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Prediction of *in vivo* organic matter digestibility of beef cattle diets from degradation parameters estimated from *in situ* and *in vitro* incubations

Pedro Del Bianco Benedeti[1,2], Sebastião de Campos Valadares Filho[1], Diego Zanetti[3], Fabyano Fonseca e Silva[1], Breno de Castro Silva[1], Herlon Meneguelli Alhadas[1], Jéssica Marcela Vieira Pereira[1], Marcos Vinicios Carneiro Pacheco[1], Pauliane Pucetti[1], Ana Clara Baião Menezes[1], Flavia Adriane de Sales Silva[1], Letícia Artuzo Godoi[1] and Stefanie Alvarenga Santos[4]

[1]Department of Animal Sciences, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil; [2]Department of Animal Sciences, Universidade do Estado de Santa Catarina, Chapecó, Santa Catarina 89815-630, Brazil; [3]Instituto Federal do Sul de Minas Gerais – Campus Machado, Machado, Minas Gerais 37750-000, Brazil and [4]Department of Animal Sciences, Universidade Federal da Bahia, Salvador, Bahia 40110-909, Brazil

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Author for correspondence:
Pedro Del Bianco Benedeti,
E-mail: pedro.benedeti@udesc.br

Abstract

The objective of this meta-analysis study was to develop and validate equations estimated from *in situ* and *in vitro* methods to predict *in vivo* ruminal digestibility of organic matter (OM) of beef cattle diets. The database was composed of individual data of 23 diets from six experiments. Information collected from these studies was: *in vivo* digestibility and degradation parameters of OM calculated from *in situ* and *in vitro* incubations. The values of estimated times for the *in situ* and *in vitro* incubations to access *in vivo* digestibility of OM, and differences between degradation at 24, 48 and 72 h (*in situ* and *in vitro*) and *in vivo* digestibility were analysed in a model that included the fixed effects of forage neutral detergent fibre level. Thereafter, a multiple stepwise regression was carried out using OM digestibility as a dependent variable and degradation parameters (A = water-soluble fraction; B = potentially degradable water-insoluble fraction; and kd = degradation rate of fraction B) as independent variables. Equation validation was performed using data from a seventh experiment that have the same methods than previous studies. Stepwise regression results showed that the kd contributed significantly in most of the algorithms derived to predict *in vivo* digestibility. Validation analysis showed that equations developed from both *in vitro* and *in situ* incubations accurately estimated the *in vivo* digestibility of OM ($P > 0.05$). Our results suggest that equations developed to estimate OM digestibility showed both precision and accuracy; however, *in situ* method presented better results than *in vitro*.

Introduction

Digestibility coefficient is an important tool for livestock production, since it is closely related to nutrient utilization, as well as intake and performance (Patterson *et al*., 2006). However, *in vivo* trials to evaluate apparent digestibility are usually time-consuming, laborious, costly and require a large number of animals to ensure repeatability (Stern *et al*., 1997). Moreover, in recent years, the scientific community has been under pressure to reduce animals' usage in research projects. Thus, alternative methods have been developed to determine accurate results that can be correlated with *in vivo* digestibility, such as *in situ* and *in vitro* evaluations (Tilley and Terry, 1963; Nocek, 1988). In *in situ* methods, samples are weighed into nylon bags and incubated in cannulated animals receiving a standard diet (Nocek, 1988). The porosity of these bags allows colonization by microorganisms and further sample degradation. On the other hand, *in vitro* methods may utilize ruminal fluid from cannulated animals to estimate degradation by sample incubation under laboratory conditions (Tilley and Terry, 1963; Weiss, 1994). Results from these techniques can be obtained faster and with lower costs, labour and animals usage than those from *in vivo* trials, since it is possible to incubate several bags with different diets using rumen inoculum from the same animal.

Research has been done using *in situ* (Rymer and Givens, 2002; Gosselink *et al*., 2004; Chaudhry and Mohamed, 2011; Krizsan *et al*., 2012; Holt *et al*., 2016) and *in vitro* (NRC, 2001; Gosselink *et al*., 2004; Chaudhry and Mohamed, 2011; Krizsan *et al*., 2012; Stalker *et al*., 2013; Ferraretto *et al*., 2015; Lopes *et al*., 2015) methods on the determination of

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feed digestibility in ruminants. However, most studies have evaluated individual feeds and not total mixed diets. Moreover, different recommended times of incubation have been proposed among studies (López, 2005). The time of incubation might differ depending on diet composition, since different feedstuffs have different degradation parameters in the rumen, such as water-soluble fraction (*A*), potentially degradable water-insoluble fraction (*B*) and degradation rate of fraction *B* (kd). Thus, utilizing these parameters might allow the proposal of a single equation to predict the digestibility of diets with different forage content. Although the effective degradability can be correlated with *in vivo* digestibility, the passage rate needs to be estimated to do so, which is a limitation. Thus, the use of degradation parameters might allow the *in vivo* digestibility estimation without passage rate utilization. However, few studies correlate the degradation parameters of *in situ* and *in vitro* methods with the ideal incubation time to reach *in vivo* digestibility.

We hypothesized that equations estimated from ruminal parameters developed using *in situ* and *in vitro* incubations with multiple time points could produce results that mimic *in vivo* digestibility of diets with different forage neutral detergent fibre (fNDF) levels. Therefore, the objective of this meta-analysis study was to develop and validate equations estimated from *in situ* and *in vitro* methods to predict *in vivo* organic matter (OM) ruminal digestibility of diets for ruminants.

Materials and methods

This study compiled data from seven experiments (six for equations development and one for validation) previously carried out at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

In vivo, in situ, *and* in vitro *trials*

Efforts were made to minimize the sources of variations among experiments by using the same cannulated animals for both *in situ* incubations and ruminal fluid collection for *in vitro* incubations. All studies (A, B, C, D, E and F) had *in vivo* and *in situ* evaluation. However, all diets from study B were not submitted to the *in vitro* evaluation. Ingredient proportion and chemical composition of the 23 experimental diets and *in vivo* OM digestibility are presented in Tables 1 and 2.

In vivo trials were performed using Nellore bulls [A ($n = 10$); B ($n = 42$); C ($n = 16$); D ($n = 18$); E ($n = 15$); and F ($n = 25$)], which were kept in individual pens equipped with water and feed troughs. For all studies, animals were fed twice daily, allowing for up to 10% refusals. Feeds and refusals were daily sampled. Total faeces collection was performed during three consecutive days to estimate dietary constituents' digestibility (Mezzomo *et al.*, 2011; Benedeti *et al.*, 2014). Feed, refusals and faeces samples were oven-dried (55°C), grounded in a knife mill using 2 and 1 mm screens sequentially, and were packed for further laboratory analyses.

Regarding *in situ* evaluation, three cannulated bulls were used for the incubation of the bags and all ingredients were previously ground through a 2 mm screen (Wiley mill; Thomson Scientific Inc., Philadelphia, PA, USA) for all studies. Diets were individually weighed into Nylon bags (Sefar Nitex, Switzerland; 50 µm porosity, 400 cm^2 surface area) and incubated in each animal. The bag surface area to mass ratio was 15 mg/cm^2. Incubation times were: 0, 2, 4, 8, 16, 24, 48, 72 and 96 h. The number of bags varied as a function of incubation time to guarantee enough residual samples after incubation (i.e. more bags per sample were incubated for the longer incubation times relative to shorter incubation times). Samples were incubated in the rumen by attaching the bags to a steel chain with a weight at the end to allow for continual immersion within ruminal contents. Bags were placed into the rumen in the reverse order of incubation hours so that all bags were removed at the same time for washing.

After the incubation period, bags were washed by hand with running cold tap water and the end-point for washing was when the rinsing water was clear. The 0 h bags were not incubated in the rumen, but they were rinsed in running water with the incubated bags. Samples were oven-dried at 55°C for 72 h. After drying, bags were placed in an oven at 105°C for 2 h and weighed. Residues of each diet were removed from nylon bags and placed in a labelled plastic bag to obtain a sample of each diet per animal/incubation time. Residual samples in the bags of different time points were used to estimate the parameters of ruminal degradation.

As regards *in vitro* evaluation, ingredients were ground to pass through a 1 mm screen (Wiley mill; Thomson Scientific Inc.). One system of four 4 litre digestion vessels (TE-150; Tecnal Lab®, Piracicaba, SP, Brazil), equipped for slow rotation and with a temperature controller was used in four consecutive 96 h fermentation batches with eight different time points: 0, 3, 6, 12, 24, 48, 72 and 96 h. Ruminal fluid was collected from three rumen cannulated bulls 2 h post-feeding, immediately filtered through four layers of cheesecloth and kept into pre-warmed thermal containers and transported to the laboratory. Furthermore, approximately 200 g of rumen solid particles were also added to the containers. For the inoculum preparation, the rumen content was blended for 2 min, followed by filtering through four layers of cheesecloth (Holden, 1999; Benedeti *et al.*, 2018*a*). The buffer mineral solution was prepared following the equipment manual and the pH was adjusted to 6.8 when needed (Holden, 1999). After preparation, 1600 ml of buffer solution was added in each vessel, which was placed into TE-150 incubator and kept at 39°C for 30 min. Then, 400 ml of rumen inoculum was added in each vessel under anaerobic conditions. Diet samples were weighed (0.5 g/bag) into filter bags (F57, Ankom technology, Macedon, NY, USA), which were heat-sealed and placed into the digestion vessels. For each incubation, each vessel received three bags of one of the diets/time point plus two bags with no samples (blanks), and 2000 ml of rumen/buffer solution. After inoculation, vessels were closed and then placed into the incubator with a temperature at 39°C for 96 h. At the end of each incubation time point, the bags were rinsed with cold water and oven-dried at 55°C for 72 h. Residual samples in the bags of different time points were used to estimate the parameters of ruminal degradation.

All ingredients used in these studies were ground to pass through a 1 mm screen (Wiley mill; Thomson Scientific Inc.) for laboratory analysis of all studies. Samples were analysed for dry matter (DM; method G-003/1), ash (method M-001/1), crude protein (method N-001/1) and ether extract (method G-005/1) according to Detmann *et al.* (2012). The OM was calculated as the difference between DM and ash contents. *In situ* and *in vitro* trial residues and faecal samples were analysed for final DM and OM.

For *in situ* and *in vitro* evaluations, the OM degradation profiles were estimated using the Ørskov and McDonald (1979)

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Table 1. Composition of the 23 experimental diets used to develop *in vivo* apparent digestibility and *in situ* and *in vitro* ruminal degradation parameters

Item	Diets composition, g/kg					
Study A	**D1**	**D2**				
Corn silage	300	300				
Dry ground corn	600	–				
Dry ground sorghum	–	600				
Soybean meal	65	65				
Urea	8.9	8.9				
Mineral mixture	26	26				
Study B	**D3**	**D4**	**D5**	**D6**	**D15**	**D16**
Sugarcane	400	400	400	400	–	–
Corn silage	–	–	–	–	580	580
Dry ground corn	298	298	482	482	267	267
Soybean hulls	210	210	–	–	–	–
Soybean meal	51	51	76	76	126	126
Urea	15	15	15	15	7.9	7.9
Mineral mixture	26	26	27	27	20	20
Study C	**D7**	**D8**	**D17**	**D18**		
Corn silage	500	–	700	–		
Sugarcane	–	500	–	700		
Soybean hulls	275	275	165	165		
Dry ground corn	194	194	116	116		
Soybean meal	21	21	13	13		
Mineral mixture	10	10	6.0	6.0		
Study D	**D9**	**D10**	**D11**			
Corn silage	500	500	500			
Dry ground corn	395	395	395			
Soybean meal	18	43	69			
Wheat meal	60	30	–			
Urea	2.9	7.0	11			
Mineral mixture	25	25	25			
Study E	**D12**	**D13**	**D14**			
Corn silage	500	500	500			
Dry ground corn	394	394	394			
Soybean meal	24	49	75			
Urea	4.8	10	15			
Wheat meal	61	31	–			
Mineral mixture	16	16	16			
Study F	**D19**	**D20**	**D21**	**D22**	**D23**	
Brachiaria grass silage	989	–	–	–	–	
Corn silage	–	983	–	–	–	
Elephant grass silage	–	–	971	–	–	
Sugarcane	–	–	–	986	–	
Tifton 85 bermuda grass	–	–	–	–	993	
Urea	11	17	29	14	7	

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Table 2. Chemical composition of the 23 experimental diets used to develop *in vivo* apparent digestibility, and *in vivo* OM digestibility

		Chemical composition									
Group[a]	Diet #	DM, g/kg	CP, g/kg of DM	NDF, g/kg of DM	fNDF, g/kg of DM	EE, g/kg of DM	NFC, g/kg of DM	OM, g/kg of DM	iNDF, g/kg of DM	OMd, g/kg	Study
Low	D1	624	128	240	158	33.5	556	943	63.2	723 ± 31	A
	D2	626	134	224	158	31.4	564	939	64.3	733 ± 35	A
	D3	506	140	376	201	29.5	427	957	116	718 ± 38	B
	D4	506	140	376	201	29.5	427	954	116	743 ± 16	B
	D5	506	137	289	201	32.3	519	962	112	742 ± 14	B
	D6	506	137	289	201	32.3	519	960	112	749 ± 10	B
Medium	D7	468	144	402	258	41.2	386	950	77.9	706 ± 21	C
	D8	446	134	396	241	32.4	434	962	96.5	723 ± 28	C
	D9	587	100	320	251	27.0	471	919	78.0	750 ± 26	D
	D10	588	118	308	251	27.0	465	919	75.0	750 ± 18	D
	D11	593	137	297	251	26.0	463	924	72.0	770 ± 23	D
	D12	574	95.1	316	258	31.1	511	948	91.8	697 ± 39	E
	D13	575	114	308	258	30.4	507	948	89.5	712 ± 42	E
	D14	575	134	301	258	29.7	503	948	87.1	729 ± 09	E
	D15	415	141	374	304	35.5	397	937	122	689 ± 19	B
	D16	415	141	374	304	35.5	397	937	122	710 ± 25	B
	D17	394	147	425	361	39.2	373	950	96.6	708 ± 38	C
	D18	368	133	417	337	26.9	439	966	123	710 ± 49	C
Forage	D19	195	109	694	694	34.8	42.6	864	219	588 ± 37	F
	D20	304	117	506	506	39.6	292	926	158	592 ± 81	F
	D21	211	117	733	733	19.5	43.0	888	239	582 ± 65	F
	D22	261	98.1	536	536	26.9	312	937	281	654 ± 61	F
	D23	835	103	798	798	14.7	20.4	922	302	618 ± 67	F

DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; EE, ether extract; NFC, non-fibre carbohydrates; OM, organic matter; fNDF, forage neutral detergent fibre; iNDF, indigestible neutral detergent fibre; OMd, *in vivo* organic matter apparent digestibility.
[a]Low (below 250 g/kg of fNDF), Medium (from 250 to 500 g/kg of fNDF) and Forage (only fNDF).

asymptotic function:

$$Y_t = A + B \times \left(1 - e^{-(kd*t)}\right)$$

where:
Y_t = fraction degraded in time '*t*', g/kg; A = water-soluble fraction, g/kg; B = potentially degradable water-insoluble fraction, g/kg; kd = degradation rate of fraction B, h^{-1}; t = time, h.

Estimated times for the *in situ* and *in vitro* incubations of OM to access the *in vivo* digestibility were obtained by the following equation:

$$t = -\left(\ln\left(1 - \left(\frac{\textit{in vivo}\ \text{digestibility} - A}{B}\right)\right)\right)/\text{kd}$$

where: t = estimated time; A = water-soluble fraction, g/kg; B = potentially degradable water-insoluble fraction, g/kg; kd = degradation rate of fraction B, h^{-1}.

All statistical procedures were carried out using SAS 9.3 PROC MIXED for Windows (Statistical Analysis System Institute, Inc., Cary, NC, USA) with $\alpha = 0.05$. Degrees of freedom denominator was estimated using the Kenward and Roger (1997) method.

Meta-analysis

A multi-study analysis was performed using data obtained from the 23 different beef cattle diets evaluated in the six experiments cited above. Collected information included *in vivo* digestibility of OM; *in situ* and *in vitro* degradation parameters (A, B and kd) of OM (Table 3).

Regarding forage NDF levels' analysis, diets were arranged in three groups, according to their fNDF level: Low (below 250 g/kg of fNDF), Medium (from 250 to 500 g/kg of fNDF) and Forage (only fNDF). The values of estimated times for *in situ* and *in vitro* incubations to access *in vivo* digestibility of OM were evaluated using a mixed model including the random effect of study and the fixed effect of fNDF level (Low, Medium and Forage). Least-squares means were contrasted using the Tukey–Kramer test. A significance level of 5% was assumed. Furthermore, the values of apparent digestibility of OM (from *in vivo* trials) were subtracted from *in situ* and *in vitro*

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Table 3. Descriptive statistics of the data used to develop and evaluate models to predict *in vivo* digestibility of dry matter and organic matter

	Evaluation	
Item	*In situ*	*In vitro*
Organic matter		
n	23	14
In vivo digestibility, g/kg	700 ± 55	711 ± 44
A	308 ± 69	254 ± 72
B	534 ± 94	593 ± 114
kd	0.046 ± 0.010	0.033 ± 0.009
t	32.1 ± 12	47.7 ± 11
Degradation, g/kg		
At 24 h	661 ± 84	548 ± 107
At 48 h	772 ± 80	686 ± 103
At 72 h	814 ± 91	751 ± 96

A, water-soluble fraction, g/kg; *B*, potentially degradable water-insoluble fraction, g/kg; kd, degradation rate of fraction *B*, h^{-1}; *t*, estimated time for *in situ* and *in vitro* incubations to access *in vivo* digestibility, h.

degradation at 24, 48 and 72 h of incubation. Then, differences between degradation (*in situ* and *in vitro*) at each of these time points and *in vivo* digestibility were analysed using the same previously described mixed model. Here, confidence levels of 95% based upon normal assumptions [mean ± (1.96 × standard error)] were used to identify if the means were different from zero. These analyses were performed with the MIXED procedure in SAS 9.4 (Statistical Analysis System Institute, Inc.).

Diets were not arranged by fNDF levels for equations development. A multiple stepwise regression was carried out for all the data using *in vivo* OM digestibility as dependent variables, whereas independent variables included *in situ* and *in vitro* degradation parameters (*A*, *B* and kd) previously described. These analyses were performed with the REG procedure in SAS 9.4 (Statistical Analysis System Institute, Inc.) assuming a significance level of 5%.

Equation validation was performed using data from a seventh experiment ($n = 14$ and 15 for *in vitro* and *in situ*, respectively) that had the same *in vivo*, *in situ* and *in vitro* methods as previous studies. Results from this experiment were not included in the database used to adjust tested equations. Table 4 provides the composition of diets utilized in this experiment.

Digestibility values of OM estimated by the equations proposed were compared with the observed values using the following regression model:

$$Y = \beta_0 + \beta_1 \times X$$

where X is the predicted value; Y is the observed value; β_0 is the intercept of the equation; and β_1 is the slope of the equation. Regression was evaluated according to the following statistical hypotheses (Mayer *et al.*, 1994):

$$H_0 : \beta_0 = 0 \text{ and } \beta_1 = 1, \text{ and } H_a : \text{not } H_0$$

Table 4. Feeds composition of experimental diets of the validation study

	Dry ground		Reconstituted	
Item	Corn	Sorghum	Corn	Sorghum
Feed, g/kg of dry matter				
Maize silage	284.4	284.4	284.4	284.4
Dry ground corn	608.3	–	–	–
Reconstituted corn[a]	–	–	608.3	–
Dry ground sorghum	–	608.3	–	–
Reconstituted sorghum[a]	–	–	–	608.3
Soybean meal	67.5	67.5	67.5	67.5
Vitamin–mineral premix[b]	29.4	29.4	29.4	29.4
Urea + ammonium sulphate[c]	10.4	10.4	10.4	10.4
Composition, g/kg of dry matter				
Dry matter, g/kg as fed	541	539	465	468
Organic matter	940	940	938	937
Crude protein	132	137	132	136
Neutral detergent fibre[d]	204	197	193	192
Non-fibre carbohydrates[e]	577	593	587	596

[a]Ground corn and sorghum were moisturized (until dry matter reach 640 g/kg) and ensiled for 90 days to form the reconstituted grains (Benedeti *et al.*, 2018*b*).
[b]Premix guarantees (per kg of DM): 200–220 g of Ca, 10 mg of Co (Min), 500 mg of Cu (Min), 22 g of S (Min), 333 mg of Fe (Min), 178.41 mg of F (Max), 10 g of P (Min), 25 mg of I (Min), 17 g of Mg (Min), 1500 mg of Mn (Min), 1100 mg of monensin, 100 × 109 CFU of *Saccharomyces cerevisiae* (Min), 6.6 mg of Se (Min), 50 g of Na (Min), 100 000 IU of vitamin A (Min), 13 000 IU of vitamin D3 (Min), 150 IU of vitamin E (Min) and 2000 mg of Zn (Min).
[c]Urea + ammonium sulphate in a 9:1 ratio.
[d]Neutral detergent fibre corrected for residual ash and residual nitrogenous compounds.
[e]Non-fibre carbohydrates = 100 − [(crude protein–crude protein from urea + urea) + neutral detergent fibre + ether extract + ash].

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Table 5. *In situ* ruminal degradation of organic matter (OM) at different time points and ruminal degradation parameters estimated from *in situ* incubations

	Ruminal OM degradation, g/kg			Ruminal degradation parameters[a]				
Diet #	at 24 h	at 48 h	at 72 h	*A*	*B*	kd	*t*	Study
D1	695	868	910	279	664	0.042	27.0	A
D2	589	844	887	286	684	0.029	36.5	A
D3	747	789	829	344	498	0.058	24.7	B
D4	746	822	839	340	511	0.063	24.9	B
D5	675	828	832	343	521	0.045	32.3	B
D6	682	814	829	342	512	0.048	33.3	B
D7	698	757	782	299	568	0.049	25.7	C
D8	646	785	833	334	464	0.054	34.0	C
D9	700	790	850	349	525	0.046	31.0	D
D10	720	790	870	325	558	0.048	29.9	D
D11	710	800	870	326	546	0.053	30.6	D
D12	749	847	879	276	610	0.064	18.2	E
D13	765	869	999	210	674	0.058	23.8	E
D14	703	827	876	284	667	0.053	20.8	E
D15	679	812	833	335	527	0.046	24.2	B
D16	650	807	824	335	530	0.042	28.2	B
D17	631	649	715	336	517	0.036	35.3	C
D18	665	739	812	378	353	0.042	67.6	C
D19	648	727	743	289	510	0.043	24.2	F
D20	658	748	797	395	453	0.032	26.2	F
D21	489	663	674	197	543	0.032	39.3	F
D22	562	629	648	401	282	0.033	33.9	F
D23	408	562	591	89	561	0.032	67.2	F

[a]*A* = water-soluble fraction, g/kg; *B* = potentially degradable water-insoluble fraction, g/kg; kd = degradation rate of fraction *B*, h^{-1}; *t* = estimated time for *in situ* incubation to access *in vivo* digestibility, h.

If the null hypothesis was not rejected, it could be concluded that equations accurately estimate the apparent digestibility of OM. Slope and intercept were separately evaluated to observe where equations have possible errors. Estimates were evaluated using the estimated value of the mean square error of prediction and its components (Bibby and Toutenburg, 1977):

$$\mathrm{MSEP} = \mathrm{SB} + \mathrm{MaF} + \mathrm{MoF} = 1/n\Sigma_{i=1}(X_i - Y_i)^2$$

$$\mathrm{SB} = (X - Y)^2$$

$$\mathrm{MaF} = (s_X - s_Y)^2$$

$$\mathrm{MoF} = 2s_X s_Y(1 - R)$$

where X are the predicted values; Y are the observed values; MSEP is the mean squared error of prediction; SB is the squared bias; MaF is the component relative to the magnitude of random fluctuation; MoF is the component relative to the model of random fluctuation; s_X and s_Y are the standard deviations of predicted and observed values, respectively; and R is the Pearson linear correlation between predicted and observed values.

For all variance and covariance calculations, total number of observations was used as a divisor since it was a prediction error estimate (Kobayashi and Salam, 2000). Prediction of efficiency was determined by estimating the correlation and concordance coefficient (CCC) or reproducibility index described by Tedeschi (2006). Validation analyses were performed with the Model Evaluation System [MES; version 3.1.16 (Tedeschi, 2006)] and significance was established at $\alpha = 0.05$.

Results

Forage NDF levels' analysis

Ruminal degradation parameters and ruminal degradation of OM at 24, 48 and 72 h are presented in Tables 5 and 6, respectively for *in situ* and *in vitro* trials. For certain diets, the models utilized did not converge due to different degradation responses. Thus, they were not adopted in this case. Regarding both methods, Low group had lower ($P < 0.01$) incubation time to access OM *in vivo* digestibility, compared to Medium and Forage.

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Table 6. *In vitro* ruminal degradation of organic matter (OM) at different time points and ruminal degradation parameters estimated from *in vitro* incubations

	Ruminal OM degradation, g/kg			Ruminal degradation parameters[a]				
Diet #	at 24 h	at 48 h	at 72 h	*A*	*B*	kd	*t*	Study
D1	753	850	892	118	806	0.050	28.0	A
D2	696	841	868	166	751	0.041	34.8	A
D7	600	741	773	261	586	0.026	47.6	C
D8	544	721	803	284	520	0.039	47.9	C
D9	580	720	780	300	576	0.039	38.6	D
D10	520	670	780	288	618	0.033	41.6	D
D11	530	710	770	298	617	0.036	39.6	D
D12	561	668	796	207	602	0.037	45.8	E
D13	520	692	–	206	643	0.026	60.3	E
D14	526	646	752	232	569	0.034	60.8	E
D17	593	684	717	231	671	0.026	48.0	C
D18	532	708	756	327	421	0.037	64.5	C
D20	499	635	690	226	551	0.026	56.7	F
D22	499	598	630	407	368	0.013	53.3	F
D23	262	406	507	–	–	–	–	F

[a]*A* = water-soluble fraction, g/kg; *B* = potentially degradable water-insoluble fraction, g/kg; kd = degradation rate of fraction *B*, h^{-1}; *t* = estimated time for *in vitro* incubation to access *in vivo* digestibility.

Table 7. Differences between *in situ* and *in vitro* degradation (at 24, 48 and 72 h of incubation) and *in vivo* digestibility of organic matter (OM)

	Residuals			SEM			*P* value[a]		
Item	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
In situ–in vivo, g/kg									
Low	54	59	84	30.2	28.5	31.1	0.09	0.01	0.05
Medium	28	68	124	19.5	18.4	20.1	0.16	<0.01	<0.01
High	46	93	120	27.5	26.0	28.4	0.11	<0.01	<0.01
In vitro–in vivo, g/kg									
Low	−201	−75	−12	39.6	34.7	31.2	<0.01	0.70	0.05
Medium	−175	−30	44	21.7	19.0	18.0	<0.01	0.03	0.15
High	−3	118	152	48.5	42.4	38.2	0.95	<0.02	0.02

[a]Values are significantly different from 0 ($P \leqslant 0.05$) with 95% confidence interval based on normal assumptions [mean ± (1.96 × standard error)] (Casella and Berger, 2002).

Results for residuals (*in situ* and *in vitro* degradation minus *in vivo* digestibility of OM) are presented in Table 7. Considering *in situ v. in vivo* evaluation, OM residuals at 48 and 72 h were positive and different from 0 ($P < 0.01$), but similar at 24 h ($P > 0.05$) for all fNDF levels. With regard to *in vitro* minus *in vivo* evaluation, Forage group displayed negative residuals for OM that were different from 0 ($P < 0.01$) at 24 and 48 h, but similar to 0 at 72 h ($P > 0.05$). Regarding Medium fNDF diets, *in vitro* minus *in vivo* OM residuals negatively differed from 0 at 24 h ($P < 0.01$), were similar at 48 h ($P > 0.05$) and positively differed from 0 at 72 h ($P < 0.01$). With respect to Low fNDF diets, residuals of OM did not differ from 0 at 24 h ($P > 0.05$), but positively differed from 0 at 48 and 72 h ($P < 0.01$).

Equations development

Figure 1 presents the comparison between observed and predicted OM digestibility values analysed in the validation study. Stepwise regression results of digestibility assays showed that kd contributed significantly in most of the algorithms derived to predict *in vivo* digestibility (Table 8). Furthermore, kd was the only significant parameter ($P < 0.05$) for the estimation of OM *in vivo* digestibility from both *in situ* and *in vitro* assays. Equations developed from both *in vitro* and *in situ* incubations accurately estimated *in vivo* digestibility of OM ($P > 0.05$). Validation analysis showed that CCC was farther from 1.0 and MSEP was lower for *in vitro* equations than for those for *in situ* equations.

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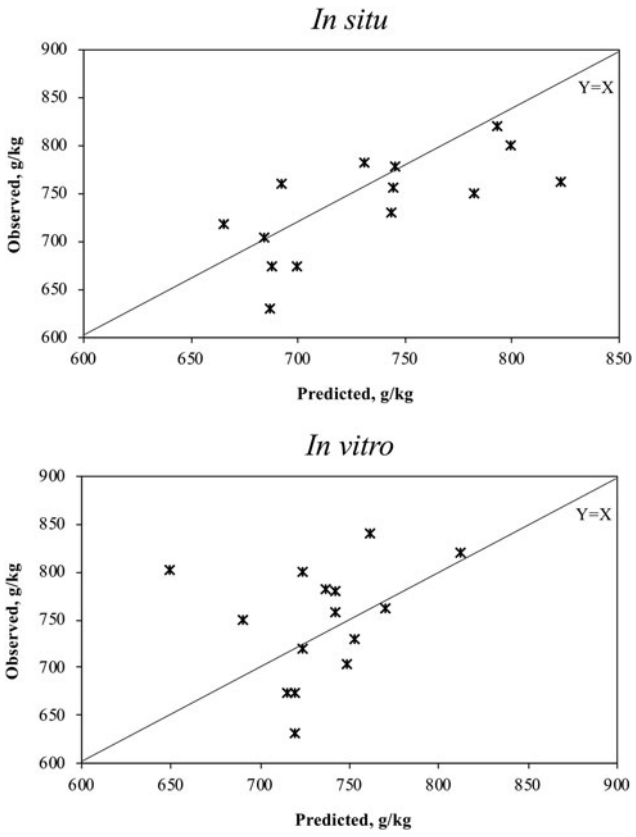

Fig. 1. Relationship among observed and predicted (*in situ* and *in vitro*) organic matter digestibility values.

Table 8. Developed equations and mean and descriptive statistic of the relationship among the observed (*in vivo*) and predicted (*in situ* and *in vitro*) values of organic matter (OM) digestibility

Item	Observed	*In situ*	*In vitro*
Equations			
OM digestibility, g/kg		(569.5204 + 2859.7612 kd)	(622.7653 + 2674.4842 kd)
R^2		0.35	0.46
Validation analysis			
Mean, g/kg	739	734	734
Standard deviation, g/kg	53.5	50.2	36.6
Maximum, g/kg	820	823	812
Minimum, g/kg	631	665	650
R	–	0.7	0.24
CCC	–	0.69	0.21
Regression			
P value (H_0: $\beta_0 = 0$ and $\beta_1 = 1$)	–	0.47	0.28
MSEP	–	15.5	37.9
SB	–	0.27	2.24
MaF	–	1.55	4.55
MoF	–	13.6	31.1

R, determination coefficient; CCC, correlation and concordance coefficient; MSEP, mean square error of prediction; SB, squared bias; MaF, magnitude of random fluctuation; MoF, model of random fluctuation; kd, degradation rate of potentially degradable water-insoluble fraction, h^{-1}.

Discussion

Forage NDF levels' analysis

Reports regarding nutrient utilization from feedstuffs are important to improve diet formulation and animal performance. Moreover, alternative methods (*in situ* and *in vitro*) on the determination of feed digestibility in ruminants have been developed to obtain faster results, with lower costs, labour and animal usage (Nocek, 1988). However, most of the studies have evaluated individual feeds (mostly forages) and proposed that times of incubation have been conflicting (Stern *et al.*, 1997; López, 2005; Krizsan *et al.*, 2012; Stalker *et al.*, 2013). Because fibre is known to be the slowly degradable or undegradable fraction of feedstuffs (Mertens, 2015), diets with different forage content might differ in degradation pattern and incubation times. Thus, we hypothesized that performing *in situ* and *in vitro* methods, Low fNDF diets present lower incubation times to reach *in vivo* digestibility than fNDF diets. Contrary to our hypothesis, *in situ* incubation times were similar among diets, regardless of fNDF levels. Moreover, OM digestibility was overestimated from 48 h of incubation by the *in situ* method used in the experiments evaluated here. Thus, it seems that because of feed grinding, rapid microbial colonization occurred, which allowed a fast and similar degradation of diets, regardless of fNDF level.

On the other hand, the lower fNDF required less time to mimic *in vivo* OM digestibility. Furthermore, OM diets digestibility might be under or overestimated at different time points, depending on fNDF level. For example, 48 h incubation time was good for digestibility determination of Medium diets, however underestimates digestibility for Forage and overestimates digestibility for Low diets. The lack of relationship between *in vitro* and *in situ* results for OM digestibility might be related with the low rumen inoculum amount used in the former method, resulting in greater lag time, especially in diets with high forage. Nevertheless, the different estimated incubation times among methods and diet components suggests that more than a single time point incubation should be used to develop equations to predict *in vivo* digestibility. Others also have suggested that the use of a single time point might not be satisfactory when using *in vitro* methods (Lopes *et al.*, 2015). In summary, 24 h incubation was suitable for *in situ* methods to estimate *in vivo* OM digestibility, regardless of fNDF level. On the other hand, *in vitro* suitable results were obtained at 24, 48 and 72 for Low, Medium and Forage groups, respectively.

Equations development

To develop equations that correctly estimate *in vivo* digestibility of diets, we utilized ruminal degradation parameters (A, B and kd) estimated from *in situ* and *in vitro* studies that have performed incubations with multiple time points. From these parameters, kd is the one associated with the degradation rate of the slowly degradable feedstuff fraction in the rumen, such as fibre components (Ørskov and McDonald, 1979). Thus, equations that utilize this parameter might correctly estimate *in vivo* digestibility, regardless of forage content, which may allow the proposal of a single equation that may be used for diets with different fNDF levels. Indeed, kd was the only variable that significantly

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contributed to all equations that estimate OM digestibility. Therefore, two equations (one for each method) are proposed here to estimate *in vivo* digestibility of OM of beef cattle diets.

To validate proposed equations, we tested them using data from an independent study that was performed by using diets composed of 19.2, 19.3, 19.7, and 20.4% of NDF content (DM basis). As expected, equations estimated from both *in situ* and *in vitro* methods were appropriate to predict *in vivo* digestibility of OM. Validation tests have demonstrated that equations that estimate these variables from *in situ* incubations were more accurate and precise than those from *in vitro* incubations, since equations from the former method had greater CCC and lower MSEP. These are parameters that indicate the model's efficiency and reproducibility (Tedeschi, 2006). Thus, models have better accuracy and precision when CCC is closer to 1.0. Furthermore, a lower MSEP is better, since it can indicate model errors associated with SB or errors related to the high dispersion of data around the mean or systematic errors concerning predicted curve direction. Thus, equations from both methods estimate digestibility correctly for both intercept and slope. *In vitro* equations presented the largest SB, which might mean that they had a smaller capacity to simulate variation around the mean than *in situ* equations. However, it is important to emphasize that R^2 values observed in the stepwise regression were not high for both equations, which may be applied to the high variation of the composition of the diets. On the other hand, R^2 needs to be analysed together with other variables in a statistical model (such as these commented above) to indicate the correctness of the regression model. Therefore, the equations proposed here (from both methods) can be considered adequate to estimate *in vivo* digestibility of OM due to their good precision and accuracy.

In summary, the current study results indicate that more than a single time point incubation should be used to develop equations to predict *in vivo* digestibility of diets with different forage levels. However, incubation times of 24 h may be adequate to estimate *in vivo* OM digestibility from *in situ* method. Furthermore, incubation times to estimate *in vivo* OM digestibility from the *in vitro* method might depend on fNDF levels and, for this study, suitable results were obtained at 24, 48 and 72 for Low, Medium and Forage groups, respectively.

Despite both developed equations have been validated by using data from an independent experiment, the *in situ* results were more precise and accurate (Greater CCC, and Lower MSEP and SB), compared to *in vitro* results. However, the NDF levels (from 19.2 to 20%, on a DM basis) of diets used in the validation study can be considered low, thus it would be recommended to test the efficacy of these equations on the OM digestibility estimation of diets with high fibre content. Nevertheless, *in situ* and *in vitro* equations developed to estimate OM digestibility exhibit both precision and accuracy and they represent an important advance in the prediction of *in vivo* digestibility.

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Ethical standards. Care and handling of all experimental animals were conducted under protocols approved by the Institutional Animal Care and Use Committee of the Universidade Federal de Viçosa (protocol numbers 95/2014, 96/2014, 17/2015, 18/2015, 42/2016 and 59/2016).

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