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In vitro and *in situ* evaluation of secondary starch particle losses from nylon bags during the incubation of different cereal grains☆

N. Seifried, H. Steingaß, M. Rodehutscord*

Institut für Nutztierwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

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ABSTRACT

Three experiments were conducted to study the loss of secondary starch particles from bags used for the *in situ* incubation of different cereal grains. Two *in vitro* and one *in situ* study were combined. Ruminally cannulated Jersey cows were used for rumen fluid collection and *in situ* degradation studies in randomly assigned repeated measures designs, each including three replicates per treatment. An *in vitro* time course study was conducted to determine whether secondary starch particle losses occur during ruminal incubation. Ground wheat was weighed and placed in bags with a pore size of 50 μm, then washed, dried, and incubated for 0.5, 1, 2, 3, 5, 8, 16, and 32 h in a modified RUSITEC-system. Bag residues and samples of freeze-dried fermenter fluids were enzymatically analyzed for starch content. Using the same technique used for the first *in vitro* experiment, but with an incubation time of only 8 h, ground wheat, barley, and corn grains were incubated in bags with pore sizes of 50, 30 (with the exception of corn), 20, and 6 μm. In the *in situ* experiment, ground wheat, barley, corn, and oats were rumen-incubated in bags with pore sizes of 50, 20, and 6 μm for different time periods. Then, the grains and bag residues were enzymatically analyzed to determine their starch content, and the degradation characteristics of the materials were calculated. The *in vitro* trials showed that incubating wheat and barley in bags with pore sizes of 50 and 30 μm leads to a substantial degree of secondary starch particle loss during incubation. These losses were not detectable when the samples were placed in bags with pore sizes of 20 and 6 μm. No secondary starch particle losses occurred in corn, regardless of pore size; thus, corn can be studied *in situ* even when using bags with 50 μm pore size. Due to the high washout losses the *in situ* method is not suitable for the measurement of starch degradation in oats using the pore sizes tested in the present study. Because of the methodological problems associated with pore sizes <50 μm, no recommendations can be provided for the evaluation of wheat and barley. Thus, caution must be taken when the *in situ* technique is used for ruminal grain starch degradation measurements, as there are substrate-related errors possible that must be taken into account.

Abbreviations: ADF, acid detergent fiber; DM, dry matter; ED, effective degradation; NDF, neutral detergent fiber; RUSITEC, rumen simulation technique; P50, pore size of 50 μm; P30, pore size of 30 μm; P20, pore size of 20 μm; P6, pore size of 6 μm.

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* Corresponding author.
E-mail address: inst450@uni-hohenheim.de (M. Rodehutscord).

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1. Introduction

The *in situ* technique was originally applied to study the degradation of forage in the rumen (Van Keuren and Heinemann, 1962; Hopson et al., 1963; Ørskov et al., 1980). In recent years, it has increasingly been used for the evaluation of concentrates. Many methodological details affect the outcomes of *in situ* studies, and published data (Nocek and Tamminga, 1991; Tothi et al., 2003; Offner and Sauvant, 2004) suggest that the rate and extent of starch degradation of rapidly degradable grains may have been overestimated. During the degradation process, starch granules are released from the surrounding protein and structural carbohydrate matrix (Huhtanen and Sveinbjörnsson, 2006). Most of the starch granules in cereal grains are smaller than 50 μm (*e.g.*, 2–36 μm for wheat and barley, 2–15 μm for oats, and 5–20 μm for corn) (Jane et al., 1994). Thus, once granules are released they can easily pass through the material of bags with 50 μm pores (P50), which are those most commonly used in *in situ* studies. When starch granules pass through the pores, they do not degrade in the bag, leading to the so-called secondary starch particle loss (Huhtanen and Sveinbjörnsson, 2006). In contrast to the washout-losses, represented in the *a*-fraction according to the exponential equation proposed by Ørskov and McDonald (1979), secondary starch particle losses may result in an overestimation of the degradation rate (*c*). If these lost starch particles are not rapidly degraded outside the bag, they may lead to the overestimation of the effective degradation (ED) of dry matter (DM) and starch, and the underestimation of the amount of bypass-starch leaving the rumen. It has often been suggested that secondary starch particle losses during ruminal *in situ* incubations may be responsible for the higher ruminal starch degradation values of soft cereal grains using the *in situ* technique compared to those measured in *in vivo* experiments (Huhtanen and Sveinbjörnsson, 2006; Nocek and Tamminga, 1991; Offner and Sauvant, 2004; Tothi et al., 2003). However, experimental data supporting this assumption have not yet been reported.

Additionally, the *in situ* technique was originally applied for studying the degradation of forages in the rumen and it was reported (Van Hellen and Ellis, 1977) that bag pore size has a major effect on the results of *in situ* degradation measurements. Therefore, in most previous studies, forage was used in order to investigate the effect of bag pore size on degradation characteristics of fiber including cellulose (Marinucci et al., 1992), neutral detergent fiber (NDF) (Huhtanen et al., 1998), and acid detergent fiber (ADF) (Lindberg and Knutsson, 1981); or, in the case of grains, the influence of pore size on N degradation (Varvikko and Lindberg, 1985) was tested. To our knowledge, only one published study evaluated the influence of bag pore size on the *in situ* ruminal starch degradation of cereal grains (Tothi et al., 2003).

Therefore, the aims of the present study were (1) to determine whether secondary starch particle losses occur during the incubation of cereal grains from bags typically used for *in situ* degradation studies, (2) to study the effects of bag pore size on secondary starch particle losses from different cereal grain types, and (3) to evaluate the effect of different pore sizes on calculated DM and starch degradation characteristics in an *in situ* ruminal degradation study.

2. Materials and methods

Three experiments were conducted in order to study the influence of bag pore size on secondary starch particle loss during the incubation of wheat, barley, corn, and oat grains. Two *in vitro* approaches and one *in situ* approach were used. The nutrient characteristics of the grains are given in Table 1.

2.1. Animals and diet

Lactating Jersey cows fitted with a rumen cannula were used for *in vitro* rumen fluid collections and ruminal *in situ* incubations. For all experiments, the cows were individually fed a total mixed ration composed of (per kg DM) 220 g corn silage, 140 g grass silage, 150 g hay, 30 g barley straw, 10 g rapeseed meal, 20 g of a mineral mixture, and 430 g of a mixed concentrate. The concentrate was composed of (per kg DM) 200 g barley, 250 g corn, 250 g field beans, 100 g peas, and 250 g rapeseed cake. The starch content of the ration was calculated from the individual feeds to be 223 g/kg DM. The feed

Table 1
Nutrient composition of the grains used in the different experiments.

	Grain type				
	Wheat	Wheat	Barley	Corn	Oats
Used in experiment	1; 2a; 3	2b	2a; 2b; 3	2a; 3	3
Nutrients (g/kg DM)					
Organic matter	983	984	976	987	971
Crude protein	140	140	118	89.8	132
Starch	631	623	547	722	594
aNDFom[a]	100	111	209	78.0	319
ADFom[b]	–	28.6	56.2	26.8	142
Ether extract	26.9	19.9	28.4	55.7	62.2

[a] aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive residual ash.
[b] ADFom, acid detergent fiber exclusive of residual ash.