Microstructure of grains as an indicator of nutritive value

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Summary

The gross chemical contents of grains explain most, but not all, of the variation in their nutritive value for livestock. It is believed that the microstructural features of grains may be responsible for this unexplained part of the variation. A light microscopy technique combined with differential staining has been used in the grain industry for some time to study the structures of starch granules, protein matrix and protein bodies, and cell wall architecture. In this paper, the application of this technique in nutritional studies will be introduced and some preliminary data presented.

Keywords: grain, microscopy, image analysis, microstructure, nutritive value

Introduction

The gross chemical composition of feed ingredients is the most important determinant of their nutritive value for livestock, and most chemical analyses are cheaper and easier to perform than feeding trials. However, the value of a given diet depends not only on the characteristics of the feed, but also the animal to which it is fed. Despite the advances that are continuously made in analytical techniques for chemical characterisation of ingredients, a large part of the variation in the nutritive value of feed ingredients still cannot be explained by quantitative analyses of their chemical constituents (Black 2001). It is believed that the physical configuration of grain cell walls is an important factor that influences the accessibility of digestive or microbial enzymes to release utilizable nutrients. In addition, the protein structure around the starch granules of sorghum grain also affects the accessibility of amylolytic enzymes. The only way to examine this hypothesis is to use microscopy to show the structural interactions between the starch granules and the surrounding cell walls and protein matrix. Microscopy, in particular, electron microscopy, has been used extensively in many fields of science to study the ultra structures of materials. However, recent advances in light microscopy combine staining techniques and powerful image analysis software that allow identification of grain structural components that are likely to influence the digestion and absorption of nutrients in animals.

Microscopy and staining techniques to assess factors affecting the nutritive value of grains

Microstructural features that may affect nutritive value

The nutritive value of grains varies widely depending on the species of grain and class of livestock. The causes are many. In pigs and poultry, for instance, the fibre fraction is a major determinant of the available energy value (King and Taverner 1975; Choct and Annison 1990). The fibre in grains is present in the cell walls and it consists predominantly of non–starch polysaccharides (NSP). Quantitative analysis of NSP can give a reasonable estimate of the nutritive value of grain, but it is tedious and time–consuming and still leaves a significant part of the variation unexplained. It is assumed that the cell wall architecture, not just its content, is a determining factor for part of that variation.

The strand of protein surrounding the starch granules of most cereal grains is referred to as protein matrix. In sorghum this matrix forms a tight structure encasing the starch granules, making the starch less accessible to amylolytic enzymes. In addition, protein bodies are also tightly associated with the starch granules, and together with the matrix form a web that inhibits amylolytic degradation of sorghum starch. The nature of these protein structures varies with grain species and cultivar and will, therefore, affect the availability of nutrients to livestock.

Microscopy

The size, shape and content of plant cells (Waldron *et al.* 1997), lamella cementing the cell boundaries, the thickness and the content of the cell walls, and the type of starch granules in the cells are potential sources of variation in the solubility of cell wall materials, particle size distribution when ground, viscosity as dough, and possibly digestibility (Autio 2001). Microscopy offers the possibility of studying these microstructural features. The technique used in animal nutrition studies involves colour staining to differentiate chemical entities within the feed ingredient and then conversion of the image of sections of feedstuffs under the microscope into electronic form. The components identifiable through differential staining include fibre (some individual components such as cellulose, pectin, lignin), protein, starch (amylose and amylopectin may be differentially stained), and fat. Using the techniques and stains described by Dürrenberger *et al.* (2001), samples can be prepared relatively easily for microscopic studies.

In the current study, wheat grains with low and high apparent metabolizable energy (AME) values for chickens, and sorghum grains, were cut in half and embedded in agar, fixed in glutaraldehyde, dehydrated through a series of ethanol treatments and embedded in Historesin (Jung, Germany). For the fluorescence microscope examinations, the sections were stained with 0.1% acid fuchsin and 0.01% Calcofluor White M2R New. The sections were rinsed with distilled water, dried and examined microscopically (Olympus BX50 Microscope) using U–filter with a maximum transmission at 400–410 nm. For bright–field microscopy, starch granules were stained with iodine and the samples were examined and photographed. For an estimation of the cell wall percentage in the grain, the samples were counterstained with 0.1% Calcofluor. The area of bright fluorescence representing thick cell walls was then measured by computerised image analysis using an Olympus Vanox–T microscope with a BH2–DMV filter set at a maximum transmission of 405–455 nm.

Microstructural differences in cell walls of wheats with differing AME values

In a preliminary study (Choct 1995) it was demonstrated that the cell wall of a low AME wheat (12.02 MJ/kg dry matter) was clearly distinguishable from a normal wheat (14.52 MJ AME/kg dry matter). The cross section of the normal wheat grains appeared evenly rounded and had a glassy appearance, whereas the width of the low AME wheat grains was severely reduced and the grains appeared chalky. In addition, the low AME wheat had thicker cell walls than normal wheat. Figure 1 shows the difference in cell wall thickness, which is indicated by the area of blue fluorescence. The area of blue fluorescence, as a percentage of the total area of the grain, was 23% for the normal wheat and 34% for the low AME wheat. The endosperm cavity of the low AME wheat was exceptional, being long and narrow compared with that of the normal grain. This example illustrates how microstructural differences can help to explain how reduced digestibility of low AME grain is related to an increase in cell wall material. The micrograph also shows that the amount as well as the distribution of NSP may be important in determining the digestibility of the starch.

Variation in the number of starch granules in wheat

The starchy endosperm of wheat at maturity contains primarily two types of starch granules: large disc–shaped A granules, and small spherical B granules. Figure 2

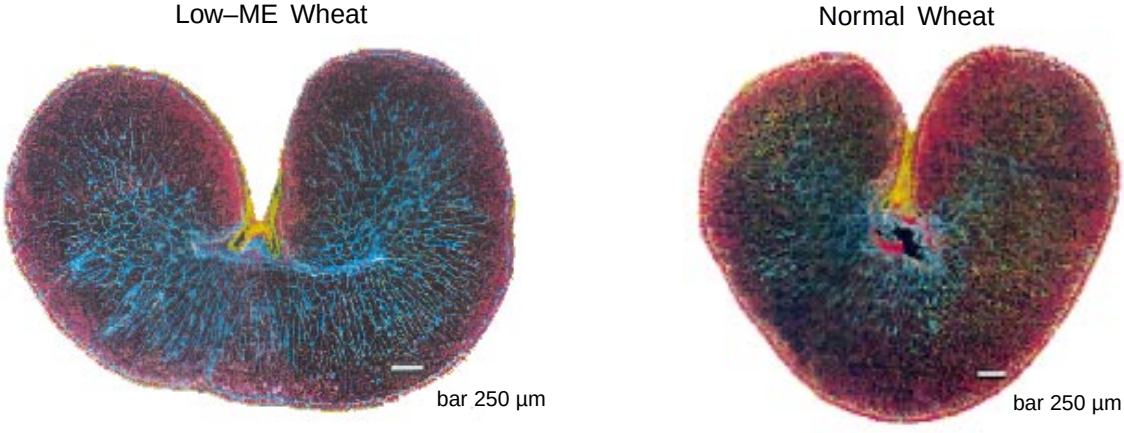


Figure 1 Cross–sections of a low AME and a normal wheat grain (produced by Dr Karin Autio, VTT, Finland; Choct 1995).

shows that there were fewer B granules in the low AME than in the normal wheat. In addition, the A granules were larger in the low AME wheat. There is evidence that the rate of starch digestibility varies widely for different wheats, digestion of low AME wheat being markedly slower (Wiseman *et al.* 2000). The finding of Zarrinkalam *et al.* (2000) that the small granules may be quicker and easier to digest than the large A granules is therefore consistent with the microscopic analysis and the *in vivo* evidence.

Effect of germination on the *in vitro* fermentability and digestibility of sorghum starch

Australia produced 2.161 million tonnes of sorghum in 2000, and in that and earlier years approximately 74% of the crop has been fed to livestock (FAO 2001). The feeding value of sorghum for cattle is low and extensive pre–treatment of the grain is necessary before inclusion in their feed (Rowe *et al.* 1999). Unprocessed sorghum is poorly fermented by rumen microbes and the digestibility of its starch is also very low (Bird *et al.* 1999). Rooney and Pfugfelder (1986) suggested that the protein matrix surrounding the starch granules could impair their accessibility for amylolytic enzymes. In practice, the feed and livestock industries use steam flaking and reconstitution treatments to overcome the problem, but specialised equipment is required and the operational costs are high. An alternative treatment is to germinate sorghum to take advantage of the natural enzymes present in the grain. Mature cereal grains contain various amounts of enzymes (Conchie *et al.* 1968; Lee and Ronalds 1972) that can be activated when moisture and temperature become suitable for germination. In a recent study, the effect of germination on the cell structure of sorghum was examined using Brightfield microscopy. Two distinct changes can be seen from the images: (a) the effect of germination on the protein bodies (red colour) cementing the starch granules (Figure 3), and (b) the erosion of the starch granules during germination (Figure 4).

It appears that allowing sorghum to germinate resulted in the erosion of the protein matrix surrounding the starch granules, which coincided with a significant increase in starch digestibility and fermentation *in vitro*. Furthermore, the starch granules in Figure 4 appeared transparent after germination, indicating the slow degradation of the cell walls and the protein matrix by the activation of the endogenous enzymes naturally present in the grain. Such an effect can only be

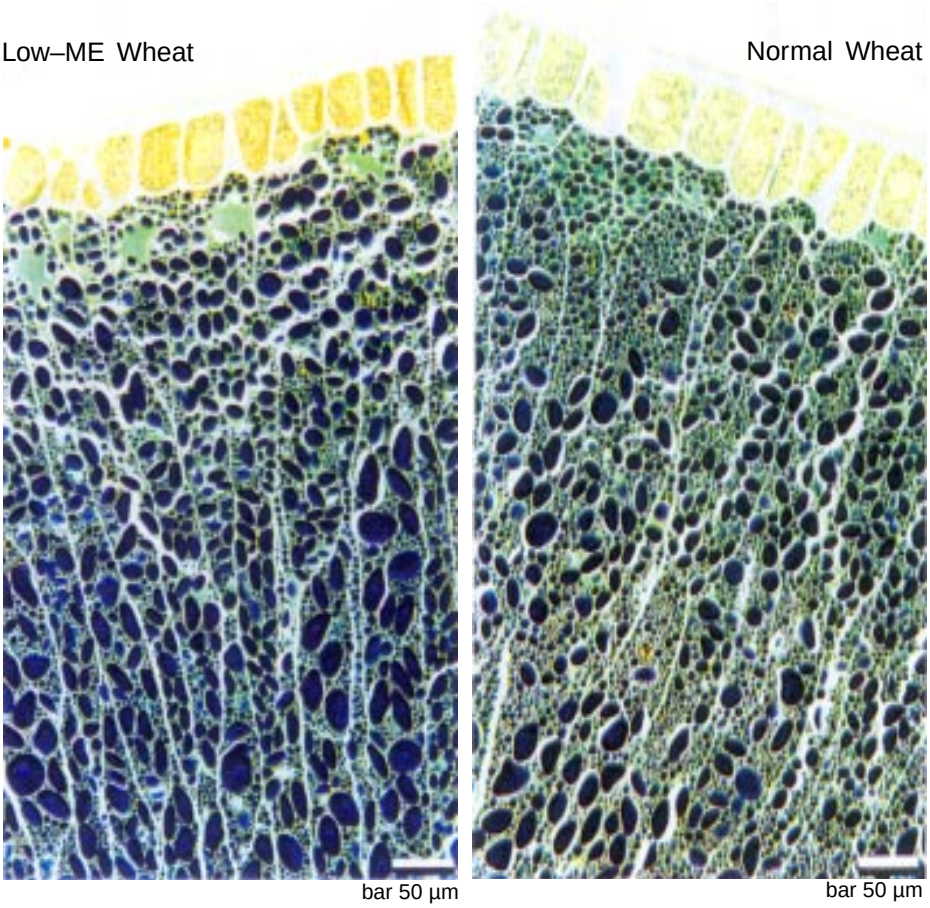


Figure 2 Images of A and B starch granules in the starch endosperm of low AME and normal wheat grains (produced by Dr Karin Autio, VTT, Finland; Choct 1995).

demonstrated using microscopy and the information obtained by this means supports the hypothesis that the protein matrix in sorghum endosperm limits the digestion of the starch.

Quantitative analysis of the microstructural features of grains in relation to nutritive value for livestock

The qualitative assessment by microscopy gives a feeling of 'seeing is believing', but many fine details and quantitative assessments are not possible using the naked eye. To make full use of the technique, it is essential to develop image analysis to segment the microstructural features that may influence the nutritive value of grains for livestock. Image analysis software has recently been developed and offers a number of possibilities:

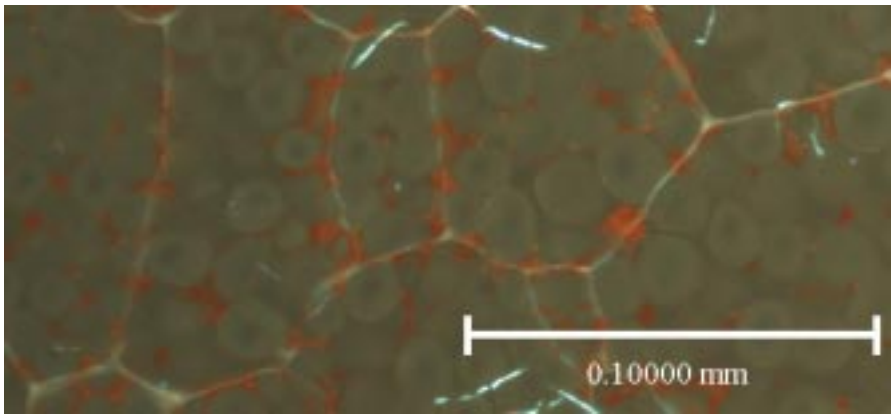
- cell wall thickness and content can be quantified from fluorescent images (Figure 5)
- starch granule distribution may be estimated using a technique termed 'granulometry' (data not shown)
- the intensity of staining may be used to determine changes in grain components as a result of processing.

Further development of image analysis software programs will enable us to quantify the various microstructural features, such as cell wall width, cell wall content, cell shape and size, and the number and size of the starch granules. This technology is rapidly developing.

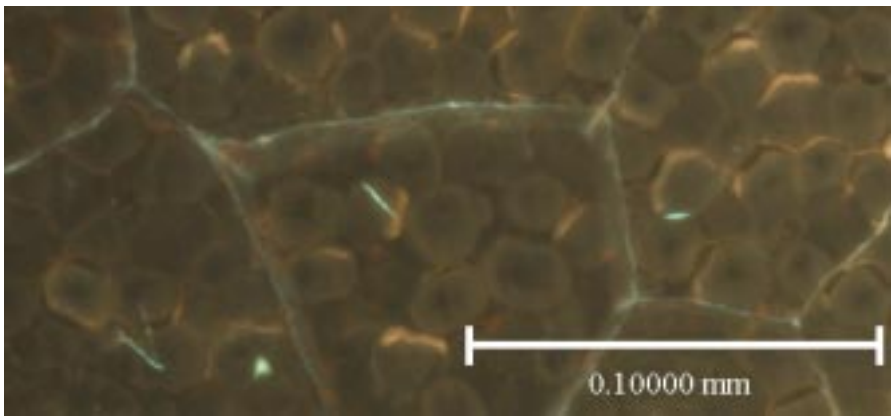
Conclusion

The results that have been obtained suggest that quantitative microscopic analysis of grain structure is a promising new tool for increasing understanding of the characteristics of grains that influence nutritive value. New information on the links between microstructure and nutritive value can be expected to give direction to

Figure 3 The effect of germination on the protein bodies cementing the starch granules in sorghum.

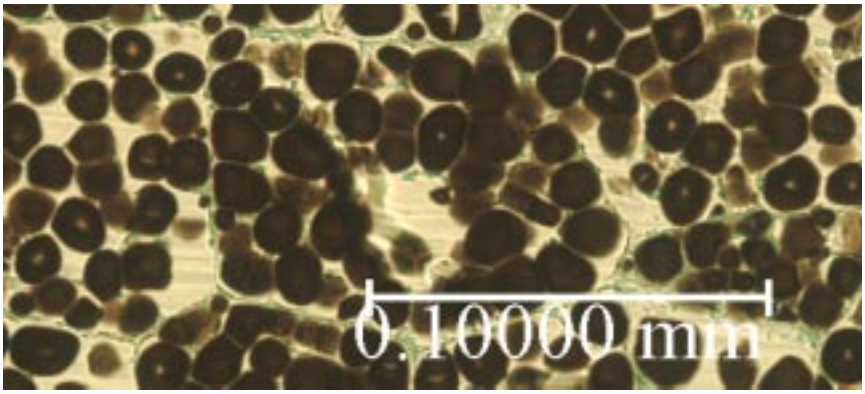


(a) Unprocessed sorghum, micrographed at 20 x magnification. Total starch content: 66%; *in vitro* starch fermentation: 15%; *in vitro* starch digestibility: 52%. Red colour represents protein bodies, which are clearly visible in this micrograph.

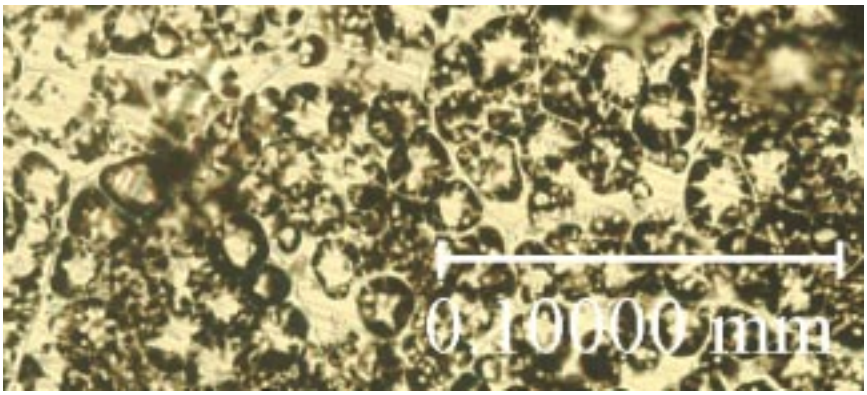


(b) Germinated 5 days at 20 x magnification. Total starch content: 66%; *in vitro* starch fermentation: 24%; *in vitro* starch digestibility: 54%. The red colour representing protein bodies has disappeared after germination.

Figure 4 The effect of germination on starch granules in sorghum.

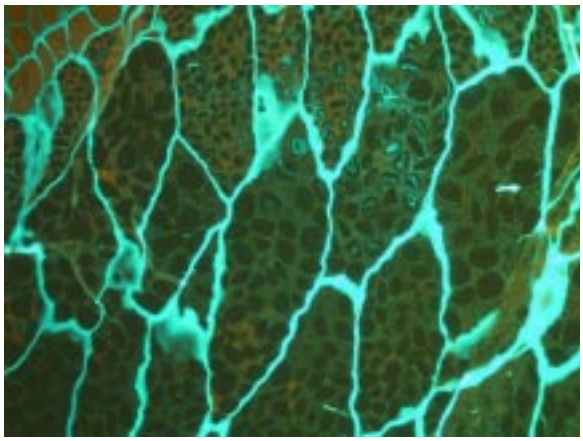


(a) Unprocessed sorghum at 20x magnification. Total starch content 66%; *in vitro* starch fermentation 15%; *in vitro* starch digestibility 52%. All starch granules are intact before germination.

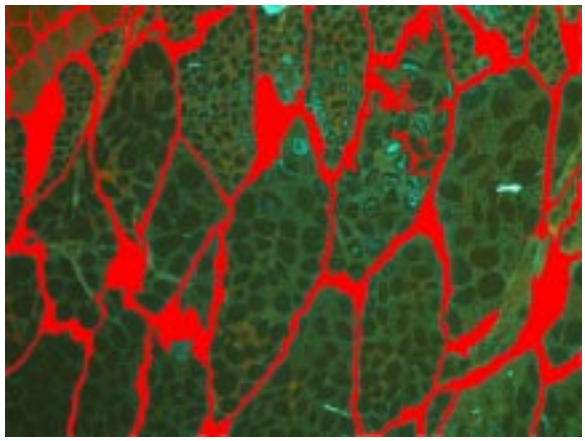


(b) Germinated 5 days at 20x magnification. Total starch content 66%; *in vitro* starch fermentation 24%; *in vitro* starch digestibility 54%. Starch granules have become less distinct, indicating that the degradation of starch has been initiated by the endogenous amylase in the grain during germination.

Figure 5 Cell walls of a barley sample before and after segmentation.



(a) Cross section of barley at 20x magnification. The cell wall is in blue.



(b) The cell wall is segmented by image analysis software specifically developed for the purpose.

plant breeders and feed processors for the production of better livestock feeds. Microstructural features cannot be characterised using conventional chemical analyses and feeding trials. However, qualitative microscopy is difficult to interpret without the expertise of a highly trained specialist because some of the microscopic differences are often very subtle. With increased availability of selective stains producing high colour differentiation coupled with the development of image segmentation software programs, the possibility of characterising physical factors in grain that affect the nutritive value for livestock is close to reality.

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