5 THE EFFECT OF GRAIN SPECIES AND PROCESSING ON SMALL INTESTINAL STARCH DIGESTION

5.1 GENERAL INTRODUCTION

This Chapter describes the objectives, methodology and results of a study designed to ascertain the effects of grain species and grain processing on small intestinal starch digestion in equines. The discussion in Chapter 2, examining starch digestion and absorption in the equine small intestine, highlighted the problems that may arise when high starch feedstuffs are included in equine diets. A small stomach (Argenzio, 1993b), relatively fast passage rate through the small intestine (Frape, 1998) and a possible deficiency of amylolytic enzymes in the pre-caecal section of the gastrointestinal tract (Comline *et al.*, 1969; Roberts, 1974) can result in starch escaping undigested, into the hindgut. Here, starch is rapidly fermented, causing radical changes to the constitution of hindgut flora and an increase in the volatile fatty acid and lactic acid concentrations in the hindgut (Garner, 1978).

Such an outcome is shown to be associated with a reduction in feed conversion efficiency (de Fombelle *et al.*, 1999: Black, 1971), behavioural problems (Johnson *et al.*, 1998; Rowe *et al.*, 1995; Willard *et al.*, 1977) and laminitis, a crippling and potentially career and life ending disease of horses (Garner *et al.*, 1975; Garner *et al.*, 1977; Pollitt, 2001a; Rowe *et al.*, 1995).

In order to improve the efficiency of grain feeding and to prevent the negative consequences associated with hindgut starch fermentation, it is desirable to increase the percentage of cereal grain starch that is digested in the horse's small intestine. One way to do this might be by selecting grains with starch that is naturally more digestible in the equine small intestine. One could also physically process cereal grains to change their gross and endosperm structure to increase the susceptibility of starch granules to enzymatic attack and improve the rate and extent of pre-caecal starch digestion. In order to investigate these possible solutions, it was decided to set up a series of three experiments. These investigate the small intestinal starch digestibility of barley, triticale, oats, corn and rice and the effectiveness of expansion, extrusion, micronising and steam-rolling as means of improving the digestion of cereal grain starch in the equine small intestine. These specific grains were selected for use during this study for two main reasons: 1. they were currently used in industry in the case of oats, corn and barley (Chapter 3); and 2. they were not currently used in industry but had shown potential for increased use in industry in the case of triticale and rice (Bird *et al.* 1999; McMeniman *et al.* 1990).

An *in vitro* assay designed to estimate small intestinal starch digestion (Bird *et al.*, 1999), a second *in vitro* assay designed to demonstrate the fermentation characteristics of cereal grain starches (Bird *et al.*, 1999) and the glycaemic and insulin responses (Loeb, 1971; Wolever *et al.*, 1991) were used to predict small intestinal starch digestibility. Faecal

parameters, including pH, dry matter, starch, nitrogen, VFA concentrations and lactic acid concentrations were also measured to allow for estimations of the extent of hindgut starch fermentation occurring on the test diets. The intention was also to evaluate these faecal parameters as simple methods for determining small intestinal starch digestion in horses.

5.2 HYPOTHESES

1. That the physical and chemical changes in cereal grain structure, caused by processing methods that involve any combination of heat, steam and pressure, will improve the *in vitro* and *in vivo* small intestinal starch digestion characteristics of cereal grains.
2. That the grain processing methods used to increase the small intestinal digestion of starch will also change the grains fermentation characteristics, increasing the rate at which grains ferment *in vitro* in bovine rumen fluid and equine caecal fluid.
3. That the *in vitro* enzyme starch digestion assay and the *in vivo* glycaemic and insulin response assays will be closely related and rank grains in the same order of digestibility.

Details of the three experiments follow.

5.3 EXPERIMENT 1

The aims of experiment one were to:

(i) investigate the *in vitro* enzyme starch digestion and fermentation characteristics and the *in vivo* small intestinal starch digestion characteristics of the cereal grains barley, triticale and oats; and

(ii) determine the effect of expansion and steam-rolling on the *in vitro* and *in vivo* small intestinal starch digestion and the *in vitro* starch fermentation characteristics of barley and triticale.

5.3.1 Methods and Materials

Six grain treatments: cracked barley; cracked triticale; cracked oats; expanded barley; expanded triticale; and steam-rolled triticale and a glucose control were used in the trial (cracked and expanded barley were the same variety, variety unknown; extruded triticale and steam-rolled triticale were the same variety, variety unknown; cracked triticale was from the variety Madonna; oats variety was unknown). The study was conducted in two 4x4 latin square periods with the glucose control diet incorporated into each latin square to allow for later comparisons between periods. Expanded barley, expanded triticale, steam-rolled triticale and glucose were fed in period one while cracked barley, cracked triticale, cracked oats and glucose were fed in period two. Treatments were rotated at random within each latin square period with each animal remaining on one treatment for three days (since the primary consideration was digestion occurring in the small intestine, a period of three days was considered long enough to allow all feed to pass through this section of the gastrointestinal tract). The study was conducted over a six-week period during which time horses were kept in stables for two 12-day periods for controlled feeding and measurement

and were maintained at pasture for two weeks between observation periods. The University of New England's Animal Ethics Committee approved the experimental protocol.

Meal sizes were adjusted to supply 670 g of starch or glucose per feed (Table 5.1). All meals, except for oats, were adjusted to a weight of 1250 g, through the addition of bran, to remove any effects of meal size. Each meal of oats weighed 1800 g.

Table 5.1: The percentage starch in each grain and the quantity fed in order to supply 670 grams of starch.

	% Starch (as is basis)	Quantity to be fed (g/meal)
Cracked barley	54.3	1232
Cracked triticale	52.6	1110
Cracked oats	37.3	1800
Expanded barley	56.5	1185
Expanded triticale	56.5	1185
Steam-rolled triticale	59.7	1122
Glucose	100.0	670

Animals and Management

Four (4) thoroughbred geldings, aged 6 to 9 years and weighing 480 ± 40 kg were used in the trial. All horses were treated with an anthelmintic and had their teeth corrected by an equine dentist prior to the experimental period. The horses were stabled in individual box stalls overnight and held in individual yards during the day for the duration of experimental feeding. Sawdust was used as bedding in the stables and the horses were exercised each evening for approximately half an hour. All animals were considered healthy and free from parasites at the commencement of the trial.

During the experimental feeding periods, horses were fed the grain portion of their diet at 0700 h and 1730 h and on the completion of eating the grain the horses were offered 4kg of lucerne hay. On days when the glycaemic and insulin responses were to be measured, only the grain portion of the diet was fed in the morning. On these days, hay was fed at the conclusion of blood sampling. The evening feed following blood sampling consisted of a 50% mix of the current test diet and the subsequent test diet. On the morning following blood sampling, the horses were offered 100% of their new test diet. All animals had access to water *ad libitum*.

Measurements

The total starch content of all grains was determined prior to the trial using the method of (McCleary *et al.*, 1997). *In vitro* enzyme digestibility of the grains was determined using the *in vitro* enzyme digestion assay of Bird *et al.* (1999), which involves the incubation of 100mg of grain, ground to 0.5 mm, at biological temperatures for 1 hour in the presence of excess α-amylase and amyloglucosidase. An incubation period of 15 minutes was also examined. A modification of the method of Bird *et al.* (1999) was used to determine the rate of *in vitro* starch digestion. The modification involved the use of several incubation times, with times of 15, 30, 45, 60, 90 and 120 minutes used to calculate of rate of enzymatic

starch digestion. The coefficient of variation for these assays was 1.45% and was calculated using the standard deviations of the replicates within the *in vitro* enzyme digestion and rate of digestion assays. These assays all used thermostable α-amylase, derived from *Bacillus licheniformis* and amyloglucosidase, derived from *Aspergillus niger* (Megazyme International Ireland Ltd).

The *in vitro* rumen fluid fermentation characteristics of cracked oats, barley and triticale and expanded barley and triticale were determined using the method of Bird *et al.* (1999), during which, 30 grams of each cereal grain, ground to 0.5mm, was fermented in 125 mL of rumen fluid at 39°C for 5 hours. At the conclusion of the 5-hour incubation the fermentation medium pH, volatile fatty acid concentration and lactic acid concentration were determined. VFA concentrations were measured on a Varian CP-3800 Gas Chromatograph fitted with a Varian CP-8400 auto-sampler (Varian Australia, 6/81 Frenchs Forest Road, Frenchs Forest, NSW, Australia). D and L lactic acid concentrations were measured using the Boehringer Mannheim UV method for determination of D and L lactic acid (Boehringer Mannheim, R-Biopharm GmbH, D-64293, Darmstadt, Germany), modified for use on the Roche COBAS-BIO auto-analyser (Roche Diagnostic Systems, Basle, Switzerland). The rumen fluid used in the fermentation assay was collected from rumen fistulated, mature low-line Angus steers that were maintained on a 50% mixed grain (oats, barley, wheat, maize and sorghum) 50% chaff diet.

In vitro equine caecal fluid fermentation characteristics of the grains used in this experiment were determined using a modification of the method of Bird *et al.* (1999, *modified by* S. Bird). The modifications involved incubating 10 g of cracked grain in one-third the volume of fermentation medium used during the original assay, in 250 mL plastic culture jars. The incubation was stopped at 4 hours upon which time the fermentation medium pH was measured and a liquid fermentation medium sample was collected for determination of volatile fatty acid and lactic acid concentrations (methods as described above). Caecal fluid was collected from the caeca of the horses that were slaughtered for collection of jejunal and stomach fluid in the experiment described in Chapter 7. Both horses had been fed a grain diet (2.8 kg oats and 2 kg extruded rice/day) for 7 days prior to their slaughter.

Crude fat content and the crude protein content of all grains were determined during laboratory analysis following the experimentation period. Crude fat content of grains was determined by chloroform solvent extraction using the Soxtec system. Total nitrogen content was determined using the LECO FP 2000 system (LECO Corporation, Michigan, USA) and multiplied by 6.25 to calculate crude protein content.

Horses were weighed on arrival at the stable complex and feed intakes and refusals were recorded each day for the duration of the trial. Faecal samples were collected fresh from the stable of each horse between 0700 and 0800 hours on the second and third day of each latin square rotation and were sub-sampled and analysed for pH, dry matter, total nitrogen and total starch, using the methods described in Section 4.2. Faecal volatile fatty acid and lactic

acid concentrations were also measured. Faecal samples were prepared for VFA and lactate analysis by mixing 10 grams of fresh faeces with an equal weight of 0.1M H_2SO_4. VFA concentrations were measured on a Varian CP-3800 Gas Chromatograph fitted with a Varian CP-8400 auto-sampler (Varian Australia, 6/81 Frenchs Forest Road, Frenchs Forest, NSW, Australia). D and L lactic acid concentrations were measured using the Boehringer Mannheim UV method for determination of D and L lactic acid (Boehringer Mannheim, R-Biopharm GmbH, D-64293, Darmstadt, Germany), modified for use on the Roche COBAS-BIO auto-analyser (Roche Diagnostic Systems, Basle, Switzerland).

On the third day of each latin square period, blood samples were taken to determine the glycaemic and insulin responses to the test diets using the methods described in Section 4.2. Blood samples were taken at 0 hours (prior to grain being fed) and then at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes following commencement of grain consumption. Plasma samples were analysed for glucose and insulin concentration as per the methods described in Section 4.2.

Curves of glucose and insulin against time for individual horses were used to calculate peak glucose/insulin concentrations, average glucose/insulin concentrations, peak minus the basal glucose/insulin concentrations, time to peak glucose/insulin and slope to peak glucose/insulin. Area under the curve and a glycaemic index were also calculated for the glucose data (Wolever *et al.* 1991). The glycaemic index (GI) was calculated as: (area under the curve for treatment ÷ area under the curve for glucose control) x 100.

Data from each latin square were analysed separately. Glycaemic and insulin response data were statistically analysed using a fixed effect ANOVA, with diet, period and horse being included in the model. Faecal data were analysed using a split plot design. Significant differences between diet means were further examined using a Fishers least square difference multi-comparisons function at a 99% confidence interval. Analysis of the consistency of ranking of horses and grains in order of glycaemic and insulin response was carried out using a coefficient of concordance (Moroney, 1968). Statistical analysis was carried out using the S-plus for Windows statistical package (Insightful Corporation, Seattle, WA. USA).

5.3.2 Results

In Vitro Starch Digestion and Fermentation Characteristics

Steam-rolled triticale had the highest percentage of starch on a dry matter basis and oats the lowest. Oats had the greatest crude fat content at 8.2%, while the steam-rolled and extruded triticale had the lowest crude fat content. Expanded triticale contained the highest level of crude protein. Steam-rolled triticale and expanded triticale exhibited the most extensive starch digestion at both 15 and 60 minutes, while cracked oats and cracked triticale were the most digestible of the unprocessed grains *in vitro* at both the 15 and 60-minute periods of incubation, with very little difference between them. The expanding of triticale increased *in*

vitro starch digestion by 19.6% and 9.3% at the end of the 15 and 60 minute *in vitro* incubations, respectively, compared to unprocessed triticale. The steam-rolling of triticale increased the *in vitro* starch digestion of this grain by 44.2% and 11.5% following 15 and 60 minute *in vitro* incubations respectively. Likewise the expanding of barley increased the starch digestibility of barley by 53.7% and 23.5% at 15 and 60 minutes (Table 5.2).

Table 5.2: The total starch, crude fat and crude protein contents and the *in vitro* starch digestibilities at 15 and 60 minutes for cracked barley, cracked triticale, cracked oats, expanded barley, expanded triticale and steam-rolled triticale (SRT).

	Cracked barley	Cracked triticale	Cracked oats	Expanded barley	Expanded triticale	SRT
Starch (%DM)	61.0	58.8	41.0	63.1	63.0	66.2
Crude fat (%DM)	3.0	3.1	8.2	3.0	2.8	3.2
Crude protein (%DM)	9.9	12.8	8.2	10.3	13.1	12.0
Starch digestion (% starch in15 mins)	25.7	41.9	40.6	39.5	50.1	60.4
Starch digestion (% starch in 1hour)	50.3	73.8	69.5	62.1	80.7	82.3

The fastest rates of starch digestion occurred within the first 15 minutes of *in vitro* incubation during the rate of enzyme digestion assay, for all grains (Table 5.3, Figure 5.1). Cracked triticale displayed the fastest rate of digestion during this time period for the unprocessed grains, with 2.8% of starch present digested/minute. The corresponding rates for expanded and steam-rolled triticale were 3.3% and 4.0% digested/min respectively. The expansion of barley increased the rate of barley starch digestion in the first 15 minutes from 1.7% digested/min to 2.6% digested/min (Table 5.3, Figure 5.1).

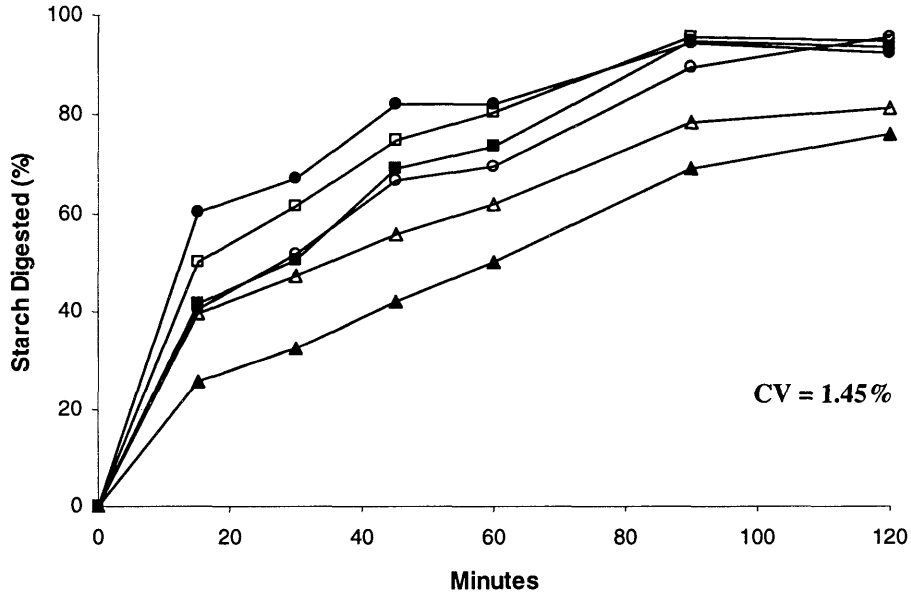

Figure 5.1: The pattern of *in vitro* digestion for (■) cracked triticale, (□) expanded triticale, (●) steam-rolled triticale, (▲) cracked barley, (△) expanded barley and (○) cracked oats.

Table 5.3: The rate of digestion (% of starch present in the grain on a dry matter basis/minute) for cracked barley, cracked triticale, cracked oats, expanded barley, expanded triticale and steam-rolled triticale (SRT).

	0-15 mins	15-30 mins	30-45 mins	45-60 mins	60-90 mins	90-120 mins
Cracked barley (% starch/min)	1.71	0.44	0.66	0.54	0.63	0.24
Cracked triticale (% starch/min)	2.79	0.57	1.26	0.29	0.69	-0.03
Cracked oats (% starch/min)	2.70	0.75	1.01	0.17	0.67	0.20
Expanded barley (% starch/min)	2.63	0.52	0.57	0.42	0.55	0.09
Expanded triticale (% starch/min)	3.34	0.75	0.91	0.38	0.50	-0.04
SRT (% starch/min)	4.02	0.45	1.02	-0.01	0.40	-0.06

Grain fermentation characteristics in bovine rumen fluid and equine caecal fluid are presented in Tables 5.4 and 5.5 respectively. Expanded triticale was the most fermentable grain in the bovine rumen fluid and expanded triticale and cracked barley were the most fermentable grains in equine caecal fluid. However, cracked oats in the rumen fluid initiated the highest concentration of lactic acid, while in the equine caecal fluid, cracked barley produced the highest concentration of lactic acid.

Table 5.4: The fermentation characteristics in bovine rumen fluid, of cracked barley, cracked triticale, cracked oats, expanded barley, expanded triticale and steam-rolled triticale (SRT) when fermented for 5 hours. The pH, volatile fatty acid (VFA) concentrations and total lactate concentrations in the rumen fluid were measured at the conclusion of five hours.

	Cracked barley	Cracked triticale	Cracked oats	Expanded barley	Expanded triticale	SRT
pH	7.0	7.0	6.5	6.8	5.8	7.0
Acetate (mmol/L)	40.9	44.5	44.5	47.0	66.4	38.7
Propionate (mmol/L)	15.1	18.1	28.7	19.3	24.3	15.3
Butyrate* (mmol/L)	6.8	8.1	4.6	7.8	13.1	6.9
Total VFA (mmol/L)	64.1	72.0	78.4	75.1	104.9	61.6
Total lactate (mmol/L)	11.0	5.6	16.1	5.1	15.6	10.5
Total acid (mmol/L)	75.1	77.6	94.5	80.2	120.5	72.1

* 1 mmol glucose = 2 mmol Acetate, 2 mmol Propionate, 1 mmol Butyrate

Fermentation characteristics varied between the bovine rumen fluid and equine caecal fluid, with VFA concentrations in the equine caecal fluid lower than in rumen fluid, but lactic acid concentrations were higher in the equine caecal fluid than in bovine rumen fluid for all grains (Tables 5.4 and 5.5). It should be noted that the rumen fermentation assay for these grains was carried out in accordance with the method of Bird *et al*, 1999 while the equine caecal fluid fermentation assay was carried out using a modification of this method. Modifications are described in Section 5.3.1.

Table 5.5: The fermentation characteristics in equine caecal fluid, of cracked barley, cracked triticale, cracked oats, expanded barley, expanded triticale and steam-rolled triticale (SRT), when fermented for 4 hours. The pH, volatile fatty acid (VFA) concentrations and total lactate concentrations in the caecal fluid are measured at the conclusion of four hours.

	Cracked barley	Cracked triticale	Cracked oats	Expanded barley	Expanded triticale	SRT
pH	6.8	7.0	7.2	7.6	6.8	7.2
Acetate (mmol/L)	23.7	26.8	17.5	17.6	28.2	20.7
Propionate (mmol/L)	9.2	7.2	5.9	6.5	8.1	6.0
Butyrate* (mmol/L)	2.7	2.7	1.8	2.0	2.9	2.1
Total VFA (mmol/L)	36.6	37.8	25.9	26.9	40.2	29.7
Total lactate (mmol/L)	38.4	17.7	22.3	20.1	33.7	24.1
Total acid (mmol/L)	75.0	55.5	48.2	47.0	73.9	53.8

* 1 mmol glucose = 2 mmol Acetate, 2 mmol Propionate, 1 mmol Butyrate

In Vivo Results – by Treatment

The glycaemic response curves measured during latin squares one and two, for each treatment diet are illustrated in Figures 5.2a and 5.2b respectively. During the first latin square, the glycaemic response curves were typical response curves as described by Loeb (1971). The glycaemic response curves measured during the second latin square however, were slightly A-typical, with the glycaemic response for the glucose control diet observed during latin square two appearing to be much lower than that observed for the control diet during the first latin square.

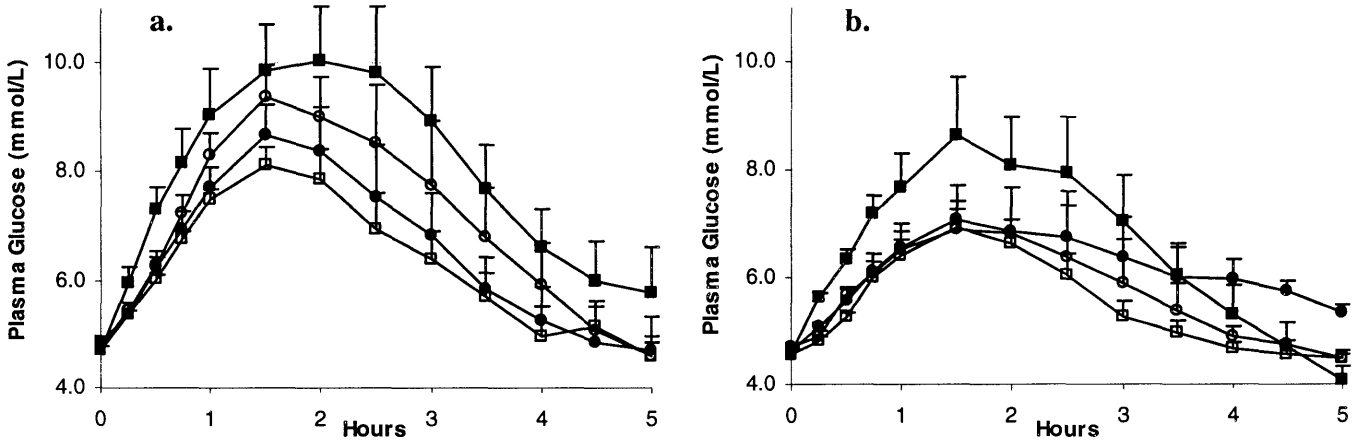

Figure 5.2: Mean glycaemic response curves for: (**a**) period 1; (□) expanded barley, (●) expanded triticale and (○) steam-rolled triticale and (■) the glucose control: and for; (**b**) period 2 (○) cracked barley, (□) cracked triticale and (●) cracked oats and the (■) glucose control.

During the first latin square period, the processed grains steam-rolled triticale, expanded triticale and expanded barley initiated peak glucose, average glucose and peak minus basal glucose concentrations were not significantly different from one another. Horses fed expanded barley displayed significantly lower ($P<0.01$) peak, average and peak minus basal glucose concentrations than those displayed by horses on the glucose control diet. Expanded triticale generated a significantly ($P<0.01$) lower average glucose concentration in comparison to the glucose control diet. Diet had no significant effect on time to peak glucose, slope to peak glucose, area under the glycaemic response curve or the glycaemic index. There were significant differences between horses for peak glucose concentrations,

average glucose concentrations and peak minus basal glucose concentrations. Period had no effect on any plasma glucose parameters measured during latin square one (Table 5.6).

Table 5.6: Mean peak glucose concentration, average glucose concentration, peak minus basal glucose concentration (P-B), time to peak glucose, slope to peak glucose, area under the curve and glycaemic index for the glucose control, expanded barley, expanded triticale, and steam-rolled triticale diets during latin square one.

	Glucose		Expanded barley		Expanded triticale		Steam-rolled triticale	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Peak glucose (mmol/L)	10.3^{a}	1.06	8.2^{b}	0.38	8.8^{ab}	0.62	9.4^{ab}	0.62
Average glucose (mmol/L)	7.8^{a}	0.72	6.2^{b}	0.26	6.4^{b}	0.41	6.9^{ab}	0.50
P-B glucose (mmol/L)	5.6^{a}	0.89	3.5^{b}	0.34	4.0^{ab}	0.60	4.7^{ab}	0.56
Time to peak glucose (hours)	2.0	0.20	1.8	0.14	1.8	0.14	1.9	0.24
Slope to peak glucose (mmol/L/h)	2.7	0.30	2.1	0.12	2.4	0.27	2.7	0.39
Area under curve (mmol/L*min)	940	150.0	509	93.9	560.0	127.1	739	162.4
Glycaemic index (%)	100	0	63	8.7	66	8.0	89	10.7

^{abc} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

During the second latin square, diet and period had no significant effect on peak glucose, average glucose, peak minus basal glucose, time to peak glucose and slope to peak glucose concentrations, or area under the glycaemic response curve. Cracked barley and cracked triticale generated significantly lower ($P<0.01$) glycaemic indices, compared to the glucose control, however there were no significant differences between grains for glycaemic index (Table 5.7). Individual horses varied significantly ($P \leq 0.01$) for average glucose and peak minus basal glucose concentrations during the second latin square.

Table 5.7: Mean peak glucose concentration, average glucose concentration, peak minus basal glucose concentration (P-B), time to peak glucose, slope to peak glucose, area under the curve and glycaemic index for the glucose control, cracked barley, cracked triticale and cracked oat diets during latin square two.

	Glucose		Cracked barley		Cracked triticale		Cracked oats	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Peak glucose (mmol/L)	8.7	1.04	7.1	0.74	6.9	0.34	7.3	0.73
Average glucose (mmol/L)	6.4	0.35	5.6	0.34	5.4	0.13	6.0	0.38
P-B (mmol/L)	4.1	1.03	2.5	0.70	2.4	0.42	2.6	0.69
Time to peak glucose (hours)	1.9	0.24	1.8	0.25	1.6	0.13	1.8	0.32
Slope to peak glucose (mmol/L/h)	2.4	0.87	1.5	55.4	1.6	0.16	1.5	0.33
Area under curve (mmol/L*min)	628	127.1	325	116.1	302	72.4	476	124.8
Glycaemic Index (%)	100^{a}	0.0	54^{b}	17.8	52^{b}	12.3	74^{ab}	12.5

^{ab} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

The observed insulin response curves (Figures 5.3a and 5.3b) are irregular in shape and highly variable between horses, as illustrated by the large stardard error bars. In agreement with the glycaemic response curves illustrated in Figure 5.2, insulin response to the glucose control diet during latin square two appears to be lower than the mean insulin response to the control diet during the first latin square.

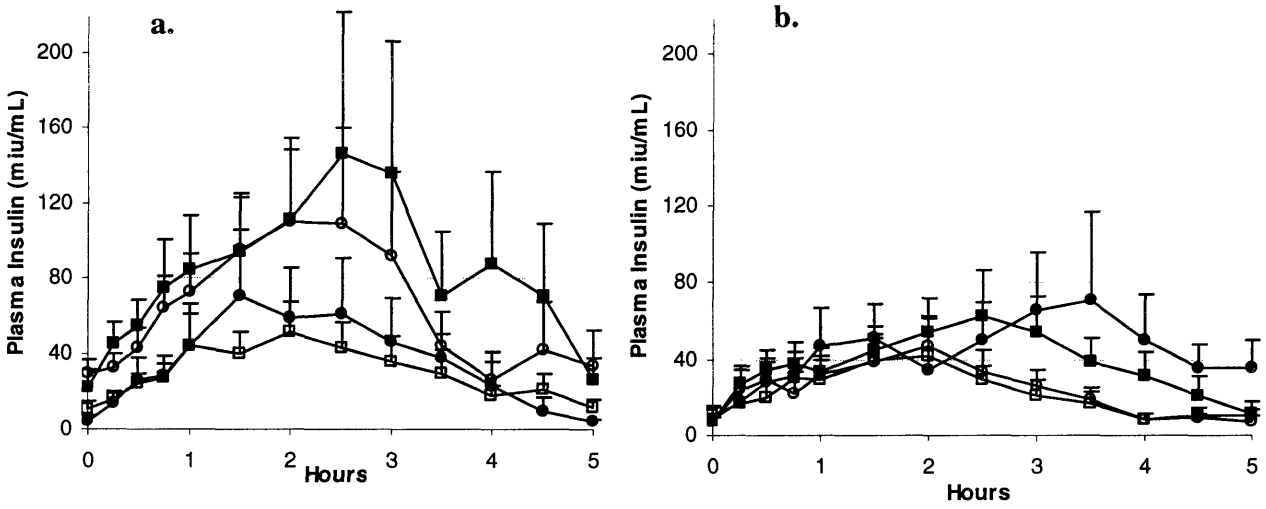

Figure 5.3: Mean insulin response curves for the: (**a**) processed grains; (□) expanded barley, (●) expanded triticale and (○) steam-rolled triticale and (■) the glucose control in latin square one and for the: (**b**) cracked grains; (○) cracked barley, (□) cracked triticale and (●) cracked oats and the (■) glucose control in latin square two.

Mean insulin responses from latin square 1 are shown in Table 5.8. Diet, horse and period had no significant effects on peak insulin concentration, average insulin concentration, peak minus basal insulin concentration or time to peak insulin during the first latin square. Peak insulin concentrations were reached on average at 1hour and 53 mins (Table 5.8). Horse had a significant effect ($P \leq 0.01$) on slope to peak insulin during latin square one.

Table 5.8: Mean peak insulin concentration, average insulin concentration, peak minus basal insulin concentration (P-BI), time to peak insulin and slope to peak insulin for the glucose control, expanded barley, expanded triticale, and steam-rolled triticale diets during latin square one

	Glucose		Expanded Barley		Expanded Triticale		Steam-rolled Triticale	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Peak insulin (miu/mL)	158	75.2	66	21.1	81	37.6	122	47.0
Average insulin (miu/mL)	83	33.6	29	8.3	33	14.9	65	22.5
P-BI (miu/mL)	135	74.1	55	16.7	76	35.1	99	44.2
Time to peak insulin (hours)	2.0	0.20	1.8	0.25	1.6	0.31	2.1	0.43
Slope to peak insulin (miu/mL/hr)	51	23.3	34	14.9	41	19.4	51	20.0

Mean insulin responses from latin square 2 are shown in Table 5.9. Diet and period had no significant effects on peak insulin, average insulin or peak minus basal insulin responses during the second latin square. Horse had a significant ($P<0.01$) effect on peak and average insulin concentrations. Peak insulin was reached on average at 2 hours and 5 mins, 12 minutes later than in the first latin square. Diet, period and horse had no significant effects on time or slope to peak insulin concentrations (Table 5.9).

Table 5.9: Mean peak insulin, average insulin, peak minus basal (P-BI), time to peak insulin and slope to peak insulin concentrations for the glucose control, cracked barley, cracked triticale and cracked oat diets during latin square two.

	Glucose		Cracked barley		Cracked triticale		Cracked oats	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Peak insulin (miu/mL)	75	20.4	55	22.6	46	19.4	86	43.0
Average insulin (miu/mL)	36	9.4	24	8.2	22	8.4	43	17.7
P-B (miu/mL)	70	16.6	50	20.2	38	14.7	82	43.4
Time to peak insulin (hours)	2.4	0.24	1.6	0.38	1.6	0.24	2.8	0.60
Slope to peak insulin (miu/mL/hr)	23	3.8	30	11.6	21	6.8	24	10.1

There was a strong positive relationship (R^2=0.77) between peak plasma glucose and peak plasma insulin concentrations for all diets during latin squares 1 and 2 (Figure 5.4).

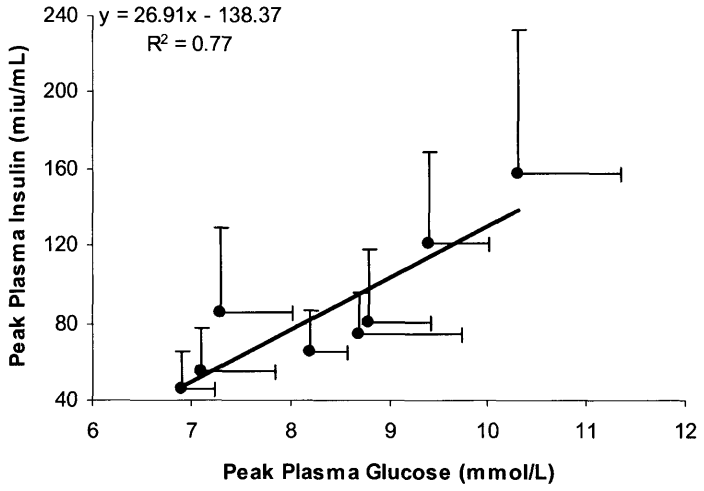

Figure 5.4: The relationship between peak plasma glucose and peak plasma insulin for each test diet.

The ranking of all grains by mean peak glucose response is shown in Table 5.10. Steam-rolled triticale produced the highest peak plasma glucose concentrations while the steam-rolled and expanded grains had greater mean peak glucose responses in comparison to the cracked grains. The consistency of ranking (coefficient of concordance) of grains in order of peak glucose concentration, by different horses, determined by a coefficient of concordance, was significant (P<0.01). The responses of individual horses to the grains were however variable. Horses 1 and 4 generally displayed higher peak glucose concentrations in response to all diets than horses 2 and 3. The consistency of ranking of horses in order of peak glucose response was also significant (P<0.01, Table 5.10).

Table 5.10: Peak plasma glucose concentrations (mmol/L) for individual horses in response to the six treatment diets.

	Peak Plasma Glucose (mmol/L)				
	Horse 4	**Horse 1**	**Horse 2**	**Horse 3**	***MEAN***
Steam-rolled triticale	11.0	9.8	8.4	8.5	***9.4***
Expanded triticale	10.2	9.1	8.8	7.2	***8.8***
Expanded barley	8.9	8.7	7.3	7.8	***8.2***
Cracked oats	8.5	8.4	6.4	5.6	***7.2***
Cracked barley	7.4	9.2	6.1	5.9	***7.1***
Cracked triticale	6.5	7.9	6.8	6.5	***6.9***
MEAN	***9.5***	***9.1***	***7.5***	***7.2***	

Ranking of the grains by mean peak insulin response is shown in Table 5.11. Steam-rolled triticale produced the highest peak insulin concentrations followed by cracked oats, expanded triticale, expanded barley and cracked barley respectively. Cracked triticale, in accordance with the lowest peak glucose response also displayed the lowest peak insulin response. The consistency of ranking of grains in order of peak insulin concentration, by different horses, was not significant ($P>0.05$). The peak insulin responses of individual horses to the treatment grains were variable. Horses 4 and 2 generally displayed higher peak insulin concentrations in response to all diets in comparison to horses 1 and 3. The consistency of ranking of horses in order of peak insulin response was significant ($P<0.01$, Table 5.11).

Table 5.11: Peak plasma insulin concentrations (miu/mL) for individual horses in response to the six treatment diets.

	Peak Plasma Insulin (miu/mL)				
	Horse 4	**Horse 2**	**Horse 1**	**Horse 3**	***MEAN***
Steam-rolled triticale	259.7	103.2	65.5	58.5	***121.7***
Cracked oats	207.4	79.9	47.5	8.8	***85.9***
Expanded triticale	126.5	162.4	21.1	12.3	***80.6***
Expanded barley	91.2	109.6	43.3	18.1	***65.6***
Cracked barley	114.6	67.4	19.2	20.2	***55.4***
Cracked triticale	55.0	97.5	21.0	11.8	***46.3***
MEAN	***142.4***	***103.3***	***36.27***	***21.6***	

The rates at which horses consumed the treatment diets were not significantly different, with the average time ranging from 12.6 minutes for horse 2 to 20.9 minutes for horse 3.

In Vivo Results - Faecal Data

During latin square one, diet had no significant effect on faecal pH, faecal dry matter or faecal nitrogen percentages. Expanded barley caused a significant increase in faecal starch when compared to the glucose control diet, however, faecal starch content from horses on the expanded barley diet did not differ from faecal starch contents observed for horses on the other grain diets during latin square one (Table 5.12). Individual horses differed significantly ($P\leq0.01$) for faecal nitrogen percentage.

Diet had no significant effect on faecal acetate, propionate, acetate: propionate ratio, butyrate, isobutyrate, valerate, isovalerate, total volatile fatty acid, total lactic acid or total faecal acid concentrations during latin square one (Table 5.12). Period had a significant effect on faecal acetate, isobutyrate, butyrate, isovalerate, valerate, total faecal volatile fatty acid and total faecal acid concentrations. Horses differed significantly during latin square one for the acetate: propionate ratio in their faeces, with horse 2 having a significantly lower acetate: propionate ratio (3.2) than horses 1 (4.0), 3 (4.2) and 4 (4.3).

Table 5.12: Mean faecal pH, faecal starch (%DM), faecal dry matter (%), faecal nitrogen (%DM), faecal acetate, propionate, acetate: propionate ratios, total VFA concentrations, total lactate concentrations and total faecal acid concentrations for horses on the glucose control, steam-rolled triticale, expanded triticale and expanded barley diets.

	Glucose		Expanded barley		Expanded triticale		SRT	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
pH	6.9	0.17	6.5	0.17	6.7	0.13	7.0	0.19
Starch (% DM)	0.3^{a}	0.03	0.8^{b}	0.19	0.3^{ab}	0.02	0.5^{ab}	0.08
Dry matter (%)	24	1.0	23	1.2	25	1.4	24	0.6
Nitrogen (% DM)	2.2	0.09	2.5	0.07	2.2	0.09	2.2	0.09
Acetate (mmol/L)	17	5.1	23	3.0	25	5.2	19	5.6
Propionate (mmol/L)	4.7	1.64	6.0	1.06	6.7	1.39	4.6	0.95
Acetate: propionate	3.9	0.29	4.0	0.27	3.8	0.30	4.0	0.48
Total VFA (mmol/L)	26	8.0	35	4.7	38	8.2	28	7.9
Total lactate (mmol/L)	0.8	0.15	0.9	0.25	1.2	0.29	1.0	0.49
Total acid (mmol/L)	27	8.0	36	4.9	40	8.2	29	8.2

[ab] Values in same row with different superscripts are significantly different ($P \leq 0.01$).

During the second latin square, diet had no significant effect on faecal pH, faecal starch, faecal dry matter or faecal nitrogen percentage's and horses did not differ significantly for any of the measured faecal parameters (Table 5.13). However, diet had a significant effect ($P<0.01$) on faecal acetate, propionate, and isobutyrate concentrations, as well as total volatile fatty acid concentrations and total faecal acid concentrations. Individual horses and period had no significant effect on these faecal parameters during latin square two (Table 5.13).

Table 5.13: Mean faecal pH, faecal starch (%DM), faecal dry matter (%), faecal nitrogen (%DM), faecal acetate, propionate, acetate: propionate ratios, total VFA concentrations, total lactate concentrations and total faecal acid concentrations for horses on the glucose control, cracked oats, cracked barley and cracked triticale diets.

	Glucose		Cracked barley		Cracked triticale		Cracked oats	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
pH	7.2	0.09	6.7	0.05	6.8	0.10	7.1	0.12
Starch (% DM)	0.3	0.04	0.4	0.05	0.4	0.03	0.3	0.03
Dry Matter (%)	24.5	1.13	25.6	0.97	24.2	0.69	25.6	0.84
Nitrogen (% DM)	2.1	0.07	2.3	0.06	2.3	0.09	1.9	0.08
Acetate (mmol/L)	15^{b}	2.1	23^{a}	2.0	25^{a}	2.3	14^{b}	2.0
Propionate (mmol/L)	4.2^{bc}	0.67	5.9^{ac}	0.48	6.2^{a}	0.74	3.8^{b}	0.48
Acetate: propionate	3.9	0.39	4.0	0.22	4.0	0.24	3.6	0.19
Total VFA (mmol/L)	24^{b}	3.3	35^{a}	3.1	37^{a}	3.7	20^{b}	2.9
Total lactate (mmol/L)	0.4	0.17	0.6	0.21	0.4	0.19	0.4	0.06
Total acid (mmol/L)	24^{b}	3.3	36^{a}	3.2	37^{a}	3.8	21^{b}	2.9

[abc] Values in same row with different superscripts are significantly different ($P \leq 0.01$).

5.4 EXPERIMENT 2

The aims of experiment two were to:

(i) examine the *in vitro* enzyme starch digestion and fermentation and *in vivo* small intestinal starch digestion characteristics of corn

(ii) investigate the effects of the grain processing techniques, extrusion and micronising, on the *in vitro* and *in vivo* digestion and fermentation characteristics of corn starch

(iii) closely examine the between horse variation observed during experiment one.

5.4.1 Methods and Materials

The study was conducted over a 12-day period during which time horses were kept in stables for controlled feeding and measurement. The University of New England's Animal Ethics Committee approved the experimental protocol.

The trial was conducted as a balanced 4x4 latin square with three grain treatments and a grain control. Unprocessed corn 1 (fed cracked), extruded corn (extruded in an Instapro single screw extruder; corn is ground then processed at 138 – 149°C for 14 – 15 seconds with no preconditioning) and micronised corn (micronised for 12 seconds at an external temperature of 89°C and assumed internal temperature of 68°C at a moisture of 22%) were the grain treatments. The cracked and extruded corns were the same variety (variety unknown), while the micronised corn was from the variety 'XL80'. Unprocessed 'XL80' corn (unprocessed corn 2) was included in all *in vitro* analyses. Steam-rolled triticale, observed as a grain that initiated a consistent and high glycaemic response in horses during experiment 1, was included as the grain control. Treatments were rotated at random with each animal remaining on each treatment for three days. Meal sizes were adjusted to supply 670 g starch/feed to each horse and were not adjusted according to body weight (Table 5.14).

Table 5.14: The percentage starch in each grain and the quantity fed to supply 670 grams of starch/meal.

Grain	% Starch (as is basis)	Quantity to be fed (g/meal)
Unprocessed corn 1	71.9	930 g
Extruded corn	67.1	1000 g
Micronised corn	70.2	954g
Steam-rolled triticale	59.7	1122 g

Animals and Management

Four (4) horses (3 thoroughbred geldings, 1 standardbred gelding), aged from 4.5 to 9 years, weighing 482 – 512 kg (average 497 kg) were used in the trial (Table 5.15). The horses were stabled in individual box stalls overnight and held in individual yards during the day for the duration of the trial. Sawdust was used as bedding in the stables. The horses were

exercised each evening for approximately half an hour except on days when blood sampling had occurred. All horses were treated with an anthelmintic 2 weeks prior to the trial and were considered to be healthy and free from internal parasites at the commencement of the trial.

Table 5.15: The age, weight, condition score and temperament of the horses used in the experiment 2. Horse 1 was a standardbred, horses 2, 3 and 4 were thoroughbreds.

	Horse 1	Horse 2	Horse 3	Horse 4
Age (years)	4.5	9	5	6
Weight (kg)	496	482	498	512
Condition score	7	6	4	4.5
Temperament	Quiet	Very quiet	Quiet/flighty	Very quiet

Horses were fed the grain portion of their diet at 0700 h and 1730 h each day. On completion of eating the grain, the horses were offered 4 kg of lucerne hay. On mornings when the glycaemic and insulin responses were measured, only the grain portion of the diet was fed. Hay was fed at the conclusion of blood sampling. The evening feed following blood sampling consisted 100% of the next diet on which the animal was to be tested, as it was not thought necessary to allow any changeover adaptation period. All animals had access to water *ad libitum*, except during the 5-hour blood sampling periods, during which time water intake was prohibited.

Measurements

Total starch content of all grains was determined prior to the trial using the method of McCleary *et al.* (1997). The *in vitro* enzyme digestibility and *in vitro* rate of enzyme digestibility was determined for all grains using methods described in Section 5.3.1. The grains fermentation characteristics in bovine rumen fluid were determined using a modification of the method of Bird *et al.* (1999 *modified by* S. Bird). The modifications involved incubating 10 g of cracked grain in one-third the volume of rumen/caecal fluid and buffer used during the original assay, in 250 mL plastic culture jars. The pH of the fermentation medium was measured at 4 hours and the incubation was stopped. A liquid fermentation medium sample was then collected for determination of volatile fatty acid and lactic acid concentrations. Grain fermentation characteristics in equine caecal fluid were determined using methods described in Section 5.3.1.

Crude fat content, and the crude protein content of all grains were determined during laboratory sample analysis following the experimentation period. Analyses were carried out as per methods described in Section 5.3.1. The gross energy content of all grains was determined using bomb calorimetry (Automatic Calorific Processor dds CP500).

Horses were weighed on arrival at the stable complex and feed intake and refusals were recorded each day. The condition score of each horse was assessed and recorded using the method of Henneke *et al.* (1983) on the final day of the trial and time taken to eat the grain portion of the diet was measured and recorded for each individual horse where practical. At

least three 'time eating' measurements for each horse on each diet were made, including on the morning of blood sampling.

Faecal samples, collected fresh from the stable of each horse between 0700 and 0800 hours on the third day of each latin square period, were sub-sampled and analysed for pH, dry matter and starch concentrations as per the methods described in Section 4.2. Faecal samples were also analysed for VFA and lactate concentrations (methods in Section 5.3.1).

On the third day of each latin square period blood sampling occurred for the measurement of the glycaemic and insulin responses using methods described in Section 6.2.2. Blood samples were taken at 0 hours (prior to grain being fed) and then at 30, 60, 90, 120, 150, 180, 240, and 300 minutes following commencement of grain consumption, making a total of nine blood samples/horse. The plasma glucose concentration was determined using the Dimension® clinical chemistry system on a DADE XL clinical auto-analyser (Dade Behring Inc, Newark, DE 19714, USA). The GLU Flex™ reagent cartridge (Cat No DF39A) was used as the *in vitro* reagent. Plasma insulin concentrations were measured using the methods described in Section 4.2.

Curves of glucose and insulin against time for each individual horse on each diet were used to calculate the peak glucose/insulin concentrations, average glucose/insulin concentrations, peak minus the basal glucose/insulin concentrations, time to peak glucose/insulin and slope to peak glucose/insulin. Statistical analysis of glycaemic and insulin response data and faecal data was carried out using a fixed effect ANOVA with diet, horse and period included in the model. Significant differences between diet and horse means were further examined using a Fishers least square difference multi-comparisons function at a 99% confidence interval. Analysis of the consistency of ranking of horses and grains in order of glycaemic and insulin response was carried out using a coefficient of concordance (Moroney, 1968). Statistical analysis was carried out on the S-plus statistical package (Insightful Corporation, Seattle, WA. USA).

5.4.2 Results

In Vitro Starch Digestion and Fermentation Characteristics

The unprocessed corns and micronised corn had the highest percentages of starch on a dry matter basis. The extruded corn had a lower starch content than its corresponding unprocessed corn 1 due to the inclusion of 12% soybean prior to extrusion. Unprocessed corn 2 and micronised corn had the greatest crude fat content, while extruded corn (due to the inclusion of soybean) and steam-rolled triticale had the highest crude protein contents (Table 5.16). The extrusion and micronising of corn increased the extent of *in vitro* enzyme starch digestion during both the 15 and 60 minute *in vitro* periods of incubation. However, the digestion of extruded corn was more extensive than the digestion of micronised corn (Table 5.16)

Table 5.16: The total starch, gross energy, crude fat and crude protein contents and the *in vitro* starch digestibility's at 15 and 60 minutes for unprocessed corn 1, extruded corn, unprocessed corn 2, micronised corn and steam-rolled triticale (SRT).

	Unprocessed corn 1	Extruded corn	Unprocessed corn 2	Micronised corn	SRT
Starch (%DM)	74.9	63.3	72.2	74.3	66.2
Gross energy (mJ/kg DM)	16.5	17.1	19.0	16.8	17.0
Crude fat (%DM)	3.6	4.4	6.5	8.8	3.2
Crude protein (%DM)	8.9	12.5	10.5	10.5	12.0
Starch digestion (% starch in15 mins)	15.8	73.9	12.3	34.0	60.4
Starch digestion (% starch in 1hour)	33.0	83.4	23.7	50.5	82.3

During the *in vitro* rate of starch digestion assay, the period of starch digestion from 0 to 15 minutes was the period that most clearly differentiated the grains. The fastest rates of digestion for all grains were also exhibited during this time period (Table 5.17, Figure 5.5). The extrusion of corn increased the rate of *in vitro* starch digestion in the first 15 minutes of incubation from 1% of starch present/minute to nearly 5% of starch present/minute. Similarly micronising corn increased the *in vitro* rate of starch digestion in the first 15 minutes from nearly 1% of starch present/minute to over 2% of starch present/minute (Table 5.17, Figure 5.5).

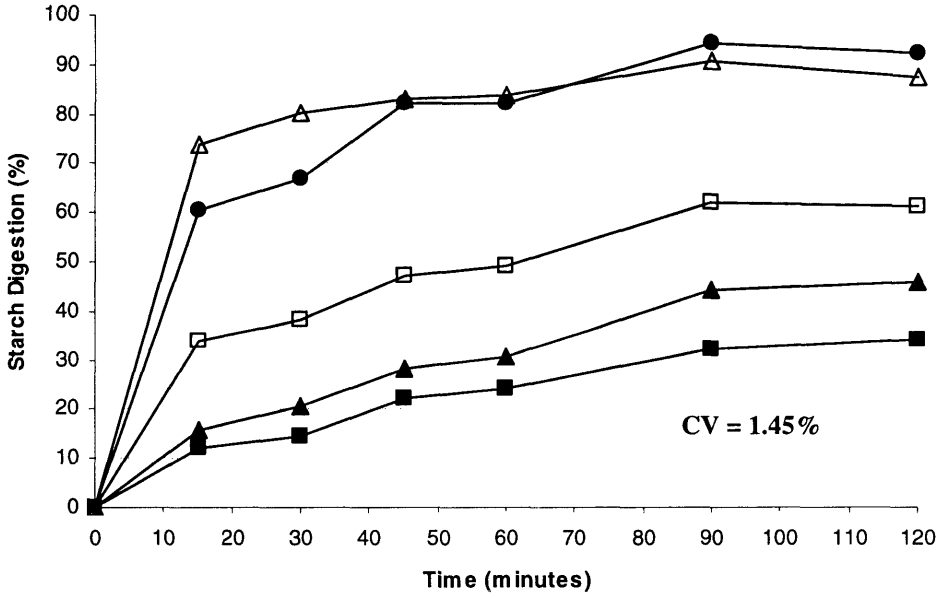

Figure 5.5: The pattern of *in vitro* starch digestion for unprocessed corn 1 (▲), extruded corn (△), unprocessed corn 2 (■), micronised corn (□) and steam-rolled triticale (●).

Table 5.17: The rate of digestion (% of starch present in the grain on a dry matter basis/minute) for unprocessed corn 1, extruded corn, unprocessed corn 2, micronised corn and steam-rolled triticale (SRT).

	0-15 mins	15-30 mins	30-45 mins	45-60 mins	60-90 mins	90-120 mins
Unprocessed corn 1 (% starch/min)	1.05	0.33	0.51	0.15	0.45	0.06
Extruded corn (% starch/min)	4.93	0.42	0.18	0.07	0.22	-0.10
Unprocessed corn 2 (% starch/min)	0.82	0.15	0.51	0.15	0.26	0.07
Micronised corn (% starch/min)	2.27	0.29	0.61	0.13	0.42	-0.02
SRT (% starch/min)	4.02	0.45	1.02	-0.01	0.40	-0.06

Extruded corn was the most fermentable grain in bovine rumen fluid, producing 100 mmol/L of VFA and 25 mmol/L of lactate during the four-hour incubation time. Unprocessed corn 1 produced 43% more VFAs than unprocessed corn 2. The extrusion of corn increased the total organic acid production in the rumen fluid medium by 108%, while micronised corn had a 15% higher acid production than unprocessed corn 2. Unlike extrusion, micronising did not induce lactic acid production in rumen fluid (Table 5.18).

Table 5.18: The fermentation characteristics in bovine rumen fluid, of unprocessed corn 1, extruded corn, unprocessed corn 2, micronised corn and steam-rolled triticale (SRT) when fermented for 4 hours. The pH, volatile fatty acid (VFA) concentrations and total lactate concentrations of the rumen fluid are measured at the conclusion of four hours.

	Unprocessed corn 1	Extruded corn	Unprocessed corn 2	Micronised corn	SRT
pH	6.8	6.8	7.3	7.2	6.8
Acetate (mmol/L)	35.2	63.4	26.0	29.2	35.8
Propionate (mmol/L)	16.2	24.8	9.7	12.4	17.1
Butyrate* (mmol/L)	7.1	10.3	5.1	5.6	6.8
Total VFA (mmol/L)	60.0	99.7	42.1	48.6	60.7
Total lactate (mmol/L)	0.0	25.1	0.0	0.0	0.0
Total acid (mmol/L)	60.0	124.8	42.1	48.6	60.7

* 1 mmol glucose = 2 mmol Acetate, 2 mmol Propionate, 1 mmol Butyrate

Fermentation characteristics of the grains in equine caecal fluid (Table 5.19) were substantially different to those observed in bovine rumen fluid. Extruded corn was again the most fermentable grain, however, for all grains, total VFA concentrations were approximately half of that observed in rumen fluid. Lactic acid concentration in contrast, was increased in equine caecal fluid by 19 to 25 mmol/L, with all grains producing lactic acid in the caecal fermentation medium. The highest concentrations of lactic acid were present in the extruded corn's fermentation medium, with 64% of organic acid present in the form of lactic acid (Table 5.19).

Table 5.19: The fermentation characteristics in equine caecal fluid, of unprocessed corn 1, extruded corn, unprocessed corn 2, micronised corn and steam-rolled triticale (SRT) when fermented for 4 hours. The pH, volatile fatty acid (VFA) concentrations and total lactate concentrations of the caecal fluid are measured at the conclusion of four hours.

	Unprocessed corn 1	Extruded corn	Unprocessed corn 2	Micronised corn	SRT
pH	7.1	6.7	7.3	7.1	7.2
Acetate (mmol/L)	22.0	18.7	20.2	20.2	20.7
Propionate (mmol/L)	6.8	6.5	6.4	6.8	6.0
Butyrate* (mmol/L)	2.4	2.0	2.3	2.2	2.1
Total VFA (mmol/L)	32.1	27.9	29.8	30.1	29.7
Total lactate (mmol/L)	23.2	50.0	19.1	24.5	24.1
Total acid (mmol/L)	55.3	77.9	48.9	54.6	53.8

* 1 mmol glucose = 2 mmol Acetate, 2 mmol Propionate, 1 mmol Butyrate

In vivo Results - by Treatment

The mean glycaemic and insulin response curves for the treatment and control diets are illustrated in figures 5.6a and 5.6b respectively. The glycaemic response curves were typical response curves as described by Loeb (1971). The insulin response curves were more regular than those observed during experiment 1, although variation between horses remained high.

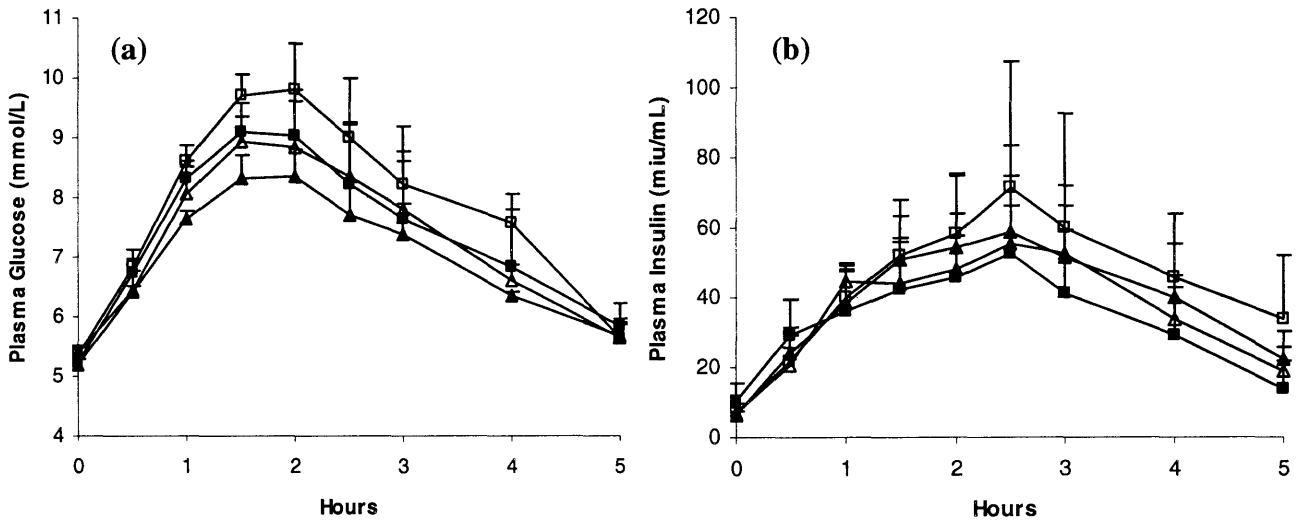

Figure 5.6: The **(a)** mean glucose and **(b)** mean insulin responses for (■) unprocessed corn 1, (□) extruded corn, (△) micronised corn and (▲) steam-rolled triticale.

Extruded corn actuated significantly higher peak glucose, average glucose and peak – basal glucose responses in the horses in comparison to the micronised corn. Unprocessed corn 1, extruded corn and steam-rolled triticale did not differ significantly for peak, average or peak – basal glucose concentration parameters, There were no differences between diets for time or slope to peak glucose concentrations (Table 5.20).

Table 5.20: Mean peak glucose concentration, average glucose concentration, peak minus basal concentration, time to peak glucose and slope to peak glucose for unprocessed corn 1, extruded corn, micronised corn and steam-rolled triticale (SRT).

	Unprocessed corn 1		Extruded corn		Micronised corn		SRT	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak glucose (mmol/l)	9.4^{ab}	0.65	10.1^{a}	0.56	8.5^{b}	0.34	9.3^{ab}	0.64
Average glucose (mmol/l)	7.6^{ab}	0.50	8.1^{a}	0.38	7.2^{b}	0.21	7.5^{ab}	0.4
Peak-basal glucose (mmol/l)	4.1^{ab}	0.69	4.7^{a}	0.69	3.3^{b}	0.41	3.9^{ab}	0.73
Time to peak glucose (hours)	1.6	0.31	1.9	0.13	1.6	0.24	1.9	0.24
Slope to peak glucose (mmol/L/h)	2.6	0.25	2.6	0.22	2.1	0.14	2.2	0.25

[ab] Values in same row with different superscripts are significantly different ($P \leq 0.01$).

There were no significant differences between diets for peak, average or peak minus basal plasma insulin concentrations. Diets did not differ in time to reach peak insulin concentrations, however unprocessed corn 1 and steam-rolled triticale had significantly lower slopes to peak insulin in comparison to extruded corn. Extruded corn and micronised corn did not differ significantly for slope to peak insulin (Table 5.21).

Table 5.21: Mean peak insulin concentration, average insulin concentration, peak minus basal insulin concentration, time to peak insulin and slope to peak insulin for unprocessed corn 1, extruded corn, micronised corn and steam-rolled triticale (SRT).

	Unprocessed corn 1		Extruded corn		Micronised corn		SRT	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak insulin (miu/mL)	55	21.8	82	33.4	65	24.5	60	10.6
Average insulin (miu/mL)	33	13.7	43	15.9	39	13.4	35	7.2
Peak-basal insulin (miu/mL)	45	16.3	75	31.2	59	23.9	53	11.0
Time to peak insulin (hours)	2.1	0.24	1.9	0.24	1.8	0.25	2.5	0.20
Slope to peak insulin (miu/mL/h)	19^{a}	6.2	34^{b}	9.1	32^{ab}	11.1	20^{a}	5.3

[ab] Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Horses on the steam-rolled triticale diet displayed the highest faecal pH and lowest concentrations of faecal starch and total faecal acid (Table 5.22). In the reverse, horses on the micronised corn diet had the lowest faecal pH and highest concentrations of total faecal acid. Extruded corn had the lowest concentrations of both VFAs and lactic acid amongst the corn diets. There were however no significant differences between diets for faecal pH, starch, VFA, and total faecal acid concentrations. Horses on the unprocessed corn diet had significantly higher concentrations of faecal lactic acid than horses on the steam-rolled triticale diet. Horses on the extruded corn, micronised corn and steam-rolled triticale diets did not differ significantly for faecal lactic acid concentrations (Table 5.22).

Table 5.22: Mean faecal pH, starch and faecal metabolites for horses on the unprocessed corn 1, extruded corn, micronised corn and steam-rolled triticale diets.

	Unprocessed corn 1		Extruded corn		Micronised corn		SRT	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Faecal pH	6.9	0.22	6.9	0.15	6.7	0.16	7.1	0.06
Faecal starch	0.9	0.19	0.7	0.21	0.5	0.04	0.4	0.04
Faecal acetate (mmol/l)	18.0	2.09	17.2	4.03	23.0	4.09	10.4	0.85
Faecal propionate (mmol/l)	4.8	0.27	4.2	0.97	5.6	0.79	3.0	0.20
Faecal butyrate (mmol/l)	2.2	0.32	1.7	0.49	3.6	0.86	1.0	0.08
Total VFA (mmol/l)	27.0	2.86	25.0	5.49	35.9	6.58	16.0	1.18
Total lactate (mmol/l)	3.6^{a}	1.3	2.1^{ab}	0.79	2.8^{ab}	0.74	1.1^{b}	0.09
Total faecal acid (mmol/l)	30.6	3.63	27.1	5.71	38.8	6.11	17.1	1.24

[ab] Values in same row with different superscripts are significantly different ($P \leq 0.01$).

There were no significant differences between diets for time taken to eat the allocated meal with horses taking 10, 15, 13 and 12 minutes to eat the cracked corn, extruded corn, micronised corn and steam-rolled triticale diets respectively.

In Vivo Results - by Horse

Horse 2 exhibited the most elevated average glycaemic response (Figure 5.7b) while horse 1 displayed the highest average insulin response (Figure 5.7a). Horse 3 had the lowest average glycaemic and insulin responses (Figure 5.7c).

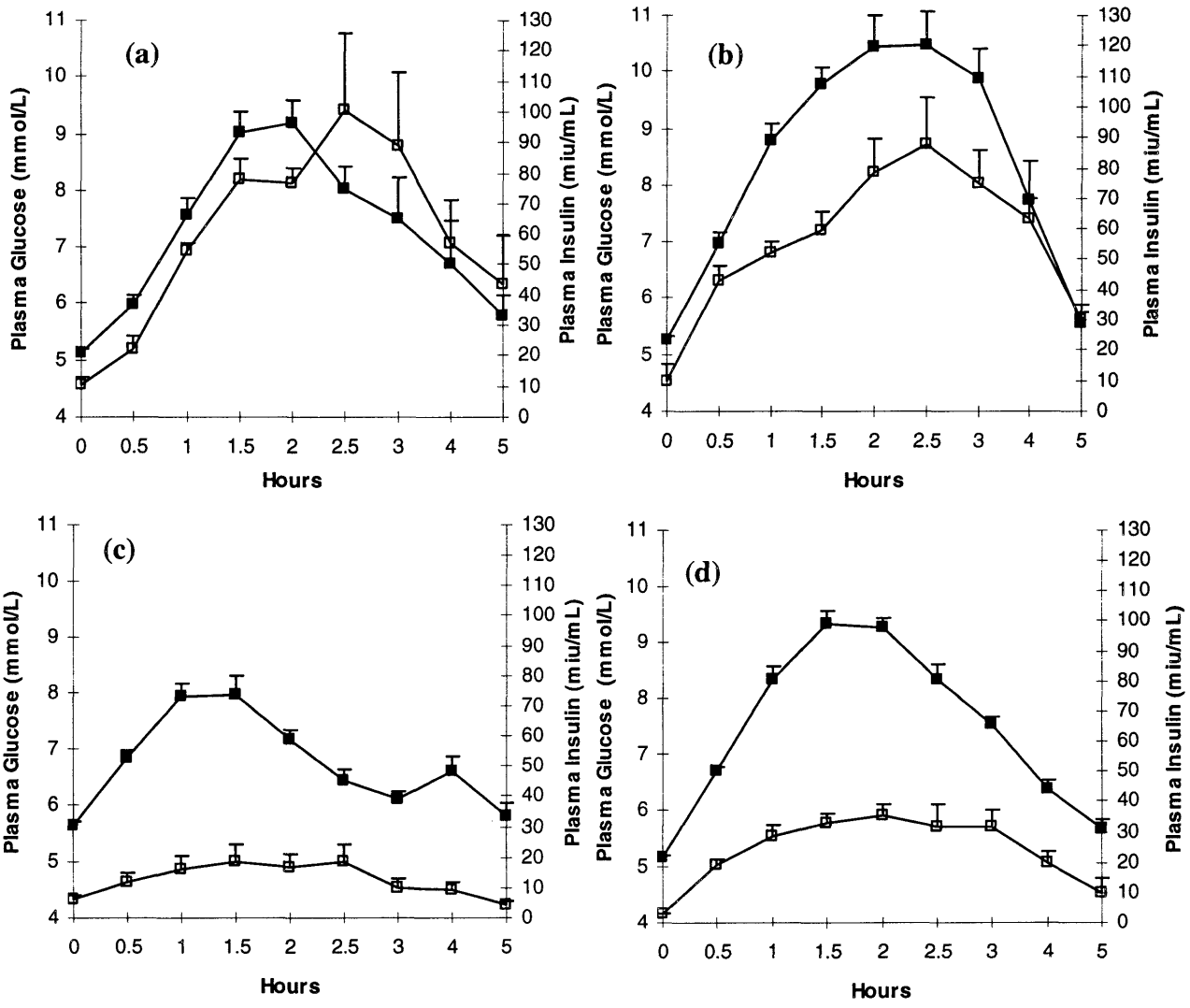

Figure 5.7: The (■) mean glucose and (□) mean insulin responses for (**a**) horse 1, (**b**) horse 2, (**c**) horse 3 and (**d**) horse 4, averaged over all diets.

Horse 3 displayed a significantly lower peak glucose response in comparison to horses 2 and 4. Horse 1 exhibited a significantly lower peak glucose concentration in comparison to horse 2 (Table 5.23). The average and peak – basal glucose responses actuated by horse 2 were significantly higher than those observed for horses 1, 3 and 4. Horse three's average glucose response was significantly lower than that observed for horse 4. Horses did not differ significantly for time or slope to peak glucose concentrations (Table 5.23).

Table 5.23: Mean peak glucose concentration, average glucose concentration, peak minus basal concentration, time to peak glucose and slope to peak glucose, averaged over all diets for horses 1, 2, 3 and 4.

	Horse 1		Horse 2		Horse 3		Horse 4	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak glucose (mmol/l)	9.2^{ac}	0.40	10.6^{bd}	0.57	8.0^{c}	0.28	9.4^{ad}	0.17
Average glucose (mmol/l)	7.4^{ac}	0.31	8.54^{b}	0.35	6.8^{c}	0.13	7.6^{a}	0.11
Peak-basal glucose (mmol/l)	4.1^{a}	0.41	5.3^{a}	0.57	2.42^{c}	0.20	4.3^{a}	0.15
Time to peak glucose (hours)	1.9	0.13	2.1	0.24	1.25	0.14	1.8	0.14
Slope to peak glucose (mmol/L/h)	2.4	0.31	2.6	0.20	2.0	0.12	2.6	0.23

^{abcd} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Horse 1 initiated a significantly higher peak insulin concentration in comparison to horses 3 and 4 (Table 5.24). Horse 2 displayed a peak insulin concentration significantly higher than that displayed by horse 3. Horse's 1 and 2 exhibited average insulin concentrations that were significantly higher than those displayed by horses 3 and 4, and significantly higher peak – basal insulin concentrations in comparison to horse 3. There were no significant differences between horses for time taken to reach peak insulin concentration. Horses 1, 2 and 4 had significantly higher slopes to peak insulin than horse 3 (Table 5.24).

Table 5.24: Mean peak insulin concentration, average insulin concentration, peak minus basal insulin concentration, time to peak insulin and slope to peak insulin, averaged over all diets for horses 1, 2, 3 and 4.

	Horse 1		Horse 2		Horse 3		Horse 4	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak insulin (miu/mL)	105^{a}	24.1	96^{ab}	11.2	23^{c}	5.8	38^{cb}	6.2
Average insulin (miu/ml)	59^{a}	9.9	55^{a}	6.2	12^{b}	3.0	23^{b}	3.3
Peak-basal insulin (miu/mL)	94^{a}	22.9	86^{a}	10.5	17^{b}	5.0	35^{ab}	4.8
Time to peak insulin (hours)	2.1	0.24	2.5	0.20	2.0	0.29	1.6	0.13
Slope to peak insulin (miu/mL/h)	44^{a}	7.4	32^{a}	5.5	9^{b}	2.9	20^{a}	2.8

^{abc} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

There was no significant difference between horses for faecal pH, starch, VFA concentrations or total acid concentrations. Horse 3 however had significantly higher faecal lactic acid concentrations than horses 1, 2 and 4 (Table 5.25).

Table 5.25: Faecal pH, starch and faecal metabolite concentrations measured in horses 1, 2, 3 and 4.

	Horse 1		Horse 2		Horse 3		Horse 4	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Faecal pH	7.1	0.10	6.9	0.21	6.8	0.08	6.9	0.23
Faecal starch	0.6	0.14	0.5	0.11	0.8	0.25	0.5	0.05
Faecal acetate (mmol/l)	17.4	2.89	15.6	3.76	17.7	2.23	18.0	6.01
Faecal propionate (mmol/l)	4.5	0.71	5.2	0.73	3.7	0.30	4.3	1.17
Faecal butyrate (mmol/l)	2.3	0.49	1.8	0.54	2.0	0.33	2.5	1.22
Total VFA (mmol/l)	26.3	3.93	24.3	5.64	25.6	2.65	27.7	9.68
Total lactate (mmol/l)	2.1^{a}	0.71	1.1^{a}	0.36	4.3^{b}	1.11	2.03^{a}	0.49
Total faecal acid (mmol/l)	28.4	4.06	25.5	5.93	30.0	3.48	29.8	9.9

^{ab} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Horse 2 took the longest to eat the allocated test diets (mean 15.2 min), while horses 3 and 4 were the fastest eaters (mean 10.6 and 10.5 min respectively). There was however, no significant difference between horses for time taken to eat the allocated test diets.

The consistency of ranking of grains, in order of peak glucose response, by different horses, determined by a coefficient of concordance was significant (P<0.05), and although the response of individual horses was highly variable, the ranking of horses in order of peak glucose response was also significant (P<0.01, Table 5.26).

Table 5.26: Peak plasma glucose concentrations (mmol/L) for individual horses in response to the expanded corn, cracked corn, steam-rolled triticale and micronised corn diets.

	Extruded corn	Unprocessed corn 1	SRT	Micronised corn	*MEAN*
Horse 2	11.5	10.8	11.0	8.9	***10.6***
Horse 4	9.8	9.5	9.3	9.0	***9.4***
Horse 1	10.2	9.4	8.5	8.6	***9.2***
Horse 3	8.8	7.7	8.2	7.5	***8.0***
MEAN	***10.1***	***9.4***	***9.3***	***8.5***	

The ranking of grains in order of peak insulin response by the different horses was not consistent and thus the coefficient of concordance was not significant (P>0.05), however the consistency of ranking of horses in order of peak insulin response was significant (P<0.01, Table 5.27).

Table 5.27: Peak plasma insulin concentrations (miu/mL) for individual horses in response to the expanded corn, cracked corn, steam-rolled triticale and micronised corn diets.

	Extruded corn	Micronised corn	SRT	Unprocessed corn 1	*MEAN*
Horse 1	175.5	92.5	82.4	68.4	***104.7***
Horse 2	85.4	117.8	69.3	110.2	***95.7***
Horse 4	35.6	40.8	52.6	22.4	***37.9***
Horse 3	32.0	9.8	33.6	17.5	***23.2***
MEAN	***82.1***	***65.2***	***59.5***	***54.6***	

5.5 EXPERIMENT 3

The aims of this experiment were to:

(i) investigate the *in vitro* enzyme digestibility and fermentation characteristics and the *in vivo* small intestinal digestibility of white rice; and

(ii) examine the effect of extrusion processing on the starch digestion and fermentation characteristics of white rice.

A test diet investigating the effect of extruded rice bran on the small intestinal digestibility of white rice was included in experiment 3 for commercial purposes and thus will not be discussed in detail.

5.5.1 Methods and Materials

The trial was conducted as a balanced 4x4 latin square with two randomly paired animals in each latin square cell. Three grain treatments; unprocessed white rice; extruded rice; and unprocessed white rice plus 0.5kg of extruded rice bran (rice bran + rice) were used in the trial. Steam-rolled triticale was included as the grain control. Treatments were rotated at

random with each animal remaining on one treatment for three days. The University of New England Animal Ethics Committee approved the experimental protocol.

Meal sizes were adjusted to supply 670 g of starch per feed and were not adjusted according to bodyweight (Table 5.28).

Table 5.28: The percentage starch in each grain and the quantity fed in order to supply 670 grams of starch.

Grain	% Starch (as is basis)	Quantity to be fed (g/meal)
Unprocessed white rice	79.1	847 g
Extruded rice*	66.2	1012 g
Unprocessed white rice + 0.5 kg extruded rice bran	52.3	1281g
Steam-rolled triticale	59.7	1122 g

* Extruded Rice was a commercial product and consisted 81.15% white rice, 12.50% rice bran and 6.35% other ingredients

Animals and Management

Eight horses, (two standardbred geldings, four standardbred mares and two thoroughbred geldings), aged from 4 to 9 years, weighing 432 – 510 kg (average 478 kg) were used in the trial (Table 5.29). The horses were stabled in individual box stalls overnight and held in individual yards during the day for the duration of the trial. Sawdust was used as bedding in the stables. The horses were exercised each evening for approximately half an hour except on days when blood sampling had occurred. All horses were treated with an anthelmintic 2 weeks prior to the trial and were considered to be healthy and free from internal parasites at the commencement of the trial.

Table 5.29: The breed, sex, age, weight and condition score characteristics of horses used in the trial.

	Pair A		Pair B		Pair C		Pair D	
	A1	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
Breed/Sex	SB/G	SB/M	SB/M	TB/G	SB/M	SB/G	SB/M	TB/G
Age (years)	4.5	4.5	9	9	4	4.5	4	7
Weight (kg)	476	432	476	450	504	494	478	510
Condition Score	6	7	3	3.5	7	5	8	6

SB – Standardbred *TB – Thoroughbred* *M – Mare* *G - Gelding*

Horses were fed the grain portion of their diet at 0700 h and 1730 h each day. On completion of eating the grain the horses received 4 kg of lucerne hay. On mornings when the glycaemic and insulin responses were measured, only the grain portion of the diet was fed. Hay was fed at the conclusion of blood sampling. The evening feed following blood sampling consisted 100% of the next diet on which the animal was to be tested, as it was not thought necessary to allow any changeover adaptation period. All animals had access to water *ad libitum*, except during the 5-hour blood sampling periods, during which time water intake was restricted.

Measurements

The total starch content of all grains was determined prior to the trial using the method of McCleary *et al.*(1997). The *in vitro* enzyme digestibility and *in vitro* rate of enzyme digestibility for grains examined during this experiment were determined using methods

described in Section 5.3.1. The grains fermentation characteristics in bovine rumen fluid were determined as per the methods described in Section 5.4.1. Grain fermentation characteristics in equine caecal fluid were determined using the methods described in Section 5.3.1. The crude fat content, crude protein content (methods described in Section 5.3.1) and the gross energy content (methods described in Section 5.4.1) of the cereal grains were determined during *in vitro* analyses.

Horses were weighed on arrival at the stable complex and feed intake and refusals were recorded each day. Condition score of each horse was assessed and recorded using the method of Henneke *et al.* (1983) on the final day of the experiment and time taken to eat the grain portion of the diet was measured and recorded for each individual horse where practical. At least three 'time eating' measurements for each horse on each diet were made, including on the morning of blood sampling.

Faecal samples collected fresh from the stable of each horse between 0700 and 0800 hours on the third day of each latin square period were sub-sampled and analysed for pH, dry matter and starch concentrations using methods described in Section 4.2. Faecal samples were also analysed for VFA and lactate concentrations, determined using methods described in Section 5.3.1.

On the third day of each latin square period, blood samples were collected for the measurement of glycaemic and insulin responses to the cereal grain test diets (methods in Section 4.2). Blood samples were taken via an indwelling catheter at 0 hours (prior to grain being fed) and then at 30, 60, 90, 120, 150, 180, 240, and 300 minutes following commencement of feeding, making a total of nine blood samples/horse. Plasma samples were analysed for glucose and insulin concentrations using the methods described in Section 5.3.1 and Section 5.4.1 respectively.

Curves of glucose and insulin against time for each individual horse on each diet were used to calculate the peak glucose/insulin concentrations, average glucose/insulin concentrations, peak minus the basal glucose/insulin concentrations, time to peak glucose/insulin and slope to peak glucose/insulin. These response variables and the faecal data were analysed using a fixed effect ANOVA. Since a latin square design was used, with two blocking variables: diet and period, and two horses per cell, data were initially analysed to see if the sampling error (variability within cells) was comparable with experimental (residual) error and hence, if it were feasible to combine the two error terms. This was valid for all response variables analysed.

Significant differences between diets and horses were further examined using Fischer's least squares difference method with a 99% confidence interval. Analysis of the consistency of ranking of horses and grains in order of glycaemic and insulin response was carried out using a coefficient of concordance (Moroney, 1968). Statistical analysis was carried out on the S-plus statistical package (Insightful Corporation, Seattle, WA. USA).

5.5.2 Results

In Vitro Starch Digestion and Fermentation Characteristics

Unprocessed white rice had the highest percentage of starch per unit on a dry matter basis. White rice had the lowest gross energy (17.4mJ/kg DM) and correspondingly the lowest crude oil content. Unprocessed white rice had the lowest percent of starch digested *in vitro* at both the 15 and 60-minute periods of *in vitro* incubation. Extruded rice was the most extensively digested with 80% of starch present, digested in 15 minutes (Table 5.30).

Table 5.30: The total starch, gross energy, crude fat and crude protein contents and the *in vitro* starch digestibility's at 15 and 60 minutes for unprocessed white rice, extruded rice, rice bran + rice and steam-rolled triticale (SRT).

	Unprocessed white rice	Extruded rice	Rice bran + rice	SRT
Starch (%DM)	90.2	69.0	57.5	66.2
Gross energy (mJ/kg DM)	17.4	17.5	19.5	17.0
Crude fat (%DM)	1.4	3.7	11.6	3.2
Crude protein (%DM)	8.2	9.2	11.7	12.0
Starch digestion (% starch in15 mins)	18.7	80.2	40.7	60.4
Starch digestion (%starch in 60 mins)	36.4	88.3	41.1	82.3

The fastest rate of starch digestion for all grains was during the first 15 minutes of *in vitro* incubation (Figure 5.8, Table 5.31). Unprocessed white rice had the slowest rate of digestion during this period with just over 1% of starch being digested/minute. Extruded rice had by contrast the fastest rate of digestion with over 5% of starch being digested/minute (Figure 5.8, Table 5.31).

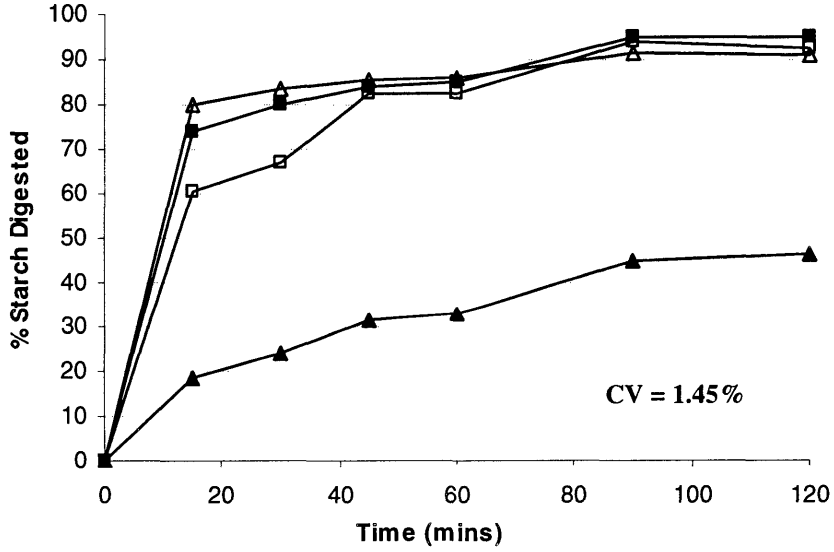

Figure 5.8: The pattern of *in vitro* digestion for unprocessed white rice (▲), extruded rice (△), extruded rice bran (■) and steam-rolled triticale (□).

Table 5.31: The rate of digestion (% of starch present in the grain on a dry matter basis/minute) for unprocessed white rice, extruded rice, extruded rice bran and steam-rolled triticale (SRT).

	0-15 mins	15-30 mins	30-45 mins	45-60 mins	60-90 mins	90-120 mins
Unprocessed rice (% starch/min)	1.3	0.3	0.5	0.1	0.4	0.0
Extruded rice (% starch/min)	5.3	0.1	0.1	0.0	0.2	0.0
Rice bran (% starch/min)	4.9	0.4	0.3	0.1	0.3	0.0
SRT (% starch/min)	4.0	0.4	1.0	0.0	0.4	0.0

Extruded rice was the most fermentable grain in bovine rumen fluid, initiating a lactic acid concentration of over 22 mmol/L and causing the pH to drop to 5.9. Unprocessed white rice was the least fermentable grain with no lactic acid present in the fermentation medium following the 4-hour incubation and the rumen fluid pH being maintained at 7.5 (Table 5.32).

Table 5.32: The fermentation characteristics, in bovine rumen fluid, of unprocessed white rice, extruded rice and steam-rolled triticale (SRT) when fermented for 4 hours (Bird *et al.*, 1999). The pH, volatile fatty acid (VFA) concentrations and lactic acid concentrations of the rumen fluid are measured at the conclusion of four hours.

	Unprocessed white rice	Extruded rice	SRT
pH	7.5	7.0	6.8
Acetate (mmol/L)	23.9	57.6	35.8
Propionate (mmol/L)	8.7	23.0	17.1
Butyrate (mmol/L)	4.7	9.9	6.8
Total VFA (mmol/L)	38.9	91.6	60.7
Total lactate (mmol/L)	0	22.4	0
Total acid (mmol/L)	38.9	114.0	60.7

The caecal fermentation pH and concentrations of fermentation end products following the 4-hour *in vitro* fermentation assay are shown in Table 5.33. As observed for the grains in experiments 1 and 2, fermentation in caecal fluid gave rise to lower concentrations of VFAs and higher concentrations of lactic acid. The fermentation of extruded rice resulted in higher concentrations of VFAs and lactic acid in comparison to unprocessed white rice (Table 5.33).

Table 5.33: The fermentation characteristics, in equine caecal fluid, of unprocessed white rice, extruded rice and steam-rolled triticale (SRT) when fermented for 4 hours (Bird *et al.*, 1999). The pH, volatile fatty acid (VFA) concentrations and lactic acid concentrations of the caecal fluid are measured at the conclusion of four hours.

	Unprocessed white rice	Extruded rice	SRT
pH	7.2	6.9	7.2
Acetate (mmol/L)	22.2	18.9	20.7
Propionate (mmol/L)	7.5	6.4	6.0
Butyrate (mmol/L)	2.4	2.1	2.1
Total VFA (mmol/L)	33.1	28.2	29.7
Total lactate (mmol/L)	18.3	42.0	24.1
Total acid (mmol/L)	51.4	70.2	53.8

In Vivo Results - by Treatment

The glycaemic and insulin response curves measured during experiment 3 are illustrated in Figures 5.9a and 5.9b respectively. The glycaemic response curves observed are typical equine glycaemic response curves as described by Loeb (1971), with the mean curve generated on the unprocessed white rice diet the only diet to cause glucose levels to fall below baseline levels within the five hour measurement period. The insulin response curves were more irregular in shape and highly variable between horses.

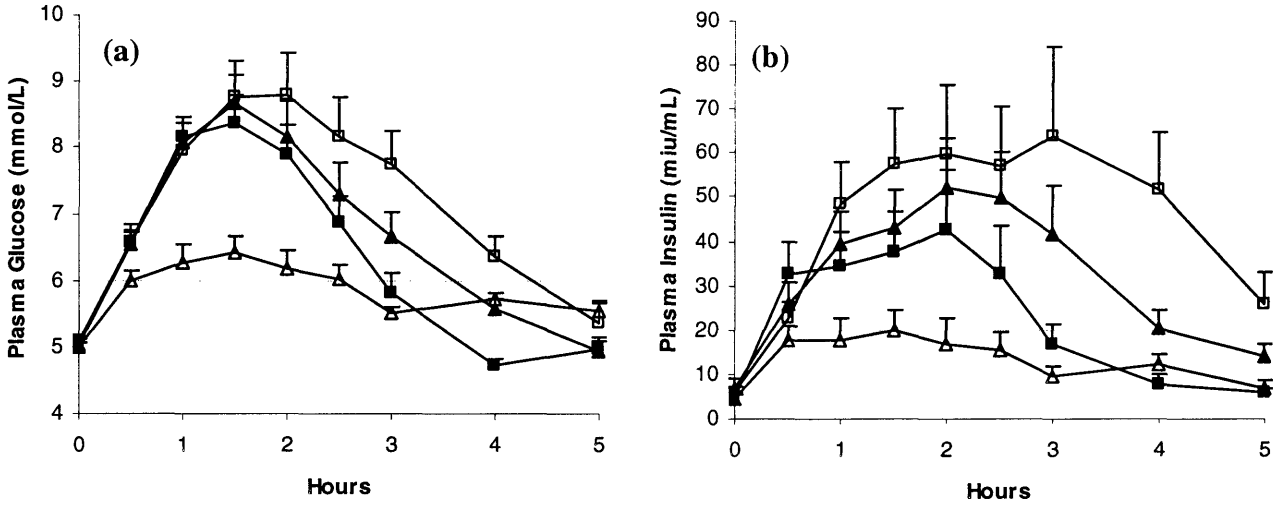

Figure 5.9: The **(a)** mean glucose and **(b)** mean insulin responses for (■) unprocessed white rice, (□) extruded rice, (△) rice bran + rice and (▲) steam-rolled triticale.

Extruded rice, steam-rolled triticale and unprocessed white rice initiated significantly higher peak glucose concentrations and peak minus basal glucose concentrations in comparison to the rice bran + rice diet but did not differ significantly from each other (Table 5.34). Extruded rice and steam-rolled triticale initiated higher average glucose responses in comparison to the rice bran + rice diet while the average glucose concentration for extruded rice was significantly higher than that for unprocessed white rice (Table 5.34). There were no significant differences between diets for the time taken to reach peak glucose. The rice bran + rice diet's slope to peak glucose was significantly lower than that for all other diets (Table 5.34).

Table 5.34: Mean peak glucose concentration (n=8), average glucose concentration (n=8), peak minus basal concentration (n=8), time to peak glucose (n=8) and slope to peak glucose (n=8) for unprocessed white rice, extruded rice, rice bran + rice and steam-rolled triticale (SRT).

	Unprocessed white rice		**Extruded rice**		**Rice bran + rice**		**SRT**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak glucose (mmol/L)	8.3^{a}	0.37	9.2^{a}	0.59	6.5^{b}	0.26	9.0^{a}	0.46
Average glucose (mmol/L)	6.4^{bc}	0.20	7.3^{a}	0.30	5.8^{b}	0.14	6.9^{ac}	0.24
Peak-basal glucose (mmol/L)	3.3^{a}	0.30	4.1^{a}	0.53	1.5^{b}	0.26	4.0^{a}	0.44
Time to peak glucose (hours)	1.4	0.12	1.7	0.13	1.3	0.21	1.6	0.13
Slope to peak glucose (mmol/L/h)	2.5^{a}	0.26	2.5^{a}	0.24	1.2^{b}	0.21	2.6^{a}	0.26

^{abc} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Extruded rice initiated significantly higher peak, average and peak minus basal insulin responses in comparison to the unprocessed white rice and the rice bran + rice diets (Table 5.35). Steam-rolled triticale initiated, peak, average and peak minus basal insulin responses that were significantly higher than those observed for the rice bran + rice diet. Extruded rice and steam-rolled triticale did not differ significantly for peak, average or peak minus basal insulin parameters (Table 5.35). There was no significant difference between diets for the time taken to reach peak insulin concentration. The rice bran + rice diets slope to peak insulin concentration was significantly lower than that observed for the extruded rice diet (Table 5.35).

Table 5.35: Mean peak insulin concentration (n=8), average insulin concentration (n=8), peak minus basal insulin concentration (n=8), time to peak insulin (n=8) and slope to peak insulin (n=8) for unprocessed white rice, extruded rice, rice bran + rice and steam-rolled triticale (SRT).

	Unprocessed white rice		**Extruded rice**		**Rice bran + rice**		**SRT**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak insulin (miu/mL)	40^{bc}	12.2	80^{a}	14.0	25^{b}	4.8	58^{ac}	11.4
Average insulin (miu/mL)	22^{bc}	5.5	44^{a}	9.0	14^{b}	2.7	33^{ac}	6.2
Peak-basal insulin (miu/mL)	36^{bc}	11.6	74^{a}	13.0	19^{b}	4.8	50^{ac}	10.9
Time to peak insulin (hours)	1.2	0.14	2.1	0.35	1.4	0.25	2.0	0.19
Slope to peak insulin (miu/mL/h)	25^{ab}	6.4	40^{a}	8.9	16^{b}	3.6	26^{ab}	5.2

^{abc} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

The unprocessed white rice and rice bran + rice diets lowered faecal pH significantly, in comparison to the control diet (Table 5.36). Faecal pH ranged from 5.91 –7.33 for the unprocessed white rice diet and correspondingly, this diet initiated the greatest production of total faecal acid (VFA + lactate), although there were no significant differences between diets for total faecal acid concentrations. There was no significant difference between diets for any other faecal parameters (Table 5.36).

Table 5.36: Mean faecal pH (n=8), faecal starch (n=8), faecal acetate (n=8), propionate (n=8), butyrate (n=8) and total faecal VFA (n=8), total faecal lactate (n=8) and total faecal acid (n=8) for horses on the unprocessed white rice, extruded rice, rice bran + rice and steam-rolled triticale diets.

	White rice		**Extruded rice**		**Rice bran + rice**		**SRT**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Faecal pH	6.8^{a}	0.18	7.0^{ab}	0.14	6.7^{a}	0.14	7.4^{b}	0.12
Faecal starch (% DM)	0.6	0.14	0.4	0.04	0.6	0.20	0.4	0.07
Acetate (mmol/L)	20.5	3.39	13.7	2.70	16.2	3.23	10.0	2.67
Propionate (mmol/L)	6.2	1.10	4.2	0.65	5.0	1.04	2.8	0.67
Butyrate (mmol/L)	2.4	0.57	1.3	0.25	1.8	0.46	1.1	0.43
Total VFA (mmol/L)	31.4	5.39	20.4	3.69	24.6	4.97	15.3	3.98
Total Lactate (mmol/L)	1.1	0.17	1.2	0.27	2.0	0.69	1.2	0.44
Total Faecal Acid (mmol/L)	32.5	5.38	21.6	3.84	26.5	5.54	16.5	4.12

^{ab} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

On average over the duration of the trial, extruded rice took significantly longer to consume (19.1 min) in comparison to the unprocessed white rice (9.4 min).

In vivo Results - by Horse

All horse pairs displayed typical glycaemic and insulin responses, with pair B displaying the highest mean glycaemic response and pair D having the highest mean insulin response (Figure 5.10).

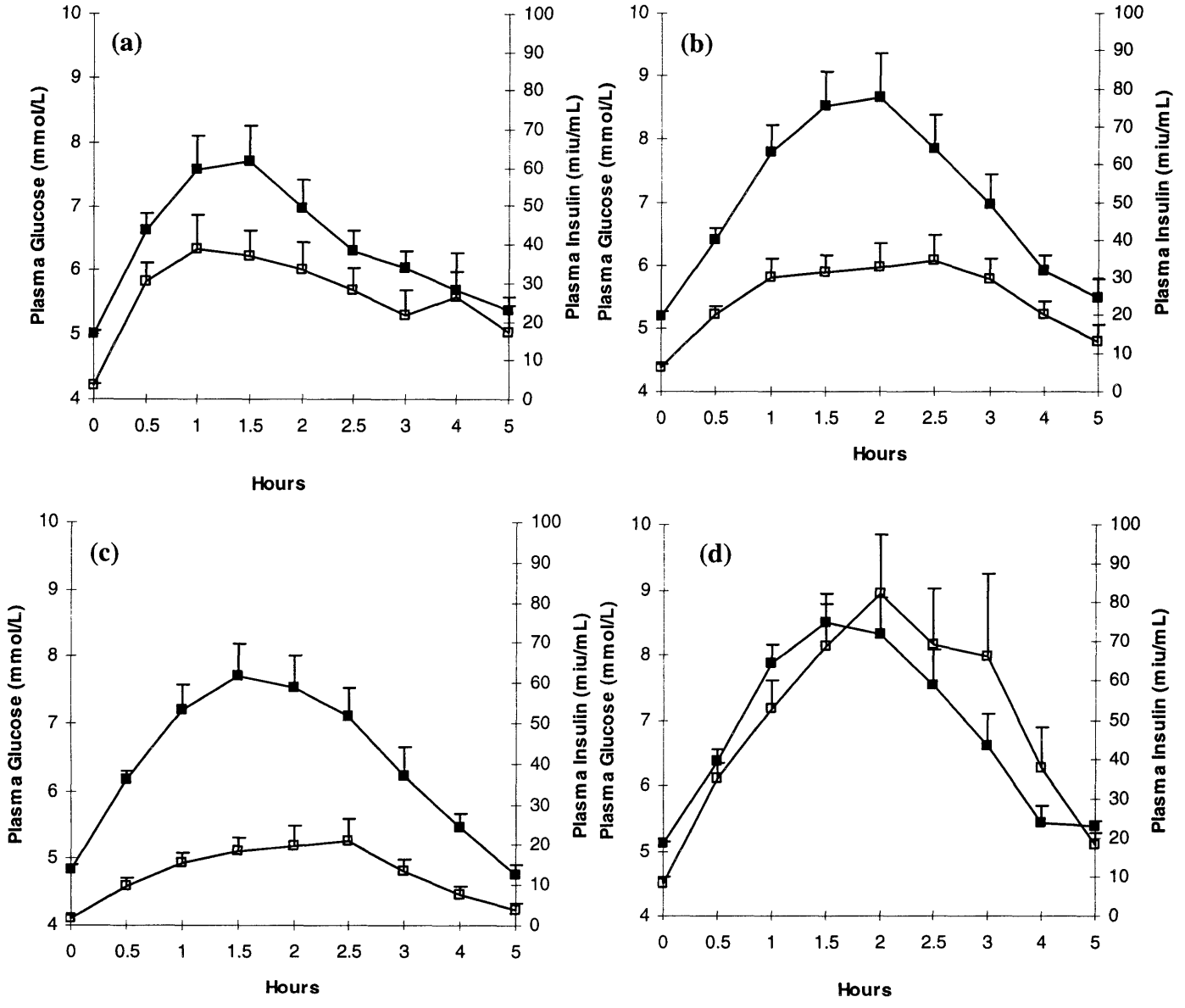

Figure 5.10: The (■) mean glucose and (□) mean insulin responses, averaged over all diets, for horses (**a**) pair A, (**b**) pair B, (**c**) pair C and (**d**) pair D.

There were no significant differences between horse pairs for peak glucose, average glucose, peak minus basal glucose, or slope to peak glucose concentrations. Pair A had a significantly slower time to peak glucose than Pair B (Table 5.37)

Table 5.37: Mean peak glucose concentration (n=8), average glucose concentration (n=8), peak minus basal concentration (n=8), time to peak glucose (n=8) and slope to peak glucose (n=8), averaged over all diets for horse pairs A, B, C and D.

	Pair A		Pair B		Pair C		Pair D	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak glucose (mmol/L)	7.7	0.59	8.8	0.69	7.7	0.47	8.7	0.52
Average glucose (mmol/L)	6.4	0.26	7.0	0.35	6.2	0.25	6.8	0.26
Peak-basal glucose (mmol/L)	2.7	0.57	3.6	0.62	2.9	0.50	3.6	0.51
Time to peak glucose (hours)	1.1^{a}	0.13	1.9^{b}	0.13	1.5^{ab}	0.12	1.5^{ab}	0.13
Slope to peak glucose (mmol/L/h)	2.4	0.49	2.0	0.32	2.0	0.32	2.4	0.22

^{ab} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Horse pair D had significantly higher, peak, average and peak minus basal insulin concentrations than pairs B and C. Time to peak insulin concentration did not differ significantly between pairs, but pair C had a significantly lower slope to peak insulin concentration than pairs A and D (Table 5.38).

Table 5.38: Mean peak insulin concentration (n=8), average insulin concentration (n=8), peak minus basal insulin concentration (n=8), time to peak insulin (n=8) and slope to peak insulin (n=8), averaged over all diets for horse pairs A, B, C and D.

	Pair A		Pair B		Pair C		Pair D	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak insulin (miu/mL)	51.0^{ab}	11.08	42.3^{a}	7.81	22.0^{a}	4.64	87.3^{b}	14.66
Average insulin (miu/mL)	26.5^{ab}	5.26	24.3^{a}	3.82	11.8^{a}	2.52	48.7^{b}	9.08
Peak-basal insulin (miu/mL)	46.8^{ab}	11.02	36.0^{a}	8.08	17.2^{a}	4.76	78.9^{b}	13.67
Time to peak insulin (hours)	1.4	0.38	1.5	0.23	1.9	0.23	1.9	0.16
Slope to peak insulin (miu/mL/h)	35.9^{a}	7.91	22.7^{ab}	3.09	8.8^{b}	2.32	39.8^{a}	6.55

^{ab} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Horse pair A had a significantly lower mean faecal pH than pair C, while pair C had a significantly lower faecal acetate concentration than pair B. There were no significant differences between horses for faecal starch, faecal propionate, faecal butyrate, total faecal VFAs, faecal lactate or total faecal acid concentrations (Table 5.39).

Table 5.39: Mean faecal pH (n=8), faecal starch (n=8), faecal acetate (n=8), propionate (n=8), butyrate (n=8) and total faecal VFA (n=8), total faecal lactate (n=8) and total faecal acid (n=8), averaged over all diets for horse pairs A, B, C and D.

	Pair A		Pair B		Pair C		Pair D	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Faecal pH	6.5^{a}	0.18	7.1^{ab}	0.16	7.3^{b}	0.11	7.0^{ab}	0.12
Faecal starch (% DM)	0.6	0.15	0.7	0.17	0.3	0.02	0.4	0.05
Acetate (mmol/L)	15.9^{ab}	3.15	19.7^{a}	2.65	7.0^{b}	1.49	17.6^{ab}	3.88
Propionate (mmol/L)	4.6	1.01	5.4	0.91	2.6	0.47	5.6	1.13
Butyrate (mmol/L)	1.6	0.39	2.0	0.39	0.6	0.12	2.3	0.64
Total VFA (mmol/L)	23.4	4.64	29.1	4.04	11.3	2.20	27.7	6.14
Total Lactate (mmol/L)	1.8	0.58	1.9	0.58	0.8	0.18	1.1	0.12
Total faecal acid (mmol/L)	25.2	4.75	31.0	4.55	12.0	2.25	28.8	6.15

^{ab} Values in same row with different superscripts are significantly different ($P \leq 0.01$).

Horse pair C were the fastest to consume the allocated test diets (mean 10.1 min) while pair A were the slowest (mean 17.5 min), however there were no significant differences between pairs for time taken to eat the meals.

The consistency of ranking of grains, in order of peak glucose response, by individual horses, determined by a coefficient of concordance was significant ($P<0.01$), however, the response of individual horses was highly variable and thus the consistency of ranking of horses in order of peak glucose response was not significant ($P>0.05$, Table 5.40).

Table 5.40: Peak plasma glucose concentrations (mmol/L) for individual horses in response to the expanded rice, steam-rolled triticale (SRT), unprocessed rice and rice + rice bran.

	Extruded rice	SRT	Unprocessed rice	Rice + rice bran	*MEAN*
B2	11.5	9.9	9.9	7.9	**9.8**
D1	10.4	10.4	8.3	6.2	**8.8**
A2	9.1	10.1	8.9	6.2	**8.6**
D2	8.4	9.2	9.3	7.2	**8.5**
C2	8.1	9.6	NA	6.6	**8.1**
B1	10.7	7.0	7.9	5.9	**7.9**
C1	9.2	8.7	6.7	6.4	**7.8**
A1	6.3	7.2	8.6	5.6	**6.9**
MEAN	**9.2**	**9.0**	**8.5**	**6.5**	

The consistency of ranking of grains, in order of peak insulin response, by individual horses, determined by a coefficient of concordance was significant ($P<0.05$) and although the between horse variation for peak insulin response was high, the consistency of ranking of horses in order of peak insulin response was significant ($P<0.01$, Table 5.41).

Table 5.41: Peak plasma insulin concentrations (miu/mL) for individual horses in response to the expanded rice, steam-rolled triticale (SRT), unprocessed rice and rice + rice bran diets.

	Extruded rice	SRT	Unprocessed rice	Rice + rice bran	*MEAN*
D2	126.7	111.5	113.7	50.8	**100.7**
D1	133.2	92.2	46.7	23.3	**73.9**
A1	102.9	34.0	57.1	15.3	**52.3**
A2	83.8	60.1	40.3	14.8	**49.8**
B2	46.5	73.6	21.7	28.8	**42.7**
B1	78.0	36.2	29.0	24.0	**41.8**
C2	39.8	32.6	NA	33.3	**35.2**
C1	28.4	21.4	6.9	6.6	**15.8**
MEAN	**79.9**	**57.7**	**45.1**	**24.6**	

5.6 DISCUSSION

In order to maximise starch digestion in the equine small intestine, we must feed grains that contain starch, which is susceptible to amylolytic enzyme attack and thus digestible. Processing cereal grains prior to feeding to disrupt endosperm and starch granule structures to improve starch digestibility is also very important and it is essential to be able to differentiate grains and processing methods on the basis of starch digestion using quantitative assays. It was seen that research into these areas could contribute to the horse industry.

The *in vitro* assay of Bird *et al.* (1999) was used during the current set of experiments to screen grains for rate and extent of potential small intestinal starch digestion. Oats and triticale were the most digestible of the unprocessed grains and this result is consistent with the *in vivo* observations of de Fombelle *et al.* (2001), Meyer *et al.* (1995), Radicke *et al.* (1991) and Arnold *et al.* (1981), who all observed that oats is more digestible than corn and barley in the equine small intestine (Table 2.4).

Grain processing methods involving heat, moisture and pressure, as previously observed by Holm *et al.* (1989) Ross *et al.* (1987) and Brand *et al.* (1985), improved the *in vitro* enzyme digestibility characteristics (rate and extent of digestion) of the grains studied, indicating that expansion, extrusion, micronising and steam-rolling disrupt endosperm and starch granule structures, increasing the susceptibility of starch to enzyme degradation and causing a corresponding increase in starch digestibility. Grain processing also increased the rate of fermentation of the studied cereal grains, with all processed grains, except expanded barley and steam-rolled triticale, giving rise to higher concentrations of VFAs and lactic acid during the *in vitro* fermentation assays than their corresponding unprocessed grains, a result consistent with the *in vivo* observations of Galyean *et al.* (1976), Theurer *et al.* (1999) and Zinn (1993). Thus it appears that while grain processing improves starch digestion in the small intestine, the potential of a grain to ferment rapidly and cause hindgut lactic acidosis is also increased.

Fermentation in horse caecal fluid gave rise to lower concentrations of VFAs but higher concentrations of lactic acid than fermentation in bovine rumen fluid for all of the grains studied. This may indicate that the equine hindgut is more prone to lactic acid production, supporting the observations of Kern *et al.* (1973), who isolated greater numbers of gram +ve rods and cocci (a majority of lactic acid producers are gram +ve bacteria) from the caecum of ponies than from the rumen of steers. The differences between the two fermentation mediums may however be explained by differences in the length of adaptation and degree of exposure to starch between the bovine rumen and equine caecum, from which fluid was collected. The cattle used were maintained on 50% grain diet and thus the rumen flora were exposed to large quantities of starch, allowing the balance of lactic acid producers and utilisers to equilibrate. This fine equilibrium was only upset by the highly digestible extruded corn and extruded rice (NB all grains used in Experiment 1 caused lactic acid

accumulation in rumen fluid, however these grains were fermented for 5 hours in a greater volume of rumen fluid and thus the results are not easily comparable). The equine caecal fluid, on the other hand, may not have been exposed to any starch. Despite the fact that the horses were consuming a grain diet prior to slaughter, it is likely that the oats and extruded rice was extensively digested in the small intestine, leaving very little starch to enter the hindgut. Thus, during the 4-hour fermentation it appears that lactic acid producers, capable of doubling their population in as little as 15 minutes (Leek, 1993) have rapidly multiplied, causing the observed accumulation of lactic acid. These observations emphasise the danger present when horses are fed high starch diets and the speed at which serious hindgut lactic acidosis may occur, particularly when processed grains are fed.

Based on results in this study from the *in vitro* enzyme digestion and fermentation assays, grains may be separated into four categories. These are:

1. grains with low enzyme digestibility but rapid fermentation characteristics (large capacity for lactic acid accumulation);
2. grains with high enzyme digestibility and rapid fermentation characteristics;
3. grains with a high enzyme digestibility but slow fermentation characteristics and;
4. grains with low enzyme digestibility and slow fermentation characteristics.

Figure 5.11 graphically demonstrates the studied grains enzyme digestion and fermentation characteristics, with grains separated into the categories 1 to 4, described above.

Cracked barley, in category 1 is potentially the most unsuitable grain for inclusion in equine diets, with the greatest capacity to cause hindgut lactic acidosis. Category 2 contains the extruded grains, which, when fed 'correctly', may be the most suitable and efficient grains for inclusion in equine diets, as a majority of starch will be digested pre-caecally. However, it could be argued that, should these grains be fed in a manner that allows substantial quantities of starch to reach the hindgut, these grains are potentially the most dangerous grains for horses, as their capacity to induce hindgut lactic acidosis is so high. Category 3 includes the grains most suitable for horses, with a superior small intestinal starch digestibility, but poor fermentation characteristics. Category 4 grains, where a majority of the studied cereal grains are found, are likely to deliver starch, undigested, to the hindgut. However, these grains ferment more slowly, giving rise to lower lactic acid concentrations during starch fermentation than grains in quadrats 1 and 2. From this point of view, these grains theoretically present a lower hindgut acidosis risk to horses than grains in categories one and possibly category two. In saying that however, all grains in category 4 had a lactic acid concentration of >15 mmol/L in the equine caecal fluid medium after the 4 hour *in vitro* fermentation and thus cannot be considered totally 'safe' (Figure 5.11).

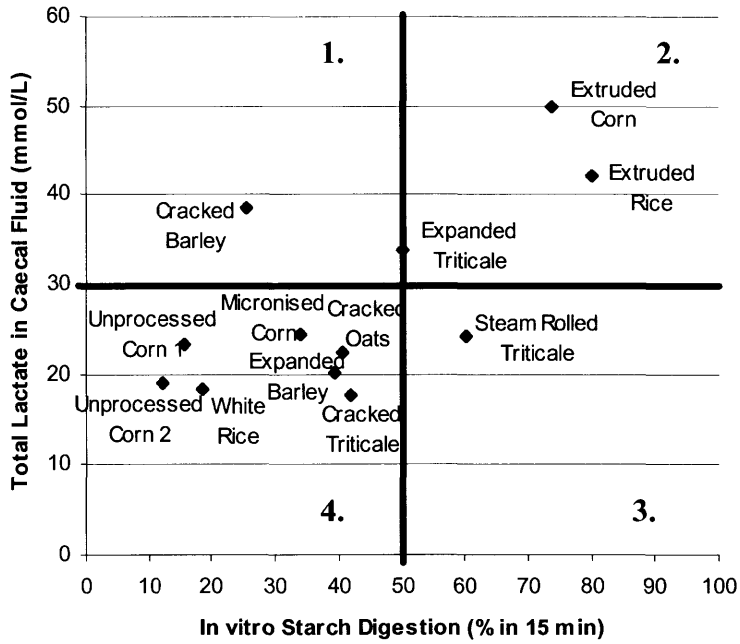

Figure 5.11: The *in vitro* enzyme digestion and fermentation characteristics of the studied grains.

The *in vivo* findings support those observed *in vitro.* Grain processing appears to improve the small intestinal starch digestibility of barley, triticale, corn and rice, with the glycaemic and insulin responses for the expanded, extruded and steam-rolled grains being higher than those in horses consuming the unprocessed grains. These results are in agreement with those of Householder *et al.*(1977), Meyer *et al.* (1993) and Hoekstra *et al.* (1999) who observed that improvements were made to small intestinal starch digestion if cereal grains were processed using methods involving any combination of heat, moisture and pressure. Micronised corn was an exception and in contrast to the extrusion of corn, micronising appeared to make little difference to the digestibility of this cereal grain, a result in stark contrast to those reported by McLean (2001). When extruded, the corn fed during this experiment was exposed to temperatures in excess of 130°C for 14 – 15 seconds while the micronised corn was processed at an external temperature of 89°C for 12 seconds. Thus in support of the findings of Meyer *et al.* (1993), it appears that by increasing the severity of processing of corn and more extensively disrupting protein matrix, cell wall and starch granule structures within the corn endosperm, we may increase small intestinal starch digestibility.

The relationship between the glycaemic response and the *in vitro* enzyme assay with a 15-minute incubation period was not strong, with peak minus basal glucose concentrations and the 15-minute *in vitro* assay displaying an R^2 of 0.30 (Figure 5.12). The weak relationship between the *in vitro* assay with a 15-minute incubation period and the *in vivo* glycaemic response may be partly attributed to the highly variable glycaemic responses observed between horses. In addition, there were several diets, including cracked corn, white rice, cracked triticale and cracked oats that appeared as outliers from the relationship trend line (Figure 5.12).

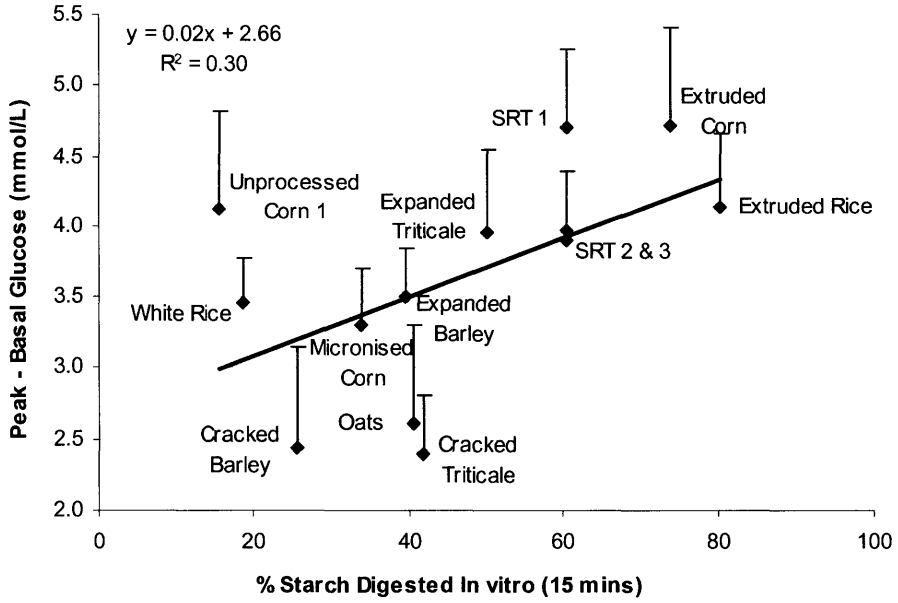

Figure 5.12: The relationship between the *in vitro* starch digestion assay with a 15 min incubation period and the mean peak – basal plasma glucose concentrations for each diet.

Cracked corn, while having an *in vitro* digestibility of around 16%, initiated a glycaemic response comparable to extruded rice. Previously reported *in vivo* starch digestibility's for cracked corn in the equine small intestine range from 29% - 94% (de Fombelle *et al.* 2001; Meyer *et al.* 1995; Arnold *et al.* 1981; Radicke *et al.* 1991; McLean 2001; Hinkle *et al.* 1983), thus it is possible that corn is extensively digested in the small intestine. However, there are several factors to indicate that the cracked corn was not being pre-caecally digested:

1. horses consuming the cracked corn diet had significantly higher concentrations of lactic acid in their faeces in comparison to horses on the control diet (Table 5.22);
2. insulin responses for horses on the cracked corn diet were lower than observed for horses on the extruded and micronised corn diets (Figure 5.8);
3. results of the thoroughbred industry survey indicated that unprocessed corn contained starch relatively resistant to enzyme attack and that inclusion of corn in thoroughbred diets was a major contributor to the problems experienced with hindgut starch fermentation; and
4. during a comparable trial, Hoekstra *et al.* (1999) recorded a peak glucose response for horses on a cracked corn diet that was 3 mmol/L lower than that observed during experiment two in this study.

After taking these factors into account, it appears reasonable to conclude that the glycaemic response observed for horses on a cracked corn diet during experiment 2, overestimates the pre-caecal digestibility of unprocessed corn and that the insulin response and the *in vitro* assay more accurately represent corn's true pre-caecal digestibility. The lactic acid accumulation in the faeces of horses consuming cracked corn during this study supports the observation made during the thoroughbred industry survey (Chapter 3) that corn is a major contributor to hindgut starch fermentation.

It also appears that the glycaemic response is overestimating the small intestinal starch digestibility of unprocessed white rice and as in the case of unprocessed corn, the *in vitro* starch digestion assay and the insulin response appear to give a more accurate representation of the true enzymatic digestibility of white rice (Figure 5.13). Although McMeniman *et al.* (1990) reports that rice has a 100% total tract organic matter digestibility in equines, the extent of small intestinal starch digestion has not been previously reported and it is suspected that, with horses consuming the white rice diet during the current experiment experiencing faecal pH's as low as 5.9, the digestion of unprocessed white rice in the equine small intestinal is not extensive.

Oats also does not appear to fit the trend line fitted between the *in vitro* assay and the *in vivo* glycaemic response (Figure 5.12). The low glycaemic response of oats, relative to the *in vitro* enzyme assays starch digestion estimation, is likely to be related to its high crude fat content (8.2%), which may slow gastric emptying (Cooke 1975; Cooke *et al* 1976; Debas *et al* 1975; Pagan *et al.* 2001), prolong starch digestion and lower the glycaemic response and thus apparent *in vivo* starch digestibility. This effect of fat on the glycaemic response has previously been reported by Pagan *et al.* (2001) and was also demonstrated by the high oil rice bran diet included in Experiment 3. On inspection of the insulin response for cracked oats (Figure 5.3), it does appear that starch digestion is occurring over a longer period of time, possibly due to delayed gastric emptying, as evidenced by insulin concentrations remaining elevated and not returning to baseline levels for more than five hours post prandial. The glycaemic and insulin response results from the cracked corn, white rice and cracked oat diets highlight the importance of measuring both the glycaemic and insulin responses to allow for correct interpretation of results.

Cracked triticale was also ranked in a different order of digestibility by the *in vitro* and *in vivo* assays, with *in vivo* digestibility appearing lower than *in vitro* digestibility. No explanations for this discrepancy are obvious, however it may be partly attributed to particle size, seed coat and cell wall structures that limit the *in vivo* access of amylolytic enzymes to the starch within triticale. Due to the fine grinding of grains prior to *in vitro* incubation, it is possible that these factors were partly removed, allowing *in vitro* estimations for the digestibility of triticale to be exaggerated. The poor *in vivo* starch digestibility of cracked triticale is also supported by faecal data, with horses eating this diet having significantly higher faecal acetate, propionate, total faecal VFA and total faecal acid concentrations than horses consuming the glucose control and cracked oat diets.

When the *in vitro* assay incubation period was extended to 60 minutes, the relationship between the glycaemic response and the *in vitro* assay weakened further (R^2=0.10). Thus the fourth hypothesis for this set of experiments is not supported, with the glycaemic response and the *in vitro* assay not ranking grains in the same order of digestibility. However, given the number of grains that the glycaemic response appears to have overestimated or underestimated the small intestinal digestibility of, it appears that the *in*

vitro assay may be the more accurate indicator of the small intestinal starch digestibility of cereal grains. It is however recommended that for the estimation of small intestinal starch digestibility in equines, the *in vitro* enzyme assay of Bird *et al.* (1999) be modified to use a 15-minute incubation period only.

The relationship between the *in vitro* assay with a 15-minute incubation period and the *in vivo* insulin response was slightly stronger (R^2=0.43, Figure 5.13) than the relationship observed with the glycaemic response, with unprocessed corn, white rice and oats appearing to be been ranked in the correct order of digestibility by the insulin response, supporting the observations of Lee *et al.* (1998) and Heaton *et al.* (1988) who reported that the insulin response may be a more accurate indicator of small intestinal starch digestion than the glycaemic response. There was, however large variability observed within the insulin response parameter (Figure 5.13), perhaps limiting the accuracy, repeatability and therefore usefulness of this parameter as an *in vivo* indicator of small intestinal starch digestion. The insulin response was also strongly related to body condition score (R^2=0.52), emphasising the importance of this measurement when interpreting insulin response data.

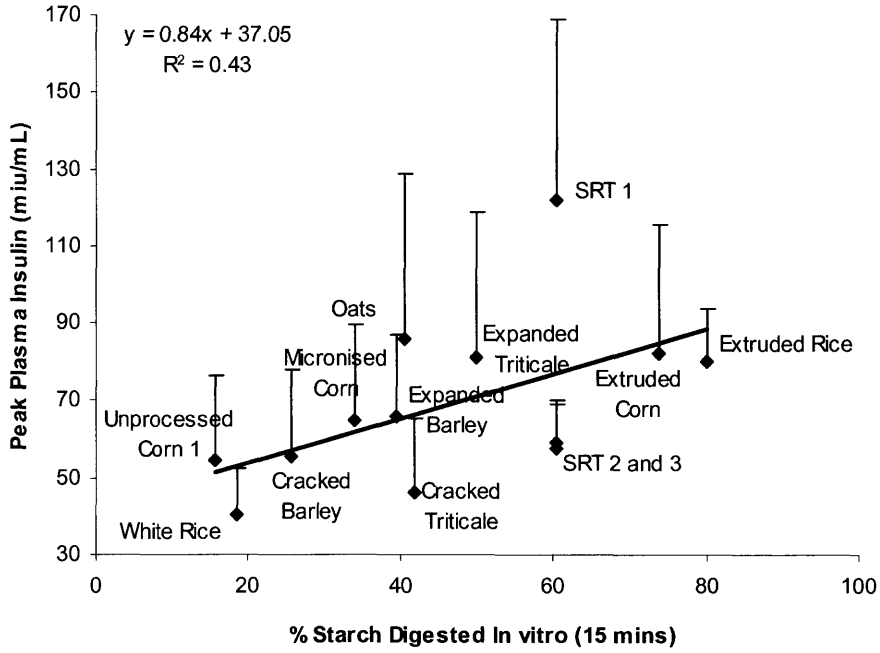

Figure 5.13: The relationship between the *in vitro* starch digestion assay with 15 min incubation period and the mean peak plasma insulin concentrations for each diet.

Faecal parameters were measured during the current set of experiments with the aim of finding an easily measured parameter that may allow the estimation of the extent of hindgut starch fermentation occurring on various cereal grain diets. Relationships between the faecal parameters (pH, starch, VFA lactate and total faecal acid concentrations) and the *in vitro* assay with a 15-minute incubation period, the peak plasma glucose and peak plasma insulin concentrations were not significant. However, it is reasonable to postulate that, with only approximately 1 kg of grain being fed per meal, quantities of starch large enough to cause significant changes to the equine hindgut would not be reaching this area of the gastrointestinal tract. To assess the relationship between faecal parameters and the *in vitro*

and *in vivo* assays used during this trial, horses may need to be fed larger meals of these cereal grains.

Significant variations between horses for glycaemic response, insulin response and faecal parameters were present during this set of three experiments. However, during all experiments, horses were ranked consistently in order of glycaemic and insulin response, to the individual grain diets (with the exception of the glycaemic response during experiment three). This consistency of ranking suggests that both metabolic responses may be useful tools for estimating an individual horse's capacity for starch digestion in the small intestine.

The variation observed between horses may be due to differences in enzyme activity in the small intestine and thus capability to break starch down into glucose molecules (Kienzle *et al.*, 1994; Roberts, 1974), differences in the capacity to absorb glucose from the small intestine (Church *et al.*, 1997; Mair *et al.*, 1991), differences in eating and chewing behaviours (Meyer *et al.*, 1995) or condition score (Jeffcott *et al.*, 1986; Mattheeuws *et al.*, 1984). These variations emphasise the need for individual diet formulation, based on a horse's ability to digest starch and the corresponding susceptibility to hindgut lactic acidosis and also the need for careful trial design when conducting grain-feeding experiments in horses. Further research needs to be conducted to define the reasons for the large variations observed. This research may also be directed toward assessing inter-horse differences in hindgut bacterial populations and its potential affect on faecal parameter variation.

5.7 CONCLUSIONS

The small intestinal starch digestibility of the unprocessed grains, studied during this set of experiments, varied greatly between grain species, with oat starch appearing to be the most digestible in the equine small intestine. The grain processing methods studied improved the *in vitro* and *in vivo* enzyme digestibility of cereal grain starch, with extrusion appearing to be the most effective method. Grain processing also increased the rate at which cereal grains fermented and gave rise to lactic acid production in bovine rumen fluid and equine caecal fluid. Thus, while grain processing may improve small intestinal starch digestion, it may also increase the risks involved with the feeding of large quantities of cereal grains to horses.

The *in vitro* and *in vivo* assays used during this study were not well related and while it appears that the glycaemic and insulin responses may be able to rank horses in order of their ability to digest starch pre-caecally, the glycaemic response appeared to overestimate or underestimate the digestibility of a number of the cereal grains studied. The reasons for the poor relationships between the *in vitro* and *in vivo* assays remain largely unexplained, however, the large variation between horses for glycaemic and insulin responses may have contributed. These large between horse variations indicate that horses do not have an equal capacity for small intestinal starch digestion. The specific physiological mechanisms causing these variations between horses are not well understood and will thus be the focus of the experiments detailed next in Chapter 6.

6 USING EXOGENOUS GLYCANASES TO IMPROVE STARCH DIGESTION IN THE SMALL INTESTINE OF THE HORSE

6.1 INTRODUCTION

Results from the previous three experiments (Chapter 5) show significant variation between horses for glycaemic and insulin responses and faecal metabolites, indicating that horses vary considerably in their ability to digest starch in the small intestine. Several factors, including a horse's rate of eating, extent of chewing and passage rates through the small intestine (Frape, 1998; Meyer *et al.*, 1995), small intestinal glucose absorption capacity (Church *et al.*, 1997; Mair *et al.*, 1991) and protease activities in the stomach and small intestine, may all contribute to the observed variation (Figure 2.15). However with numerous reports that the activities and concentrations of α-amylase, in the equine small intestine, are low in comparison to dogs, pigs and cats and highly variable between horses (Alexander *et al.*, 1958; Comline *et al.*, 1969; Kienzle *et al.*, 1994; Roberts, 1974), a deficiency of this small intestinal glycanase may be a major contributing factor to the horses' variable and often poor ability to digest starch pre-caecally.

In contrast to these findings regarding α-amylase, Roberts *et al.* (1974) and Kienzle *et al.* (1993) have reported activity levels of brush border glycanases in the equine small intestine that are comparable to those observed in humans, pigs and dogs. It therefore appears unlikely that a deficiency of brush border glycanases will limit pre-caecal starch digestion in the horse.

It was decided to carry out an experiment to investigate the effect of the addition of exogenous α-amylase and/or amyloglucosidase (AMG) to a cereal grain diet, on the digestion of starch in the equine small intestine. The hypothesis was that the addition of α-amylase or a combination of α-amylase and AMG, to diets containing digestible starch, would improve the digestion of starch in the equine small intestine. The hypothesis also suggests that α-amylase is the primary limiting factor and that the addition of AMG alone would have a non-significant effect on small intestinal starch digestion. The design of the experiment was based on the assumption that an improvement in small intestinal starch digestion will be reflected in glycaemic and insulin responses that are elevated above those observed for the control diet.

6.2 METHODS AND MATERIALS

Treatments and Animals

Three treatment diets and a control diet were used in the experiment. Steam-rolled triticale (662 g/kg starch, 120 g/kg non-starch polysaccharide, 120 g/kg crude protein, 32 g/kg crude fat, 25 g/kg crude fibre, 17.0 MJ/kg, all figures presented on a dry matter basis), was used as the control grain, to which exogenous enzymes were added. Steam-rolled triticale was chosen as the control grain to which enzymes would be added because it had a high *in vitro* starch digestibility (Table 5.2), therefore ensuring that it would be susceptible to enzyme

attack *in vivo*. The control diet consisted of 1.12 kg of steam-rolled triticale, which corresponded to 670 g of starch and meal sizes were not varied according to body weight.

Treatment 1 was 1.12 kg steam-rolled triticale and 3 mL of heat stable α-amylase, derived from *Bacillus licheniformis* (450 Kilo Novo α-amylase units, a quantity capable of digesting 2.4 kg starch; Termamyl® Classic, Novozymes A/S. DK-2880, Bagsvaerd), treatment 2 was 1.12 kg steam-rolled triticale and 1 mL AMG, derived from *Aspergillus niger* (300 amyloglucosidase units, a quantity capable of digesting 6.2 kg of starch; AMG 300L, Novozymes A/S. DK-2880, Bagsvaerd) and treatment 3 consisted of 1.12 kg steam-rolled triticale, 1 mL AMG (AMG 300L) and 3 mL of heat stable α-amylase (Termamyl® Classic). All enzymes were diluted to 25 mL with distilled water and sprayed over the steam-rolled triticale meal using a hand held spray nozzle to thoroughly cover the grain. Enzymes were added to the grain meal 10 minutes prior to feeding.

Twelve horses (eight standardbred horses; 4 geldings and 4 mares and four thoroughbred horses; 4 geldings), aged 4 to 9 years and weighing 432 – 512 kg were used in the study. Horses were randomly divided into four groups of three horses (Table 6.1). Group A was allocated to the control diet, group B to the α-amylase diet, group C to the AMG diet, and group D were placed on the α-amylase + AMG diet. Horses were allowed one and a half days to acclimatize to the stables and daily routine. Horses were fed the experimental diets from the evening feed of the second day. Blood sampling to measure the glycaemic and insulin responses took place on the morning of the fifth and eighth days. The University of New England Animal Ethics Committee approved the experimental protocol.

Table 6.1: The breed, sex, age, weight and condition score (CS) characteristics of the horses used in the trial.

	Group A			Group B			Group C			Group D		
	A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**	**D1**	**D2**	**D3**
Breed/Sex	S/G	T/G	T/G	T/G	S/M	S/M	S/M	T/G	S/G	S/G	S/M	S/G
Age (years)	4.5	9	5	6	4	4.5	9	8	4.5	4.5	4	6
Weight (kg)	476	482	498	512	504	432	476	490	496	494	478	482
CS	6	6	4	4.5	7	7	3	5	7	5	8	7

S – Standardbred *T – Thoroughbred* *M – Mare* *G - Gelding*

The horses were stabled in individual box stalls overnight and held in individual yards during the day for the duration of the trial. Sawdust was used as bedding in the stables. The horses were exercised each evening for half an hour. All animals had been previously maintained on pasture and treated with an anthelmintic two weeks prior to the trial. Horses were considered to be healthy and free from internal parasites at the commencement of the trial.

During the trial horses were fed the grain portion of the diet at 0700 h and 1730 h each day. On completion of eating the grain the horses received 4 kg of lucerne hay. Some horses had their night allocation of hay reduced on the days prior to blood sampling to ensure they ate the test diet the following morning. On mornings when the blood sampling was to take place, only the grain portion of the diet was fed. Hay was fed at the conclusion of blood

sampling. All animals had access to water *ad libitum,* except during the two five-hour blood sampling periods when water was not available.

Measurements

Horses were weighed on arrival at the stable complex and condition score was assessed and recorded for each horse using the method of Henneke *et al.* (1983). Feed intake and refusals were recorded each day. Fresh faecal samples were collected from the stable of each horse between 0700 and 0800 hours on the fifth day. Faecal samples were sub-sampled and analysed for faecal pH, total starch content, volatile fatty acid (VFA) and lactic acid concentrations using methods described in Sections 4.2 (pH and starch) and 5.3.1 (VFA and lactic acid).

On the morning of the fifth and eighth days blood samples were collected for the measurement of glycaemic and insulin responses to the test diets using the methods described in Section 4.2. Blood samples were taken at 0 hours (prior to grain being fed) and then at 30, 60, 90, 120, 150, 180, 240, and 300 minutes following commencement of eating, making a total of nine blood samples/horse. Plasma samples were analysed for glucose concentration (methods described in Section 5.4.1) and insulin concentration (methods described in Section 4.2).

Statistical Methods

Changes in plasma glucose and insulin concentrations with time were used to determine the peak glucose/insulin concentrations, average glucose/insulin concentrations, peak minus the basal glucose/insulin concentrations, time to peak glucose/insulin and slope to peak glucose/insulin. Statistical analyses of glucose and insulin data were carried out using an ANOVA with a split plot design, split over period to look at the diet × period interaction. Although this was a repeated measures study, it was feasible to use a split plot design for analysis, as there were only two periods. Confidence intervals (CI) were calculated using the method for split plots (Cochrane *et al.*, 1957) and were used to determine significant differences ($P \leq 0.008$, determined using Bonferroni's adjustment) between the means. If the calculated confidence intervals encompassed 0, means were considered to be not significantly different. Faecal data was analysed using a fixed effect ANOVA with diet included in the model. Statistical analysis was carried out using the S-plus 6 for Windows Professional statistical package (Insightful Corporation. Seattle, WA. USA).

6.3 RESULTS

Feed Refusals

Horse C2, in the α-amylase + AMG group, refused 130g of the test diet on the morning of the first blood sampling (day 5) and 84 g on the morning of the second blood sampling (day 8). Horse D3, in the control group, refused 600 g and 406 g on the morning of the first and second blood sampling days respectively. There were no other feed refusals.

Glycaemic and Insulin Responses

The glycaemic response curves for the test diets, shown in Figure 6.1a, were typical glycaemic response curves as described by Loeb (1971). Similarly the insulin response curves, shown in Figure 6.1b appeared to be normal. Plasma glucose and insulin concentrations for horses on the α-amylase + AMG diet failed to return to baseline levels five hours after consumption of the test diet.

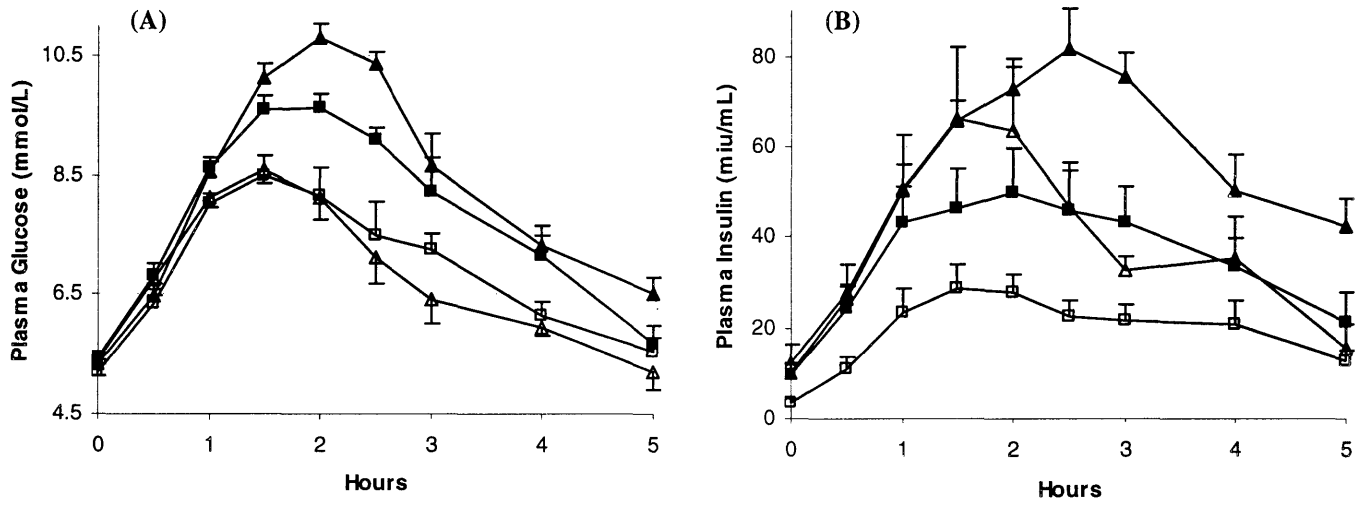

Figure 6.1: The **(A)** mean glucose responses and **(B)** mean insulin responses for the (■) α-amylase, (□) amyloglucosidase (AMG), (▲) α-amylase + AMG and (△) control diets.

The addition of α-amylase + AMG to steam-rolled triticale significantly ($P \leq 0.008$) increased the peak plasma glucose responses in comparison to the peak responses initiated by the AMG and control diets (Table 6.2). The average glucose concentrations over the five-hour sampling period were significantly ($P \leq 0.008$) higher for horses fed the α-amylase and the α-amylase + AMG diets than for horses on the AMG or control diets. Horses fed the α-amylase + AMG diet had a significantly ($P \leq 0.008$) higher peak - basal plasma glucose concentration compared to animals on the control diet (Table 6.2). Horses given the AMG or control diets did not differ from each other for the peak, average or peak - basal glucose concentration parameters. Similarly, horses on the α-amylase and α-amylase + AMG diets were not different for any parameters involving plasma glucose concentration. There were no differences between diets for time or slope to peak glucose (Table 6.2).

Table 6.2: Mean peak glucose concentration, average glucose concentration, peak minus basal glucose concentration, time to peak glucose and slope to peak glucose for the control, α-amylase, amyloglucosidase (AMG) and α-amylase + AMG diets.

	Control		α-Amylase		AMG		AMG + α-Amylase	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak glucose (mmol/L)	8.8^{a}	0.19	10.0^{ab}	0.56	8.8^{a}	0.30	10.8^{b}	0.22
Average glucose (mmol/L)	6.9^{a}	0.08	7.9^{b}	0.41	7.0^{a}	0.19	8.4^{b}	0.11
Peak-basal glucose (mmol/L)	3.3^{b}	0.24	4.6^{ab}	0.54	3.6^{ab}	0.31	5.5^{a}	0.23
Time to peak glucose (hours)	1.6	0.20	1.9	0.20	1.6	0.15	2.2	0.11
Slope to peak glucose (mmol/L/h)	2.2	0.16	2.5	0.33	2.4	0.12	2.8	0.16

Values in same row with different superscripts are significantly different ($P \leq 0.008$).

There were no differences between diets for peak, average, or peak – basal insulin concentration parameters. Time and slope to peak insulin did not differ significantly between diets (Table 6.3).

Table 6.3: Mean peak insulin concentration, average insulin concentration, peak minus basal insulin concentration, time to peak insulin and slope to peak insulin for the control, α-amylase, amyloglucosidase (AMG) and α-amylase + AMG diets.

	Control		α-Amylase		AMG		AMG + α-Amylase	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Peak insulin (miu/mL)	74.5	17.84	58.8	11.50	33.6	4.41	87.2	7.88
Average insulin (miu/mL)	39.1	7.23	36.2	6.76	20.6	2.95	52.8	3.90
Peak-basal insulin (miu/mL)	62.1	20.99	46.3	16.10	26.0	6.71	77.0	9.35
Time to peak insulin (hours)	2.0	0.22	2.4	0.42	1.9	0.30	2.1	0.30
Slope to peak insulin (miu/mL/h)	36.0	9.98	20.7	4.39	20.2	6.32	40.5	6.50

Sampling period significantly ($P \leq 0.008$) affected the average glucose response. Horses given α-amylase had a significantly reduced average glucose response during the second sampling period (day 8) compared to that observed during the first sampling period (day 5, Figure 6.2a). A significant diet × period interaction was also observed for average ($P \leq 0.02$) and peak ($P \leq 0.03$) glucose concentration parameters, with the control diet increasing both average and peak glucose responses over time, while the α-amylase and the AMG diets had a decreasing response between sampling periods one and two (Figure 6.2a and 6.2b).

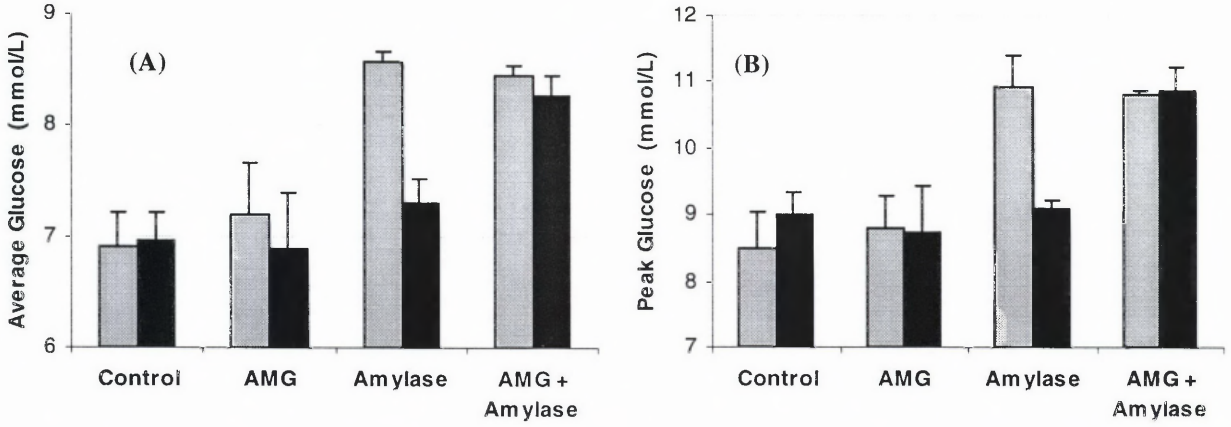

Figure 6.2: The significant effect of sampling period and the sampling period × diet interactions for (**A**) peak plasma glucose concentration and (**B**) average plasma glucose concentration for the diets α-amylase, amyloglucosidase (AMG), α-amylase + AMG and the control diet in (▒) period 1 and (■) period 2.

Faecal Parameters

Diet had no effect on faecal pH, faecal starch concentration, faecal VFA concentration, total faecal lactate or total faecal acid concentrations (Table 6.4).

Table 6.4: Mean faecal pH, faecal starch, faecal acetate, propionate, butyrate and total faecal volatile fatty acid (VFA) concentration, total faecal lactate and total faecal acid concentrations for horses on the control, α-amylase, amyloglucosidase (AMG) and α-amylase + AMG diets.

	Control		α-Amylase		AMG		AMG + α-Amylase	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Faecal pH	7.1	0.12	7.4	0.14	7.4	0.06	6.9	0.17
Faecal starch (% DM)	0.4	0.03	0.5	0.09	0.3	0.03	0.5	0.06
Acetate (mmol/L)	24.6	3.84	17.5	1.23	20.8	2.26	22.7	4.38
Propionate (mmol/L)	4.8	0.94	3.2	0.14	4.2	0.51	4.2	0.90
Butyrate (mmol/L)	2.0	0.48	1.0	0.26	1.4	0.42	1.9	0.54
Total VFA (mmol/L)	33.5	5.41	22.6	1.59	27.9	3.34	30.5	6.13
Total lactate (mmol/L)	0.4	0.03	1.1	0.33	0.6	0.11	0.6	0.27
Total faecal acid (mmol/L)	33.8	5.44	23.7	1.91	28.5	3.44	31.1	6.39

Values in same row with different superscripts are significantly different ($P \leq 0.05$).

All horses, except Control horse 3 and Amylase + AMG horse 2, were placed on the steam-rolled triticale control diet within 12-days of this trial, during corn and rice digestion experiments (that were for logistical reasons conducted after this study) and the individual horse's responses to the steam-rolled triticale control diet during these experiments are illustrated below in Figure 6.3. The addition of α-amylase to steam-rolled triticale increased the individual peak glucose response of two horses, indicating that for these animals the addition of α-amylase improves small intestinal starch digestion. For horse 2, the addition of α-amylase to steam rolled triticale made no difference to peak glucose response, suggesting that this horse has a naturally high α-amylase activity in the small intestine (Figure 6.3b). The addition of α-amylase and AMG to the steam-rolled triticale diet substantially increased each individual horses glycaemic response to this diet, suggesting that large improvements to pre-caecal starch digestion were made through the addition of these enzymes.

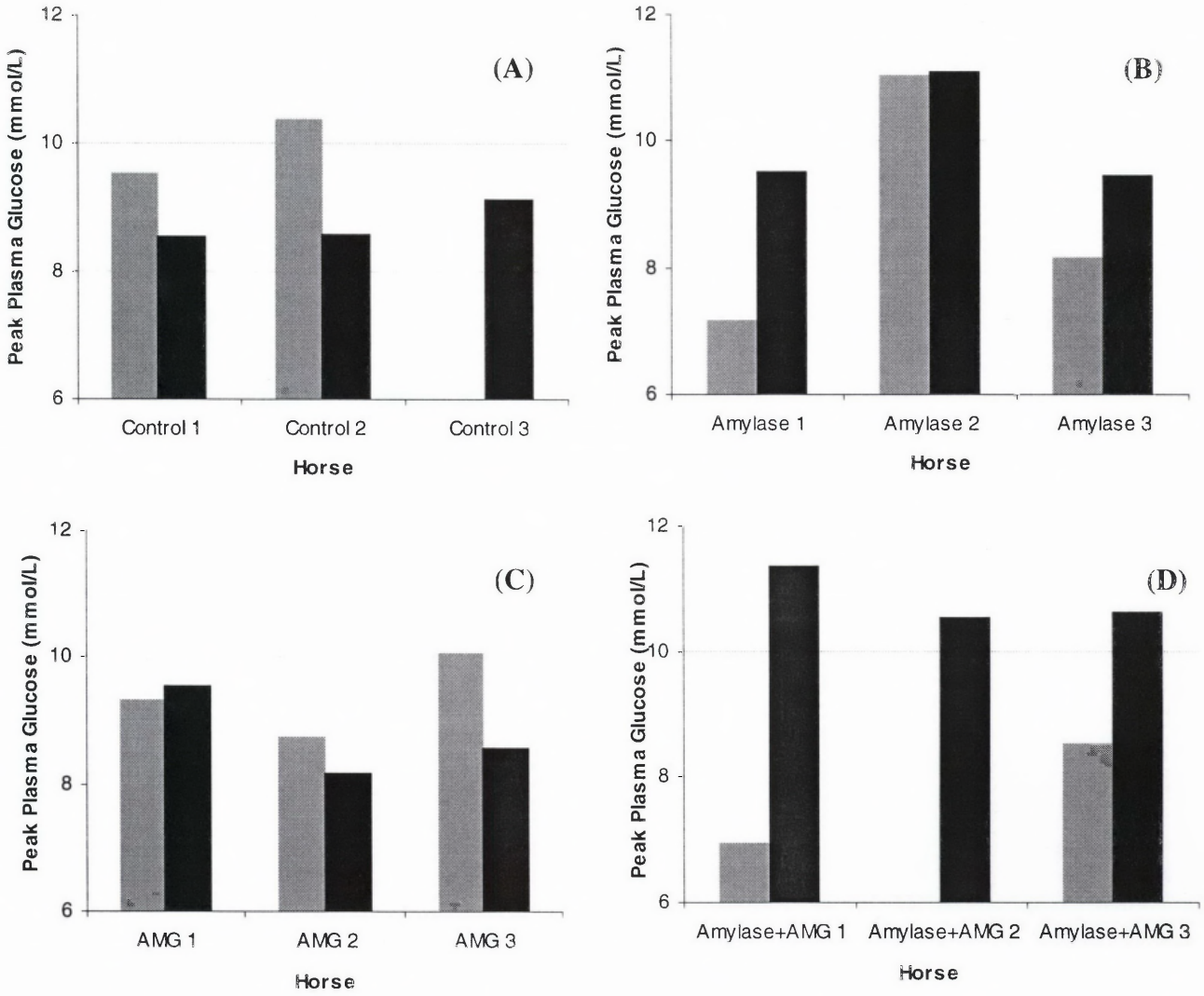

Figure 6.3: The peak plasma glucose responses of individual horses during the (■) enzyme trial on the (**A**) control diet, (**B**) α-amylase diet, (**C**) AMG diet, and (**D**) the α-amylase + AMG diet in comparison to their (▒) peak glucose response to the steam-rolled triticale diet with no addition of enzyme measured up to 12 days later during subsequent trials (corn and rice digestion trials). NB, Control horse 3 and Amylase + AMG horse 2 were not included in subsequent trials due to their tendency not to eat the test diets.

6.4 Discussion

Addition of α-amylase or a combination of α-amylase and AMG to steam-rolled triticale significantly ($P \leq 0.008$) elevated glycaemic responses above that initiated by the control diet, indicating an improvement in small intestinal starch digestion. These findings are in accordance with Meyer *et al.* (1993) who observed a 12% improvement in the pre-caecal digestion of cracked corn following the addition of powdered α-amylase to the diet. The improvement in starch digestion when α-amylase is added to the diet strengthens the theory that a deficiency of α-amylase in the equine small intestine limits small intestinal starch digestion in horses. The addition of α-amylase to the diet of horse 2 in the α-amylase group however, made no improvement to this horse's glycaemic response, perhaps indicating that this horse has naturally higher levels of endogenous α-amylase than horses 1 and 3 in the

same treatment group. This observation supports those of Kienzle *et al.* (1994) and Roberts (1974) who reported highly variable concentrations of small intestinal α-amylase between horses.

The addition of AMG to the control diet on its own appeared to make no improvement to the digestion of starch in the small intestine, with similar glycaemic response curves observed for horses given the control and AMG diets. This suggests that with normal levels of α-amylase, activities of AMG naturally present in the small intestine are adequate to break down the products of α-amylase digestion to glucose. Further addition of AMG did not improve starch digestion, presumably because in this situation the breakdown of starch to maltose, maltotriose and α-dextrins by α-amylase was the primary limiting step in the starch digestion process. This observation is in agreement with Kienzle *et al.* (1993) and Roberts *et al.* (1974), who suggested that horses possess naturally high levels of brush border glycanases, including AMG.

Average and peak glucose concentrations for the control diet increased from period 1 (day 5) to period 2 (day 8). As the horses had only been placed on grain 2.5 days prior to the first testing period, it is possible that this increase in glycaemic response to the control diet over time was due to a rise in the concentration of endogenous glycanases in the small intestine of the horses. Increases in endogenous glycanase activities, presumably stimulated by the addition of starch to the diet, would allow more extensive digestion of starch and thus an elevated glycaemic response. Similar increases in the production of starch digesting enzymes, stimulated by the addition of starch or sucrose substrates to the diet, have been observed in rats, horses, pigs, sheep and cattle (Clary *et al.*, 1969; Corring, 1977; Harmon, 1992; Johnson *et al.*, 1977; Kienzle *et al.*, 1994; Snook, 1971). Thus it is possible that the need for exogenous glycanases for the efficient digestion of starch in the small intestine of the horse will be reduced over time.

Although not significantly different, the α-amylase + AMG diet appeared to actuate a higher and more consistent response across both testing periods than α-amylase alone. Thus the addition of a combination of α-amylase and AMG may be desirable, as endogenous AMG appears to be insufficient to cope with the surplus maltose, maltotriose and α-dextrin substrate that may be produced through the addition of α-amylase to the diet, or through adaptation to grain based diets. Glycaemic response in horses given the α-amylase diet actually decreased from the first to the second sampling period. The addition of α-amylase in the second period made no improvement to the glycaemic response compared to the control (Figure 6.2). As indicated above, it is possible that this result is explained by the increasing endogenous quantities of α-amylase between the first and the second sampling periods, in conjunction with exogenous α-amylase, overwhelming the ability of the endogenous brush border glycanases to rapidly hydrolyse maltose, maltotriose and α-dextrin units to glucose, for absorption. High concentrations of these sugars in the small intestine may create a strong osmotic gradient in the gut, drawing water into the small

intestine. Consequently passage rate will be increased and apparent digestibility could decrease. This theory is supported by the fact that animals on the α-amylase diet had 2.75 times more faecal lactate than animals on the control diet (Table 6.4), perhaps as a consequence of large quantities of readily fermentable maltose, maltotriose and α-dextrin units reaching the hindgut.

It is not clear why brush border glycanase activity does not appear to adapt to increasing quantities of substrate. Studies in sheep and cattle show no increase in small intestinal brush border glycanase activity, even after prolonged adaptation to a high starch diet (Janes *et al.*, 1985; Russell *et al.*, 1981). In contrast, however, Kienzle *et al.* (1993) reported an increase in sucrase and maltase activity in the small intestine of ponies when placed on a maize or high sugar diet, compared to ponies on a hay diet. This experiment was conducted over a six week period, however, leaving the possibility that brush border enzymes may take longer to respond to dietary stimuli than pancreatic α-amylase, which can take as little as three days for an increase in activity to occur in monogastric animals (Corring, 1977).

An alternative explanation for the observed decrease in plasma glucose response between period one and period two for horses supplemented with α-amylase may be the effect of adaptation to a grain diet and thus glucose absorption and metabolism. Jacobs *et al.* (1982) reported that horses adapted for one week to a grain based diet, prior to being subjected to an oral glucose tolerance test, displayed lower glycaemic response curves than horses that were maintained on pasture prior to the test. These observations are supported by the findings of Argenzio *et al.* (1972) who report that ponies adapted to a grain diet for one month had lower plasma glucose concentrations following an intravenous glucose tolerance test compared to horses maintained on a diet of roughage alone. However, Garcia *et al.* (1986) found the opposite, with their horses adapted to a grain based diet displaying higher plasma glucose responses than horses adapted to a hay diet following an intravenous glucose tolerance test. The reduction in plasma glucose response following an oral glucose tolerance test in horses adapted to a grain diet may be due to greater removal of glucose by the liver or decreased absorption of glucose from the small intestine (Jacobs *et al.*, 1982). However, it is reported frequently in the literature that animals are able to rapidly up-regulate glucose absorption from the small intestine when α-linked carbohydrates are supplied in the diet (Cheeseman *et al.*, 1991; Diamond *et al.*, 1984; Ferraris *et al.*, 1992). Thus, it appears that increased removal of glucose by the liver is the more likely explanation. During this experiment, adaptation to grain for six days may therefore, have contributed to the reduced glycaemic response for horses supplemented with α-amylase.

6.5 CONCLUSIONS

A deficiency of α-amylase in the small intestine of the horse appears to limit starch digestion when a supply of digestible starch is first included in the diet. The addition of α-amylase or a combination of α-amylase and AMG to a digestible starch source elevated the glycaemic response above that observed for horses on the control diet. The elevation of the

glycaemic response suggests more extensive digestion of starch in the small intestine. Between horse variation was evident, supporting the theory developed in Chapter 5 that the glycaemic and insulin response variation observed between horses reflects their ability to digest starch pre-caecally. For horses with insufficient endogenous glycanases, the addition of exogenous glycanases to digestible starch in their diets may reduce the risk of hindgut starch fermentation, lactic acidosis and acidosis related disorders when consuming cereal grains.

The addition of AMG alone did not improve the digestion of starch, confirming previous reports that a deficiency of brush border glycanases is unlikely to be a major limiting factor for pre-caecal starch digestion. However, endogenous AMG appears to be insufficient to break down the products of α-amylase when exogenous α-amylase is added to the diet. Thus it is possible that the addition of α-amylase alone may cause the delivery of highly fermentable sugars to the hindgut and, in this case, the risk of hindgut acidosis will be increased. It is therefore recommended that the two exogenous enzymes be used together to improve the safety and efficiency of grain feeding for horses.

Having confirmed that the addition of exogenous glycanases to a digestible starch source improves the digestion of starch in the equine small intestine *in vivo*, the following chapter outlines an experiment undertaken which examines the concentration of α-amylase in the small intestine of two horses and the capacity of this endogenous equine α-amylase to digest various sources of starch *in vitro*.