diet throughout the study.

RDP content (Table 2) was highest in GN cake (40.14%) and lowest in wheat bran (8.96%). Obviously, the very high RDP content for GN cake is because of its highly degradable nature of protein, which is present in high concentration in the cake. RDP values for rapeseed cake, soybean meal and cotton seed cake were in medium range. The UDP value followed a somewhat reverse trend. Highest UDP value was seen for maize gluten (35%) and the lowest was observed for rice polish (4.00%). The feed ingradients, which had lower effective CP degradability and higher CP content, like maize gluten, soyabean meal and to some extent cotton seed cake, had higher UDP content, values being 35.5, 19.84 and 14.5 percent, respectively. All these three feeds are good sources of bypass protein, with maize gluten being the best source. The values obtained for UDP are in agreement with respect of maize gluten and soybean[6] and cotton seed cake[5].

Data on N solubility (Table 3) shows that N from rice polish was highly soluble in phosphate buffer (59.28%), followed by wheat bran (48.12%), rapeseed cake (32.28%) and GN cake (23.65%). The values were lower for sunflower seed cake, cotton seed cake, soybean meal, maize gluten and tamarind seed cake, ranging from 12.50 per cent in tamarind seed cake to 16.04 percent in sunflower seed cake. The values for N solubility in the present investigation were comparable to the values reported earlier with respect to soybean meal, sunflower seed cake and wheat bran[9]. However, in another study[21], the values for rice polish were quite low and were quite high for GN cake, contrary to our findings. Further more, for the same feeds, the N solubility has been shown to give different values when tested in different solvents[22,23].

Table 3 Protein solubility and *in vitro* NH_3 release values for various feed ingredients.

Feed Ingredient	N solubility in Phosphate buffer (%)	*In vitro* NH_3 release (mg/100 ml SRL)
Tamarind seed cake	12.50	3.62
Rapeseed cake	38.28	13.09
GN cake	23.65	15.89
Sunflower cake	16.04	13.02
Cotton seed cake	14.07	17.06
Soybean meal	14.30	8.90
Wheat bran	48.12	14.12
Rice polish	59.28	10.62
Maize gluten	15.42	7.12

A strong relationship between N solubility and protein degradability of purified protein sources has been demostrated for short incubation times[22,24] however, this relationship diminishes with extended time of ruminal fermentation. A poor correlation ($r = 0.41$ and $r^2 = 0.16$)

between N solubility in buffer and the extended ruminal fermentation (*in situ* degradation) in the present study could probably be due to microbial contamination of undigested feed residues[25], the several N fractions that very considerably in their degradability in relation to the quantity of soluble protein[26], and the degradability in relation to the protein configuration and structure than solubility.

Tamarind seed cake accounted for lowest *in vitro* NH_3 release (Table 3), being 3.62 mg/ 100 ml SRL followed by maize gluten (7.12mg) and soybean cake (8.90 mg). The highest NH_3 release (17.06 mg) was observed in cotton seed cake, which had comparatively lower effective protein degradability and N solubility in phosphate buffer. In the present experiment, correlation between *in vitro* NH_3 release and effective protein degradability also was not significant ($r = 0.56$, $r^2 = 0.31$). Although it has been observed[28], that proteins which are highly soluble in rumen are subjected to rapid microbial attack and degradation, associated with sharp rise in NH_3 level.

The present investigation has shown that the effective protein degradability may not be accurately predicted on the basis of *in vitro* N solubility or *in vitro* NH_3 release. Through this study, it has been further confirmed that maize gluten has the highest bypass protein value followed by soybean cake and cotton seed cake and the GN cake and rapeseed cake have a low bypass protein.

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