In vitro fermentation of grain and enzymatic digestion of cereal starch

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Summary

Fermentation of grain in the rumen and the digestion of starch in the small intestine are influenced by both animal and grain characteristics. Two assays were established to determine the importance of grain characteristics on fermentation and enzyme digestion. To simulate microbial fermentation in the rumen finely milled samples of grain were incubated in rumen fluid and the end products of fermentation and starch disappearance were measured. The second assay was based on amylase and amyloglucosidase and was established to simulate starch digestion in the small intestine. The assays identified large differences between and within grains with respect to both susceptibility of grain to microbial fermentation and enzyme digestibility of starch. Ranking grains on the basis of total acid production identified expected differences between grains. Lactic acid was positively correlated to total acid production, which implies that strategies aimed at increasing the rate of fermentation of grain inevitably increase the risk of rumen acidosis. The assays identified a sorghum cultivar with a higher rate of fermentation and enzyme digestibility compared to other sorghum varieties tested. Triticale was also identified as a grain with a high enzyme digestibility of starch.

Introduction

Cereals are widely used in the ruminant livestock industries to increase digestible energy intake. Grains are also used as drought reserves and fed as survival rations in times of pasture shortage. In Australia barley, sorghum, oats and wheat are the grains most commonly fed to ruminants with triticale and maize being of lesser importance. Of the legume seeds, lupins are the most important.

The nutritional value of grains fed to ruminants differs between grain types and this variation is not necessarily related to starch content. For example when rolled barley (60% starch) and rolled sorghum (75% starch) are fed to cattle under comparable conditions barley is used more efficiently than sorghum (Saba *et al*. 1964). While it is recognised that the availability of protein and minerals from grains are important for animal nutrition, the efficiency with which the carbohydrate component of the grain is utilised is considered to be the major determinant of nutritive value (Theurer 1986). Microbial digestion of feed in the rumen reduces the amount of energy available to the animal because of energy losses associated with methane production and heat of fermentation (Hungate 1966). Based on theoretical calculations Black (1971) suggested that the efficiency of energy utilisation would be maximised if the intestinal digestible component of feed offered to ruminants escaped rumen fermentation and was digested in the small intestine. Strategies aimed at shifting the site of digestion of starch from the rumen to the small intestine will only be successful if starch is extensively digested in the small intestine. Currently the factors limiting starch digestion in the small intestine of sheep and cattle have not been clearly established. From a series of studies with sheep in which starch was infused directly into the abomasum it was concluded that the supply of endogenous enzymes may limit the digestion of starch in the small intestine of sheep (Mayes and Ørskov 1974). However there was no indication from a review of results reported in the literature (Owens *et al*. 1986) that the intestine had a finite capacity to digest starch in cattle fed sorghum and maize diets. An understanding of the factors limiting starch digestion in the intestine would help resolve these conflicting results. The merits of maximising starch fermentation in the rumen are further confounded by the likelihood that this strategy will lead to the rapid production and accumulation of lactic acid which may result in health problems and lower productivity. Starch undigested in the small intestine will pass to the caecum and colon (hind gut) where rapid fermentation can lead to lactic acid accumulation and low pH (Lee 1977; Godfrey *et al*. 1993). Acidosis in the hind gut may also affect animal health and productivity. Clearly the efficiency of starch utilisation in the ruminant will be influenced by a combination of: whole tract digestibility;

the extent of starch digestion in the rumen and small intestine; the amount of lactic acid produced in the rumen; and the amount of acid produced in the hind gut.

The fermentation of grain in the rumen and the digestion of starch in the small intestine will be influenced by both animal and grain characteristics. To determine the effects of grain characteristics on digestion, grains were assayed under a set of standard *in vitro* conditions. Two separate *in vitro* assays were developed to simulate fermentation of grain in the rumen and digestion of starch in the small intestine. Grains examined included cultivars of barley, sorghum, wheat, triticale, oats and maize and the results from these assays are discussed.

Development of an *in vitro* assay to simulate fermentation in the rumen

The digestive processes in the rumen are complex and cannot be accurately simulated by either the *in situ* (nylon bag) or the *in vitro* procedures used to estimate the digestibility of feed. In comparison with *in vitro* methods the *in situ* method more closely simulates the *in vivo* situation because it involves digestive processes that occur in the rumen of a living animal. The *in situ* method however cannot account for the effects of rumination and the environment in the nylon bag is unlikely to simulate the effects of mixing the feed with other components of the ration. In addition the method does not provide any information on the end products of fermentation of the test–feed. The conditions of the *in vitro* procedure differ significantly from the *in vivo* environment. Rumen fluid inoculum is diluted with buffer and the products of fermentation accumulate in the incubation vessel. For this reason Stern *et al*. (1997) suggested that *in situ* and *in vitro* assays should be used to obtain relative estimates of digestibility among feeds rather than absolute values. The *in vitro* assay was chosen in preference to the *in situ* method for these studies because the method provided the opportunity to measure the products of fermentation. In particular the measurement of lactic acid production was considered to be important because it is commonly associated with the fermentation of grain and accumulation in the rumen of grain fed animals can have detrimental effects on rumen function and animal health.

Published *in vitro* and *in situ* techniques use time–series sampling to obtain the data necessary to characterise digestion curves. Mertens (1993) suggested that 9–12 sampling times were required to obtain accurate estimates for digestion kinetics of rapidly digesting components (i.e. starch) and that a final measurement at 48–72 h is needed to establish the potential extent of digestion. As a separate incubation vessel is required for each sampling time, the number of feed samples that can be assayed in a run is severely limited and chemical analysis of the fermentation residues and end products is time consuming and expensive. Richards *et al.* (1993) established digestion curves from time–series measurements for the disappearance of starch from milled sorghum varieties in vitro and determined a fractional rate constant of digestion for each grain. These authors reported that ranking grains according to a single measure of starch disappearance at 8h gave a comparable ranking to that obtained using the fractional rate constant of digestion. A single fermentation time (5 h) was chosen because a large number of grain samples were assayed. A shorter fermentation time (5 h) was used in this assay because the ratio of grain to buffer was greater than the ratio used by Richards *et al.* (1993) and buffering capacity was often exhausted at 5h for the more readily fermentable grains.

The *in vitro* fermentation assay included measurements of gas production, incremental change in pH, volatile fatty acid and lactic acid production and the disappearance of starch during incubation (5 h). Although gas production was easy to measure results were confounded by the amount of CO_2 released from the buffer and changes in the proportion of acids produced. Total acid production was considered to be a more reliable estimate of grain fermentability and starch disappearance was measured to obtain a direct estimate starch digestion. Total acid production is inversely correlated to the yield of microbial cells so results may be confounded by variation in yield. Lactic acid was expected to be an important end product of grain fermentation and is related to rate of fermentation (Russell 1998). Surprisingly Opatpatanakit *et al*. (1994) detected no lactic acid during the *in vitro* fermentation of grain.

Initial work on the development of an *in vitro* fermentation assay concentrated on the published method of Opatpatanakit *et al.* (1994). This method was based on the disappearance of a starch and some of the end products of fermentation (gas and volatile fatty acids). Incubations were conducted with small amounts of feed (0.1 g) in a sealed tube containing buffered fluid. Several adaptations to these methods were made to meet the requirements of this assay. The first adaptation was to increase the size of the fermentation vessel and the amount of grain sample assayed. The size of the fermentation vessel was increased to one litre and the sample size to 30 g to allow the system to handle whole processed grain samples. The second important change was the removal of glucose from the incubation mixture. Published methods generally included glucose in the incubation mixture but in our studies it was found that the fermentation of glucose had a confounding effect on the fermentation of grain.

During the development of this assay it became apparent that the measurement of a single end product of fermentation was unlikely to provide an adequate description of the fermentation of grain. Therefore the *in vitro* fermentation assay included the measurements of gas production, change in pH, volatile fatty acid and

lactic acid production and the disappearance of starch during incubation at 39ºC for 5 h.

Protocol for *in vitro* fermentation assay

(i) Unprocessed grain samples were hammer–milled (0.5 mm screen)

(ii) A sample of milled grain (30 g) was added to a culture jar (1 l). One hour prior to the commencement of the assay 375 ml of McDougalls buffer and urea (1.2 g/l) were added to the jar and stirred with a rod until the grain was completely wet. The jar was sealed and flushed with CO_2 (via a 3–way gas tap) and placed in a shaking water bath at 39ºC

(iii) Rumen fluid (2l/animal) was collected from 2 steers (fitted with permanent rumen cannula) prior to feeding. The diet of the cattle (fed once daily, am) contained approximately 50% mixed grain (oats, barley, wheat maize and sorghum) and 50% chaffed hay

(iv) The assay was initiated with the addition of 125 ml of rumen fluid to the culture jar. This occurred approximately 30 min after the collection of rumen fluid from the cattle

(v) The incubation was stopped after 5 h with the addition of H_2SO_4 (15 ml 20% w/w) and a liquid sample was collected for the determination of volatile fatty acids and lactic acid. The remainder of incubation contents were dried and assayed for starch.

Development of an *in vitro* assay to simulate intestinal digestion of starch

Starch occurs in two forms: a–amylose and amylopectin. Amylose is a linear polymer of α–1,4–linked glucose units. Amylopectin is a much larger polymer consisting of linear chains of α–1,4–linked glucose units with α–1, 6 branch points occurring on average every 12 glucose units (Lehninger 1970). Enzymatic digestion of starch in the small intestine of ruminants proceeds in a similar fashion to intestinal starch digestion in monogastric animals (Huntington 1997). Amylose and amylopectin are hydrolysed into small oligosaccharides of two or three glucose units by α–amylase secreted into the duodenum by the pancreas. In the ruminant the oligosaccharides are hydrolysed to produce glucose by maltase (amyloglucosidase) and isomaltase (Harmon 1992). The strength of the enzyme assay developed for these studies was that it was based on two of the major enzymes responsible for the digestion of starch in the duodenum. In addition the assay was conducted under similar physiological conditions of pH (7) and temperature (39ºC found in the small intestine. Therefore ranking grains according to the amount of starch hydrolysed to glucose in this assay can be expected to provide a reliable *in vivo* index of intestinal starch digestibility for the test grains. A potential weakness of the assay is that it does not account for the effects of pre–digestion in the rumen. While separate assays were developed to simulate fermentation in the rumen and starch digestion in the duodenum the *in vivo* processes are not independent (Owens *et al*. 1986). Pre–digestion in the rumen will influence the quality and quantity of starch reaching the duodenum with the more readily accessible starch in the grain being fermented in the rumen. In addition some of the products of fermentation (microbial protein) flowing to the duodenum may influence secretion of enzymes in the duodenum (Taniguchi *et al*. 1995). Therefore the extent of grain digestion in the rumen may indirectly affect starch digestion in the intestine.

The *in vitro* enzyme assay developed for these studies was adapted from the established method for starch determination (McCleary *et al*. 1997). The method of McCleary *et al.* (1997) is based on two enzymes: amyloglucosidase (AMG) and α–amylase (AA) and high temperature treatment to gelatinise the starch. Starch digestion was determined from the amount of starch hydrolysed to glucose. Various combinations of incubation times, pH conditions and temperature were examined (Table 1). There were clear differences between the enzyme digestibility of sorghum, oats and barley and these differences were evident for all combinations of pH, temperature and incubation time (Table 1). The conditions selected for our assay were: an incubation time of 1 h; pH 7; and temperature 39ºC.

Protocol for *in vitro* enzyme digestion of starch

(i) Unprocessed grain samples were hammer–milled (0.5 mm screen)

(ii) A sample of milled grain (0.1 g) was added to a pre–weighed 30 ml culture tube

(iii) Ethanol (0.2 ml) was added to the tube to wet the grain

(iv) Thermostable α–amylase (1 ml) was added to MOPS ($C_7H_{14}NO_4SNa$) buffer (30 ml). The amylase/MOPS mixture (3 ml) and amyloglucosidase (0.1 ml) were added to the tube, mixed and placed in water bath at 39ºC for 60 min. The tube was gently shaken at 15 minute intervals

(v) After the tube was removed from the water bath and dried the volume was adjusted to 10 g using distilled H_2O

(vi) Tubes were shaken and centrifuged at 3000 rpm for 10 min

(vii) An aliquot of the supernatant (0.5 ml) was withdrawn from the tube and diluted with deionised H_2O (4.5 ml)

(viii) Duplicate aliquots (0.1 ml) of the diluted supernatant were added to a test tube containing 3.0 ml of GOPOD (glucose determination reagent). The tubes were vortexed and incubated in a water bath at 50°C for 20 min.

(ix) Tubes were allowed to cool for 15 min, remixed and the absorbance read at 510 nm against deionised H_2O

Fermentation and enzyme digestion of starch

There was a large variation both between and within grain types for starch fermentation and enzyme digestion (Table 2). The *in vitro* results for barley and sorghum (Table 2) are consistent with *in vivo* observations. Barley and sorghum are the grains most commonly fed to feedlot cattle and it is well known that dry–rolled barley is used more efficiently than dry–rolled sorghum (Saba *et al*. 1964). Incomplete whole–tract digestion of sorghum starch is the primary reason for the poor utilisation of this grain by cattle. Starch content of faeces collected from steers fed either dry–rolled sorghum or barley was 25% and 4% respectively (Watts and Tucker 1993). Using the values for barley and sorghum as benchmarks the relative merits of the other grains in Table 2 can be assessed. In comparison with barley the rumen and intestinal digestibility of starch in the oats and triticale varieties is likely to be comparable or superior. The result for oats was expected as it is generally accepted that utilisation of starch in whole oat grain by cattle is efficient and processing is considered unnecessary (Owens *et al*, 1997). The result for triticale was unexpected. This grain is not commonly fed to beef cattle yet the *in vitro* starch fermentation and enzyme digestion characteristics suggest that triticale may be a grain superior to either wheat or barley. Of particular significance is the high enzyme digestibility of starch in triticale (70%) which is clearly superior to that of barley (45%) and wheat (43%) and implies that the digestion of starch in the small intestine will be more efficient for triticale.

Table 1 Effect of temperature, pH and incubation time on the enzymatic (AA/AMG) digestion of starch in milled grain.

Grain	Incubation time	Starch digestion (percent of original)			
		pH 2.5		pH 7.0	
	(min)	39°C	50°C	39°C	50°C
Sorghum	15	15.3	35.3	15.4	40.6
	60	33.8	65.5	35.0	75.9
	120	54.9	89.8	61.6	91.1
Barley	15	19.6	80.0	22.3	87.2
	60	43.0	91.4	48.7	96.3
	120	73.7	94.7	74.2	95.5
Oats	15	42.2	87.9	44.8	98.5
	60	72.5	91.7	76.4	93.6
	120	89.2	91.0	87.0	95.3

Table 2 *In vitro* fermentation and enzyme digestion of starch in 54 finely milled (0.5 mm screen) samples of grain.

		Starch digestibility (percent of original)*			
		Fermentation		Enzyme digestion	
Grain type	Number of cultivars	Mean	Range	Mean	Range
Barley	20	67	52–76	45	37–53
Wheat	7	48	35–63	43	37–47
Oat	4	72	70–77	61	57–66
Sorghum	20	44	35–51	28	23–33
Triticale	3	60	52–78	70	65–76
Maize	1	42		29	

*Values are the mean for each grain type

Barley and wheat are generally regarded as being highly fermentable and accordingly the processing of these grains for cattle is normally restricted to dry–rolling. However (Owens *et al*. 1997) reported that compared with dry–rolled wheat, steam–rolling increased the average (mean response measured in 12 studies) metabolisable energy (ME) value of wheat by 13%. Therefore the low fermentation of starch in wheat (48%) relative to barley (67%) was not totally unexpected (Table 2). The individual results for the wheat varieties show a considerable range in starch fermentation (Table 3) that suggests dry–rolling may not be a satisfactory method of processing for all wheat varieties. Fermentation of starch in the wheat varieties Swift (35%) and Sunland (42%) was lower than the mean value for sorghum (44%). The wheat variety Swift also had the lowest enzyme starch digestibility value of the wheats examined (Table 3).

There was a weak positive correlation ($r^2 = 0.46$) between the enzyme digestibility of starch and starch fermentation (Figure 1). However two varieties of triticale (Abacus and Madonna) appear to be outside this general trend. These two grains had a much higher enzyme digestibility of starch relative to their starch fermentation. Further investigation is warranted as the *in vitro* results suggest that a greater proportion of starch in these triticale varieties may be digested in the small intestine while maintaining a high whole tract digestibility. Shifting the site of digestion of starch from the rumen to the intestine should increase the efficiency of starch utilisation (Black 1971).

Lactic acid and total acid production

Accumulation of lactic acid in the rumen is associated with the ingestion of large amounts of readily fermentable carbohydrate (Owens *et al*. 1998). In the presence of these readily available sources of carbohydrate the rate of glycolysis increases and some rumen bacteria change their fermentation products from acetate to lactate (Russell 1998). The accumulation of lactic acid *in vitro* therefore should provide a sensitive index of fermentation rate. Lactic acid was produced from the *in vitro* fermentation (5 h) of barley wheat oats

Table 3 *In vitro* fermentation and enzyme digestion of starch in finely milled (0.5 mm screen) samples of wheat.

Wheat variety	Starch (DM%)	Starch digestibility (percent of original)	
		Fermentation (5 h)	Enzyme (AA/AMG, 1 h)
Sunland	69	42	43
Swift	70	35	37
Declic	69	58	47
Ouyen	69	63	44
Currawong	70	54	44
Rosella	71	53	41
Kelallac	70	56	47

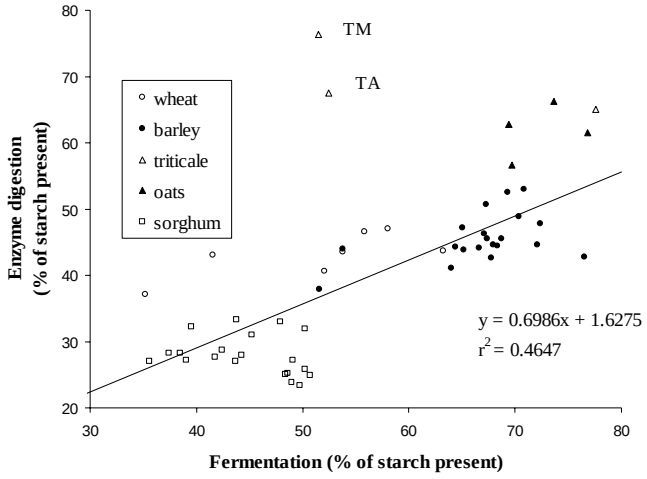

Figure 1 Relationship between starch fermentation and enzyme digestibility of starch in finely milled (0.5 mm screen) samples of grain; TM = Triticale Madonna, TA = Triticale Abacus.

and lupins but was not measured in sorghum or maize fermentations (Table 4). Comparing the results for lactic acid and fermentation of starch (Table 3) it is clear that these two variables are positively correlated. This result suggests that the amount of readily fermentable carbohydrate in sorghum and maize is low relative to the other grains. Steam flaking has been reported to increase the ME value of sorghum, maize and wheat but have no effect on the ME value of barley or oats (Owens *et al*. 1997). The *in vitro* production of lactic acid therefore may provide a guide to the method of grain processing required to optimise fermentation in the rumen.

It was significant that after a 5 h incubation period the lowest pH recorded for the fermentation of sorghum was 6.3. This pH value contrasts with the final pH values recorded for the fermentation of barley varieties that were frequently below 5.0 (Table 4). These results suggest that there will be a constant risk of rumen acidosis associated with barley but little or no risk associated with dry–rolled sorghum. If the pH in the rumen falls below 5.5 it is likely that rumen function will be impaired and an acidotic condition may develop (Owens *et al.* 1998). Therefore it may be possible to increase the fermentation rate of sorghum by fine grinding without increasing the risk of acidosis.

The large amount of lactic acid produced from the fermentation of lupins was unexpected. Lupins are generally regarded as being a safe grain to feed to ruminants because they contain no starch. However this result suggests that over–processing lupins should be avoided and supports a number of unconfirmed reports of acidosis in cattle fed lupin–based rations.

The considerable range in both total acid (30–64 mmol) and lactic acid (2–21 mmol) produced from the fermentation of the wheat varieties was consistent with the results obtained for starch fermentation (Table 3). The range in the amount of lactic acid produced suggests that the risk of rumen acidosis associated with feeding these wheat varieties will also be variable. However the highest amount of lactic acid produced from the fermentation of wheat was lower than the amount of lactic acid produced from the fermentation of the barley varieties assayed.

There was a strong positive correlation ($r^2 = 0.95$) between total acid and lactic acid production (Figure 2) Therefore lactic acid production would appear to be a sensitive indicator of fermentation rate of grain. An important implication of this relationship is that strategies aimed at increasing the rate of fermentation of grain in the rumen will inevitably increase the risk of acidosis.

Starch gelatinisation

The high temperature associated with steam flaking results in the gelatinisation of some or all of the starch in the grain. To determine the effects of gelatinisation of starch samples of finely milled grain were cooked prior to *in vitro* fermentation. The amount of acid produced from the fermentation of cooked sorghum (67 mmol) was significantly higher than for the

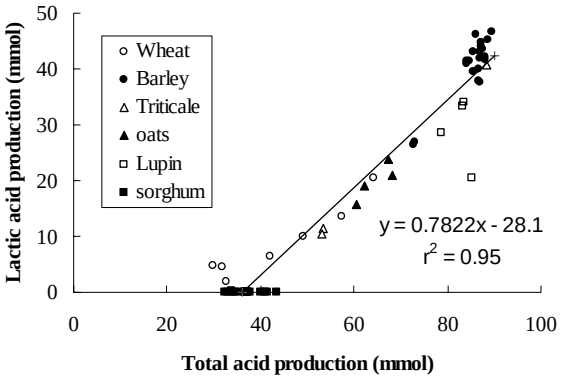

Figure 2 Relationship between production of lactate and total acid production from the *in vitro* fermentation of grain.

Table 4 Total acid and lactic acid production from *in vitro* fermentation of finely milled (0.5 mm screen) samples grain.

	Lactic acid production (mmol)		Total acid production (mmol)		Final pH	
Grain type	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Barley	41	27–47	86	73–90	4.8	4.7–5.4
Wheat	9	2–21	44	30–64	6.5	5.6–7.0
Oat	20	16–24	65	61–68	5.7	5.4–6.0
Sorghum	0	0	37	33–44	6.6	6.3–6.7
Triticale	21	11–41	65	53–89	6.1	5.1–6.7
Lupin	29	21–34	83	79–85	5.8	5.6–6.0
Maize	0	0	47	47	6.5	6.5

*Values are the mean for each grain type

uncooked grain (30 mmol) indicating that the rate of fermentation was increased (Table 5). In contrast to uncooked sorghum (3 mmol) a large amount of lactic acid was produced from the fermentation of cooked sorghum (37 mmol). The increase in the production of lactic acid is a clear indication that cooking has increased the proportion of readily fermentable carbohydrate in sorghum. Cooking also resulted in an increase in the production of lactic acid from the fermentation of barley. The final pH of cooked sorghum (5.3) was significantly lower than the final pH of uncooked sorghum (6.7). Final pH was low for uncooked oats and barley (less than 5.0) and not affected by cooking. These results suggest that steam flaking sorghum will increase the rate of fermentation in the rumen but also significantly increase the risk of rumen acidosis. The value of steam flaking barley must be questionable as ME value is not generally improved (Owens *et al*. 1997) and the risk of acidosis is increased.

Particle size

The relationship between particle size (four screen sizes) and grain type (three grains) was examined. Particle size had a significant effect on the fermentation rate of barley. As particle size increased from 0.5 mm to 4 mm total acid production declined from 79 mmol to 41 mmol (Figure 3a), lactic acid production declined from 44 to 3 mmol (Figure 3b) and starch fermentation declined from 79% to 46% (not shown). The decline in total acid production as particle size increased was less pronounced for either sorghum or oats (Figure 3a). Lactic acid was not produced from the fermentation of sorghum and an increase in particle size of oats resulted in moderate decline in lactic acid production (19 to 10 mmol). These results suggest that the rate of fermentation of barley starch in the rumen can be partially controlled through particle size while particle size has a much smaller influence on the rate of fermentation of either sorghum starch or oat starch. Fermentation of sorghum was slow for all particle sizes.

A large proportion of the sorghum grain produced each year is fed to livestock. Although sorghum is a popular feed for cattle it is recognised that extensive processing is required to realise its full nutritive value. The starch granules are located in a protein matrix that is thought to limit access by digestive enzymes (Rooney and Pflugfelder 1986). To improve the digestibility of sorghum, starch must be gelatinised and/or the protein matrix disrupted. The methods most commonly used to process sorghum for cattle are steam flaking and reconstitution. Both methods are expensive and require a large capital investment in specialised equipment. Steam flaking and reconstitution are therefore restricted to large cattle feedlot operations. The identification of a sorghum variety that can be efficiently utilised by cattle without extensive processing is a priority for the sorghum feed grain industry. The assays appear to have identified just such a grain. Two new varieties of sorghum (sorghum 1and 2) were obtained from plant breeders and the assay results are presented in Table 6. Also included in the table are values for a sorghum and a barley 'control' that were run on the day of the assay. The barley and sorghum 'control' grains (from the original grain collection) were run with each assay. For comparison the mean values for the sorghum varieties (n = 20) are included. Sorghum 1 clearly stands out from the other sorghum varieties. Total acid production (63 mmol) was greater than either the control (33 mmol) or the mean value for sorghum (37 mmol), a result that indicates that fermentation rate of sorghum 1 was superior to the other varieties. Importantly, enzyme digestibility of sorghum 1 (56%) was significantly higher than either the control (34%) or the mean sorghum value (28%). A comparison of the fermentation characteristics of sorghum 1 and the barley control suggest that dry–rolling should be an adequate method of processing this sorghum cultivar for cattle.

Table 5 Total acid and lactic acid production from *in vitro* fermentation of cooked and uncooked samples of barley, sorghum and oats.

		Acid production (mmol)		
Grain	**Treatment**	**Lactate**	**Total**	**pH**
Sorghum	Uncooked	3	30	6.7
	Cooked	37	67	5.3
Barley	Uncooked	37	62	4.9
	Cooked	51	73	4.8
Oats	Uncooked	51	62	4.9
	Cooked	49	73	4.8

Conclusion

Results from the two assays indicate that there are large differences between grains with respect to susceptibility to microbial fermentation and enzyme digestibility of starch. Ranking grains on the basis of total acid production identified expected differences between grains that indicates the assay may provide a tool for screening grains with respect to fermentability. Lactic acid production appears to be a sensitive indicator of the readily fermentable component of the grain and is likely to provide a reliable guide to the method of processing required. For example the ME value of sorghum and maize (no lactic acid) is increased by steam flaking while the ME value of oats and barley (high lactic acid) is not improved by steam flaking.

It is apparent that there are inefficiencies associated with grain feeding irrespective of the level of processing employed. Processing grain to maximise fermentation of starch in the rumen increases lactic acid production that is likely to lead to an increased incidence of health problems and loss of production. However reducing fermentation of starch in rumen will reduce the efficiency of starch utilisation because post–rumen digestion of starch may be incomplete. Hind–gut acidosis may also be a problem if a significant amount of starch reaches the large intestine. Therefore increasing starch digestion in the small intestine should be a critical component of any strategy aimed at improving the efficiency of starch utilisation and the identification of grains with high enzyme digestibility of starch a research priority. Of the grains tested, the enzyme digestibility was highest for triticale. Interestingly two varieties of triticale appeared to have a high enzyme digestibility of starch relative to starch fermentation.

The identification of a sorghum variety with high fermentability and enzyme digestibility has significant implications for the cattle industry. An *in vivo* study is required to determine whether this grain can be

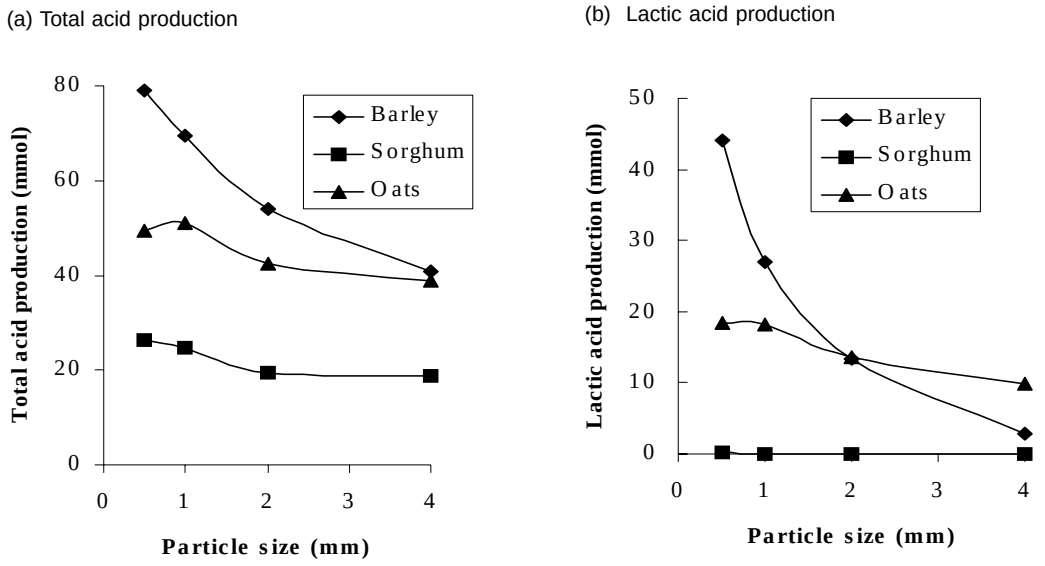

Figure 3 The effect of particle size and grain type on *in vitro* acid production.

Table 6 *In vitro* fermentation characteristics and enzyme digestibility of sorghum varieties (n = 20) and new cultivars nos. 1 and 2, and of sorghum and barley standard reference controls.

Grain type	Starch content (% DM)	Acid production (mmol)			Final pH	Starch	
		VFA	Lactate	Total acid		F* (%)	E# (%)
Varieties mean	67	37	0	37	6.6	44	28
Sorghum 1	70	21	43	63	5.3	76	56
Sorghum 2	73	25	0.3	26	6.8	54	30
Sorghum 'control'	70	33	0.2	33	6.7	46	34
Barley 'control'	61	27	50	77	4.8	85	48

* Fermented
#Enzyme digestibility

efficiently utilised by cattle without extensive processing. The use of these assays in collaboration with plant breeders has the potential to improve the selection of feed grains. Identifying the chemical and/or physical characteristics of grains that limit digestion will also assist in the selection and breeding of feed grains.

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