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RESEARCH ARTICLE

In Situ Assessment of Ruminal Dry Matter Degradation Kinetics and Effective Rumen Degradability of Feedstuffs Originated from Agro-Industrial By-Products

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ABSTRACT

In the tropical Asian countries, information on nutrients availability from various by-products of agro-food industries remains scarce and even less is known about their application in the feed evaluation systems. The objective of this study was to generate renewed data on *in situ* rumen dry matter (DM) degradability of by-products from oilseeds, cereal grains, and animal origin, commonly fed to animals in tropical Asian countries. The data were used to derive regression equations to understand the relationships between effective rumen degradability of DM (ED_{DM}) and the rumen degradation characteristics of the by-products. Sixty four samples of five oilseed by-products, seven cereal grain by-products and four animal by-products were used. From each feed, 4 samples (~1 kg each) were collected from dairy farms (n=1), local markets (n=1) and different agro-industries (n=2). The feeds were incubated in the rumen for 2, 4, 8, 12, 24 and 48 h, using nylon bag technique. There was a significant ($P<0.01$) variation in the rumen degradation kinetics and ED_{DM} within by-products of oilseeds, cereal grains and animal origin. Regression analysis showed strong relationships (R^2 values above 0.90) between the ED_{DM} (g/kg DM) calculated using three rumen passage rates (0.04, 0.06 and 0.08 h^{-1}) and the washout (*W*) and rumen undegradable (*U*) contents (g/kg DM) of oilseed and cereal grain by-products. The renewed database is more reliable because all the by-product feeds were evaluated under uniform experimental conditions and the variation among sources of these feeds were also covered.

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INTRODUCTION

To increase the economical profitability of dairy farms in terms of animal performance and health, information is required on nutrient requirements of animals and the nutrient digestibility of feedstuffs that are commonly fed to animals. By-products from oilseeds, cereal grains and animal origin are commonly fed to the dairy animals as protein supplement in the tropical Asian countries. In these countries, information on nutrient availability of various concentrates remains scarce and even less is known about their application in the feed evaluation system.

Alternative techniques (*in situ*, *in vitro* and *in vivo*) have been used to measure the nutrient availability of different feedstuffs (Habib *et al.,* 2013). During the last three decades, the *in situ* (*in sacco*) techniques are extensively used for the determination of ruminal degradation of different chemical components like dry matter (DM), crude protein (CP), neutral detergent fibre, organic matter and trace elements (Piri *et al.,* 2012; Kokten *et al.,* 2012). The *in situ* nylon bag technique is used as a reference method in the evaluation of rumen degradation characteristics of feedstuffs (De Boever *et al.,* 2002; Habib *et al.,* 2013). Main factors affecting the results of *in situ* DM degradability are bag size, pore size, sample size, bag material, rumen incubation protocol and mathematical models (Vanzant *et al.,* 1998; Mesgaran *et al.,* 2010). There was no significant effect of animal, day of incubation and period of experimentation on ruminal disappearance of DM and nitrogen (N), whereas there was a significant effect of the type of bag material and the porosity of bag on the ruminal disappearance of DM and N of soybean meal (Weakley *et al.,* 1983; Sahu, 2011).

The database about the DM degradability of by-products from oilseeds, cereal grains and animal origin, which is currently in use in tropical Asian countries, was developed from different *in situ* experiments. These experiments were performed at different places with different incubation protocols (rumen incubation procedure, rumen incubation time period, etc.) and under different experimental conditions (animals, particle size of samples, chemical analysis procedures, nylon bag quality, etc.). This makes the database unreliable as there appears to be large variation due to incubation protocols and experimental conditions. Therefore, there was a need to develop renewed database on the DM ruminal degradability of concentrate ingredients commonly fed in the tropical Asian countries.

The objective of this study was to generate renewed data on *in situ* rumen degradability of DM of by-products from oilseeds, cereal grains, and animal origin, commonly fed to animals in tropical Asian countries. The renewed data were used to derive regression to investigate the relationships between the effective rumen degradability of DM (ED_{DM}) and the degradation characteristics of by-products.

MATERIALS AND METHODS

Samples collection and processing: Sixty four samples of five oilseed by-products, seven cereal grain by-products and four animal by-products were used in the present study. From each feed, 4 samples (~1 kg each) were collected from commercial dairy farms (n=1), local markets (n=1) and different agro-food industries (n=2) in Pakistan. After collection, the samples were transported to the Animal Nutrition Laboratory of the University of Agriculture Peshawar, Pakistan. The samples of each by-product feed were homogenized, divided into roughly two equal parts; one half was oven dried (70°C for 16 h) for chemical analyses while the other half was stored at -20°C for *in situ* nylon bag incubations.

In situ **rumen incubations:** Three buffalo steers (age, 5 years ± 2 months; 400-450 kg body weight), fitted with permanent rumen fistulas (13 cm internal diameter; Bar Diamond, USA) were used in this experiment. The animals were fed maize fodder (DM, 23.36±4.23; CP, 11.81±2.26) *ad libitum*, and had 24 h/d access to fresh water. Concentrate mixture (DM, 88.6±2.11; CP, 18.31±1.38) at the rate of 2 kg/day was fed.

The air dried samples were ground to 2 mm particle size in a Thomas-Willey laboratory mill. About 5 g air dried sample of each feed was weighed into nylon bags (bag size 90 × 140 mm; porosity 26 %; pore size 40 µm) and incubated in the rumen for 2, 4, 8, 12, 24 and 48h according to the procedure described by Ali *et al.* (2012). Six bags of each by-product feed, combined with 2 reference samples per series, were incubated in the rumen of three steers (2 bags per steer per incubation time). After rumen incubation, the bags were taken out from the rumen and the loose material adhering to the outer surface of the bags was rinsed with tap water. Then the bags were stored at -20°C. The frozen bags were thawed and washed in a washing machine (AEG-Electrolux Öko Turnamat 2800, Stockholm, Sweden) for 40 min using tap water at 25°C. The 0 h bags were washed in the washing machine without incubation in the rumen according to the above mentioned procedure and residues were used to determine the *W* fraction. The washed bags were oven dried to constant weight at 70°C. The rumen incubated residues of each feed sample from the 3 steers were pooled per incubation time. The pooled residues were ground over a 1 mm sieve, using a hammer mill.

Chemical analysis: The oven dried ground samples of each feed were analysed for DM, CP, crude fat and ash. The rumen incubated residues were also analysed for DM and ash. The DM content was determined by oven drying at 103°C (ISO 6496; ISO 1983) and ash by incineration at 550°C (ISO 5984; ISO, 1978). The N content was determined by Kjeldahl method (ISO 5983; ISO, 2005) and CP was calculated as 6.25×N. Crude fat was determined gravimetrically after 6 h extraction with petroleum ether (40/60) (ISO 6492; ISO 1999).

Calculations: The ED_{DM} was calculated according to the equation of Ørskov and McDonald (1979):

$$ED_{DM} = W + \left(\frac{k_d}{k_d + k_p}\right) \times D \qquad (1)$$

Where, k_d is degradation rate (h^{-1}) of fraction *D*, k_p is passage rate (h^{-1}) and *D* is potentially rumen degradable fraction. The ED_{DM} was calculated using three values of passage rates (0.04 h^{-1}, ED_{DM4}; 0.06 h^{-1}, ED_{DM6}; and 0.08 h^{-1}, ED_{DM8}). The k_d of DM was calculated using the following modified model of Ørskov and McDonald (1979):

$$Y_t = U + D_t\,(1 - exp^{-k_d t}) \qquad (2)$$

Where, Y_t is degradation at time t and D_t is potentially degradable fraction in the rumen at time t.

Statistical analysis: Data on chemical composition of the by-products from oilseeds, cereal grains and animal origin were summarized by descriptive statistics using Statistical Analysis System Software (SAS®, 2009). The variation in the rumen degradation kinetics and ED_{DM} was computed between by-products of oilseeds, cereal grains and animal using PROC MIXED procedure of SAS® (2009), using the following model:

$$Y_{ijk} = \mu + PS_i + S_j + \epsilon_{ijk}$$

Where, Y_{ijk} is the dependent variable; µ the general mean; PS_i is the fixed effect of feedstuff; S_j is the random steer effect; ϵ_{ijk} is the residual effect.

The regression equations were derived between the ED_{DM} and *W*, *D* and *U* of oilseeds, cereal grains and animal by-products using PROC REG procedure of SAS® (2009). The backward stepwise procedure was used to derive regression equations with the significant predictors ($P<0.05$). For animal by-products, no regression equation was presented as none of the predictors proved to be significant.

RESULTS

Nutrient composition: The nutrient composition of by-products from oilseeds, cereal grains and animal origin is summarized in Table 1. There was a large variation in the composition of by-products from oilseeds, cereal grains and animal origin. The CP content (g/kg DM) of oilseed by-products varied from 185 (maize oil cake) to 519 (soybean meal). The CP content (g/kg DM) of cereal grain by-products varied from 120 (rice meal) to 610 (corn gluten meal), while the CP content (g/kg DM) of animal by-products varied form 267 (bone meat meal) to 543 (blood meal and fish meal). The fat and ash contents also varied between the oilseed, cereal grain and animal by-products.

Rumen degradation kinetics and effective rumen degradability: The rumen degradation kinetics and ED_{DM} of oilseed by-products are shown in Table 2. The *W* content (g/kg DM) of DM of oilseed by-products varied from 193.3 (soybean meal) to 336.6 (cottonseed meal), while the *D* content (g/kg DM) varied from 431.8 (rapeseed meal) to 770.2 (soybean meal). Soybean meal showed the highest values (g/kg DM) for ED_{DM4} (771), ED_{DM6} (706.8) and ED_{DM8} (655.5). The rumen degradation kinetics and ED of DM of cereal grain by-products are presented in Table 3. The *W* content (g/kg DM) of DM of cereal grains by-products varied from 164.0 (corn gluten meal) to 299.7 (toria meal), while the *D* content (g/kg DM) varied from 268.5 (rice polishing) to 629.2 (guar meal). Among the cereal grain by-products, guar meal had the highest (P<0.05) values (g/kg DM) for ED_{DM4} (671.2), ED_{DM6} (614) and ED_{DM8} (570). The values for rumen degradation characteristics and ED_{DM} of animal by-products are presented in Table 4. The *W* content (g/kg DM) of DM of animal by-products varied from 12.8 (bone meat meal) to 304.8 (blood meal), while the *D* content (g/kg DM) varied from 170.2 (fish meal) to 293.2 (bone meat meal). Among the animal by-products, blood meal had the highest values (g/kg DM) for ED_{DM4} (499.6), ED_{DM6} (473) and ED_{DM8} (452.8).

Table 1: Nutrient composition (g/kg DM) of by-products from oilseed, cereal grains and animal origin

Feedstuff	Dry matter		Crude protein		Crude fat		Ash	
	Mean	SD[a]	Mean	SD	Mean	SD	Mean	SD
Oilseed by-products								
Soybean meal	910	5.7	519	30.4	72	2.4	97	21.6
Sunflower meal	912	5.9	378	21.6	238	41.0	104	42.7
Cottonseed meal	923	6.8	282	67.0	242	51.5	102	41.2
Maize oil cake	934	31.7	185	54.2	111	25.6	116	34.9
Rapeseed cake	895	30.6	350	37.7	179	27.4	125	7.6
Cereal grain by-products								
Corn gluten feed	880	12.8	272	8.5	30	1.8	61	3.1
Corn gluten meal	903	16.6	610	20.9	5.7	1.2	30	25.0
Guar meal	931	5.8	430	9.5	131	12.6	60	9.8
Toria meal	910	12.2	411	18.1	151	4.3	98	9.4
Rice bran	901	8.2	151	1.6	13	2.5	130	10.4
Rice meal	909	15.0	120	1.1	61	7.3	134	29.1
Rice polishing	900	4.9	139	1.1	166	14.2	116	30.7
Animal by-products								
Blood meal	926	21.4	543	32.4	20	11.1	335	31.3
Bone meat meal	922	1.4	267	1.8	11	0.7	592	56.2
Fish meal	909	16.7	531	32.1	47	16.1	298	14.8
Feather meal	915	1.1	478	85.3	22	9.8	180	18.3

[a] Standard deviation

Table 2: Rumen degradation kinetics and effective rumen degradability of dry matter of oilseed by-products

Feedstuff	Degradation kinetics[a]			Effective rumen degradability [b]		
	W (g/kg DM)	*D* (g/kg DM)	k_d (h^{-1})	ED_{DM4}	ED_{DM6}	ED_{DM8}
Soybean meal	193.3	770.2	0.120	771.0	706.8	655.5
Sunflower meal	218.6	451.9	0.117	555.4	517.3	487.0
Cottonseed meal	336.6	488.6	0.088	671.9	626.4	591.8
Maize oil cake	214.6	659.7	0.104	690.9	632.8	587.3
Rape seed cake	306.2	431.8	0.098	613.0	574.3	544.2
_SEM	28.32	66.08	0.006	36.45	31.64	27.92
P-value	**	***	**	***	***	***

[a] *W*, the content disappeared by washing the rumen unincubated bags in washing machine; *D*, potentially rumen degraded content; k_d, degradation rate; [b] Effective rumen degradability was calculated using three passage rates: 0.04 h^{-1} (ED_{DM4}), 0.06 h^{-1} (ED_{DM6}) and 0.08 h^{-1} (ED_{DM8}); **, P< 0.001; ***, P<0.001.

Table 3: Degradation kinetics and effective rumen degradability of dry matter of cereal grains by-products

Feedstuff	Degradation kinetics[a]			Effective rumen degradability [b]		
	W (g/kg DM)	*D* (g/kg DM)	k_d (h^{-1})	ED_{DM4}	ED_{DM6}	ED_{DM8}
Corn gluten feed	198.4	417.7	0.093	490.6	452.4	423.1
Corn gluten meal	164.0	589.2	0.081	559.1	503.2	461.2
Guar meal	228.4	629.2	0.095	671.2	614.0	570.0
Toria meal	299.7	423.4	0.089	591.8	552.6	522.7
Rice bran	253.6	344.3	0.136	519.8	492.7	470.6
Rice meal	187.6	429.1	0.067	456.3	414.0	383.2
Rice polishing	278.5	268.5	0.078	456.3	430.6	411.4
_SEM	18.85	48.17	0.01	29.66	26.76	24.73
P-value	***	***	**	***	***	***

[a] *W*, the content disappeared by washing the rumen unincubated bags in washing machine; *D*, potentially rumen degraded content; k_d, degradation rate; [b] Effective rumen degradability was calculated using three passage rates: 0.04 h^{-1} (ED_{DM4}), 0.06 h^{-1} (ED_{DM6}) and 0.08 h^{-1} (ED_{DM8}); **, P< 0.001; ***, P<0.001.

Table 4: Rumen degradation kinetics and effective rumen degradability of dry matter of animal by-products

Feedstuff	Degradation kinetics[a]			Effective rumen degradability [b]		
	W (g/kg DM)	*D* (g/kg DM)	k_d (h^{-1})	ED_{DM4}	ED_{DM6}	ED_{DM8}
Blood meal	304.8	285.1	0.086	499.6	473.0	452.8
Bone meat meal	12.8	293.2	0.090	215.4	188.2	167.5
Fish meal	257.2	170.2	0.145	390.6	377.6	366.9
Feather meal	286.4	282.1	0.117	496.6	472.9	453.9
SEM	68.2	29.2	0.014	66.7	67.1	67.4
P-value	***	**	**	***	***	***

[a] *W*, the content disappeared by washing the bags in washing machine; *D*, potentially rumen degraded content; k_d, degradation rate; [b] Effective rumen degradability was calculated using three passage rates: 0.04 h^{-1} (ED_{DM4}), 0.06 h^{-1} (ED_{DM6}) and 0.08 h^{-1} (ED_{DM8}); **, P<0.01; ***, P<0.001.

Regression equations: Regression equations were derived to describe the relationships between the ED_{DM} and the rumen degradation characteristics of by-products of oilseeds and cereal grains (Table 5). The ED_{DM4}, ED_{DM6} and ED_{DM8} of oilseed by-products were influenced by *U* content in oilseed by-products. The ED_{DM4}, ED_{DM6} and ED_{DM8} of cereal grain by-products were influenced by the contents of *W* and *U* in cereal grain by-products. There was no relationship found between the rumen degradation characteristics and ED_{DM} of animal by-products.

Table 5: Relationship between the dry matter effective rumen degradability (g/kg DM) calculated using passage rates of 0.04 h^{-1} (ED_{DM4}), 0.06 h^{-1} (ED_{DM6}) and 0.08 h^{-1} (ED_{DM8}), and the washout (*W*, g/kg DM) and rumen undegradable (*U*, g/kg DM) contents of by-products from oilseeds, and cereal grains origin

Regression equation	R^2	RMSE[a]
Oilseed by-products		
$ED_{DM4} = 792.02\ (\pm 8.25) - 0.71\ (\pm 0.04)\ U$	0.99	8.91
$ED_{DM6} = 724.94\ (\pm 11.36) - 0.61\ (\pm 0.05)\ U$	0.98	12.27
$ED_{DM8} = 672.04\ (\pm 14.32) - 0.53\ (\pm 0.07)\ U$	0.95	15.49
Cereal grains by-products		
$ED_{DM4} = 662.61\ (\pm 41.32) + 0.46\ (\pm 0.16)\ W - 0.71\ (\pm 0.07)\ U$	0.96	19.46
$ED_{DM6} = 566.51\ (\pm 46.92) + 0.57\ (\pm 0.18)\ W - 0.62\ (\pm 0.08)\ U$	0.94	22.09
$ED_{DM8} = 494.76\ (\pm 49.09) + 0.64\ (\pm 0.19)\ W - 0.53\ (\pm 0.07)\ U$	0.92	23.11

[a] Root mean square error.

DISCUSSION

To meet the nutrient requirements of animals in Pakistan and other tropical South Asian countries, information is required on nutrient availability from different concentrate feedstuffs that are commonly fed to animals in that area. In the present study, new data on the rumen DM degradability of by-products from oilseeds, cereal grains and animal origin was developed. The new developed data is more reliable because all the feeds were evaluated under uniform experimental conditions (same standard incubation protocol, same steers, and same chemical analysis procedures) and the variation among the sources of these feedstuffs was covered (the feedstuffs were collected from local markets, agro-food industries and dairy farms). This information on rumen degradation kinetics of the concentrate feeds can be used to optimize diet formulation of dairy animals in the tropical Asian countries. The rumen degradation data of toria meal and guar meal was very scarce; these feedstuffs were evaluated for the first time under uniform experimental conditions for the rumen degradation of DM. The large variation among the by-products of oilseed, cereal grain and animal origin was due to the broad range in the chemical composition and different sources of these feeds.

In the present study, the *W* content (g/kg DM) recorded for soybean meal (193.3), sunflower meal (218.6), cottonseed meal (336.6) and rapeseed meal (306.2) differed from previous reports (Woods *et al*., 2003; Kamalak *et al*., 2005). Woods *et al*. (2003) reported values of 327.0, 283.5, 315.0 and 271.6 for *W* content (g/kg DM) of DM of soybean meal, sunflower meal, cottonseed meal and rapeseed meal respectively. Whereas, Kamalak *et al*. (2005) reported values of 251, 216, 195 and 276 for *W* content (g/kg DM) of DM of soybean meal, sunflower meal, cottonseed meal and rapeseed meal, respectively. The difference in the values might be due to different rinsing procedures and rinsing times used. In the present study, the nylon bags were washed in the washing machine for 40 min whereas nylon bags were washed under running water for unsepcified time in the study of Kamalak *et al.* (2005) and for 65 minutes (40 min wash and 25 min rinse) in the study of Woods *et al.* (2003). Kendall *et al.* (1991) reported variation in the ED_{DM} of different sources of canola meal. The ED_{DM} of all the feedstuffs decreased with increased passage rates. Similar relationship between the ED_{DM} and passage rates was reported in the previous studies (Arieli *et al.,* 1996; Khan *et al.,* 2009). In the present study, the values of ED_{DM} at the three passage rates (0.04, 0.06 and 0.08 h^{-1}) were higher for soybean meal than cottonseed meal, sunflower meal, maize oil cake and rapeseed cake. Kamalak *et al.* (2005) and Woods *et al.* (2003) also reported similar trend for ED_{DM} values at rumen passage rates of 0.02, 0.05 and 0.06 h^{-1}; the ED_{DM} values were higher for soybean meal than cotton seed meal, rapeseed meal and sunflower meal.

In the present study, the rumen degradation kinetics and ED_{DM} of blood meal, bone meat meal, fish meal and feather meal showed large variation (P<0.001). Kamalak *et al.* (2005) also reported significant (P<0.001) variation in the rumen degradation kinetics and ED_{DM} between the animal by-products. Chaudhry and Mohamed (2011) stated that the *in situ* rumen degradation characteristics and ED_{DM} of concentrates significantly (P<0.01) differed. The *W* and *D* contents (g/kg DM) of DM were lower for animal by-products compared to the plant by-products (oilseed and cereal grain by-products). The ED_{DM} of plant sources (oilseed and cereal grain by-products) at three rumen passage rates was significantly higher than that of animal by-products.

There was a relationship found between the ED_{DM} (g/kg DM) calculated using three rumen passage rates (0.04 h^{-1}, ED_{DM4}; 0.06 h^{-1}, ED_{DM6} and 0.08 h^{-1}, ED_{DM8}) and the *W* content (g/kg DM) of oilseed by-products. The R^2 values of 0.99, 0.98 and 0.95 for oilseed by-products showed strong relationships between the ED_{DM4}, ED_{DM6} and ED_{DM8}, and *U* content of oilseed by-products. Regression analysis also showed relationship between the ED_{DM4}, ED_{DM6} and ED_{DM8}, and the *W* and *U* contents of cereal grain by-products. The R^2 values of 0.96, 0.94 and 0.92 showed strong relationships between the ED_{DM4},

ED_{DM6} and ED_{DM8}, and *W* and *U* contents of cereal grains by-products. These regression equations give an acceptable estimate to determine the ED_{DM} of the concentrate feedstuffs.

Conclusions: The present study reports a renewed database on the *in situ* rumen DM degradation kinetics and ED_{DM} of by-products from oilseeds, cereal grains and animal origin commonly fed to ruminants in the tropical Asian countries. The renewed database is more adequate because it was obtained under uniform experimental conditions and the variation between the sources of these feedstuffs was also covered. The database of these agro-industrial by-products can be used to optimize ration formulation for dairy animals. The ED_{DM} of plant by-products calculated using different rumen passage rates was significantly higher than that of animal by-products. The developed regression equations can be used for the accurate and rapid estimation of ED_{DM} of oilseed and cereal grain by-products from their rumen degradation characteristics.

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