

Chapter 8. Genetic Variation in Faecal Parameters of Feedlot Cattle and the Association with Efficiency of Feed Utilisation

8.1 Introduction

Factors such as the amount and type of feed eaten, breed, age, sex and environmental conditions are all known to contribute to between-animal variation in the efficiency of feed utilisation for maintenance and growth. More recently it has been shown that animals of the same type, eating the same diet, can still vary in their efficiency of nutrient utilisation (Archer *et al.* 1999). Residual feed intake (RFI) is a measure of feed conversion efficiency used to describe variation in feed intake that is independent of an animal's size and growth rate. It is the difference between what an animal actually eats and its predicted feed intake based on LW and growth rate. Animals with lower RFI values are deemed to be more efficient. Conversely, less efficient animals have a higher RFI and eat more than expected. Cattle selected for lower RFI have been shown to require less feed without any compromise in growth performance (Herd *et al.* 1997; Richardson *et al.* 1998).

The biological basis for variation in RFI between similar animals is still largely unknown (Archer *et al.* 1999). If the mechanisms controlling variation in RFI can be more completely understood, then it may be possible to identify and directly select for parameters that make individuals more efficient. Whilst a combination of mechanisms is likely to influence RFI (Archer *et al.* 1999), it is possible that hindgut fermentation could be a contributing factor and this was investigated in the current study.

As previously discussed, the hindgut is the least desirable site of starch digestion in the gastrointestinal tract of ruminants (Lee 1977; Orskov 1986; Godfrey *et al.* 1992). Measuring starch flow to the hindgut and hindgut fermentation is difficult but it is likely that quantitative measurement of hindgut fermentation provides an indirect way of determining between-animal variation in the efficiency of starch digestion in the rumen and small intestine. Chapter 2 established that measurement of faecal pH, DM and nitrogen content should theoretically provide a good indication of hindgut fermentation.

The hypothesis tested in this experiment was that variation in hindgut fermentation contributes to variation in RFI in beef cattle. By using steers of known genetic background, the hypothesis that variation in hindgut fermentation has a genetic basis was also tested.

8.2 Materials and Methods

8.2.1 Animals and management

The cattle used in this experiment were progeny of lines selected for high or low RFI. Cattle were bred and tested for postweaning RFI at the NSW Agriculture Research Centre, Trangie, NSW, Australia. Postweaning RFI tests were conducted on Angus bulls and heifers bred at Trangie, and on Angus, Shorthorn, Hereford and Poll Hereford heifers purchased from industry herds. Details of these tests are given in Arthur *et al.* (1997). At the end of each test, heifers of the four breeds and the Angus bulls were ranked for RFI. The top 50% of heifers were then mated to the top five Angus bulls, and the bottom 50% of heifers mated to the bottom five Angus bulls to produce High Efficiency (“HE”; low RFI) and Low Efficiency (“LE”; high RFI) progeny. For this experiment the calves were considered to be either Angus or crossbred (Angus x Hereford, Angus x Poll Hereford or Angus x Shorthorn). Calves were born in March 1998 and all of the steer progeny were available for this study. The group comprised 58 HE and 72 LE steers. They were weaned from their dams at about seven months of age and transported to Glenn Innes, NSW, where they were backgrounded on pasture.

In March 2000 they were transported to the Cooperative Research Centre for Cattle and Beef Quality’s Research Feedlot “Tullimba”, located 50 km west of Armidale, NSW - for description see Bindon (2001). The cattle were rested on pasture for one week with hay being made available before entering the feedlot. The average weight of the 24-month-old steers was 520 kg at feedlot entry. Cattle were held in small feedlot pens with between seven and nine steers per pen. The animals were fed for 121 days.

Over a two-week period the steers were accustomed to rations of increasing grain content fed in a long feeding trough (bunk). From day 15 they were fed a finisher diet that consisted of 75% rolled barley, 10% sorghum hay, 5% protein pellets, 2% mineral additives and a proprietary mixture of molasses, urea, vitamins, minerals and water (8%). The diet had an ME content of 12.0 MJ/kg DM and 15.8% crude protein content.

On day 29 the feeding system was changed. Steer groups were placed in new pens (12 m x 20 m), each pen containing a single, automated self-feeder. The feeders were designed to measure

individual animal feed intake (Bindon 2001). The steers were allowed 15 days to adapt to feeding from the self-feeders before experimental measurements commenced.

8.2.2 Feed efficiency study

The ten-week feed efficiency study began on day 45 in the feedlot. LW (no fasting) was measured fortnightly throughout the period and individual daily feed intake was measured using the automated self-feeders. This information was used to calculate DMI, ADG, FCR and RFI following the procedures described by Richardson *et al.* (2001). The study concluded on day 121.

8.2.3 Faecal sampling and analysis

Faecal samples were taken directly from the rectum on days 66, 80, 94 and 108, coincident with the cattle being weighed. A complete set of faecal samples was collected from 75% of the steers, three samples only were collected from 22% of steers, and two samples only from 3% of steers. Samples were placed in 20 mL sample collection vials and kept in an ice-filled esky until processed.

Faecal pH was measured approximately 2 h after collection as the rate at which the cattle were weighed and sampled meant that it was not possible to measure faecal pH immediately after collection.

Faecal DM content was determined for the samples taken on days 80, 94 and 108. Labour constraints meant that this measurement was not made on samples from day 66.

Faecal nitrogen content was determined on sub-samples of the dried faecal samples taken on days 80 and 94. Results from these two sampling times indicated that there were no significant differences between the progeny groups and on this basis it was decided that further analysis of samples from days 66 and 108 was not justified.

8.2.4 Carcass measurements

At the end of the feed efficiency study, subcutaneous fat thickness at the rump (Australian P8 site) and rib (12/13th) was measured in the live animal by a trained technician using an Aloka 500 ultrasound scanner. The area of the eye-muscle (LD; at the 12/13th rib) was measured subsequently by computer analysis of stored images.

8.2.5 Statistical analysis

Eight steers (5 HE: 3 LE) had to be withdrawn from the experiment for reasons such as digestive upset or failure to adapt to the automated feeders. This was done at the discretion of feedlot management. Data for these animals was not used.

Feed intakes were converted to a DM basis. Weights for each steer taken over the ten-week feed efficiency study were regressed against time (day of RFI test) to calculate ADG and mid-test weight (MWT) for individual steers. RFI was calculated by using individual animal data in the multiple regression equation:

(actual) feed intake = $a \times \text{MWT}^{0.73} + b \times \text{ADG} + \text{residuals}$, where residuals equal RFI.

The coefficients 'a' and 'b' represent kg DMI per day required to sustain each kg of MWT and required for each kg of ADG, respectively. Calculated in this way, RFI was the difference between actual feed intake for each animal and feed intake predicted on the basis of size and growth rate.

FCR was calculated as DMI/ADG for the study period. The automated self-feeders also stored data from which the number of feeding sessions per day and the total duration of time spent feeding per day were derived. Feeding behaviour could also be described in terms of the average length of time per feeding session (seconds), the average rate of DMI (g/second) and the average DMI per feeding sessions (kg).

Relationships between faecal pH, DM and nitrogen content were investigated by pair-wise regressions in a repeated measures model using the mixed procedure of SAS (2001). Breed (Angus or crossbred) and day of sampling (for pH: day 66, 80, 94, 108; for DM content: day 80, 94, 108; for nitrogen content: day 80 and 94) were fitted as fixed effects. Regression coefficients between faecal pH, DM and nitrogen content were determined from the "solution" option available in this procedure.

Differences between sire progeny groups were taken as preliminary evidence for genetic variation. Sire effects were analysed for the nine sires that each contributed at least 10 offspring to the experiment. One sire was omitted from this analysis because it only contributed one offspring. Animal production data were analysed for differences between sire progeny groups with the generalised linear model (GLM) procedure of SAS (2001) with breed fitted as a fixed effect and sire fitted as a random effect. A repeated measures model was initially used to assess differences in faecal pH, DM and nitrogen content between the sire progeny groups. This was done using the mixed procedure of SAS (2001) with a model that

included breed, sire, sample day and sire-by-sample day. This analysis showed no re-ranking of sires over days (i.e. no sire-by-sample day interaction). The mean value for each sire was then used in a GLM procedure with sire fitted as a random effect. Least squares means for sire progeny groups were calculated using the LS-means statement available in the GLM procedure. This analysis was only constructed to demonstrate sire differences. For this reason, relationships between sires were ignored and selection line was not fitted in the models.

Phenotypic associations between the factors associated with hindgut fermentation and variation in animal production performance over the feed efficiency study were investigated in a repeated measures analysis within the mixed procedure of SAS (2001). The model contained breed and sampling day as fixed effects and the production trait being examined was as an independent, continuous variable. Regression coefficients were calculated using the “solution” option within the mixed procedure.

Confirmatory evidence of genetic associations between parameters describing hindgut fermentation and animal production traits was sought by determining if there was a statistical association between genetic variation in the parents of the steers with variation in fermentation parameters measured on their progeny. Regressions were determined for this purpose using the mid-parent Estimated Breeding Value (EBV) for selected postweaning traits. The sire and dam EBVs were determined using the results from the progeny tests for postweaning RFI conducted at the Trangie Agriculture Research Centre and genetic parameters reported by Arthur *et al.* (2001). The mixed procedure of SAS (2001) was used to conduct a repeated measures analysis of the fermentation parameters in a model that included breeding and sampling day as fixed effects and the mid-parent EBV as an independent variable. The regression coefficients were calculated using the “solution” option available.

8.3 Results

The mean performance and faecal parameters for the progeny of the nine different sires are presented in Table 8.1. There were significant differences between sire progeny groups for faecal pH, faecal DM content, DMI, RFI, FCR, carcass rib fat and carcass rump fat but not for faecal nitrogen content or ADG. Eating rate was the only measure of feeding behaviour that differed significantly between sire progeny groups. It was possible that differences in feed intake could have explained the observed variation in faecal pH and DM content. To test this possibility, DMI was included as a covariate in the models testing for sire progeny group differences. There was a tendency for DMI to affect faecal pH ($P < 0.10$) (positive association) but differences between sire progeny groups were still significant ($P < 0.05$). Similarly, DMI

was affecting faecal DM content ($P < 0.05$) (negative association) but the sire progeny group differences were still significant ($P < 0.05$). This information shows that whilst DMI was associated with variation in the faecal parameters, it did not explain all of the variation between the sire progeny groups. These differences between sire progeny groups provided evidence for genetic variation in faecal pH, faecal DM content and the other traits.

Table 8.2 shows that faecal DM content was phenotypically negatively correlated with DMI and RFI. This indicates that the more efficient animals tended to have a higher faecal DM content. Although eating rate was shown to differ between sire progeny groups, the regression coefficients revealed that this was not associated ($P > 0.05$) with the faecal parameters for pH, DM and nitrogen content. Total DMI, rather than rate of consumption, was more closely related to faecal DM content.

Variation in faecal pH was positively associated with DMI, but not with variation in the other traits measured in the feedlot. Variation in the animal production traits was not associated with variation in faecal nitrogen content.

The statistically significant regression coefficients for faecal pH and DM content with mid-parent EBVs for the efficiency of feed utilisation and body composition (Table 8.2) were evidence of genetic associations. There was a significant, negative relationship between faecal pH and mid-parent EBVs for RFI, carcass rump fat and carcass rib fat. Faecal DM content was significantly negatively associated with mid-parent EBVs for DMI, RFI, carcass rump fat and carcass rib fat. This indicates that parents with EBVs for low (more efficient) RFI and low subcutaneous carcass fat were likely to have progeny with a high faecal pH and DM content. Faecal nitrogen content was not associated with variation in mid-parent EBVs for any production trait.

Faeces from the cattle varied in pH, DM and nitrogen content. The CV for faecal pH ranged between 3.7 and 4.6% on the four days samples were collected. As pH is a logarithmic measurement, these CVs represent considerable between-animal variation in faecal pH. The CVs for faecal DM content were 15.1, 11.8 and 20.4%, and for nitrogen content were 13.2 and 17.7%.

Faecal DM content varied inversely with faecal nitrogen content (regression coefficient = -0.03 DM content/nitrogen content; $P < 0.01$). There was no evidence of a phenotypic association between faecal pH and faecal DM content, or between faecal pH and faecal nitrogen content (regression coefficients not different from zero; $P > 0.05$).

Table 8.1 Differences between sire progeny groups in faecal parameters, feedlot performance, carcass characteristics and feeding behaviour. Values are sire group least squares means.

Sire number	Number of progeny	Efficiency line	Faecal pH	Faecal nitrogen (%)	Faecal DM (%)	ADG (kg/d)	DMI (kg DM/d)	RFI (kg DM/d)	FCR (kg DM/kg)	Carcass rib fat (mm)	Carcass rump fat (mm)	Rate of DMI (g/second)
Q002	17	Low	6.85 ^a	2.30	14.61 ^a	1.52	14.08 ^a	0.87 ^a	9.55 ^{ac}	14.31 ^{ac}	19.51 ^a	2.98 ^c
Q018	14	Low	6.91 ^{ab}	2.21	15.97 ^{bcd}	1.63	13.26 ^{ab}	0.13 ^{bc}	8.25 ^{ade}	14.93 ^a	19.68 ^a	2.21 ^a
Q048	12	Low	6.91 ^{ab}	2.25	15.68 ^{abc}	1.65	12.91 ^{bcd}	-0.27 ^b	7.92 ^{bd}	13.47 ^{ab}	15.49 ^{bcd}	2.35 ^{ab}
Q167	14	Low	7.00 ^{bc}	2.26	14.99 ^{ad}	1.42	12.92 ^b	0.16 ^b	9.89 ^c	15.55 ^a	19.78 ^a	2.47 ^{ab}
Q312	12	Low	6.94 ^{abc}	2.41	14.73 ^{ac}	1.72	13.34 ^{ad}	-0.21 ^b	7.93 ^{bd}	13.31 ^{ab}	16.41 ^{ac}	2.45 ^{ab}
Q006	10	High	6.98 ^{bc}	2.20	16.36 ^{bc}	1.60	13.48 ^{ab}	-0.12 ^{bc}	8.54 ^{acde}	10.70 ^b	14.10 ^{bc}	2.40 ^{ab}
Q072	13	High	6.90 ^{ab}	2.35	15.54 ^{abc}	1.60	11.85 ^c	-0.44 ^b	7.50 ^{be}	12.17 ^{bc}	16.09 ^{bcd}	2.36 ^{ab}
Q106	11	High	6.96 ^{abc}	2.25	15.83 ^{bcde}	1.49	12.93 ^{bc}	-0.14 ^{bc}	9.34 ^{acd}	13.53 ^{ab}	18.68 ^{ad}	2.41 ^{ab}
Q198	19	High	7.04 ^c	2.22	15.75 ^{bde}	1.66	13.97 ^a	0.37 ^{ac}	8.66 ^{abc}	13.68 ^{ac}	16.63 ^{ac}	2.70 ^{bc}

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

Table 8.2 Phenotypic and genetic regression coefficients between faecal parameters and animal production traits.

Repeated measures	DMI (kg DM/d)	RFI (kg DM/d)	FCR (kg DM/kg)	ADG (kg/d)	Carcass rump fat (mm)	Carcass rib fat (mm)	Rate of DMI (g/second)
Phenotypic regression ¹							
Faecal pH	0.02*	0.01	0.00	0.06	0.00	0.01	-0.01
Faecal DM (%)	-0.20*	-0.47**	-0.09	0.03	-0.01	0.01	-0.37
Faecal nitrogen (%)	0.02	-0.02	0.02	-0.03	0.00	0.00	0.04
Genetic regression ²							
Faecal pH	-0.01	-0.07*	-0.04	0.17	-0.03*	-0.04**	
Faecal DM (%)	-0.49*	-0.80**	-0.34	-0.39	-0.35*	-0.48**	
Faecal nitrogen (%)	-0.01	0.03	0.03	-0.21	0.04	0.04	
SD of mid-parent EBV	0.56	0.45	0.45	0.08	0.94	0.80	

*(P<0.05); **(P<0.01); indicates that the regression coefficient is significantly different from zero

¹Phenotypic associations between steer faecal and production parameters.

²Genetic associations between faecal parameters in progeny and mid-parent EBV for selected production traits. A mid-parent EBV was not available for rate of DMI.

8.4 Discussion

This study identified differences between sire progeny groups in RFI which supports previous findings that genetic variation in RFI exists in feedlot steers (Archer *et al.* 1999). Genetic variation in faecal pH and DM content was also evident by the significant differences between sire progeny groups for these parameters. Given that these faecal parameters are likely to reflect the extent of starch fermentation in the hindgut (low pH and DM content indicating more fermentation), this may provide preliminary evidence of genetic differences in hindgut fermentation. The range in mean faecal pH for the sire progeny groups was 6.85-7.04 and appears small, however, these differences may be important from a biological perspective as a consistently lower pH may reflect a higher susceptibility to hindgut acidity and reduce the efficiency of microbial digestion.

There was an association between faecal pH and DM content and mid-parent EBV for RFI. High efficiency parents (selected for low RFI) generally produced progeny that had higher faecal pH and DM content compared to the progeny of low efficiency sires. Although speculative, a high faecal pH and DM content may be an indication that starch is being more extensively utilised in the rumen and small intestine, with less starch digested in the hindgut.

Faecal DM content was negatively correlated with faecal nitrogen content which is a trend that should theoretically be indicative of hindgut fermentation. Faecal pH was not correlated with either faecal nitrogen or DM content. Endogenous secretions (Mason 1984) and buffering (Dougherty *et al.* 1975c; Ding 1997) arising in response to hindgut fermentation may have influenced faecal nitrogen content and pH. The between-animal differences in faecal pH and DM content were significant in this study but without this possible influence these differences might have been even greater. It is acknowledged that a direct measurement of whole tract starch digestibility would have been useful to relate to the chosen faecal parameters. Given the outcomes of this initial trial there is reason to proceed with further work to evaluate the extent of genetic variation in starch digestion in cattle.

A genetic association between body composition and RFI was reported by Richardson *et al.* (2001). Less than 5% of the variation in sire RFI was explained by variation in the body composition of the steer progeny in that study. Results from the current experiment show a negative association between faecal pH and DM content and mid-parent EBV for rib and rump fat. This suggests that a low level of starch fermentation in the hindgut (higher faecal pH and DM content) may be genetically associated with lower levels of subcutaneous fat, however,

there were no significant phenotypic associations between these parameters in the steers. Therefore, definite conclusions cannot be made about the data.

The possibility of genetic differences in extent of hindgut fermentation inevitably leads to attempts to understand the mechanisms involved. It is believed that it may involve a combination of factors associated with starch digestion and absorption as discussed in chapter 2. Differences in digesta flow rates may account for some of the observed variation in hindgut fermentation and this could be a useful measurement to make in future experiments. As all cattle in this experiment were fed the same diet, variation in feed composition or particle size ('as-fed') would not have been contributing to differences in digesta flow rates. Rather, if there were any differences in flow rate this would have been due to inherent variation within the animals or in their pattern of eating. Differences in feed intake may also have influenced digesta flow rates (Grofum and Hecker 1973) with higher feed intakes commonly associated with reduced rumen retention time. Genetic differences in DMI and eating rate were evident in this experiment. Eating rate did not influence faecal parameters but there was a significant association with DMI. Exploratory statistical analysis confirmed that significant genetic variation in faecal pH and DM content was still evident even when differences in DMI were accounted for. This result supports the hypothesis that variation in hindgut fermentation has a genetic basis.

8.5 Conclusions

This experiment has provided evidence of genetic differences between animals in the level of hindgut fermentation and it appears that these differences may also contribute to between-animal differences in the efficiency of feed utilisation.

Fermentation in the hindgut may contribute to lower efficiency of feed utilisation and could also be genetically associated with traits such as fatness.

It is possible that differences in hindgut fermentation are related to genetic variation in starch digestion and experiments designed to measure between-animal differences in whole tract and pre-caecal starch digestibility appear to be justified.

Chapter 9. Processing Wheat to Alter the Site of Starch Digestion

9.1 Introduction

The steam flaking of wheat may provide a means to alter the site of starch digestion from the rumen to the small intestine. Whereas steam flaking corn and sorghum increases rumen digestibility of starch (Zinn *et al.* 1995; Theurer *et al.* 1999b), there is evidence from *in vitro* (chapter 5), *in sacco* (Kreikemeier *et al.* 1990a) and *in vivo* (Zinn 1994) studies that the fermentability of wheat is not necessarily increased when it is steam flaked. On the other hand, as reported in chapter 5, there is evidence that the starch in steam flaked wheat has a much higher *in vitro* enzymatic digestibility than the starch in dry rolled wheat. The improved starch digestion resulting from steam flaking (Osman *et al.* 1970; Theurer 1986) may help to overcome limitations to starch digestion in the small intestine (Zinn 1994).

If the supply of starch to the small intestine is increased, it is important to know if this causes more starch to reach the caecum. The most appropriate way to assess the pattern of hindgut fermentation and the propensity for different diets to cause hindgut acidosis (Diez-Gonzalez *et al.* 1998; Rowe 1999) is to use cattle fitted with caecal cannulas.

The hypothesis for this experiment was that steam flaked wheat will ferment less in the rumen than dry rolled wheat and supply more digestible starch to the small intestine and that different degrees of steam processing (flake density) will affect both fermentative and intestinal digestion to a variable extent. The effect of wheat processing on whole tract starch digestion and the pattern of rumen and hindgut fermentation was evaluated in beef cattle (including the propensity to cause acidosis).

9.2 Materials and Methods

9.2.1 Experimental design

Three treatments were fed to each of six crossbred cows in a Latin square design experiment. The cows were Low-line x Angus, aged 28 months and weighed 398 kg LW at the beginning of the experiment. Treatment diets were based on 1) dry rolled wheat; 2) poorly flaked wheat (high flake density), and 3) well flaked wheat (low flake density). Treatment allocation to periods for each cow followed a Latin square arrangement as shown in Table 9.1.

Table 9.1 Treatment diets allocated to cow x period.

Period	Cow					
	1	2	3	4	5	6
1	DR	PF	WF	DR	PF	WF
2	PF	WF	DR	WF	DR	PF
3	WF	DR	PF	PF	WF	DR

DR=dry rolled wheat diet; PF=poorly flaked wheat diet; WF=well flaked wheat diet

Cows were fitted with cannulas in the rumen and caecum (MacRae *et al.* 1973). The caecal cannula was placed midway between the blind pole of the caecum and the ileo-caecal junction.

Each period was 10 days with the final 5 days used for sampling. A 5-day period of adaptation to each diet was considered adequate as the three diets contained 70% grain and were all based on wheat. Measurements were conducted to assess the patterns of fermentation in the rumen, caecum and faeces. Whole tract and *in vitro* starch digestibility and *in sacco* DM digestibility was also determined for the three treatment diets.

9.2.2 Diets

Steam flaked grain was produced in a steam flaking plant at a commercial feedlot. Tension of the rollers was adjusted to provide the desired flake densities of 52 kg/hL (poorly flaked) and 40 kg/hL (well flaked). Measurements of flake density were made on grain sampled directly beneath the rollers. Retention time of the grain in the steam chamber was approximately 30 min for the poorly flaked wheat and 40 min for the well flaked wheat. Dry rolled wheat was prepared by cracking the grain in a roller mill (roller gap of 0.5 mm) in the absence of moisture. The variety of wheat used in the experiment was unknown but all grain was from the same batch. After processing, all grain samples were mixed, bagged and placed into storage at -18°C to prevent spoilage of the moist, steam flaked diets. Feed samples were taken at the beginning of each period and bulked for analysis. An analysis of the dietary ingredients is listed in Table 9.2.

The enzymatic digestibility of starch *in vitro* was determined for each grain type. Flake thickness was determined by breaking the flake in half and measuring the thickness in millimetres (using vernier callipers) of the flattest spot near the centre of the grain flake (Zinn 1993). The estimate of flake thickness was the average of 10, randomly selected, air-dried, grain flakes.

Table 9.2 Ingredient and nutrient composition of experimental diets.

Item	Dry Rolled	Poorly Flaked	Well Flaked
Ingredient (% inclusion)			
Dry Rolled wheat	70.0		
Poorly Flaked wheat		70.0	
Well Flaked wheat			70.0
Oaten chaff	22.3	22.3	22.3
Molasses	1.7	1.7	1.7
Lime	1.4	1.4	1.4
Ammonium sulphate	0.5	0.5	0.5
Salt	0.2	0.2	0.2
Minerals and vitamins ^a	3.9	3.9	3.9
Nutrient analyses			
DM composition (%)			
Protein	14.2	13.6	13.2
NDF	21.2	22.1	23.7
ADF	10.4	12.7	14.7
Ash	5.8	5.9	6.0
Starch ^b	53.7	51.2	49.8
ME (MJ/kg)	12.3	12.4	12.4

^aAnalysis: Min. Total Crude Protein 70%, Min. True Protein 18%, Min. Equivalent Crude Protein 52%, Urea 18%, Min. Calcium 8%, Max. Calcium 10%, Min. Phosphorus 1%, Max. Phosphorus 2%, Min. Salt 5%, Max. Salt 7%, Min. Sulphur 1%, Min. Copper 250 mg/kg, Min. Cobalt 4 mg/kg, Min. Iodine 12.5 mg/kg, Min. Manganese 1000 mg/kg, Min. Selenium 6 mg/kg, Min. Zinc 1000 mg/kg, Min. Vitamin A 72,000 IU/kg, Min. Vitamin D3 5,500 IU/kg, Min. Vitamin E 600 mg/kg (Rumevite 'Stud n' Sale' concentrate)

^bMegazyme method described in chapter 3

9.2.3 Animal management

Cows were housed indoors in individual pens with slatted floors (4 m x 3 m) and given exercise in an outside yard for 3 h, every second day. Water was available *ad libitum*. Individual feed intake was set at 1.6% of LW (DM basis) with feed offered once daily at 0900 h. Cows were adapted to a 70% concentrate diet before the start of the trial. On day 1 of each period, cows were weighed before feeding and feed intakes for that period were adjusted according to LW. The daily feed given to each animal was at room temperature as the frozen feed was weighed into individual bins the night before. At the time of feeding, 1.5 g of chromic oxide (Cr₂O₃) powder was added directly into the rumen as a digestibility marker. Acid insoluble ash was also used to allow a comparison of the two methods.

9.2.4 In vivo starch digestion

After the 5-day adjustment period, faecal samples were taken from the cows four times daily, for three days. Collection times were advanced by 2 h every 24 h to provide 12 samples, representing each 2 h of a 24 h period. Individual samples consisted of 100 g (wet basis) of faeces and represented a composite of faecal material that accumulated on the slatted floor during each collection interval. These samples were immediately frozen but prior to analysis, samples from each cow and within each period were composited (equal weight, wet basis) to form a single representative sample for marker and starch analysis. Bulk samples were dried at 60°C and ground (0.5 mm screen) prior to analysis. Milled subsamples of faeces were ashed and digested (Stevenson and DeLangen 1960) and the Cr⁺⁺⁺ content determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 360, Norwalk, Connecticut, USA) with an acetylene-nitrous oxide flame. Acid insoluble ash content of faeces and grain was determined by the method described by Vogtmann *et al.* (1975) and Van Keulen and Young (1977) using 4 M HCl. Faecal starch content was determined and whole tract starch digestibility for each cow-diet combination was calculated using the marker ratio technique based on either Cr₂O₃ or acid insoluble ash.

9.2.5 In vivo fermentation patterns

On day 9 of each treatment period, rumen, caecal and faecal samples were collected every 3 h for 24 h. Rumen fluid samples were obtained using a 20 mL syringe and a metal probe (with end covered in gauze) inserted via the rumen cannula. Rumen fluid pH was measured immediately and approximately 8 mL of rumen fluid was acidified (pH 2-3) and frozen for later VFA and lactate analysis. Caecal digesta were collected by inserting a plastic spoon into the cannula and scooping out a sample when it became available and fresh faecal samples were collected from the floor of the pens. Faecal and caecal pH was determined immediately before a sub-sample of diluted caecal digesta and faeces was acidified and frozen for later VFA and lactate analysis. In addition, approximately 150 g of fresh faeces was placed in a pre-weighed foil dish, weighed and oven-dried for DM determination.

9.2.6 In vitro fermentation

System 2 was used to incubate the grains in the 'as-fed' form. Rumen fluid (300 mL) was taken from each cow at 0900 on day 10 of each period and used to incubate two replicates of the specific grain that the cow was eating. After 5 h incubation, samples were collected to

determine total VFA concentration, acetate to propionate ratio, fluid pH and starch digestibility. Only negligible amounts of lactic acid were detected in the liquor samples.

9.2.7 *In sacco* fermentation

The *in sacco* rumen digestion kinetics of the three different grain types was determined in each of the six cows with bags placed into the rumen at 0900 (0 h) on day 10. Replicate bags were removed from the rumen after 2, 4, 8, 12 and 24 h of fermentation. Two, 0 h bags were also soaked in water (37°C) for 30 min before being processed with the other bags.

9.2.8 *Statistical analysis*

A simple model of direct and carry-over effects, with random cow effects was used to analyse starch digestibility and *in vitro* fermentation data. A more complex repeated measures model was used for the 24 h fermentation pattern data. The models are described in the following paragraphs.

The statistical model used to compare responses from the cross-over design included direct effects of grain and the carry-over effects that one diet may have on the following diet. The model was fitted as a mixed model where the direct and carry-over effects are the predicted part and the cow and cow x diet effects are the random part. In Tables 9.4 and 9.6, the means are those derived from the model for the cross-over experiment which accounts for carry-over effects of preceding diets and the correlations due to repeated measures from the same cows (Ihaka and Gentleman 1996).

The repeated measures data; which had the extra effect of sampling time (0, 3, 6, 9, 12, 15, 18, 21 and 24 h), were analysed by determining the mean profiles of each measurement over 24 h by fitting a semi-parametric regression including random cow effects and a first order auto correlation for the errors. The 24 h fermentation pattern results were interpreted by inspecting the graph of the mean profiles and their 95% confidence intervals. On these graphs (Figures 9.3 – 9.6), the raw means are also plotted as points but it is important to appreciate that the mean profiles are weighted averages of individual cow responses over time. They are not simply fits to the unadjusted means (Ihaka and Gentleman 1996).

The results describing *in sacco* DM loss over the 24 h interval (0, 2, 4, 8, 12 and 24 h) were analysed as a non linear mixed model. The predictive regression model was:

$$y = y_1 + (y_2 - y_1) \times \frac{(1 - k^{(n-1)\frac{(x-x_1)}{(x_2-x_1)}})}{(1 - k^{n-1})}$$

where x_1 was set as 0 h and x_2 as 20 h and y_1, y_2 were the expected values of the responses at x_1, x_2 . The amount of curvature is represented by k and for these data $n=6$ (the number of pairs of data points). The mean responses are compared by inspection of the fitted regressions and their 95% confidence interval.

The large number of samples taken simultaneously from the caecum and faeces allowed an investigation of the relationship between mean VFA concentration, VFA proportions, pH and DM concentration within and between each site of measurement. Relationships were also investigated between measured caecal and faecal parameters and faecal starch content, DM content and starch digestibility. Regression analysis was performed using the linear regression method.

9.3 Results

9.3.1 *In vitro* enzymatic digestion and fermentation

Table 9.3 shows the characteristics of the processed wheat grains. The difference in average flake thickness was significant ($P<0.05$; two-tailed t-test) with poorly flaked wheat being thicker than well flaked wheat.

In vitro enzymatic starch digestibility was substantially improved for both poorly and well flaked wheat compared with dry rolled wheat.

Table 9.3 Characteristics of dry rolled, poorly flaked and well flaked wheat.

	Total starch in grain (% DM)	Flake thickness (mm)	Flake density (kg/hL)	<i>In vitro</i> enzymatic digestibility* (% of total starch)
Dry rolled	69.8			46.7
Poorly flaked	69.4	1.9	52.0	83.9
Well flaked	68.5	1.6	40.0	88.3
Average	69.2	1.7	46.0	73.0

* All samples milled through a 0.5 mm sieve for assay

Table 9.4 Fermentation characteristics *in vitro* for 'as-fed' dry rolled, poorly flaked and well flaked wheat (predicted means \pm se).

	VFA concentration (mmol/L)	Acet/Prop ratio	pH	Starch digestibility (% of total starch)
Dry rolled	85.8 \pm 1.1	1.8 \pm 0.3	6.4 \pm 0.0	32.2 \pm 0.9
Well flaked	65.4 \pm 7.0	1.6 \pm 0.1	6.8 \pm 0.1	23.5 \pm 3.0
Poorly flaked	41.7 \pm 4.0	2.0 \pm 0.3	7.0 \pm 0.1	13.2 \pm 2.1
Average	64.3	1.8	6.7	23.0

Table 9.4 shows that the three grains had different fermentation rates as reflected in concentrations of VFA and starch digestibility. Dry rolled wheat was most rapidly fermented followed by well flaked then poorly flaked wheat ($P < 0.001$). In producing more VFA, the pH was lower for dry rolled wheat than the two steam flaked grains ($P < 0.001$). Differences between grains in the acetate to propionate ratio were not significant. There was a significant negative correlation between mean *in vitro* VFA concentration and pH ($P < 0.01$; $R^2 = 1.0$) and a significant positive correlation between mean *in vitro* starch digestibility and VFA concentration ($P < 0.01$; $R^2 = 1.0$).

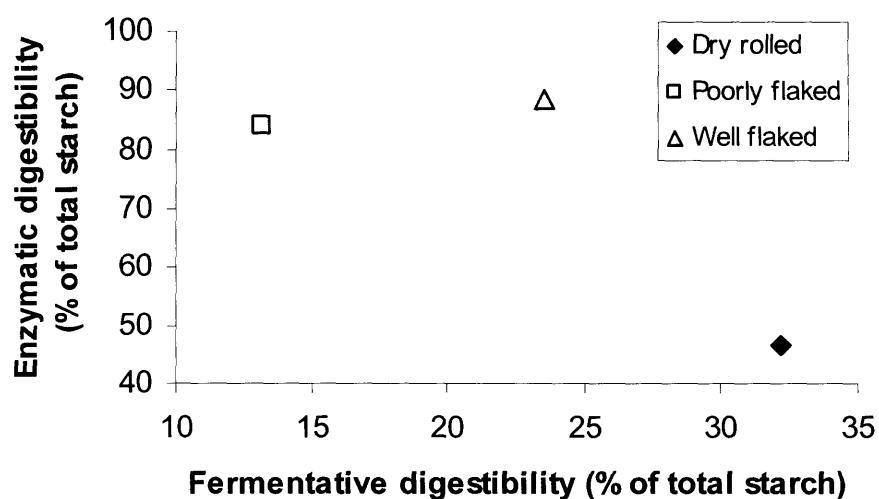
**Figure 9.1** Relationship between fermentative digestibility (% of total starch) *in vitro* and enzymatic digestibility (% of total starch) *in vitro* of starch in dry rolled, poorly flaked and well flaked wheat.

Figure 9.1 shows that poorly flaked wheat had a relatively low level of fermentation in relation to dry rolled and well flaked wheat, yet a high enzymatic digestibility of starch. In contrast, the

starch in dry rolled wheat was extensively fermented but had the lowest enzymatic digestibility whilst well flaked wheat fermented less than dry rolled wheat and had the highest enzymatic digestibility of starch.

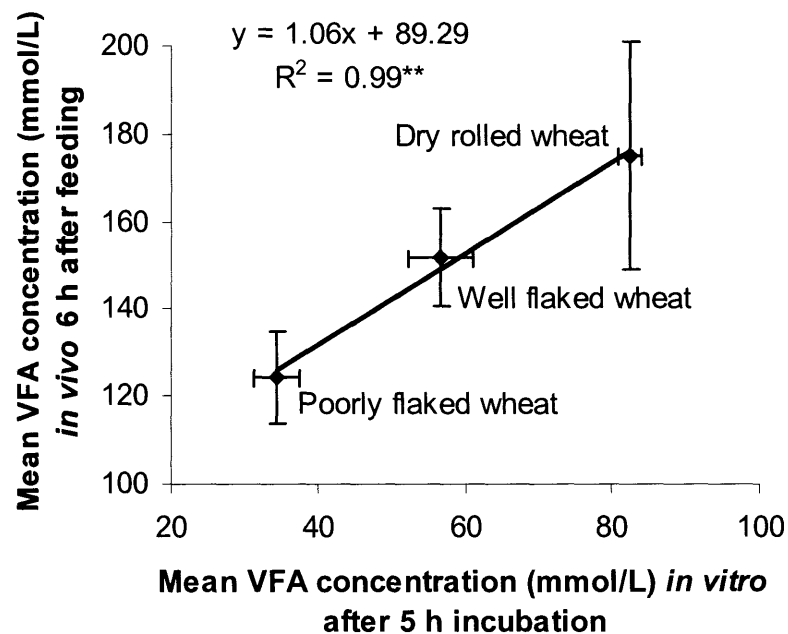


Figure 9.2 Relationship between mean VFA concentration (mmol/L) *in vitro* for dry rolled, poorly flaked and well flaked wheat (after 5 h incubation) and mean VFA concentration (mmol/L) *in vivo* for diets based on these three grains (6 h after feeding). Error bars \pm SEM. **($P < 0.01$).

Figure 9.2 shows that there was a strong correlation between results obtained in the *in vitro* fermentation assay and the pattern of *in vivo* fermentation of diets based on the same grains.

9.3.2 *In vivo* fermentation and starch digestion

In the rumen, there were no significant differences amongst treatments in the profiles of rumen pH, lactic acid and butyrate concentrations. Only negligible amounts of lactic acid were detected at the three sites of measurement. In the rumen, lactic acid concentration averaged 0.43 mmol/L across treatments. Significant profile differences in the rumen were noted for total VFA concentration and the relative proportions of acetate and propionate. In the caecum and faeces, the only significant differences amongst treatments were for faecal DM content. The specific differences are discussed below in Figures 9.3 - 6. In each of these figures, the shaded 95% confidence interval is shown on the two most extreme curves. Significant differences between treatments occurred where the shaded areas do not overlap.

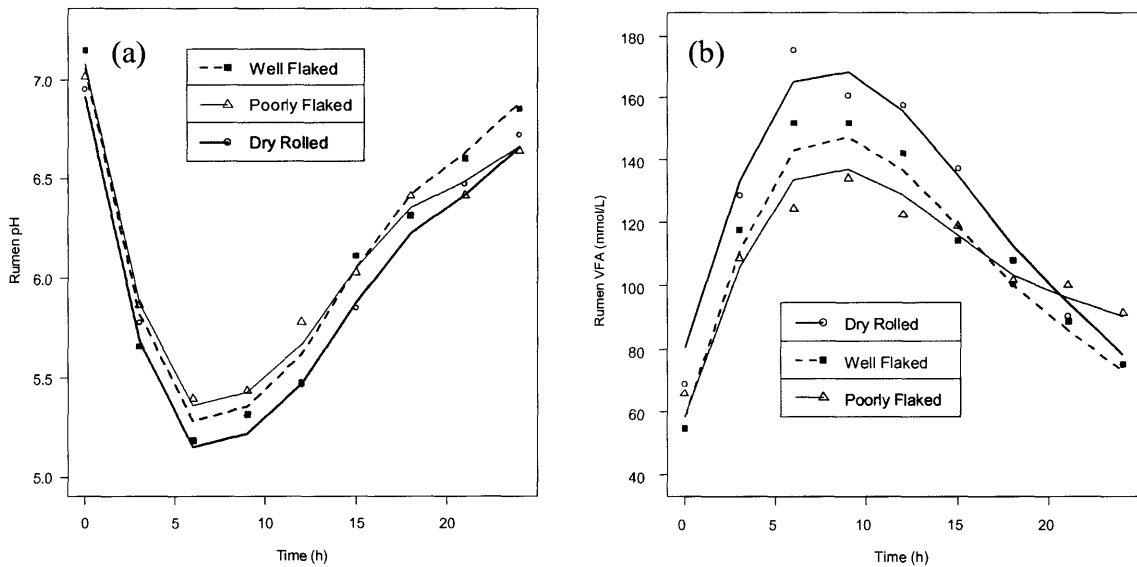


Figure 9.3 Diurnal profiles of the means of rumen (a) pH and (b) VFA concentration (mmol/L); and 95% confidence intervals for dry rolled, well flaked and poorly flaked wheat over a 24 h interval. Cattle fed at 0 h.

Figure 9.3 shows that there were no significant differences between treatments in rumen pH. The mean pH for each treatment was below 5.5 at 6 h and 9 h after feeding. In general, rumen pH was lowest in cattle fed dry rolled wheat and highest in cattle fed poorly flaked wheat. For all diets, the lowest pH was measured 6 h after feeding.

Dry rolled wheat was associated with a higher concentration of VFA in the rumen between 3 h and 12 h after feeding than poorly flaked wheat. The highest VFA concentrations occurred between 6 and 9 h after feeding.

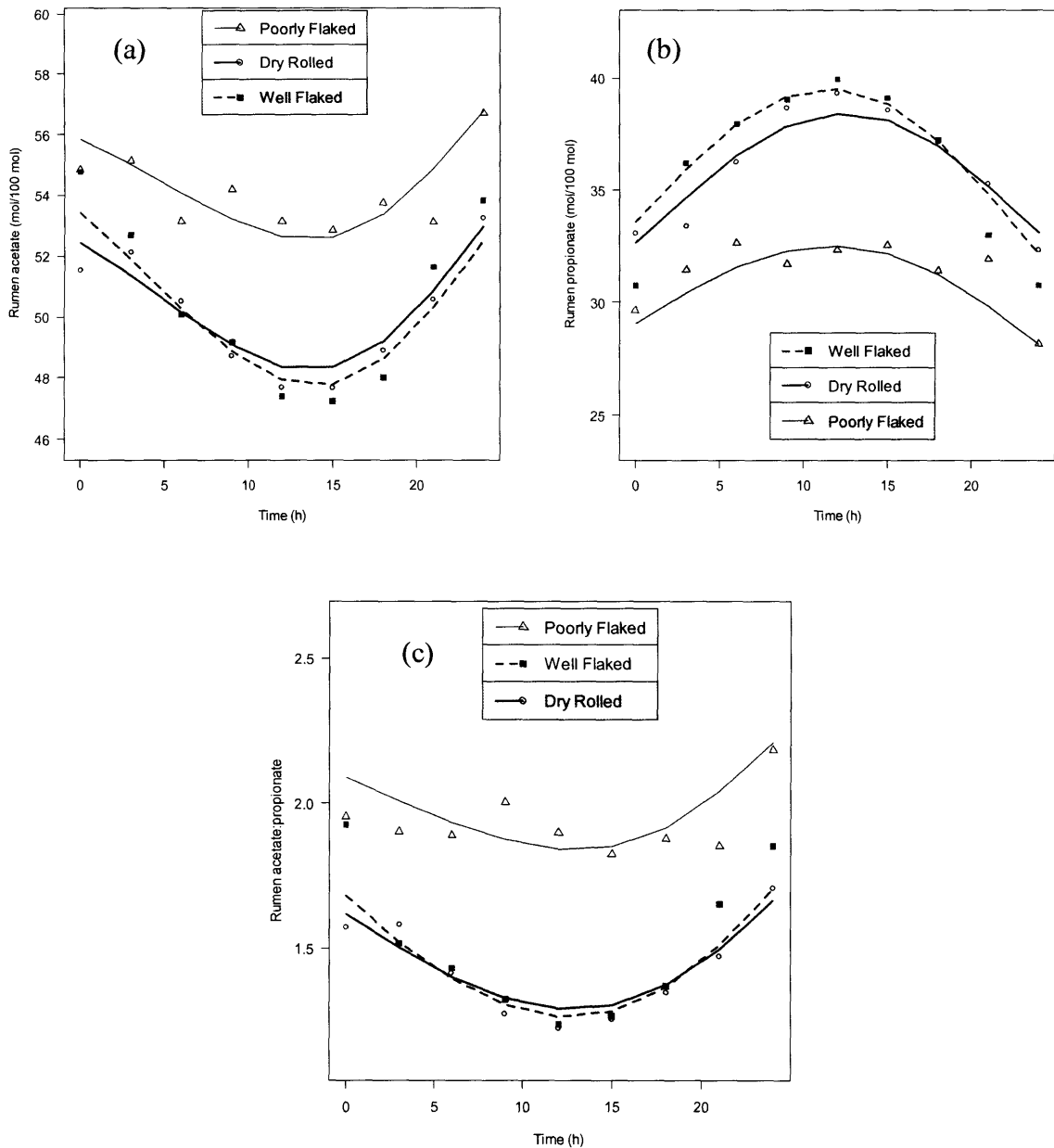


Figure 9.4 Diurnal profiles of the means of the (a) proportion of rumen acetate (mol/100 mol) (b) proportion of rumen propionate (mol/100 mol) and (c) rumen acetate to propionate ratio; and 95% confidence intervals for dry rolled, well flaked and poorly flaked wheat over a 24 h interval. Cattle fed at 0 h.

Figure 9.4 shows that poorly flaked wheat was associated with a higher proportion of acetate in the rumen than well flaked wheat between 6 h and 21 h and a lower proportion of propionate in the rumen between 6 h and 18 h. Thus, poorly flaked wheat was associated with a higher acetate to propionate ratio in the rumen between 3 h and 21 h after feeding than dry rolled and well flaked wheat.

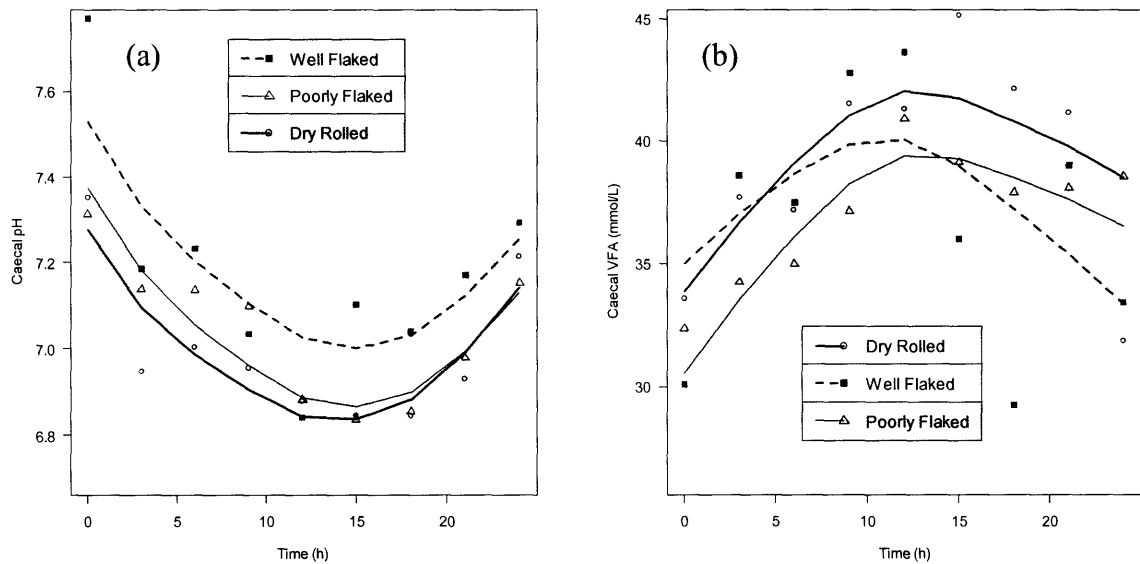


Figure 9.5 Diurnal profiles of the means of caecal (a) pH and (b) VFA concentration (mmol/L); and 95% confidence intervals for dry rolled, well flaked and poorly flaked wheat over a 24 h interval. Cattle fed at 0 h.

Figure 9.5 shows that there were no significant differences between treatments in caecal pH or VFA concentration.

Caecal pH remained between 6.7 and 7.8 over the 24 h interval. The relatively high mean caecal pH of 7.8 at 0 h for well flaked wheat occurred because of an unusually high pH of 8.7 for one sample. There was nothing noticeably unusual about this sample.

Diurnal variation in the parameters was difficult to detect but the lowest pH and highest VFA concentrations appeared to occur around 12 h.

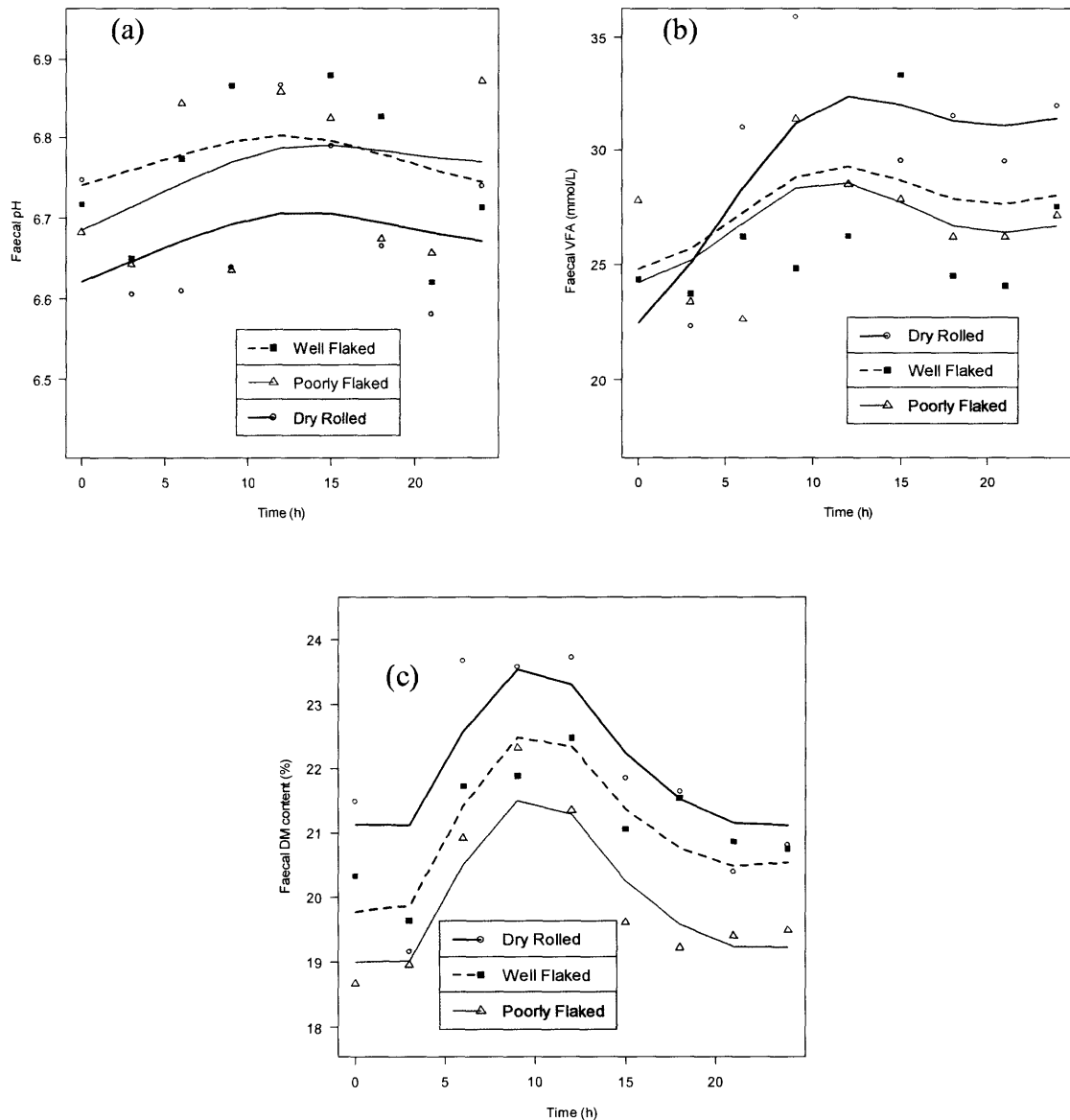


Figure 9.6 Diurnal profiles of the means of faecal (a) pH (b) VFA concentration (mmol/L) and (c) DM content; and 95% confidence intervals for dry rolled, well flaked and poorly flaked wheat over a 24 h interval. Cattle fed at 0 h.

Figure 9.6 shows that there were no significant differences between treatments in faecal pH or VFA concentration. There was no ($P>0.05$) diurnal variation in either parameter.

Poorly flaked wheat had lower faecal DM contents between 3 h and 21 h than dry rolled wheat. For all treatments, faecal DM content peaked between 9 h and 12 h after feeding.

Table 9.5 Means over a 24 h interval for pH and VFA concentration (mmol/L) in the rumen, caecum and faeces of cattle fed three diets differing in source of wheat.

	Rumen		Caecum		Faeces	
	pH	VFA (mmol/L)	pH	VFA (mmol/L)	pH	VFA (mmol/L)
Dry rolled	6.0	121.6	7.0	39.5	6.7	31.2
Well flaked	6.1	111.6	7.2	37.1	6.8	26.0
Poorly flaked	6.1	107.5	7.1	36.9	6.7	26.9
Average	6.1	113.6	7.1	37.8	6.7	28.0

Table 9.5 provides a summary of the general pattern of fermentation for the different grain types at the three sites of measurement. Due to the curvilinear (cyclical) nature of the data, statistical analysis was not performed to identify differences between these raw means.

Average caecal VFA concentration of each cow x treatment combination was negatively correlated with average caecal pH ($P < 0.01$; $R^2 = 0.59$) but average faecal VFA concentration was not significantly associated with average faecal pH.

Average caecal VFA concentration was not correlated with average rumen VFA concentration.

Average caecal pH tended to be positively associated with average faecal pH ($P = 0.06$; $R^2 = 0.20$) but this relationship was not strong. Caecal pH was usually higher than faecal pH.

Average faecal DM content was not associated with average caecal VFA, faecal VFA or faecal pH but was negatively correlated with the average faecal acetate to propionate ratio ($P < 0.01$; $R^2 = 0.42$).

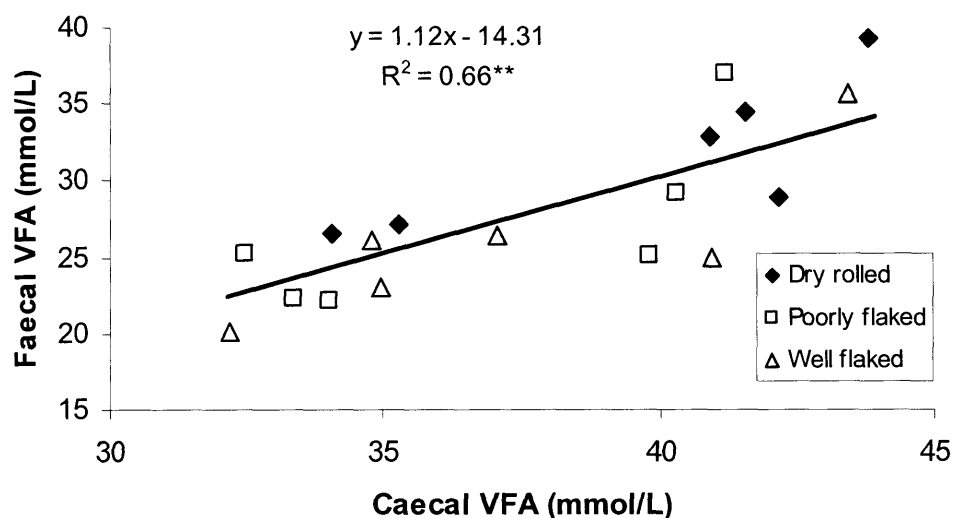


Figure 9.7 The relationship between average caecal VFA concentration (mmol/L) and average faecal VFA concentration (mmol/L). $^{**}(P < 0.01)$. The mean value for each cow x treatment combination is shown.

Figure 9.7 shows that there was a positive correlation between average caecal and faecal VFA concentrations.

Table 9.6 Whole tract starch digestibility (% of starch intake) in cows fed diets based on dry rolled, poorly flaked and well flaked wheat (using Cr_2O_3 as a marker) (predicted means \pm se).

	Starch intake (kg/d)	Faecal starch flow (kg/d)	Whole tract digestibility (% of starch intake)
Dry rolled	3.42	0.10	97.3 \pm 0.4
Poorly flaked	3.24	0.05	98.7 \pm 0.3
Well flaked	3.18	0.04	98.8 \pm 0.1
Average	3.28	0.06	98.3

Table 9.6 shows that whole tract starch digestibility was marginally reduced for dry rolled wheat compared with well flaked wheat ($P < 0.001$). However, there was no significant difference between poorly flaked wheat and well flaked wheat, or between poorly flaked wheat and dry rolled wheat.

Whole tract starch digestibility was also determined using acid insoluble ash and results obtained with this method correlated with results obtained by Cr_2O_3 ($R^2=0.93$).

Faecal starch content (FS) was very closely correlated with whole tract starch digestibility (TTSD) ($P<0.01$; $R^2=0.94$; $\text{TTSD} = -0.45\text{FS} + 99.82$) but not associated with faecal VFA, pH or DM content. Whole tract starch digestibility was not associated with any of the parameters measured in the rumen, caecum or faeces.

9.3.3 In sacco fermentation

The disappearance of grain DM *in sacco* over time for dry rolled, poorly flaked and well flaked wheat is presented in Figure 9.8.

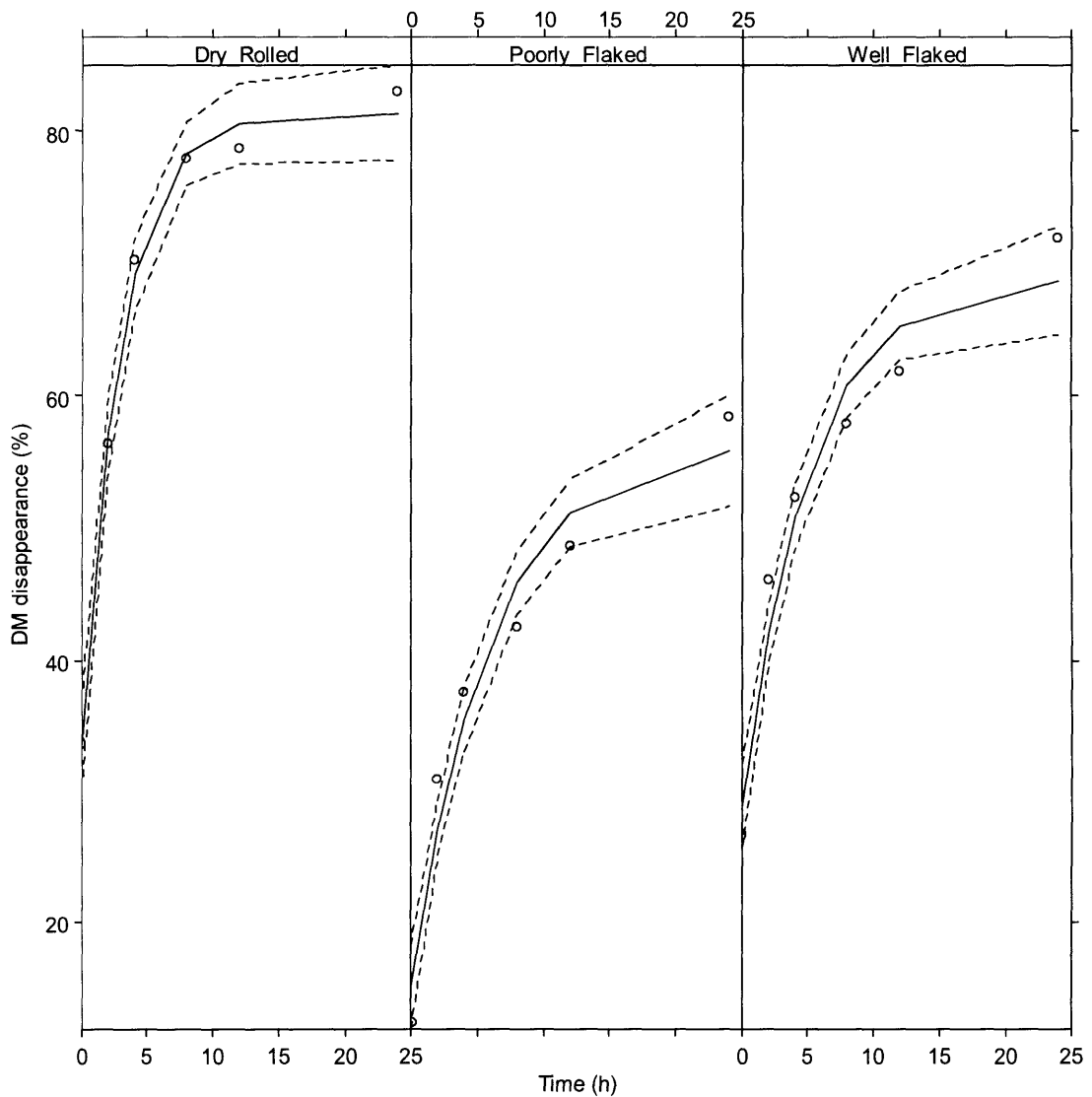


Figure 9.8 Disappearance of grain DM (% of total DM) *in sacco* over 24 h and 95% confidence intervals for dry rolled, well flaked and poorly flaked wheat. The lines are fitted regressions.

Dry rolled wheat fermented at a faster rate *in sacco* than the steam flaked grains whilst poorly flaked wheat fermented the least.

9.4 Discussion

The *in vivo*, *in vitro* and *in sacco* results support the hypothesis that poorly flaked wheat will ferment to a lesser extent in the rumen of cattle than dry rolled wheat. Although starch digestion in rumen was not directly measured *in vivo*, there was a greater concentration of VFA as well as a tendency for lower rumen pH values in cattle fed dry rolled wheat. The relatively slow fermentation rate of poorly flaked wheat was also associated with higher levels of acetate and lower levels of propionate than the other grains and this was consistent with reports that the rate of fermentation is inversely related to the acetate to propionate ratio (Murphy *et al.* 1982; Orskov 1986). Therefore, it appears that the poorly flaked wheat-based diet would have been supplying the most starch to the small intestine but this cannot be substantiated without the measurement of starch digestibility in the rumen.

Average VFA concentration for each grain in the *in vitro* assay was strongly correlated with the VFA concentration *in vivo*, after a similar period of fermentation (Figure 9.2). This was an encouraging result providing confirmation that the *in vitro* fermentation assay originally described by Bird *et al.* (1999), can be reliably used to predict the *in vivo* consequences of feeding particular grains. It should be noted that the differences between the fermentability of grains *in vitro* were not as large, *in vivo*. Fermentation over a longer period of time (24 h) in the animal is likely to have resulted in smaller differences between the diets than when they were fermented for 5 h *in vitro*. Furthermore, only the grains were incubated in the *in vitro* assay whereas the animals were eating complete diets based on these grains. It may have been useful to ferment the complete diets *in vitro* although this has not been done before with this assay.

The results of the *in vitro* enzyme assay suggested that starch from the steam flaked grains would be far more digestible in the small intestine than starch from dry rolled grain. The fact that the dry rolled wheat-based diet had a slightly lower whole tract starch digestibility than the well flaked wheat-based diet, despite the dry rolled wheat being more fermentable, supports the theory that the post-ruminal digestibility of dry rolled wheat was lower than that of the steam flaked grains. As there were no differences between the treatments in the pattern of hindgut fermentation, this suggests that poorly flaked wheat was more digestible in the small intestine than dry rolled wheat.

The physical nature of starch reaching the caecum for each of the three wheat-based diets is likely to have been different. For this reason, if there were different quantities of starch reaching the hindgut for each diet, this may not have been reflected by the pattern of hindgut fermentation. Starch content of caecal digesta was not measured because of the small sample sizes obtained but a digestibility study using cattle fitted with ileal cannulas would be useful to detect differences between these diets in pre-hindgut starch digestion.

Diurnal fluctuations in rumen pH may be important with respect to sub-clinical acidosis (Beauchemin 2000) and it is known that the major cellulolytic bacteria cannot function properly when the pH falls below 6.0 (Russell and Wilson 1996). Figure 9.3 showed that rumen pH fell below 5.5 for all diets. As the duration of below-optimal rumen pH is important (Beauchemin 2000), the tendency for dry rolled wheat to ferment at a faster rate and cause a lower pH than the steam flaked grains suggests that the risk of acidosis would be greatest for dry rolled wheat.

Intake was fixed at 1.6% of LW because this level of feeding was found to be the maximum that these cattle would eat in one meal. Had more feed been offered, some cattle would still have eaten it all at once but others would have had 2-3 meals throughout the day. An irregular pattern of feed intake would have influenced digesta flow rates and complicated the fermentation patterns being measured. Galyean *et al.* (1979) reported that DM, OM and starch digestibility were reduced with increasing DMI (from 0.9% LW to 1.9% LW) in steers fed an 84% corn-based diet. Higher feed intakes may have also provided more of a challenge to the digestive capacity of the animals and supplied more starch to the hindgut (Grovmum and Hecker 1973; Diez-Gonzalez *et al.* 1998). It is therefore likely that, if the cattle in this experiment had been fed under *ad libitum* feedlot conditions, starch digestibility may have been lower and the differences between diets greater.

There was a higher faecal DM content in cattle fed dry rolled wheat than in those fed poorly flaked wheat but this was not associated with other parameters measured in the faeces or caecum. Differences in faecal DM content and the peak between 9 h and 12 h after feeding are difficult to explain. Faecal pH was not associated with faecal VFA concentrations and tended to be only weakly associated with caecal pH. There was a correlation between mean faecal and caecal VFA concentrations, which suggests that faecal VFA levels may provide a more reliable indication of hindgut fermentation than faecal pH or DM content alone whilst the combination of faecal starch content and VFA concentration may provide some measure of pre-caecal starch digestibility.

The close correlation between faecal starch content and whole tract starch digestibility was consistent with the relationship reported by Zinn (1994) for wheat-based diets. Faecal starch content could be a useful tool for assessing the efficiency of grain processing and for identifying between-animal differences.

Both Cr_2O_3 and acid insoluble ash gave similar results for starch digestibility which indicates that acid insoluble ash can be used as a reliable internal marker for ruminant digestibility studies. The results are in agreement with previously reported starch digestibility values for dry rolled (96.6%) and steam rolled wheat (98.6%) (Zinn 1994). No attempt was made to measure diurnal variation in the appearance of Cr_2O_3 but we accounted for this possibility by taking 12 samples; each sample being representative of every 2 h of a 24 h period. Van Keulen and Young (1977) found no evidence of a diurnal pattern in the excretion of acid insoluble ash. The implication is that measurements could theoretically be made on single samples of feed and faeces.

9.5 Conclusions

Poorly flaked wheat was less fermentable than dry rolled or well flaked wheat but appeared to have a higher level of small intestinal starch digestibility than dry rolled wheat under the conditions of this experiment.

Steam flaking appeared to change the site of wheat starch digestion from the rumen to the small intestine without increasing the supply of starch to the hindgut or reducing whole tract starch digestibility.

A significant correlation between faecal and caecal VFA concentrations indicated that VFA levels in the faeces may be a more reliable indicator of fermentation in the hindgut than faecal pH or DM content.

Chapter 10. General Discussion

The literature review and the experiments reported in this thesis support the hypothesis that the characteristics of the grain and the processing of that grain can be used to improve starch digestion in cattle. It may also be possible to achieve more effective use of starch and to reduce animal-health and environmental problems associated with feedlot beef production by selecting animals that ferment less starch in the hindgut.

There are several reasons why improving the efficiency of starch digestion by endogenous enzymes in the small intestine needs to be a priority. The major disadvantage of fermentation in the rumen and hindgut is the loss of energy associated with methane and heat production (Harmon and McLeod 2001). Hindgut fermentation has an added disadvantage in that microbial protein produced during fermentation is lost in the faeces (Orskov *et al.* 1970b). Under certain feeding scenarios, the accumulation of acid in the rumen and hindgut of cattle may pose a problem in terms of animal health and productivity (Zinn 1990a; Zinn 1993; Reinhardt *et al.* 1997). Although hindgut acidosis can occur in ruminants (Zust *et al.* 2000) the prevalence and significance of this problem in feedlot cattle is less well understood than is rumen acidosis.

Efficient whole tract starch digestibility is usually associated with high levels of starch digestion in the rumen. In addition, high whole tract starch digestibility is usually related to improved efficiency of feed utilisation (Spicer *et al.* 1986; Theurer 1986). However, the risk of excessive starch fermentation in the rumen means that whole tract starch digestibility should not be the only criterion used for assessing the efficiency of starch digestion. A reduction in whole tract starch digestibility may be acceptable if energy is conserved by a greater proportion of starch being digested in the small intestine and if acidic conditions in both the rumen and hindgut are reduced. The challenge is to increase the proportion of starch digested in the small intestine without delivering more starch to the hindgut.

Starch digestion in the small intestine is often incomplete but it is not clear what is the first limiting factor. Possible factors include starch structure and availability to endogenous enzymes (Kreikemeier *et al.* 1991), α -amylase activity (Orskov 1986) and the capacity of the gut for glucose absorption (Huntington 1997). It appears that if the structure and availability of starch is improved through grain processing, limitations associated with the activity of starch-degrading enzymes in the small intestine can be reduced. It is also likely that factors such as

amylolytic enzyme activity and absorptive capacity can vary between animals and that the capacity for starch digestion in the small intestine can be increased by animal adaptation to high-starch diets.

Particular grain processing techniques can be used to increase whole tract starch digestibility as well as to manipulate the site of starch digestion. Changes in starch digestibility with grain processing occur through cracking and reduction in particle size in the case of dry rolling (Galyean *et al.* 1981) and through additional changes in protein matrix structure, cellular distribution and starch gelatinisation with steam flaking (Rooney and Pflugfelder 1986; Theurer 1986).

In vitro experiments reported in this thesis suggested that poorly (coarsely) steam flaked wheat and barley are likely to ferment less in the rumen than the equivalent dry rolled grains but have a substantially higher level of starch digestibility in the small intestine. *In sacco* studies also showed that dry rolled wheat and barley fermented at a faster rate in the rumen than poorly flaked wheat and barley. Experiments with cannulated cattle confirmed that results obtained from *in vitro* studies of fermentation and digestion correlated with the pattern of starch fermentation and digestion *in vivo*. The moderate steam flaking of wheat appeared to alter the site of starch digestion from the rumen to the small intestine without increasing the supply of starch to the hindgut. These findings are important because the common objective of commercial grain processing should be to restrict the rate and extent of starch digestion in the rumen. Given the benefits of starch digestion in the small intestine and the problems associated with rumen acidosis when a grain ferments too quickly, the ability to process a grain to control fermentation in the rumen while at the same time achieving efficient digestion in the small intestine would be very useful.

It appears that the larger particle size (and smaller available surface area) of steam flaked wheat and barley compared to dry rolled grain, is partly responsible for the reduced rate of rumen fermentation. On the other hand, the effects of 'cooking' appear to be more important than particle size in determining starch digestibility in the small intestine. The *in vitro* enzyme assay used in the experiments reported in this thesis does not account for the effect of rumen fermentation prior to enzymatic digestion of starch in the small intestine but it still appears to be a useful method for estimating differences between grains and between processing methods.

The microbial population in the hindgut readily responds to a highly fermentable substrate such as starch (Orskov *et al.* 1970b; Allison *et al.* 1975) and it is reasonable to assume that changes in the hindgut will be reflected by changes in the faeces. For this reason, faecal characteristics

were used in many of the experiments reported in this thesis as indirect indicators of hindgut fermentation. Significant between-animal variation in faecal pH and DM content suggested that measurement of these parameters may reflect differences between individuals in the pattern and extent of hindgut fermentation. Between-animal variation in faecal parameters should theoretically increase in cattle ingesting diets containing high levels of starch which will increase the supply of starch to the hindgut (Grovmum and Hecker 1973; DeGregorio *et al.* 1982; Siciliano-Jones and Murphy 1989b; Siciliano-Jones and Murphy 1989a). Diets containing starch of different digestibility may not produce measurable differences in the extent or pattern of hindgut fermentation if feed intake is controlled. This may have been the situation in the experiment reported in chapter 9 where cows were given restricted rations rather than being fed *ad libitum*, as is commercial feedlot practice. In this case, lower intake resulted in similar levels of hindgut fermentation with diets known to marginally differ in whole tract starch digestibility.

The value of faecal pH, DM and nitrogen content as indirect measures of hindgut fermentation was questionable in a number experiments and the associations between these parameters were generally inconsistent. It appeared that the relationship between faecal pH and DM content was poor which was interesting because it is considered that extensive hindgut fermentation and acidity are partly responsible for lowering faecal DM content (Lee 1977; Godfrey *et al.* 1992). It was also interesting that there were no correlations between faecal pH and faecal starch content in any of the studies, even though relationships of varying strengths have previously been reported for these two parameters (Wheeler and Noller 1977; Russell *et al.* 1980). Using faecal pH as a measurement of starch fermentation in the hindgut assumes that starch reaching the hindgut will ferment enough to cause a decrease in pH and that pH accurately reflects the amount of acid without the possibility of variable buffering.

In certain situations, starch that reaches the hindgut may be resistant to fermentation and this could explain why sometimes the correlation between faecal pH and faecal starch content is poor or non-existent. An increase in faecal starch content indicates that more starch is entering and passing out of the hindgut but is not a measure of the amount of starch fermented there. Therefore, faecal starch cannot be used by itself to reliably predict the level of fermentation in the hindgut. Similarly, increased faecal starch content may also cause faecal DM content rise; as suggested by the positive correlation between these parameters for the finisher cattle studied in the work described in chapter 4.

It appears likely that the effects of buffering and mucosal secretions in response to acidity in the hindgut may influence faecal pH and nitrogen content. Many faecal samples taken

throughout the cattle trials reported in this thesis were observed to contain mucous and what appeared to be gut linings. This was particularly evident in the cattle experiencing hindgut acidosis in the experiment reported in chapter 7. Measurement of VFA concentration in the faeces is likely to be one of the more accurate ways of predicting the extent of starch fermentation in the hindgut. Studies in chapter 9 showed that, in grain-fed cattle, VFA concentrations in the faeces were correlated with VFA concentrations in the caecum. It is possible that faecal VFA concentration together with faecal starch may provide the most accurate indirect predictor of starch entering the caecum.

Faecal nitrogen content was not an accurate measure of between-diet or between-animal differences in hindgut fermentation. Methods of measuring specific bacterial components in faeces could provide a more accurate indication of the extent of hindgut fermentation. In future, real-time PCR techniques (Tajima *et al.* 2001) may be useful for quantifying bacterial species present in the faeces. Alternatively, methods that can separate microbial protein fractions from other sources of faecal nitrogen may be useful. Orskov *et al.* (1970b) used this approach to demonstrate an increase in bacterial and endogenous debris nitrogen in the faeces of sheep receiving infusions of starch into the caecum. However, all of these measurements are time consuming and potentially too expensive for routine screening of feedlot animals.

The experiment described in chapter 7 was designed to study hindgut acidosis as a potentially important factor in the overall pathogenesis of grain-poisoning. It was interesting that, in this experiment, the initial site of acidosis was the hindgut and that changes measured in the faeces were correlated with an early reduction in DMI. This was consistent with findings in sheep (Lee 1977; Godfrey *et al.* 1992). However, the experimental model involved rapid and artificial conditions for introduction of grain into the rumen which may have caused a faster passage rate of digesta to the small intestine and hindgut than when grain is ingested by adapted animals.

Lactic acid was found to be more important than VFA in influencing pH in both rumen and faecal contents, however, the increase in VFA alone can also lower rumen pH during bouts of sub-clinical acidosis (Reinhardt *et al.* 1997). Some animals also appeared to resist acidosis by maintaining normal rumen and faecal pHs under carbohydrate overload. Differences between animals in their ability to resist rumen acidosis may be associated with factors such as microbial adaptation, saliva production/buffering capacity and rumen turnover time. Digesta flow rate and the capacity for pre-caecal starch digestion may also determine an animal's susceptibility to hindgut acidosis.

Little research has been undertaken to assess the extent of and mechanisms behind genetic variation in starch digestion in cattle. Chapter 8 provided evidence of genetic variation in faecal parameters that are likely to reflect the level of hindgut fermentation. Differences in hindgut fermentation may also contribute to between-animal differences in the efficiency of feed utilisation. Further experimental work is required to measure variability in starch digestion and to assess whether it is likely to be of practical importance. If hindgut fermentation and/or starch digestion are linked with the efficiency of feed utilisation, these parameters may potentially be used to identify and select for animals that are more efficient. Such a tool could be extremely useful as measuring and selecting for RFI directly is currently a very labour intensive and expensive process (Archer *et al.* 1998).

10.1 Conclusions

This thesis has shown that both the characteristics of the grain and the animal can influence the efficiency of starch digestion in cattle. There are three factors that can be managed to improve these important aspects with respect to feedlot beef production. The first two factors are grain selection and grain processing and the *in vitro* techniques investigated in this thesis can be used to improve these management options. The third factor represents a new approach towards identifying those animals that are more efficient with respect to starch digestion. Knowledge of the capacity of individual animals to digest starch could assist in more accurate diet formulation for different groups of animals and lead to long-term benefits through genetic selection.

Industry-relevant conclusions arising from this thesis are:

1. Moderate steam flaking of wheat and barley may be recommended as a reliable method to improve the efficiency of starch digestion while reducing the risk of acidosis.
2. Cattle unadapted to high-grain diets may be susceptible to the problem of hindgut acidosis that can occur even when conditions in the rumen are normal.
3. Genetic variation in faecal parameters in cattle are associated with between-animal differences in the efficiency of feed utilisation.

There are a number of recommendations for future work arising from the experiments reported in this thesis.

- It would be useful to repeat the experiment reported in chapter 9 using cattle fitted with duodenal and ileal cannulas. Digestibility measurements are required to quantitatively determine if pre-caecal starch digestibility is higher for poorly flaked wheat than dry

rolled wheat. There is also a need to study the efficiency of feed utilisation in cattle given *ad libitum* access to diets based on dry rolled, poorly flaked and well flaked wheat. An evaluation of the economic cost of LW gain in cattle fed these different diets would be valuable from an industry perspective.

- It is important to determine the optimal cooking time and flake thickness of various steam flaked grains. If acceptable digestibility and feed efficiency levels can be obtained with lower processing costs, this will undoubtedly benefit the industry. *In vitro* analysis of different grains and processing methods not assessed in this thesis would also be useful to identify other combinations that could potentially supply readily digestible starch to the small intestine. This is important because a preferred source of grain may not always be available to a beef feedlot.
- There may be value in developing an *in vitro* enzyme starch digestibility assay that takes into account the effect of rumen fermentation before small intestinal digestion. It could be possible to combine the *in vitro* fermentation and enzyme assays to develop one test to rank grains in terms of pre-caecal starch digestibility.
- Future acidosis studies in ruminants should include measurement of hindgut, as well as rumen fermentation, in order to accurately assess the overall risk of acidosis with particular feeding regimes. It is still important to assess the significance of hindgut acidosis in cattle adapted to high-grain diets and/or voluntarily ingesting large quantities of grain.
- The existence, extent and cause of genetic variation in starch digestion is important to determine as well as the possibility of using this attribute as a basis for genetic selection. Experiments should involve feeding high levels of a relatively poorly digestible starch-source such as sorghum in order to highlight between-animal differences. Such diets could provide more of challenge to the rumen and intestinal digestive systems than a readily digestible grain like wheat. There may be value in using invasive experiments to measure differences in rumen retention time and digesta flow rates as these are possible mechanisms behind between-animal variation in starch digestion.