7 THE CONCENTRATION OF EQUINE SMALL INTESTINAL α-AMYLASE AND ITS *IN VITRO* STARCH DIGESTION CAPACITY

The previous experiment confirmed the hypothesis that endogenous small intestinal α-amylase activity is not sufficient in horses to allow for extensive pre-caecal starch digestion, even when digestible starch is supplied in the diet. Although estimated concentrations of α-amylase in the equine small intestine have been previously reported (Kienzle, 1994; Roberts, 1974), the starch digesting capacity of equine α-amylase has never been examined. It was decided to conduct an experiment to address this omission.

The experiment aimed to:

(i) investigate and compare the activity of α-amylase and amyloglucosidase (AMG) in the small intestine of two horses;

(ii) examine, *in vitro,* the capacity of the endogenous α-amylase, present in equine jejunal supernatant, to digest various cereal grain starches;

(iii) determine if the stomach plays an important role in small intestinal starch digestion in horses; and

(iv) determine if the ranking of cereal grains in order of *in vitro* starch digestibility by the jejunal fluid more closely represents the ranking of grains in order of *in vivo* starch digestibility, determined using the glycaemic and insulin responses.

The hypotheses for this experiment were that:

1. the presence of both glycanase and protease enzymes in the equine jejunal supernatant would result in a more extensive degradation of cereal grain starch *in vitro* in comparison to that digested during the standard enzyme digestion assay of Bird *et al.* (1999) that utilises glycanase enzymes only;

2. that the pre-incubation of cereal grain starch in equine stomach fluid would increase the quantity of starch digested *in vitro* by equine α-amylase; and

3. the *in vitro* digestibility estimates for the various cereal grain starches, obtained through the digestion of the cereal grains in equine jejunal supernatant, with and without a pre-incubation in equine stomach fluid, would be closely related to the glycaemic and insulin response data presented in Chapter 5.

7.1 METHODS AND MATERIALS

Samples of small intestinal contents were collected from two horses following slaughter at a commercial abattoir. The concentration of α-amylase and AMG in the collected samples was measured. The jejunal digesta supernatant of horse A was then used as a source of α-

amylase for use in a modification of the *in vitro* starch digestion assay of Bird *et al.* (1999) to assess the ability of equine α-amylase to digest starch from various cereal grain sources.

Sample Collection

Two horses (1 thoroughbred gelding aged 9 and 1 mare, breeding unknown, aged >20) that were being sent to slaughter by their respective owners were used during this trial. The owners gave permission to collect gastrointestinal tract samples from these horses at the abattoirs. The thoroughbred gelding was previously owned by the University of New England and was Horse 2 during experiment 2 and Horse 2 in the Amylase treatment group during the experiment described in Chapter 6.

The horses had been fed a cereal grain diet for 7 days prior to slaughter, with each horse consuming 2.8 kg of oats and 2 kg of extruded rice in two meals/day. Lucerne hay was fed *ad libitum* as the only roughage component of the diet. On the evening prior to slaughter, horses were fed only lucerne hay. On the morning of slaughter, horses were transported to a commercial pet food abattoir, where they were fed 2 kg of lucerne chaff. On completion of eating, horses were slaughtered using a captive bolt gun and the gastrointestinal tract was immediately removed. Cotton ties were promptly placed at the junctions of the: stomach and duodenum; duodenum and jejunum; jejunum and ileum; and the ileum and caecum to segregate these sections.

Caecal contents were immediately removed and strained through cheesecloth into a plastic container with a hole at the top to allow gas to escape. Caecal fluid from both horses was pooled and maintained at 39°C in a water bath until return to the laboratory, where it was used for fermentation innoculum for the *in vitro* hindgut fermentation simulation assay (described in Section 5.3.1). The stomach from each horse was weighed full and then had the contents removed and strained through nylon gauze. Fluid from the stomach of each horse was individually bottled, labelled and placed on ice. The stomachs were then reweighed empty.

The small intestine of each horse was cut into the duodenal, jejunal and ileal sections. Each section was weighed individually, full, then the contents were emptied from each section into labelled plastic containers and each section was reweighed empty. All small intestinal digesta was immediately placed on ice. On return to the laboratory all digesta samples were centrifuged at 8000 *g* and the supernatant and pellet were stored separately at -18°C prior to experimental analysis.

Measurement and Analytical Procedure

α-Amylase concentration in the digesta supernatant was measured using the Megazyme Ceralpha Method (Megazyme International Ireland Ltd, Ireland) using the Amylase HR reagent (specific for α-amylase) and non-reducing-end blocked *p*-nitrophenyl maltoheptaoside (BPNPG7) as substrate. AMG concentration in the small intestinal digesta

supernatant was determined using the Megazyme assay for amyloglucosidase using *p*-nitrophenyl-β-maltoside (4 mM) and β-glucosidase (25 U/ml) as substrate.

A series of *in vitro* starch digestion assays, using the jejunal supernatant from horse A to supply α-amylase, were carried out. The method of Bird *et al.* (1999), which utilises 300 U of α-amylase, derived from *Bacillus licheniformis* and 20 U of AMG derived from *Aspergillus niger,* per grain sample, was modified for these assays. All grains used in the experiments described in Chapter 5 were included in the assays. Assay 1 and 2 involved the incubation of 100 mg of cereal grain in 5.3 ml of equine jejunal fluid (300 U α-amylase and 5.6 U AMG) and 14.4 U of AMG, derived from *Aspergillus niger* (Megazyme). The incubation periods were 15 min and 60 min for assays 1 and 2 respectively.

Assays 3 and 4 involved a 60 minute pre-incubation of 100 mg grain samples in 3 mL of stomach fluid collected from horse A (pH 3.7) to investigate the influence of the stomach on the digestion of starch in the small intestine. Following the 60 minute pre-incubation the pH was adjusted 6.9 with 50 μL of 5M NaOH and the assays were continued as described above for assays 1 and 2, with assays 3 and 4 having 15 and 60 minute incubation periods in jejunal supernatant, respectively.

Assay 5 involved a 60-minute pre-incubation in pH 3.7 HCl acid, after which time, pH was adjusted to 6.9 with NaOH and the assay was then continued as described for assay 1. Tubes with no grain sample were run as blanks for all assays.

7.2 RESULTS

Using weight as an indication of size, horse B had a larger pre-caecal gastrointestinal tract than horse A and also had more digesta resident within this section of the gastrointestinal tract (Table 7.1).

Table 7.1: The full and empty weights of the pre-caecal sections of the digestive tracts from horses A and B and the quantity of supernatant (mL) collected from each section.

		HORSE A	HORSE B
Stomach	**Full Wt (g)**	4025	9810
	Empty Wt (g)	996	1525
	Supernatant (mL)	411	381
Duodenum	**Full Wt (g)**	511	1103
	Empty Wt (g)	400	534
	Supernatant (mL)	7.5	181.1
Jejunum	**Full Wt (g)**	6875	13258
	Empty Wt (g)	3085	6939
	Supernatant (mL)	840	880
Ileum	**Full Wt (g)**	400	750
	Empty Wt (g)	339	530
	Supernatant (mL)	18	55

Table 7.2 shows the concentrations of α-amylase in the three segments of the equine small intestine. While the relatively crude digesta collection process, the centrifugation of digesta

to remove particulate matter and the freezing and thawing of the digesta supernatant prior to analysis may have affected measured α-amylase concentrations, horse A had higher concentrations of α-amylase/mL in all three segments of the small intestine than horse B. However, owing to a greater digesta pool size in the gastrointestinal tract of horse B, the total number of units of α-amylase did not differ between horses in the duodenum and ileum.

Table 7.2: The α-amylase concentration (IU/mL) and the total units of α-amylase in the digesta supernatant collected from the duodenum, jejunum and ileum of horses A and B.

	α-Amylase Concentration			
	HORSE A		HORSE B	
	IU/mL	Total Units (IU)	IU/mL	Total Units (IU)
Duodenum	166	1238	8	1450
Jejunum	57	47 870	28	24 630
Ileum	80	1400	22	1210

Similarly, AMG concentrations in the small intestine of horses A and B are shown in Table 7.3 and again, horse A tended to have higher concentrations of AMG/mL in all sections of the small intestine. However, horse B had a greater number of total units of AMG than horse A in the duodenum and ileum due to a greater digesta pool size.

Table 7.3: The amyloglucosidase (AMG) concentration (IU/mL) and the total digesta supernatant units of AMG in the duodenum, jejunum and ileum of horses A and B. Total units were calculated by multiplying U/mL by the total supernatant volume for each segment of the small intestine.

	AMG Concentration			
	HORSE A		HORSE B	
	IU/mL	Total Units (IU)	IU/mL	Total Units (IU)
Duodenum	1.68	13	0.94	170
Jejunum	1.00	840	0.60	528
Ileum	1.71	30	0.70	38

The percent of starch digested during incubations in jejunal fluid was typically lower than that digested during the standard *in vitro* assay for both the 15 and 60 minute incubation times. The 60 minute pre-incubation in stomach fluid increased the percentage of starch digested in jejunal fluid during the 15 and 60 minute incubations, however, with the exception of oats for both incubation times and unprocessed corn 1 and white rice for the 60 minute jejunal fluid incubation, the quantity of starch digested was still greater during the standard assay (Table 7.4). Pre-incubation in pH 3.7 HCl increased the quantity of starch digested during the 15 minute incubation in jejunal fluid, however, the increases were not to the same magnitude as those observed following pre-incubation in stomach fluid (Table 7.4).

Table 7.4: The % starch digested from the specified grains during the standard assay (Bird *et al.*, 1999), employing bacterial derived enzymes with 15 and 60 minute incubation periods and the % starch digested during the jejunal fluid assays employing equine α-amylase with 15 and 60 minute incubation periods, with and without a 60 min pre-incubation in stomach fluid (pre-inc) or pH 3.7 hydrochloric acid (HCl).

	***In vitro* starch digestion (% starch digested in specified time)**						
	Standard assay (15 min)	**Jejunal fluid (15 min)**	**Pre-inc in stomach - Jejunal fluid (15 min)**	**Pre-inc in pH 3.7 HCl - Jejunal fluid (15 min)**	**Standard assay (60 min)**	**Jejunal fluid (60 min)**	**Pre-inc in stomach – Jejunal fluid (60 min)**
Cracked barley	***25.7***	12.7	22.7	17.2	***50.3***	30.7	49.0
Expanded barley	***39.5***	19.2	28.6	22.9	***62.1***	39.5	58.2
Cracked triticale	***41.9***	16.7	34.1	20.5	***73.8***	37.1	71.0
Expanded triticale	***50.1***	21.3	37.3	25.5	***80.7***	45.0	75.0
Oats	***40.6***	27.2	45.2	-	***69.5***	61.6	81.3
Unprocessed corn 1	***15.8***	8.2	12.2	9.4	***30.6***	21.6	34.8
Extruded corn	***73.9***	40.4	53.8	47.1	***84.0***	63.4	73.2
Unprocessed corn 2	***12.3***	5.8	10.4	7.8	***24.4***	15.2	21.7
Micronised corn	***34.0***	13.2	18.9	14.8	***49.4***	28.8	38.5
Unprocessed white rice	***18.7***	10.8	16.2	11.6	***32.8***	29.5	38.7
Extruded rice	***80.2***	44.9	52.7	46.5	***86.2***	58.0	70.3
SRT	***60.4***	25.1	45.2	33.6	***82.3***	52.5	76.7

The glycaemic response and the *in vitro* starch digestion assay using equine jejunal fluid (15-minute incubation) were weakly related (Figure 7.1a) with the 60-minute pre-incubation in stomach fluid further weakening the relationship between the assays (Figure 7.1b).

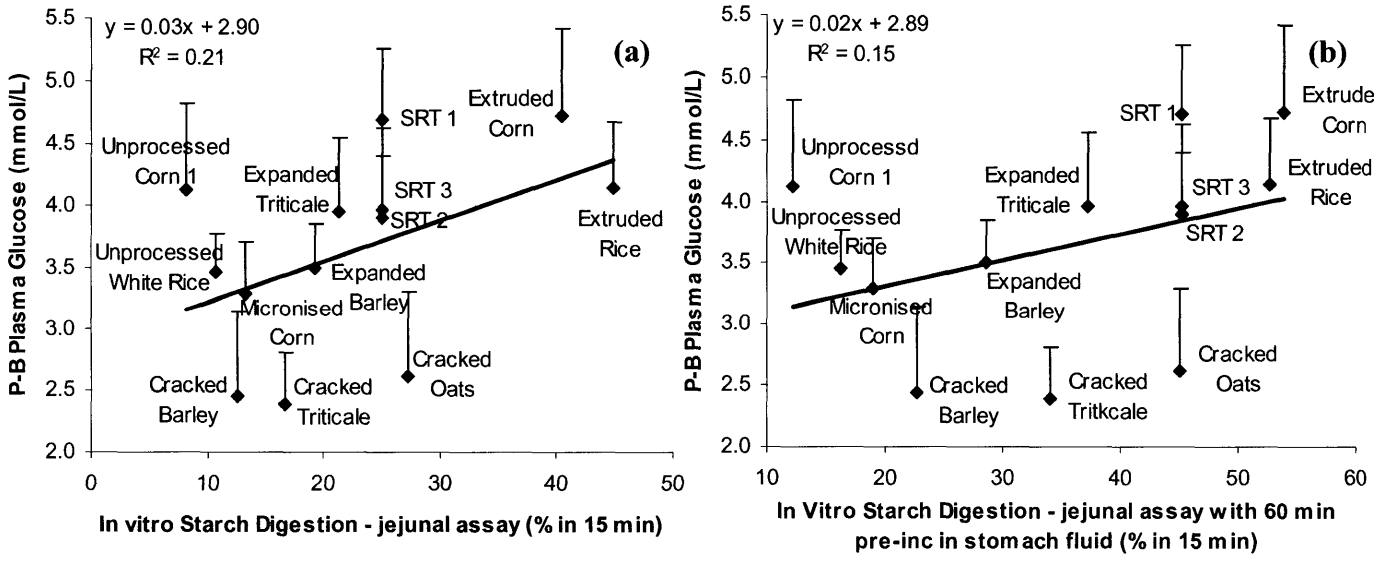

Figure 7.1: The relationship between the glycaemic response (peak – basal glucose concentration, P-B) and the jejunal digesta assay with **(a)** 15 min incubation period and **(b)** with a 60 min pre-incubation in stomach fluid followed by a 15 min incubation in jejunal fluid.

The jejunal fluid assays with both 15 and 60-minute incubations were strongly and positively related to their respective standard *in vitro* starch digestion assays (R^2= 0.91 and 0.79 respectively, Figures 7.2a and 7.2c). Pre-incubation in stomach fluid, while it increased the quantity of starch digested during the 15-minute incubation in jejunal fluid, did not change the relationship with the standard assay (Figure 7.2b). The stomach fluid pre-incubation did, however improve the relationship observed between the jejunal fluid assay with a 60-minute incubation and the standard assay (Figure 7.2d).

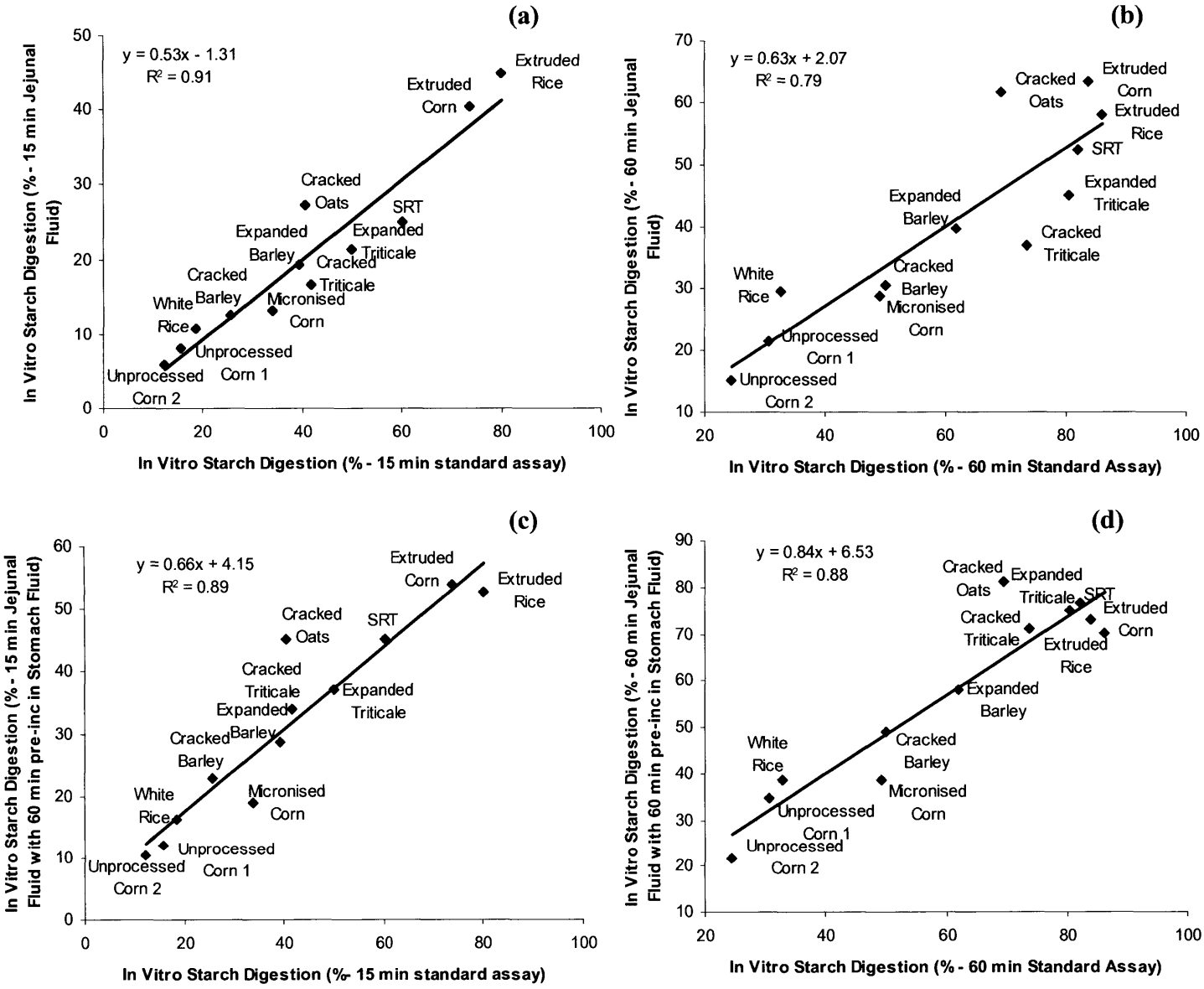

Figure 7.2: The relationship between the: **(a)** standard *in vitro* enzyme assay with a 15 min incubation period and the jejunal digesta assay with a 15 min incubation; **(b)** standard *in vitro* enzyme assay with a 60 min incubation period and the jejunal digesta assays with a 60 min incubation period; **(c)** standard *in vitro* enzyme assay with a 15 min incubation period and the jejunal digesta assay with a 60 min pre-incubation in stomach fluid followed by a 15 min incubation in jejunal fluid; and **(d)** standard *in vitro* enzyme assay with a 60 min incubation period and the jejunal digesta assays a 60 min pre-incubation in stomach fluid followed by a 60 min incubation in jejunal fluid.

7.3 DISCUSSION

The α-amylase concentrations measured in these two horses, if calculated on a units/g wet mucosa basis, are comparable to those reported by Kienzle *et al.* (1994). Also in support of earlier observations (Kienzle *et al.*, 1994; Roberts, 1974), the concentration of enzymes appears to be variable between these horses, with Horse A having a higher concentration of α-amylase, on a units/mL basis, in all three segments of the small intestine in comparison to Horse B. This observation is supported by data collected during the experiment described in Chapter 6 (Horse A was Horse 2 in the Amylase diet group) when the addition of exogenous α-amylase to this horse's diet resulted in no increase to an already high glycaemic response. This lack of response to enzyme supplementation can almost certainly be attributed to the high concentrations of endogenous α-amylase present within Horse A's small intestine.

Despite reports from Kienzle *et al.* (1993) and Roberts *et al.* (1974) indicating that small intestinal brush border glycanase activity in the equine small intestine is high and comparable to other monogastrics and the observation that the addition of exogenous AMG to equine cereal grain diets makes no improvement to small intestinal starch digestion (Chapter 6), the AMG activity in the small intestine of these horses appears to be low. However, brush border glycanases, such as AMG, are attached to the intestinal brush border by a short terminal hydrophobic section of their protein chain (Gray, 1992) and thus it is likely that a majority of AMG remained attached to the small intestinal brush border. The pattern of distribution of AMG throughout the small intestine was, however, consistent with the pattern of distribution of α-glucosidases reported by Roberts *et al.* (1974).

It was hypothesised that due to the greater array of protease and glycanase enzymes assumed to be present in the equine jejunal fluid, *in vitro* starch digestion within jejunal fluid would be higher than that which was observed in the standard *in vitro* starch digestion assay employing only *Bacillus licheniformis* α-amylase and *Aspergillus niger* derived AMG. However, the extent of starch digestion in jejunal fluid was up to 57% lower than that achieved by the standard starch digestion assay, suggesting that perhaps the substrate specificity or activity of the equine α-amylase is inferior to that of the *B. licheniformis* α-amylase. Such variation between amylases in ability to degrade starch has been previously reported by Anindyawati *et al.* (1998) who found that, of the three forms of α-amylase produced by the fungi *Aspergillus awamori* KT-11, only two had the ability to degrade raw corn starch and of these two, one was more effective than the other. The substrate specificity or enzyme activity of specific α-amylases is thought to be related to the number of sub-sites (an area within an active site capable of interacting with one glucose molecule) at each of the enzymes active sites and the affinity of each of these sub-sites for a glucose molecule (MacGregor, 1993; MacGregor *et al.*, 2001). Interestingly, equine α-amylase appeared to have greater substrate specificity for oat starch (Table 7.4), which may possibly explain the superior pre-caecal digestibility and thus 'safety' of oats for horses (Chapter 5).

In order to confirm the trends observed during this study, the kinetics of equine α-amylase must be studied. Determination of the Michaelis-Menton constant (K_m) and maximal velocity (V_{max}) of pancreatic derived equine α-amylase would give a clear indication of the enzymes activity and substrate affinity. Measurement of these parameters would also allow objective comparisons of the activity of equine α-amylase to the activity of other mammalian and bacterial α-amylases.

A 60 minute pre-incubation in stomach fluid (pH of 3.7) improved the extent of *in vitro* starch digestion when cereal grains were incubated in jejunal fluid, by between 15% and >100%. This draws attention to the importance of the stomach during starch digestion in the equine. With pre-incubation in pH 3.7 HCl improving *in vitro* starch digestion, but not to the extent achieved by stomach fluid, it is likely that the observed positive effect of stomach fluid on *in vitro* starch digestion is due to a combination of the low pH of the fluid and the protease activity within the stomach fluid (Argenzio, 1993a; Fox, 1991). These affect the macro and micro-structures of the cereal grains, removing cell wall and protein matrix barriers from around the starch, making access for amylolytic enzymes easier.

The standard *in vitro* starch digestion assay and the jejunal fluid assays were strongly and positively related, with both assays ranking a majority of the grains in the same order of digestibility. The pre-incubation of the grains in stomach fluid did not largely alter the assays relationships with one another. Thus, it appears that the standard *in vitro* assay may be used as an accurate tool for ranking grains in order of small intestinal starch digestion for equines.

The relationship between the jejunal assay and the glycaemic response was, however poor. with the glycaemic response appearing to almost certainly be overestimating the *in vivo* digestibility of unprocessed corn and white rice and underestimating the *in vivo* digestibility of oats and cracked triticale, again indicating the limited usefulness of the glycaemic response for determining and ranking cereal grain digestibility in the small intestine of equines. The precise reasons for the apparent overestimation/underestimation of the digestibility of these grains remains unclear (Chapter 5), however, given the relatively unpredictable nature of the glycaemic response and the strong relationship which has been observed between the *in vitro* assay employing equine glycanases and the *in vitro* assay employing bacterial glycanases, more confidence must be placed on the standard *in vitro* assay of Bird *et al.* (1999) with a 15-minute incubation period, as a reliable and repeatable tool for ranking grains in order of small intestinal starch digestion in horses.

7.4 CONCLUSIONS

The horses' seemingly limited ability to digest cereal grain starch in the small intestine may be partly attributed to a low concentration of α-amylase in the pre-caecal section of the equine gastrointestinal tract. However, it also appears that the activity or substrate specificity of equine α-amylase is inferior to that of α-amylase produced by *Bacillus*

licheniformis. It is possible then that poor substrate specificity is also contributing to the horses' limited ability to digest starch pre-caecally.

Despite these differences between bacterial and equine derived α-amylase and the influence of the stomach on the digestion of starch in the small intestine, the standard *in vitro* starch digestion assay, employing enzymes of bacterial origin, appears to be capable of ranking grains in the same order of *in vitro* digestibility as the same assay employing equine derived α-amylase. Thus the standard *in vitro* assay with a 15-minute incubation period may be used as a reliable and repeatable tool for ranking cereal grains in order of starch digestibility in the small intestine of horses.

8 GENERAL DISCUSSION

There is clear evidence that serious problems in equine health, feed conversion efficiency and behaviour can result from grain feeding and hindgut starch fermentation. The development of an energy dense, yet 'safe' diet for performance horses is thus a major priority in the equine industry, where a wide range of cereal grains and grain based diets are currently used without a sound understanding of the relative risks involved. The present study found that thoroughbred trainers' still use non-science based methods to guide the feeding practices and choice of grains for their horses. Evidence was found that these feeding practices can be inefficient and are potentially dangerous in that they can result in the fermentation of starch in the caecum and colon. Qualitative estimation of the starch digestibility of different grains and examination of the factors limiting a horse's capacity to digest starch, using non-invasive methodologies during this study, provide an improved basis with which grain selection and feeding practices for horses may be guided.

The grain species studied varied considerably in estimated small intestinal starch digestion. Oats and triticale appeared to contain the most digestible starch, in an unprocessed form, while unprocessed corn contained starch that was least susceptible to degradation by amylolytic enzymes. The grain processing methods investigated improved the small intestinal digestion of cereal grain starch, with extrusion appearing to be the most effective method for maximising pre-caecal starch digestion. However, grain processing also increased the rate at which starch fermented in equine caecal fluid. This trend indicates that processed cereal grains potentially have more capacity to induce hindgut lactic acidosis than unprocessed grains, if fed in a manner that allows substantial quantities of starch to reach the hindgut undigested, for example: if fed to hasty eaters that do not chew their food adequately or; if fed in meal sizes that exceed the small intestines capacity for starch digestion.

Irrespective of grain processing and the small intestinal digestibility of cereal grain starch, horses varied considerably in their capacity to digest starch in the pre-caecal region of their gastrointestinal tract. This between-horse variation suggests that physical and physiological attributes of individual horses are placing limitations on the extent of starch digestion that can occur in the small intestinal. The addition of exogenous α-amylase (from *Bacillus licheniformis*) to equine diets containing a digestible starch source, improved the digestion of starch in the small intestine. It was subsequently observed that equine α-amylase has a lower substrate specificity or activity than α-amylase derived from *Bacillus licheniformis.* Thus, providing that a digestible starch source is fed, it appears that the major factor, explaining differences between horses and limiting pre-caecal starch digestion, is a low concentration and/or poor substrate specificity/activity of the α-amylase found in the equine small intestine.

Thus, in support of the general hypothesis presented in the introduction to this thesis, it appears that both, the attributes of cereal grains, which govern starch digestibility and the capacity of individual horses to digest starch, will determine how much cereal grain starch may be safely fed, before problems with hindgut starch fermentation and acid accumulation will be experienced. The feeding of cereal grains to horses, therefore, must become a precisely balanced science, with the capacity of an individual horse to digest starch being matched to the digestibility and quantity of starch being fed. Such a precise approach to cereal grain feeding has not previously been possible as there have been no definitive and reliable methods for:

(i) determining a horses capacity to digest starch in the small intestine; and

(ii) assessing grains on a starch digestibility basis.

The glycaemic response and *in vitro* assay used during this study may provide these tools for use in the industry.

The ability of the glycaemic response to consistently rank horses in order of response to a grain diet make it a promising tool for assessing a horses capacity for small intestinal starch digestion. Faecal parameters such as pH and organic acid concentrations may also be used as support for the glycaemic response, with a low glycaemic response, low faecal pH and high faecal organic acid concentrations indicating a limited ability to digest starch pre-caecally.

The ability of the standard *in vitro* assay with a 15 minute incubation period (Chapter 7) to rank grains in the same order of *in vitro* starch digestion as the *in vitro* assay using the endogenous enzymes present in equine jejunal fluid, suggests that this *in vitro* assay may be a reliable and repeatable tool, capable of ranking grains in order of pre-caecal starch digestibility for horses, despite the obvious differences in substrate specificity/activity between bacterial and equine α-amylases.

With these tools potentially available for use in industry and with evidence presented in Chapter 3 showing that hindgut starch fermentation is a problem within the Australian thoroughbred industry, thoroughbred trainers should be encouraged to take a more scientific approach toward the grain feeding of their horses. This would firstly involve the selection of grains with superior starch digestibility characteristics using the standard *in vitro* starch digestion assay with a 15-minute incubation period.

Following the selection of grains containing the most digestible starch, all horses should be then be characterised on the basis of their ability to digest starch using the glycaemic response and faecal parameters such as pH and organic acid concentrations. Once the animals are classified on this basis, their diets may be formulated individually, with the animals displaying a seemingly limited ability to digest starch perhaps being fed in smaller meals with α-amylase

and AMG being used to overcome an endogenous enzyme deficiency, which is likely to be the major factor limiting starch digestion in the small intestine of these animals. If the glycaemic response, faecal parameters and the *in vitro* assay utilised during this study can be used effectively, to precisely formulate diets for individual horses, hindgut starch fermentation and the negative consequences associated with it may be largely avoided, making grain feeding to horses a safer and more efficient practice than it has been in the past.

It is generally recognised that obtaining, financing and managing large numbers of horses in an experimental situation is challenging, making the collection of meaningful data on which you can conduct statistical comparisons difficult. Where possible, a latin square approach was used during the studies presented in this thesis to counteract these effects. In studies where time and funding was limited, a factorial design was used with as large a number of replicates as could be reasonably funded and managed within animal welfare guidelines, in an attempt to overcome many of the problems associated with small numbers of replicates. The generally small number of replicates used during the studies does however raise some concerns with respect to how representative the animals used were to the broader equine population. For this reason, further research needs to be conducted to confirm the trends observed in this thesis.

Future research should be directed toward the validation of the glycaemic response as a non-invasive method for assessing a horse's capacity to digest starch. A broader population and larger sample of horses needs to be investigated and following the initial glycaemic response testing which should utilise a standard, commercially available and digestible diet (such as the extruded rice used during this study), meal size should be increased to a level high enough to cause measurable changes to the hindgut fermentation process in horses with a limited capacity to digest starch. This will allow the validation of glycaemic response data with faecal parameters such as pH and lactic acid concentrations and may also potentially allow the glycaemic response, which is a relatively labour intensive measure that needs professional intervention, to be replaced by faecal parameters that are non-invasive and may, in part, be measured by the trainers or horse owners themselves. The addition of α-amylase and AMG to the same standard diet should also be used to determine if a low glycaemic response to a digestible cereal grain diet is due primarily to an endogenous enzyme deficiency or if there are several factors that affect an individual horses glycaemic response.

Further research should also be directed toward the apparent deficiency of α-amylase in the equine small intestine to confirm previous research and to allow an estimation of what percentage of the equine population have low concentrations of small intestinal α-amylase. The development of a rapid method for non-invasively assessing α-amylase concentrations in a specific horse's small intestine, perhaps by characterising a 'marker' for α-amylase that may be

measured in the faeces should also be considered. The poor substrate specificity of equine α-amylase needs further investigation to confirm the preliminary observations made in this thesis. Dietary supplementation with exogenous enzymes must be investigated using longer adaptation periods, with a range of grains at various feeding levels, to determine if the effects observed during this study are maintained: over longer feeding periods; across a range of grain species and digestibilities; and at higher feeding levels, before exogenous enzyme supplementation may be applied at a commercial level. Rates of inclusion and commercial application of exogenous enzymes to equine diets also needs further study.

8.1 CONCLUSIONS

If cereal grain selection for horses within the Australian thoroughbred industry is based on starch digestibility attributes, thoroughbred horses are characterised by their ability to digest starch and fed accordingly and exogenous enzymes are used to correct small intestinal enzyme deficiencies, many of the problems presently experienced with hindgut starch fermentation, may be eliminated. A reduction in the quantity of starch being fermented in the equine hindgut will improve feed conversion efficiency for intensively fed equines, which should theoretically reduce the quantity of grain that needs to be fed. Likewise, behavioural abnormalities and diseases related to hindgut starch fermentation and acid accumulation should be reduced, improving animal welfare. While feeding a safe and digestible diet of cereal grains will not necessarily make a horse perform at a level higher than it is capable, it will remove any limiting factors associated with hindgut starch fermentation and allow them to perform to their potential.