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Variation between individual cows in *in situ* rumen degradation characteristics of maize and grass silages

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ABSTRACT

Different numbers of animals have been used in different studies to cover the variation between individual animals in *in situ* rumen degradation characteristics of maize and grass silages. The objective of this study was to determine whether three cows are sufficient or not to cover the variation between individual cows in *in situ* rumen degradation characteristics of dry matter (DM), organic matter (OM), crude protein (CP), starch and neutral detergent fibre (NDF) for maize and grass silages. Fifteen maize and 15 grass silage samples, with a broad range in chemical composition, were selected. The maize and grass silage samples were incubated in the rumen for 2, 4, 8, 16, 32, 72 and 336 h, using the nylon bag technique. Three cows were used for nylon bag incubation of maize silages and three other cows for grass silages. The variation between individual cows was found significant ($P < 0.05$) for degradation rate (k_d) of DM, OM and CP, and the effective rumen degradation (ED) of DM and CP of maize silages whereas non-significant ($P > 0.05$) differences were found for all other parameters of DM, OM, CP, starch and NDF. The variation between individual cows was found non-significant ($P > 0.05$) for rumen undegradable fraction (U), potentially rumen degradable fraction (D), k_d and ED of DM, OM, CP and NDF of grass silages. The results of this study indicate that the use of three cows for nylon bag incubations of grass silages is sufficient whereas the use of three cows for nylon bag incubations of maize silages is not sufficient to cover variation between the individual cows.

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1. Introduction

Dietary nutrient bioavailability of feed or feed ingredient is essential information to ensure that the nutrient requirements of animals are met. Several *in situ*, *in vitro* and *in vivo* techniques have been developed to measure nutrient digestibility and over the past decades, much research has focussed on determining the nutritive value of major forages and concentrate ingredients used in dairy cow nutrition. The *in situ* (*in sacco*) technique has been extensively used for the determination of the rumen degradation of different chemical components in the rumen [1–3]. Factors affecting the results of *in situ* technique include bag and pore size, sample size, bag material, bag insertion and removal procedures, rumen incubation time, number of replicate animals, animals, diet of the experimental animals, feeding level, feeding frequency, rinsing procedure, mathematical models, and microbial contamination [4,5]. A number of these factors have been extensively studied [6–8]. Less focus has been directed on investigating the variation between individual animals for rumen incubations of forages.

In the past, many studies have been conducted to determine the *in situ* rumen degradability of dietary fractions or nutrients of maize and grass silages. In many of these studies, an arbitrary assumption was made that two [9,10], three [11–13], four [14,15] or six [16,17] animals are sufficient for *in situ* rumen incubations of forages. How-

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ever, little data exists in the scientific literature to determine the validity of this assumption for *in situ* rumen incubations of forages. Various ruminant feed evaluation systems such as the DVE/OEB_{2010} system [18] in the Netherlands and the NorFor – the Nordic feed evaluation system [19] recommend to use three cows for rumen incubations of feeds and allow pooling of rumen incubated residues to perform chemical analysis. The use of three cows for *in situ* rumen incubations has not been validated for maize and grass silages. In the past, few studies were performed with other feed ingredients to determine the variation between individual cows. Weakley et al. [6] observed no significant differences between individual animals (n = 4), days of incubation and periods of experimentation on ruminal disappearance of dry matter (DM) of soybean meal whereas significant differences between individual animals in *in situ* nitrogen (N) disappearance were observed. Figroid et al. [20] found significant differences between steers (n = 2) in *in situ* rumen DM degradability for barley and sorghum. Recently, an *in situ* study conducted by Castillo-Gallegos et al. [8] with king grass (*Pennisetum purpureum*) leaves showed no significant differences between cows (n = 3) for DM disappearance and concluded that two cows are sufficient for *in situ* rumen incubations. As the experimental costs increase significantly with increasing animal numbers, therefore, knowledge of between-animal variation is essential to obtain cost-effective estimates for *in situ* rumen degradation of maize and grass silages.

This study was designed to determine whether three cows are sufficient or not to cover the variation between the individual cows in *in situ* rumen degradation characteristics of DM, organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and/or starch of maize and grass silages.

2. Materials and methods

2.1. Samples collection and processing

Fifteen maize and 15 grass (mainly *Lolium perenne*) silage samples (~5 kg per silage) were obtained during 2007, 2008 and 2009 from various commercial farms located in different regions in the Netherlands. The samples were collected by trained technicians from a feed analysis laboratory (Blgg Research, Wageningen, The Netherlands) using a hollow drill. After collection, the samples were stored at −20 °C until processing. The frozen samples were cut using a bread slicer (JAC Duro BEL 450; ABO, Leek, The Netherlands) having a distance of 11 mm between the discs, thoroughly mixed by hand and divided into three parts; one part (~2.5 kg) was subjected to chemical analyses after freeze drying and grinding (3 mm), another part (~1.5 kg) was stored at −20 °C for later *in situ* rumen incubations, and the third part (~1.0 kg) was stored (−20 °C) as a reserve for possible future analysis.

2.2. In situ *rumen incubations*

Six multiparous (second or third lactation) Holstein Friesian cows, producing >15 kg milk per day and fitted with permanent rumen cannulas, were used in this experiment. Cows were fed a total mixed ration (Table 1) and had 24 h/day access to fresh water. Information on age, lactation number, milk production, milk protein, and milk fat production of the six cows used for the maize and grass silage rumen incubations is presented in Table 2. Three cows (Cow 1–3) were used for *in situ* rumen incubations of maize silages whereas other three cows (Cow 4–6) were used for grass silages. The maize and grass silage samples (~5 g DM) were weighed into 10 cm × 19 cm nylon bags (porosity 24%; pore size 37 μm; Nybolt, Zürich, Switzerland) and incubated in the rumen for 2, 4, 8, 16, 32, 72 and 336 h according to the procedure used by Ali et al. [21]. The

Table 1
Components of the total mixed ration fed to the cows during rumen incubations.

Feed ingredient	g/kg DM
Maize silage	237.4
Grass silage	395.8
Grass hay/wheat straw	13.5
Soybean meal	47.4
Sweet syrup	9.5
Wet distillers grains with solubles	84.3
Soybean meal (rumen protected)	18.6
Vitamin and minerals premix[a]	10.9
Concentrate[b]	182.6

[a] Contained: calcium 175 g/kg; phosphorous 0.2 g/kg; magnesium 130 g/kg; sodium 50 g/kg; chloride 78 g/kg; vitamin A 600,000 IE/kg; vitamin D 120,000 IE/kg; vitamin E 8000 IE/kg.
[b] Contained: maize, 30%; palm kernel, 22%; rapeseed solvent extract, 20%; citrus pulp, 10%; soybean meal (rumen protected), 5%; Beet molasses, 5%; molasses, 2%; wheat, 2%; chalk (CaO), 0.6%; urea, 0.6%; salt, 0.5%; Magnesium oxide (80% MgO, 0.5%; palm oil, 0.2%; vitamin and minerals premix, 0.3%.

Table 2
Information about the cows used for maize and grass silage rumen incubations.

Variable	Maize silage rumen incubations			Grass silage rumen incubation		
	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6
Age (days)	1754	1717	1654	1388	1397	1271
Lactation number	2	3	2	2	2	2
Milk production/day (kg)	29.7	27.2	30.9	28.0	29.7	27.6
Milk protein/day (kg)	1.1	1.1	1.0	1.0	1.1	1.0
Milk fat/day (kg)	1.5	1.4	1.2	1.2	1.3	1.3

0 h bags were washed in a washing machine (AEG-Electrolux Öko Turnamat 2800, Stockholm, Sweden) for 40 min using tap water at 25 °C without incubation in the rumen [22]. The washed bags were stored at −20 °C for at least 24 h, subsequently freeze dried and the residues were used to calculate the washout (*W*) fraction. Six bags of each maize and grass silage sample were incubated in the rumen of the three cows (2 bags per cow per incubation time) for 2, 4, 8, 16, and 32 h. Because of the low recovery of incubated residues per nylon bag for the 72 and 336 h incubation periods, 9 bags of each silage sample were incubated in the rumen of the three cows (3 bags per cow per incubation time) for these incubation periods. After removal from the rumen, bags were frozen at −20 °C for at least 24 h, after which the bags were thawed and washed in the washing machine as described above. The washed bags were stored at −20 °C and subsequently freeze dried. Rumen incubation residues of each sample collected from each cow after each rumen incubation period were pooled and ground separately over a 1 mm sieve, using a hammer mill (Pepping, 200 AN-797002, Deventer, The Netherlands).

2.3. Chemical analysis and calculations

All the ground freeze dried samples were analysed for DM, ash, CP, crude fat, sugar, NDF, acid detergent fibre and acid detergent lignin. Incubation residues were analysed for DM, ash, CP and NDF. Additionally, starch was analysed in maize silage samples and rumen incubated residues of maize silages.

The DM content was determined by oven drying at 103 °C for 4 h (ISO 6496) and ash content by incineration at 550 °C for 4 h (ISO 5984). The N content was determined using the Kjeldahl method (ISO 5983) and CP was calculated as N × 6.25. Starch was determined using the amyloglucosidase method (ISO 15914) after dissolving in 100% dimethyl sulfoxide. The NDF was determined according to the modified method of Van Soest et al. [23], using amylase and expressing without residual ash (ISO 16472). Acid detergent fibre was analysed by boiling with acid detergent

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