

## ARTICLE

# Dry Matter Degradation Kinetics of Selected Tropical Forage in Nili-Ravi Buffalo and Cholistani Cows at Heifer and Lactating Stages Using NorFor in Situ Standards

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### ABSTRACT

Current methods of ruminant ration formulation in Pakistan use foreign-based nutrient availability values. These values may not be optimal for all geographic areas, as variation in environment, agronomic factors, animal species, and diet characteristics may not be considered. The aim of present study was to establish a database of the chemical composition and dry matter degradation parameters of tropical forage commonly fed to ruminants in Pakistan and South Asian countries using Nili-Ravi buffalo and Cholistani cattle at heifer and lactating stages. Six cereal grain and four legume species were grown in 3 locations under standard agronomic conditions and sampled at booting and at 50% flowering stage for cereal and legumes, respectively. Dried and milled feeds were analyzed for chemical composition and in situ dry matter degradation parameters using 1 g samples in bags placed in the rumen of 2 Nili-Ravi buffalo heifers, 2 lactating Nili-Ravi buffaloes, 2 Cholistani heifers, and 2 lactating Cholistani cows. The forage family (cereal vs. legumes), species, and geographic location of growth significantly influenced ( $P < 0.001$ ) chemical composition and in situ degradation fractions. Animal species and developmental stage showed no effect on degradation fractions ( $P > 0.05$ ). Legume-by-heifer interactions significantly increased ( $P < 0.05$ ), and legume-by-lactating cow interaction tended ( $P = 0.065$ ), to increase the rate of degradation (Kd). The selected forages were degraded to a similar extent independent of animal species or developmental stage, and legumes are degraded at higher rates and to a greater extent than are cereals. A moderately significant relationship between Kd and effective dry matter degradability (DMD) suggests that Kd could be the single most important predictor of forage degradability in the rumen.

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## 1. Introduction

The rate and extent of dry matter (DM) degradation in the rumen is a major determinant of energy and nutrient supply to ruminants from fiber-rich forage. This information forms the basis for ration formulation and for the prediction of metabolizable nutrient and energy intake in feed evaluation systems such as that of the National Research Council<sup>[1]</sup>, the Cornell Net Carbohydrate and Protein System<sup>[2]</sup>, and the Nordic Feed Evaluation System<sup>[3]</sup>. The pattern of rumen degradation has been reported to affect rumen function and fiber digestion, microbial protein and milk fat synthesis, and overall animal performance and health<sup>[4-5]</sup>.

Current ration formulation methods in Pakistan<sup>[6]</sup> are based on nutrient availability values reported in feed evaluation systems developed for feeds grown in temperate conditions and fed to animals common to those areas. These values may not be optimal in other geographic locations, as variations in environment, agronomy, animal species, and diet characteristics are not considered. As a consequence, animals may be under- or over-fed, resulting in lower feed efficiency and economic losses. Accurate estimates of the coefficients of nutrient degradation of locally produced feeds in the rumen are required. The in situ technique is widely used to study the fractional rate of ruminal degradation of feed DM and nutrients<sup>[7]</sup>. Despite some limitations, it utilizes the ruminal environment<sup>[8]</sup> and is considered to produce a more reliable measure of rumen degradation than do in vitro techniques.

In situ degradation of forage is primarily influenced by plant genotype, agronomic conditions, climate, and post-harvest processing<sup>[9]</sup>. Various animal-related factors that affect the in situ degradation of forage have been reported including intake level, forage to concentrate ratio, nutrient composition and degradation rate of the concentrate feeds, feeding frequency<sup>[10]</sup>, and animal species<sup>[11]</sup> and developmental stage<sup>[12]</sup>. Internationally, substantial research has been conducted to quantitatively evaluate the effects of these factors<sup>[13-15]</sup>, but information is lacking for tropical areas such as Pakistan.

The objectives of the present study were to: 1) evaluate chemical composition of commonly used forage plants; 2) assess the effects on DM degradability of forage family, geographic location of growth, and animal species and developmental stage; and 3) determine the relationship between rate and extent of degradation in situ.

## 2. Materials and Methods

The animals were kept at the Livestock Farm of Islamia University of Bahawalpur (IUB) and maintained accord-

ing to the criteria of Animal Care and Management Committee (The IUB Bioethics and Animal Use Committee, 2015).

### 2.1 Forage Sampling

Ten forage species, comprising 6 cereals and 4 legumes, were evaluated (Table 2). Summer (*maize Zea mays*, *millet Pennisetum glaucum*, *sorghum sorghum bicolor*, *lucerne Medicago sativa* and *jantar sesbania bispinosa*) and winter (*barley Hordeum vulgare*, *oats Avena sativa*, *wheat Triticum aestivum*, *berseem Trifolium alexandrinum*, and *mustard brassica napus*) crops were used for the analyses. Summer crops were sown on the same date in mid March 2015 and winter in late November and grown under uniform recommended agronomic conditions. Each species was sown in 3 plots separated by ~100 m apart with the trial replicated in Rawalpindi (33.598° N, 73.04° E), Lahore (31.55° N, 74.35° E), and Bahawalpur (29.39° N, 71.68° E) representing northern, central, and south-ern regions of Punjab Province of Pakistan, respectively. Herbage samples (~10 kg each) from locations within an area were harvested on the same date, when the cereals were at booting and legumes were at 50% flowering stage, chopped with a chaff cutter (Toka 510, Patiala Agri-Industries, Faisalabad, Pakistan) to a nominal length of 20 mm, and spread under shade to reduce moisture content within the recommended range for drying of 3 to 7 days. The dried samples were transported to the Livestock Production and Management section at the IUB, forced through a 2-mm screen using a hammer mill (POLYMIX PX-MFC, Kinematica AG, Germany) and stored in small plastic jars at room temperature for in situ experiments. The samples for chemical analyses were ground through a 1 mm screen.

### 2.2 Maintenance of Cannulated Animals

Eight rumen-cannulated (Bar Diamond, Parma, ID, USA) animals including 2 lactating Nili-Ravi buffaloes, mean live weight (LW) = 509 ± 43.4 kg, milk yield = 5.63 ± 0.207 kg/day, age = 2225 ± 49.5 days; 2 Nili-Ravi heifers LW = 531 ± 48.8 kg, age = 1913 ± 123.7 days; 2 lactating Cholistani cows, LW = 289 ± 29.4 kg, milk yield = 3.34 ± 0.271 kg/day, age = 1115 ± 21.9 days; and 2 Cholistani heifers LW = 312 ± 35.4 kg, age = 867 ± 64.3 days were used for the in situ incubations. The animals were offered a standard diet at maintenance level as per NorFor standards for cannulated-animals throughout the experiment<sup>[3]</sup>. Ingredients and mean chemical composition of the diets are presented in Table 1. The animals were confined to individual stalls, individually fed and given access to fresh clean water as per requirements.

Tables and figures

**Table 1.** Ingredients and mean chemical composition of the diets offered to rumen-cannulated animals (g/kg DM unless otherwise stated).

Item	Lactating cows	Heifers
No. of samples = 5, no. of statistical replicates = 2, Total no. of observations per feed = 10		
Ingredients (as fed basis)		
Sorghum	844	771
Lucerne hay	88	195
Cotton seed cake	30	0
Concentrate mixture	37	34
Forage to concentrate ratio (DM)	80:20	90:10
Chemical composition (DM)		
DM	302	360
CP	58	60
EE	18	17
NDF	583	567
NFC	227	241
Ash	113	113

DM = dry matter; CP = crude protein; EE = ether extract; NDF = amylase-treated neutral detergent fiber; NFC = non-fiber carbohydrates

**Table 2.** Effect of forage family, species, and geographic location of growth on chemical composition of Cereal and legumes fodder sown in 3 locations in Punjab Province. The values are presented as least square means (g/kg DM) with Standard error of mean (SEM) unless otherwise stated .

Items <sup>1</sup>	DM	CP	EE	NDF	Ash	NFC
No. of samples = 3, no. of statistical replicates = 2, Total no. of observations per feed = 6						
Cereal fodders						
Barley	954.1 <sup>a</sup>	53.5 <sup>c</sup>	16.3 <sup>b</sup>	614.3 <sup>a</sup>	127.2 <sup>b</sup>	189.3 <sup>cd</sup>
Oat	946.0 <sup>b</sup>	62.3 <sup>b</sup>	21.4 <sup>a</sup>	549.4 <sup>c</sup>	110.2 <sup>bc</sup>	257.2 <sup>a</sup>
Wheat	954.2 <sup>a</sup>	74.5 <sup>a</sup>	15.4 <sup>b</sup>	532.4 <sup>d</sup>	149.3 <sup>a</sup>	228.3 <sup>b</sup>
Maize	945.1 <sup>b</sup>	65.7 <sup>b</sup>	10.3 <sup>c</sup>	605.2 <sup>a</sup>	94.0 <sup>d</sup>	225.3 <sup>b</sup>
Millet	939.3 <sup>c</sup>	66.6 <sup>b</sup>	15.3 <sup>b</sup>	606.2 <sup>a</sup>	102.5 <sup>c</sup>	209.4 <sup>c</sup>
Sorghum	935.1 <sup>d</sup>	50.6 <sup>c</sup>	17.0 <sup>ab</sup>	584.3 <sup>b</sup>	88.3 <sup>d</sup>	260.4 <sup>a</sup>
Legume fodders						
Barseem	948.2 <sup>b</sup>	133.0 <sup>a</sup>	18.8 <sup>ab</sup>	405.4 <sup>c</sup>	183.6 <sup>a</sup>	260.2 <sup>b</sup>
Lucerne	940.3 <sup>c</sup>	135.5 <sup>a</sup>	18.2 <sup>ab</sup>	410.3 <sup>c</sup>	120.3 <sup>b</sup>	316.3 <sup>ab</sup>
Mustard	956.0 <sup>a</sup>	110.3 <sup>c</sup>	23.2 <sup>a</sup>	498.2 <sup>a</sup>	123.9 <sup>b</sup>	245.1 <sup>b</sup>
Jantar	943.1 <sup>c</sup>	119.6 <sup>b</sup>	20.8 <sup>ab</sup>	448.2 <sup>b</sup>	74.3 <sup>c</sup>	337.3 <sup>a</sup>
SEM	4.93	5.37	2.02	17.20	8.03	19.90
Family						
Cereals	945.1	62.2	15.9	581.8	111.9	228.2
Legumes	946.2	113.9	18.3	481.9	120.5	265.3
Location						
Bahawalpur	934.6	109.6	19.1	482.0	116.2	273.1
Lahore	951.8	78.0	15.8	583.8	116.3	206.1
Rawalpindi	951.1	76.6	16.4	529.8	116.1	261.0
Significance						
Forage specie	0.044	<0.001	0.016	<0.001	<0.001	<0.001
Family	0.514	<0.001	0.047	<0.001	0.007	0.023
Location	<0.001	<0.001	0.168	<0.001	0.287	<0.001
Interactions <sup>2</sup>						
T × L	<u>0.467</u>	<u>&lt;0.001</u>	<u>0.904</u>	<u>0.032</u>	<u>0.016</u>	<u>0.594</u>

<sup>1</sup> For abbreviations see Table 1

<sup>2</sup> Effect of main factor interactions (Family × Location).

Different lower-case superscripts in a column indicate significant difference ( $P < 0.05$ ).

### 2.3 In Situ Incubations and Degradation Profiles

The incubations continued from June to October, 2016. The assessment of DM degradation of fodder samples was conducted according to NorFor standards<sup>[3,16]</sup>. In brief, air dried and milled (2 mm screen; POLYMIX PX-MFC, Kinematica AG, Germany) fodder samples (1 sample per incubation period and animal) were incubated in the rumen of each rumen-cannulated animal in a sewn and glued polyester (Dacron) bag measuring 11 × 8.5 cm (10 × 7.5 effective size), pore size 33 μm (PES material 140/37) with 25% open bag area (Sefar AG, Hinterbissastrasse 12, 9410 Heiden, Switzerland). Samples of approximately 1 g, allowing 15 mg of sample per cm<sup>2</sup> of bag surface area, were incubated for 0, 4, 8, 16, 24, or 48 h. All bags were placed in the rumen at the same time and removed according to specified duration of incubation (all-in system). At the conclusion of each incubation period, the bags were removed, washed with tap water, and stored at -18 °C. After all bags from all incubation periods had been retrieved, they were thawed and washed in a washing machine twice for 12 min each with tap water at 25 °C. The residues in the bags were dried at 100 °C for 24 h to determine DM loss.

### 2.4 Chemical Analyses

Fresh forage and dry feed including commercial concentrate, lucerne hay and cotton seed cake fed to animals were sampled fortnightly throughout the experiment. The DM content of fresh chopped forage was determined at 60 °C for 48 h and that of dry feeds at 105 °C for 16 h [Association of Official Analytical Chemists (AOAC)<sup>[17]</sup>, method 7.003]. Ash was analyzed by incinerating the samples at 525 °C for 6 h (<sup>[17]</sup>; method 923.03), crude protein (CP) (6.25 × N) by Kjeldahl method (<sup>[17]</sup>; method 7.015), and ether extract (EE) by 6-h extraction with petroleum ether (<sup>[17]</sup>; method 7.062). The amylase-treated neutral detergent fiber (NDF) was determined using the method of Van Soest et al.<sup>[18]</sup> as modified by Mertens et al.<sup>[19]</sup> with the addition of sodium sulfite and heat-stable alpha-amylase (CAS No. 9000-90-2, Junsei Chemicals, Japan). The non-fiber carbohydrates were calculated as [NFC (g/kg DM) = 1,000 - (CP + EE + NDF + ash)].

### 2.5 Data Analysis and Curve Fitting

The in situ degradation data was categorized as particle loss or washable fraction (*a*, 0 h values for washed samples) and non-washable fraction. The non-washable fraction was sub-divided into the potentially degradable (*b*) and the indigestible fraction, represented as the degradation and residue at the final incubation interval, respectively as described by Ørskov and McDonald<sup>[7]</sup>. The

in situ degradation data were fitted to a first-order kinetic model (Equation 1) assuming the steady state degradation and passage conditions

$$Y_t = a + b(1 - \exp(-K_d t)) \quad \text{Equation 1.}$$

The model was fitted using Table Curve 2D (ver. 5.0, SPSS Inc. NY).  $Y_t$  denotes the degraded fraction at a given time  $t$ , and  $K_d$  denotes the fractional degradation rate of fraction  $b$ . Effective ruminal DM degradability (DMD) was calculated according to Ørskov and McDonald<sup>[7]</sup> as

$$\text{DMD} = a + b \times K_d / (K_d + K_p) \quad \text{Equation 2}$$

assuming the fractional rate of passage ( $K_p$ ) to be 0.05/h for forage (a 20-h rumen retention time) as used in several protein evaluation systems<sup>[20]</sup>. A second-order (DMD1) was calculated from the in situ data according to a 2-compartment model (Equation 3) as suggested by Allen and Mertens<sup>[21]</sup>

$$\text{DMD1} = a + [(b \times K_d) / (K_d + K_p) \times (1 + K_p) / (K_d + K_r)] \quad \text{Equation 3}$$

where  $K_p = [1 / (0.6 \times 20) = 0.083/\text{h}]$ , and  $K_r$  is the fractional rate of release from the non-escapable fraction to the escapable fraction [ $1 / (0.4 \times 20) = 0.125/\text{h}$  was used]. This implied a total rumen residence time of 20 h for forage distributed between the 2 compartments at a ratio of 40:60.

### 3. Statistical Analyses

The statistical analyses were performed using the GLM procedure of Minitab 16.1.1.0. The data on chemical composition of rations were analyzed using the model (Equation 4)

$$Y_{ijkl} = \mu + F(T)_i + T_j + L_k + E_{ijk} \quad \text{Equation 4}$$

The data on in situ parameters were analyzed considering each buffalo and cow/heifer an experimental replicate using the model (Equation 5)

$Y_{ijklmn} = \mu + F(T)_i + T_j + L_k + A_l + P_m + E_{ijklm}$  (Equation 5) in which  $Y_{ijklmn}$  is the dependent variable,  $\mu$  is the overall mean,  $F(T)_i$  shows the effect of *i*th forage species nested under forage family,  $T_j$  shows the effect of the *j*th family of forage (cereal vs. legume),  $L_k$  shows the effect of *k*th location,  $A_l$  shows the effect of *l*th animal species,  $P_m$  shows the effect of *m*th developmental stage of the animal, and  $E_{ijklm}$  is the residual error. Results were considered significant when  $P \leq 0.05$  and are presented as least square means with standard error of the means. The pairwise comparisons were made using Tukey's test.

### 4. Results

#### 4.1 Chemical Composition

Table 2 shows the chemical composition of forage by family, species, and growing location of collected samples. The CP ranged from 50.6 (sorghum) to 74.5 g/kg

DM (wheat) for cereals; and from 110.3 (mustard) to 135.5 g/kg DM (lucerne) for legumes, and was significantly influenced ( $P < 0.001$ ) by family, species, location, and family by location interaction. The NDF ranged from 532.0 (wheat) to 614 g/kg DM (barley) for cereals, and from 405.0 (berseem) to 498.0 g/kg DM (mustard) for legumes and was significantly influenced ( $P < 0.001$ ) by family, species, and location. The ash and NFC averaged 112.0 and 228.0 g/kg DM, respectively, for cereals and 126.0 and 289.0 g/kg DM, respectively, for legumes, and varied significantly ( $P < 0.05$ ) by family and species.

#### 4.2 Degradation Parameter Estimates and Effective DMD as Influenced by Forage Family, Species, and Growing Location

Table 3 shows the DMD parameters of the forages. The forage family, species, and location significantly influenced ( $P < 0.001$ ) all degradation fractions. The a-fraction ranged from 0.26 (maize) to 0.34 (wheat) in cereals and from 0.28 (jantar) to 0.46 (lucerne) in legumes. The b-fraction ranged from 0.50 (wheat and millet) to 0.59 (oats) in cereals and from 0.31 (mustard) to 0.44 (jantar) in legumes (mean 0.36). The Kd ranged from 0.05 to 0.06/h for cereals and from 0.09 to 0.12/h for legumes. The DMD varied from 0.53 (millet) to 0.61 (oats) for cereals and from 0.56 (mustard) to 0.68 (lucerne) for legumes. The DMD1 varied from 0.79 (millet) to 0.93 (oats) for cereals and from 0.70 (mustard) to 0.82 (lucerne) for legumes. The forage species ranked in order of decreasing DMD and DMD1 are oats > wheat > barley > maize > sorghum > millet (cereals) and lucerne > berseem > jantar > mustard (legumes).

#### 4.3 Degradation Parameter Estimates and Effective DMD as Influenced by Animal Species and Developmental Stage

The Kd was significantly influenced ( $P < 0.05$ ) by animal species, but other fractions were not ( $P > 0.05$ ). The DMD and DMD1 did not differ ( $P > 0.05$ ) between heifers and lactating animals although fraction b and Kd differed significantly ( $P < 0.05$ ). Legume-by-heifer interactions significantly increased ( $P < 0.05$ ) the value of Kd, whereas legume-by-lactating cow interaction tended ( $P = 0.065$ ) to increase the value of Kd however, the interaction effects for other analyzed parameters remained non-significant ( $P > 0.05$ ).

#### 4.4 Relationship of DMD to Degradation Parameters

Figures 1 and 2 show relationships of  $K_d$  with DMD and DMD1 respectively. We found a moderate ( $R^2 = 0.43$ ) but significant ( $P < 0.001$ ) positive relationship in cereals,

and a low ( $R^2 = 0.02$ ) but significant ( $P < 0.001$ ) positive relationship in legumes, between DMD and  $K_d$  (Fig. 1). Figure 2 shows a borderline ( $R^2 = 0.05$ ) significant ( $P < 0.01$ ) relationship between DMD1 and  $K_d$  in cereals but no correlation in legumes. The relationship of DMD to  $a$  and  $b$  fractions was also investigated and showed a low and no correlation, respectively (data not shown).

## 5. Discussion

### 5.1 Chemical Composition

The CP values for cereals agree with Sarwar et al.<sup>[22]</sup>, whereas those for legumes were lower. Sarwar et al.<sup>[22]</sup> may have harvested leguminous crops at more advanced stages of growth. For all forages, the content of EE was less than 30 g/kg DM, which is typical of forage plant material. With the exception of CP, the nutrient composition of the analyzed forage plants fell within the range of typical ruminant diets<sup>[1]</sup> (Table 2).

### 5.2 Degradation Parameter Estimates and Effective DMD as Influenced by Forage Family, Species, and Growing Location

Our observation that the legumes were degraded more rapidly and to a greater extent than were cereals agree with results of Sarwar et al.<sup>[22]</sup> in cannulated Nili-Ravi buffalo calves. The values for  $K_d$  and DMD reported in our study were comparable to those of Sarwar et al.<sup>[22]</sup> for sub-tropical cereal and legume forage plants, although differences from Sarwar et al.<sup>[22]</sup> in CP values were found. The  $K_d$  and DMD values for legumes were also in agreement with findings of Aufrère et al.<sup>[23]</sup> for temperate lucerne.

### 5.3 Degradation Parameter Estimates and Effective DMD as Influenced by Animal Species and Developmental Stage

A review of literature data reporting rumen degradability and  $K_d$  in situ in species such as *Bubalus bubalis* and *Bos taurus* showed varying results that were not consistent with respect to either feedstuffs or animal species. Sarwar et al.<sup>[11]</sup> compared digestibility characteristics of cattle and buffalo for various forage plants and agro-industrial by-products using the in situ nylon bag technique. They reported greater digestibility and  $K_d$  for DM and NDF of grasses in rumen-cannulated buffaloes than in cattle during 48 h incubation. For leguminous forage and agro-industrial by-products, no differences in extent and rate of DM and NDF degradation with respect to animal species were reported. Similarly, Bhatia et al.<sup>[24]</sup> reported higher DM and NDF in situ digestion rates of berseem hay in buffaloes compared to those in cattle; however, overall effective degradability values did not differ with

**Table 3.** Effect of plant and animal factors on in situ dry matter degradation kinetics and effective degradability of cereal and legume fodder sown at 3 locations in Punjab Province. The values are presented as least square means (g/kg DM) with standard error of mean (SEM) unless otherwise stated.

In situ <sup>1</sup> items		<i>a</i>	<i>b</i>	<i>K<sub>d</sub></i>	DMD <sup>2</sup>	DMD1 <sup>3</sup>
No. of samples = 3, no. of statistical replicates = 8, Total no. of observations per feed = 24						
Cereal fodders						
Barley		0.30 <sup>ab</sup>	0.54 <sup>b</sup>	0.05	0.57 <sup>ab</sup>	0.86 <sup>b</sup>
Oat		0.31 <sup>ab</sup>	0.59 <sup>a</sup>	0.06	0.61 <sup>a</sup>	0.93 <sup>a</sup>
Wheat		0.34 <sup>a</sup>	0.50 <sup>c</sup>	0.06	0.61 <sup>a</sup>	0.87 <sup>b</sup>
Maize		0.26 <sup>b</sup>	0.54 <sup>b</sup>	0.06	0.55 <sup>b</sup>	0.84 <sup>b</sup>
Millet		0.27 <sup>b</sup>	0.50 <sup>c</sup>	0.05	0.53 <sup>b</sup>	0.79 <sup>c</sup>
Sorghum		0.26 <sup>b</sup>	0.52 <sup>bc</sup>	0.06	0.54 <sup>b</sup>	0.81 <sup>c</sup>
Legume fodders						
Barseem		0.42 <sup>b</sup>	0.36 <sup>b</sup>	0.12	0.67 <sup>a</sup>	0.81 <sup>a</sup>
Lucerne		0.46 <sup>a</sup>	0.34 <sup>b</sup>	0.12	0.68 <sup>a</sup>	0.82 <sup>a</sup>
Mustard		0.36 <sup>c</sup>	0.31 <sup>c</sup>	0.09	0.56 <sup>b</sup>	0.70 <sup>b</sup>
Jantar		0.28 <sup>d</sup>	0.44 <sup>a</sup>	0.10	0.56 <sup>b</sup>	0.75 <sup>ab</sup>
SEM		0.008	0.014	0.007	0.008	0.011
Family	Cereals	0.29	0.53	0.06	0.57	0.85
	Legumes	0.38	0.37	0.11	0.62	0.77
Location	Bahawalpur	0.36	0.48	0.09	0.66	0.87
	Lahore	0.29	0.47	0.07	0.55	0.78
	Rawalpindi	0.37	0.40	0.08	0.58	0.79
Animal species	Bubalus bubalis	0.34	0.45	0.08	0.59	0.82
	Bos taurus	0.34	0.44	0.09	0.60	0.81
Developmental stage	Heifers	0.34	0.44	0.09	0.59	0.80
	Lactating animals	0.33	0.46	0.08	0.60	0.82
	Forage species	<0.001	<0.001	0.042	<0.001	<0.001
Significance	Family	<0.001	<0.001	<0.001	<0.001	<0.001
	Location	<0.001	<0.001	0.001	<0.001	<0.001
	Animal Species	0.562	0.153	0.021	0.337	0.199
	Developmental stage	0.231	0.020	0.040	0.378	0.131
Interactions <sup>4</sup>	T × L	0.001	<0.001	0.008	<0.001	<0.001
	T × A	0.902	0.699	0.065	0.973	0.568
	T × P	0.989	0.786	0.011	0.986	0.754
	A × P	0.315	0.134	0.037	0.222	0.296

*a* = washable fraction representing the portion of dry matter (DM) that had disappeared at time 0; *b* = potentially degradable DM fraction. The estimate of *K<sub>d</sub>* from the in situ method represents the fractional rate of degradation of fraction *b*; DMD = dry matter degradability.

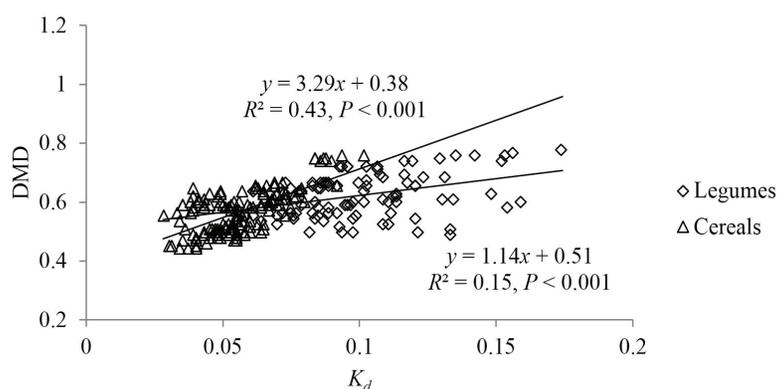
<sup>1</sup> Degradation parameters described according to the model by Ørskov and McDonald<sup>[7]</sup>

<sup>2</sup> Effective DMD calculated from data assuming the fractional rate of passage (*K<sub>p</sub>*) to be 0.05/h for forage as used by protein evaluation system of Hvelplund and Weisbjerg<sup>[20]</sup>

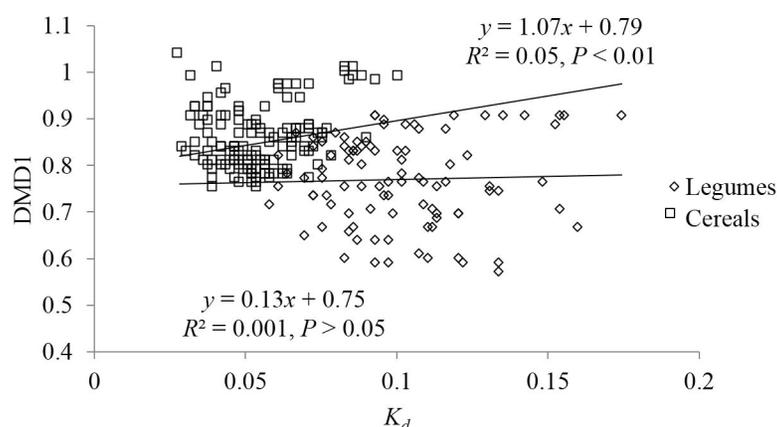
<sup>3</sup> Effective DMD1 calculated according to a 2-compartment model as suggested by Allen and Mertens (1988).

<sup>4</sup> Effect of main factor interactions (Family × Location), (Family × Animal species), (Family × Developmental stage) and (Animal species × Developmental stage).

Different lower-case superscripts in a column indicate significant difference (*P* < 0.05).



**Fig. 1.** Relationship between dry matter degradability (DMD) and rate of degradation (Kd/h) for cereal and legume fodders.



**Fig. 2.** Relationship between dry matter degradability (DMD1) and rate of degradation (Kd/h) for cereal and legume fodders.

animal species. Franzolin and Dehority<sup>[25]</sup> reported no differences with respect to rumen DM or NDF degradation in cannulated riverine buffaloes vs. cows feeding on tropical forage grasses, however,  $K_d$  values were higher in buffaloes than in cows.

Nandra et al.<sup>[26]</sup> reported that parameters of DM degradation for forage and concentrate feeds showed no differences between sheep and cattle with no significant species effect and no interaction of species with either feed or experimental period. They further suggested that a single curve for each test feed in both sheep and cattle may be used to represent DM degradation in the rumen. The results of the present study are also in accordance with the findings of Huntington and Givens<sup>[27]</sup> who observed no differences between host species on in situ DM degradation of hay, soybean- and fish-meal. Uden and Van Soest<sup>[28]</sup> also found that mature ruminant species degrade the fiber fraction of feeds similarly.

Lactating animal energy and protein requirements are different from those of heifers<sup>[1]</sup>. We hypothesized that the lactating animals would better utilize the available feed resources, based on their requirements for milk and

their developed capacity to extract nutrients from within the rumen digesta<sup>[29]</sup>. The present study did not support the hypothesis, but suggest that the feedstuffs studied are equally nutritionally important for different ruminant species at different developmental stages. The energy and protein requirements of the studied species and developmental stages might be a reflection of body mass. Further data of feed consumption and production parameters can be combined with the in situ degradation data to assess feed efficiency.

#### 5.4 Relationship Between DMD and In situ Parameters

Our data and that of other in situ studies<sup>[9]</sup> show that  $K_d$  is the single most important parameter describing ruminal degradability of tropical forages. Many feed evaluation systems using  $K_d$  values in predicting the feed intake and nutrient utilization in dairy cows are inaccurate when legumes form a substantial portion of the forage fraction<sup>[1-2]</sup>. This reflects atypical degradation in the rumen. Although they are characterized by larger quantities of soluble and rapidly degradable protein<sup>[2]</sup> and greater  $K_d$ <sup>[3]</sup>, legumes

present a lower extent of degradation compared to grasses due to the greater  $K_p$ <sup>[30]</sup>.

### 5.5 Comparison of In situ DMD and In vivo Data

All methods of nutritive evaluation in ruminants attempt to mimic in vivo methods, because they are reliable and preferred for a range of ingredients and nutrients. Despite limitations described by several researchers<sup>[31-32]</sup>, in situ is considered a more accurate and reliable method for quantifying rumen degradation parameters than other techniques<sup>[33-34]</sup>. It is not only a powerful tool for ranking the relative degradation of feedstuffs, but may also be used to increase understanding of the processes of rumen fermentation, although the in situ method has rarely been validated in vivo. Madsen and Hvelplund<sup>[35]</sup> observed a close relationship between in vivo and in situ measurements of protein degradation in concentrate feedstuffs. However, Offner and Sauvant<sup>[36]</sup> presented a high slope bias for predicting starch digestion in the rumen from in situ effective degradability data of Offner et al.<sup>[37]</sup>. The in situ method tends to overestimate starch degradation rates, and, consequently, effective degradation values for rapidly-degraded feedstuffs, and to underestimate the rate of starch degradation in feedstuffs that are degraded slowly<sup>[36,38]</sup>.

Vanzant et al.<sup>[39]</sup>, Gosselink et al.<sup>[33]</sup> and Di Marco et al.<sup>[40]</sup> did not observe significant difference between in vivo and in situ degradability of forage, although their in vivo data contained large standard errors and mean prediction errors. Adesogan et al.<sup>[41]</sup> and Di Marco et al.<sup>[42]</sup> found a poor relationship and large prediction error for DM degradation between in vivo and in situ methods for whole plant wheat and maize silage, and sweet sorghum<sup>[40]</sup> but their in vivo values determined in sheep are similar to our DMD findings. Comparing in vivo and alternative techniques, Damiran et al.<sup>[43]</sup> found greater values of forage DMD and lower of NDF degradation with the in situ technique in wether sheep and steers compared to in vivo data.

## 6. Conclusions

Availability of nutrient components such as CP and NFC in tropical forage were highly influenced by forage family and species along with geographic location of growth. No differences were found between buffaloes and cattle or their developmental stages, suggesting that these feedstuffs can be equally efficiently utilized by various species of large ruminants at different life stages. A moderately strong relationship between  $K_d$  and DMD suggests that the  $K_d$  may be the single most important predictor of rumen degradation of forage plants. The in situ technique, with all its limitations, more closely mirrors in vivo measurements than do other common techniques.

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