Chapter 4. The Effect of Variable Levels of Steam Flaking on Faecal Parameters Under Commercial Feedlot Conditions

4.1 Introduction

The concentration of starch in cattle faeces is an important issue for the feedlot industry. High levels of faecal starch signal inefficient use of grain-based diets and pose environmental problems in terms of odour pollution as well as the amount of manure that accumulates in feedlot pens. Faecal starch content may comprise 2-8% of faecal DM (Zinn 1994) in most situations but can be as high as 35% (Galyean *et al.* 1979) depending on the type of grain fed and processing method used.

Zinn (1994) reported that the correlation (R^2) between faecal starch and whole tract starch digestibility was 0.95 for cattle fed concentrate-based diets. In addition to faecal starch, the literature review presented in this thesis identified the opportunity to use faecal pH, DM and nitrogen content as measures of fermentation in the hindgut to provide an indirect estimate of the efficiency of pre-caecal starch digestion.

The experimental work reported in this chapter was undertaken as a preliminary investigation of the effect that different grains and processing methods may have on hindgut fermentation and whole tract starch digestibility in feedlot cattle. In addition, the information gained was also used to gauge the level of between-animal variation in various faecal parameters. The investigation was based on a monitoring program in collaboration with a commercial feedlot in which various changes were made to diet composition and grain processing.

4.2 Materials and Methods

4.2.1 Cattle

The breeds of cattle used in the feedlot were mainly Angus and Murray Grey along with a small number of Wagyu. Cattle were held in standard feedlot pens with approximately 250 cattle per pen.

Standard feedlot procedures were followed for cattle feeding, care and monitoring. Cattle were fed twice per day with around 40% of the feed given in the morning and the 60% given in the afternoon.

There were three categories of feeding: 'starter', 'grower' and 'finisher' cattle. Each group was fed a different ration; the finisher cattle were fed a ration containing roughly 70% grain (Table 4.1).

4.2.2 Diets

The composition of the grower and finisher diets was changed on three occasions during the study. The details of the diets are summarised in Table 4.1. An analysis of the component grains was conducted and is reported in Chapter 5.

Key: High level of inclusion of tub ground, high moisture corn: **HC**

Low level of inclusion of tub ground, high moisture corn: **LC**

High flake density barley (Poorly flaked)(49.8 kg/hL*): **PFB**

Low flake density barley (Well flaked)(36.8 kg/hL): **WFB**

High flake density wheat (Poorly flaked)(56.9 kg/hL): **PFW**

Low flake density wheat (Well flaked)(46.5 kg/hL): **WFW**

*kg per hectolitre.

Table 4.1 Inclusion rates (%) of different grains in diets fed to starter, grower and finisher groups of feedlot cattle.

Diet	Starter	Grower	Finisher
Diet 1: HC, PFB, PFW			
Poorly flaked barley	31.5	10.3	18.2
Poorly flaked wheat		14.7	22.0
Tub ground, high moisture corn		29.7	31.5
Total grain in diet	31.5	54.7	71.7
Diet 2: LC, PFB, PFW			
Poorly flaked barley	31.5	23.7	35.0
Poorly flaked wheat		14.8	24.0
Tub ground, high moisture corn		15.1	14.3
Total grain in diet	31.5	53.6	73.3
Diet 3: HC, WFB, WFW			
Well flaked barley	31.5	10.3	16.7
Well flaked wheat		14.7	28.2
Tub ground, high moisture corn		29.7	26.0
Total grain in diet	31.5	54.7	70.9

Roughage was provided in the form of corn stover for the finisher and grower cattle.

Corn silage was used as the roughage for the starter cattle and this was fed together with barley.

4.2.3 Sampling and analysis

Grain samples were taken before and after processing and immediately frozen for later analysis (see chapter 5).

A period of at least two weeks was allowed for cattle to become accustomed to each new ration. This was followed by a day of sampling and on-site, faecal measurements.

Faecal samples were collected by walking among the cattle in the pens and sampling fresh faecal material. Care was taken to avoid picking up dirt with the faeces which were placed in

labelled, 70 mL specimen containers and stored in an ice-filled esky to arrest microbial fermentation. Three pens were sampled for each class of animal (starter, grower and finisher).

Ten separate samples were taken in each of the grower pens (total of 30 samples) to examine the level of individual variation between animals. In each of the starter and finisher pens, one composite sample was prepared from ten individual samples (approximately equal weight; wet basis).

Faecal score

Discrete faecal outputs from grower animals were given an objective visual score from 1 to 3 with half unit graduations (1, 1.5, 2, 2.5 and 3)(Plates 4.1-2). A score was not given to any of the composite samples because it was impossible to identify any visual differences in faecal consistency.

Other faecal analysis

Approximately 2 h after collection, faecal pH was determined for each of the discrete grower samples and for the combined starter and finisher samples. The limited time we could spend in the feedlot yards meant that we were unable to measure pH immediately after collection. All faecal samples were also analysed for DM content. Nitrogen and starch content (%) was determined for each of the combined starter and finisher samples but not for discrete faecal samples from grower cattle because of the cost and time associated with the analyses. Furthermore, given there were no differences between diets in faecal nitrogen content for starter and finisher cattle (section 4.3), analysis of discrete faecal samples from grower cattle was not justified.

4.2.4 Statistical analysis

Analysis of variance was used to determine differences in faecal parameters within each class of livestock. Models contained grain and pen number as fixed effects. Differences between means were determined using the least significant difference (LSD) method ($P<0.05$).

Regression analysis was carried out using the linear regression method available in S-plus (MathSoft 1999).

Plate 4.1 Faecal score 1. Relatively firm and has held shape.

Plate 4.2 Faecal score 3. Runny liquid.

4.3 Results

There were significant differences between diets in faecal DM content for the starter cattle (Table 4.2). There was a significant positive correlation between faecal nitrogen and DM content ($P<0.05$; $R^2=0.58$) and a significant negative correlation between faecal nitrogen and starch content ($P<0.01$; $R^2=0.82$) in the starter cattle.

Table 4.2 Faecal characteristics of starter feedlot cattle. Each value represents the average of three combined samples taken from three different pens.

	pH	DM (%)	Nitrogen (%)	Starch (%)
Diet 1 (PFB)	7.3	19.4[a]	2.5	4.1
Diet 2 (PFB)	7.1	15.7[b]	2.1	9.4
Diet 3 (WFB)	7.1	17.5[c]	2.3	5.5
Average	7.2	17.5	2.3	6.4
SE	0.2	0.6	0.1	1.8

Means within a column with different superscripts (a, b, c) differ significantly ($P<0.05$)

There were significant differences between diets in faecal pH, DM content and score for the grower cattle (Table 4.3). There was a significant negative correlation between faecal DM content and score ($P<0.01$; $R^2=0.13$) in the grower cattle. Faecal DM content was not correlated with pH.

Table 4.3 Faecal characteristics of grower feedlot cattle. Each value represents the average of 30 animals (10 samples x three pens).

	pH	DM (%)	Score
Diet 1 (HC, PFB, PFW)	6.9[a]	23.4[a]	2.0[a]
Diet 2 (LC, PFB, PFW)	6.9[a]	19.3[b]	1.7[b]
Diet 3 (HC, WFB, WFW)	7.0[b]	19.0[b]	1.7[b]
Average	6.9	20.6	1.8
SE	0.1	0.8	0.2
CV* (%)	2.9	17.8	33.9

Means within a column with different superscripts (a, b) differ significantly ($P<0.05$)

* Coefficient of variation

There were significant differences between diets in faecal pH, DM and starch content for the finisher cattle (Table 4.4). There was a significant positive correlation between faecal pH and DM content ($P<0.05$; $R^2=0.45$) and a significant positive correlation between faecal starch and DM content ($P<0.05$; $R^2=0.55$) in the finisher cattle.

Table 4.4 Faecal characteristics of finisher feedlot cattle. Each value represents the average of three combined samples taken from three different pens.

	pH	DM (%)	Nitrogen (%)	Starch (%)
Diet 1 (HC, PFB, PFW)	7.3^a	26.6^a	2.1	12.3^a
Diet 2 (LC, PFB, PFW)	6.9^b	19.5^b	2.1	8.9^a
Diet 3 (HC, WFB, WFW)	7.1^a	19.7^b	2.1	3.6^b
Average	7.1	22.0	2.1	8.3
SE	0.1	0.8	0.1	1.9

Means within a column with different superscripts (a, b) differ significantly ($P<0.05$)

4.4 Discussion

The major difference between diets 1 and 3 was the flake density of the wheat and barley. Data obtained for the finisher cattle showed that a diet based on poorly flaked wheat and barley resulted in 4 times more faecal starch than a diet based on well flaked wheat and barley. As faecal starch content is a reliable indicator of starch digestibility (Zinn 1994), this suggests that diet 1 had a lower whole tract starch digestibility than diet 3. The result is consistent with other findings for well flaked vs poorly flaked corn (Zinn 1990a; Theurer *et al.* 1999b), sorghum (Swingle *et al.* 1999; Theurer *et al.* 1999b) and barley (Yang *et al.* 2000). In contrast, Zinn (1994) reported no differences in whole tract starch digestibility of diets based on high (0.39 kg/L) and low (0.30 kg/L) flake density wheat. Wheat and barley were fed in combination in the current experiment making it impossible to identify the main source of faecal starch. It is likely that wheat and barley were digested to different extents.

The major difference between diet 1 and diet 2 was the level of inclusion of tub ground, high moisture corn. Diet 1 tended to produce a greater amount of faecal starch than diet 2 for finisher cattle. Therefore, increasing the proportion of corn in the diet appeared to lower whole tract starch digestion. It was interesting that for diet 3, faecal starch content was low despite the level of corn in the diet being relatively high. It is possible that any reduction in starch digestibility, caused by an increase in corn content, was compensated for by an increase in

starch digestibility through heavily steam flaking wheat and barley. Corn has been shown to be less digestible than wheat and barley in cattle (Orskov 1986; McAllister *et al.* 1990; Rowe *et al.* 1999). Within the ration, particle size of corn varied considerably from whole grain to flour whilst whole corn was observed in some of the faeces in the feedlot pens. This supports the view (McAllister *et al.* 1993; McAllister *et al.* 1994) that if corn is not adequately cracked, microbial and intestinal enzymes will not be able to fully digest the grain.

The information obtained for the starter cattle is difficult to interpret. As this class of cattle were not fed wheat or high moisture corn, diets 1 and 2 were essentially identical. However, Diet 2 tended to have a slightly higher level of faecal starch. It is possible that some of the starter cattle had not fully adapted to eating a grain-based diet at the time of sampling. Supporting this argument is the fact that faecal starch values of the starter cattle were comparable to those of the finisher cattle - even though the starter ration contained 31% grain as opposed to 72% grain in the finisher ration. This may help explain the variability in the results obtained for the starter cattle.

The positive correlation between faecal pH and DM content in finisher cattle was expected but there was no evidence of any association between these parameters for starter or grower cattle. The data for faecal pH and DM content for grower cattle was considered to be the most reliable because the analyses were made on individual samples and there was a large amount of variation for these faecal parameters. Faecal DM content varied inversely with faecal score but the association was not strong. This suggests that a subjective, visual score may not be a reliable estimate of faecal DM content. The relationship may have been improved if a greater range of faecal scores were given (eg 1-10 instead of 1-3).

Faecal nitrogen content would be expected to increase in response to a greater level of microbial fermentation the hindgut (Orskov *et al.* 1970b; Mason *et al.* 1981). Measurement of faecal nitrogen content did not identify differences between diets for the starter or finisher groups however, for the starter cattle, faecal nitrogen content varied inversely with faecal starch content. This implies that diets that produced a low amount of faecal starch in the starter cattle, such as diet 1, were in fact supplying fermentable starch to the hindgut. Hindgut fermentation may have increased faecal nitrogen content and simultaneously reduced the amount of starch reaching the faeces however this was not reflected by a lower faecal pH or DM content. Dietary nitrogen or rumen microbial protein may have been having more of an influence on faecal nitrogen content than any microbial protein arsing from hindgut fermentation.

Feed intake data were not available for this experiment but this information would have been useful to relate to the measured faecal parameters. If the intake of starch is low, such as for the starter cattle, the digestibility of starch in different diets may be equal (Xiong *et al.* 1991). On the other hand, the higher starch intakes of the finisher cattle may have challenged the digestive capacity of the animals and were more likely to highlight differences between diets and animals.

4.5 Conclusions

Diets based on poorly steam flaked wheat and barley are likely to result in more faecal starch than similar diets based on well steam flaked wheat and barley. This is probably due to reduced starch digestion associated with less extensive steam treatment and coarser flakes.

Faecal starch content from animals fed diets containing steam flaked wheat and barley is likely to rise with an increase in the proportion of tub ground, high moisture corn.

There was significant between-animal variation in faecal pH and DM content. The relationship between these faecal parameters and their value in predicting hindgut fermentation and faecal starch levels was not clearly defined.

Faecal nitrogen content is difficult to interpret since it is likely to be affected by dietary nitrogen, microbial growth and nitrogen losses into the gut. On its own, this measurement may be an unreliable predictor of bacterial activity in the hindgut.

Chapter 5. Use of *In vitro* and *In sacco* Assays to Compare Different Cereal Grains and Processing Methods

5.1 Introduction

The site of digestion may be important in determining the overall efficiency of starch utilisation and the characteristics of an ideally processed grain are slow digestion and fermentation in the rumen with efficient/complete starch digestion in the small intestine. A processing technique to achieve this may be difficult to identify given that attempts to improve starch digestibility in the small intestine normally lead to a concomitant increase in the rumen fermentability of the grain (Owens *et al.* 1986; Plascencia and Zinn 1996).

The larger particle size of steam flaked grain results in a smaller available surface area than dry rolled grain. As fermentation is inversely related to particle size (Galyean *et al.* 1981) the steam flaking of grain could theoretically reduce microbial attachment and the rate of fermentation in the rumen. Once in the small intestine, the chemically altered starch may still be readily digestible.

In practice, the steam flaking of corn and sorghum has been repeatedly shown to increase both starch digestion in the rumen and small intestine compared with dry rolled grain (Theurer 1986; Huntington 1997; Swingle *et al.* 1999). This conflicts with the theory that steam flaking might reduce rumen fermentation. Less is known of the effects of steam flaking barley and wheat. The *in sacco* rate of starch digestion of steam rolled wheat has been shown to be lower than that of dry rolled wheat (Kreikemeier *et al.* 1990a) which may be beneficial in terms of reducing the risk of acidosis. There is also evidence that the steam flaking of wheat (Zinn 1994) and barley (Yang *et al.* 2000) may increase starch digestibility in the small intestine.

Compared to diets based on low flake density (well flaked) grain, diets of high flake density (poorly flaked) grain increase the amount of starch reaching and subsequently being digested in the small intestine (Zinn 1990a; Theurer *et al.* 1999b). The result is reduced whole tract starch digestibility for corn and sorghum because of incomplete digestion before the hindgut. Varying flake density of wheat has been reported to have little effect on whole tract starch digestibility in cattle (Zinn 1994) which suggests that varying steam flaking parameters may be used to shift the site of starch digestion from the rumen to the small intestine without decreasing overall starch digestibility or adversely affecting the hindgut.

The experiments reported in this chapter were conducted to compare the rumen and small intestinal digestibility of different cereal grains and the effects of different processing methods with the overall objective of identifying grain/processing combinations that result in a shift in the site of starch digestion from the rumen to the small intestine. The hypothesis was that moderately steam flaked wheat and barley will ferment less in the rumen and supply more readily digestible starch to the small intestine than dry rolled grain.

5.2 Materials and Methods

5.2.1 Experimental design

In vitro and *in sacco* techniques were used to compare the rate of rumen fermentation and the *in vitro* enzyme assay was used to estimate the small intestinal digestibility of starch of different cereal grains and the effect of different processing methods.

Nine grain/processing method combinations tested were:

1. Dry rolled barley (roller gap of 0.5 mm)
2. Poorly steam flaked barley (49.8 kg/hL)
3. Well steam flaked barley (36.8 kg/hL)
4. Dry rolled wheat (roller gap of 0.5 mm)
5. Poorly steam flaked wheat (56.9 kg/hL)
6. Well steam flaked wheat (46.5 kg/hL)
7. Tub ground, high moisture corn
8. Dry rolled sorghum (roller gap of 0.5 mm)
9. Well steam flaked sorghum (37.8 kg/hL)

The six grain/processing method combinations that used moisture are shown in Plate 5.1.

5.2.2 In vitro *fermentation*

System 1

Grains were incubated in the 'as-fed' form using system 1. Whole wheat and barley and high moisture corn were also finely ground (0.5 mm screen) for the assay but sorghum grains were not included as they were unavailable at the time. The assay was repeated on two separate days to provide two replicates.

System 2

Grains were incubated in both the 'as-fed' and finely ground (0.5 mm screen) form using system 2. Grains were finely ground to determine if processing method altered digestibility for reasons in addition to available surface area. Grains were fermented for 4 h instead of 5 h and the assay was repeated on two separate days, with two replicates of each grain tested on each day.

Analysis

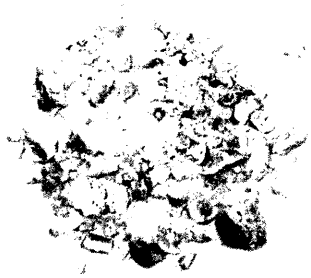
All grains were analysed for DM and starch content.

A statistical analysis was conducted to determine the main effects of grain and processing method in both fermentation systems on total acid production *in vitro*. Models also contained the grain by processing interaction, replicate and the replicate by day interaction where appropriate. The additional effect of grain treatment before fermentation (fine grinding vs 'as-fed') and interactions with grain and processing method was tested in the analysis of data from system 2. Only data for wheat and barley were subjected to full analysis. Data were initially analysed using a multifactor analysis of variance to determine significant main and interaction effects. Differences between means were determined using the least significant difference (LSD) method ($P<0.05$).

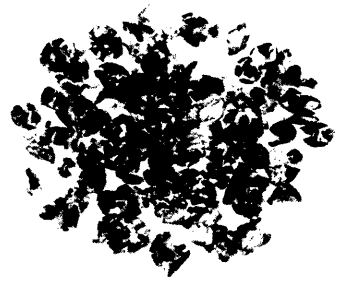
Regression analysis was carried out using the linear regression method available in S-plus (MathSoft 1999). High moisture corn was included in the regression analysis of the data in system 1.

5.2.3 In vitro *enzymatic digestion*

The enzymatic digestibility of starch *in vitro* was determined for each grain.



Ground Maize



Well Flaked Sorghum

Poorly Flaked Barley

Well Flaked Barley

Poorly Flaked Wheat

Well Flaked Wheat

Plate 5.1 Processed cereal grains.

5.2.4 In sacco *fermentation*

The *in sacco* rumen digestion kinetics of the three barley and three wheat grains were determined by their incubation in the rumens of the same two steers from which rumen fluid was collected for the *in vitro* fermentation assays (chapter 3). Bags were placed into the rumen at 0900 h (0 h) when the steers were fed and removed after 6, 12, 24, 48 and 72 h of incubation. Replicate measurements were made over two weeks.

Data from each fermentation period were analysed separately using analysis of variance. Models contained replicate, animal, grain and processing along with the appropriate interactions. The multiple comparison function was used to test for significance among treatment means by use of the Tukey method with a 95% confidence interval (MathSoft 1999). Owing to the consistency of the differences at each time, only data from the 6 h and 72 h exposure periods are presented. The data for 6 h were analysed in order to assess the differences between grains and processing methods after a relatively short fermentation period. The 72 h data were analysed to assess if the difference identified at 6 h still persisted after this relatively long incubation period.

In sacco fermentation studies of high moisture corn, dry rolled sorghum and well flaked sorghum were conducted in parallel with the barley and wheat grain studies. Statistical analyses were not performed on the results for these three grains because they did not fit into the balanced experimental design used for wheat and barley.

In a separate study, an opportunity arose to measure the *in sacco* rumen digestion kinetics of dry rolled wheat, barley, sorghum and oats. The wheat, barley and sorghum grains were not from the same source as the grains used in all other experiments described in this chapter but the data obtained in the study was relevant because it highlighted differences between grains.

The procedure was exactly the same as described above, with some important modifications. Three steers were used instead of two and replicates were made on the same day. As there were four different grains, five removal times and two replicates for each time, 40 bags were placed in each rumen. Grains and bag residues were analysed for starch content and starch disappearance *in sacco* was calculated in the same way as DM disappearance.

The statistical analysis was as described above with data from each fermentation exposure period analysed separately. Models included replicate, animal, grain and the animal by grain interaction.

5.3 Results

5.3.1 In vitro *fermentation – system 1*

There were no significant differences in total acid production *in vitro* for wheat and barley and no interaction between grain and processing method. There were differences between processing methods ($P<0.01$) and measurement day ($P<0.05$). The fermentation characteristics of the grains are presented in Table 5.1. While not statistically analysed, the data for high moisture corn was included for the purposes of comparison.

Table 5.1 Fermentation characteristics *in vitro* for different grains and processing methods – system 1.

Grain	Processing method	Total starch in grain (% DM)	Acid production (mmol)		pH	Rate of gas production (mL/min)
			Lactate	Total acid		
Wheat	Poorly flaked	67.3	1.9	13.7	7.0	3.6
	Dry rolled	69.3	1.5	16.5	6.9	4.1
	Well flaked	66.5	4.6	24.3	6.9	5.3
	Ground*	69.3	11.2	42.7	5.9	7.7
Barley	Poorly flaked	59.6	0.3	11.2	7.1	3.0
	Dry rolled	59.3	1.1	17.6	7.0	4.2
	Well flaked	59.8	3.0	20.3	6.9	5.0
	Ground*	59.3	19.6	53.0	5.4	7.7
Corn	Tub ground, high moisture	70.3	0.3	16.0	6.9	3.5
	Ground*	70.3	0.4	35.1	6.1	8.2
	Average	65.1	4.4	25.0	6.6	5.2

*Ground through a 0.5 mm screen

There was a positive correlation between the rate of gas and total acid production ($P<0.01$; $R^2=0.82$) and between lactate and total acid production ($P<0.01$; $R^2=0.75$). When corn was omitted from the analysis, the relationship between lactate and total acid production improved ($P<0.01$; $R^2=0.95$).

Differences between grain processing methods in the total amount of acid produced *in vitro* are presented in Figure 5.1. Finely ground, unprocessed grain produced significantly more acid *in vitro* than steam flaked or dry rolled grain ($P<0.05$) whilst well flaked grain produced more acid *in vitro* than poorly flaked grain ($P<0.05$). Dry rolled grain fermented to a level between the poorly and well flaked grains.

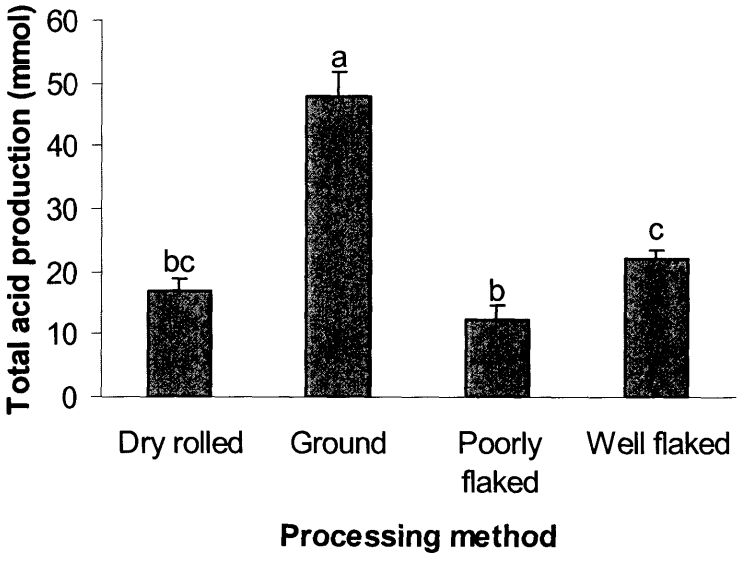


Figure 5.1 The average effect of processing method on total acid production (mmol) *in vitro* (combined data for wheat and barley). Means with different superscripts (a, b, c) differ significantly ($P<0.05$). Error bars ±SEM.

5.3.2 In vitro *fermentation - system 2*

Ground grains produced over twice as much acid *in vitro* as the 'as-fed' grains ($P<0.001$) (Table 5.2). While not statistically analysed, the data for corn and sorghum were included in Table 5.2 for the purposes of comparison.

For wheat and barley, there was an interaction ($P<0.001$) between grain processing method and pre-fermentation treatment but no significant variation between days. Figure 5.2 shows that, when grains were fermented 'as-fed', the well flaked grains produced 40% more acid *in vitro* than the dry rolled grains. In contrast, when grains were all finely ground before being fermented, the dry rolled grains produced 24% more acid *in vitro* than the well flaked grains.

Table 5.2 Total acid production (mmol) *in vitro* for different grains, processing methods and treatments before fermentation – system 2.

		Pre-fermentation treatment	
Grain	**Processing method**	**As-fed**	**Finely ground***
Wheat	Poorly flaked	2.9	7.7
	Dry rolled	3.6	10.6
	Well flaked	4.7	8.7
Barley	Poorly flaked	3.9	9.4
	Dry rolled	3.5	11.8
	Well flaked	5.3	9.3
Corn	Tub ground, high moisture	5.2	7.7
Sorghum	Dry rolled	1.9	6.0
	Well flaked	6.1	6.5
	Average	4.1	8.6

*All grains finely ground (0.5 mm screen)

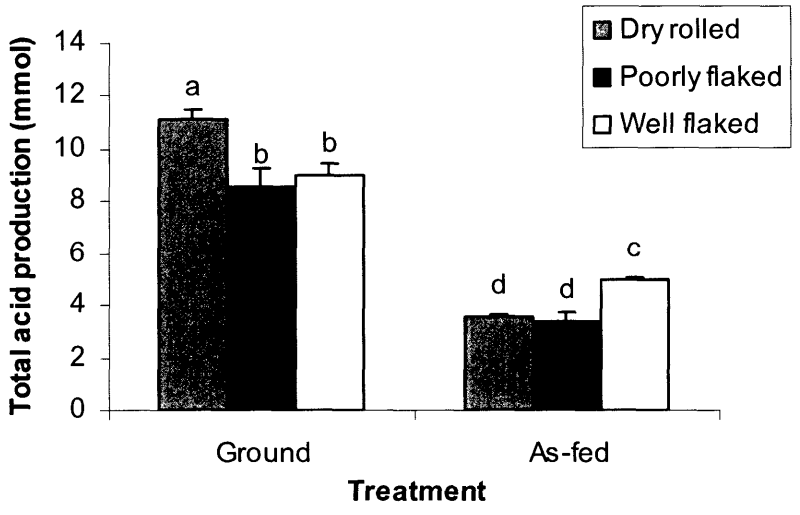


Figure 5.2 The interaction between grain processing method and pre-fermentation treatment on total acid production (mmol) *in vitro* (combined data for wheat and barley). Means with different superscripts (a, b, c, d) differ significantly ($P<0.05$). Error bars ±SEM.

5.3.3 In vitro *enzymatic digestion*

Table 5.3 shows that well flaked grains had the highest enzymatic digestibility of starch *in vitro*, followed by poorly flaked grains and finally dry rolled grains. The poorly flaked grains were far more digestible than the corresponding dry rolled grains. Well flaked wheat and barley were both approximately twice as digestible as the dry rolled equivalents. The greatest improvement in enzymatic digestibility of starch (over dry rolling) was achieved when sorghum was well flaked (82.4 vs 28.0%).

Table 5.3 Enzymatic digestibility (% of total starch) of starch *in vitro* for different grains and processing methods.

Grain	Treatment	Total starch in grain (% DM)	Enzymatic digestibility (% of total starch)
Wheat	Dry rolled	69.3	43.3
	Poorly flaked	67.3	76.9
	Well flaked	66.5	85.7
Barley	Dry rolled	59.3	39.0
	Poorly flaked	59.6	66.7
	Well flaked	59.8	83.7
Corn	Tub ground, high moisture	70.3	46.5
Sorghum	Dry rolled	73.9	28.0
	Well flaked	75.5	82.4
	Average	66.8	61.4

5.3.4 In sacco *fermentation*

The disappearance of grain DM *in sacco* over time for processed barley and wheat is presented in Figure 5.3. A significant interaction between grain and processing method was observed at 6, 12, 24 and 72 h for DM disappearance *in sacco*.

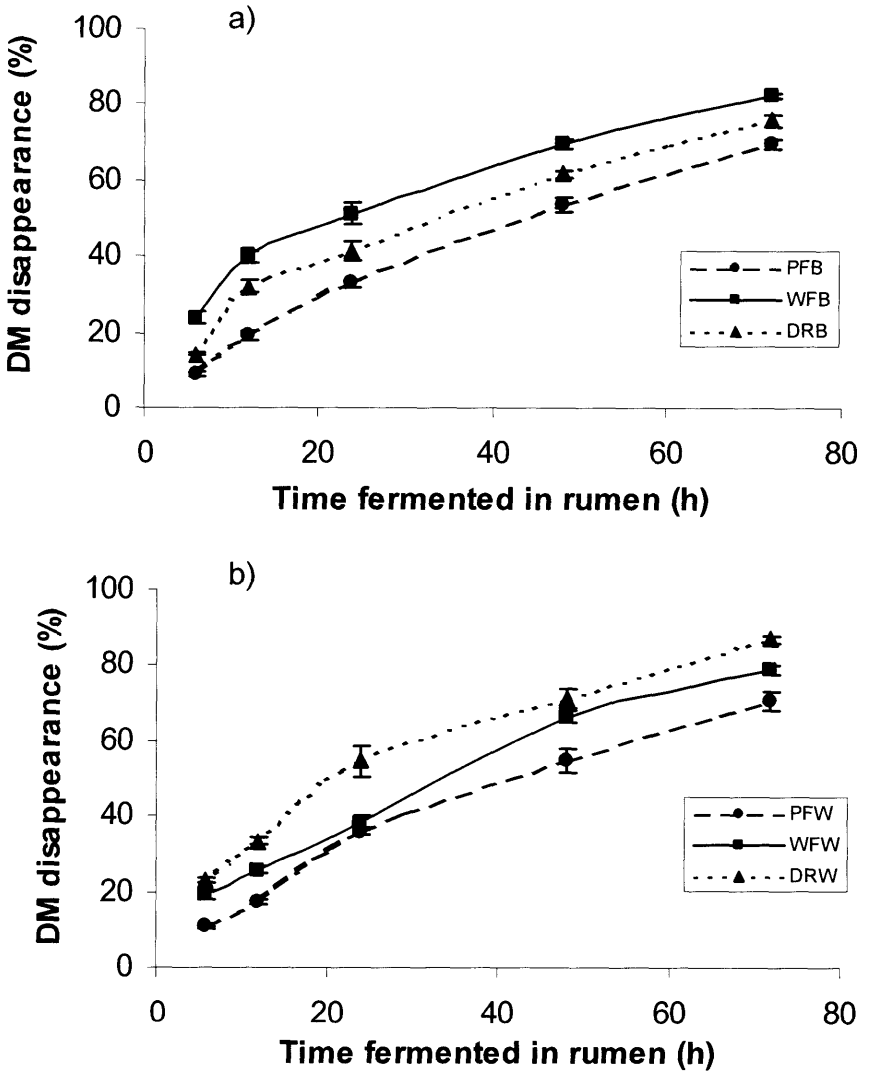


Figure 5.3 Disappearance of grain DM (% of total DM) *in sacco* over time for (a) dry rolled barley (DRB), poorly flaked barley (PFB) and well flaked barley (WFB) and (b) dry rolled wheat (DRW), poorly flaked wheat (PFW) and well flaked wheat (WFW). Each value is the mean of four observations (1 bag/d x 2 d/steer x 2 steer). Error bars ±SEM.

Table 5.4 Disappearance of grain DM (% of total DM) *in sacco* after 6 h in the rumen.

	Dry rolled	**Poorly flaked**	**Well flaked**
Barley	$14^{A,c}$	$9^{A,c}$	$24^{A,d}$
Wheat	$23^{B,c}$	$11^{A,d}$	$19^{A,c}$

Means within a column with different superscripts (A, B) differ significantly ($P<0.05$); SE=2.2

Means within a row with different superscripts (c, d) differ significantly ($P<0.05$); SE=2.2

Table 5.4 shows that there was an interaction ($P<0.01$) between grain and processing method at 6 h. Dry rolled wheat had fermented to a greater extent than dry rolled barley ($P<0.05$) and poorly flaked wheat ($P<0.05$). Well flaked barley had fermented to a greater extent than dry rolled and poorly flaked barley ($P<0.05$). There were significant differences between the extents of fermentation measured one week apart ($P<0.01$) but no variation between the steers.

Table 5.5 Disappearance of grain DM (% of total DM) *in sacco* after 72 h in the rumen.

	Dry rolled	**Poorly flaked**	**Well flaked**
Barley	$76^{A,d,c}$	$69^{A,c}$	$82^{A,d}$
Wheat	$87^{A,d}$	$71^{A,c}$	$79^{A,d,c}$

Means within a column with different superscripts (A, B) differ significantly ($P<0.05$) SE=3.4

Means within a row with different superscripts (c, d) differ significantly ($P<0.05$) SE=3.4

Table 5.5 shows that there was an interaction ($P<0.05$) between grain and processing method at 72 h. Well flaked barley had fermented more extensively than poorly flaked barley ($P<0.05$) whilst dry rolled wheat had fermented more than poorly flaked wheat ($P<0.05$). For this period of fermentation, there was no significant variation between time of the test but there were differences between steers ($P<0.05$).

Additional *in sacco* fermentation work

Figure 5.4 shows that well flaked sorghum had a greater grain DM disappearance *in sacco* than dry rolled sorghum. Tub ground, high moisture corn appeared to lose more DM than dry rolled sorghum.

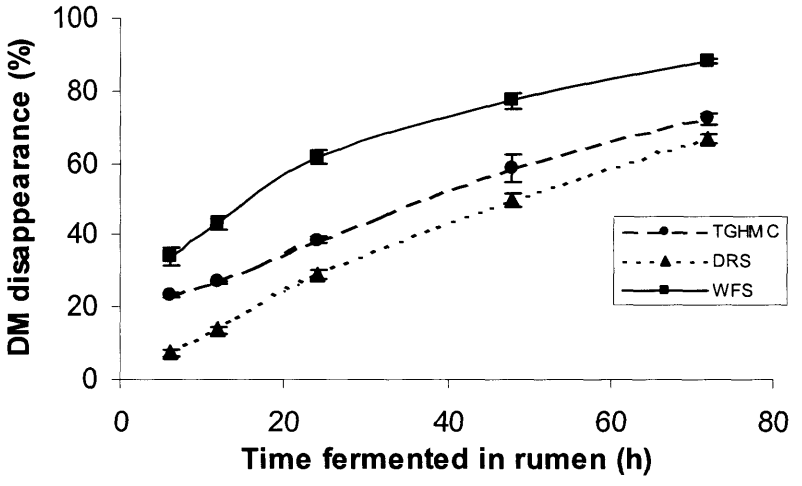


Figure 5.4 Disappearance of grain DM (% of total DM) *in sacco* over time for dry rolled sorghum (DRS), well flaked sorghum (WFS) and tub ground, high moisture corn (TGHMC). Each value is the mean of four observations (1 bag/d x 2 d/steer x 2 steer). Error bars ±SEM.

In the second study, using 4 grains (Table 5.6), dry rolled wheat and barley lost a large proportion of DM in the first 24 h compared with dry rolled oats and sorghum. Dry rolled oats lost more DM than dry rolled sorghum in the first 24 h but had the lowest DM loss at 72 h. There were significant differences between animals within treatments at 6, 12, 48 and 72 h.

Table 5.7 shows that for dry rolled wheat, barley and oats, most of the starch fermented over the 72 h had disappeared within the first 24 h. In contrast loss of starch from dry rolled sorghum was evident between 48 and 72 h. There were significant differences between animals within treatments at 6, 12, 48 and 72 h. There was a significant interaction between grain and animal for starch disappearance at 12, 48 and 72 h.

Table 5.6 Disappearance of grain DM (% of total DM) *in sacco* over time for different dry rolled grains.

Grain	DM in grain (%)	Time fermented in the rumen (h)				
		6	12	24	48	72
Wheat	87.9	68[a]	79[a]	87[a]	90[a]	92[a]
Barley	88.2	54[b]	69[b]	79[b]	82[b]	88[a]
Oats	88.2	45[c]	52[c]	60[c]	62[c]	68[b]
Sorghum	87.6	35[d]	48[c]	54[d]	70[d]	83[c]
Average	88.0	51	62	70	76	83
SE		1.7	1.4	1.5	2.1	1.6

Means within a column with different superscripts (a, b, c, d) differ significantly (P<0.05)

Table 5.7 Disappearance of grain-starch (% of total starch) *in sacco* over time for different dry rolled grains.

Grain	Total starch in grain (% DM)	Time fermented in the rumen (h)				
		6	12	24	48	72
Wheat	66.5	85[a]	93[a]	98[a]	98[a]	99[a]
Barley	61.1	70[b]	85[b]	94[b]	95[a]	99[a]
Oats	31.3	68[b]	76[c]	86[c]	83[b]	88[b]
Sorghum	78.6	44[c]	54[d]	59[d]	76[c]	87[b]
Average	59.4	67	77	84	88	93
SE		1.1	0.8	1.0	1.3	1.1

Means within a column with different superscripts (a, b, c, d) differ significantly (P<0.05)

5.4 Discussion

5.4.1 In vitro *fermentation and enzymatic digestion*

The *in vitro* assays allow examination of the combined attributes of fermentability (total acid production) and enzymatic starch digestibility and the relationship is shown in Figure 5.5. The grid has been arbitrarily added to the figure to highlight the differences between processing methods and to aid in discussion. The points where the grid lines cross the axis of the figure are of no significance. Grains that fall into the top-left quadrant of the grid are considered to be the most desirable for supplying digestible starch to the small intestine. Poorly flaked wheat and barley produced relatively low amounts of acid *in vitro* (reflecting a slow rate of fermentation) yet maintained a high enzymatic digestibility of starch *in vitro*. In contrast, tub ground, high moisture corn fell into the bottom-right quadrant of the grid and would be least likely to supply digestible starch to the small intestine.

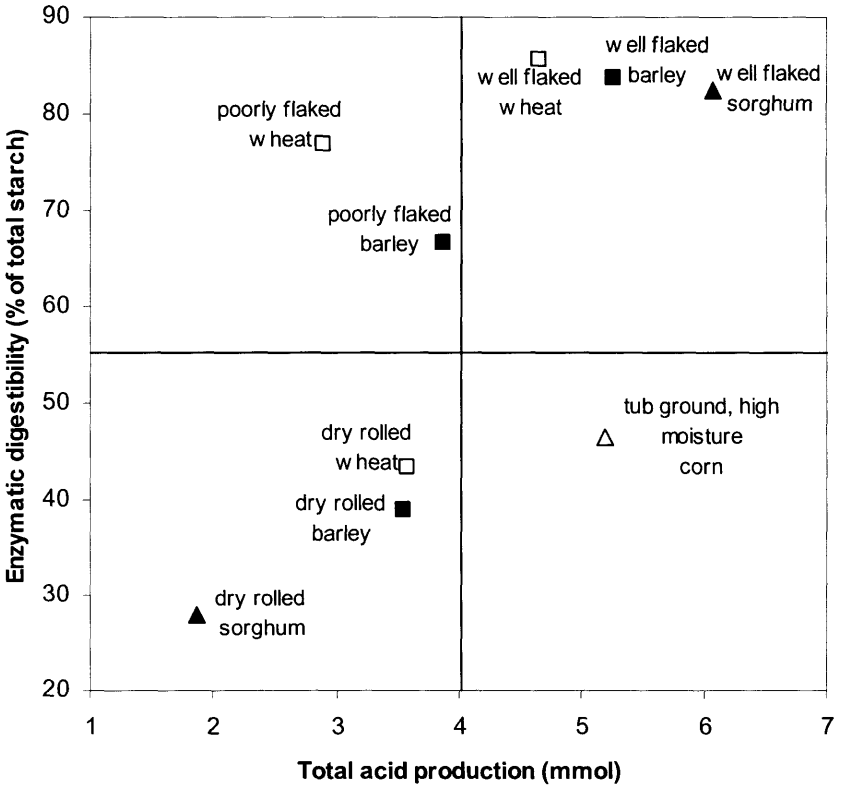


Figure 5.5 Relationship between fermentative (mmol acid produced in system 2) and enzymatic digestibility (% of total starch) of starch *in vitro* for different grains and processing methods.

When grains were fermented 'as-fed,' system 1 and system 2 produced similar results. Well flaked grains fermented at a faster rate than poorly flaked grains and this finding is consistent with results for sorghum (Xiong *et al.* 1991; Theurer *et al.* 1999b) and corn (Zinn 1990a). Faster fermentation of the well flaked grains is likely to be due to a larger surface area (thinner flake) and/or greater alteration of the starch and protein matrix structure (Rooney and Pflugfelder 1986).

If the only objective of grain processing is to maximise starch digestibility in the rumen, the results for system 1 indicate that fine grinding will be effective. However, the risk of acidosis means that the fine grinding of wheat and barley would not be recommended in practice. The high total acid and gas production for finely ground grains was associated with an increase in lactic acid and a lower fluid pH. Bird *et al.* (1999) studied a range of grains and found that lactic acid was positively correlated with total acid production in this *in vitro* assay and their results are consistent with our data when high moisture corn is excluded. As the build-up of lactic acid is closely associated with the incidence of acidosis (Rowe 1999; Al Jassim and Rowe 1999) finely ground wheat and barley carry a greater acidosis risk than the other 'as-fed' grains.

In contrast to finely ground wheat and barley, finely ground corn produced a relatively large amount of total acid in system 1, without significant amounts of lactic acid or a major depression in pH. As fine-grinding removes particle size effects, this variation is a result of inherent differences between the grains. Bird *et al* (1999) also reported this pattern of fermentation for ground corn and sorghum. It is therefore likely that there is a larger margin of safety when feeding corn or sorghum, even finely ground, than when feeding wheat and barley.

The interaction observed between method of grain processing and fine-grinding prior to fermentation in system 2 was unexpected and is difficult to explain. The fact that finely ground unprocessed wheat and barley produced more acid than finely ground well or poorly flaked wheat and barley, suggests that the steam flaking treatment reduced the accessibility and/or digestibility of starch. There may have been some retrogradation of starch during the time that the steam flaked grain was oven-dried and hammer-milled but there is no data to support this suggestion. Furthermore, such an explanation is not consistent with the results for the *in vitro* enzyme assay (discussed below) obtained with grains that were treated in the same way. This confirms that different aspects determine digestion in the rumen and small intestine.

The *in vitro* enzymatic digestibility of the steam flaked grains was clearly higher than the dry rolled grains and is consistent with the findings of Zinn (1994). As the assay uses a finely

ground grain sample, the effects of steaming and flaking were not only related to changes in surface area. It is likely that the improved digestibility of starch in steam flaked grain was related to chemical and/or microscopic changes to starch and cellular structures.

The results also highlighted differences between grains with the digestibility of dry rolled barley being substantially higher than that of dry rolled sorghum. This is consistent with the findings of Osman *et al.* (1970) who showed that the *in vitro* digestibility of starch in untreated barley was higher than starch in untreated sorghum. In our study, the digestibility of starch in well flaked barley was similar to that of well flaked sorghum. However, Osman *et al.* (1970) reported that steam processed barley was more digestible than a similarly treated sorghum. It is likely that differences in the degree of steam flaking led to discrepancies between these two sets of results. The evidence indicates that the extent of improvement in enzymatic starch digestion is potentially much larger for sorghum than for barley or wheat – a conclusion that was also reached by Huntington (1997).

5.4.2 In sacco *fermentation*

The general conclusion drawn from the *in sacco* results is that poorly flaked grains fermented less than dry rolled and well flaked grains and this is consistent with the *in vitro* fermentation results. The observation that the rate of digestion of steam flaked wheat *in sacco* tended to be lower than that of dry rolled wheat is in agreement with the work of Kreikemeier *et al.* (1990a). There are two possible explanations for this difference. Firstly, the greater surface area created in the dry rolling process (smaller particles) may have allowed greater attachment by rumen bacteria. Secondly, the steam flaking treatment may reduce the accessibility to and/or digestibility of starch.

Significant variation between the two steers was observed at the 72 h removal time and variation between weeks was noted at 6 h. Weakley *et al.* (1983) studied *in sacco* DM and nitrogen disappearance of different feedstuffs and reported no effect due to animals or between-day variation. In contrast, Mehrez and Orskov (1977) reported variation in substrate disappearance between animals and days.

The DM disappearance *in sacco* of well flaked sorghum appeared to be greater than that of dry rolled sorghum and this supports the view that the steam flaking of sorghum will result in large improvements in rumen digestibility (Theurer 1986; Rooney and Pflugfelder 1986) compared with dry rolling sorghum.

The comparison of dry rolled grains revealed the fact that DM loss does not accurately represent starch loss for oats because of the relatively low proportion of starch in this grain. Although oat-starch is extensively digested, the fibrous hull means that the DM is more slowly digested. On the other hand, starch and DM loss occur at similar rates for wheat and barley. The results also showed that sorghum needs more fermentation time to approach the DM and starch digestibility levels that are obtained relatively quickly by wheat and barley.

5.4.3 General

The most important finding from the studies reported in this chapter is that the enzymatic digestibility of wheat and barley-starch *in vitro* was increased by poorly steam flaking, without causing an increase in the extent of fermentation *in vitro* or *in sacco*. If this pattern of digestion occurs *in vivo*, the steam flaking of wheat and barley may have important practical applications. Generally, processing techniques have only been utilised to increase starch digestibility in the rumen but the possibility of using steam flaking to maintain or even reduce fermentation may be just as useful provided that starch digestibility in the small intestine is not compromised.

It was clear that well flaked wheat and barley fermented more rapidly than poorly flaked wheat and barley. However, in contrast to our *in vitro* and *in sacco* results, the digestibility of starch in the rumen *in vivo* has been reported by others to be unaffected by flake thickness of barley (Yang *et al.* 2000) and wheat (Zinn 1994). Therefore, more work is needed to clarify the effect of flake density on the fermentability of these particular grains *in vivo*.

While the main benefit of the *in sacco* technique is considered to be the ability to compare feeds within a functioning rumen (Kitessa *et al.* 1999), confinement within the bags does not truly reflect normal feeding conditions. Weakley *et al.* (1983) suggested that bag pores may be blocked by bacterial slime which accumulates in the rumen of animals fed concentrate based diets. This would effectively block the entry of digestive microbes and enzymes. *In sacco* measurements should also be interpreted with some caution as material that is small enough to leave the bag is not necessarily completely degraded (Uden and Van Soest 1984; Perdok 1986). Fine material and additional microbial biomass can also enter and remain in the bag from the rumen (Uden and Van Soest 1984) which may again influence the result. A further limitation of the *in sacco* technique is that the end-products of fermentation cannot be collected and assessed.

The *in vitro* fermentation systems offer a relatively quick and inexpensive test suited to large numbers of samples but as with the *in sacco* technique, there is no mixing with saliva or initial

chewing of grain. It is possible that the larger particle size of the poorly flaked grains would have been reduced through the effects of chewing and rumination by the steers. Given the shortcomings associated with both methods, it is useful to look at *in sacco* and *in vitro* results together to develop the best assessment of the *in vivo* fermentation characteristics of a grain.

The major limitation of the *in vitro* enzyme assay is that finely ground grain is not a true reflection of the grain in the digesta that normally reaches the small intestine. The assay takes no account of the effects of pre-digestion in the rumen even though fermentation is known to affect the quality and quantity of starch that reaches the small intestine. An assay where grain is initially fermented by microbes before being tested for enzymatic digestibility of starch may have more relevance.

5.5 Conclusions

Surface area appears to be the main factor influencing microbial fermentation whereas 'cooking' and its effect on cellular and chemical structures seems to more strongly influence digestibility by endogenous enzymes in the small intestine.

Under the conditions of these experiments, poorly steam flaked wheat and barley did not ferment more extensively than the equivalent dry rolled grains, yet appeared to have a higher level of endogenous enzyme digestibility. Varying the degree of steam flaking may provide the means for supplying increased amounts of readily digestible starch to the small intestine without necessarily increasing the rate of fermentation in the rumen.

Chapter 6. The Relative Importance of Cooking Time and Flake Thickness on the Digestibility of Steam Flaked Barley

6.1 Introduction

The most desirable level of steam flaking is likely to differ between grain species, cultivar and even between batches of the same grain. Given the theoretical benefits of increasing small intestinal starch digestion (Harmon and McLeod 2001), the challenge is to identify the level of steam flaking that optimises intestinal digestion of starch without increasing the risk of acidosis in the rumen or hindgut.

The major objective of the experiment in this chapter was to determine changes in microbial and enzymatic digestion of barley grain, using the *in vitro* assays described in chapter 3, in response to cooking and physical pressure. The hypothesis was that well cooked, thick flakes may promote efficient enzymatic digestion of starch with less increase in the rate of starch fermentation *in vitro* than grain cooked for the same amount of time and pressed to a finer flake.

6.2 Materials and Methods

6.2.1 Grain

Barley is a widely used grain in Australian feedlots and the commonly grown malting barley cultivar, Tantangara, was used in this experiment because of its availability.

6.2.2 Experimental design

To simulate different levels of steam flaking a 3x3 treatment design comprising three different cooking times (0, 15 and 30 min) and three different flake thicknesses (1.0, 1.55 and 1.9 mm) was developed. The cooking times were set after careful consideration of industry practice and visual assessment of the moisture content and softness of grain cooked for different periods.

In addition, the digestive characteristics of unprocessed, finely ground (0.5 mm screen), barley (Tantangara) were examined along with two samples of commercially steam flaked barley. These industry samples, referred to as 'well flaked barley' (36.8 kg/hL) and 'poorly flaked barley' (49.8 kg/hL) were included to allow the experimentally flaked grains to be compared with products prepared under commercial conditions.

6.2.3 Steam flaking simulation

To simulate the steaming process, barley (90% DM) was submerged in boiling water. Boiling was used instead of steaming (industry procedure) because equipment could not be accessed to safely steam a small amount of grain under controlled conditions. The water was pre-boiled using a gas burner and heating was continued during cooking. Approximately 1.5 kg of grain was placed in a stainless steel mesh container and submerged in 10 L of boiling water for the designated time.

To simulate the flaking process, grain was pressed through a meat carver press. Immediately following cooking, the grain was placed between the plates and pressed to the desired thickness by placing galvanised iron gauges between the plates. The gauge thickness was determined using vernier callipers. The grains for each cooking time were prepared on three separate days and immediately frozen before later analysis.

6.2.4 In vitro *fermentation*

Grains were incubated in the 'as-prepared' form using system 1. Gas production was not measured in this experiment because total acid production was used as the principal measure of the rate of rumen fermentation, as suggested by Bird *et al.* (1999). Two replicates were obtained for each grain.

6.2.5 In vitro *enzymatic digestion*

Ground grain

The enzymatic digestibility of starch *in vitro* was determined for each grain. Two replicates were obtained for each treatment grain.

'As-prepared' grain

The *in vitro* enzyme assay was modified to test the digestibility of 'as-prepared' grain. The procedure remained the same except that 0.5 g of whole grain was used instead of 0.1 g of ground grain. More whole grain was used in order to provide a similar surface area for enzymatic digestion as that provided by the smaller sample of finely ground grain. Four replicates were obtained for each treatment grain.

6.2.6 Analytical analysis

All grains were analysed for DM and starch content.

6.2.7 Statistical analysis

Differences in total acid production *in vitro* and enzymatic digestibility of starch *in vitro* for steam flaked grains were analysed via multifactor analysis of variance (MathSoft 1999). Analyses were used to identify the existence and type of any interactions between cooking time and flake thickness. Differences between means were determined using the least significant difference (LSD) method ($P<0.05$). Regression analysis was performed on the *in vitro* fermentation data using the linear regression method (MathSoft 1999).

6.3 Results

6.3.1 In vitro *fermentation*

There was a quadratic relationship ($P<0.01$) between cooking time and flake thickness (Figure 6.1) for total acid production *in vitro*. The magnitude of this interaction was greatest ($P<0.05$) for the 1 mm flake thickness with the highest fermentation rates for the uncooked grain and the grain cooked for 30 min. In all cases, grains cooked for 15 min had the lowest rate of acid production.

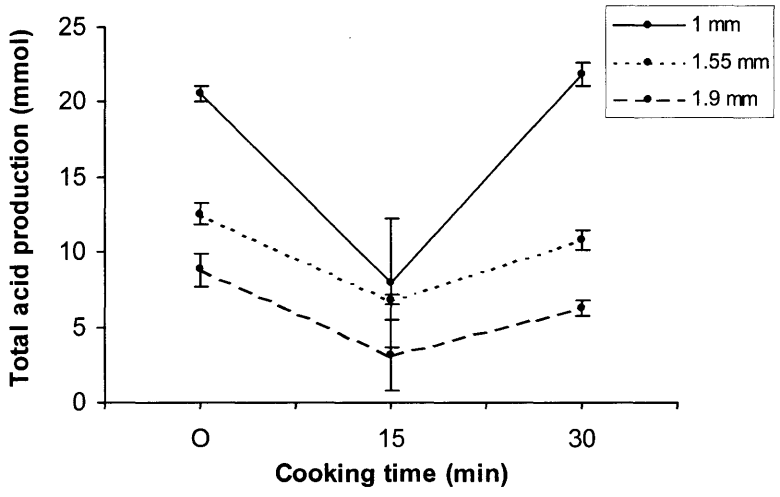


Figure 6.1 The effect of cooking time (0, 15 or 30 min) and flake thickness (1, 1.55 or 1.9 mm) on the total acid production (mmol) *in vitro* of barley. Error bars ±SEM.

Table 6.1 shows that finely ground barley fermented more than any of the other, 'as-prepared' grains (indicated by the highest total acid production and lowest pH). Some of the experimental barleys (made in steam flaking simulation) fermented to a similar extent as the poorly flaked barley (made under commercial conditions). No experimental barleys produced sufficient acid to cause a large depression in incubation fluid pH. Across all grains, total acid production was positively correlated with lactic acid production ($P<0.01$; $R^2=0.84$) and negatively correlated with pH ($P<0.01$; $R^2=0.88$).

Table 6.1 Fermentation characteristics *in vitro* of experimental and commercially steam flaked barley.

Cooking time (min)	Flake thickness (mm)	DM in grain (%)	Total starch in grain (% DM)	Acid (mmol)		pH
				Lactate	Total Acid	
0	**1**	88.6	61.9	1.4	20.5	7.1
0	**1.55**	88.3	61.4	0.2	12.5	7.3
0	**1.9**	88.7	63.3	0.0	8.8	7.4
15	**1**	62.6	62.3	0.0	7.9	7.2
15	**1.55**	62.6	59.7	0.0	6.8	7.4
15	**1.9**	63.1	60.9	0.0	3.1	7.6
30	**1**	56.1	60.3	1.2	21.9	7.0
30	**1.55**	55.5	61.8	0.1	10.8	7.3
30	**1.9**	56.3	61.1	0.0	6.3	7.3
Ground barley*		95.7	58.7	18.0	53.5	5.8
Well flaked barley**		77.6	64.0	3.4	29.1	6.8
Poorly flaked barley**		79.1	58.9	0.4	15.1	7.2
Average		72.9	61.2	2.1	16.4	7.1

*Unprocessed Tantangara barley (finely ground in a cyclotec mill through a 0.5 mm sieve)

** Commercial samples

6.3.2 In vitro *enzymatic digestion – ground grain*

Results of the *in vitro* enzyme assay indicated differences between cooking time ($P<0.001$) and flake thickness ($P<0.01$) but no significant interaction (Figure 6.2). Cooking had a much larger effect on starch digestibility than flake thickness.

Starch digestibility increased ($P<0.01$) with cooking time from 15 to 30 min with mean digestibility values of 82.9% and 87.8% respectively. A thinner flake thickness (1 mm vs 1.9 mm) also improved starch digestibility ($P<0.05$) with mean digestibility values of 88% and 83% respectively.

While not shown in Figure 6.2, the *in vitro* enzymatic digestibility of starch for ground, unprocessed Tantangara barley was 38.6%; poorly flaked barley was 68.8% and well flaked barley was 79.7%.

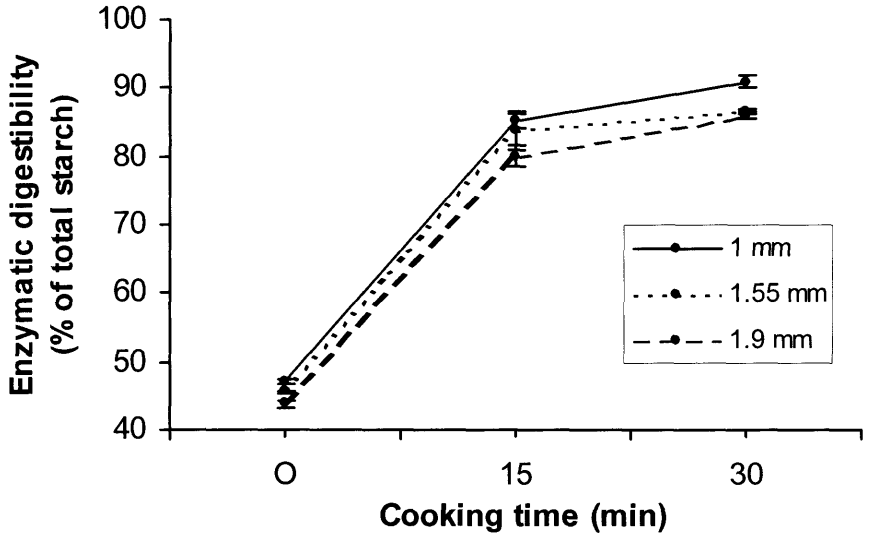


Figure 6.2 The effect of cooking time (0, 15 or 30 min) and flake thickness (1, 1.55 or 1.9 mm) on the enzymatic digestibility (% of total starch) *in vitro* of barley-starch (ground). Error bars ±SEM.

6.3.3 In vitro *enzymatic digestion – 'as-prepared' grain*

There was a quadratic relationship ($P<0.001$) between cooking time and flake thickness for enzymatic digestion of starch in 'as-prepared' grain (Figure 6.3). All values were much lower than those obtained for ground grain in this assay (section 6.3.2). Furthermore, cooking appeared to have a smaller effect on starch digestibility when grain was digested 'as-prepared' as opposed to being finely ground.

The enzymatic digestibility of starch for 'as-prepared' poorly flaked barley was 4.8% and well flaked barley was 32.8%.

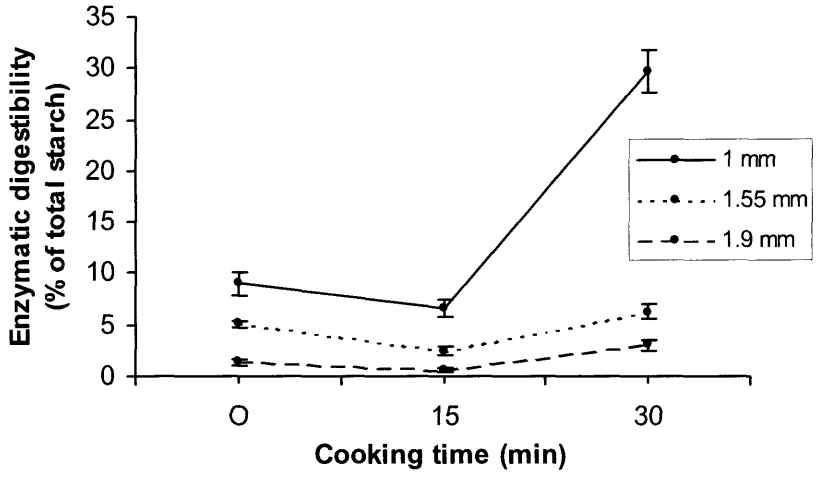


Figure 6.3 The effect of cooking time (0, 15 or 30 min) and flake thickness (1, 1.55 or 1.9 mm) on the enzymatic digestibility (% of total starch) *in vitro* of barley-starch ('as-prepared'). Error bars ± SEM.

Table 6.2 shows that for uncooked barley, fermentative and enzymatic digestibility of starch *in vitro* were inversely related to flake thickness.

Table 6.2 The influence of increasing flake thickness (1, 1.55 or 1.9 mm) on acid production (mmol) *in vitro* and enzymatic digestibility (% of total starch) of starch *in vitro* of uncooked barley.

Thickness of uncooked barley (mm)	Total acid production (mmol)	Enzymatic digestibility ('as-prepared')	Enzymatic digestibility (ground)
1	20.5^{a}	9.0^{a}	47.1^{a}
1.55	12.5^{b}	5.1^{b}	45.6^{b}
1.9	8.8^{c}	1.4^{c}	43.8^{c}
Average	13.9	5.2	45.5
SE	0.4	0.9	0.5

Means within a column with different superscripts (a, b, c) differ significantly ($P<0.05$)

6.4 Discussion

Zinn *et al.* (2002) suggested that the digestibility of starch in steam flaked grain may be more dependent on flake density than on moisture concentration before rolling but the results of the current experiment suggest that cooking time and flake density are both critical factors in determining the digestibility of steam flaked grain.

When cooking time was held constant, decreasing the flake thickness of barley increased the rate of fermentation and the extent of enzymatic starch digestion *in vitro*. However, changes in the fermentability of barley due to flake thickness were smallest for the 15 min cooking time. Similarly, Zinn (1990a) reported that when corn was steamed for a constant time (34 min at 105°C), increasing the pressure on the rolls to create grains of decreasing flake densities caused an increase in rumen and whole tract starch digestibility.

When flake thickness was held constant, increasing the cooking time from 15 min to 30 min also increased the rate of fermentation and the extent of enzymatic digestion *in vitro*. Changes in digestibility due to cooking time were greatest for the 1 mm flake thickness. Zinn (1990b) showed that enzymatic digestibility of corn-starch *in vitro* was linearly increased with steaming time (34, 47 and 67 min) but the increase in steaming time did not result in increased rumen, small intestinal or whole tract starch digestibility in steers fed diets based on these grains. In fact, steaming time had a quadratic effect on rumen starch digestion with corn steamed for 47 min having a 7% lower digestibility than the other two steaming times. Zinn (1990b) was not certain of the reason for this effect but the pattern is similar to the data in the current experiment where the fermentability of barley cooked for 15 min was relatively low.

Results from the *in vitro* fermentation assay alone suggest that there may be no benefit in cooking barley for 30 min because the same level of fermentative digestion can be achieved through pressing dry grain. It is possible that the pressure exerted by the press on the dry, uncooked grain, shattered the seed coat and disrupted starch granules. However, the benefit of cooking grain was seen in the enzyme assay (ground grain) as cooked grain had substantially higher starch digestibility than dry grain (Figure 6.2). Similarly, Zinn (1993) found that the enzymatic digestibility of ground, steam flaked barley was much higher than that of ground, dry rolled barley. This suggests that the benefit of 'cooking' is achieved at the molecular level.

Although the effect was greatest for the 30 min cooking time, the improvement in enzymatic digestibility over 15 min was relatively minor. Future work should examine the effect of intermediate cooking times between 0 and 15 min. If an enzymatic digestibility of starch around 80% could be achieved by cooking for less time, this would reduce the cost of

producing the steam flaked product and may also decrease the rate of fermentation in the rumen. Zinn *et al.* (2002) reported that as little as 5% moisture uptake during steaming allowed the adequate steam flaking of corn but this may be different for other grains.

The enzymatic digestibility of starch in ground, unprocessed Tantangara barley was lower than that of the three pressed samples for the zero cooking time. This demonstrates that even without added moisture, the pressure exerted by the press stimulated intestinal starch digestibility in ways beyond a change in surface area. Pressing uncooked grain to successively smaller flake thicknesses also improved fermentative and enzymatic starch digestion. Similar findings were reported by Bird *et al.* (1999) for the *in vitro* production of acid from grains of decreasing particle size.

When 'as-prepared' grain was used in the *in vitro* enzyme assay, the uncooked grains generally had a similar digestibility to grains cooked for 15 min (Figure 6.3). The digestibility of whole grain cooked for 30 min with a flake thickness of 1 mm was far higher than any of the other grains which highlights the importance of matching the correct cooking time and flake thickness. Finely ground and whole ('as-prepared') grain are both not truly representative of the grain in the digesta that reaches the small intestine of the ruminant. A more realistic test may use a grinding level that is intermediate of these two extremes.

Extrapolation of these findings to commercial conditions requires caution because boiling and steaming are different cooking procedures and the grain was squashed instead of being passed through heated rollers. Boiling the grain resulted in a greater increase in moisture content than observed with steaming. Steam flaked grain may have a DM content of 80% (Zinn 1990b) with moisture uptake averaging around 5%. In contrast, the DM contents of the 15 and 30 min cooked barley were 63% and 56% respectively (Table 6.1). Nevertheless, the cooking treatment applied in this experiment should still have caused irreversible swelling of the starch as this is known to occur when starch is heated in excess of 60°C in the presence of moisture (French 1973). Further, the poorly flaked commercial barley was fermented and digested in the assays to a similar extent as many of the experimental samples and this adds credibility to the steam flaking simulation.

The commercially well flaked barley was more fermentable *in vitro* than any of the experimentally flaked grains yet similar to grains cooked for 15 min in the enzyme assay for ground grain. On this evidence alone, boiling and squashing the barley appeared to be more effective than steam flaking at increasing small intestinal starch digestibility without an

accompanying increase in rumen fermentability. However, in the enzyme assay for 'as-prepared' grain, well flaked barley was similar to grain cooked for 30 min and flaked to 1 mm.

6.5 Conclusions

The *in vitro* assays described in chapter 3 provided an informative evaluation of grains altered through cooking and physical pressure, however, the *in vivo* starch digestibility of the experimental grains can only be predicted from these assays.

Fermentative and enzymatic digestion of experimentally steam flaked barley was highest when grain was cooked for 30 min and pressed to the smallest flake thickness of 1 mm. Cooking time and flake thickness were both found to influence the digestion characteristics of barley.

Moderately flaked barley (1.55 and 1.9 mm) tended to ferment less than barley of a thin flake (1 mm) but had a similar enzymatic digestibility of starch in ground grain. It is possible that barley may be steam flaked to improve starch digestibility in the small intestine without increasing fermentation in the rumen.

Chapter 7. Hindgut Acidosis in Cattle Following Carbohydrate Overload

7.1 Introduction

Lactic acidosis in the rumen of cattle is a relatively well-understood problem (Nocek 1997; Al Jassim and Rowe 1999) but less is known about the consequences of readily fermentable starch passing into the hindgut. Evidence suggests that hindgut acidosis may be important in sheep (Lee 1977; Godfrey *et al.* 1992) but very little comparable work has been done in cattle. Zust *et al.* (2000) showed that acidosis can occur in the bovine hindgut but this experiment did not indicate whether or not it is a problem under practical conditions.

Few studies have reported simultaneous measurements taken from both the rumen and hindgut in cattle following carbohydrate overload. However, even in normal grain-based feeding scenarios, acidic conditions can arise in the hindgut when the proportion of grain in the diet and/or feed intake is increased (Grovum and Hecker 1973; Diez-Gonzalez *et al.* 1998). Under these conditions, there is good reason to expect that faecal pH will fall (DeGregorio *et al.* 1982) and faecal lactate concentration will increase (Siciliano-Jones and Murphy 1989b).

The study reported in this chapter was designed to investigate the effects of a carbohydrate overload on rumen and hindgut fermentation in beef cattle and the possible effects of acidosis on DMI. The hypothesis was that hindgut acidosis plays an important role in the overall pathogenesis of grain-poisoning and should be considered under conditions of grain feeding where small intestinal starch digestion is likely to be incomplete.

7.2 Materials and Methods

7.2.1 Animals and management

Eighteen Angus heifers (24 months, 355 kg) were used in the experiment. These animals had been grazing dry, annual pastures for at least six weeks prior to being moved into individual pens (100 m^2) with a weighable feed bin and free access to water. For the initial two weeks in the pens they were fed a diet comprising 85% hay and 15% barley *ad libitum*.

Animals were weighed just prior to being given an artificial carbohydrate overload where ground wheat was administered into the rumen via a stomach tube at a rate of 20 g/kg LW on two occasions, separated by 24 h. Each dose of grain was given in approximately 30 seconds.

The experiment was constrained to operate within the boundaries of a larger acidosis trial however the data was considered valuable in that it involved simultaneous sampling from the rumen and faeces. This model was chosen to induce acidosis to (a) ensure that acidosis was created, to (b) control exactly how much grain each animal received and to (c) attempt to avoid complicating factors such as an animal having a feed of hay or drink of water.

Cattle continued to be offered the pre-challenge diet *ad libitum* during the grain challenge period and for five days after the second dose of wheat. Daily feed intake was recorded for the entire period that the cattle were kept in the pens.

7.2.2 Sampling

Rumen fluid and faeces were sampled immediately prior to the administration of the first dose of wheat (0 h) and again at 12, 24, 36, 48 and 72 h. To facilitate this sampling, animals were moved along a race and held in a squeeze chute in order to obtain rumen fluid via a stomach tube fitted to a brass sampling bulb with a blind end and small holes (0.5 mm diameter).

The stomach tube apparatus and method have been found to give similar pH results to samples taken via rumen cannula in sheep and cattle (J. Rowe, pers. comm.). The sampling bulb is effectively swallowed by the animal and moves quickly down the oesophagus. Negative pressure was not applied to the sampling tube until the bulb was completely in the rumen contents. A sample of rumen fluid was then drawn into a 60 mL syringe. There was no negative pressure on the sampling tube as the bulb was withdrawn from the oesophagus. Examination of rumen fluid gave no indication of salivary contamination.

Rumen fluid pH was immediately determined and approximately 8 mL of rumen fluid was acidified (pH 2-3) and frozen. Faecal samples were taken directly from the rectum and faecal pH was immediately determined before samples were acidified (pH 2-3) and frozen. Rumen and faecal samples were later analysed for lactic acid and VFA concentrations.

7.2.3 Animal well-being

Animals displaying signs of acidosis, particularly ataxia, were treated immediately with. intramuscular injections of long-acting Benicillin (Ilium Veterinary Products, 10-20 mL), Vitamin B_{12} (Troy Laboratories Pty Ltd, 1 to 4 mL) and an anti-inflammatory (Dexapent, Ilium Veterinary Products, 10 to 30 mg). In addition, approximately 4 L of rumen fluid taken from fistulated animals (pasture grazed) was administered orally. If after treatment the rumen pH of any animal remained below 6.0, sodium bicarbonate (1 kg) was mixed with a further rumen fluid inoculum.

Any animal that had failed to resume eating by 72 h after the grain challenge was also treated with B vitamins and rumen fluid as described above. Sodium bicarbonate was again given to any animals with a rumen fluid pH below 6.0.

Animals that developed severe acidosis and failed to respond to subsequent treatment were euthanased.

7.2.4 Statistical analysis

Data was analysed using analysis of variance in S-plus (MathSoft 1999). Mean pH and lactate concentrations in the rumen and faeces were compared using the 5% least significant difference (LSD) method. Regression analysis was performed using the linear regression method.

7.3 Results

The carbohydrate overload model used in this experiment produced severe acidosis as indicated by 14 of the 18 animals developing rumen pH<5.5. Between 72 and 96 h after the initial grain challenge, seven of the heifers required veterinary treatment and two of these animals were euthanased.

The changes in rumen and faecal pH and lactate concentrations over time are presented in Figure 7.1. Faecal pH had decreased significantly by 12 h and was associated with a marked increase in faecal lactate concentration. In contrast, it was 36 h after the first grain challenge before similar changes were noted in the rumen. By this time, faecal pH was increasing and lactate concentrations were decreasing.

Lactic acid concentration (mmol/L) was closely associated with pH in the rumen ($P<0.01$; $R^2=0.94$) and faeces ($P<0.01$; $R^2=0.89$).

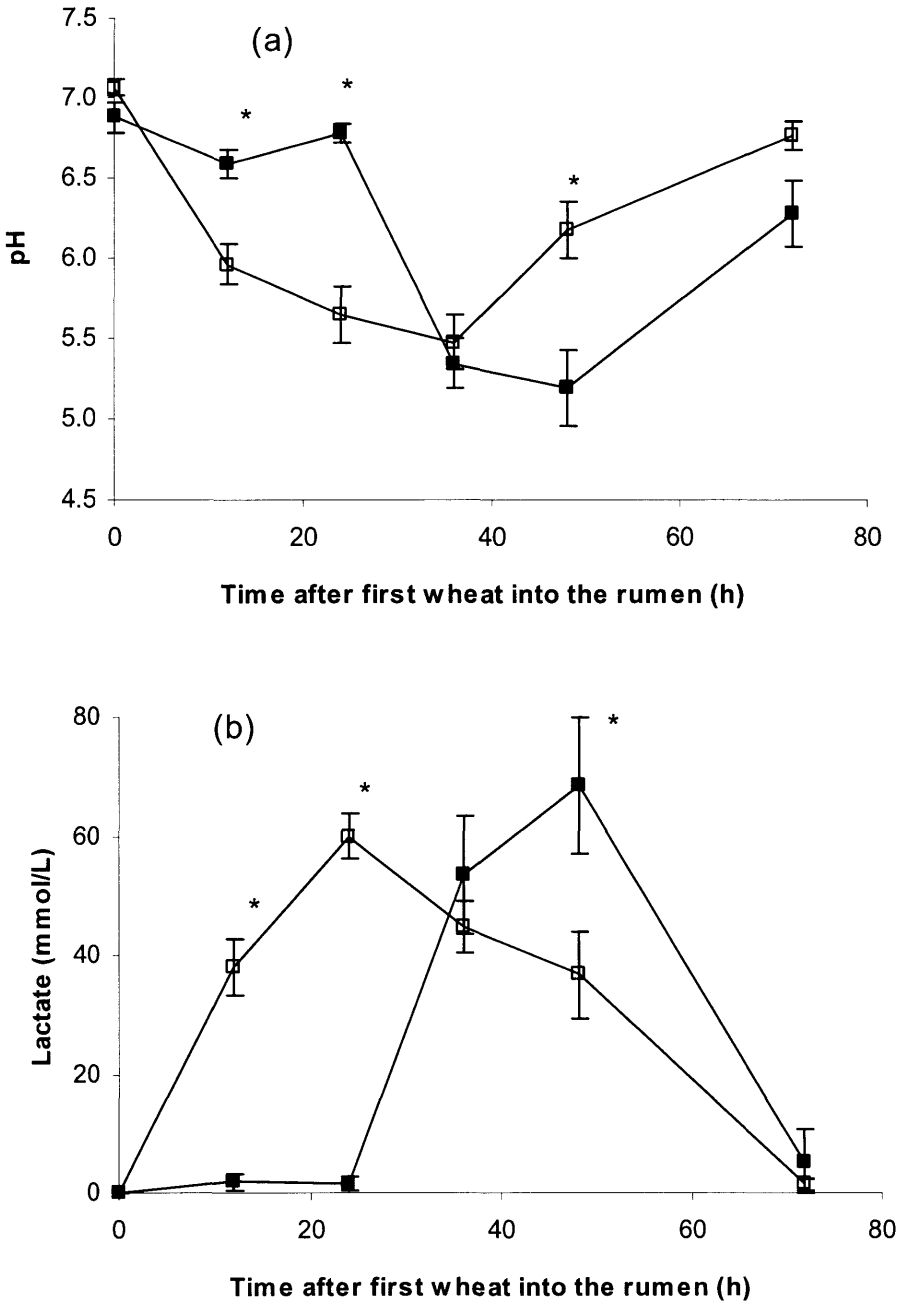


Figure 7.1 Changes in (a) pH and (b) lactate (mmol/L) in the rumen (■) and faeces (□) following carbohydrate overload at 0 and 24 h. Error bars ± SEM. * Rumen differs ($P<0.05$) from faeces.

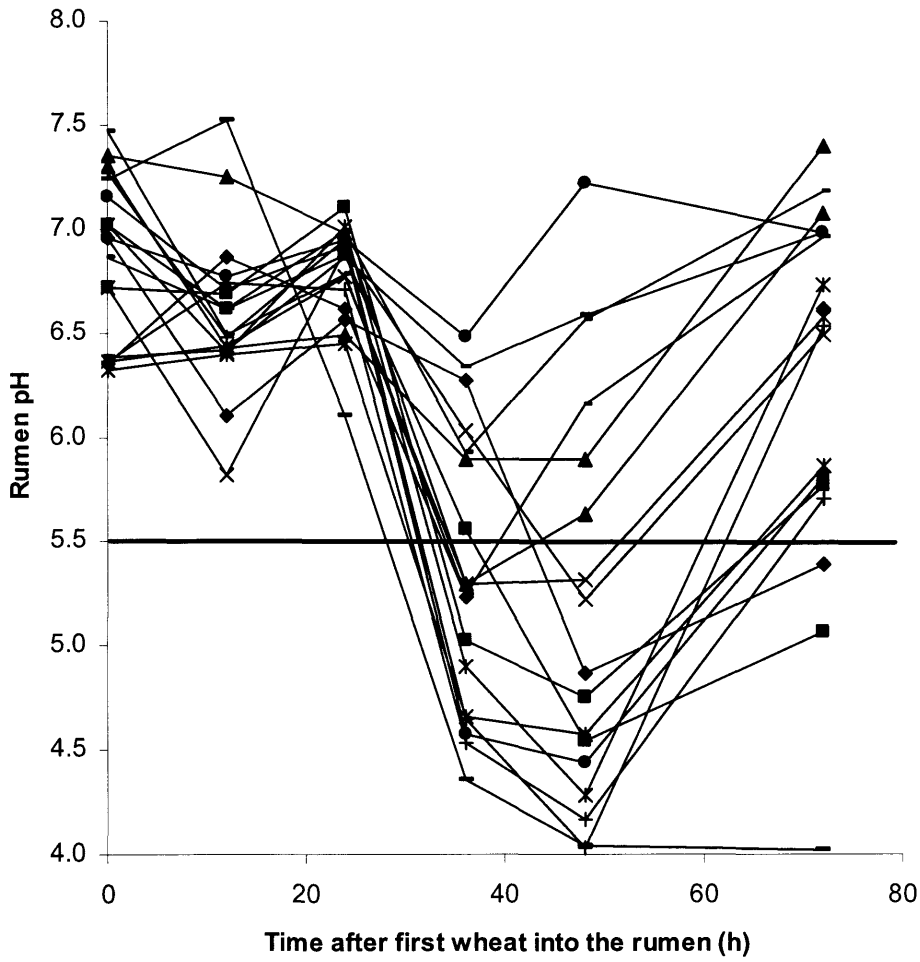


Figure 7.2 Variation in rumen pH for individual animals (each with a different legend) following carbohydrate overload at 0 and 24 h. The black line indicates the theoretical pH threshold before the onset of sub-clinical acidosis (Slyter 1976).

Figure 7.2 shows the between-animal variation in rumen pH over time. The extent of the variation was greatest at 48 h (CV=19.1%), at which time the average pH was the lowest and ranged between 4.03 to 7.22. In comparison, the CV at 0 h was 5.6%. Likewise, rumen lactate levels ranged from 0 to 141.2 mmol/L at 48 h (data not presented). It is interesting to note that some animals experienced a prolonged period of time when their rumen pH was below 5.5 whereas in other animals, rumen pH never fell below 5.5.

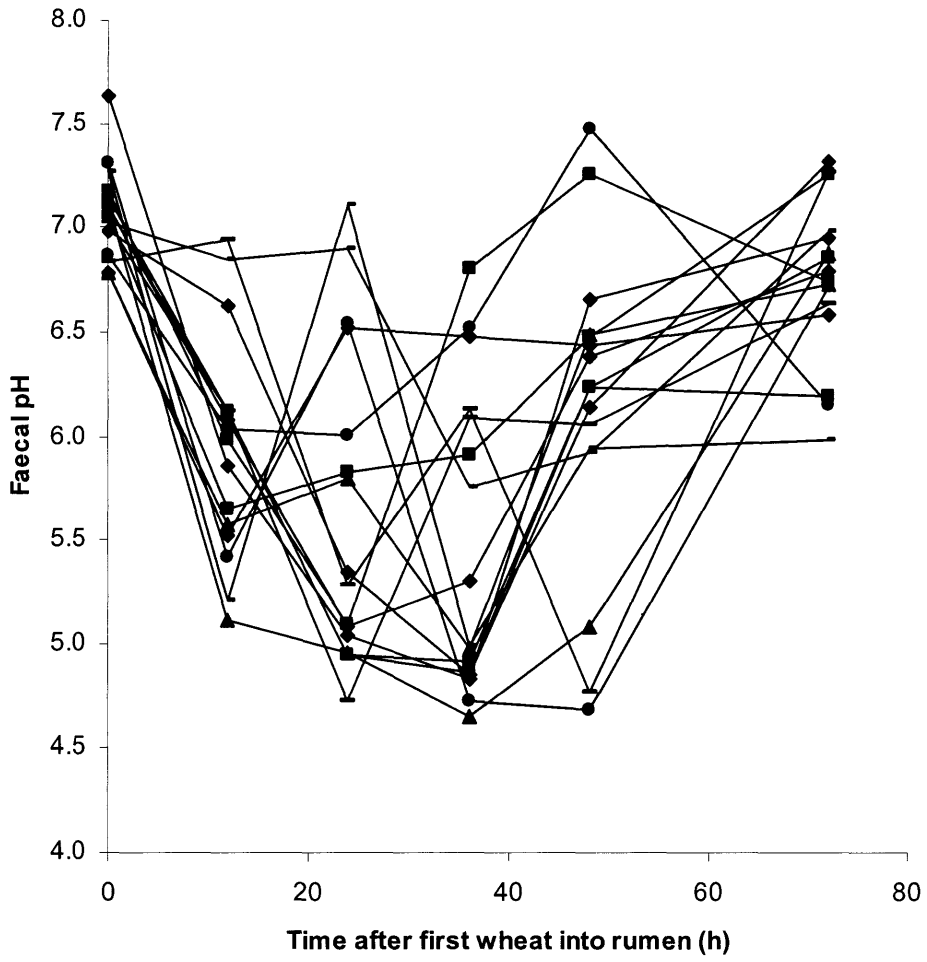


Figure 7.3 Variation in faecal pH for individual animals (each with a different legend) following carbohydrate overload at 0 and 24 h.

Figure 7.3 shows the between-animal variation in faecal pH over time. The extent of the variation was greatest at 24 h (CV=13.2%) and 36 h (CV=12.7%) whilst the CV of faecal pH at 0 h was 3.0%. At 36 h, faecal pH ranged from 4.65 to 6.80 and faecal lactate ranged from 0.4 to 74.8 mmol/L (data not presented).

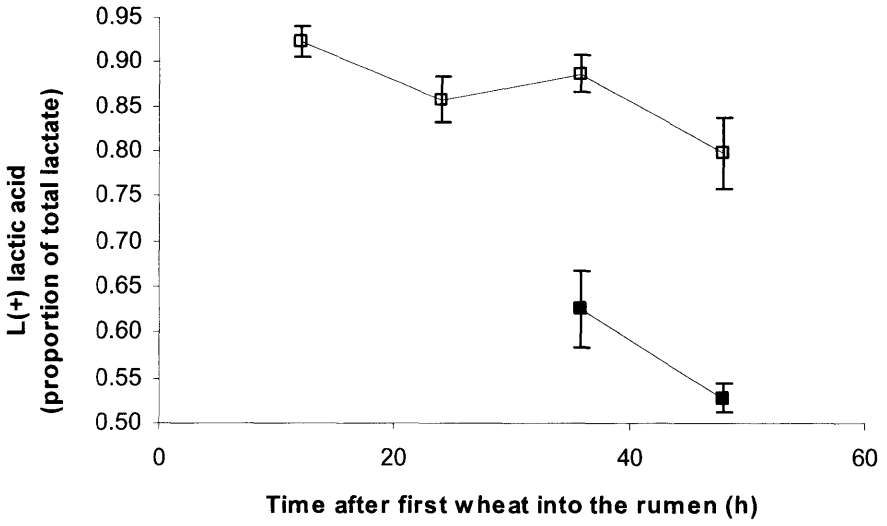


Figure 7.4 Differences in the proportion of lactic acid present in the L(+) form in the rumen (■) and faeces (□) following carbohydrate overload at 0 and 24 h. Error bars ± SEM.

Figure 7.4 shows the proportion of lactic acid present in the L(+) form in the rumen and faeces. There was no lactic acid detected in the rumen before 36 h. In the rumen, approximately half of the total lactate was present as the L(+) isomer whereas in the faeces, L(+) lactate comprised nearly 90% of total lactate.

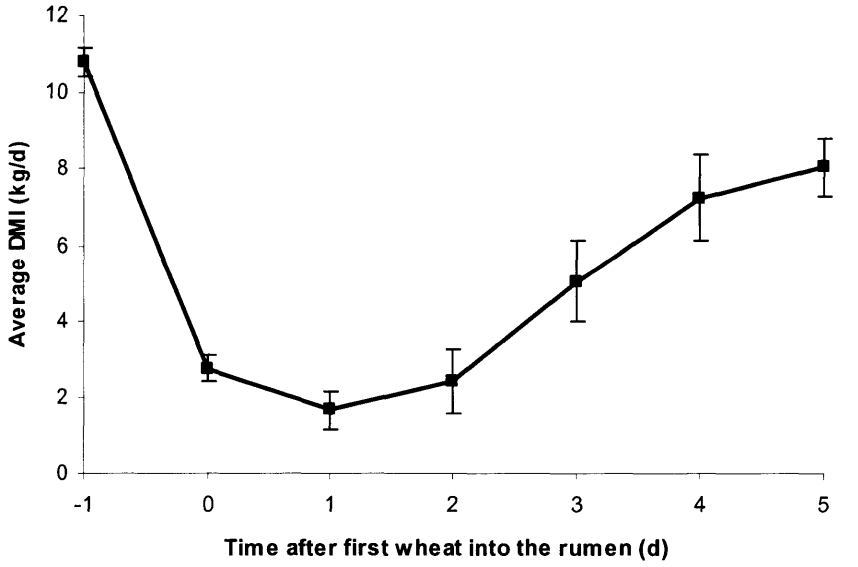


Figure 7.5 Pattern of DMI (kg/d) following carbohydrate overload at 0 and 24 h (days 0 and 1). Error bars ± SEM.

Figure 7.5 shows the changes in feed intake over time (days 0 to 5) after the grain challenge, based on the feed intake data available for the 11 animals that did not require treatment after 3

days. Data for treated animals was not used because of the potential for bicarbonate and rumen fluid inoculum to confound the results. The level of feed intake decreased significantly at 24 h after the challenge and had only returned to about 80% of the pre-challenge level by day 5.

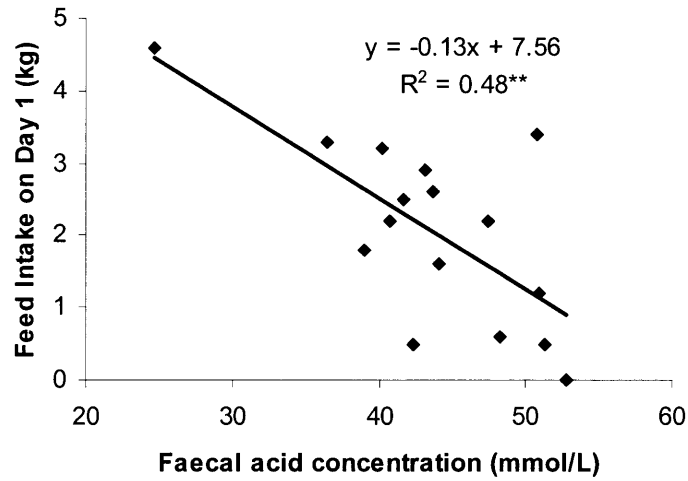


Figure 7.6 Relationship between faecal acid concentration (mmol/L) (mean of 0, 12 and 24 h values for each animal) and feed intake (kg) on the day after the challenge. ** ($P<0.01$).

Figure 7.6 shows that feed intake over the 24 h following the challenge was negatively associated with faecal acid concentration, expressed as the average VFA + lactate in faecal contents of the heifers at 0, 12 and 24 h. Over the first 24 h, total faecal acid concentration was largely due to faecal lactate concentration ($P<0.01$; $R^2=0.88$). Measured rumen parameters were not associated with feed intake on the day following the challenge.

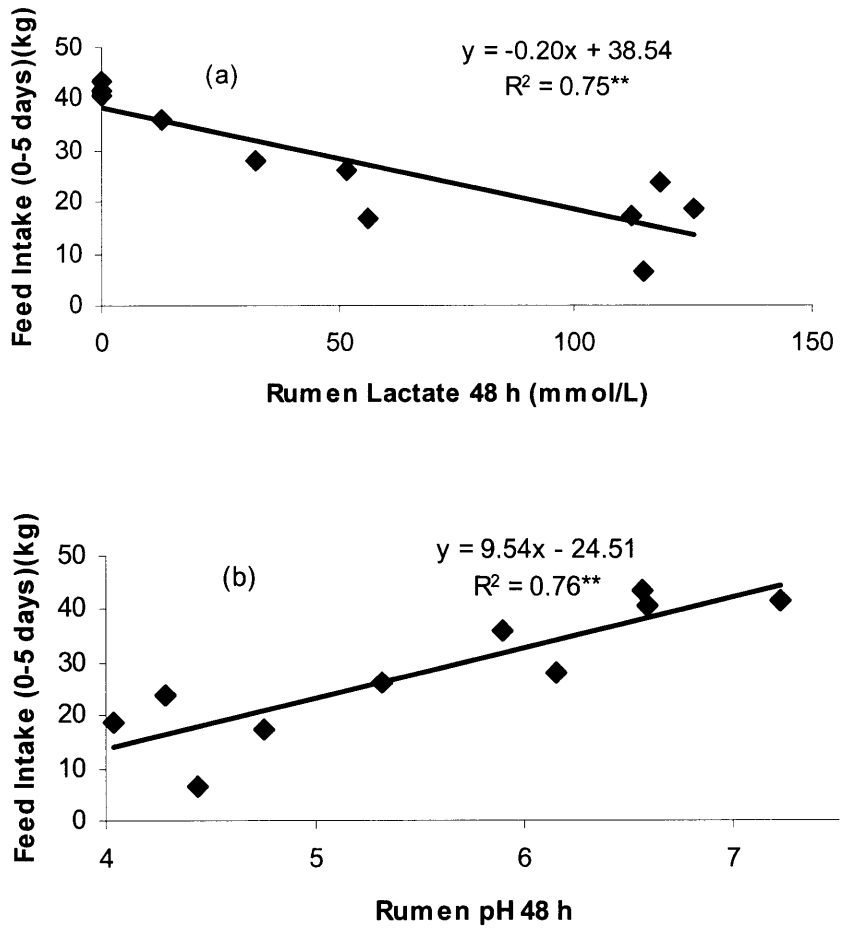


Figure 7.7 Relationship between 48 h rumen (a) lactate concentration (mmol/L) and (b) pH, with total feed intake (kg) from the day of the challenge (day 0) to day 5 after the challenge. ** ($P<0.01$).

Figure 7.7 shows that for the 11 untreated animals, feed intake (days 0 to 5) was negatively associated with 48 h rumen lactate concentration and positively associated with rumen pH. There was no association between 48 h VFA or total acid concentration in the rumen and feed intake (day 0 to 5). Total feed intake was not correlated with any of the measured faecal characteristics and there was also no relationship between any of the faecal and rumen parameters.

Rumen pH at 48 h was negatively associated with total acid ($P<0.01$; $R^2=0.84$) and lactic acid ($P<0.01$; $R^2=0.80$) concentrations. Eleven animals had a rumen pH<5.5 at 48 h, whilst 10 of these animals had rumen lactic acid concentrations greater than 50 mmol/L.

7.4 Discussion

The initial site of acidosis in cattle subjected to this grain-challenge model was the hindgut (as indicated by faecal changes). The data also suggests that without the second dose of wheat at 24 h, there would have been no rumen acidosis (Figure 7.1). In this case, hindgut acidosis would have been the only sign of grain overload. This result is in agreement with the findings of Godfrey *et al.* (1992) who found that acidosis occurred in the hindgut of unadapted sheep consuming large amounts of barley, even when rumen fermentation was relatively 'normal'. Figure 7.6 shows that the average amount of acid in the faeces was correlated with DMI on the day following the initial challenge. The parameters measured in the rumen during this first day appeared 'normal' and had no association with DMI. The result supports the hypothesis that hindgut acidosis may play an important role in the overall pathogenesis of grain-poisoning under certain feeding conditions.

The carbohydrate overload model used in this study effectively reduced feed intake in all of the cattle whilst individual variation in the severity of induced acidosis was remarkable. This ranged from a mild depression in feed intake to extremely severe, clinical acidosis requiring euthanasia. It is difficult to explain why apparently similar animals varied so considerably in their ability to cope with this grain challenge. The reasons for this variation in response are not clear but could involve differences in the microbial population (before and after the challenge), varying efficiency in small intestinal digestion, differences in the immune response to tissue damage/disease or variable changes in the gut wall characteristics and the extent of inflammation and leakage. Feed intake after the grain challenge would also have affected saliva production, the buffering capacity of the rumen and digesta transit time. Figure 7.7 suggests that animals with a higher feed intake (0-5 days) had a higher rumen pH and lower rumen lactate concentrations than animals with a low feed intake.

Figures 7.2 and 7.3 indicate that a mean rumen or faecal pH at each sampling time does not reflect the variability between animals. Similarly, an average pH or a single sample for the entire period following the grain challenge would not reflect the extent of the pH fluctuations over time in the rumen and faeces. To overcome this limitation, other researchers (Barajas and Zinn 1998; Cooper *et al.* 1999) have opted to measure the area below the pH/time curve in an effort to create a better measurement of the extent of acidosis.

When rumen pH drops below 5, onset of acute acidosis can be expected whereas sub-acute acidosis is likely to be a problem when rumen pH falls below 5.5 (Slyter 1976; Nocek 1997).

Some animals experienced prolonged periods of low rumen pH whilst pH for other individuals remained consistently at or above 5.5 (Figure 7.2).

A higher proportion of L(+) lactate was observed in the faeces (hindgut) than there was in the rumen (Figure 7.4). This suggests that either the population of lactic acid-producing bacteria differed in the two sections of the digestive tract or that there was differential absorption of the different lactic acid isomers in the rumen and hindgut.

The results suggest that lactic acid was more important than VFA in influencing feed intake and pH. In contrast, Reinhardt *et al.* (1997) used a sub-clinical acidosis model and found that a low rumen pH (<5.0) was associated with high concentrations of VFA and relatively low levels of lactic acid (<5 mmol/L). Reinhardt *et al.* (1997) only reported results for VFA and L(+) lactic acid in rumen fluid and did not present data for D(-) lactic acid or the effect of acidosis on feed intake. The relative importance of VFA and lactate in fermentative acidosis remains unclear but most likely depends on the level of acidosis created and the level of animal adaptation to the challenge grain.

Animals in this experiment were not adapted to a concentrate-based diet before being challenged with the dry rolled wheat. The results of Godfrey *et al.* (1992) indicated that compared to unadapted animals, sheep already adapted to high-grain diets would experience smaller changes within the hindgut following a grain challenge – presumably due to a greater capacity for fermentation in the rumen and/or adaptation of the small intestine. Hence, there is still a need to study the importance of hindgut acidosis in cattle adapted to grain-based diets.

Transit time of digesta was not measured in this experiment but it was clear that the rapid introduction of grain during the first carbohydrate overload caused a faster rate of passage of material from the rumen than when grain is normally ingested by the animal. This is an unfortunate limitation of the model used but was unavoidable given the timing and logistics of administering the grain. The early changes in faecal pH and lactate concentrations (Figure 7.1) indicate that it took less than 12 h for the bulk of grain to reach the hindgut. It is also difficult to explain why the second carbohydrate overload caused acidosis in the rumen when conditions in the faeces were returning to 'normal'. It is possible that the initial grain overload caused normal gut movement to become inhibited (Slyter 1976; Huber 1976) but no measurements were made to determine if this was the case. If rumen motility was reduced, this may partly explain why the second dose of grain appeared to be fermented in the rumen instead of being quickly moved through the gastrointestinal tract to the hindgut.

7.5 Conclusions

In unadapted cattle given a carbohydrate overload, the initial site of acidosis appeared to be the hindgut as indicated by a high faecal lactate concentration and low faecal pH. Similar changes within the rumen generally occurred 24 h after the faecal changes and in response to a second carbohydrate overload.

Although an initial decrease in feed intake coincided with the changes in the hindgut, rumen pH and lactic acid concentration 48 h after the grain challenge were more closely correlated with feed intake over the 5-day experimental period.

Total lactic acid in the rumen had a greater effect than VFA on rumen pH and feed intake.

Given the abnormal method of grain administration, the conclusions from this study should be applied with caution to understand implications for commercial grain feeding.