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Scanning Electron Microscopic Examination of Different Varieties of Oat Grains in Comparison with the Analyzed Degree of Starch Breakdown and Glycaemic Responses in Horses

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ABSTRACT

Starch granules of different varieties of oat grains were observed by a scanning electron microscope (SEM) in order to provide information about morphologic characteristics e.g. the surface and interconnections of the granules to evaluate a possible enzyme attack and degradation, respectively. Additional information about the analyzed degree of starch breakdown and the glycaemic responses in horses after feeding the oat grains were used as parameters for comparison. The SEM has been used as an effective tool in determination of shapes and surface structures of various starch granules, in particular the description of differences within one variety.
Keywords: starch granules, scanning electron microscope, oat grains, glycaemic response, horse

I. INTRODUCTION

Starch from cereal grains is the most abundant energy source for the majority of domestic animals. A high small intestinal digestibility of cereal starch is the precondition for maximal starch utilization in monogastric animals [1]. Particularly in horses, the amylase activity and the capacity for starch digestion in the small intestine is critically low [2].
Starch granules have a complex and highly ordered semi-crystalline structure. Besides other important morphological characteristics the last-mentioned appears to be an important barrier to starch digestion. More details about starch microstructure and digestion can be found elsewhere [3]. It can be distinguished between exo-corrosive alterations where signs of starch destruction can be found at the granules' surface only and endo-corrosive alterations where digestive enzymes have access through small pin holes in the granules [4]. These types of corrosion are largely independent from the amylase source [5], but the starch granules' particle size and the structure and integrity of the surface area play an important role for the efficacy of enzymatic hydrolysis [6].

Evidence exists that starch characteristics according to scanning electron microscopy (SEM) may have a predictive value regarding the small intestinal digestibility of different starch sources in horses [7]. Previous studies investigated whether small intestinal starch digestibility corresponds to the microscopic starch structures in feed and chyme of horses, but the proven feedstuffs included either native or processed cereal grains (e. g. whole, broken, grounded, expanded) or corn silage [2]. To our knowledge a comparison of SEM-pictures from starch granules deriving from different oat grain varieties has not yet been published and certainly not a comparison of those pictures with laboratory scale and *in vivo* results, however, related to starch digestibility in the small intestine of horses.

The aim of the study was to characterize and compare starch granules from quite distinct oat grain varieties *via* SEM and to compare these with results from the degree of starch breakdown (DSB) measured in the laboratory and the *in vivo* determined postprandial (ppr.) glycaemic response to starchy meals in adult healthy horses.

II. METHODS AND MATERIAL

Starch granules and their embedding in surrounding structures deriving from four varieties of oat grains were visualized by SEM and the pictures further interpreted (German Patent and Trademark Office; Brief disclosure for the Patent Application 10 2013 016 050.2). Prior to SEM, grains were crushed, spread out on a microscope slide, air-dried and sputtercoated with gold. The studied oat varieties can be characterized as follows: 'Energie', high fat content; 'Melody', high content of ß-glucans; 'Scorpion', high starch-content; naked oats 'Sandokan', particularly low fiber content. DSB was determined according to [8]. SEM and DSB results were compared with the mean glycaemic response of six adult healthy horses. For this, the horses received crushed oat grains from the above mentioned varieties according to a cross over design where further 1.0, 1.5, and 2.0 g starch/kg body weight were fed from each variety and the overall mean from these ingested quantities was taken [9]. The respective area under the plasma glucose curve was than calculated starting from the baseline level at time point 0 min up to 300 min ppr. [AUC_{gluc}].

III. RESULTS AND DISCUSSION

The starch grains from different oat varieties were individual regarding their size and the occurrence of bondings, coverings, matrix structures, particularly well-defined structures and interconnections (Tab. 1, Fig. 1 – 4). Giant granules (GG; 22.2 – 29.1 µm diameter) were observed in the varieties 'Scorpion' and 'Melody' (Fig. 2 and 3), which might suggest delayed digestion. For example a large and smooth surface explains the resistance of potato granules to enzymatic breakdown [10]. Smaller granule sizes on the other hand indicate higher susceptibility for enzymatic breakdown regardless of the botanical origin [11]. A high percentage of such small sized granules were found in 'Energy' and 'Sandokan' (Fig. 1a and 1b, Fig. 4a and 4b).

In previous studies 95% ethanol were used to suspend individual starch granules [12] and wash out potential coverings or matrix structures. In the present study not any solvent was used, the preparation was limited to rough crushing and drying of the grains only, because an excess of water and high temperature during processing alter the original structure including coverings and any kind of embedding and may cause starch gelatinization [13].

Table 1: DSB, AUC_{gluc} and morphologic characteristics of starch granules from different oat grain varieties and their embedding in surrounding structures

	Variety of oat grains			
	Energie	Melody	Scorpion	Sandokan
DSB [%]	6.8	19.0	10.0	8.7
AUC_{gluc} [mmol/ L min^{-1}]	1,635^{c}	1,648^{c}	1,926^{a}	1,800^{b}
morphologic characteristics of starch granules				
BO	x	-	-	x
GG	-	x	x	-
DS	-	x	x	-
CS	x	x	x	x
IN	xxx	x	x	xxx

BO, bondings; CS, coverings and/or matrix structures; DS, well defined structures; GG, giant granules; IN, interconnections; -, not-existent; x, weak; xxx, strong; ^{a,b,c} Means with unlike superscripts are significantly different ($P < 0.05$).

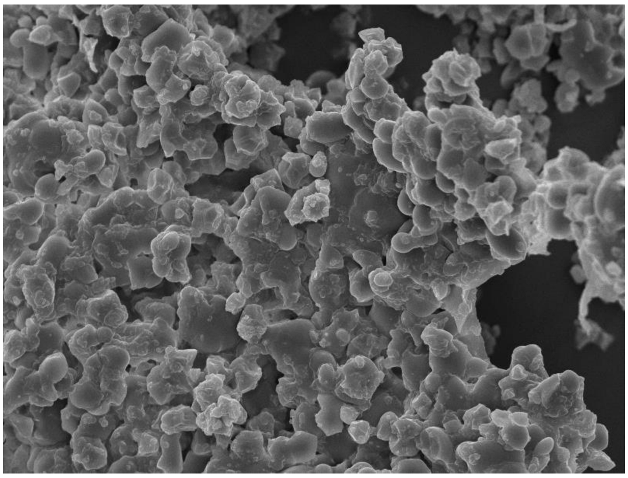

Figure 1a: SEM-picture from oat grains 'Energıe' (x 1000)

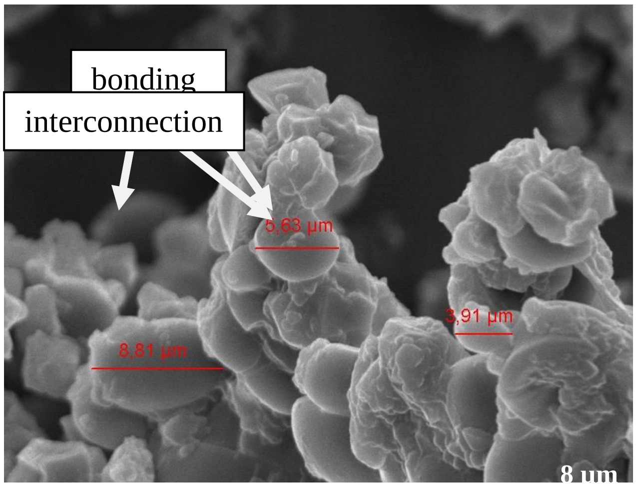

Figure 1b: SEM-picture from oat grains 'Energie' (x 3000)

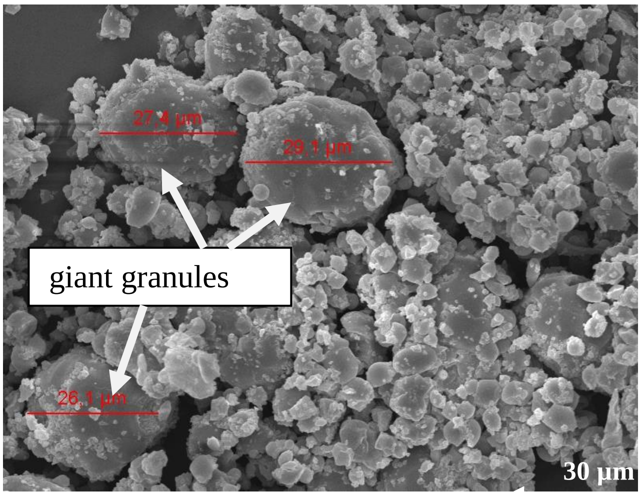

Figure 2: SEM-picture from oat grains 'Melody" (x 1000)

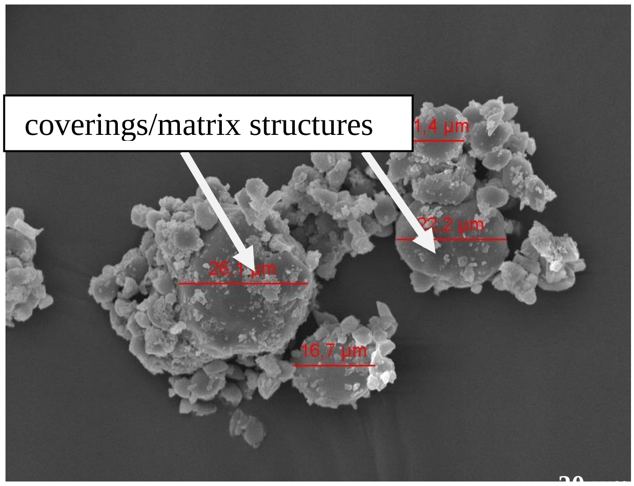

Figure 3: SEM-picture from oat grains 'Scorpion' (x 1000)

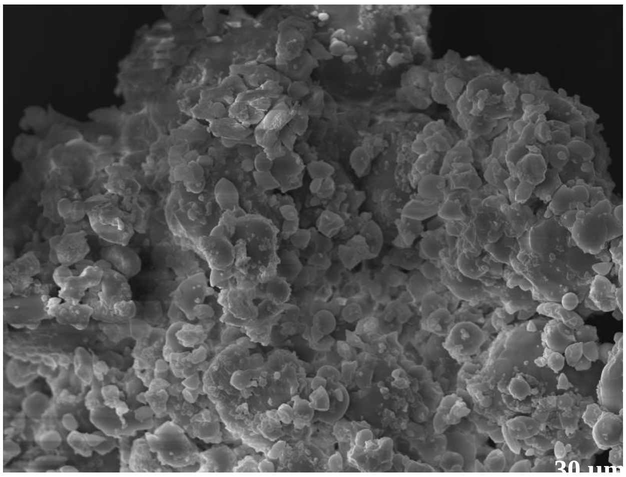

Figure 4a: SEM-picture from oat grains 'Sandokan' (x 1000)

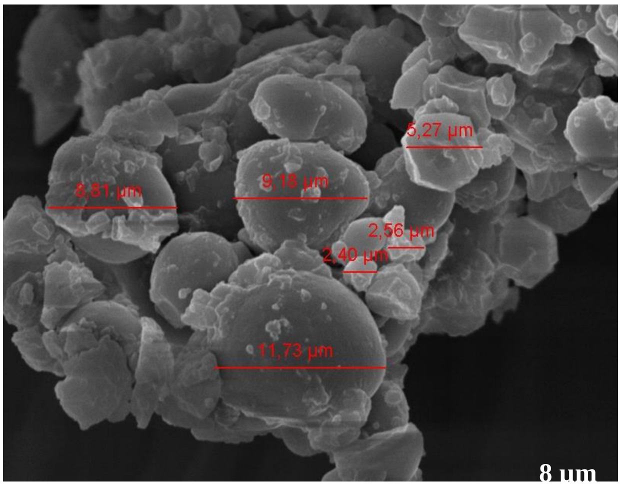

Figure 4b: SEM-picture from oat grains 'Sandokan' (x 3000)

For example, the surface of 'Melody' and 'Scorpion' did not appear smooth, but coverings and/or matrix structures were clearly present (Table 1).

SEM-pictures and starch characteristics (Fig. 1 - 4, Tab. 1) showed highest similarity between ‘Energie’ and ‘Sandokan’ (occurrence of bonding) on the one hand and ‘Scorpion’ and ‘Melody’ (giant granules; well defined granule structures) on the other. The particularly high glycaemic response following feeding of ‘Scorpion’ and ‘Sandokan’ was not reflected by the interpretation of the morphological characteristics of starch granules and further did not correspond to DSB.

IV.CONCLUSION

Within the different varieties of oat grains no apparent relationship has been determined between SEM-based characterization of starch granules, the DSB and the *in vivo* measured glycaemic response. The nevertheless demonstrated differences between the individual varieties of oat grains regarding their morphometric characteristics should motivate further research.

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