



## Particle size alterations of feedstuffs during in situ neutral detergent fiber incubation

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### ABSTRACT

Particle size alterations during neutral detergent fiber (NDF) determination and in situ rumen incubation were analyzed by dry sieving and image analysis to evaluate the in situ procedure for estimation of NDF degradation parameters and indigestible NDF concentration in terms of particle size. Early-cut and late-cut grass silages, corn silage, alfalfa silage, rapeseed meal, and dried distillers grains were examined. Treatments were (1) drying and grinding of forage samples and grinding of concentrates; (2) neutral detergent-soluble (NDS) extraction; (3) machine washing and NDS extraction; (4) 24-h rumen incubation, machine washing, and NDS extraction; and (5) 288-h rumen incubation, machine washing, and NDS extraction. Degradation profiles for potentially degradable NDF were determined and image analysis was used to estimate particle size profiles and thereby the risk for particle loss. Particle dimensions changed during NDF determination and in situ rumen incubation and variations depended on feedstuff and treatment. Corn silage and late-cut grass silage varied most in particle area among feedstuffs, with an increase of 139% between 0 and 24 h and a decrease of 77% between 24 and 288 h for corn silage and a decrease of 74% for late-cut grass silage between 24- and 288-h in situ rumen incubation. Especially for late-cut grass silage residues after 288 h in situ rumen incubation, a high mass proportion in the critical zone for escape was found. Particle area decreased linearly with increasing incubation time. Particle loss during in situ rumen incubation cannot be excluded and is likely to vary among feedstuffs.

**Key words:** Dacron bag, rumen, method, image analysis

### INTRODUCTION

Fiber is a major energy source in ruminant feedstuffs. Fiber digestion and passage behavior have a great influence on the net energy value of a ration, as cell contents are almost completely digestible. Chemically, fiber can be defined as the residue after boiling feedstuffs in neutral detergent (ND) solution and is indicated as NDF (Van Soest and Wine, 1967; Mertens et al., 2002). Potential digestibility and degradation rate of potentially digestible NDF (**pdNDF**) determine the availability of NDF in the rumen and thereby the energy availability for the ruminant (Huhtanen et al., 2006). Indigestible NDF (**iNDF**) is an important input factor in new feed evaluation systems (NRC, 2001; Volden, 2011) and can be determined by in situ rumen incubation of feedstuffs in Dacron bags, followed by machine washing and analysis of the residual NDF. Reliability of results from in situ rumen incubation, however, depends on the complete retention of iNDF in the Dacron bags. Grinding of feedstuffs before in situ incubation might produce small particles that can be lost from the Dacron bags due to their dimensions. Immediate particle loss of small particles can be taken into account when estimating NDF degradation rate through correction for particles lost from bags during machine washing (Åkerlind et al., 2011). Particle loss during rumen incubation is not accounted for in the in situ approach. Akin (1993) suggested that chewing mainly reduces particles in size, whereas degradation by rumen microorganisms was not expected to affect particle size. Microbial degradation, however, was shown to weaken plant structures (Akin, 1993), which subsequently could be mechanically broken during rumen contractions and, therefore, reduced in particle size. Differences among feedstuffs in particle dimension alterations during rumen incubation and thereby potential particle loss could be due to intrinsic plant characteristics such as plant origin and maturity (Akin, 1989; Wilson and Kennedy, 1996).

The hypothesis was that mean particle length, width, and area remain constant during rumen incubation. Particle loss from Dacron bags was, therefore, not expected during in situ rumen incubation. The objective

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was to describe changes in particle dimensions during NDF determination and in situ rumen incubation.

## MATERIALS AND METHODS

### Feedstuffs

Forage and concentrate feedstuffs were investigated. Forages were corn silage, early- and late-cut grass silage, and alfalfa silage. Corn silage was harvested on October 26, 2010. Early-harvested, primary growth grass silage was cut on May 26, 2010. Late-harvested, primary growth grass silage was cut on June 15, 2010. Both grass silages contained less than 10% clover. Re-growth alfalfa silage was cut on June 24, 2009. Grass and alfalfa were prewilted before ensiling.

Concentrates were rapeseed meal and dried distillers grains (DDG). Dried distillers grains consisted of 80% wheat, 10% triticale, and 10% rye on a DM basis and were delivered by Lantmännen Agroetanol AB (Norrköping, Sweden). Rapeseed meal was delivered by DLG (Viborg, Denmark).

Forages were dried for 48 h at 60°C in a forced-air drying cabinet. All dried forages and concentrates were ground through a 1.5-mm screen using a cutter mill (Pulverisette 15; Fritsch GmbH, Idar-Oberstein, Germany). The nutrient composition of the feedstuffs is given in Table 1.

### Degradation of Fiber

Degradation in the present study is the term used for disappearance of particles from Dacron bags. Dried and ground feed samples (2 g each) were incubated in Dacron bags. Bags were incubated for 0, 24, 48, 96, and 288 h in the rumen of 3 nonlactating cows. Zero-hour bags were only presoaked and machine washed. The NDF degradation profile and iNDF determination for each feedstuff were determined using Dacron bags with both 12- $\mu\text{m}$  (Saatifil PES 12/6,  $10.5 \times 8 \text{ cm}^2$  in size,  $0.06 \text{ mm}^2/\text{mm}^2$  open surface area; SaatiTech S.p.A., Como, Italy) and 38- $\mu\text{m}$  pore size (Saatifil PES 38/31,  $9 \times 7.5 \text{ cm}^2$  in size,  $0.31 \text{ mm}^2/\text{mm}^2$  open surface area;

SaatiTech S.p.A.). All in situ incubations followed the protocol of Åkerlind et al. (2011).

A maximum of 6 bags were mounted on 1 rubber stopper using a cable tie. Bags incubated for NDF degradation profile were mounted on rubber stoppers equipped with hooks and fixed on a plastic tube. At each end, plastic tubes were equipped with a weight of 200 g and a 40-cm string fixed to a plastic ring placed outside the rumen cannula to maintain bags in the rumen mat and to allow movement with rumen contractions. Bags were presoaked for 20 min in 39°C warm tap water without agitation before rumen incubation. For 288-h rumen incubation (iNDF determination), a maximum of 9 rubber stoppers were gathered in a household washing net with 2 weights, each of 912 g. A maximum of 16 rubber stoppers were incubated in the ventral rumen of each cow. The weights in the washing net were used to maintain rubber stoppers in the ventral rumen during the whole incubation period.

All bags were incubated at the same time of the day (0800 h) before the morning feeding and retrieved according to their respective incubation times. After rumen incubation, bags on the rubber stopper were rinsed with cold tap water and machine washed (AEG, Fredericia, Denmark) twice for 5 min with 22 L of water at 25°C. Subsequently, bags were frozen at  $-20^\circ\text{C}$  until analysis.

Three nonlactating Danish Holstein cows were used for rumen incubations. Cows were fed at maintenance level with a diet consisting of 2 kg of artificially dried grass-clover hay, 4 kg of barley straw, and 2.8 kg of pelleted concentrate mixture as fed. The daily feed allowance was distributed between 2 meals of equal size. The concentrate consisted of 40 kg of barley grain, 40 kg of oat grain, 10 kg of soybean meal, 3 kg of rapeseed meal, 3 kg of sugar beet molasses, and 4 kg of a commercial mineral mixture (6 g of Ca/100 g, 10 g of P/100 g, 12 g of Mg/100 g, and 5 g of Na/100 g; Type 3; Vitfoss A/S, Gråsten, Denmark) per 100 kg of fresh mixture. The forage-to-concentrate ratio in the ration was 670:330 on a DM basis and the CP concentration was 139 g/kg of DM.

**Table 1.** Nutrient composition of the feedstuffs

Nutrient, g/kg of DM	Grass silage		Corn silage	Alfalfa silage	DDG <sup>1</sup>	Rapeseed meal
	Early cut	Late cut				
Ash	94.0	81.5	35.9	116	47.7	76.0
CP	177	128	82.2	216	338	383
NDF	380	496	479	324	223	251
ADF	251	330	298	305	148	210
ADL	16.3	28.0	30.6	58.7	46.7	93.3
ADL:NDF ratio	0.043	0.056	0.064	0.181	0.209	0.372

<sup>1</sup>Dried distillers grains.

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