v. 5, n. 5, 2025

Journal of Agricultural Sciences Research

Acceptance date: 22/07/2025

UNDERESTIMATION OF FORAGE DIGESTIBILITY COEFFICIENTS DUE TO THE EFFECT OF OMITTING DETERGENT EXTRACTION OF *IN SITU* RESIDUES

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Abstract.: Accurate determination of ruminal degradability is essential for evaluating the nutritional value of forages. The in situ technique is widely used for such assessments; however, microbial contamination (MC) can significantly underestimate digestibility parameters. This study evaluated the extent of underestimation caused by omitting detergent extraction during mechanical washing of in situ residues. Samples of four chemically distinct forages (alfalfa hay, corn stover, oat hay, and cactus silage) were incubated in the rumen of two cannulated bovines for 48 hours. After incubation, residues were mechanically washed either with or without sodium lauryl sulfate and analyzed for digestibility of dry matter (DMD), organic matter (OMD), and crude protein (CPD). The omission of detergent led to underestimations of DMD and OMD by 2.8 and 2.3 percentage units, respectively, while CPD was markedly affected—up to 17.9 percentage units in corn stover. These findings emphasize the importance of incorporating detergent-based microbial correction in in situ protocols to ensure accurate forage evaluation.
Key words: *In situ* Digestibility, Microbial correction, Detergent.

INTRODUCTION

Ruminal degradability varies within and among food groups; thus, its determination is of utmost importance in food evaluation. The *in situ (in sacco)* technique is the most commonly used in the studies of digestibility. In this method, feed samples are incubated in bags with pores that allow the exchange of liquids, the entry and exit of bacteria, and, ideally, limit the exit of undigested particles. During fermentation, bacteria adhere to and penetrate the food particles to digest them; at the end of incubation, bacteria remain attached to the particles and, since bacteria are constituted by organic matter and protein, their presence constitutes a possible contamination factor that affects food evaluations (Vanzant *et al.*, 1998). It has been estimated that microbial contamination (MC) with markers (usually ^{15}N) can constitute up to 28% of the dry matter and 95% of the nitrogen of residues *in situ* (Beckers *et al.*, 1995; Vanzant *et al.,* 1998), but microbial contamination of residues is not uniform among different substrates or feeds. Others have reported that microbial contamination means 6 to 7% and 60 to 70% of the dry matter and protein of residual forage particles. In contrast, Menezes *et al.* (2017) suggest that the effect of microbial contamination may be irrelevant to the degradability of concentrates in high protein and low fiber. Several methods and compounds that are effective in correcting MC have been compared (Beckers *et al.*, 1995). However, it is not uncommon for studies to fail to report whether any correction for MC was made (Vanzant *et al.*, 1998).

OBJECTIVE

The objective of this trial was to determine the extent of the underestimation of forage digestibility by the omission of detergent extraction during mechanical washing of *in situ* residues.

MATERIAL AND METHODS

The study was conducted at the Centro Nacional de Investigaciones Disciplinarias en Fisiología y Mejoramiento Animal-INIFAP, located in Ajuchitlán, Colón, Querétaro, México. Four types of forages were selected that varied in crude protein content: alfalfa hay, 2) corn stover, 3) oat hay, and cactus silage (Table 1). Samples of these forages were ground with a Wiley-type laboratory mill (Thomas Scientific, Swedesboro, NJ) through a 2 mm sieve. A sample of 5 ± 0.1 g was placed in *in situ* dacron bag (10 x 20 cm; Ankom Technologies Corp, Macedon, NY), achieving a ratio of 13.5 mg of dry matter per cm^2 of bag surface area.

Subsequently, the bags were heat-sealed using a pulse sealer (American International Electric). For each type of forage, 16 bags were used, which were divided into two runs and two bovines with a cannula in rumen. Thus, in each bovine, 16 bags (4 bags per type of forage) were incubated per run (block). Before insertion in rumen, in situ bags were placed in a 45 x 40 cm nylon bag and then immersed in water at 37°C for 10 to 15 min. The bags were incubated in the rumen for 48 h. Cattle were kept grazing in a natural pasture with free access to water and a mineral block. In addition, the animals received a mixture of alfalfa hay and corn stover and another mixture of soybean and canola meal. The guidelines of the Mexican Official Standard (NOM-062-ZOO-1999) and CIOMS (1985) were observed. At the end of the incubation period, the bags were removed from the rumen and washed without (WND) or with (WWD) sodium lauryl sulfate (1 g detergent / g fresh residue) in a volume of water of 40 L, using a washing machine. The bags were then dried at 55°C for 72 h in a forced-air oven and weighed. The samples and residues were analyzed for dry matter (100°C; Method 967.03, AOAC, 2000), organic matter (600°C; AOAC, 1990), and crude protein by Kjeldahl method (Nx6.25; Method 984.13, AOAC).

Thus, the *in situ* digestibility of dry matter (DMD), organic matter (OMD), and crude protein (CPD) was calculated as the percentage of the weight of the original sample that disappeared from the bag after the incubation time:

$$D\ (\%) = 100 * (1 - \frac{Residue\ weight\ (g)}{Initial\ sample\ weight\ (g)})$$

A randomized complete block experimental design was used. The statistical model was mixed and included the effects of block (βi; run), forage (Ƒj), detergent extraction (τ_k) and interaction (Ƒ*τ), for which, PROC MIXED of the SAS 9.4 statistical package (SAS Institute Inc., Cary, NC, 2016). The effect of the block was considered random. Thus, the statistical model was:

$$y = \mu + \beta i + \gamma j + \tau k + \gamma * \tau jk + \varepsilon(ijk)$$

RESULTS AND DISCUSSION

The chemical composition of the forages used in the trial is shown in Table 1. It can be appreciated the chemical variability among forages. Table 2 shows that for DMD and OMD the main effects of forage and detergent washing are significant. On the other hand, for CPD there was interaction between the main factors. The interaction can be explained by the fact that the underestimation for CPD of stubble was 17.9 percentage units (PU), compared to 2.6, 7.1, and 5.1 PU for alfalfa hay, oat hay and cactus silage, respectively. The graph shows the effect of detergent use by box-and--whisker plots for DM, OM and OC digestibility by forage. Vanzant *et al.* (1998) point out that only 12 of 61 reports evaluating dry matter (DM), organic matter (OM), or crude protein (CP) degradation mentioned some method to correct MC. Rodriguez and Gonzalez (2006) evaluated the effect of MC on the effective degradability of various feeds with the use of ^{15}N. They report that the MC of DM in forages can be between 5 to 10% of the dry matter of the residue and 10 to 90% of the nitrogen of the residue. MC correction increased the degradability coefficients of dry matter and feed protein, with the underestimates being lower for DM than for CP. The results of the present study agree with the results of Rodriguez and Gonzalez (2006). The underestimation of degradability for CP of alfalfa hay can be up to 8.1 percentage units per MC, which contrasts with only 2.6 percentage units reported in this study (Table 2). Beckers *et al.* (1995) compared different methods (chilling at 4°C for 6 h plus NaCl solution, commercial detergent,

sodium lauryl sulfate, methyl cellulose, or no compound) to correct for MC in the estimation of degradability of wheat bran (dST) incubated for 24 h in the rumen. They reported that dST was lower if any method for correcting MC was omitted. However, the use of one chemical compound and applying cooling, dST was similar when MC is corrected with the marker ^{15}N. In the study by Beckers *et al.* (1995), MC reduced the underestimation of degradability by 3.3 and 7.1 percentage units for DM and CP degradability, respectively.

CONCLUSIONS

Omitting detergent in the mechanical washing of in situ bags with residues leads to the underestimation of in situ digestibility coefficients in forage samples. The most pronounced effect occurs in crude protein digestibility, while those for dry matter and organic matter are comparatively less affected.

IMPLICATIONS

For its easy application and low cost, microbial correction with detergent should always be considered in the studies of in situ digestibility. Furthermore, from an environmental standpoint, future research should explore the development and validation of biodegradable or naturally derived detergents for use in digestibility protocols.

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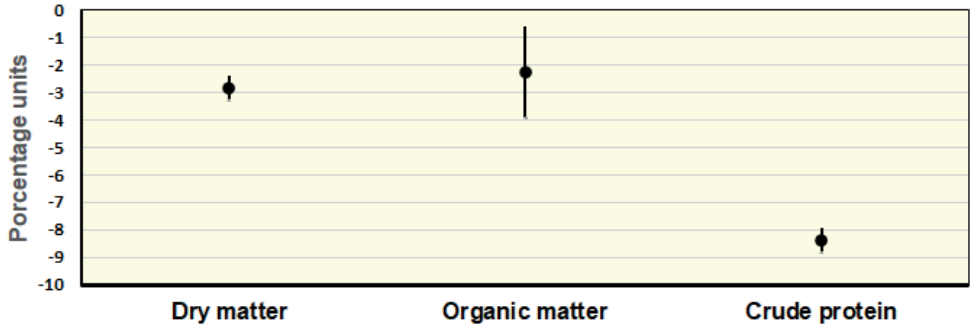
SOURCE OF FINANCING

The work was carried out with self-generated resources from INIFAP.

Chemical component (%)			
Forage	Dry matter (%)	Organic matter (% dry basis)	Crude protein (% dry basis)
Alfalfa hay	82.36	88.34	18.37
Corn stover	94.42	67.36	3.45
Oat hay	92.22	90.95	10.12
Cactus silage	93.06	83.34	5.73

Table 1. Chemical composition of forages.

Graph. Underestimation of digestibility of dry matter, organic matter, and crude protein of forages when detergent extraction is omitted in the mechanical washing of *in situ* residues. (Confidence interval, α= 95%)



VR	TRT	Alfalfa hay	Corn stover	Oat hay	Cactus silage	Media TRT	eem	F	TRT	Int
In situ digestibility of dry matter (DMD), %										
	WND	82.7	62.7	71.7	71.5	72.2	0.79	0.01	0.01	0.10
	WWD	85.1	66.4	74.6	73.8	75.0				
In situ digestibility of organic matter (OMD), %										
	WND	81.2	53.6	66.0	75.8	69.1	3.03	0.01	0.01	0.87
	WWD	83.8	56.5	68.3	77.0	71.4				
In situ digestibility of crude protein (DPC), %										
	WND	94.4	57.5	88.4	84.2	81.1	0.89	0.01	0.01	0.01
	WWD	97.0	75.4	95.5	89.3	89.4				

WND=Mechanical washing without detergent; WWD=Mechanical washing with detergent; F=-Forage effect; TRT=Detergent effect (LSD; Int=Interaction between mechanical washing and type of forage.

Table 2. Least square means (LSM) of *in situ* digestibility coefficients of forages mechanical washed without detergent (WND) or with detergent (WWD).