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# Ruminal degradability of bypass fat and protein of certain commonly used feedstuffs in dairy rations

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#### ABSTRACT

Studies were conducted to determine the rumen degradability of protein of certain commonly used feed ingredients and degradability of bypass fat used in dairy rations. *In situ* crude protein (CP) disappearance of groundnut cake, soybean extraction, cottonseed cake, maize gluten meal, wheat bran, rice bran, maize, 0.3, 0.6 and 0.9% (of CP) formaldehyde treated soybean extraction, and *in situ* fat disappearance of bypass fat in the rumen at different incubation periods were determined by nylon bag technique using 3 adult crossbred steers. The effective degradability was calculated for an assumed outflow rate of 5%/h. The effective CP degradability of groundnut cake, soybean extraction, cottonseed cake, maize gluten meal, wheat bran, rice bran, maize, 0.3, 0.6 and 0.9% (of CP) formaldehyde treated soybean extraction was 86, 74, 66, 21, 73, 65, 63, 66, 48 and 26%, respectively. The effective fat degradability of bypass fat was 86%. The results indicated that maize gluten meal is a good source of bypass protein. Soybean extraction although extensively degraded in the rumen, contribute fairly more amount of undegradable protein than rumen degradable protein at an outflow rate of 5%/h, when it is treated with 0.6 and 0.9% (of CP) formaldehyde. The bypass fat supplement prepared from rice bran acid oil is a good source of energy in which about 86% of fat would be available at the lower tract of ruminants. Therefore, maize gluten meal, 0.9% formaldehyde treated soybean extraction and protected fat can be included in the rations of dairy animals for improved productive performance.

Keywords: Bypass fat, Energy supplements, Protein and fat degradability, Protein supplements

Better utilization of available feed resources has become a key theme in the recent years all over the world, especially in developing countries including India. Most of the supplements (concentrates, grains and other agro-industrial by-products) became major and essential components of feed formulation process in ruminant rations. The introduction of newer varieties of feedstuffs and different processing techniques to which these feedstuffs are subjected before being made available for incorporation in the ruminant feeding leads to considerable variation in the chemical composition and digestibility. During early lactation, the dairy animals, particularly high yielding animals will be in negative energy balance because of lower feed intake, which may not meet the demand for higher milk yield. The situation will further aggravate in animals fed on poor quality roughages. Under these conditions, feeding with protected nutrients viz. proteins and fats will be helpful to some extent in avoiding or reducing the incidence of negative energy balance in dairy animals.

The protein requirement of ruminants and protein value of feedstuffs have for a long time been expressed

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as digestible crude protein (DCP). The metabolizable protein systems (ARC 1980, NRC 1985) proposed as an improvement to the digestible crude protein (DCP) system for protein evaluation of feedstuffs and for expression of protein requirement of ruminants, require the description of dietary crude protein in terms of rumen degradable protein (RDP) and undegradable protein (UDP). This not only applies to the protein supplements but also to the cereal grains and other agro-industrial byproducts which make up a considerable proportion of compounded feeds for ruminants. On the other hand, excessive feeding of grain or oil/fat in meeting energy requirements of animals may affect rumen fermentation and may lead to further metabolic disorders. In order to avoid these complications, protected fat can be included in the ration of dairy animals. The degradability of protein or fat may vary considerably due to the source of raw materials and the processing methodology. Therefore, it is essential to have more data on degradability of certain commonly used feedstuffs including protected fats to have a comprehensive knowledge. The present study was, therefore, undertaken to assess the degradability of some commonly used feedstuffs and protected fat, which can be used in ruminant rations.

## MATERIALS AND METHODS

Animals: Adult crossbred steers (3) of ~500 kg body

weight fitted with large rumen canula were used to estimate the degradability of feedstuffs. Animals were fed a concentrate mixture (maize 30%, groundnut cake 25%, wheat bran 42%, mineral mixture 2% and salt 1%), green grass (para grass) and ragi straw (Roughage to concentrate ratio 70:30) to meet the nutrient requirement for maintenance (ICAR 1985).

Feed ingredients: Commonly available feed ingredients such as soybean extraction, groundnut cake, wheat bran, maize gluten meal, cotton seed cake, rice bran and maize were used for the study and were screened for DM and CP. Though the groundnut cake and soybean extraction are good sources of protein for ruminants, but they are highly degradable in the rumen. Further, the soybean extraction has good amount of amino acids in its protein content when compared to groundnut cake (Chandrasekharaiah et al. 2003). Hence, the soybean extraction was selected for the study and was treated with different levels of formaldehyde (0.3, 0.6 and 0.9% of CP) in order to select optimum level of treatment for protecting protein from rumen degradation.

Fats/oils: Different oils (acid oils from sunflower oil processing, rice bran oil processing, and palm oil) were procured from the local market in Bengaluru and used for preparation of bypass fat. Standardized the bypass fat preparation with different levels of oils (25, 50 and 100 g), acid (1.5, 2.0 and 3.0), lime viz. laboratory, technical grade and commercially available sources of lime (40 and 50 g), temperature (100, 120 and 150°C), time (45, 50 and 100 min), washings (6-8 times) and drying period (2 days) and the bypass fat so prepared (Fig. 1) was stored in a cool and dark place in air tight containers till further analysis.

Preparation of bypass fat: The bypass fat prepared from rice bran acid oil was selected for further studies and kept in air tight container in a cool place after mixing with butylated hydroxyl toluene (BHT) @ 0.05% as an antioxidant (Naik et al. 2007).

Determination of degradability of different feedstuffs and bypass fat: The samples (about 5 g each) were placed in nylon bags (pore size 40  $\mu$ m) of size 100 mm  $\times$  170 mm and suspended in the rumen of fistulated animals for 3, 6, 9, 12 and 24 h. Nylon bags were introduced into the rumen in descending order (i.e. 24 h was introduced first), so that all bags were removed and washed at the same time. Control samples (0 h) were washed without incubation in









Fig. 1. Bypass fat prepared from different oils

the rumen in a washing machine (LG make, Model no. WP - 9241, Spinning speed 740 rpm for 15 min) and dried to a constant weight at 60°C as was also practiced for nylon bags removed after specific incubation period in the rumen. Each sample was tested in duplicate for 2 consecutive days in the rumen of 3 animals. The dry matter (DM) and crude protein (CP) disappearing from different feed ingredients, and DM and fat disappearing from bypass fat samples at different incubation periods were estimated (Mehrez and Orskov 1977). The DM, CP and fat degradability values after different incubation times were fitted to the model suggested by Ørskov and McDonald (1979). The effective degradability was calculated for an assumed outflow rate of 5%/h. The chemical composition of samples used in the study was estimated (AOAC 1995), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were assayed without a heat stable amylase and sodium sulphite and expressed inclusive of residual ash, as per Van Soest et al. (1991). Fat content of bypass fat was estimated after acid hydrolysis (Folch et al. 1957).

For analysis of fibre fractions, NDF and ADF were assayed without a heat stable amylase and sodium sulphite and expressed inclusive of residual ash, as per Van Soest *et al.* (1991).

#### RESULTS AND DISCUSSION

Dry matter and protein degradability of feed ingredients: The chemical composition of different feed ingredients

Table 1. Chemical composition of feedstuffs/supplements (% on DM basis)

Parameter	Dry matter	Organic matter	Crude protein	Ether extract	Total ash	Neutral detergent fibre	Acid detergent fibre
Groundnut cake	95.25	91.29	43.26	0.698	8.71	28.1	19.61
Soybean extraction	95.36	91.57	48.08	1.26	8.43	27.87	19.43
Cottonseed cake	94.56	93.49	35.74	0.377	6.51	36.82	26.45
Maize gluten meal	96.45	97.04	63.2	0.607	2.96	20.83	9.34
Wheat bran	92.1	93.99	13.8	2.31	6.01	40.01	12.01
Rice bran	89.85	86.94	15.5	1.55	13.06	55.25	23.89
Maize	97.98	98.77	8.95	1.17	1.23	14.14	3.79
Bypass fat	97.03	70.77	-	56.84	29.23	-	-

and bypass fat prepared from rice bran acid oil is given in Table 1. The CP content of the feedstuffs used in the present study ranged from 8.95 (maize grain) to 63.2% (maize gluten meal) (Table 1). The percentage of DM and CP disappearing from feedstuffs at various hours of rumen incubation is given in Table 2. In protein supplements and cereals and byproducts, the trend of disappearance was similar for both dry matter and protein except for cotton seed cake. The DM disappearance of cotton seed cake was much lower (41%) than that of protein disappearance (73%) during 24 h of incubation in the rumen. The lower rate of DM disappearance may be attributed to higher ADF content in cotton seed cake. Sampath and Sivaraman (1985) and Chandrasekharaiah et al. (2001) also observed a poor correlation between protein and DM disappearance in high fibrous feeds. Although soybean extraction and groundnut cake are good sources of protein for ruminants, these feeds do not provide sufficient amount of metabolizable protein for high yielding animals due to extensive degradation in the rumen. These protein sources need to be fed in protected form in high yielders. Further, the soybean extraction has good amount of amino acids in its protein content when compared to groundnut cake (Chandrasekharaiah et al. 2003). Hence, the soybean extraction was selected for the study and was treated with different levels of formaldehyde (0.3, 0.6 and 0.9% of CP) in order to select optimum level of treatment for protecting protein from rumen degradation.

Among the feedstuffs studied, the groundnut cake, soybean extraction and wheat bran were highly susceptible to rumen microbial degradation with ruminal protein

degradability values of 86, 74 and 73%, respectively, at an outflow rate of 5%/h, while the maize gluten meal and 0.9 and 0.6% formaldehyde treated soybean extraction was least degraded with 21 and 26 and 48% degradability, respectively at the same outflow rate (Table 3). Feedstuffs such as maize grain, rice bran, cottonseed cake and 0.3% formaldehyde treated soybean extraction had the degradability values in the range of 63 to 66%. Thus the maize gluten meal and 0.9% formaldehyde treated soybean extraction provided the highest amount of UDP/kg DM, while the groundnut cake provided the highest amount of RDP/kg DM. Low degradability value of 22% for maize gluten meal and degradability value of 38 and 54% for soybean extractions were reported by Chaturvedi and Walli (1995), Garg (1997) and Prasad (1997), respectively. It is difficult to fix absolute protein degradability values for feedstuffs. Feed/feed composition etc. affects degradability of feedstuff in situ in a particular category of animal. Several factors introduce considerable variations into what is supposed to be standard for comparison. It is realistic therefore, to determine the relative protein degradability values for different feedstuffs and to rank them relative to one another. The protein degradability values for wheat bran, maize gluten meal and rice bran obtained in this investigation are in the range of earlier reported values (Sampath et al. 1999, Chandrasekharaiah et al. 2001). However, in maize grain, the 63% ruminal protein degradability recorded in this study is within the range of reported mean value of 62% for different maize varieties by Seifried et al. (2016), but higher than the value of 30%

Table 2. Percent of DM and protein disappeared from feedstuffs incubated in the rumen for different periods of time (Average of 12 observations)

Feedstuff	Attribute		Period of incubation (h)					
		3	6	9	12	24		
Groundnut cake	DM	56.12±5.19	63.95±1.91	71.32±2.48	76.86±0.57	81.69±4.02		
	Protein	$56.89 \pm 6.43$	72.45±1.61	91.63±3.35	$96.76\pm0.501$	$98.70 \pm 0.28$		
Soybean extraction	DM	25.54±1.60	$50.38\pm4.90$	61.74±5.53	75.91±6.46	$97.52\pm0.67$		
	Protein	$30.25\pm2.00$	$55.40\pm5.94$	64.90±6.80	90.41±0.92	99.45±0.14		
Cottonseed cake	DM	$11.20\pm0.73$	$15.95\pm0.86$	32.93±1.66	$38.00\pm2.51$	$41.20\pm3.29$		
	Protein	41.44±4.20	52.92±3.58	69.10±5.21	$71.79\pm5.40$	$72.76\pm6.20$		
Maize gluten meal	DM	14.35±0.98	18.30±1.08	21.79±1.18	28.32±1.40	43.84±1.95		
	Protein	3.15±1.04	5.21±1.07	8.88±1.16	15.06±1.29	30.75±1.93		
Wheat bran	DM	$27.52\pm2.91$	45.24±3.62	52.96±3.15	52.25±2.56	$63.41\pm6.26$		
	Protein	$14.37 \pm 1.20$	64.81±4.48	67.63±5.88	$78.42 \pm 5.04$	85.10±3.26		
Rice bran	DM	19.79±1.86	45.63±3.47	48.68±3.05	$67.92\pm4.3$	$74.73 \pm 4.91$		
	Protein	$30.54 \pm 3.05$	45.36±4.81	67.38±2.17	$74.86\pm6.31$	85.95±5.21		
Maize	DM	24.42±1.52	41.29±2.31	58.28±3.16	60.37±3.98	85.81±2.31		
	Protein	$28.87 \pm 2.02$	$39.91\pm2.84$	55.63±4.89	$57.02\pm4.60$	83.25±3.29		
Soybean extraction (0.3%)	DM	$5.83 \pm 0.5$	$12.04 \pm 1.77$	29.45±2.81	43.51±2.91	$83.81 \pm 1.4$		
	Protein	$7.67 \pm 0.64$	11.88±1.98	24.32±3.12	$36.43\pm3.92$	83.81±2.18		
Soybean extraction (0.6%)	DM	4.23±1.29	$8.85\pm2.34$	18.48±1.91	27±1.26	$68.24 \pm 5.08$		
	Protein	$5.71\pm1.41$	$9.7 \pm 2.83$	11.57±1.07	19.94±1.6	$64.3 \pm 6.47$		
Soybean extraction (0.9%)	DM	$7.58\pm0.6$	$8.73 \pm 0.44$	12.97±0.35	$18.72\pm0.90$	$42.39\pm3.02$		
	Protein	$6.38\pm0.54$	$6.93\pm0.53$	$7.34 \pm 0.50$	$11.58\pm0.58$	34.66±3.34		

Feedstuff	CP (%)	Degradability (%)	RDP (g/kg DM)	UDP (g/kg DM)
Groundnut cake	43.26	86	372	61
Soybean extraction	48.08	74	356	125
Cottonseed cake	35.74	66.01	236	121
Maize gluten meal	63.20	21	133	499
Wheat bran	13.80	73	101	37
Rice bran	15.50	65.29	101	54
Maize	8.95	63.18	57	33
Soybean extraction (0.3%)	48.82	65.60	320	168
Soybean extraction (0.6%)	48.12	48.40	236	252
Souhean extraction (0.0%)	48.02	25.90	126	362

Table 3. Crude protein, effective degradability, RDP, UDP and content of feedstuffs at an outflow rate of 5%/h

reported by Sampath *et al.* (1999) and lower than the value of 77 and 68% reported by Lamba *et al.* (2014) and Lei *et al.* (2018), respectively. The degradability of soybean extraction observed in the study (74%) was in the range of value (74%) reported by Lee *et al.* (2016), but higher than the reported values of 60% (Sampath *et al.* 1999), 63% (Lei *et al.* 2018) and 54% (Chandrasekharaiah *et al.* 2001, 2008). Sampath *et al.* (1999) and Gao *et al.* (2015) reported that the effective degradability of cotton seed cake was about 52% and 39%, which were lower than the values reported in the present study.

The variations observed in the present study may be attributed to the temperature and time to which the cakes are subjected during oil extraction process. These processing factors affect the susceptibility of feedstuff for ruminal degradation. The in situ results from different studies and laboratories are relatively difficult to compare as even a small differences in methodological details can have high impact on estimated degradation parameters in in situ approaches. Further, the introduction of newer varieties of feedstuffs and different processing techniques to which these feedstuffs are subjected before being made available for incorporation in the ruminant feeding leads to considerable variation in the chemical composition and digestibility. Therefore, it is essential to have more data on chemical composition and its in vitro digestibility to have comprehensive knowledge.

Information is limited about the ruminal protein degradability values of certain Indian ruminant feedstuffs viz. formaldehyde treated soybean extractions and also cereal and their byproducts such as maize, rice bran and wheat bran. Although, the contribution of cereal and their byproducts in terms of crude protein is lower as compared to protein supplements, they make up a considerable

proportion of the ration of concentrate mixture in the diet of ruminants in southern parts of India. The CP content of brans may vary from 10 to 16%. These byproducts form the important ingredients of the concentrate mixture of lactating animals because of their bulky nature.

DM and fat degradability of bypass fat: Acid oils from sunflower oil, rice bran oil and refined palm oil appeared black, dark brown and yellow in colour, respectively. The particle size /shape and colour of bypass fat prepared from sunflower acid oil, rice bran acid oil and refined palm oil observed in this study ranged from small granules to medium to large chunks with creamish yellow, grey and white in colour respectively. The bypass fat prepared from sunflower acid oil and refined palm oil was sticky in nature with large chunks and was posing handling and drying problems. Hence, bypass fat prepared from the rice bran acid oil alone which appeared in granular form was taken for further *in sacco* studies. The literature with regards to the degradability of bypass fat at different time intervals is either scanty or limited.

The percentage of DM and fat disappearing from bypass fat prepared from rice bran acid oil at various hours of rumen incubation is given in Table 4. The trend of disappearance was similar for both dry matter and fat at different hours of rumen incubation.

The fat % of the rumen protected fat prepared from rice bran acid oil in this study was 57%. The fat content of bypass fat obtained in the present study was lower than the findings of the earlier workers, which were 85% (Garg and Mehta 1998), 84% (NRC 2001), 86.1% (Alexander *et al.* 2002), 82 to 84.5% (Anonymous 2002, Naik *et al.* 2007) and 70.6% of (Sanz Sampelayo *et al.* 2004), but higher than 40%, reported by Haland *et al.* (1999). The total ash content of bypass fat was about 30% which was higher than

Table 4. Percent DM and EE disappeared from bypass fat incubated in the rumen for different periods of time (Av. 12 observations) and effective degradability of bypass fat at an outflow rate of 5%/h

Attribute	3 hours	6 hours	9 hours	12 hours	24 hours
DM	$0.746 \pm 0.084$	2.493±0.087	5.496±0.242	12.03±0.97	$13.45 \pm 0.795$
Fat	$0.832 \pm 0.0273$	$3.696\pm0.781$	5.496±0.657	$10.72 \pm 0.781$	$14.98 \pm 0.357$
Effective DM degradability	-	-	-	-	9.80
Effective Fat degradability	-	-	-	-	14.1

the values reported by earlier workers (Garg and Mehta 1998, Alexander *et al.* 2002, Naik *et al.* 2007). Generally, after preparation of bypass fat, the product is washed several times to remove unbound calcium. The differences in washing process might have resulted in higher ash content (30%) than the observations of earlier reports and would have contributed for lower fat percentage of bypass fat prepared in this study.

The mean degradability of the DM and fat of bypass fat observed in the present study was 10 and 14%, respectively indicating the undegradability (protection) of the same with 90 and 86%, respectively, which will be available at the lower tract of ruminants for absorption. The undegradability of bypass fat observed in this study was higher than the values of the earlier workers who reported as 76% (Haland *et al.* 1999) and 79-80% (Garg *et al.* (2002a, b), but similar (84-88%) to the findings of Naik *et al.* (2007).

The acid oil used in this study is the byproduct of rice bran oil extraction which contain higher amount of free fatty acids and variable amounts of triglycerides (Naik *et al.* 2007). The digestibility of nutrients and reproductive performance of crossbred heifers will not be affected when fed with bypass fat in concentrate mixtures up to 3.5% of the total DM intake (Saijpaul *et al.* 2001). The bypass fat was supplemented by about 200-300 g per day or @ 2.5% to 4.0% of the total DM intake in the dairy cattle and buffaloes rations by many workers (Naik *et al.* 2009, Tyagi *et al.* 2009a, 2009b, Thakur and Shelke 2010, Sirohi *et al.* 2010, Mudgal *et al.* 2012, Wadhwa *et al.* 2012) in India. Therefore, the bypass fat prepared in this study can be included in the rations of dairy animals for improved productive performance.

The results indicated that maize gluten meal is a good source of bypass protein. The soybean extractions treated with 0.6% and 0.9% formaldehyde provide fairly good amount of bypass protein. The bypass fat supplement prepared from rice bran acid oil is a good source of energy in which about 86% fat would be available at the lower tract of ruminants. Therefore, maize gluten meal, 0.9% formaldehyde treated soybean extraction and protected fat can be included in the rations of dairy animals for improved productive performance.

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