

In vitro assessment of starch digestion in pigs

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Summary

Application of *in vitro* feed evaluation assays that simulate digestion in the pig have limited potential for routine application at the feedstuff producer's site of ingredient receipt. If the assays are to have a role, it will be the strategic assessment of grain characteristics that may influence digestion in the small intestine of pigs and subsequent nutrient yield. Starch composition and factors affecting access to starch within different grains may have a significant influence on energy yield, but it is difficult to detect these differences using traditional two-step enzymic procedures based on pepsin and pancreatin. An *in vitro* assay that focuses on starch digestion alone is likely to be more sensitive to how differences in starch composition and starch access may influence energy digestion. This paper discusses the application of the 'glucose release index' as a means of assessing factors that may influence starch digestion *in vitro*, and how factors such as grain hardness and the protein matrix surrounding starch granules in cereals may be influencing starch digestion in the small intestine in pigs. Results suggest that the protein matrix in barley presents less of a physical barrier to starch digestion than in wheat and sorghum and that roller milling may be a far more effective way of preparing sorghum for use in non-pelleted pig diets.

Keywords: pigs, starch digestion, *in vitro* assays, glucose release index

Introduction

Application of *in vitro* feed evaluation assays that simulate digestion in the pig have limited potential for routine application at the feedstuff producer's site of ingredient receipt. If the assays are to have a role, it will be the strategic assessment of grain characteristics that may influence digestion and subsequent nutrient yield. The general application of *in vitro* methods for the analysis of feed ingredient quality has been widely promoted by countries such as Denmark where such methods have been in general use for the evaluation of

pig feeds since 1995 (Boisen 2000). In that case the methods have been implemented to fine-tune energy and amino acid supply according to predicted actual requirements defined using pig growth models. The shortcomings of this approach include:

- the *in vitro* models utilised are difficult to apply at the point of grain receipt due to the time taken to complete the analysis;
- given the difficulty in simulating the dynamic processes that occur in the gastrointestinal tract of an animal, it is somewhat unrealistic to expect a simplistic laboratory assay to adequately define the nutritional quality of all ingredients (Moughan 1999);
- in the case of pigs, simulation of whole tract energy digestion assumes that digestible energy is the most reliable measurement of available energy for the animal.

Based on criteria of speed, ease of use, accuracy, and cost it is difficult to envisage any form of routine feed ingredient analysis exceeding the current potential near infra-red spectroscopy (NIRS). Application of NIRS is already widely accepted as the basis for grain classification and payments using measurement of parameters such as crude protein and moisture and potential already exist to measure *in vivo* parameters such as pig digestible energy (DE; van Barneveld *et al.* 1999). In addition, recent research and development undertaken in the Premium Grains for Livestock Program involving grains, livestock and feed milling sectors suggests that NIRS calibrations are capable of simultaneously measuring a suite of chemical parameters. These include proximates, anti-nutritional factors, amino acids, selected soluble and insoluble non-starch polysaccharide components, starch characteristics and fatty acids plus a range of *in vivo* measurements such as ileal and faecal digestible energy for pigs, or apparent metabolizable energy for broiler chickens and laying hens, and may soon be available for routine use

(R.J. van Barneveld, unpublished). NIRS also holds significant potential for real-time analysis of grain composition that may flag a need to adjust diet formulations and consider strategic application of feed additives such as exogenous enzymes. That may not be so for routine application of *in vitro* assays based on wet chemistry unless they can be applied for determinations not suited to NIRS.

In broad terms, a nutritional quality definition system is adequate if it provides a means of discrimination between ingredients, and reflects the level of accuracy that can influence commercial responses in a livestock production system (van Barneveld 2001). There will be improvements in the ability to separate the potential supply of net energy from similar grain types if techniques increase the focus on energy digestion in the small intestine of the pig. An understanding of those factors that have the greatest influence on digestion in the small intestine will increase our capacity to manipulate these grain characteristics and it is here that *in vitro* assays may have a very useful strategic role.

The objective of this paper is to demonstrate the potential for *in vitro* assays to identify factors that may influence energy supply from grains in the small intestine of pigs and thus provide a basis for more detailed assessment using *in vivo* measurements. The 'glucose release index' will be used as a specific example of strategic application of an *in vitro* assay for this purpose.

The basis for strategic application of *in vitro* assays

Moughan (1999) and Boisen (2000) described traditional approaches to simulation of pre-caecal and total tract digestion in pigs. Most of these *in vitro* systems are based on two-step (in the case of pre-caecal digestion) or three-step (in the case of total tract digestion) enzymic procedures (Figure 1). Comparative application of these procedures by Boisen (2000) demonstrates clearly the lack of sensitivity of whole tract digestion versus pre-caecal digestion (Figure 2) when assessing ingredients varying widely in composition. However, the capacity of two-step and three-step enzymic procedures applied by Boisen (2000) to discriminate between similar grain types is not clear. While the Boisen (2000) assays focus on simulating digestion, it is difficult to apply them in a way that allows an assessment of how specific chemical and physical characteristics of the grain may influence digestion.

Black (2001) suggested that differences in the nutritional quality of grains could be largely attributed to combinations of the following factors:

- gross chemical composition;
- cell wall quantity, structure and composition;

- fatty acid type and content;
- starch composition;
- protein matrix;
- grain hardness; and
- lignin bound to phenols and proteins.

If we focus specifically on energy supply from the small intestine of pigs, the starch composition and the access to starch granules within different grains may have a significant influence of energy yield, but it is difficult to detect these differences using traditional two-step enzymic procedures based on pepsin and pancreatin (Figure 1). An *in vitro* assay that focuses on starch digestion alone is likely to be more sensitive to how differences in starch composition may influence energy digestion in the small intestine of pigs.

The 'glucose release index' as an *in vitro* assay for starch digestion

Several *in vitro* starch digestibility assays have been developed and used across a variety of species including humans, ruminants and poultry (Zarrinkalam 2002). The basic principle applied to most *in vitro* starch digestibility assays is similar and aims to partially mimic the digestive conditions present in the small intestine by using similar starch digestion enzymes and pH conditions. Englyst *et al.* (1992) used a more rapid approach to measure the digestibility of starch and other carbohydrates such as fructose by designing a single time point multi-enzyme assay. This assay has been successfully used for rapidly assessing variations in starch digestibility between different foodstuffs (Englyst *et al.* 1996). A 'starch digestion index' was utilised and defined as the percentage of rapidly digested starch (following a 20 minute incubation) to total starch (Englyst *et al.* 1992). Zarrinkalam (2002) refined this methodology specifically for cereal grains. Only two digestive enzymes (heat stable α -amylase and amyloglucosidase; Figure 1) out of the 6 enzymes originally used by Englyst *et al.* (1992) were considered necessary to investigate the variation in starch digestibility between cereal grains, since almost all of the digestible carbohydrate in cereal grains is starch. Data from this modified procedure of determining the ratio of the released glucose in the initial stage of starch digestion to the released glucose at the completion of starch digestion was defined as the glucose release index (GRI). The procedure accounts for the fact that differences in the amount of glucose released from the digestion of starch in grains are maximal during the initial rapid phase of starch digestion. Restricting measurements of the progression of an enzymic reaction to a period where less than 20–50% of total substrate consumption has occurred, provides a value that reflects the conversion rate of a substrate (starch) to its product (glucose).

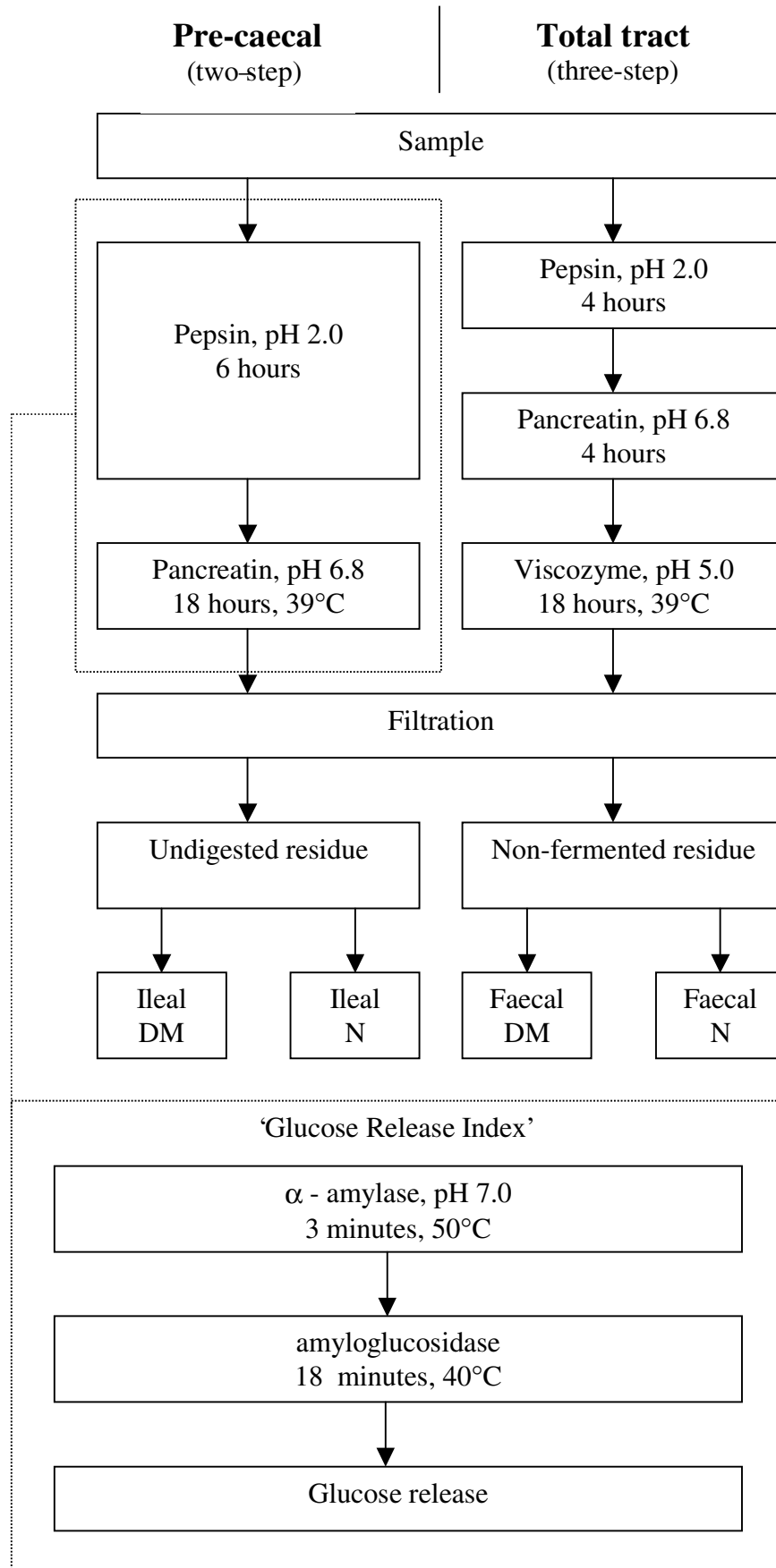


Figure 1 Flow diagram depicting traditional two-step and three-step *in vitro* incubations and the comparative application of the 'glucose-release' assay.

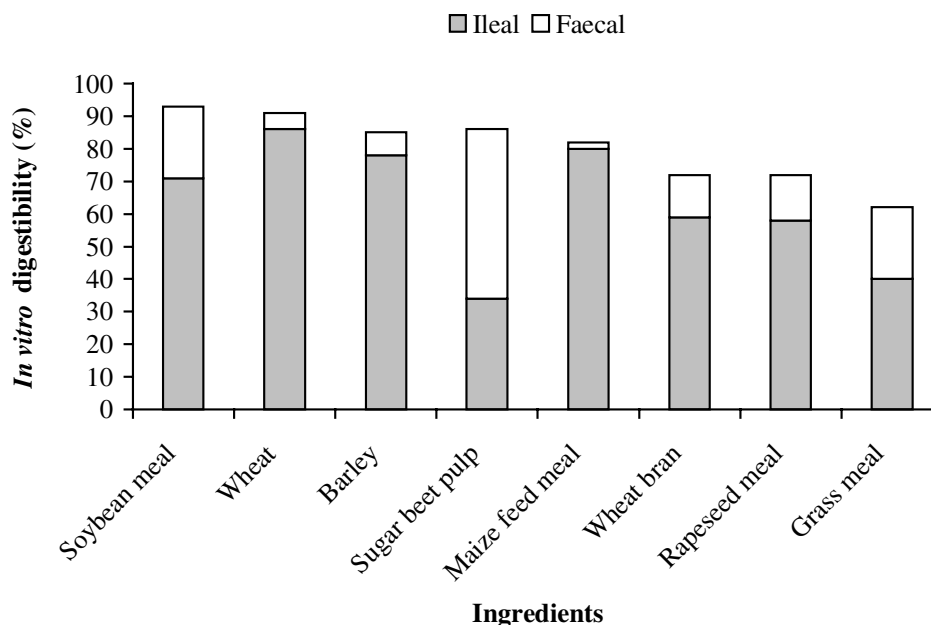


Figure 2 *In vitro* digestibility of dry matter of common feedstuffs after simulation of pre-caecal and whole tract digestion (Boisen 2000).

Grain factors influencing starch digestion that can be assessed using the GRI

Wheat and sorghum will be used as example cereals to discuss grain factors influencing starch digestion in pigs, due to their significantly different cell structure and configuration. Pigs appear to digest similar amounts of energy from cereal grains such as wheat and sorghum in the small intestine, but the lower starch and fat content in wheat may mean that enzymic access is superior with a higher energy yield per unit of those substrates (van Barneveld *et al.* 2001). Examination of the cellular characteristics of wheat and sorghum may identify some of the reasons for differences in the efficiency of starch digestion in cereals, within and between species. Wheat consists of elongated cells containing a mixture of two-thirds large, lenticular starch granules (8–30 μm ; A granules) and one-third near spherical granules of <8 μm in diameter (B granules). In contrast, sorghum is characterised by small, tightly packed, angular cells containing uniform-sized starch granules encapsulated in a protein matrix. It is reasonable to suggest that the capacity to break these sorghum cells and disrupt the associated protein matrix may have a significant influence on starch digestion. The elongated cells of wheat are likely to be easier to rupture through chewing or processing prior to feeding, making the cell contents more accessible to enzyme degradation, whereas the nature of the cells in sorghum means they are more likely to remain intact following chewing or processing. To this end, assessment of the potential influence of the protein matrix surrounding starch cells and grain hardness, respectively, may provide some insight into how the efficiency of starch digestion may be improved in the small intestine of pigs.

Application of the GRI to assess the influence of protein matrix on starch digestion

The influence of protein matrix on the GRI of starch was determined by digesting grain protein with pepsin at pH 2 for 60 minutes at 39°C prior to conducting the GRI assay (Zarrinkalam 2002). The use of pepsin protease, and the pH and incubation conditions were chosen to simulate the digestive conditions present in the stomach of monogastric animals.

Crude protein content of the grain was not related to the influence of the protein matrix on GRI (Figure 3). There were different responses between grain types to pre-treatment with pepsin which has an ability to remove protein matrix (as revealed by scanning microscopy). Pre-treatment of barley with pepsin resulted in a consistent decrease in GRI compared with wheat and sorghum where increases in GRI were observed in all cases. Zarrinkalam (2002) suggested that the protein matrix in barley presents less of a physical barrier to starch digestion than in wheat and sorghum and that the protease activity may actually impede starch digestion in barley. This conclusion is further supported by the result that non pepsin-treated barley grains exhibited the highest average GRI value compared to sorghum and wheat. Also, in sorghum, despite a significant increase in the average GRI value after protease treatment, this value was still significantly lower than in barley and wheat samples treated with pepsin. This may be related to the lower surface area to volume ratio of starch granules in sorghum, which could restrict digestive enzyme accessibility to starch. Another important difference between sorghum and other grain types such as barley and wheat is its prolamine-rich

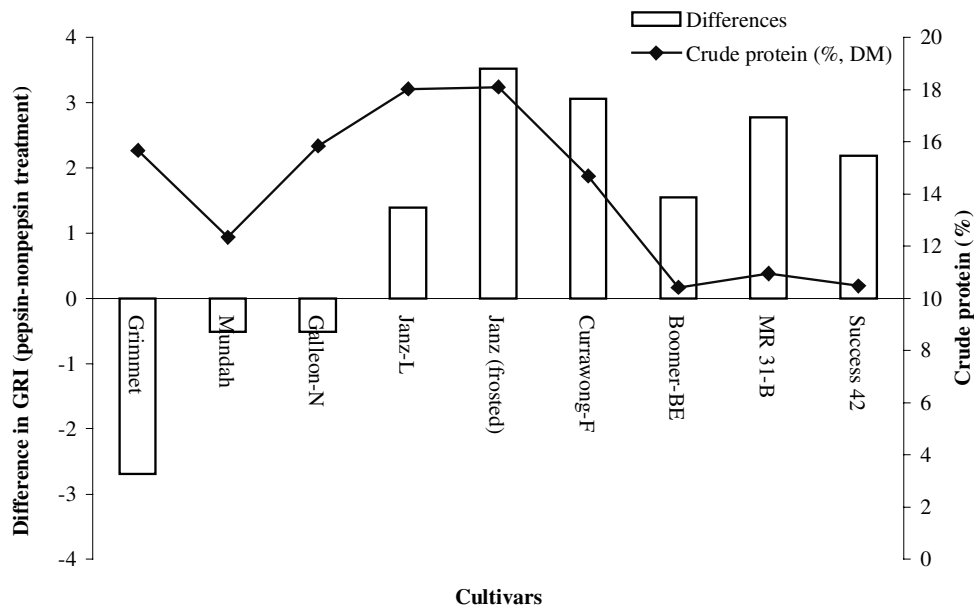


Figure 3 Difference between the glucose release index of untreated barley, wheat and sorghum and pepsin pre-treated barley, wheat and sorghum (Zarrinkalam 2002).

protein matrix (β - and γ -kaffarins). The protein matrix in sorghum, particularly in the peripheral area of the corneous endosperm region, may not have been completely digested by the pepsin treatment, restricting enzyme accessibility to starch granules.

The above results are supported by *in vivo* responses by pigs to application of exogenous enzymes containing an active protease (Figure 4). The ileal:faecal DE ratio of wheat fed concurrently with an exogenous enzyme was significantly improved relative to barley and sorghum, possibly due to an increase in energy yield from starch in the small intestine (R.J. van Barneveld, unpublished data). In this instance the sorghum sample tested was a waxy sorghum (which was not tested using the GRI), and hence the response to an exogenous enzyme may have been atypical.

Application of the GRI to assess the influence of grain hardness on starch digestion

Zarrinkalam (2002) applied the GRI to investigate the influence of milling process on starch digestion in barley. The GRI values determined for samples milled through a 2 mm screen and samples roller-milled were subtracted from the corresponding GRI values of samples milled through a 0.5 mm screen and the difference expressed as a proportion of the corresponding 0.5 mm-milled value (Figures 5 and 6). Zarrinkalam (2002) reported a significant ($P < 0.05$) linear relationship between the grain hardness index (determined on whole grain using single kernel analysis; Bread Research Institute, North Ryde), and the change in GRI that occurred when grains were either milled

through a 2 mm screen or roller milled relative to the 0.5 mm-milled samples. As the grain hardness increased, the GRI generally decreased when the grains were roller milled or more coarsely ground. Softer barley cultivars such as Sloop and Schooner actually had a higher GRI relative to 0.5 mm-milled samples when they were roller-milled (Figure 6). Hard barley cultivars such as Tantangara consistently had a higher GRI when they were finely ground. It should also be noted that in all cases, the GRI of roller milled barley was closer to the GRI of 0.5 mm-milled samples than the 2 mm-milled samples (Figures 5 and 6). Information of this nature could be extremely valuable to feed milling systems where savings in the operation of roller mills relative to hammer mills may also be enhanced by improvements in available energy for the target species.

Extending the above example with barley, Zarrinkalam (2002) also demonstrated that increases in GRI were greatest when sorghum was 2 mm-milled relative to 0.5 mm-milled compared with wheat and barley, yet the change in GRI was the lowest when sorghum was roller-milled. Based on the suggestion by van Barneveld *et al.* (2001) that capacity exists to increase the energy yield from sorghum for pigs, this application of the GRI suggests that roller milling may be a far more effective way of preparing sorghum for use in non-pelleted pig diets.

Conclusions

A primary focus of *in vitro* assays in feed evaluation systems for pigs (and other livestock) has been to simulate digestion and to provide a rapid and more cost-effective means of distinguishing between the nutritional quality of feed ingredients. Adoption of this approach

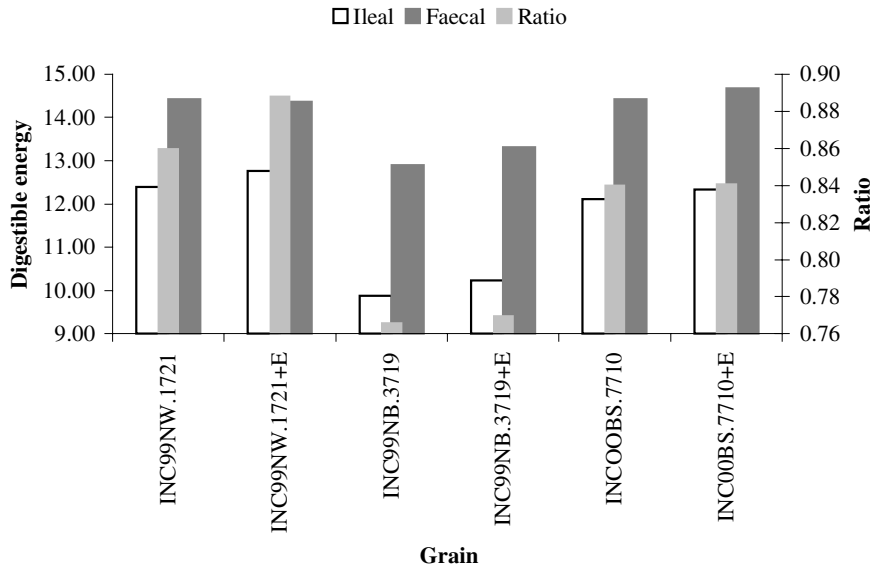


Figure 4 Influence of exogenous enzyme application on the *in vivo* ileal and faecal digestible energy (MJ/kg, as received) content of wheat, barley and sorghum fed to growing pigs (R.J. van Barneveld, unpublished data).

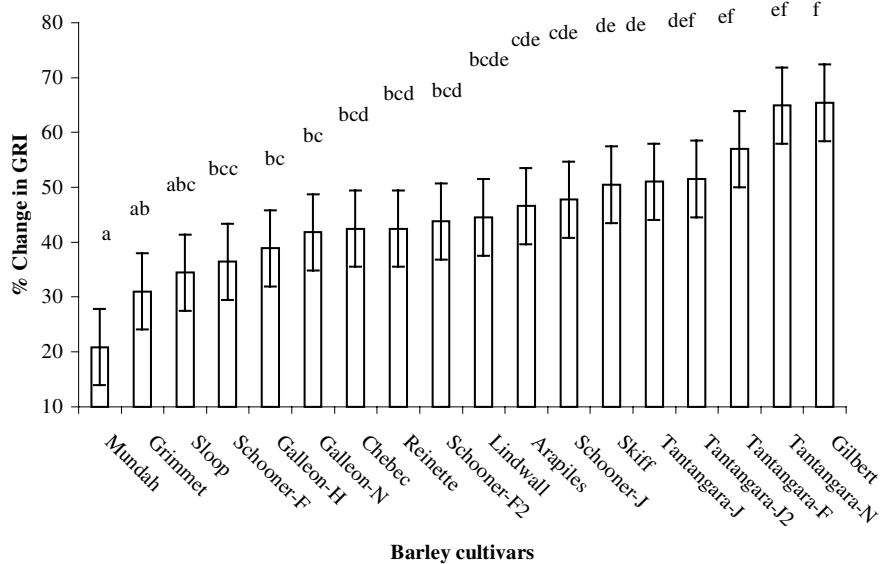


Figure 5 Change in glucose release index (%) of barley samples milled through a 2 mm screen relative to samples milled through a 0.5 mm screen. Standard error bars with different superscripts differ significantly ($P < 0.05$) (Zarrinkalam 2002).

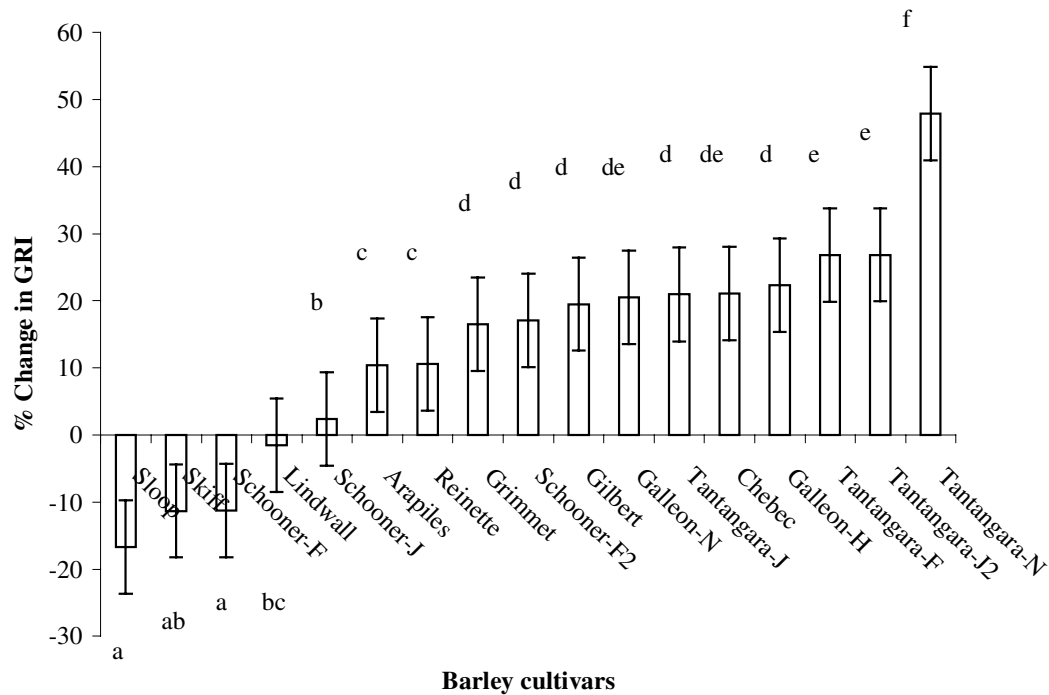


Figure 6 Change in glucose release index (%) of barley samples roller-milled relative to samples milled through a 0.5 mm screen. Standard error bars with different superscripts differ significantly ($P < 0.05$) (Zarrinkalam 2002).

has been limited, largely due to a lack of agreement between the results arising from *in vitro* systems and *in vivo* measurements. In addition, other forms of analysis such as those involving near infra-red spectrophotometry hold far greater potential as a means of routinely assessing the nutritional quality of grains than any method based on wet chemistry. Despite this, if we are to improve the efficiency of use of nutrients from grains for livestock we not only have to revisit the way we assess nutritional quality, focussing on more sensitive measures of energy and amino acid availability, but we have to be able to increase our capacity to assess a wider range of hypotheses associated with ways to improve nutritional quality. It is here that opportunities exist to strategically apply *in vitro* assays with a view to identifying those subtle changes or inherent characteristics of grains (and other feed ingredients) that may result in an improvement in livestock production efficiency. Using the glucose release index as an example, it has been demonstrated in this paper that a simple two-step enzymic procedure can be applied to identify grain components that may influence the digestion of starch in the small intestine of pigs. Further research is required to assess the extent that these influences can be observed *in vivo* and their subsequent effects on commercial pig production.

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