3 Current Grain Feeding Practices in the Australian Thoroughbred Industry

A survey of thoroughbred trainers in several regions of NSW was conducted in order to better define and understand current grain feeding practices, the range of cereal grains fed and the risks involved with grain feeding in the Australian Thoroughbred Industry. That there is potential of serious risk when feeding cereal grains to horses, has been demonstrated in earlier chapters and the goal was to determine if thoroughbred trainers undertake feeding practices that minimise this risk, and whether certain feeding practices appeared to be safer than others.

Previous surveys of feeding practices in the Australian thoroughbred industry have been conducted by Bourke in 1968 and by Southwood *et al.* in 1993. Bourke (1968), in a survey of 45 Victorian thoroughbred trainers, ascertained that oats was the most widely used cereal grain and was commonly fed whole, or occasionally rolled. Oats was partly replaced in some diets, by crushed corn, sorghum, linseed or barley. He noted that thoroughbreds were fed 14 to 20 quarts of a concentrate mix, with a ratio of grain to chaff of approximately 60: 40, per day, which equates to 5.7 kg of grain/horse/day and 1.5 kg chaff/horse/day. Some trainers also fed an additional 0.9 – 1.8 kg of protein rich concentrate. Horses in these Victorian stables were commonly fed up to four times per day, with meals offered after work in the morning, at noon, in the late afternoon and occasionally a small feed was given in the evening. In addition, most horses were provided with around 4.5 kg of hay/day. Thoroughbred trainers considered that most diets would be deficient in calcium, salt, iron and cobalt, and thus these minerals were commonly added to the diet.

In 1993, Southwood *et al.* conducted a survey of 25 thoroughbred trainers in metropolitan and country areas of NSW and estimated that trainers were feeding an average of 7.8 kg of grain concentrate/horse/day. This represents an increase of around 2 kg of grain/horse/day compared to Bourke's survey results in 1968. Again, oats was the most commonly fed grain, with 84% of surveyed trainers feeding an average 3.6 kg of oats/day. Oats was the 'preferred' grain as it was perceived to be 'traditional, cheaper, higher in protein and more palatable' than other grains. Despite the obvious popularity of oats, corn appeared to have increased in popularity amongst trainers, with 84% feeding an average 1.7 kg of corn/day. Twelve percent of trainers fed barley, however it was fed in small quantities, with 0.1 – 0.2 kg being fed/horse/day. In 1993 it appeared that it was becoming common for trainers to feed some form of commercial premixed diet, with 44% of trainers using these feeds. However, only 4% of trainers fed commercial premixed feeds as the sole concentrate component of the diet. Trainers also fed up to 2.55 kg of protein-rich legume and oilseed supplements, with sunflower seeds and faba beans being most popular.

Southwood *et al.* (1993) found that chaff was still a common ingredient in thoroughbred diets, with 88% of trainers feeding an average of 0.48 of kg lucerne chaff/horse/day and 64% of trainers feeding an average of 0.56 kg cereal chaff/horse/day. In contrast to the feeding practices reported by Bourke (1968), Southwood *et al.* (1993) found that trainers fed horses only twice/day, with time limitations cited as the main reason for less frequent feeding of the horses.

Even though the surveys of Bourke and Southwood *et al.* were extensive, their major focus was not to study the type and quantity of cereal grains fed and the likely consequences for starch digestion and fermentation. Thus the present survey was conducted to provide detailed and current information on grain feeding practices within the industry and the likely impact of these practices on small intestinal starch digestion and hindgut starch fermentation for thoroughbred horses.

A series of questions was developed to investigate: the types or species of cereal grains being fed; the quantities of grains being fed to thoroughbred horses and; the methods by which grains were processed and/or prepared prior to feeding. Grain and faecal samples were also collected to allow us to determine if incomplete small intestinal starch digestion and hindgut starch fermentation were problems being experienced.

3.1 Grain Feeding in the NSW Thoroughbred Industry 2001/2002 Survey

Methods

North-eastern NSW was selected as the target geographical area for the survey. The area stretched north to Grafton, south to Sydney and east to Tamworth.

This area was chosen as it incorporated sub-tropical and temperate climatic conditions and country (any area outside of the NSW central coast or Sydney), provincial (NSW central coast) and metropolitan (Sydney) thoroughbred training centres, with both small (<10 horses) and large (>10 horses) training establishments. It was anticipated that these areas would give a representative sample of trainers within the thoroughbred industry. The sample consisted of approximately 12% of NSW thoroughbred trainers and 3% of thoroughbred trainers Australia wide. The University of New England Human Ethics Committee approved the protocol for the survey.

The survey questionnaire contained 18 questions (Appendix 1), that covered the general areas of: type and operation of the training establishment; factors that influenced choice and level of feeding of grains; which grains were being fed; what quantities of grains were being fed; how the grains were processed prior to being fed; how grains were fed to horses; and what were the costs involved?

Names, phone numbers and addresses of potential participants were taken from the trainers list in the NSW Racing Magazine. Each potential participant received by mail, a copy of the questionnaire, a participant information sheet and a cover letter (Appendix 1). Following receipt of the letter, each potential participant was contacted by phone to determine his or her willingness to participate in the survey. If the trainer was unwilling to participate the trainer's name and details were removed from the participant list. If the trainer was willing to take part, a suitable time and place to meet was arranged. The survey visit was used to complete the survey form, weigh the grain and chaff portions of the diet and for the random collection of grain(s) and horse faecal samples.

Survey information was entered into excel spreadsheets for later statistical analysis. Statistical analysis of the following information was undertaken using binomial data and analysed with generalised linear models with binomial link functions to determine effects of region (country; provincial; metropolitan) and establishment size (less than 10; greater than or equal to 10):

- whether a commercial premix diet was fed
- if chaff was included with the grain
- if diets were formulated for each individual horse
- whether grain was fed with or without hay and
- whether trainers considered grain costs to be a major expense;

Grains data were classified into cereal and legume/oilseed groups. Cereal grain data were transformed using a natural log function to obtain a normal (gaussian) distribution. Legume/oilseed data were not transformed. A two-way ANOVA was used to determine any significant effects of region, size of establishment and grain type (when appropriate) on the following sets of data:

- quantity of cereal and legume/oilseed grains fed
- number of times horses were fed hay
- number of times horses were fed grain
- weight of chaff fed with the grain
- total grain quantity fed/horse/day
- number of days adaptation to a grain diet the horses were allowed; and
- the cost of grain feeding/horse/week

Significant differences between means were determined using a Fisher's least square difference model. All confidence intervals were adjusted according to the number of comparisons being made using Bonferroni's adjustment method. Statistical analysis was carried out using the S-plus for Windows statistical package (Insightful Corporation, Seattle, WA. USA). All means are presented as actual values and not least square means.

Grain samples collected during the survey, were analysed for total starch content (McCleary *et al.*, 1997), *in vitro* enzymatic starch digestibility (Bird *et al.*, 1999), dry matter and nitrogen content. Forty nine faecal samples consisting of approximately 50 grams of faeces, collected randomly from the stables of participating trainers, were analysed for pH, dry matter, starch content, nitrogen content, volatile fatty acid and lactic acid concentrations. Methods for faecal pH, dry matter, starch and nitrogen contents are described in Section 4.2. Methods for faecal VFA and lactic acid concentrations are described in Section 5.3.1. These analyses were conducted to ascertain whether there was any relationship between grain characteristics and faecal parameters, in order to establish which grains held the greatest risk of hindgut starch fermentation.

3.2 RESULTS

3.2.1 General

Seventy-two trainers took part in the survey. This was 25% of those initially contacted. It is possible that the trainers who agreed to take part in the survey were more interested and further advanced in their methods used to feed their horses than the non-responders. However, it was not possible to take into account the effect of responders versus non-responders when collating and analysing the results.

The minimum number of horses in training per trainer surveyed was one while the maximum number was 35. On average trainers had 9.6 ± 0.99 horses in training and trainers in provincial and metropolitan regions had on average an extra 1.4 horses in training compared to trainers in country regions. A total of 690 horses were covered by the survey, which is approximately 2.2% of horses in training Australia wide (ARB, 2001). Of the horses covered by the survey, 8.4% of horses in training were colts/stallions, 43.3% were fillies/mares and the remaining 48.3% were geldings.

3.2.2 Faecal Analysis

Mean faecal pH was 6.5, however, faecal pHs as low as 5.5 and as high as 7.9 were observed. Mean faecal starch content was 1.2% DM, but in some horses faecal starch was over 7% DM. Faecal VFA concentrations were variable, with a range of 10 to over 60 mmol/L. The mean acetate to propionate ratio was 3.7, however the minimum ratio observed was 2.3. Faecal lactate concentrations were also variable, ranging from nearly 0 mmol/L to over 3 mmol/L (Table 3.1).

Table 3.1: The mean, standard error (SE), minimum and maximum results for the faecal parameters pH, starch, nitrogen, acetate, propionate, butyrate, acetate: propionate ratio, total volatile fatty acids (VFA), L-lactate, D-lactate, total lactate and total faecal acid concentrations (n=49).

Faecal Parameter	Mean	SE	Minimum	Maximum
pH	6.5	0.07	5.52	7.85
Dry Matter (%)	24.3	0.47	15.65	33.63
Starch (% DM)	1.2	0.18	0.28	7.35
Nitrogen (% DM)	1.6	0.06	0.56	2.88
Acetate (mmol/L)	20.5	1.29	6.24	40.99
Propionate (mmol/L)	5.6	0.34	1.64	11.03
Butyrate (mmol/L)	3.0	0.32	0.36	9.33
Acetate: propionate ratio	3.7	0.11	2.30	5.90
Total VFA (mmol/L)	30.9	2.01	10.23	63.37
L-Lactate (mmol/L)	0.33	0.06	0.02	1.82
D-Lactate (mmol/L)	0.36	0.04	0.03	1.37
Total lactate (mmol/L)	0.69	0.09	0.05	3.19
Total faecal acid (mmol/L)	31.6	2.06	10.49	65.85

There was a strong negative relationship between faecal propionate concentration and faecal pH (R^2=0.76; Figure 3.1), with high propionate concentrations at low faecal pH.

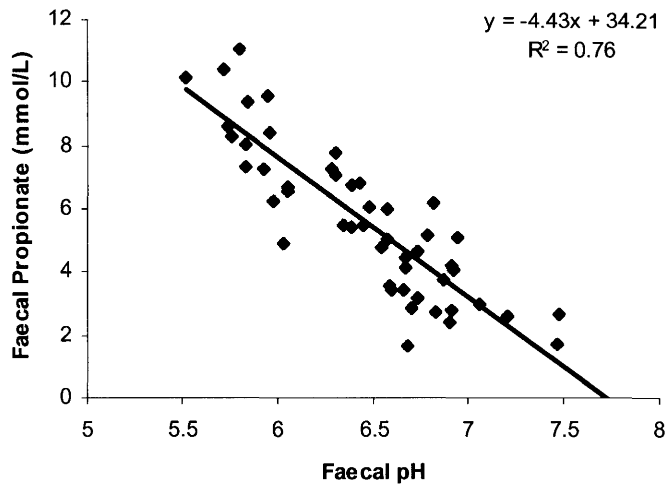

Figure 3.1: The negative relationship between faecal propionate concentration and faecal pH.

Faecal pH also held weaker, negative relationships to faecal starch and faecal lactate concentrations (R^2=0.17 and R^2=0.13 respectively).

3.2.3 Basis for Grain Selection by Trainers

A majority of trainers (77.8%) indicated that the perceived "quality" of grain (based primarily on subjective parameters independent of nutritional quality such as colour, smell and cleanliness) was the most important factor that influenced their choice, when buying grain for thoroughbreds. The remaining 22.2% made decisions based on tradition, nutritional advice, price, other reasons, availability and research (Table 3.2).

Table 3.2: The percentage of trainers who indicated that quality, tradition, nutritional advice, price, availability, research or other was the most important factor influencing their choice of grain when buying grains for their thoroughbreds.

	Indicated as the most important factor (% of trainers surveyed)
Quality	77.8
Tradition	6.9
Nutritional Advice	5.6
Price	2.8
Other	2.8
Availability	1.4
Research	1.4
Non-response	1.3

3.2.4 Grains Fed in Industry

Oats, corn and commercial premixed diets were the grains most commonly fed to the thoroughbred horses, with 80.6%, 73.6% and 73.6% of trainers feeding these grains respectively (Table 3.3). Barley was fed by one third of thoroughbred trainers surveyed.

Wheat, rice and triticale were not included in any of the diets formulated by the trainers surveyed, however there may have been wheat, rice and triticale in some of the commercial premixed diets.

Table 3.3: The percentage of trainers who fed each individual grain.

GRAIN	Trainers feeding grain (%)
Oats	80.6
Corn	73.6
Commercial premixed diet	73.6
Barley	33.3
Sunflower	27.8
Faba Beans	13.9
Soybean	12.5
Lupins	9.7
Chick Peas	2.8
Other	2.8

3.2.5 Quantities of Grain Fed to Horses

The age and sex of thoroughbred racehorses were not considered to be major factors determining the quantity of grain to be fed to individual horses by any of the trainers (Table 3.4). Fifty eight percent of trainers considered that the current workload a horse was under was the most important factor for determining how much grain it should be fed, while 26% of trainers perceived that a horse's appetite was the most important factor for determining how much grain a horse should be fed. A further 8.3% and 4.2% of trainers respectively, thought that the animals' temperament and body weight were the most important factors to be considered when deciding how much grain to feed to a horse. Less than 2% of trainers fed a standard diet that remained unchanged from year-to-year.

Table 3.4: The percentage of trainers stating which factor was the most important in deciding the quantity of grain to be fed to individual horses.

	Indicated as the most important factor (%)
Workload	58.3
Appetite	26.4
Other	8.3
Body weight	4.2
Feeding is standard	1.4
Non response	1.4
Age	0
Sex	0

Table 3.5 summarises the amount of grain and commercial premixes fed and shows that oats, premixed diets and corn were fed in the greatest quantities with averages of 3.58 kg, 2.48 kg and 1.72 kg of these grains being fed/horse/day respectively (Table 3.5). When used, barley was fed at an average of 1.25 kg/day, while legumes and oilseeds were

typically fed in much smaller quantities, in comparison to the cereal grains, with average quantities ranging from 0.2 kg/day to 0.7 kg/day. Total quantities of grain fed/horse/day for all trainers ranged from 3.8 kg to 13.2 kg of grain per day, with a mean of 7.3 kg of grain fed/horse/day (Table 3.5).

Table 3.5: The minimum, maximum and mean quantities (in kg/day) of grains fed by trainers and the minimum, maximum and mean total quantity of grain fed/horse/day.

GRAIN	Minimum (kg/day)	Maximum (kg/day)	Mean (kg/day)
Oats	1.00	9.45	3.58
Commercial premixed diet	0.44	10.00	2.48
Corn	0.25	4.35	1.72
Barley	0.40	3.8	1.25
Lupins	0.50	1.00	0.74
Faba Beans	0.20	1.20	0.66
Chick Peas	0.50	0.50	0.50
Other	0.50	0.50	0.50
Sunflower	0.10	1.20	0.43
Soybean	0.15	0.25	0.20
TOTAL GRAIN IN DIET	**3.80**	**13.20**	**7.33**

Statistical analysis of data on the quantity of grains fed required the assumption that all trainers had equal access to the same grains. Grain type significantly affected the individual quantities of cereal grain fed, with oats and premixed diets being fed in significantly greater quantities than corn and barley (Table 3.6). Grain type had no significant effect on the quantity of legume/oilseed grains fed. Size of establishment and geographic region had no significant effects on the quantities of cereal grains or legume/oilseed grains fed.

Table 3.6: Average quantities of cereal grains and premixed diets fed/horse/day. Figures were derived on the assumption that all trainers had access to the same grains and non-inclusion of a grain into a trainer's diet was included as a 0 kg result. Data is presented using actual values.

	Quantities of grains fed, averaged over all trainers (kg/day)	
	Mean	**SE**
Oats	2.85^{a}	0.240
Commercial Premix	2.51^{a}	0.271
Corn	1.32^{b}	0.147
Barley	0.40^{b}	0.095

^{ab} Values in same column with different superscripts are significantly different ($P \leq 0.005$).

In general, establishments with less than 10 horses fed a mean of 7.2 kg of grain/horse/day, which was an average of 140 g of grain/horse/day more, than fed in establishments with 10 horses or more.

Trainers in country regions fed an average of 7.5 kg (± 0.28 SE) of grain/horse/day, which was significantly more ($P<0.016$) than fed by provincial based trainers (6.4 ± 0.54 SE). Country trainers also fed 0.76 kg more grain/horse/day than metropolitan based trainers, however the difference was not significant.

3.2.6 Grain Processing

The most common form of grain processing for all grains (excluding those in commercial premixed diets) was physically cracking the grain, with 40.1% of grains fed cracked. Cracking was by far the most common form of processing for corn, faba beans and chickpeas. Around 27% of grains were not processed in any manner and were fed to the horses' whole. The majority of the grains fed whole were oats and sunflower, with smaller quantities of corn also fed whole. Ten percent of grains were fed ground while 9.2% of grains were micronised, with barley and lupins the most common grains to be micronised. Only 6.3% of grains fed were steam flaked, 4.6% extruded and 2.0% boiled. Approximately 4% of barley was processed in some other manner such as soaking (Table 3.7).

Table 3.7: Summary of the relative occurrence of various methods of processing for each grain.

	Cracked (%)	**Whole (%)**	**Ground (%)**	**Micronised (%)**	**Steam flaked (%)**	**Extruded (%)**	**Boiled (%)**	**Other (%)**
Oats*	17.0	84.0	-	-	2.0	-	-	-
Corn*	83.0	11.3	1.9	5.7	-	5.7	-	-
Barley*	4.2	8.3	-	33.3	50.0	-	16.7	4.2
Faba beans	90.0	10.0	-	-	-	-	-	-
Chickpeas	100.0	-	-	-	-	-	-	-
Soybean	-	-	80.0	-	-	20.0	-	-
Lupins	37.5	12.5	-	37.5	-	12.5	-	-
Sunflower	-	100.0	-	-	-	-	-	-
TOTAL	**40.1**	**27.3**	**9.9**	**9.2**	**6.3**	**4.6**	**2.0**	**0.5**

* Do not add to 100% - trainers indicated that they feed the grain processed using two or more methods.

Commercial premixed diets normally contained a variety of grains and in some cases different grains in a premixed diet were prepared using different processing methods. A combination of whole and cracked grain within a premixed diet was most common, with 43% of commercial premixes containing whole and cracked grains only. Thirty one percent of premixed diets used by trainers in this survey, were micronised, making it the most common processing method for premixes. A further 16% were pelleted and 9.5% contained combinations of grains processed in a variety of ways, including cracking, steam flaking, extruding and micronising (Table 3.8).

Table 3.8: The relative occurrence of different methods of processing for grains in premixed diets

Processing Method(s)	**% of Premixed Diets Fed**
Whole and cracked	43.0
Micronised	31.0
Pelleted	16.0
Whole, steam flaked and extruded	5.4
Cracked, extruded and micronised	2.7
Whole and steam flaked	1.4

3.2.7 Diet Formulation

Seventy two percent of trainers indicated that they formulated diets for each individual animal, while the remaining 28% of trainers fed a standard diet to horses at equivalent levels of training. Trainers formulated diets using an average of three to four different grains. Over 73% of trainers included at least one commercial premixed feed, 23.6% of trainers fed two premixed feeds and 6.9% of trainers fed three premixed feeds in the same diet. Only 12.5% of trainers fed premixed diets as the sole grain(s) in a horse's diet. Where premixed feed(s) were included in the ration, they made up an average of 49.9% of the total diet (minimum 4.5%, maximum 100%).

Almost all of the trainers surveyed mixed chaff with their grain diets, with a mean of 0.77 kg/horse/day of lucerne chaff and 0.85 kg/horse/day of oaten or wheaten chaff added to the diets. There were no significant effects of establishment size or geographical region on the quantity of lucerne or wheaten/oaten chaff added to the diet (Tables 3.9 and 3.10). Likewise there were no significant effects of establishment size, or geographical region, on whether or not trainers formulated diets individually or fed a standard diet, whether they included a premixed feed or not and whether or not they included chaff in the diet.

Table 3.9: Quantities of lucerne and cereal chaff included in the grain rations of horses in establishments with less than 10 horses or with greater than or equal to 10 horses.

	<10 Horses		**≥ 10 Horses**	
	Mean	**SE**	**Mean**	**SE**
Wt lucerne chaff (kg/day)	0.80	0.08	0.70	0.08
Wt cereal chaff (kg/day)	0.92	0.07	0.73	0.06

Table 3.10: The quantity of lucerne and wheaten/oaten (cereal) chaff included in the grain rations of horses in country, provincial and metropolitan regions.

	Country		**Provincial**		**Metropolitan**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
Wt lucerne chaff (kg)	0.77	0.07	0.98	0.19	0.59	0.14
Wt cereal chaff (kg)	0.88	0.06	0.90	0.18	0.69	0.12

The majority of trainers (98.6%) added one or more dietary supplements to their feeds, with the most commonly used supplements being salts and electrolytes, vitamins and minerals, oil, molasses and iron (Table 3.11). Virginiamycin (Founderguard ™) and enzymes were the least used supplements.

Table 3.11: The percentage of trainers who added each supplement to their horses' diets.

Supplement	% of trainers feeding supplement
Salts and electrolytes	84.7
Vitamins and minerals	73.6
Oil	72.2
Molasses	62.5
Iron	58.3
Other	31.9
Tying up formula	29.2
Garlic	23.6
Protein	11.1
Virginiamycin (Founderguard ™)	6.9
Enzymes	4.2
None	1.4

None of the thoroughbred trainers surveyed formulated their diets to meet specific energy, protein, vitamin or trace minerals specifications.

3.2.8 Feeding Frequency, Adaptation Period and Cost

Eighty two percent of trainers supplied a grain meal to their horses twice a day, 15.3% of trainers fed horses a grain meal three times/day and 1.4% of trainers fed grain four times/day. Trainers with 10 or more horses in training tended to feed their horses more frequently, however the difference between establishment size, with respect to this practice, was not significant (Table 3.12). Trainers in metropolitan regions fed their horses' grain meals at a significantly higher frequency than trainers in country and provincial regions (Table 3.13). The number of times horses were fed hay ranged from nil to being constantly available. There was no significant relationship between establishment size and the frequency that hay was fed/day (Table 3.12), although horses in metropolitan regions were fed hay at significantly higher frequencies than horses in country regions (Table 3.13). Hay was fed at the same time as the grain concentrate meal by 44.4% of trainers, while 54.2% of trainers fed their hay, either, an average of 1.3 hours before, or 2.8 hours after, the grain meal. The remaining 1.4% did not feed hay at all.

Table 3.12: The relationship between establishment size and feeding frequency of grain meals and hay, the adaptation period a horse is allowed from the time it is placed on grain to when it is on its full grain ration and the cost of feeding a horse grain/week.

	<10 Horses		≥ 10 Horses	
	Mean	SE	Mean	SE
No times fed grain	2.15	0.05	2.25	0.10
Times fed hay	1.59	0.16	1.54	0.23
Adaptation	22.20	2.00	20.50	2.67
Cost/horse /week ($)	30.42	1.57	24.81	4.44

Table 3.13: The effect of geographical region on the feeding frequency of grain meals and hay, the adaptation period a horse is allowed from the time it is placed on grain to when it is on its full grain ration and the cost of feeding a horse grain/week.

	Country		Provincial		Metropolitan	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
No times fed grain	2.13[a]	0.05	2.00[a]	0.00	2.54[b]	0.18
Times fed hay	1.22[a]	0.08	1.79[ab]	0.46	2.72[b]	0.46
Adaptation	21.54	1.78	18.25	4.85	24.08	4.81
Cost/horse/week ($)	25.73	1.78	28.46	3.53	28.62	3.14

Values in same row with different superscripts are significantly different ($P \leq 0.016$).

Thoroughbred racehorses were allowed an average of 21.6 days (range 1 – 60 days), from the time they were bought in to the racing stables, to build up to their full grain ration. There was no effect of establishment size or geographical region on the grain adaptation period horses were allowed.

3.2.9 *In vitro* Grain Analyses

Total starch content and *in vitro* enzyme digestion data are summarised in Table 3.14. Oats had the lowest average starch content of the individual cereal grains, however the enzyme digestibility of oaten starch was greater than that of corn or barley. In contrast to oats, corn had the greatest percentage of total starch, but the lowest enzyme starch digestibility. Both oats and corn varied within each grain type by more than 10 percentage points for starch content and starch digestibility. Mean starch content of commercial premixed diets was lower than oats, corn and barley, while mean starch digestion of these diets was higher than that for corn and barley. Premixed diets displayed the greatest range in starch content and starch digestibility, due to their highly diverse composition (Table 3.14).

Table 3.14: The average and range of starch content and *in vitro* starch digestibility for oats (n=29), barley (n=2), corn (n=20) and commercial premixed diets (n=35). CV for starch digestion assay = 1.45.

	Starch content (%)		Starch digestibility * (%)	
Grain	*Mean*	*Range*	*Mean*	*Range*
Oats	41.3	36.4 – 46.8	60.5	52.0 – 66.3
Barley	60.0	58.5 – 61.5	42.3	41.0 – 43.5
Corn	70.6	66.2 – 76.8	35.6	29.1 – 41.5
Premix	32.6	6.4 – 52.2	58.7	32.2 – 92.1

* Gives an indication of the amount of starch digested in 1hr, incubated at biological temperature with amylase and AMG.

Micronising and steam flaking improved the *in vitro* digestibility of barley from 42% digested in 1 hour to 69% and 77% digested in 1 hour respectively. Likewise micronising and extrusion increased the *in vitro* digestibility of corn from 36% to 61% and 75% respectively (Table 3.15).

Table 3.15: The average starch digestibility (%) of unprocessed (n=2), micronised (n=3) and steam flaked (n=1) barley and of unprocessed (n=20), micronised (n=3) and extruded (n=2) corn. CV for starch digestion assay = 1.45.

Grain	Unprocessed (%)	Micronised (%)	Steam flaked (%)	Extruded (%)
Barley	42.3	69.0	77.0	-
Corn	35.6	60.5	-	75.1

3.2.10 Relationships Between Feeding Practices and Faecal Data

The total quantity of grain fed to individual horses/day had no relationship to the concentration of organic acids in the faeces (Figure 3.2a). Likewise the total quantity of oats and commercial premixed diets had no relationship to the concentration of total faecal acids (Figure 3.2c and 3.2d). In contrast however, the total quantity of corn fed to individual horses/day was positively related to total faecal acid concentrations (R^2=0.28, Figure 3.2b).

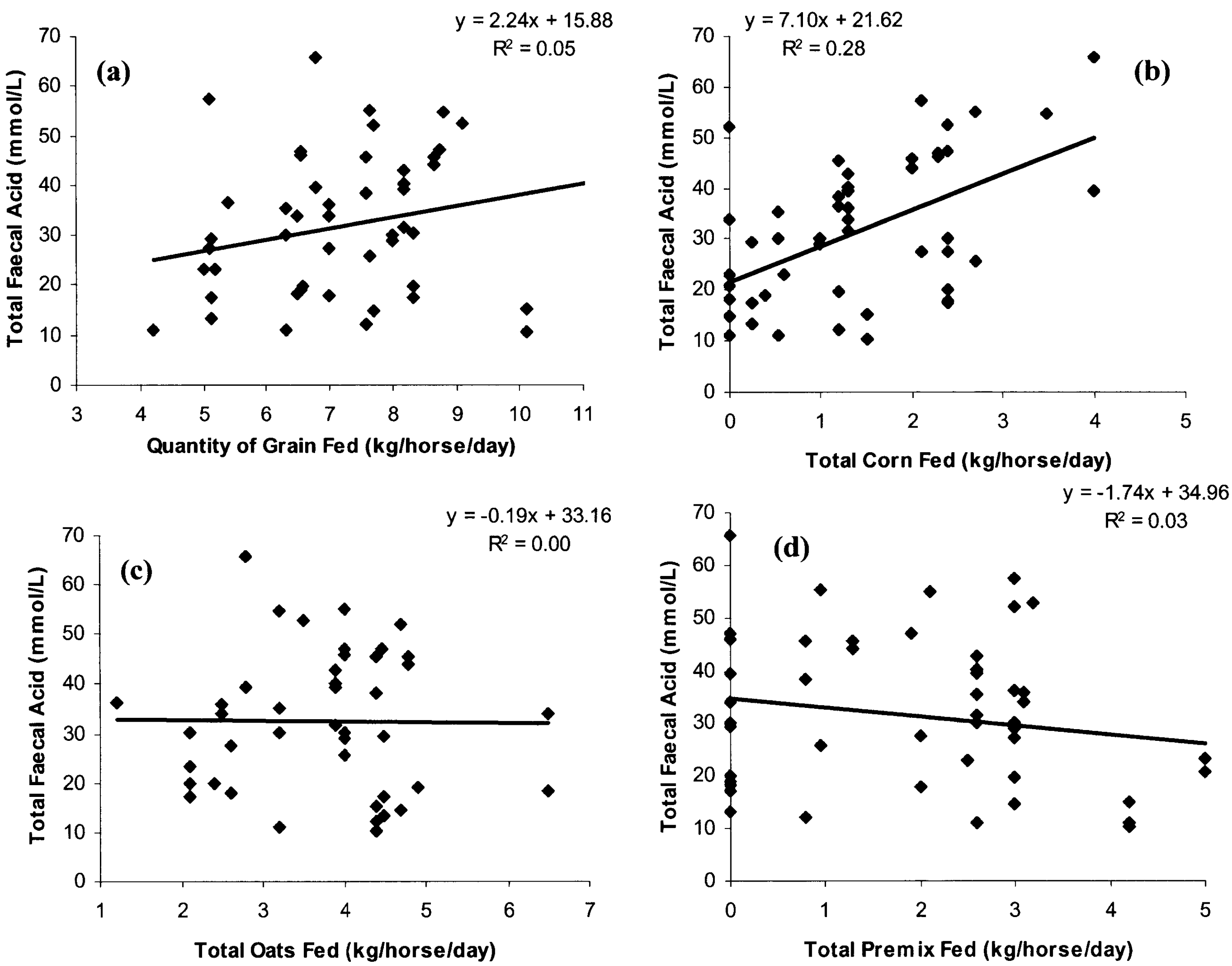

Figure 3.2: The relationship between total faecal acid concentration (n=49) and (**a**) the total quantity of grain fed/horse/day, (**b**) the quantity of corn fed/horse/day, (**c**) the quantity of oats fed/horse/day and (**d**) the quantity of commercial premix fed/horse/day.

The quantity of total indigestible starch fed/horse/day (determined using the results from the *in vitro* starch digestion assay) was positively related to total faecal acid concentrations (Figure 3.3a). As observed with the total quantity of individual grains fed, only the quantity of indigestible corn starch fed/horse/day displayed a relationship with total faecal acid concentrations (Figure 3.3b). The quantity of indigestible starch from oats and commercial premix diets fed/horse/day had no relationship with total faecal acid concentrations (R^2=0.01 and 0.04 respectively).

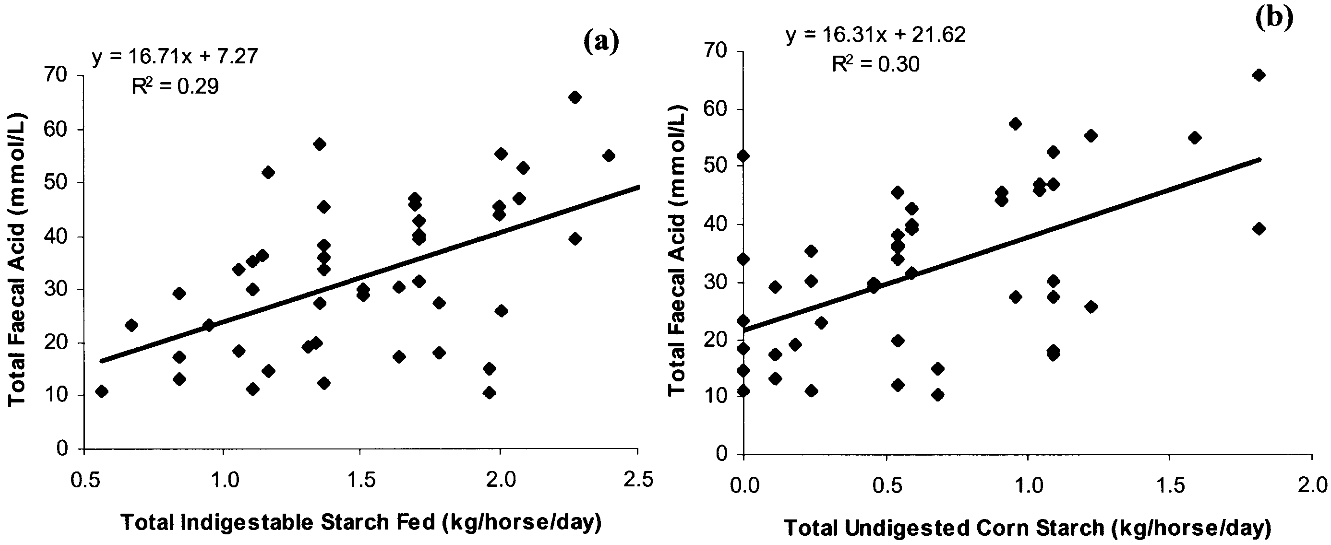

Figure 3.3: The relationship between total faecal acid concentration (n=49) and (**a**) the total quantity of indigestible starch fed/horse/day and (**b**) the quantity of indigestible corn starch fed/horse/day.

The total quantity of chaff fed to horses with their grain concentrates held no relationship with total faecal acid concentrations (Figure 3.4). There was no significant relationship between the quantity of grain fed and the quantity of chaff fed (R^2=0.00).

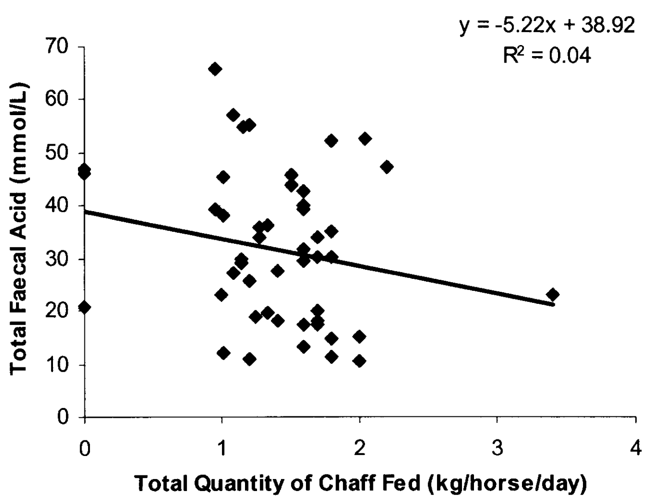

Figure 3.4: The relationship between total faecal acid concentrations (n=49) and the total quantity of chaff fed/horse/day.

3.3 Discussion

As already noted, high starch cereal grains are not a natural part of an equines diet (Hubbard *et al.*, 1976; Salter *et al.*, 1979; Waring, 1983). However, this survey suggests that horses in the Australian thoroughbred industry are fed an average of 7.3 kg of grain concentrate/day, with oats, commercial premixed diets and corn being the most commonly fed grains. Although these cereal grains provide an essential source of energy for racing thoroughbreds, results presented in this survey indicate that some negative consequences of grain feeding are evident within the industry. Hindgut starch fermentation and hindgut acidosis are examples of such negative consequences.

Approximately 27% of faecal samples collected had a pH <6.2 which is below that considered optimal for cellulolytic and lactate utilising bacteria (Leek, 1993). Faecal pH was negatively related to faecal propionate (R^2=0.76, n=49), indicating that these declines in faecal pH are caused via the fermentation of starch by amylolytic bacteria in the hindgut, which characteristically produce more propionate during carbohydrate fermentation than cellulolytic bacteria (Table 2.5, Leek, 1993).

Faecal starch concentrations were also negatively related to faecal pH (R^2=0.17, n=54) providing further evidence that hindgut starch fermentation was a problem and also suggesting that faecal pH may be a useful tool for monitoring starch digestion in horses fed high levels of grain concentrate. In addition, more than 80% of faecal samples had an acetate: propionate ratio lower than 4.7: 1, which is the ratio expected during fibre fermentation (Leek, 1993) and all faecal samples collected had lactic acid present (Table 3.1).

Of considerable importance in the surveys findings was the fact that three feeding practices, which appear to be relatively common within the thoroughbred industry, have been identified as ones, which could result in incomplete small intestinal starch digestion and thus starch fermentation in the caecum and colon. These are:

- feeding large quantities of grain in a limited number of meals/day,
- feeding chaff with the grain concentrate meal; and
- feeding large quantities of grain that contain starch of low small intestinal digestibility (as determined using the *in vitro* enzyme digestibility assay).

These factors are discussed in more detail in the following paragraphs.

Due to the horse being primarily a grazer and eating for up to 65% of a 24-hour period, their digestive tract has been designed to accommodate small meals, taken at a high frequency (Section 2.1.1). However, more than 80% of thoroughbred horses in this study were fed large meals, only twice/day. It has been observed that as meal size and starch intake increase, the quantity of starch that will escape digestion in the small intestine will rise (Chapman *et al.*, 1985). Potter *et al.* (1992) suggested an upper limit to starch digestion in the equine small intestine at around 3.5 – 4 g starch/kg BW/meal. Thus, feeding grain in meals that exceed this upper limit (approximately 7 to 8 kg of a 45% starch, grain mix, fed in two meals/day for a 450 – 500 kg horse) should theoretically negatively affect pre-caecal starch digestion. However, during the current survey, no relationship between the quantity of grain fed/day and total faecal acid (used as an indicator for the degree of hindgut starch fermentation occurring, Section 2.5.3) was observed (Figure 3.2a), suggesting that the quantity of grain being fed is having no effect on pre-caecal starch digestion.

However, while the total quantity of grain fed/horse/day appears to have no effect on faecal acid concentrations, the total quantity of corn fed/horse/day was related to total faecal acid concentrations (R^2=0.28, n=49, Figure 3.2b) with faecal acid concentrations rising with the increasing inclusion of corn in the diet. Thus it appears that corn is a major contributor to the incidence of hindgut starch fermentation in the thoroughbred industry. In contrast to corn, the total quantity of oats (average *in vitro* starch digestibility 61%) and commercial premixed diets (average *in vitro* starch digestibility 59%) fed/horse/day had no effect on total faecal acid concentrations (R^2=0.00 and 0.03 respectively, Figures 3.2c and 3.2d), suggesting that these grains have a higher *in vivo* pre-caecal digestibility and are thus 'safer' for inclusion in equine diets (NB commercial premixed diets ranged in *in vitro* starch digestibility from 32 – 92 %).

Based on reports in the literature that the inclusion of chaff in equine diets may reduce pre-caecal starch digestion (Meyer *et al.*, 1993; Kleffken 1993 *in* Kienzle, 1994), it was postulated that a positive relationship between the quantity of chaff fed/horse/day with the grain concentrates and total faecal acid may be observed. However, no evidence was found during this survey to support this theory, with the quantity of chaff fed having no relationship to total faecal acid concentrations (Figure 3.4).

The theoretical quantity of 'indigestible' starch being fed/horse/day was calculated for each individual training stable, using the results from the *in vitro* enzyme digestibility assay. In contrast to the total quantity of grain fed/horse/day, it appears that the quantity of indigestible starch fed/horse/day does have an effect on total faecal acid concentrations and thus on the incidence of hindgut starch fermentation (Figure 3.3a). More specifically, it appears that the quantity of indigestible corn starch (determined using results from the *in vitro* starch digestion assay) fed in the diet is the major factor affecting the incidence of hindgut starch fermentation and thus total faecal acid concentrations, with the quantity of indigestible corn starch fed/horse/day and total faecal acid concentrations, showing a positive relationship with an R^2 of 0.30 (n=49). Again, the quantity of indigestible oat starch and indigestible commercial premixed diet starch (determined using results from the *in vitro* starch digestion assay) fed/horse/day held no relationship with total faecal acid concentrations.

Thus, in order to reduce the incidence of hindgut starch fermentation, it appears that trainers must adopt a more specific method of grain selection, with a heavy emphasis placed on the digestibility of starch within the grains, in place of the rather non-specific and subjective measure of perceived "quality" that is presently used by almost 80% of trainers.

The feeding of grains processed using methods such as micronising, steam flaking and extrusion was uncommon amongst the surveyed trainers. The *in vitro* assay used (Bird *et al.*, 1999) indicated that the digestibility of barley and corn was more than doubled following processing that involved a combination of heat, moisture and pressure. Likewise, reports in the literature detail substantial improvements in *in vivo* pre-caecal starch digestibility, achieved via grain processing (Hoekstra *et al.*, 1999; Householder *et al.*, 1977; Meyer *et al.*, 1993). Thus it is reasonable to assume that large improvements in pre-caecal starch digestion in thoroughbred horses may be achieved through the greater use of processed grains.

3.4 CONCLUSIONS

It appears that horses in the Australian thoroughbred industry are fed about 7.3 kg of grain/day, with oats, commercial pre-mixed diets and corn being the grains most commonly fed. While the feeding of high energy feedstuffs is essential for these horses, to provide them with their daily energy requirements, the feeding of cereal grains is not without consequence, with hindgut starch fermentation and acid accumulation appearing to be common problems. The feeding of substantial quantities of 'indigestible' starch and in particular the feeding of corn starch, appears to the major contributor to the hindgut starch fermentation problems. To prevent hindgut starch fermentation and the negative consequences associated with it, there needs to be a greater emphasis placed on the selection of cereal grains on the basis of starch digestibility.

However, methods of assessment of grains on a pre-caecal starch digestibility basis, using *in vitro* and *in vivo* tools need to be developed. The next chapter describes an experiment conducted to develop the glycaemic and insulin responses and measures of faecal pH, dry matter, nitrogen and starch contents as *in vivo* measures of starch digestibility in the equine small intestine and starch fermentation in the equine hindgut, respectively.

4 Pilot Study – *In Vivo* Predictors of Small Intestinal Starch Digestion

4.1 Introduction

As noted at the conclusion of the previous Chapter, there appears to be a need for *in vivo* measures of small intestinal starch digestion and hindgut starch fermentation in equines to allow the assessment of grains on a pre-caecal starch digestibility basis. Possible methods appear to be measures of glycaemic and insulin response and of faecal pH, dry matter, nitrogen and starch content. However, given the observed effects of meal size on glycaemic response (Section 2.5.1), an optimum meal size for the generation of clear and repeatable glycaemic response curves in horses needs to be established. It is also yet to be established if the insulin response in horses closely resembles the glycaemic response and thus if it may be used as a predictor of small intestinal starch digestion.

In addition to the glycaemic and insulin responses, measures of faecal pH, faecal dry matter, faecal nitrogen (N) and faecal starch concentrations may be possible indicators of the extent of small intestinal starch digestion and hindgut starch fermentation occurring in horses. Therefore, it needed to be established if the trends reported in the literature (Section 2.5.3) are be present in horses on grain-based diets. If they are, then valuable tools for monitoring hindgut starch fermentation could perhaps be developed.

The aims of the following trial therefore were:

(i) to determine a level of feeding that will allow the generation of clear and repeatable glycaemic and insulin response curves in horses;

(ii) to verify if the glycaemic and insulin responses in horses are well related;

(iii) to ascertain whether faecal parameters such as pH, starch content, nitrogen content and dry matter may be used in horses to estimate extent of hindgut starch fermentation.

The hypothesis was that an increase in grain consumption/meal from 2.5 g triticale/kg BW (1.47 g starch/kg BW) to 5 g triticale/kg BW (2.94 g starch/kg BW) will result in a non-linear increase in plasma glucose concentrations (Jenkins *et al.*, 1981; Lee *et al.*, 1998; Wolever *et al.*, 1991) but will cause a linear increase in plasma insulin concentrations (Lee *et al.* 1998). It was also hypothesised that as grain intakes increase, the quantity of grain reaching and fermenting in the equine hindgut will increase and thus faecal pH and faecal dry matter will fall, while faecal starch and nitrogen concentrations will rise.

4.2 Methods

In order to examine the effect of meal size on the glycaemic and insulin responses following a grain meal, two diets were used; **diet 1** was lucerne chaff (5 g/kg BW/meal) plus 2.5 g/kg BW/meal of cracked triticale; and **diet 2** was lucerne chaff (3.5 g/kg BW/meal) plus 5 g/kg BW/meal of cracked triticale. Cracked triticale is a cereal grain with a moderate starch digestibility (Bird *et al.* 1999) and was therefore considered suitable for use in this experiment.

Three horses (thoroughbred geldings) aged from 6 to 8 years, weighing 430 – 500 kg (average 455 kg) were used in the trial (Table 4.1). 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 morning for approximately half an hour, except on days when blood sampling occurred. All horses had their teeth corrected by an equine dentist prior to the trial and were treated with an anthelmintic 2 weeks prior to the trial.

Table 4.1: The age, weight, condition score and temperament of the horses used in the trial.

	Horse 1	Horse 2	Horse 3
Age (years)	6	6	8
Weight (kg)	435	500	430
Temperament	Quiet	Quiet	Quiet/Flighty

The horses were first placed on a lucerne chaff only diet (9 g/kg BW/meal) for days 1 - 7 to allow adaptation to daily routine and surrounds. On day 8 they were placed on 1.25 g/kg BW/meal cracked triticale for one day after which they were placed on diet 1 for days 9 – 15 inclusive. All horses were then fed 3.75 g/kg BW/meal triticale for days 16 – 18 before being placed on diet 2 for days 19 - 25. The University of New England Animal Ethics committee approved the experimental protocol.

Horses were fed the grain portion of their diet at 0830 and 1730 each day. On completion of eating the grain the horses received their allocation of lucerne chaff. On mornings when the glycaemic and insulin responses were measured (days 15 and 25) only the grain portion of the diet was fed initially. Chaff was fed at the conclusion of blood sampling. All animals had access to water *ad libitum.*

Measurements

Body weight was measured on days 1, 14 and 25. Feed intakes and refusals were recorded daily. Faecal samples were collected on the final three days of each seven-day feeding period to determine faecal pH, faecal dry matter, faecal nitrogen and faecal starch content. Faecal pH was determined by combining 10 mL of distilled water with 10 g of faeces (James Rowe, University of New England *personal communication*), mixing well and measuring with an EcoScan pH 5/6 meter (Eutech Instruments Pty Ltd, Singapore). The remaining faecal sample was weighed and then dried to constant weight at 105°C before being weighed again to determine dry matter. The dried faecal sample was then ground through a 0.5mm screen in a Cyclotech mill (Tecator, Höganäs, Sweden) for use in starch and nitrogen content assays. Total starch content of the dried faecal samples was determined using the total starch determination assay of McCleary *et al.* (1997). Total faecal nitrogen was determined using the LECO FP 2000 system (LECO Corporation, Michigan, USA). On days 15 and 25 blood samples were collected via an indwelling catheter for measurement of the glycaemic and insulin response. A 1 mL injection of Lignocaine was administered subcutaneously over the jugular vein at the site where a 14-gauge catheter (Surflo® Terumo Corporation, Tokyo, Japan) was to be inserted. Catheters were inserted approximately 5 min later. Once the catheters were inserted, a pre-feeding blood sample was taken and catheters were flushed with a heparin (50 i.u./mL) saline (0.09%) solution. On completion of catheter insertion the horses were fed the grain portion of their diet. Blood samples were collected every 30 min for 4 h following consumption of the grain portion of the diet. The catheters were flushed with heparin saline after each blood sample was taken. All blood samples consisted of 10 mL of venous blood and were collected into lithium heparinised blood collection tubes that were immediately placed on ice. On return to the laboratory, samples were centrifuged at 2000 *g* for 15 min at 4°C and the plasma was transferred to labelled plastic vials. Plasma samples were stored at –16°C until analysed for glucose and insulin concentration.

Prior to glucose and insulin analysis, plasma samples were thawed and re-centrifuged at 2000 *g* for 10 min to remove plasma proteins that had precipitated from solution on thawing. Plasma glucose concentrations were determined using the Unimate 5 GLUC HK *in vitro* diagnostic reagent system (Roche Diagnostic Systems) on a COBAS BIO auto-analyser (Roche). Plasma insulin concentrations were determined using a radioimmunoassay (RIA; Albano *et al.* 1972). The polyclonal antiserum (Sigma-Aldrich. St Louis, Missouri, USA) was raised in guinea pigs against purified bovine insulin. Bovine insulin was used as both standard and tracer. Tracer was iodinated using the iodogen method (Pierce. Rockford, Illinois, USA). Separation of bound insulin from free insulin was achieved using polyethylene glycol 6000.

Peak glucose/insulin concentrations, average glucose/insulin concentrations, peak minus basal glucose/insulin concentrations and the time and slope to peak glucose/insulin concentrations were calculated from each individual glycaemic and insulin response curve generated (Figure 4.1).

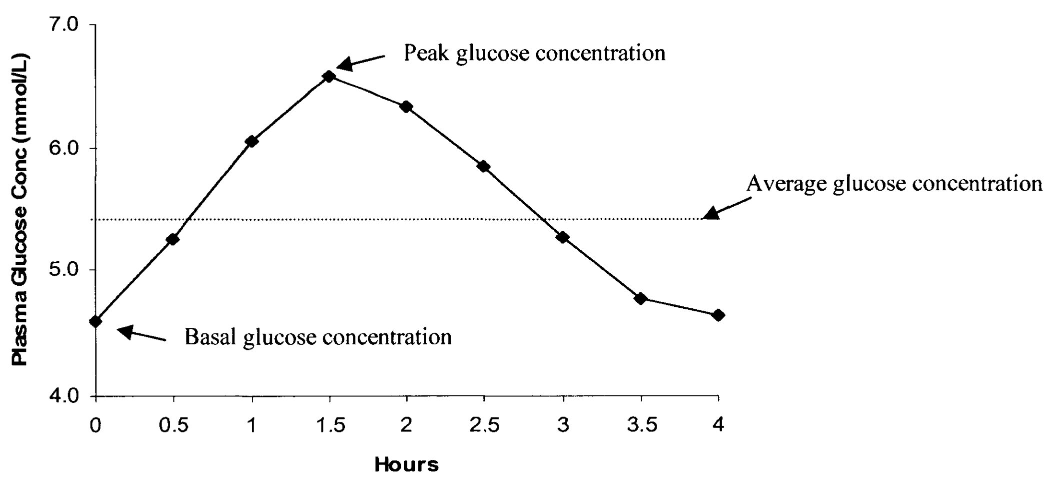

Figure 4.1: Important points on the observed glycaemic response curves. The same points are used on insulin response curves. Hours represents the number of hours after feeding commenced.

4.3 Results

Feed Refusals

Horse 1 refused 100% of the grain component of his diet on the morning that the glycaemic response was to be measured at the 5 g/kg BW/meal triticale feeding level (day 25) and data from this animal was excluded from all calculations for glycaemic and insulin responses for that diet.

Glycaemic and insulin response data

Diet 1 initiated the most elevated glycaemic and insulin responses in all horses. In addition, the curves generated on diet 1 were more 'normal' than those observed at the higher grain feeding level, with clear increases and decreases in plasma glucose and insulin evident (Figure 4.2 and Figure 4.4).

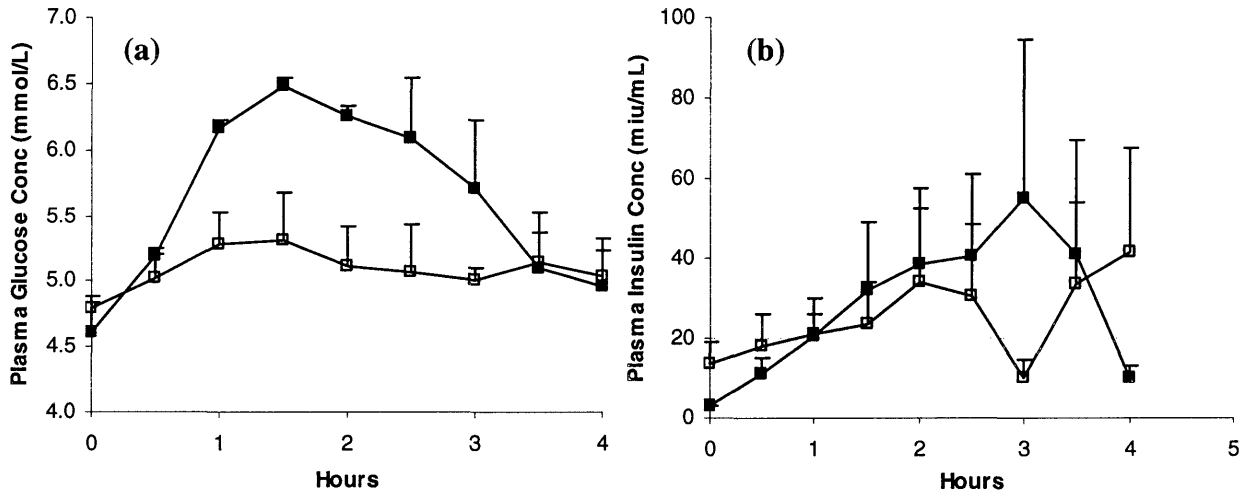

Figure 4.2: The **(a)** average postprandial glycaemic responses and **(b)** average postprandial insulin responses to the (■) 2.5 g/kg BW triticale/meal diet and to the (□) 5 g/kg BW triticale/meal diet.

Peak, average and peak minus basal glucose concentrations for diet 1 were greater than those observed for diet 2 (Table 4.2). Time to peak glucose was increased with increasing meal size while slope to peak glucose was reduced (Table 4.2).

Table 4.2: Mean peak glucose concentration, average glucose concentration, peak minus basal glucose concentration, time to peak glucose and slope to peak glucose for horses consuming 2.5 g/kg BW triticale/meal.

	2.5 g/kg BW Triticale		**5 g/kg BW Triticale**	
	Mean	*SE*	*Mean*	*SE*
Peak glucose conc (mmol/l)	6.7	0.18	5.8	0.15
Average glucose conc (mmol/l)	5.6	0.23	5.3	0.02
Peak-basal glucose conc (mmol/l)	2.1	0.07	0.9	0.30
Time to peak glucose (hours)	1.8	0.33	2.0	0.50
Slope to peak glucose	1.2	0.23	0.5	0.30

Insulin responses of horses on diet 1 were higher than those observed for horses when consuming diet 2. Plasma insulin response was highly variable between horses as demonstrated by the large standard errors (Table 4.3). Time to peak insulin was the same for both diets while slope to peak insulin was reduced for horses at the higher grain feeding level (Table 4.3). Horse 2 displayed the most elevated glycaemic and insulin responses (Figure 4.4).

Table 4.3: Mean peak insulin concentration, average insulin concentration, peak minus basal insulin concentration, time to peak insulin and slope to peak insulin for horses consuming 5 g/kg BW triticale/meal.

	2.5 g/kg BW Triticale		**5 g/kg BW Triticale**	
	Mean	*SE*	*Mean*	*SE*
Peak insulin conc. (miu/mL)	59.0	37.49	51.3	41.00
Average insulin conc. (miu/mL)	33.4	19.32	31.0	23.56
Peak-basal insulin conc. (miu/mL)	49.8	32.95	37.5	34.40
Time to peak insulin (hours)	2.5	0.50	2.5	1.50
Slope to peak insulin	22.7	10.51	11.3	5.26

There was no significant relationship between the observed glycaemic and insulin responses (Figure 4.3). Individually, horse 3 tended to have stronger plasma glucose: insulin relationships than horses 1 and 2.

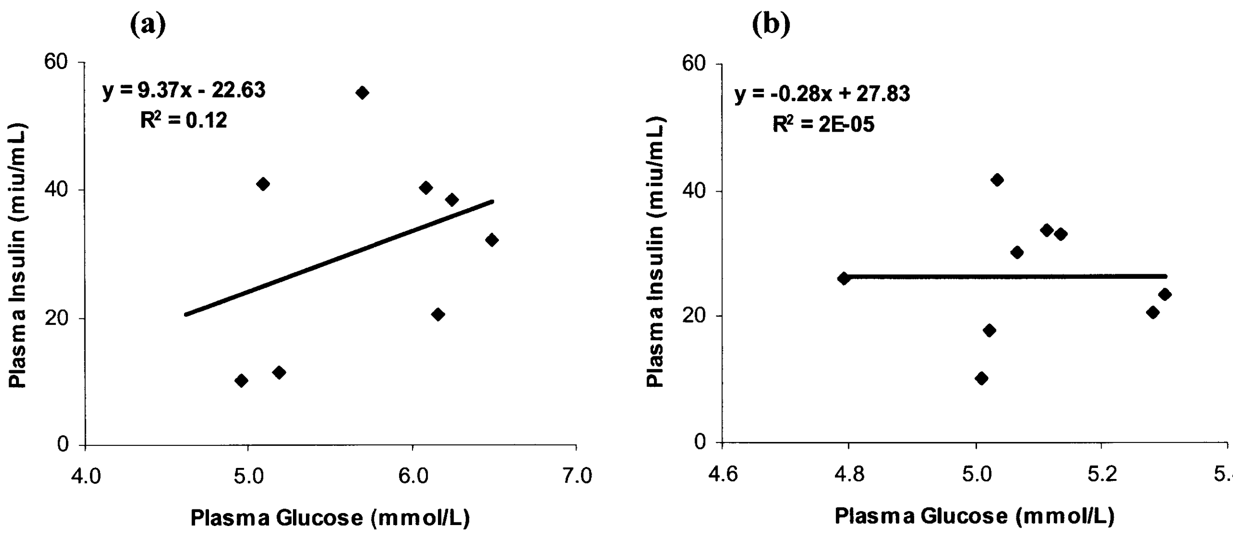

Figure 4.3: The relationship between plasma glucose and plasma insulin for (**a**) diet 1 (2.5 g/kg BW/meal triticale diet) and (**b**) diet 2 (5 g/kg BW/meal triticale diet).

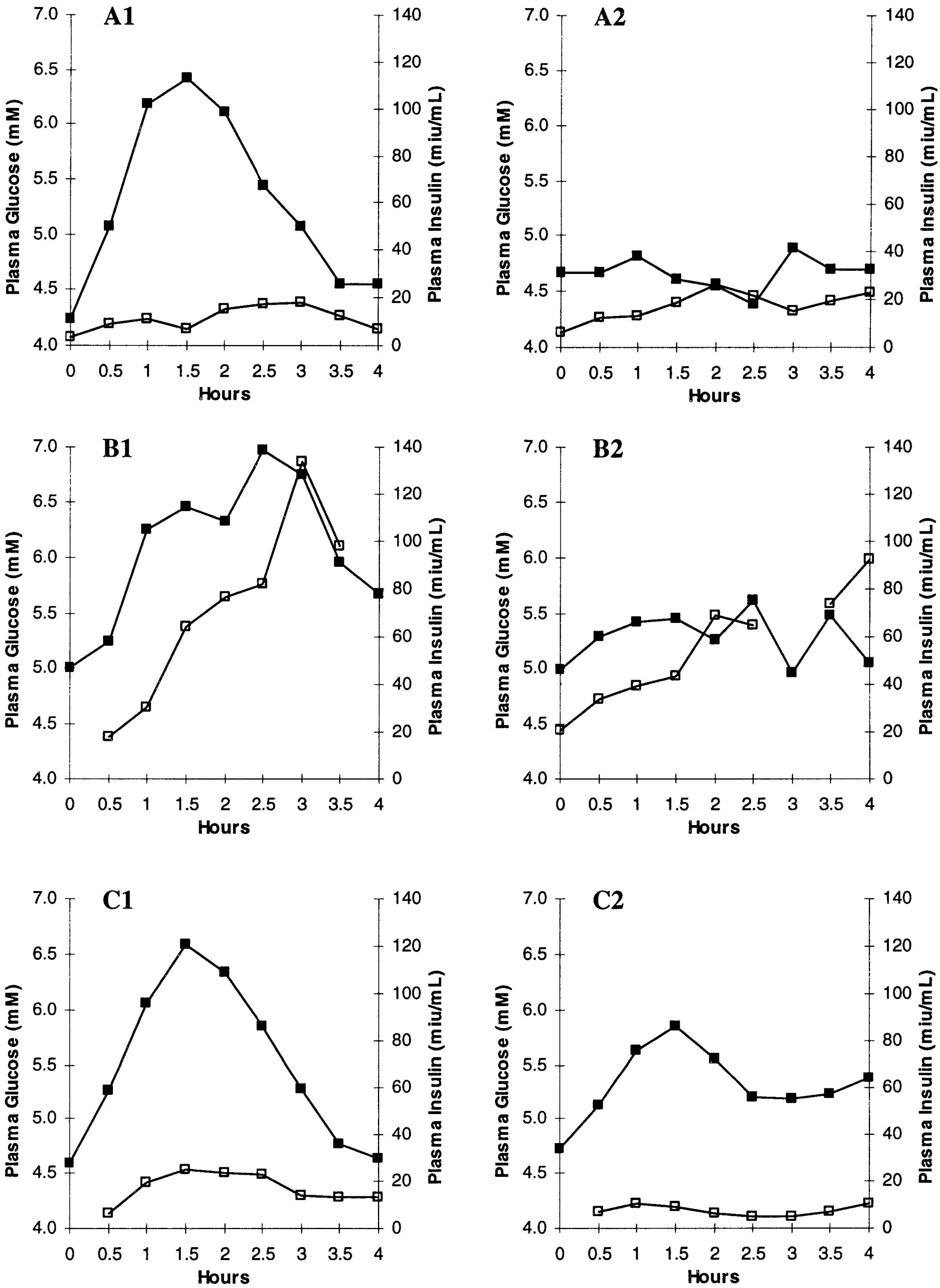

Figure 4.4: The (■) glucose and (□) insulin responses following consumption of 2.5 g/kg BW cracked triticale for (**A1**) horse 1, (**B1**) horse 2 and (**C1**) horse 3 and glucose (■) and insulin (□) responses following consumption of 5 g/kg BW cracked triticale for (**A2**) horse 1, (**B2**) horse 2 and (**C2**) horse 3.

Faecal Data

At the higher level of grain intake, faecal pH and faecal dry matter were lower and faecal N and faecal starch content were higher than that observed for horses on the lower level of grain intake (Table 4.4).

Table 4.4: The effect of varying levels of grain intake on faecal pH, faecal dry matter (FDM), nitrogen content (N) and starch content at various intakes of cracked triticale (g/kg BW/meal).

Triticale (g/kg BW/meal)	pH	SE	FDM (%)	SE	N (% DM)	SE	Starch (% DM)	SE
0	8.5	0.08	33.7	0.96	1.7	0.03	0.62	0.02
2.5	7.2	0.08	33.6	1.18	1.8	0.10	0.39	0.02
5	6.7	0.07	24.8	2.57	2.1	0.11	0.74	0.08

There was a strong negative relationship between grain intake and faecal pH (R^2=0.83) (Figure 4.5a) and a strong positive relationship between grain intake and faecal nitrogen content (R^2=0.67) (Figure 4.5c). Relationships between grain intake and the faecal dry matter and starch content parameters were poor (Figure 4.5b and 4.5d).

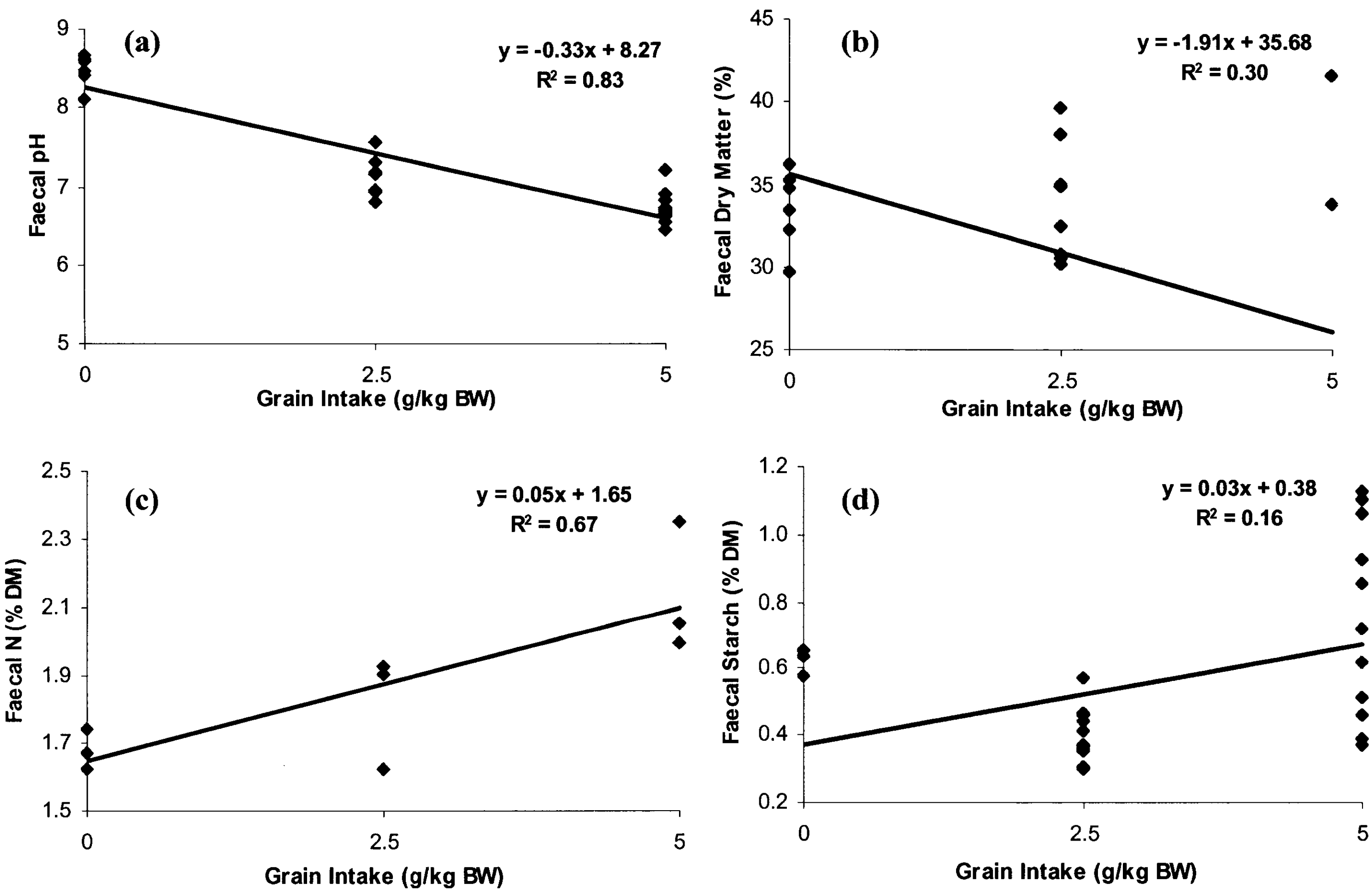

Figure 4.5: The relationships between increasing grain intake and **(a)** faecal pH, **(b)** faecal dry matter (%), **(c)** faecal nitrogen (% DM) and **(d)** faecal starch (% DM).

4.4 DISCUSSION

The results observed during this experiment are difficult to interpret and explain, especially given the very small number of experimental animals used. Clear glycaemic response curves were generated when horses were fed 2.5 g/kg BW/meal of triticale. However, when grain quantity was increased to 5 g/kg BW/meal of triticale, the observed glycaemic response was virtually non-existent with peak glucose responses not reaching those observed for horses on the lower level of grain intake. These findings are similar to those previously reported by Pagan (1999) for horses fed an oats diet and highlight the importance of meal size if glycaemic response is to be measured accurately.

It has been proposed that lowered glycaemic responses observed at high grain feeding levels are due to regulation of blood glucose levels by insulin (Radicke *et al.*, 1994) and that the insulin response will tend to increase linearly with increasing meal size (Lee *et al.*, 1998). If this was so in this the case, we would expect to see insulin responses on the higher grain feeding level elevated above those observed at lower grain intakes. However, the insulin responses of horses consuming 5 g/kg BW/meal of triticale were lower than the insulin responses measured in horses consuming 2.5 g/kg BW/meal of triticale, again a result that is difficult to explain. Two speculative possibilities that may explain this seeming absence of a glycaemic or insulin response at higher levels of grain feeding are:

(i) that following an 18-day adaptation to a grain diet and at the higher level of grain feeding, the liver is removing greater quantities of glucose from the blood in the hepatic portal vein, as it passes directly through the liver from the small intestine (Fox, 1991), thus reducing the observed glycaemic response and negating the need for an elevated insulin response (Jacobs *et al.*, 1982);

(ii) that an increased small intestinal passage rate caused by the larger grain meal is decreasing the extent of pre-caecal starch digestion occurring (Chapman *et al.*, 1985) and thus reducing the observed glycaemic and insulin responses.

It is possible that feeding grain on a g/kg BW basis when measuring glycaemic and insulin responses will also present problems when it comes to interpreting data. For example, horse 2, the heaviest horse, displayed the most elevated glycaemic and insulin response curves when fed. It is possible that:

(i) horse 2 is an efficient digester of starch and thus displays the highest glycaemic and insulin response curves due to the most extensive digestion of starch in the small intestine

(ii) horse 2 may have some form of insulin resistance or insensitivity, which will allow an exacerbated elevation of the glycaemic and insulin responses for this horse (Jeffcott *et al.*, 1986); or

(iii) horse 2 is displaying the highest glycaemic and insulin responses because he is consuming larger quantities of grain than the other horses because of a heavier bodyweight.

To ensure that the third possibility is eliminated, it appears advisable that grain meals should be fed on a grams of starch/meal basis and meal size should not be varied according to bodyweight. This approach will also allow starch digestibility comparisons between grain species using the glycaemic and insulin responses, as starch content/meal will not vary between grains as it would if they were fed on a weight only basis. A grain feeding level of 2.5 g/kg BW/meal of triticale appears to be suitable for the generation of clear glycaemic response curves and thus a quantity of 670 g starch/meal (average BW 455 kg @ 1.47 g starch/kg BW/meal) appears to be optimal for the generation of clear and repeatable glycaemic and insulin response curves in horses.

The insulin response was measured during this experiment to determine if it is a more accurate indicator of small intestinal starch digestion than the glycaemic response. The absence of a relationship between the glycaemic and insulin responses indicates that in addition to blood glucose concentration, there are other factors that have major effects on the insulin response in the horse. Some proposed factors may include the presence of elevated concentrations of hormones that antagonise the actions of insulin, including growth hormone, cortisol, adrenalin, progesterone and glucagon, as well as stress, pregnancy, obesity and angina (Corke, 1986; Freestone *et al.*, 1992; Williams *et al.*, 2001). Thus the alteration of the insulin response by these and other factors may limit the usefulness of this response as an *in vivo* predictor of small intestinal starch digestion However, it should be measured in further experiments to enable a more accurate evaluation of glycaemic response curves.

Several reports in the literature have indicated that as starch intake increases, faecal pH and dry matter decrease while faecal starch and nitrogen concentrations increase (Godfrey *et al.*, 1992; Hintz *et al.*, 1971; Karr *et al.*, 1966; Lee, 1977; Orskov, 1970; Pluske *et al.*, 1998; Rowe *et al.*, 2001; Wheeler *et al.*, 1977). These observations are supported by the results reported here, with declining faecal pH and dry matter values and increasing faecal starch and nitrogen contents observed as grain quantity in the diet was increased. Faecal starch concentrations measured when horses were consuming the lucerne hay only diet were less than 1%. Thus any concerns involving starch of bacterial origin significantly influencing faecal starch concentrations were removed. The strong relationships observed between faecal pH and grain intake and between faecal nitrogen and grain intake make these measures promising non-invasive parameters for gauging small intestinal starch digestion and hindgut fermentation of starch on high grain diets.

4.5 CONCLUSIONS

The results from this study have not allowed us to draw any conclusions as to why the glycaemic response is lowered when grain intake levels increase. Even though the study utilised a small number of experimental animals, it has indicated that a feeding level equivalent to 2.5 g/kg BW/meal of triticale will generate clear glycaemic and insulin response curves that appear to be consistent between horses. The study also demonstrated that the faecal parameters pH, dry matter, nitrogen and starch have potential for use as non-invasive *in vivo* indicators of hindgut starch fermentation in equines.

The following chapters describe a series of experiments, where, using the methods developed here, as well as an *in vitro* assay designed to estimate the small intestinal digestibility of cereal grains, the effect of grain species, grains processing and the addition of exogenous enzymes on the small intestinal digestion of cereal grain starch will be examined.