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One Point *In situ* Incubation Estimation of Undegraded Protein in Forages

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ABSTRACT : To estimate undegraded intake protein (UIP) fraction in feeds and fodders, on the basis of their neutral detergent insoluble N content was studied. Samples of six feeds and forages were incubated *in situ* for a time equivalent to their mean retention time, estimated on the basis of their digestibility plus 10 h (to account for a lag in passage of particles from the rumen). The samples were incubated for 0, 25, 50, 75 and 100% of the estimated total mean retention time. UIP value of leguminous forages, obtained from the fractional rates of degradation and passage, were highly correlated with those estimated from samples incubated for 75% of total mean retention time, while incubating the non-leguminous forages and groundnut cake for this point over estimate the UIP fraction. (**Key Words :** One Point Estimation, Undegrdable Protein Content, Forages)

INTRODUCTION

According to the Cornell Net Carbohydrate protein system (CNCPS), the feed CP can be divided into five fractions including b_3, which is the neutral detergent insoluble protein or rumen undegradable protein (UIP). The UIP fraction of the feeds is generally estimated using standard model of Ørskov and McDonald (1979). This first order disappearance model assumes ingested particles are capable of passing immediately out of the rumen. This may not be the case of particles that are too large or buoyant to reach the reticulo-omasal orifice and escape the rumen. The consequence of not accounting for a lag in passage, time during which the particles may be digested but can not escape may result in overestimation of UIP content (Lamothe and Klopfestein, 2003). Ellis et al. (1994) suggested that lag in passage is relatively constant to approximately 10 h. Ørskov and Mc Donald model for predicting the UIP fraction of feeds requires a number of ruminally cannulated animals besides frequent insertion and retrieval of large number of nylon bags in their rumen at different time intervals, which is laborious and time consuming. Therefore, Lamothe and Klopfestein (2003) developed a simple one point *in situ* incubation technique to determine the forage protein quality in terms of UIP.

In spite of the fact that forages supply sizeable part of the dietary proteins to ruminants in India, their rumen degradable protein (RDP) and UIP fractions is not available. The objective of the study was therefore, to estimate the RDP and UIP fractions of various forages using one point incubation technique and their comparison with those obtained using the model given by Ørskov and Mc Donald (1979).

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MATERIALS AND METHODS

Feed samples

Six feed samples of berseem (*Trifolium alexandrinum*), lucerne (*Medicago sativa*), maize, oat (forages), subabool (*Leucaena leucocephala* L.) leaf meal (unconventional protein supplement) and expeller pressed groundnut cake (a conventional protein source) were evaluated for their UIP fraction. Samples of both the leguminous forages were collected from their second and third cut in the month of January-February and after drying at 80°C and grinding (1.5-2.5 mm), mixed in equal proportion to get the representative sample of each forage. Leafy portion of non leguminous forages, subabool leaves and groundnut cake (GNC), collected in the month of Jan./Feb. from Karnal, were dried and ground like to the particle size (1.5-2.5 mm). Each sample was evaluated for their organic matter and CP contents as per AOAC (1990) and cell wall constituents as per Goering and van Soest (1970) prior to *in situ* and *in vitro* evaluation.

Animals and their feeding

Two crossbred bulls and two buffalo bulls of similar age

and body weight, fitted with permanent rumen canulae, were taken as donor animals. All the four animals were fed concentrate mixture and roughage (fresh berseem/oat+ wheat straw+conc. mix) according to their maintenance requirements (NRC, 2002) two times a day (6.00 am and 2.00 pm) for 21 days for their adaptation prior to the starting of *in vitro* and *in situ* trials. The time schedule of feeding, watering, and collection of rumen liquor were kept similar throughout the experimental period to minimize the error.

***In vitro* study**

In vitro dry matter digestibility of the feed and forages samples was determined using single stage technique of Tilly and Terry (1963). Rumen fluid was collected from different parts of rumen three hours post feeding. After checking the pH, rumen fluid was brought into the laboratory, strained through four layer of muslin cloth, and placed in water bath at 39°C. Incubation flasks, containing feed samples (0.5 g) in triplicate, 10ml strained rumen liquor and 40 ml buffer (Mc Doughall, 1948) were incubated at 39°C in an incubator for 48 h with occasional stirring and maintaining the anaerobic conditions. Blanks were also run simultaneously. At the end of the incubation period, 1 ml toluene was added to each flask to stop the fermentation. The contents of the flasks were filtered and the residue was kept overnight at 60°C. The dried residue was weighed for the estimation of DM digestibility.

***In situ* study**

Protein degradability of experimental feeds and fodder in rumen was determined by incubating pre-weighed nylon bags (9×15 cm with 40 μm pore size), containing about 5 g samples (ground to pass through 2.5 mm sieve) as per Mehrez and Ørskov (1977). The bags in triplicate for each feed, fastened on iron chain, were placed in the rumen of cattle and buffalo bulls (2 each) through rumen fistula in each animal. The incubation was carried out at 0, 25, 50 and 75 percent of TMRT (calculated from *in vitro* study), which varied for different feeds. For concentrate ingredients (GNC and subabool leaf meal) and forages, rumen digestion end point was taken as 48 and 96 h, respectively. All the bags were retrieved at the same time (though introduced in the rumen at different time points) and washed thoroughly in cold tap water until rinse was clear. Excess water was squeezed out. The nylon knot was loosened and the bags were kept in oven at 60-70°C overnight followed by drying at 90°C till constant weight. The dried bags were weighed again to determine the dry matter loss at different hours from each of the feed sample. The residual material in each bag was subjected to N estimation (AOAC, 1990) for measuring the protein disappearance.

The fractional passage rate (k_P) was estimated by using the following equation proposed by Klopfenstein et al. (2001)

$$k_P\ (\%/h) = 0.07\ IVDMD\ (\%) - 0.20$$

Mean retention time was calculated by inversing the value of passage rate.

$$MRT\ (h) = 1/0.01\ k_P\ (\%/h)$$

Total Mean retention time (TMRT) was calculated by adding lag phase of 10 h to MRT TMRT = MRT+10, (Ellis et al., 1994).

The residual material in each nylon bag was also analyzed for neutral detergent fibre (NDF) as per van Soest (1968) and its N content as per micro Kjeldahl method (AOAC, 1990) to estimate NDIN fraction. The potentially degradable fraction (b) of feeds was calculated by subtracting the NDIN of residual material from the NDIN of the original feed sample. The undegraded protein (NDIP) fraction at each incubation time point was calculated by multiplying the NDIN with 6.25 to convert N to CP equivalent. Values were not corrected for microbial contamination. The specific time point, where most of the potentially degradable CP is degraded, was estimated.

The degradation rate (kd) of rumen degradable protein fraction of each feed was taken as the slope of regression of the natural log of residual NDIN (corrected for the 96 h indigestible fraction) against time.

The data were analyzed using MS Excel with correlation and regression equations. The regression model used for the analysis is

$$Y_{ijk} = \mu + F_i + I_j + e_{ijk}$$

Whereas Y^{ijk} = UIP content of k^{th} feed sample of i^{th} forage incubated for j^{th} time

μ = Overall mean

Fi = Effect of i^{th} forage (berseem, lucerne, maize, oat, subabool or groundnut cake)

I_j = Effect of j^{th} incubation period (25, 50, 75, 100 percent TMRT and 96 h)

E_{ijk} = Error associated with Y_{ijk}

R^2 = Sum of the square due to regression/total sum of squares

Correlations between methods (one point vs Ørskov and Mc Donald, 1979) were also worked out.

From the protein disappearance at various incubation intervals, constants a, b, and c were estimated from the following equation given by Ørskov and McDonald (1979)

$$P = a + bc/c + k\ (1 - e^{-(c+k)t})$$

Table 1. Chemical composition of different feeds and forages (%DM basis)

Feed	DM	OM	CP	NDF	ADF	ADL	Cellulose	Silica	ADF-N
GNC	90.90±0.1	92.12±0.1	39.05±0.2	35.11±0.0	27.53±0.3	17.62±2.9	7.94±2.2	2.2±0.0	1.32±0.2
Berseem	15.02±0.8	89.20±0.1	13.64±0.2	54.59±1.2	42.74±2.2	8.32±0.7	21.64±6.1	2.76±0.4	2.72±0.3
Lucerne	16.28±0.3	88.40±0.2	14.65±0.0	49.72±2.4	26.72±2.9	7.88±0.0	18.05±4.5	1.40±0.0	1.57±0.0
Maize	22.36±0.6	91.91±0.1	07.79±0.0	48.52±2.2	31.33±1.6	8.48±2.1	18.40±0.0	3.71±0.7	1.44±0.1
Oats	23.38±1.5	93.01±0.0	17.66±0.0	49.05±0.0	40.6±1.7	8.6±1.7	28.06±5.2	4.22±0.0	1.25±0.0
SLM	8.14±0.1	89.73±0.2	26.93±0.3	55.06±2.0	18.23±2.8	25.0±2.8	2.89±0.9	1.62±0.2	3.41±0..3

Table 2. *In vitro* DM digestibility (IVDMD), k_p and total mean retention time of different feeds

Feed	IVDMD (%)	k_p (%/h)	MRT (h)	TMRT (h)
GNC	58.43±0.69	3.89	25.70	35.70
Berseem	57.02±0.08	3.79	26.37	36.37
Lucerne	56.94±0.57	3.78	26.41	36.41
Maize	55.21±0.31	3.66	27.28	37.28
Oats	57.49±0.55	3.82	26.15	36.15
SLM	49.03±0.79	3.23	30.93	40.93

k_p (%/h) = 0.07 IVDMD (%)-0.20.

Where 'a' is readily soluble fraction, 'b' is digestible fraction, and 'c' is degradation rate. Effective protein degradability (EPD) for different feeds from the exponential equation was derived after putting the CP disappearance data at each incubation time point.

The effective degradability (ED) was calculated as per following formula:

$$ED = a+bc/c+k$$

Where 'k" is the fractional rumen outflow rate or passage rate (k_p)

The RDP and UIP values for a feed (% of CP) by this model were computed using the equations RDP = a+b (c/k+c) and UIP = b (k/c+k)+c.

The RDP and UIP values of feeds, obtained from the Ørskov and McDonald (1979) model, were compared with those of the adopted method. For the comparison of UIP values of particular feed in cattle and buffaloes, student's 't' test was applied (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

Chemical composition of feedstuffs

The CP content of forages (Table 1) except that of oats forage was similar to the values reported by Sen et al. (1978). Higher CP content of oats forage than that reported by Sen et al. (1978) may be attributed to the fact that only leafy portion of the fodder was taken for this study. The CP content of subabool leaf meal (SLM) was similar to that reported by Samanta et al. (1994). NDF content of fodders varied in a narrow range, irrespective of their nitrogen content.

Dry matter digestibility

In vitro dry matter digestibility (IVDMD) values for GNC, berseem, lucerne, maize and oats fodder, and SLM were 58.43, 57.02, 56.94, 55.21, 57.49 and 49.03, per cent, respectively (Table 2). Similar IVDMD of GNC and forages may be attributed to the longer incubation period and higher ADF content of GNC (27.53%). The IVDMD of SLM was lower than that of GNC and fodders and values varied in the range of 42-70 per cent. Lower IVDMD value for SLM can be attributed to its tannins content, which is negatively correlated to IVDMD (Terill, 1989) and to non-adaptation of ruminal microbes to mimosine (toxic amino acid). The fractional passage rate of digesta (kp) of all feeds except for SLM was considered as 4 per cent (nearest whole number) while the value for later was considered as 3 per cent.

TMRT

The estimated mean retention time (MRT) for most of the samples was about 26 h except for SLM where it was 30.9 h. Therefore, total mean retention time (TMRT) for GNC, berseem, lucerne, maize, oats fodders and SLM was calculated to be 35.7, 36.37, 36.41, 37.28, 36.15 and 40.93 h, respectively. For the practical purposes, TMRT of all the feeds except that of SLM was taken as 36 h and for later it was taken as 40 h. Variation in TMRT value of different feeds was in a narrow range as recorded by Klopfenstein and Lamothe (2003) for pasture and range grasses.

Protein disappearance

Crude protein disappearance of feeds at various incubation intervals (25, 50, 75 and 100 per cent of TMRT and 96 h) in crossbred cattle and buffaloes have been presented in Table 3.

The disappearance of protein from the rumen is the sum of degradation and passage. The CP disappearance curve was found similar for all the feeds in both species. Most of the CP disappeared during the incubation period up to 25 per cent of TMRT and the value was 63, 56, 68, 50 and 61 per cent for GNC, berseem, lucerne, maize, and oats, respectively, however value for SLM was only 22 per cent. Thereafter, the disappearance rate declined gradually. In maize fodder, the residual CP was constant after 75 and 100 per cent of total mean retention time as well as at 96 h of incubation. In oats, SLM and GNC, the CP was degraded

Table 3. Residual CP (% DM) of feeds at different TMRT points in cattle and buffalo bulls

Feed	Species	25%	50%	75%	100%	96 h
GNC	Cattle	13.73±0.02	11.05±0.04	9.39±0.01	6.91±0.03	3.84±0.02
	Buffalo	13.54±0.01	10.9±0.02	10.61±0.02	6.7±0.02	4.23±0.01
Berseem	Cattle	5.85±0.05	3.63±0.01	3.32±0.02	2.12±0.02	1.77±0.02
	Buffalo	5.72±0.02	4.1±0.01	3.52±0.04	2.22±0.03	2.0±0.01
Lucerne	Cattle	4.51±0.01	3.51±0.01	2.97±0.02	2.63±0.01	2.27±0.02
	Buffalo	4.7±0.01	3.82±0.01	3.1±0.01	2.8±0.01	2.6±0.00
Maize	Cattle	3.76±0.01	3.42±0.02	3.15±0.05	3.11±0.01	1.26±0.01
	Buffalo	3.93±0.01	3.38±0.01	3.18±0.01	3.0±0.01	1.08±0.01
Oats	Cattle	6.56±0.01	5.65±0.02	4.26±0.01	3.84±0.02	2.1±0.01
	Buffalo	6.56±0.01	5.65±0.02	4.94±0.02	4.58±0.03	1.79±0.01
SLM	Cattle	20.3±0.02	18.23±0.01	15.92±0.02	13.92±0.02	8.78±0.03
	Buffalo	19.91±0.03	17.96±0.01	15.73±0.03	14.1±0.02	9.02±0.02

Table 4. Degradability parameters of protein in feeds measured *in situ* according to Ørskov and McDonald (1979)

FEED	Species	a	b	c	Potential degradability	Effective degradability ED
GNC	Cattle	5.97	79.28	0.1267	85.26	66.2
	Buffalo	5.94	78.25	0.1326	84.19	66.1
Berseem	Cattle	2.61	91.81	0.0923	94.42	66.7
	Buffalo	2.74	90.48	0.094	93.22	66.2
Lucerne	Cattle	4.44	86.87	0.1311	91.32	71
	Buffalo	4.63	86.69	0.1224	91.32	70
Maize	Cattle	2.9	77.2	0.1501	80.1	63.9
	Buffalo	2.93	76.74	0.1553	79.68	64
Oats	Cattle	4.01	76.93	0.1397	80.94	63.8
	Buffalo	4.01	76.41	0.1418	80.42	63.6
SLM	Cattle	5.56	66.89	0.0262	72.45	36.7
	Buffalo	5.95	63.86	0.0283	69.81	37

a: soluble fraction, b: not soluble, but potentially degradable fraction, c: fractional rate of degradation (%).

even after 100 per cent TMRT but at decreasing rate and the values of residual CP at 100 per cent TMRT and 96 h incubation were 4.48 and 1.79, 14.1 and 9.02, 6.7 and 4.23, respectively in cattle and buffaloes (Table 3) as recorded by Klopfenstein and Lamothe (2003). The difference may be due to variation in protein solubility of different feeds (Chalupa, 1975; Tamminga, 1979), which, in turn depends upon the affinity of bacteria towards the soluble protein fraction. The modest decrease in the extent of CP degradation after 25 percent TMRT was largely associated with the increased proportion of N recovered as NDIN or UDN.

Protein degradation characteristics

The effective protein degradability (EPD) of feeds (Table 4) was determined at the fractional passage rate of digesta (kp) estimated by i*n vitro* study at 4%/h for all the feeds except SLM, for which it was 3%/h. Highest EPD was recorded for lucerne (71%) followed by berseem and GNC (66%), maize and oats (64%) and lowest for SLM (36.7%) and the values were similar in both species. These results indicated that protein content of GNC, berseem and lucerne was more susceptible to microbial degradation in the rumen than that of maize and oats forages and SLM was having the lowest susceptibility, possibly due to its tannin content. Therefore, SLM may be considered as a better source of UIP than forages. Availability of UIP from SLM at lower tract appeared to be high as replacement of 50 percent of dietary CP with it improved the growth in buffalo calves (Akbar and Gupta, 1985), however, higher level may be detrimental (Kurar et al., 1984) due to its mimosine content (Akbar and Gupta, 1985).

Similar to present observations, Sampath et al. (1999) recorded that the protein degradability value of expeller GNC, maize fodder and SLM was 67, 58.62 and 25 per cent, respectively.

The soluble fraction ‘a’ for six feeds in the increasing order were: berseem (2.61), maize (2.9%), oats (4.01%), lucerne (4.44%), SLM (5.56%) and GNC (5.97%) and insoluble but degradable fraction ‘b’ in increasing order were: SLM (66.89%), oats (76.93%), maize (77.2%), GNC (79.28%), lucerne (86.87%) and berseem (91.81%). These differences may be attributed to the variations in protein characteristics of different feeds and forages. Total potentially degradable CP fraction (a+b) was highest in berseem followed by lucerne, groundnut cake, oats, maize

Table 5. NDIN (% DM) of feeds at different TMRT periods in cattle and buffalo bulls

Feed	Species	25%	50%	75%	100%	96 h
GNC	Cattle	9.44±0.00	9.35±0.09	8.68±0.06	6.89±0.10	3.81±0.03
	Buffalo	9.79±0.34	8.90±0.19	7.58±0.24	6.63±0.36	4.19±0.36
Berseem	Cattle	2.72±0.04	2.41±0.00	2.23±0.17	2.09±0.00	1.75±0.00
	Buffalo	2.67±0.02	2.27±0.18	2.25±0.16	2.08±0.36	1.92±0.18
Lucerne	Cattle	2.95±0.15	2.82±0.06	2.66±0.11	2.61±0.03	2.25±0.19
	Buffalo	3.07±0.13	2.96±0.52	2.88±0.25	2.77±0.03	2.60±0.19
Maize	Cattle	3.46±0.04	3.31±0.17	3.13±0.01	3.08±0.37	1.21±0.16
	Buffalo	3.59±0.07	3.32±0.17	3.13±0.34	2.96±0.17	1.04±0.01
Oats	Cattle	5.94±0.00	5.59±0.01	4.18±0.01	3.82±0.03	2.06±0.34
	Buffalo	6.29±0.70	5.54±0.41	4.87±0.01	4.54±0.34	1.73±0.01
Subabool	Cattle	16.17±0.08	16.09±0.01	14.99±0.04	12.98±0.99	8.70±0.05
	Buffalo	15.73±0.34	15.38±0.35	14.37±0.38	13.76±0.22	8.98±0.24

Table 6. Rate of CP degradation (kd %/h) of feeds

Feed sample	Animal species	0-25% TMRT	25-75% TMRT	75-100% TMRT
GNC	Cattle	1.03	0.81	5.08
	Buffalo	0.91	2.8	2.65
Berseem	Cattle	3.7	3.92	3.67
	Buffalo	5.97	4.56	7.99
Lucerne	Cattle	2.76	2.96	1.39
	Buffalo	4.06	2.76	5.73
Maize	Cattle	2.94	0.87	0.31
	Buffalo	2.44	1.32	0.94
Oats	Cattle	1.68	3.35	2.12
	Buffalo	0.51	2.08	1.24
Subabool	Cattle	0.22	0.86	3.85
	Buffalo	0.5	1.13	1.2

and lowest in SLM (Table 4). The degradation rate 'c' was highest in maize followed by oats, berseem, lucerne, and lowest for SLM, and the variation between species was not significant.

Fractional degradation rate of protein

Lower NDIN degradation rate of all the feeds during 75 to 100 per cent of TMRT (Table 7) indicated that most of the potentially degradable NDIN or UIP was already degraded by the 75 per cent of TMRT, however, degradation continued slowly. Klopfenstein and Lamothe (2003) also reported the same trend of degradation rate of range grasses and pastures. The differences in rate of CP degradation among different feeds were possibly related to their ADF bound N (Table 1).

The degradation rate of leguminous forages (berseem and lucerne) was faster, irrespective of incubation interval, than those of non-leguminous ones (maize and oats). Berseem CP was degraded faster than that of lucerne and highest degradation rate was recorded during 25 to 75 per cent of TMRT in both the forages. However, highest degradation rate for maize and oats forages was recorded during 0 to 25 per cent and 25 to 75 per cent of TMRT, respectively. Such variations may be attributed to the difference in structure and solubility of protein of different feeds. Lowest degradation rate of SLM further confirmed its lowest IVDMD, recorded in an earlier experiment.

Table 7. UIP content (% DM) of feeds estimated by three different approaches

Feed	Animal species	Equation[a]	75% TMRT[b]	TMRT[b]
GNC	Cattle	5.29	8.68	6.89
	Buffalo	5.5	7.58	6.63
Berseem	Cattle	2.16	2.23	2.09
	Buffalo	2.31	2.25	2.08
Lucerne	Cattle	2.46	2.66	2.61
	Buffalo	2.77	2.88	2.77
Maize	Cattle	1.83	3.13	3.08
	Buffalo	1.72	3.13	2.96
Oats	Cattle	3.07	4.18	3.82
	Buffalo	2.78	4.87	4.54
Subabool	Cattle	11.54	14.99	12.98
	Buffalo	11.66	14.37	13.76

[a] UIP = Pot. Dig. b * (kp/(kp+kd))+96 h value.
[b] *In situ* incubation for 75% of TMRT.

It was also evident that CP degradation was higher in buffaloes than in crossbred cattle, for all the feeds except that of GNC and oat fodder. In general, average kd (%/h) values (Table 6) were higher in buffaloes than in crossbred cattle, however, values were less in buffaloes for GNC and SLM, which may be attributed to the difference in ruminal micro flora (Pradhan et al., 1991).

Undegraded intake protein (UIP)

The values of neutral detergent insoluble nitrogen (NDIN) or UIP (% DM) of different feeds and fodders at various TMRT incubation intervals and the corrected NDIP (value at 96 h subtracted from UIP value at each TMRT incubation interval) are presented in Figure 1 and Table 5, respectively.

The UIP value (undegraded protein fraction at 96 h incubation) of GNC, berseem, lucerne, maize, oats and SLM was 3.81, 1.75, 2.25, 1.21, 2.06, and 8.70 per cent, respectively, which indicated a wide variation among all the

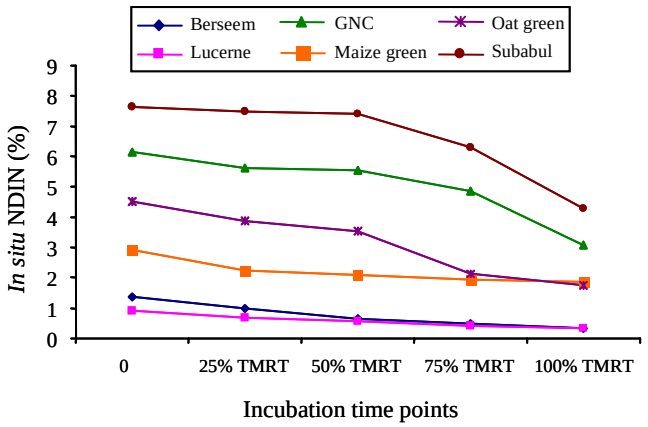

Figure 1. *In situ* CP degradation of feeds at different incubation time point in cattle.

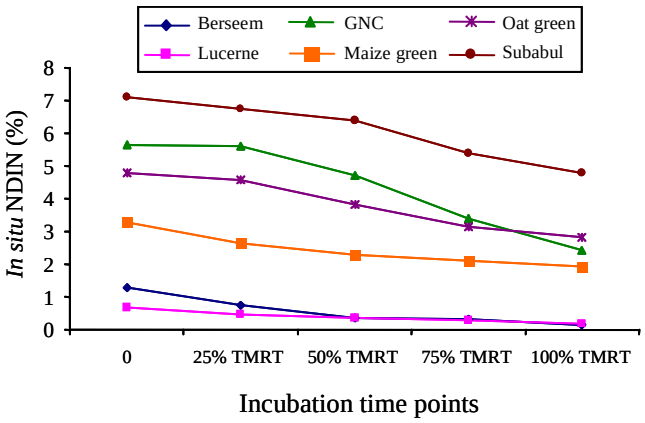

Figure 2. *In situ* CP degradation of feeds at different incubation time points in buffalo. [a] Undegraded protein (% DM) corrected for 96 h values.

feeds and forages, however, variation between animal species was narrow (15.53 to 32.39 per cent in cattle vs 13.35 to 33.33 per cent in buffaloes). Similar difference in UIP content of pasture and range grasses was reported by Klopfenstein and Lamothe (2003). Protein degradation in buffaloes was higher than in crossbred cattle except that of maize and oats forages.

From these results it can be interpreted that at different TMRT incubation periods, part of UIP fraction was still available for degradation by the next TMRT incubation point. The decrease in UIP content of feeds continued till 96 h of incubation, however, values at 100 per cent of TMRT and 96 h incubation period were almost similar. These observations indicated that UIP fraction at 96 h incubation was constant and was not expected to degrade further. It was therefore, evident that CP of all the feeds under study was degraded with in 100 per cent TMRT incubation period, calculated on the basis of *in vitro* experiment.

Difference in UIP content (% DM) of feeds and forages by different methods

The UIP content (% DM) of feeds estimated by three different methods (Ørskov, 75% TMRT and TMRT level) is shown in Table 7. The UIP content of berseem and lucerne determined at 75 and 100 per cent of TMRT were highly correlated (r^2 = 0.98) with those calculated as per Ørskov model. In SLM, the estimate of UIP from 100 per cent of TMRT incubation was close to that calculated as per Ørskov's model (42.84). However, for GNC, maize and oats fodder, values beyond 100 per cent TMRT have to be considered, irrespective of animal species.

CONCLUSION

The overall results of this study suggested that the UIP values of different feeds obtained by using single point and multi point incubation methods for crossbred cattle and buffaloes were highly correlated (r^2 = 0.98) for berseem, lucerne and SLM while single point incubation of GNC, maize and oats forages, overestimated their UIP content. Therefore, single point incubation technique for the estimation of UIP of feeds cannot be used for all type of feeds.

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