Identifying nutritive, physical and volatile characteristics of oaten and lucerne hay that affect the short-term feeding preferences of lactating Holstein Friesian cows and Thoroughbred horses

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Doctorate of Philosophy

By

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Abstract

Feeding behaviour is controlled by the integration of both perceived cues from the plant and post-ingestive signals received by the animal. In general practice, dairy cattle are presented with a variety of feedstuffs throughout each day, in addition to changing feedstuffs over time. In the Australian context, the feeds generally include fodder (usually hay) and pasture whilst grazing, as well as various concentrates. For horses that are stabled for prolonged periods, their diet consists of fodder (again usually hay or chaff) and concentrates, in addition to the pasture consumed whilst allowed access to graze. In such situations, where animals are presented with a range of feedstuffs, some of them novel and for discrete periods of time, it is important that they readily accept the feeds when they are first presented to them. For both lactating dairy cows and performance horses, for example, it is important to avoid periods of low intake, as this can have immediate and sometimes longer-term consequences to animal performance.

The daily rate of food intake is the single most important factor affecting animal performance and productivity (Illius et al. 2000) and an animal's responses to a feedstuff can be considered the ultimate measure of its quality. The animal's first response to the presentation of a new feed, which in this particular study was oaten or lucerne hay, is its level of voluntary intake, which depends in part on palatability. Palatability is an integrative term (Provenza 1995); to provide a quantitative measure of the acceptability of a feedstuff, a '*preference value'* can be obtained by describing the preference of one hay relative to an alternate hay also on offer.

The Australian fodder industry is increasingly adopting more objective measures of hay quality to improve marketing opportunities, especially in the export industry, and to meet the demands and expectations of local and overseas purchasers of hay. Being able to efficiently and reliably predict the preference value of any particular hay would be beneficial to processors, exporters, users (purchasers) of hay, and possibly plant breeders, to make more informed decisions. This thesis describes a comprehensive analysis of the chemical and physical characteristics of a selected number of oaten and lucerne hays and their relationship to the acceptability or 'preference value' of the hays for lactating Holstein Friesian cows and Thoroughbred horses.

The project aimed to (i) quantify preference values for a large number of oaten hays with dairy cows and horses and a similarly large number of lucerne hays with horses only, and (ii) develop predictive equations for animal preferences based on the chemical and physical properties of the hays and the

animal's short-term rate of consuming the hays. A total of approximately 8,500 preference tests were conducted with 85 oaten hays offered to lactating Holstein Friesian cows and Thoroughbred horses, and 70 lucerne hays offered to Thoroughbred horses. The hays were selected to cover a range of nutritive values. The intake rate and preferences for all the hays were quantified in a series of tests replicated over time and with different animals. Each 'trial' hay was offered with four 'standard' hays (in separate tests). A suite of chemical (nutritive value) traits and physical traits were quantified for the hays. An assessment of the 'odour profile' was also included in the characterisation of the hays. The nutritive, physical and volatile traits were then related to hay preference values in multiple and simple linear regression models and equations generated to predict hay preference values. A prediction of hay preference made directly from the spectra obtained by near infrared reflectance spectroscopy (NIRS) was also developed.

Overall, the average preference value of an oaten hay (i.e., that obtained using the comparisons with all four standard hays) could be predicted from a range of nutritive value traits, typically the contents of acid detergent fibre (ADF), hemicellulose, crude protein (CP) and water soluble carbohydrates (WSC) - with a correlation co-efficient of about 79% with cows and 61% for horses. Acid detergent fibre and CP contents tended to have the biggest influence on preference value for both cows and horses, followed by WSC and hemicellulose content. Examination of lucerne hays offered to horses increased the correlation co-efficient to 74%, with ADF, hemicellulose and CP each having a similar magnitude of effect. This means that these traits, with an appropriate weighting for each, could account for up to about three-quarters of the variation in hay preference values for cows and horses. The *in vitro* digestibility of dry matter (IVD) of oaten and lucerne hay was nearly as accurate in predicting preference values as using the four nutritive value traits of ADF, hemicellulose, CP and WSC, which was anticipated as digestibility is a function of these chemical traits. Hay physical traits could also be used to predict preference values, although not as accurately as the nutritive value traits or IVD. Of the physical traits, shear energy had the largest effect (co-efficient approximately -1.15 compared to an average of -0.35 for the other traits in the equation). The use of NIRS to directly predict preference values was also encouraging, with the best calibration model yielding a correlation co-efficient of 61 to 81%, depending on the hay (oaten or lucerne) and the animal (cows or horses).

Of the total 120 volatile compounds isolated from the oaten hays, six had some relationship with cow preferences and there were also six oaten hay volatiles related to horse preferences. Of the oaten hay volatiles significantly related to preference, four were positively related to cow preference whilst two were negatively related to cow preference. Similarly there were four volatiles positively related to horse

preference and two consistently negatively related. Of the six oaten hay volatiles found to influence the preferences of dairy cows and horses, four were common to both species. Of the total of 147 volatile compounds isolated from the lucerne hays, 15 had some relationship with horse preference, with nine positively related to horse preference and six negatively related.

The data reported here suggest that a predicted preference value for a hay can be generated using commonly measured quality traits, which would improve a buyer’s confidence of animals responding favourably when first offered the hay. More specifically, for an average preference value, the most reliable prediction equation was based on nutritive value traits: ADF, hemicellulose, crude protein and water soluble carbohydrates solely. Alternatively a combination of nutritive and physical traits can be used to predict preference: *in vitro* digestibility, crude protein, water soluble carbohydrates and shear energy. The visible and near infrared spectra obtained by NIRS was also a promising method of prediction, and given the speed and affordability of NIRS, this technology could be further refined and used for routine measurement of predicted hay preference values. The volatile compounds shown to influence preference value of the hay should be identified and further research undertaken to investigate novel approaches to manipulate the preference of hays. Caution should be taken when attempting to use these prediction equations on a single specific hay in a single specific situation. The predictions and relationships investigated in this thesis are based on populations of hays and preferences of a group of animals and therefore some care should be taken when applying them to a particular situation with difference circumstances. Other factors that can influence feed preferences include an animal’s feeding experiences, basal diet and diet history and various environmental factors unaccounted for here.

Declaration

This thesis is a record of original work and contains no experimental material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Sarah Jean Pain
November 2008

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Abbreviations

A	Standard oaten hay A
ADF	Acid detergent fibre
AFIA	Australian Fodder Industry Association
BC	Standard oaten hay BC
CIR	Lactating Holstein Friesian cow oaten hay intake rate (g/min)
CP	Crude protein
CPrefA	Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard A (log ratio trial hay eaten:standard hay eaten)
CPrefave	Average of CPrefA, CPrefBC, CPrefD and CPrefE
CPrefBC	Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard BC (log ratio trial hay eaten:standard hay eaten)
CPrefD	Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard D (log ratio trial hay eaten:standard hay eaten)
CPrefE	Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard E (log ratio trial hay eaten:standard hay eaten)
CSIRO	Commonwealth Scientific and Industrial Research Organisation
D	Standard oaten hay D
E	Standard oaten hay E
GCMS	Gas chromatography mass spectroscopy
GE	Gross energy
Hem	Hemicellulose
HIR	Thoroughbred horse oaten hay intake rate (g/min)
HLIR	Thoroughbred horse lucerne hay intake rate (g/min)
HPrefA	Thoroughbred horse preference when fed in combination with oaten hay standard A (log ratio trial hay eaten:standard hay eaten)
HPrefave	Average of HPrefA, HPrefBC, HPrefD and HPrefE
HPrefBC	Thoroughbred horse preference when fed in combination with oaten hay standard BC (log ratio trial hay eaten:standard hay eaten)
HPrefD	Thoroughbred horse preference when fed in combination with oaten hay standard D (log ratio trial hay eaten:standard hay eaten)
HPrefE	Thoroughbred horse preference when fed in combination with oaten hay standard E (log ratio trial hay eaten:standard hay eaten)

HPrefLA	Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio trial hay eaten:standard hay eaten)
HPrefLave	Average of HPrefLA, HPrefLB, HPrefLC and HPrefLP
HPrefLB	Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio trial hay eaten:standard hay eaten)
HPrefLC	Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio trial hay eaten:standard hay eaten)
HPrefLP	Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio trial hay eaten:standard hay eaten)
IVD	*In vitro* dry matter digestibility
LA	Standard lucerne hay LA
LB	Standard lucerne hay LB
LC	Standard lucerne hay LC
LMM	Log ratio of maximum and minimum stem diameter measurements
LP	Standard lucerne hay LP
ME	Metabolisable energy
NDF	Neutral detergent fibre
nir (prefix)	Hay trait determined using near infrared spectroscopy (eg, nirADF)
NIR	Near infrared
nirs (prefix)	Hay trait determined using near infrared spectroscopy and the value standardised (eg, nirsADF)
NIRS	Near infrared spectroscopy
nm	Nanometres
RIRDC	Rural Industries Research and Development Corporation
SA	South Australia
SD	Stem diameter
SE	Shear energy
SPME	Solid phase micro extraction
UWA	University of Western Australia
VIC	Victoria
WA	Western Australia
wc (prefix)	Hay trait determined using near wet chemistry (eg, wcADF)
WC	Wet chemistry

wcs (prefix)	Hay trait determined using near wet chemistry and the value standardised (eg, wcsADF)
WSC	Water soluble carbohydrate

Literature Review

1 The Australian Fodder Industry

Fodder has been defined as the range of crop and pasture species that are grown, harvested and lightly processed to facilitate both on farm use and domestic and export trade (Flinn and Heazlewood 2000). The Australian fodder industry has an estimated 20,000 producers on a total of 46,000 properties across the country, which combined produce between 5.5 and 6.5 million tonnes of hay annually (Zwer and Faulkner 2006). The majority of hay produced in Australia is mixed pasture hay predominantly for on-farm and hobby farm use (Figure 1). The more costly pure hay products, such as oaten and lucerne hays are produced primarily for sale on the domestic and export markets.

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Figure 1. Proportions of hay produced in Australia during the 2002/2003 season (Zwer and Faulkner 2006).

The Australian domestic market supplies a number of industries (Figure 2). The dairy industry accounts for about 40% of the total domestic market demand, the horse industry approximately a further 25%, followed by beef feedlots at 20% (Stubbs 2000). The remaining 15% encompassing various other livestock industries including sheep and beef graziers, stock feed manufacturers and for use as horticulture products.

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Figure 2. Total hay demand for Australian fodder by the dairy industry, horse industry, beef feedlotting industry and other, on-farm uses (Stubbs 2000).

The largest domestic market segment is by far the dairy industry. Most dairy farms, because of their relatively intensive systems, high stocking rates, high feed demands and need to maintain animal condition and production, are heavy users of conserved fodder. An estimated 400,000 tonnes, 54%, of the fodder consumed by the dairy industry, is purchased from off-farm sources (Stubbs 2000). Despite increasing use of grain as a feed supplement, these figures are likely to continue to grow as industry deregulations result in larger herds and increased pressure on available grazing land. The dairy industry is a large consumer of oaten hay, with interest in oaten hay in particular increasing due to the high quality of the hay currently being grown domestically (Zwer and Faulkner 2006). The horse industry is regarded as the second most important market segment in most parts of Australia. The market constitutes approximately 0.5 million horses consuming 250,000 tonnes of hay and chaff annually. This market segment is also likely to grow as high-cost rations are replaced with increased quantities of good quality hay. The horse industry is a large consumer of both oaten and lucerne hay.

Export demand is predominantly for cereal hay, with Australia exporting an average of over 500,000 tonnes of cereal hay per annum over the past five years (Zwer and Faulkner 2006). The majority of hay exported is premium oaten hay for the Japanese dairy market. The requirement is for visually attractive, highly digestible hay. The export market for Australian-grown fodder has increased in recent years and this growth has spurred an increasing demand for consistent quality testing from both fodder consumers and fodder producers.

Oaten and lucerne hay is currently traded on the basis of basic nutritive value, such as protein or fibre, falling into various quality categories depending on the levels of these particular nutrients in the hay. Subjective criteria such as colour, texture, and botanical composition, as judged by a human observer, are also taken into consideration when quality is estimated. However, these traditional measures are not always reliable indicators of quality, meaning that the claimed “quality” of the fodder may not be reflected in the performance of the animals consuming it. For example, animals may reject so-called “good quality” hays for reasons unknown to the producer, resulting in reduced intake and poor subsequent performance.

An animal’s responses to a feedstuff can be considered the ultimate measure of its quality. The animal’s immediate response to the presentation of a new feed is its level of voluntary intake, which depends in part on palatability. Palatability is a relative term, but to provide a quantitative measure of the acceptability of a feedstuff, a ‘*preference value’* can be obtained by describing the voluntary intake of one hay relative to an alternate hay also on offer. The Australian fodder industry is coming to view an animal’s preference of a particular type of hay as a more accurate estimation of that hay’s quality. However, this is not straight forward, as the preferences for (or against) a particular feed is likely to change as other components of the diet, the animal’s physiological status, or the animal’s environment change. That is, preferences (or palatability) for a feed is not fixed. Nevertheless, for intensively managed animals such as dairy cows and performance horses where delivery of feedstuffs is largely under human control, we may be able to identify key plant traits that consistently influence preferences between different ‘batches’ of a particular type of hay. Indeed, the industry desires a reliable method to predict the preference value of hays to improve the marketing of fodder (especially, but not exclusively, export hay) and to help define animal responses to a particular hay that is offered.

2 Diet selection and feed preferences

Animals can only demonstrate which food they prefer by which food they eat when given a choice. Thus the feed preferences of an animal can only be determined by monitoring their voluntary feed intake and diet selection when presented with a range of feedstuffs.

2.1 Regulation of voluntary feed intake and diet selection

Animals are commonly confronted with a wide variety of foods that contain concentrations of nutrients and toxins that can vary considerably, not just between plant species but even within different parts of the same plant. Nonetheless, animals usually manage to select and voluntarily consume amounts of feeds higher in nutrients and lower in toxins than the average on offer (Freeland and Janzen 1974, Provenza et al. 1998). This indicates that diet selection is not random (Newman et al. 1992, Illius and Gordon 1993) and that animals exhibit preferences for particular feeds that may better suit their requirements or simply be more 'palatable' than others on offer based on the animal's own innate preferences and/or previous dietary experiences.

There have been many theories proposed over the years to explain how the body regulates feed intake. Recent research has suggested that that the ability of an animal to make dietary choices and exhibit feed preferences are better understood as a learned process involving complex interrelationships between a food's flavour and its post-ingestive effects exerting psychological, physiological and metabolic response within the animal (Provenza 1995 and 1996). Animals can recognise various feed characteristics prior to ingestion and use them as cues to make 'nutritionally wise' diet selection decisions that are based on communication between digestive processes and the central nervous system (CNS). The CNS can interpret signals and adjust responses in relation to nutritional requirements and current body stores (Blundell and Halford 1994; Figure 3).

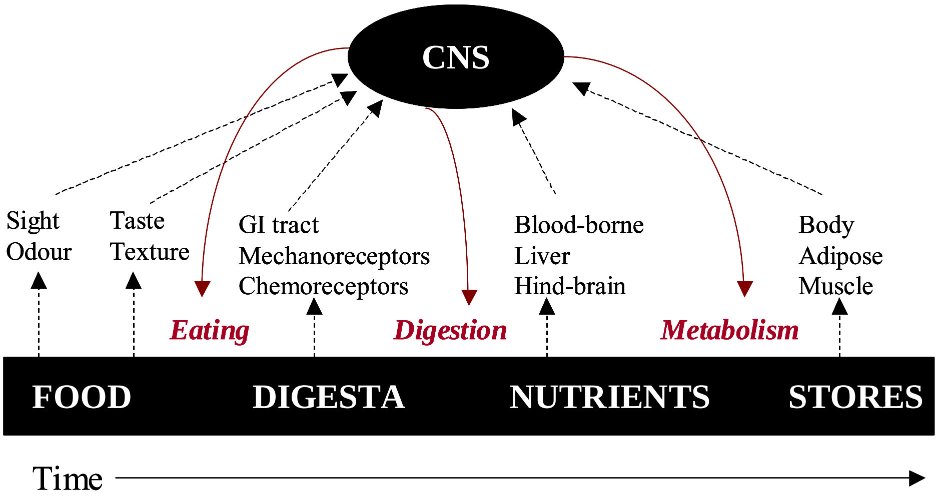

Figure 3. Satiety cascade (adapted from Blundell and Halford 1994) showing feeding behaviour as the central nervous system (CNS) exerting control at all points of feeding from eating, through digestion and metabolism, integrating signals from the food itself, the digesta, the absorbed nutrients and body stores.

Mayer (1953) proposed the "glucostatic theory", which hypothesised that the animal attempted to maintain a relatively constant blood glucose level through a central nervous monitoring system. The concept that the animal attempts to maintain the supply of energy to the body by feeding is logical and it has been observed that rats and other simple-stomached animals reduce their food intake when additional glucose is supplied. Ruminant feed intake, however, is unaffected by glucose infusions but it does respond to infusions of the volatile fatty acid propionate, presumably because the volatile fatty acid propionate produced by rumen fermentation is the main glucose precursor for fed ruminants (Forbes 1995). Balch and Campling (1962) hypothesised that the capacity of the digestive tract was an important limiting factor of feeding, especially for ruminants in which foods remain in the rumen for long periods of time undergoing fermentation. The “physical limit theory” showed a positive relationship between the rate and extent of digestion of a forage and its level of voluntary intake. The chemical and osmotic properties of the digesta also allow a degree of metabolic intake control. The “lipostatic theory” was hypothesised in 1953 by Kennedy who proposed a longer-term influence on diet selection and voluntary food intake, whereby the size of fat stores is monitored by the central nervous system (CNS), and that this information is used to selectively control and direct food intake.

The external environment presented to the animal can also influence voluntary feed intake. Changes in the thermal environment modify energy requirements and food intake normally responds accordingly. Brobeck (1948) hypothesised that animals eat to maintain constant body temperature and stop eating to prevent hyperthermia. There are heat receptors in the anterior hypothalamus and peripherally in the skin. The “thermostatic theory” of food intake control hypothesises that the heat increments produced during digestion and metabolism of food could be used as a short term indicator of intake. Evidence to support this theory can be seen in a number of species when feed intake decreases in hot temperatures and increases in cold temperatures. Changes in photoperiod also affect feed intake, whereby intake of grazing herbivores generally declines with declining day length, where the short days are coincident with a shortage of food (McDonald et al. 1995). This is thought to be a survival mechanism to prolong the limited supplies of food during critical periods of the year. This behaviour is most evident in deer and to a lesser extent sheep, whereas cattle do not seem to be as affected by changing photoperiod (McDonald et al. 1995). Feed intake in horses is also affected by day length (Kern and Bond 1972), but it is not linked to season (Dulphy et al. 1997).

There is also a strong body of evidence supporting the theory of “choice feeding” to maintain nutritional homeostasis. That is, the body sends signals regarding its nutritional requirements at any given time and the animal sets out to select a diet that best meets these requirements in order to remain metabolically comfortable. Nutritional requirements change in response to a wide variety of factors and this presents a dynamic challenge for the animal to meet at each meal. Requirements can change quite dramatically during periods of growth, stages of pregnancy, stages of lactation, as a result of deficient or excess mineral/vitamin supply, or because of the presence of gastrointestinal parasites, external parasites and/or other infections. There is a substantial amount of evidence (Emmans 1991; Illius and Gordon 1993; Forbes 1995) to support the concept that animals are capable of choosing a balanced diet from two (or more foods), one of which has more, the other less, of an essential nutrient that is required for optimum metabolism. For example, Hills et al. (1999) showed that sheep replete or deplete in sulphur when offered foods with different contents of sulphur. Replete sheep, given high and low sulphur foods, initially ate at random but within two days reduced the proportion of the high sulphur food to achieve a sulphur concentration in the total diet very close to that thought to be optimal. Conversely, the sheep deplete in sulphur initially ate a high proportion of the high sulphur food but later reduced their high sulphur intake until it stabilised at the optimal level. A study by Villalba et al 2008, demonstrated that sheep can develop learned appetites' for calcium, phosphorous and sodium, and self regulate their intake in order to meet their requirements.

Feeding behaviour is regulated on a short-, medium- and long-term basis. Generally short-term factors influence feeding behaviour and diet selection prior to ingestion, namely plant visual, olfactory, flavour and tactile attributes (McDonald et al. 1995 and Faverdin et al. 1995). Medium-term factors refer primarily to signals related to immediate ingestion responses, including stretch and mechanical receptors in the stomach and/or rumen wall. Long-term regulation signals are generally related to post-ingestive processes, nutrient demands due to physiological status and current body reserves (Faverdin et al. 1995). Medium- and long-term regulatory factors are most commonly described as being derived from the animal, whilst short-term regulatory factors are more often derived from dietary factors. An animal's future feed preference and aversions are established by way of sampling experience indicating that short-term responses to feeds can be good predictors of longer-term selection (Provenza and Balph 1987). However, in some circumstances the factors that influence long-term feed preferences can override the signals stimulated by short term factors (Romney and Gill 2000).

The primary feeding centre in the brain is situated in the cerebrum. It comprises two main centres of activity: first, the feeding centre located in the lateral hypothalamus, which drives an animal to eat unless inhibited by the second, the satiety centre in the ventromedial hypothalamus, which upon signalling from the body after consumption of food, negatively feeds back to curtail appetite (McDonald et al. 1995). It is here that signals prior to and during consumption, during and after digestion are all collected, integrated and ultimately stored in the animal's memory.

The brain receives signals from the body regarding levels of current fuel stores (glycogen and adipose tissue) and the animal's current physiological status (eg. pregnant, lactating, etc.) which dictates nutritional requirements. Those signals, if adequate, stimulate appetite and cause the animal to seek food. The animal's peripheral physiology and special senses (smell, vision, taste, and mouth and muzzle texture sensors) relay information to the brain about the environment and the food in front of the animal prior to ingestion, influencing short-term control of food intake and diet selection and allowing the animal to make a preliminary judgement as to the suitability of the food. After ingestion, physical and metabolic signals sent via neural pathways from the viscera relay post-ingestive information, such as stomach acidity, concentrations of absorbed nutrients, abdominal temperature and distension of various gastrointestinal parts, to the brain (Blundell and Halford 1994). These post-ingestive feedback responses that occur after consuming a particular food are commonly referred to as long-term influencers of intake and diet selection (Forbes 1995). These short- and long-term signals are delivered to and integrated in the brain, causing a corresponding cascade of hormones, enzymes and other secretions that affect the animal's behaviour, digestive processes, feeding patterns and forage acceptability (Figure 4).

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Figure 4. Relationship between forage characteristics (short-term factors), physical and metabolic post-ingestive signals, feeding behaviour and forage acceptability (adapted from Lindstrom 2000)

2.2 Significance of voluntary feed intake

The daily rate of food intake is the single most important factor affecting animal performance and productive efficiency (Illius et al. 2000). Knowledge of food intake is necessary for formulating diets, predicting animal performance and designing and controlling production systems. The consumption of feed is the first step in the process which converts feed into milk and meat for human consumption (Ketelaars and Tolkamp 1992), or energy for expenditure in the case of elite performance animals such as race horses. The amount of feed animals voluntarily consume has a profound impact on the efficiency of this conversion process.

The introduction of new feeds and sudden changes in diet can alter voluntary feed intake (Figure 5). After experiencing a sudden change in diet from oaten chaff to barley straw, sheep dramatically reduced their voluntary intake of the new feed (Forbes and Provenza 2000). Animals experience neophobia on the presentation of a new food, and eat very little until they have ascertained the consequences of eating it. If the small amounts consumed have not caused illness, intake will increase steadily.

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Figure 5. Mean daily intakes for 32 sheep (solid line) and two individual sheep (lines with symbols), where on September 6th the oaten chaff was replaced with barley straw (Forbes and Provenza 2000)

In general practice, dairy cattle are an intensively managed animal, presented with a variety of feedstuffs throughout each single day, in addition to changing feedstuffs throughout their productive lifetime. The main feedstuffs are fodder (usually hay) and pasture whilst grazing, as well as various concentrates. Horses by nature are a free ranging herbivore adapted to eating large volumes of high-fibre feeds (Bennet 1980). However the vast majority of horses are stabled for prolonged periods and their diet consists of offered fodder (again usually hay or chaff) and concentrates. Both the dairy cow and the horse are highly ‘managed’, with their supply of nutrients more often than not regulated by the management regime devised by the owner.

In situations where highly productive animals are presented with a range of feedstuffs, some of them novel, for discrete periods of time, it is important that they readily accept the feeds when they are first presented to them. For both lactating dairy cows and performance horses, for example, it is important to avoid short periods of low intake, as this can have immediate and sometimes longer term consequences to animal performance. Ulyatt and Waghorn (1993) and Muller (1993) indicated that limitations to cow productivity in pasture-based dairy systems often arise from a low voluntary intake of fodder. Dalley et al. (1999) showed that milk production was directly proportional to intake of herbage (Figure 6). Elite performance horses have an energy expenditure well over maintenance and often have suboptimal feed intake. Racehorses in training may only receive short and discrete feeding periods and the promotion of intake is critical to achieving the high levels of athletic performance demanded (Gallagher and Hughes 1993).

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Figure 6. Relationship between herbage allowance and milk production (■ , solid line) and potential milk production without liveweight change (x, dashed line) of cows grazing rainfed perennial pastures in spring (Dalley et al. 1999).

2.3 Factors affecting diet selection and feed preferences

An enormous range of factors influence voluntary feed intake and diet selection. Generally those factors are derived from one of three categories: (1) management, housing and environmental factors, (2) animal-derived factors and (3) feed-derived factors (Ingvartsen 1994).

2.3.1 Management, housing and environmental factors that influence diet selection and feed preferences

Management and housing factors relate to the influences of such things as: length of time and time of day when given access to feed; the frequency of feeding; the type of feeds and the combination and order of diets fed (eg. separate forage and concentrate versus total mixed rations); the type of housing (eg, grouped versus individual housing and pens versus stable boxes versus paddocks); stocking densities that determine the ease with which an individual can access feed (Ingvartsen 1994). Environmental factors include photoperiod, humidity and temperature (Ingvartsen 1994).

2.3.2 Animal-derived factors that influence diet selection and feed preferences

Animal-derived factors that can influence longer-term diet selection and feed preferences are generally those things that influence nutrient requirements: breed, sex, live weight, body condition, age, parity, disease status, level of production, pregnancy, lactation and digestive physiology (eg, monogastric versus hind-gut fermenter versus ruminant). The need to consume a particular array of nutrients to sustain oneself, cause animal’s to seek and eat particular amounts of various feedstuffs. As previously discussed, ingestion and subsequent digestion of food causes changes in the body, which are monitored by the CNS and used to determine when feeding should cease. These changes and the routes by which information concerning them is carried to the brain are referred to as negative feedback pathways.

Upon ingesting a meal, mechano- and chemoreceptors throughout the stomach and intestines relay information to the brain regarding the extent and stage of digestion. In order for gut capacity to impose a physical limit on feeding it is necessary for there to be mechanoreceptors in the stomach/rumen wall with afferent fibres connecting to the central nervous system. The rumen is innervated and Leek and Harding (1975) have described rapidly adapting mechanoreceptors in the epithelial lining of the reticulorumen. There are slowly adapting tension receptors within the muscle layers of the entire gastrointestinal tract, which are of particular importance in the control of gastrointestinal motility.

Chemoreceptors in the gastrointestinal tract also respond to chemical stimuli, primarily to changes in pH and nutrient concentrations, and are critical in maintaining optimal rumen and gut function. Of particular importance is the need for ruminants to maintain a stable rumen environment if the resident microbes are not to be threatened. To this end, ruminants to a certain extent exhibit an appetite for “effective” fibre (Campion and Leek 1997). Given free choice between forage and concentrates, most ruminants will take about 20% of their dry matter intake as forage. Campion and Leek (1997) offered sheep a diet free of long fibre and found that the animals ate a significant amount of fibre when it was made available separately. They also found that a polyethylene fibre pompom, introduced via a rumen cannula and placed so as to brush the reflexogenic areas of the reticulorumen, reduced voluntary intake of polyethylene fibre. Of equal importance is the need to maintain stable rumen conditions (Forbes 1995). Sheep decrease their consumption of high-energy feed whilst maintaining their consumption of low-energy feed, when infused with acid or alkali, in order increase rumen osmolarity and achieve stable rumen conditions (Engku Azahan and Forbes 1992 and Cooper et al. 1996). Additionally, the inclusion of sodium bicarbonate ($NaHCO_3$) in the diet of sheep given a choice between high- and low-energy density feeds, increased the proportion of the high energy feed selected (Cooper et al. 1996). This suggested that the $NaHCO_3$ was able to balance the low rumen pH resulting from the rapid fermentation of the energy dense feed. When fed diets of varying grain content, ruminants self regulate their intake of $NaHCO_3$ in order to maintain the stability of conditions in the rumen (Phy and Provenza 1998). In the racehorse industry concentrates are fed to meet the additional demands of extensive exercise and of the still growing young race horses. However this enforced pattern of offering energy-dense (eg, wheat, barley, oats) or protein-rich (soya, peas, beans) concentrates is opposite to the horses natural dietary adaptations and has implications on digestive tract health, namely disturbances to gut microflora and potential onset of colic. Therefore, similarly to ruminants, horses must also consume an adequate amount of fodder, but are less sensitive than ruminants to physical appetite controlling mechanisms (Faverdin et al. 1995).

Recent evidence (Villalba and Provenza 1997, Kyriazakis and Oldham 1997, Hills et al. 1999, Villalba and Provenza 1999, Villalba et al. 1999 and 2002, Villalba and Provenza 2000, Provenza et al. 2003, Villalba et al. 2006, Villalba et al. 2008) has given strength to the argument that animals learn to associate the post-ingestive consequences of eating a food with the sensory properties of that food. Furthermore, animals can then use such conditioned preferences and aversion to direct their selection between foods in order to maintain an optimal balance of nutrients in their diets. That is, an animal will develop a learned preference for particular food flavour when paired with a nutrient addition that corrects deficiency and conversely, when the added nutrient is given in excess it will lead to an avoidance of the associated food flavour. Villalba and Provenza (1999) gave lambs one flavour paired with rumen infusions of starch (2.5-9.4% daily digestible energy intake) and another flavour with the control. Subsequent preference was strongly for the starch-paired flavour, even up to eight weeks after the infusions had stopped. Lithium chloride (LiCl) has been used widely throughout conditioned aversion studies, as sheep find LiCl, injected or in the feed, to be highly toxic and unpleasant. It induces a conditioned taste aversion, the strength of which is proportional to the dose administered (Dutoit et al. 1991). Feeding neophobia (a fear of "new things") also increases as a function of LiCl dose associated with the last novel food encountered. These conditioned preferences and aversions can help the animal to make choices between feeds in order to avoid excessive intakes of toxins and to ensure adequate intakes of essential nutrients. It's important to note that all dietary compounds are capable of acting as toxins, if present in great excess above the required levels (Provenza 1995).

The preference for the flavour of a food decreases after an animal eats the food to satiation (sensory-specific satiety) and the degree of that decrease depends on the nutritional characteristics of that food (nutrient-specific satiety; Provenza 1996). Post-ingestive feedback thus calibrates a food's flavour (aroma and taste) with its homeostatic utility. For example, foods containing either inadequate or excessive levels of nutrients or toxins may be avoided more strongly following a meal, than foods that are adequate nutritionally.

2.3.3 Feed derived factors that influence diet selection and feed preferences

Even before feed is consumed, there are a number of short-term factors that influence an animal's behaviour towards a feedstuff, predominantly plant sensory factors including visual structure, smell, taste and texture (McDonald et al. 1995). These sensory stimuli are relayed to the central nervous system where they are integrated and an impression, positive or negative, is developed in response to the stimulus. A sensory map is established and learned responses to foods with particular characteristics are generated.

Visual discrimination between foods is more highly relied on by avian species, where mammals tend to rely more heavily on taste and smell (Werner et al. 2008). Despite this however, ruminants have an acute sense of vision (Piggins 1992), and are able to distinguish between different hues of colour (Riol et al. 1989, Uetake 1991). A number of mammalian species can be trained to associated inanimate objects with food rewards. Forbes (1995) describes sheep that were trained to associate coloured non-food objects with food rewards. He also showed that nerve cells responsible for receiving signals from the sight of food, located in the lateral hypothalamus, will respond to palatable food but not to foods sheep find unpalatable (Forbes 1995). When sheep ate excessive amounts of rolled oats, resulting in stomach upsets, the firing rate of their sensory neurons that stimulate appetite decreased when the animals were presented with rolled oats a second time (Forbes 1995). This suggests that the integrating centres of the brain respond more strongly to rewarding aspects of stimuli rather than negative ones.

The animal's visual interpretation of the spatial arrangement of the forages can also affect preference. High bulk density forages are generally preferred, by grazing animals in particular, as they alter the rate of consumption and total dry matter intake (Distel et al. 1995). Animals can use vision to locate and make some selection of feeds at a distance and once close enough, can make more detailed selections by smell and taste. Bazely (1988) showed that sheep can use sight to locate patches of forage created in monocultures of perennial ryegrass (*Lolium perenne* L.) which are more nutritious than the background sward. In an experiment where sheep were allowed to choose between a dark green and a light green tray of perennial ryegrass, sheep had a significant preference for the darker green grass (Bazely 1988). Rather than being able to distinguish between different hues of colour, Bazely and Ensor (1989) suggest that sheep are better able to distinguish between different levels of brightness. Brightness is correlated significantly with protein content in perennial ryegrass (*Lolium perenne* L.), suggesting that sheep can use brightness discrimination as a mechanism to select forages with higher protein content (Bazely 1988, Bazely and Ensor 1989). Cahn and Harper (1976) showed that sheep are capable of selecting clover based on leaf markings, indicating that they must also be able to distinguish patterns at a very fine level. Horses can also successfully discriminate between patterned visual cues (Mader and Price 1980).

When given sufficient access to food, animals will selectively eat to reach a nutritionally optimum diet, to the point where they will even choose between different parts of the same plant, such that their resulting intake is more nutritious than that which the overall composition of the forage would provide as a whole (Forbes 1995). The particle size of the food on offer also affects ease of eating. Chopping lengths of high fibre hay increased voluntary intake by cows, but did not alter the intake of better quality hays (Susmel et al. 1991). Ease of eating is likely to be correlated positively with digestibility and non-structural carbohydrate content of the fodder and inversely with shear force, structural fibre and lignin content.

There are several types of fibre in most forage plants. Lignin is indigestible and so its content is inversely related to digestibility but it has no consistent relationship with voluntary intake (Forbes 1995). Cellulose and hemicellulose are degraded by rumen microbes, but the rate of digestion is variable as is the time spent by the particles in the rumen, so no close relationship between digestible fibre and voluntary intake has been established. The cell wall fraction of forages can be determined using the neutral detergent fibre method (NDF), and Mertens (1973) found an almost constant intake of NDF in sheep for a wide range of grasses, suggesting that feed intake of ruminants is better correlated with the intake of NDF than with the weight of feed eaten. Dulphy et al. (1980) found that increasing the crude fibre of forages decreased food intake, with fewer meals and less time spent eating, as well as increased rumination. Although highly fibrous forages are eaten in lower amounts than those with lower NDF content, there is a danger of feeding too little fibre. The amount of milk fat produced decreases when the food on offer is too low in fibre (Forbes 1995).

Digestibility is the product of the retention time in the rumen, in the case of dairy cows, and in the hindgut, in the case of horses, and the degradation characteristics of the food, of which fibre (particularly the ratio of indigestible to digestible fibre content) is a major contributor. Hovell et al. (1986) demonstrated a close linear relationship between the potential degradability of the dry matter and the voluntary intake of four hays by sheep. The rate of degradation of forages in the rumen is largely dependent on microbial activity, which produces gas. Blummel and Orskov (1993) have shown a high correlation between total gas production and food intake.

Dietary protein content has little to no effect on voluntary intake when the protein levels of the food on offer are within the normal range. High producing dairy cows select a diet with a protein content that reflects their protein output in milk (Lawson et al. 2000). At low or very high protein concentrations, however, voluntary feed intake becomes depressed, most likely due to the metabolic discomfort associated with the post-ingestive consequences of consuming a feed with deficient or excess protein levels (Villalba and Provenza 1997a, 1997b, 1997c). Protein deficiency in ruminants reduces the activity of the rumen microflora and therefore the rate of cellulose digestion. Excessive levels of protein in the diet can lead to increased heat production from deamination of the excess amino acids and may depress intake if heat dissipation becomes limiting and body temperatures rises or if the products of deamination, namely ammonia, become toxic (Kyriazakis and Oldham 1993).

Sheep, beef cattle and dairy cows prefer forage with high total non-structural carbohydrate and high soluble carbohydrate contents (Fisher et al. 1999 and 1999, Orr et al. 1997, Ciavarella et al. 2000, Mayland et al. 2000). This preference may be related to higher concentrations of fructose that in the short-term the animal is able to detect through taste (sweetness). Dairy cows prefer tall fescue harvested in the afternoon over the same fodder harvested in the morning (Fisher et al. 1999, Mayland et al. 2000). Mowing the hay in the afternoon versus the morning effectively increased the content of non-structural carbohydrates. Orr et al. (1997) also identified a diurnal correlation between soluble carbohydrate content and feeding behaviour in sheep.

Research into the area of feed preferences and feed intake regulation is primarily focused on the ability of an animal to develop a learned association between a food’s flavour and its nutritional utility. Palatability has a major influence on feed intake in ruminants and sensory perception is highly developed in these animals (Albright 1993). Ruminants acquire preferences for flavours artificially applied to foods or solutions that meet their needs for energy and protein (Provenza 1995, 1996, Provenza et al. 1996). Lambs develop preferences based on administered flavour cues associated with reward incentives of intraruminal infusions of energy or protein (Villalba and Provenza 1997a, 1997b, 1997c, Villalba and Provenza 1999, Villalba et al. 1999). Lambs also more readily accept unfamiliar foods when fed with flavour cues in common with familiar foods. The preference of the unfamiliar food was then calibrated and adjusted according to its homeostatic utility, based on long-term post-ingestive feedback from nutrients and toxins present in the unfamiliar food (Villalba and Provenza 2000). That is, the association with the food’s flavour was either reinforced due to favourable post-ingestion or negated due to an unfavourable post-ingestion experience.

Little is known about the smell and taste responses of ruminants to the various naturally occurring chemical constituents found in herbaceous plants (Arnold et al. 1980). Plants contain a wide variety of chemicals, some of which stimulate appetite, whilst others deter feeding. Mammalian herbivores come into contact with these phytochemicals through their senses of taste and smell (Provenza et al. 2000). An experiment by Estell et al. (1998) showed an aversion of sheep to specific terpenes found on the leaf surface of Tarbush. Ruminants exhibited differential selection among plants with different quantities of epicuticular wax on the leaf surfaces and preference increased upon removal of leaf compounds with organic solvents (Estell et al. 1998). Volatile compounds can have an important influence on preference, but the compounds responsible have not been identified clearly. There is also evidence that some compounds in forage are rejected strongly. It is probable that several factors interact to determine the relative preference for any specific hay over another.

The chemical senses of taste (gustation) and smell (olfaction) provide a "quality control" checkpoint for substances available for ingestion. Stimulation of taste or smell receptors induces pleasurable or objectionable sensations and signals the presence of something to seek, such as a nutritionally useful (or good-tasting) food, or something to avoid, such as a potentially toxic (or bad-tasting) food (Sherwood 1997). The sensations of taste and smell in association with food intake can also influence the flow of digestive juices and affect appetite (Sherwood 1997).

The sense of smell (olfaction) is one of the most important means by which animals (including humans) receive information about the environment, and is much more highly developed in animals than in humans. It is well recognised that odours (or pheromones) are important in animal reproduction behaviour (in sheep, cattle and pigs, rodents and insects; Rekwot et al. 2001) neonate-mother interactions (eg, Distel and Hudson 1985), and detection of predators. The combination of olfaction with central nervous processing allows animals to develop learned behaviours based on associations between the sensory characteristics of feedstuffs and metabolic experiences. The next section, parts of which have been published previously by Pain et al. (2005) and Pain and Revell (2007), consider in greater detail the little understood role of odours in assisting herbivores to find, recognise and discriminate foods.

2.4 Importance of odour in the process of food selection

There are four reasons why detecting odours can be beneficial in the process of food selection. First, odour can be rapidly detected and thereby provide a means to influence feeding behaviour in the short term. Rapid decision-making may be important if a feed source is only temporarily available, such as a competitive feeding situation where other animals may consume the feed if one individual is not quick enough. Secondly, the decision to select or reject a particular feedstuff can be made without actually consuming the feed, and thereby avoiding the risk of toxicity. In an experiment on the capacity of roe deer to select between different plant species, animals used odour to recognise and avoid undesirable plants (arum and euphorbia) once they had learnt the consequences of eating these plants (Tixier et al. 1998). In the same way, once smelled, preferred plants were hardly ever refused. Thirdly, the physiology of odour detection allows animals to integrate a complex suite of odours that may reflect the biochemical composition of the food. Although animals can detect individual odorants, the way in which the olfactory system processes information also allows animals to 'generalise' the inputs to the central nervous system from a mixture of odours. Thus, the olfactory sense is able to distinguish among a practically infinite number of chemical compounds at very low concentrations (Leffingwell 2002).

Fourthly, neural processes link the detection of odour with memory, and hence the odour profile of a feedstuff can be used in learning and demonstration of learnt behaviour. These latter two points, relating to the physiology of odour detection, will be discussed in more detail in the following paragraphs.

The perception of gas phase molecules involves the combined function of the olfactory and trigeminal systems. The trigeminal system is responsible for the perception of sensations such as irritation, stinging, burning, tickling, warm, cool and painful (Doty and Commetto-Muiz 2003). Trigeminal perception occurs via free nerve endings in the nasal and oral cavities, with the nasal cavity being the more sensitive of the two (Silver and Finger 1991). Odorants are volatile chemical compounds carried into the nasal cavity with inhaled air and come into contact with the olfactory epithelium. Odorants dissolve in the mucous layer on the epithelium, in some cases aided by odorant binding proteins. The receptors are highly sensitive and act through a standard G protein cascade, causing cation channels to open and action potentials to be fired. Individual cells respond to a range of actual odours, each odour has a characteristic ‘fingerprint’ of activity across the entire epithelium. Odorant receptor (OR) genes comprise the largest gene family in the mammalian genome. Humans posses about 350 OR genes and 560 OR pseudogenes (Glusman et al. 2001), mice possess 1000-1300 OR genes, and other mammals may posses over 4000 receptors. Given that nearly three-quarters of human OR genes may be dysfunctional (Rouquier et al. 1998), we may be much less sensitive to smell than many animals, including livestock, and there is the risk that we have underemphasised the importance of odour detection in feed preferences of livestock.

A key feature of the physiology of odour detection is that each odour does not require its own receptor. Instead, it is the pattern of bound receptors in the olfactory epithelium that provide the brain with the information to recognise a specific smell. Only slight changes in the chemical structure of odours can activate different combinations of receptors. For example, octanol smells like oranges, but the chemically similar compound octaoic acid smells like sweat (Leffingwell 2002).

Research into the area of feed preferences and feed intake regulation have primarily focused on the ability of an animal to develop a learned association between additives that affect the sensory characteristics of a food and the ultimate nutritional utility of the food. However, little is known about the smell and taste responses of ruminants to the various naturally occurring chemical constituents found in herbaceous plants (Arnold et al. 1980). Arnold et al. (1980) showed evidence of differences in feeding behaviour due to odour, as various herbaceous odorants affected the food choice for normal sheep but had no effect on the food choice of anosmic sheep.

One of the most striking features of the work of Arnold et al. (1980) was the large short-term effects on feed preference due to a number of volatile compounds; eg, cedarwood oil, tannic acid, propionic acid, and glycine (Figure 7). These odours reduced feed preference during the first hour of testing and sometimes for the first six hours of testing, but over the ensuing three days the aversion was usually reduced. Of the odorants tested, the aversion remained significant for 72 hours only for glycine. This suggests that some odours act as powerful regulators of short-term preference, but if the odours are not associated with any 'metabolic discomfort' (Forbes and Provenza 2000), the aversion is temporary. As many other novel odours used by Arnold et al. (1980) did not induce this short-term aversion, the result summarised in Figure 7, cannot be attributed to a general aversion to a novel smell (neophobia). The odours that did not affect feed preference presumably did not trigger the right combination of odorant receptors to be recognised by the central nervous system as a cue to avoid the food. Arnold et al. (1980) also provided evidence that plant odour can markedly effect voluntary feed intake in ruminants, not just feed preference, as feed intake over a series of 18 three-day periods was significantly increased in the presence of butyric acid and amyl acetate, but significantly decreased in the presence on coumarin and glycine.

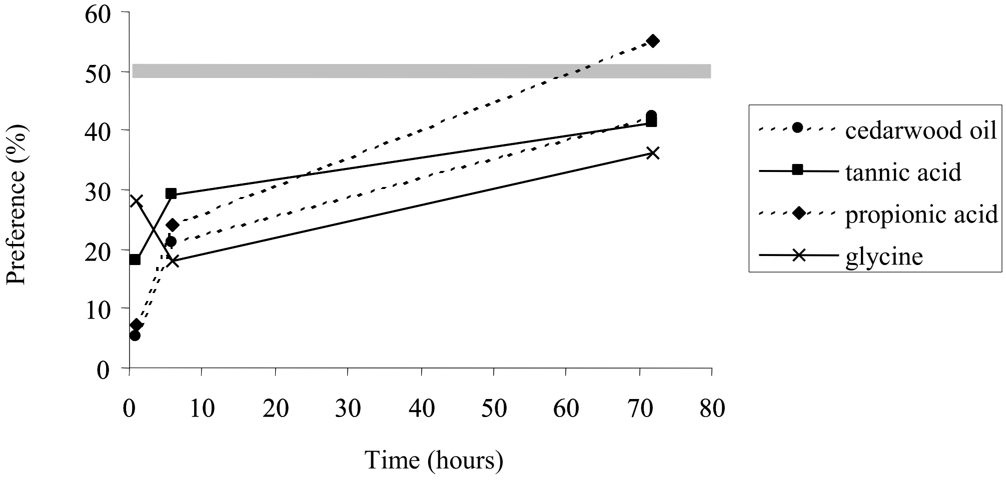


Figure 7. Changes in feed preference of sheep in response to the odours from four compounds (taken from Arnold et al. 1980). The horizontal shaded bar indicates a preference value of 50%, which implies no preference for or against the odour.

In grazing herbivores, odour is used strategically in feed selection. Field experiments often highlight the phenomenon of marked selectivity of animals towards plant species or components. Cattle grazing eight endophyte-free tall fescue cultivars moved through the different plots with their muzzles in the forage canopy, occasionally taking a bite, apparently detecting volatile components (Shewmaker et al. 1997). Mayland et al. (1997) aimed to quantify compounds emitted from fresh tall fescue cultivars that differed in grazing preference. Preference scores were significantly correlated (r^2= 0.97) with emissions of 6-methyl-5-hepten-2-one, (Z)-3-hexenyl propionate and acetic acid.

Cox (2004) showed oaten hay preference (short or longer-term) was not related to any single nutritive value trait except crude protein content (r^2=0.72). However, preference was strongly related to the abundance of two volatile compounds emitted from the hay (Table 1). One of the volatile compounds was negatively correlated (r^2=0.77) to both preference and crude protein content of the hays, suggesting that the horses may have used the odorant to identify and avoid low-protein hays. Such a phenomenon would be consistent with the finding that rats can self-select for dietary protein based on olfactory stimuli (Heinrichs et al. 1990). This compound has not yet been identified, but may be naphthalene. The other volatile compound of interest was positively related to hay preference based on its gas chromatograph spectra (r^2=0.83). The chemical identification of this compound is also yet to be confirmed, but appears to be a decane. Decanes have been linked to odours from peaches that attract insects (Natale et al. 2003), and it is conceivable that horses also find the odour attractive, or it is positively related to a favourable nutritional trait of the hay.

Table 1 Correlations between volatile compounds with gas chromatography retention times of 5.59, 19.13 minutes (RT 5.59 and RT 19.13), crude protein content and preferences of hays by thoroughbred horses (Cox 2004).

NOTE:
This table is included on page 19 of the print copy of the thesis held in the University of Adelaide Library.

In complex situations where animals are presented with a wide range of feedstuffs, each with multiple stimuli (positive and/or negative), it has been proposed that animals will require substantial pre-ingestive cues to perceive the whole value of a given food (Ginane et al. 2005). Given that herbivores select diets that are higher in nutrient concentrations and lower in toxin concentration than the average available plant material, it follows that the pre-ingestive cues must relate to post-ingestive consequences (Provenza 1995). There will be survival and production advantages in animals being able to detect antinutritional factors from the odour profile, and indeed many antifeedants are volatile, even at low temperatures (Bryant et al. 1992). For example, volatile sulphur compounds can deter feeding due to the association with predator odours (Bullard et al. 1978, Nolte et al. 1994, Provenza et al. 2000). Volatile compounds can be inhaled directly into the lungs and transported into the bloodstream, posing a direct risk of toxicosis at high concentrations and long exposures.

Less attention has been directed to considering whether the odour fingerprint of plant material is used by animals to help select plants (or plant components) of high nutritional value. Possessing this skill would provide production advantages to animals in the same way that being able to detect and avoid antinutritional factors would be beneficial. Volatile organic acids detected by an animal's olfactory system may provide a valuable cue to the energy content of the feed (eg, the odour from butyrate was found to be favourable by sheep; Arnold et al. 1980) and aromatic amino acids may also be useful indicators of the protein content. Linking the odour fingerprint to the nutritional value of forages or fodder warrants further investigation. Whilst olfaction is only one of many senses used to evaluate feeds, it may be a powerful driver of diet selection and learning behaviour in grazing/foraging herbivores.

Taste and smell combined are responsible for the generation of food flavour, a powerful cue with which the animal can learn to associate the nutritional properties of food. Villalba et al. (1997a and 1999) showed that lambs prefer flavoured wheat straw when accompanied by an energy reward in the form of intraruminal infusions of starch. Conversely lambs showed an aversion to flavours associated with high concentrations of the volatile fatty acids (VFA), propionate and acetate, the primary end products of ruminal fermentation (Villalba and Provenza 1997c), presumably because excess VFA ultimately lead to a decrease in ruminal pH. Cattle are sensitive to bitter, sour, sweet and salty tastes (Goatcher and Church 1970) to a greater degree than sheep, showing a general preference for sweet flavours in the form of added molasses, licorice or milk chocolate buds. Gherardi et al. (1991) studying the short- and long-term responses in intake of sheep to additions of chemical thought to influence preference, concluded that palatability effects are not important in determining the level at which a single forage is eaten, but can have marked effects on the relative intakes when two forages are on offer. Thus, although flavours are able to initially influence preference and intake they soon lose this ability if not accompanied by some sort of nutritional distinction.

Horses have specific taste preferences that may affect food intake. Randall et al. (1978) showed a preference for sucrose ("sweet") solutions between 1.25 and 10 g/100mL over plain water, whilst concentrations above and below this range were treated indifferently. Low concentrations of sodium chloride ("salty"), acetic acid ("sour") and quinine ("bitter") flavours were also treated indifferently, but high concentrations were rejected. Olfaction also plays a significant role in the feeding behaviour of horses. Horses avoid feed and pasture areas contaminated with faeces, possibly on the basis of odour alone. Ott et al. (1979) found that adding a citrus odour resulted in the strong rejection of the feedstuff by 75% of the horses.

Whilst in the main animals are capable of learning to associate the post-ingestive consequences of eating a particular food with the sensory properties of that food and they use these conditioned preferences and aversions to direct their selection between foods (Forbes and Provenza 2000), horses appear less well adapted than other animals in regard to learned aversions. In some circumstances horses continue consuming palatable feeds regardless of the consequences (Zahorik and Houpt 1981, Ralston 1983). Pfister et al. (2002) observed that some horses would not develop an aversion to particular palatable foods even after pairing with repeated doses of lithium chloride. It could be argued that this is a result of the general management and lifestyle of the majority of horses kept domestically. This lifestyle is largely unlike cattle or sheep, where animals are reared and live the majority of their lives in social groups and have relatively free access to forage, and are able to select their own feedstuffs. Social interaction and learning by watching and mimicking parents and other group members is very valuable in relation to developing a diet history and memory to aid lifelong diet selection. An individual can benefit from the nutritional wisdom of its social counterparts and in that way expand its own diet memory. Horses removed from their mothers at an early age and reared individually or in small groups, with highly "managed" feed sources, may not develop as broad a range of learned dietary associations as animals routinely kept in large herds, having a direct impact on the whole-life preferences of the horse. Research by Provenza et al. (1993) supports the theory that there is strong cultural transmission of food preferences from the mother to the offspring.

3 Use of NIRS technology in quality assessment of fodder

Although there are several factors that influence animal productivity, the nutritional quality of feed consumed stands out as one of the most important factors. Forage analysis by near infrared spectroscopy (NIRS) has attracted a great deal of interest since the 1970s. The near infrared (NIR) region of the electromagnetic spectrum extends from the red end of the visible region at 800 nanometres (nm) to 2500 nm (Murray 1993). Norris (1965) first used NIRS to measure cereal grain moisture content. Further investigation showed it was possible to measure both moisture and protein in wheat simultaneously using NIRS (Williams and Norris 1982). The use of NIRS technology was not applied to forage plants until 1976 when Norris et al. (1976) demonstrated that it was possible to use computer-assisted NIRS to estimate forage composition and even quality attributes associated with the animal's response to a diet, such as digestibility and intake. Several further studies using sheep (Ward et al. 1982) and cattle (Holechek et al. 1982, Abrams et al. 1987) added credence to the use of NIRS to estimate animal responses to forages. Indeed, Abrams et al. (1987) showed that NIRS could more accurately predict animal responses, such as voluntary feed intake, than any single reference laboratory method at the time or any combination of these traditional methods.

The strength of NIRS comes from its ability to provide an integrated profile of the chemical bonds in the forage material being tested. Given that an animal's response to a particular feedstuff does not depend on a single chemical entity, but rather the incorporation and balance of a number of feed characteristics, the ability of NIRS to incorporate many different wavelengths (at which each feed characteristic might absorb different levels of energy) into a single test, allows certain animal responses to be predicted with speed and precision.

4 Scope of project

In an increasingly competitive fodder market it is now acknowledged that (i) more objective measures of hay quality are needed, and (ii) ultimately, hay should be traded on the basis of feeding value, i.e. in terms of animal behaviour towards the feed and subsequent performance. Feed preferences originate from the functional interrelationship between smell, taste and sight and post-ingestive feedback. This interrelationship is dependent on the animal's physiological condition (pregnant, lactating, working, etc) and a food's chemical characteristics (Provenza 1995, 1996, Provenza et al. 1996). An animal uses the senses of smell, taste and sight to discriminate among foods and the feedback from ingested nutrients and/or toxins calibrates the smell, flavour, visual and texture sensations to a food's homeostatic utility. This enables an animal to acquire preferences for foods that are nutritious and become adverse to foods that are deficient in nutrients or toxic (Provenza 1995, 1996, Provenza et al. 1996). Animals must learn to associate sensory perceptions of food to their nutritional value because sensory receptors operate at a molecular level and do not respond to combined fractions such as protein, soluble carbohydrates, fat and fibre (Arnold et al. 1980). Thus an animal must be able to seek out and identify food with the desired protein, energy and fibre content indirectly by using learned association with various sensory cues.

However, in situations where animals are intensively managed their ability to select an adequate diet can be substantially compromised. Dairy cattle are one such intensively managed animal. They are presented with a variety of feedstuffs throughout each single day, in addition to changing feedstuffs throughout their productive lifetime. Also the vast majority of horses, which by nature are a free ranging herbivore adapted to eating large volumes of high fibre feeds (Bennet 1980), are intensively managed and stabled for prolonged periods of time. Both the dairy cow and the horse are a highly “managed” species, meaning that their supply of nutrients is more often than not regulated by the management regime devised by the owner. In situations where highly productive animals, such as lactating dairy cows and performance horses, are presented with a range of feedstuffs, some of them novel, for discrete periods of time, it is important that they readily accept the feeds when they are first presented to them. Reductions in feed intake due to neophobia or palatability can have immediate and longer lasting impacts on an animal’s production and performance. Understanding the role of specific plant chemicals in the mediation of plant-animal interactions is crucial for developing ways to alter the behaviour and selectivity of livestock towards feed on offer. Once this is established, additional studies could link this information to the longer-term measures of animal production or performance.

The dairy industry is the single largest consumer of fodder in both the export and domestic markets, with oaten hay being the dominant hay consumed by dairy cows domestically and exported to dairy enterprises overseas. The horse industry is the second largest consumer of hay within Australia, consuming large quantities of oaten hay but also relying on lucerne hay as a source of high quality roughage. Given their relevance and importance to industry, the following experimental work will focus on these two particular animal species and these two particular types of fodder.

Thesis of Work

5 Preamble

The domestic and export markets for oaten and lucerne hay are driven largely by the quality of hay produced. In order for Australia to compete in an international market, a clear, simple and uniform objective quality grading system for the marketing and use of hay should be established. The project reported here aimed to quantify preference values for a large number of oaten hays with dairy cows and horses and a similarly large number of lucerne hays with horses only, and to develop predictive equations for animal preferences based on the chemical (nutritive and volatile) and physical properties of hays. The dairy industry is the single largest consumer of fodder in both the export and domestic markets, with oaten hay being the dominant hay consumed by dairy cows domestically and exported to dairy enterprises overseas. The horse industry, within Australia, is the second largest consumer of hay. Horses consume large amounts of oaten hay, but also rely on lucerne hay as a source of high-quality roughage.

At present the assessment of fodder quality relies heavily on plant of origin, subjective criteria, such as colour, odour and general appearance and, on occasion certain analytical parameters such as protein, fibre and energy content. In an increasingly competitive market it is now acknowledged that (i) more objective measures of hay quality are needed and (ii) ultimately, hay should be traded on the basis of feeding value in terms of animal response and performance. The broad aims of the project detailed in this thesis are first, to investigate the characteristics of hay that affect its “acceptability” by animals, and second, to develop a method to predict an animal’s preference using those characteristics. For the purpose of this work, “acceptability” is quantified as a preference using a function of the relative intake of hay by animals offered a choice. The chemical and physical traits of the hay can then be related to this observed short-term preference behaviour. Once this is established, additional studies could link this information to the longer-term measures of animal production or performance.

5.1 Aims and objectives

The specific aims of the project reported here were to develop reliable predictors of ‘preference values’ for oaten hay consumed by lactating dairy cows, and oaten and lucerne hay consumed by horses, based on a “fingerprint” (or profile) of chemical and physical characteristics of the fodders. Additional objectives of this work were to compare oaten hay preference values and prediction equations for lactating dairy cows with those for horses and ultimately to deliver industry-acceptable approaches to predict the preference values of oaten and lucerne hay.

5.2 Thesis structure

This thesis is presented with all of the experimental methodology described in Chapter 6, covering all the work conducted with oaten and lucerne hays, and dairy cows and horses. All the results are presented in Chapter 7, in sequential sections covering the chemical, physical and volatile traits of oaten and lucerne hay and their influence on the preferences of dairy cows and horses. A discussion of specific findings and the broader general implications of this work are presented in Chapter 8.

6 Experimental Methodology

6.1 Quantifying preference

The term “preference” implies a behavioural trait exhibited by an animal whereby it selects something preferentially over another. With regard to the influence of preference on diet selection, an animal would be regarded as preferring one feed over another if an encounter with that particular feed were more likely to result in an act of voluntary intake than an encounter with the other. In this sense “preference” is a qualitative measure; however, it can be rendered quantitative by defining an animal’s preference for a particular set of feeds as its likelihoods of accepting those feeds when on offer.

The term “preference” used throughout this report refers to the measure of preference shown for a “trial” hay when fed against a “standard” hay, expressed in a log-ratio form, determined from the natural logarithm (LN) of the ratio between the trial hay eaten and the standard hay eaten:

$$\text{Preference} = \text{LN}\ [(\text{total trial hay eaten}) / (\text{total standard hay eaten})]$$

An equal preference corresponds to a preference log-ratio of zero. Negative numbers correspond to low preferences and positive numbers correspond to high preferences. Using the log-ratio transformation means that there is no constraining limits on the possible values of preference, unlike a percentage preference which would be limited to values 0 – 100 %.

In order to cope with occasions where one or both of the amounts of hay eaten is zero, a small constant was added to both numerator and denominator, to enable the logarithm to be calculated. The constant was the value 5; a number chosen to be less than the smallest unit measured to determine feed intake (10 g). Therefore the final calculation for the measure of preference that will be used for the remainder of this report is:

$$\text{Preference} = \text{LN}\ [(\text{total trial hay eaten} + 5) / (\text{total standard hay eaten} + 5)]$$

The log transformation allows for a more informative measure of preference than an untransformed value. For example, if an animal chooses not to consume either feed on offer, or if the animal does not select one feed preferentially over the other, the log-ratio returns a preference equal to zero (ie. equal preference). Further, in cases where an animal consumes only one of the two feeds on offer, suggesting a total aversion to one of the feeds on offer, the log-ratio calculation provides an indication as to the strength of that aversion. For example, if 0 g of a trial hay and 20 g of the standard hay were consumed, there is much weaker evidence that the trial hay is less preferable than the standard hay, compared to a situation where 0 g of a trial hay and 2000 g of the standard hay were consumed. The log-ratio calculation would give a preference value further from zero for the trial hay when more of the standard hay was consumed.

Henceforth, the term “preference” in relation to a ‘trial’ hay, refers to the log-ratio measure of preference, where a value of zero represents equal preference to the standard hay being simultaneously co-offered, positive numbers indicate that the animals favoured the trial hay over the standard hay, and negative numbers indicate the animals’ favoured the trial hay less than the standard hay.

6.2 Selection and preparation of trial and standard oaten hays

The selection criteria for the oaten hay used in the feeding trial was based on the nutritive value characteristics of *in vitro* dry matter digestibility (IVD), neutral detergent fibre (NDF) and water-soluble carbohydrates (WSC). NDF estimates total cell wall content in a feed and is predominantly cellulose, hemicellulose and lignin. WSC is a measure of the total soluble sugars present in the forage; including glucose, fructose, sucrose and fructans. This approach was taken in consultation with the RIRDC Fodder Advisory Committee. There were five target categories for IVD; 55, 58, 61, 64 and 67%. Within each IVD category, three target NDF contents were selected to cover the range available (i.e., low, medium and high), and within each NDF category, three target WSC contents were selected to cover the available range. In this way a ‘selection matrix’ was developed to cover the widest possible available range in each of the three traits (Table 2) and used to standardise the selection of trial hays from the 2003 and 2004 seasons.

Table 2. ‘Selection matrix’ used to select test hays in both the 2003 and 2004 seasons, categorising the broad range of values for IVD, NDF and WSC available.

IVD %DM	54-56									n = 9
NDF %DM	50-52			58-60			64-66			n = 3 in each cell
WSC %DM	~ 8	~ 20	~ 30	~ 8	~ 20	~ 30	~ 8	~ 20	~ 30	n = 1 in each cell

IVD %DM	57-59								
NDF %DM	50-52			56-58			58-60		
WSC %DM	~ 15	~ 22	~ 31	~ 15	~ 22	~ 31	~ 15	~ 22	~ 31

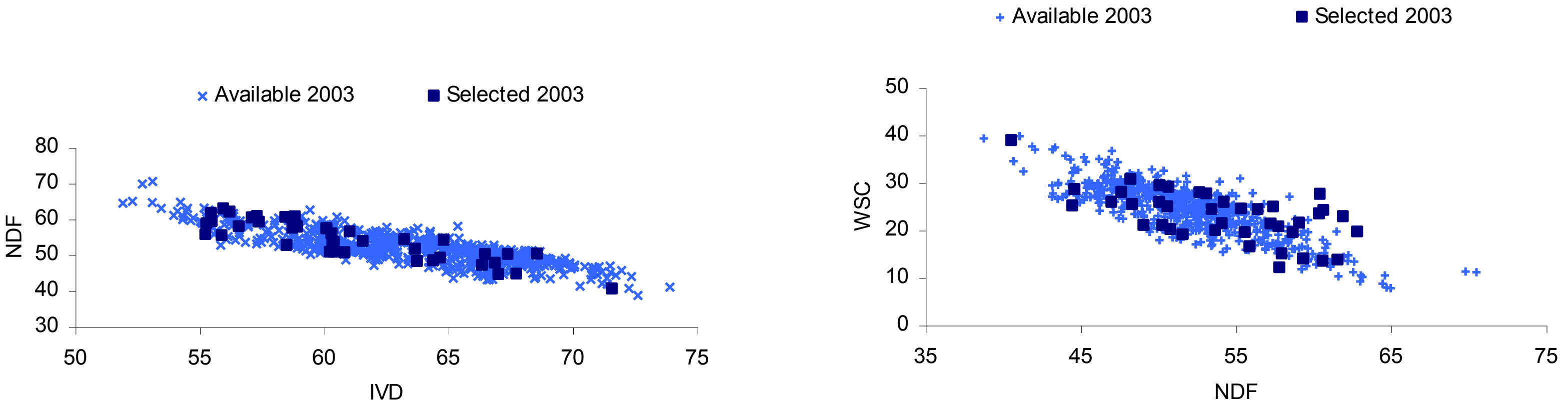
IVD %DM	60-62								
NDF %DM	48-50			52-54			56-58		
WSC %DM	~ 17	~ 24	~ 31	~ 17	~ 24	~ 31	~ 17	~ 24	~ 31

IVD %DM	63-65								
NDF %DM	46-48			50-52			54-56		
WSC %DM	~ 18	~ 25	~ 32	~ 18	~ 25	~ 32	~ 18	~ 25	~ 32

IVD %DM	66-68								
NDF %DM	46-48			48-50			50-52		
WSC %DM	~ 18	~ 25	~ 32	~ 18	~ 25	~ 32	~ 18	~ 25	~ 32

The nutritive value data for approximately 700 batches of oaten hay per season (i.e., 2003 and 2004 seasons) were made available by collaborating export companies (Balco Australia Pty Ltd and Gilmac Pty Ltd), from which the hays to be used in the trial were selected. There were 40 hays selected from the 2003 season and 45 from the 2004 season (Figure 8). The hays were sourced from South Australia (n = 49), Western Australia (n = 24) and Victoria (n = 12). The nutritive value traits of the 700 batches available relative to that of the selected oaten hays for each season are shown in Figure 8.

(a) 2003 Oaten hay season



(b) 2004 Oaten hay season

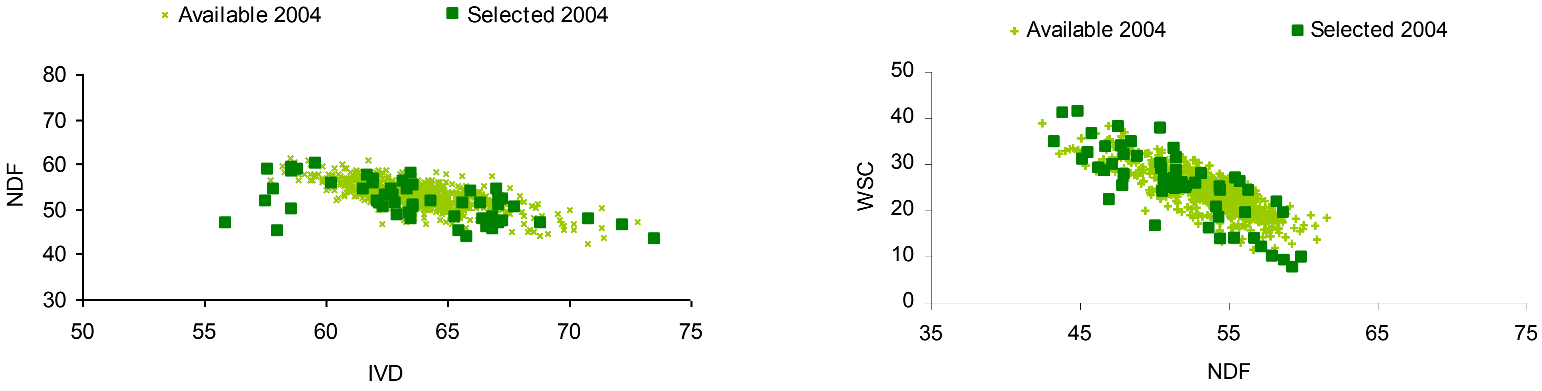


Figure 8. "Available" (x) and "selected" (■) trial oaten hays across the two seasons; **(a)** 2003 and **(b)** 2004

From the hay available in the 2003 season, five standard hays (A, B, C, D and E) were selected to possess a range of nutritive values that would represent the population of hays selected for the entire trial (Figure 9). The required amount of standards B and C could not be delivered due some bales being excessively weather damaged and therefore a combined (mixed) standard of B and C was made and the standards relabelled A, BC, D and E.

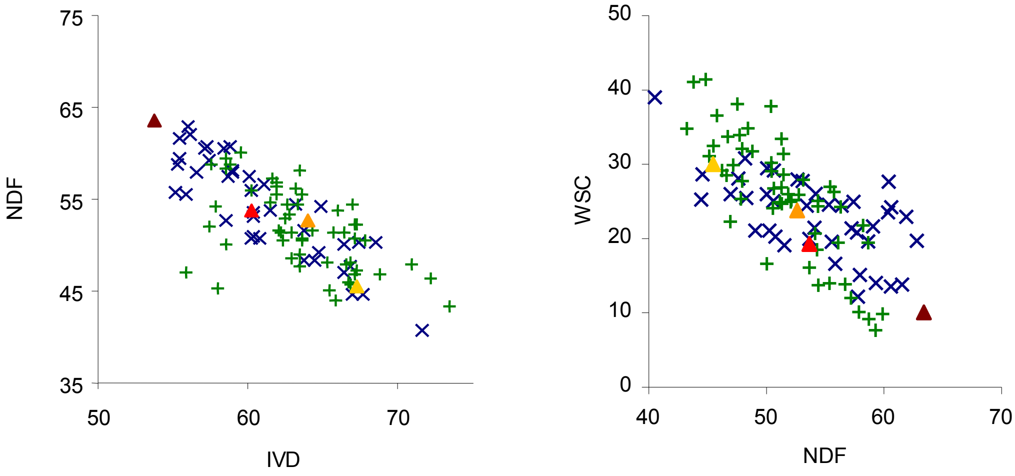


Figure 9. Standard oaten hays (A▲, BC▲, D▲ and E▲) selected in comparison to the trial oaten hays selected in the 2003 season (x) and 2004 season (+)

The intention was for the selected standard hays to provide a range of intake rate and preference values, as well as differing nutritive value, against which to test the trial hays. This was confirmed prior to commencing the main oaten hay feeding trial using both the lactating Holstein Friesian cows and the Thoroughbred horses. The nutritive value of each individual oaten hay was measured and confirmed during the course of the feeding trial.

The oaten hay was delivered as compressed bales to the Roseworthy Campus of the University of Adelaide. There were approximately six compressed bales of each trial hay and 72 compressed bales of each standard. To minimise individual bale variation, the group of bales comprising each of the trial hays and standards were mixed together by hand using pitchforks. The final mix was collected and stored in chaff bags until their use in the feeding trial. The chaff bags were stored in modified shipping containers to ensure the hay remained protected against weather and rodents.

6.3 Selection and preparation of trial and standard lucerne hays

It was originally intended that the selection of the lucerne trial hays would follow a similar approach as used with the oaten hay. However, due to a lack of readily accessible data describing the nutritive value of available lucerne hays, this wasn't possible. Instead, 50 hays were sourced and purchased from Fodder King Ltd, Victoria, across each of their five quality grades; stock, budget, medium, prime and choice (Table 3).

Table 3. Nutritive value range of Fodder King Pty Ltd quality grades of stock budget, medium, prime and choice.

	Nutritive value range advertised[1]		
Fodder King Quality Grade	**Protein %DM**	**Energy MJ/kg DM**	**Digestibility (IVD) %DM**
Choice	> 19 - 26	> 9.5 - 11.5	> 65 - 78
Prime	> 16 - 19	> 8.5 - 9.5	> 60 - 65
Medium	> 12 - 16	> 7.5 - 8.5	> 54 - 60
Budget	> 9 - 12	> 7.0 - 7.5	> 50 - 54
Stock	< 9	< 7	< 50

[1] values provided by the supplier as being the range that covers each quality category of luernce hay

An additional 20 hays were purchased from various fodder stores around South Australia, under the assumption that the different sources and variable visual characteristics would be associated with differences in nutritive value traits. In this way a total of 70 trial lucerne hays were selected that covered a range in quality and nutritive value (Figure 10).

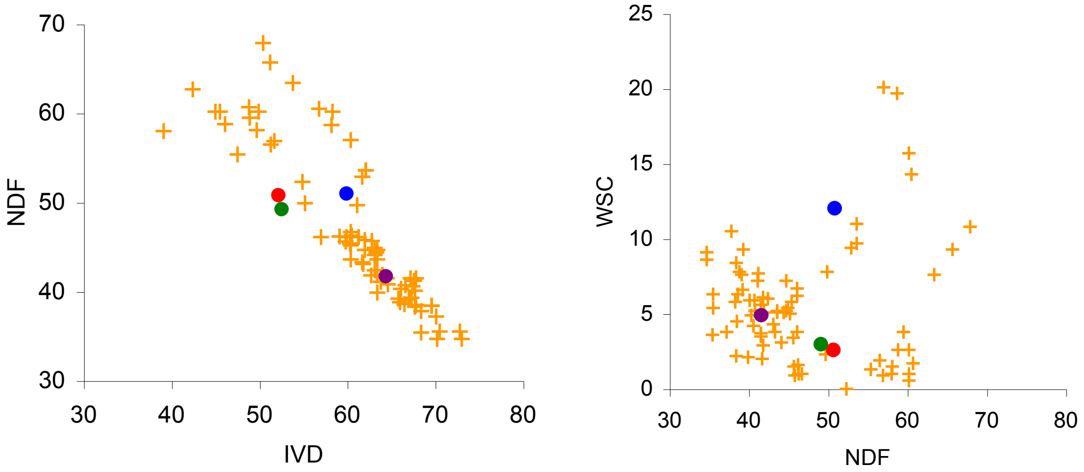


Figure 10. *In vitro* digestibility, neutral detergent fibre and water soluble carbohydrates of the selected trial (+) and standard lucerne hays (LA ●, LB ●, LP ● and LC ●)

Lucerne standard hays were sourced from Fodder King, Victoria. Four standards were selected from each of the commercial quality grades of budget, medium, prime and choice lucerne hay and were identified as LA, LB, LP and LC, respectively, in an attempt to represent the range of nutritive values of the trial hays (Figure 10). As for the oaten hay standards, the lucerne standard hays were selected with the intention of providing a range of intake rates and preference values, as well as differing nutritive value, against which to compare the trial hays. The nutritive value of each lucerne hay, both standard and trial hays, was measured during the course of the feeding trial.

The lucerne hay was delivered to the Roseworthy Campus of the University of Adelaide as either midsized (350 kg) or small (25 kg) bales. The lucerne hay bales were not compressed and were mixed and stored in the same manner as described for the oaten hay.

6.4 Determining intake rates and preferences for oaten hays by lactating Holstein Friesian cows and oaten and lucerne hays by Thoroughbred horses

A total of 28 lactating Holstein Friesian cows and a total of 22 Thoroughbred horses were used in the feeding trials. Oaten hay was tested with both cows and horses as it is used in substantial amounts by both industries. Lucerne hay was tested with horses only as they are the single largest consumer of lucerne hay in Australia. The intake rate and preference tests were designed such that each individual trial hay and each preference combination was tested over three different animals, and replicated three times with each of the three animals. The tests were split into morning (AM) and afternoon (PM) sessions. This design allowed examination of hay intake rates (g/min) and preference values averaged across the four standards, as well as investigation of the preference of trial hays against each specific standard hay.

All feeding trials were conducted with approval from the University of Adelaide's Animal Ethics Committee (W-18-2003A).

6.4.1 Holstein Friesian cow component

6.4.1.1 Experimental animals

Each day a maximum of 24 lactating Holstein Friesian cows, ranging in age from three to ten years old, were used at any given time. A total of 28 cows were used throughout the duration of the experimental period. The cattle yards at the Roseworthy Campus were modified extensively for the trial to include extra holding pens, shaded areas and an improved drafting yard (Figure 11). The holding yards fronted onto a concrete yard containing a small brick hut in which the tests for intake rate and preference were conducted.

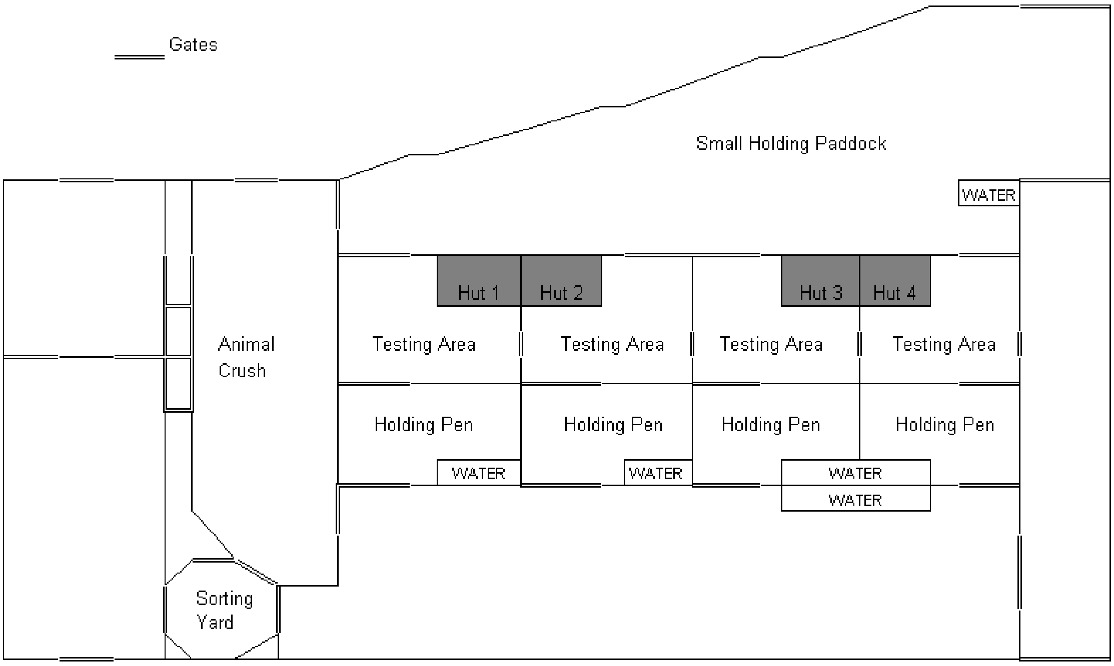


Figure 11. Layout of cattle yards where intake rate and preference testing took place

The animals underwent a training period to ensure they were comfortable in the yards where the trial was conducted, and familiar with the procedure used for preference testing. The cows were managed as part of the University of Adelaide's Roseworthy Campus dairy herd and were routinely milked twice daily at 6am and 4pm. Intake rates and preference values of the hays were tested in between these milking sessions. During the animal's first lactation on site, hay preference and intake were determined for the 2003 season hay. During this period the cows underwent an oestrus synchronisation program according to Roseworthy Dairy standard practices, to prepare for the second lactation necessary for testing of the 2004 season hays. At the end of the first lactation a number of animals were culled as they failed to become pregnant in the required time frame and suitable replacements were sourced from within the Roseworthy dairy herd. Consequently, some of the cows were used for the duration of the oaten hay preference and intake rate testing (i.e., underwent testing with both 2003-season and 2004-season hays), whilst others were used for only a portion of the time; this is detailed in Figure 12. All animals used to complete the testing of the 2004 season hays were trained, or re-trained as described above. Water was available *ad libitum* for the duration of the trial, both in the holding pens and in the paddocks. All preference test and measurements of hay intake rate were made with lactating cows. The majority of the 2003-season oaten hays were tested for preference and intake rate with the lactating Holstein Friesians between March and June 2004, with the remainder undergoing final testing in February 2005. The total 45, 2004-season oaten hays, underwent preference and intake rate testing between February and August 2005. The 2003-season oaten hays underwent testing for intake rate and preference with the Thoroughbred horses between June and November 2004, and the 2004-season oaten hays were tested between January and July 2005.

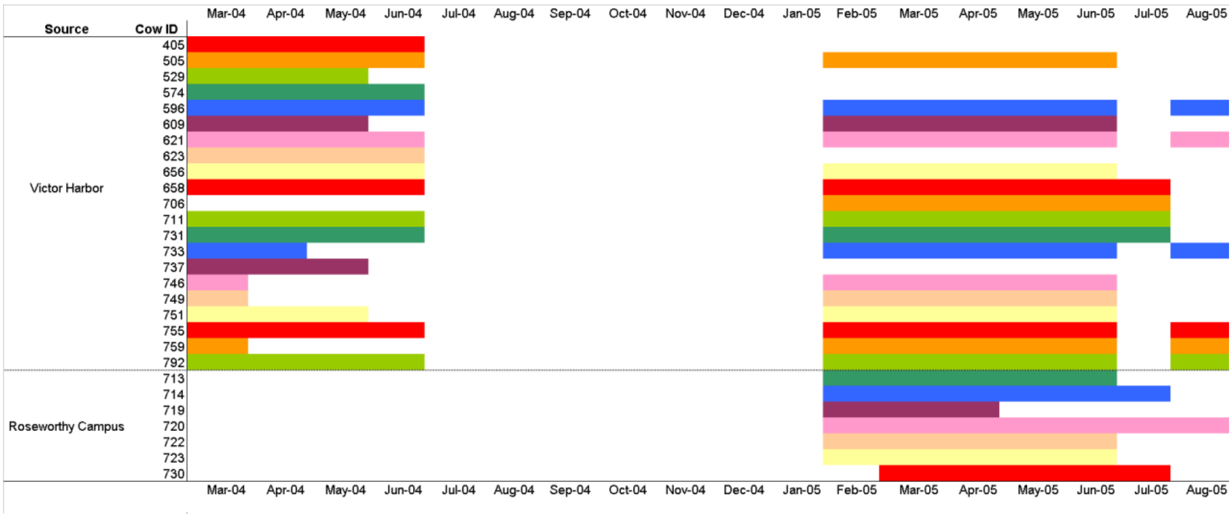


Figure 12. The feeding trial period and timing of cow usage for the oaten hay tests

Outside of the testing periods, cows were fed according to standard management practices atf the Roseworthy dairy, which entailed the *ad libitum* provision of cereal hay and fresh pasture, predominately annual grasses, and 4 kg of a cereal-based concentrate ration fed twice a day, at each milking. Cows were supplemented with pasture silage when there was insufficient pasture on offer. The basal diet of the animals was maintained as consistent as possible, allowing for minor variation due to seasonal pasture growth and the quality of fodder/concentrate supplied to the Roseworthy Dairy. The oaten hay fed in the paddock was never the same as any used in the experimental work for preference or intake rate. Any veterinary treatments were administered as required and animals temporarily removed from the experiment if necessary.

6.4.1.2 Preference of oaten hay with lactating Holstein Friesian cows

At the conclusion of each morning milking at approximately 8:00am, the cows were taken to the cattle yards. The cattle were then drafted into ‘morning’ (AM) and ‘afternoon’ (PM) groups, and the morning cows further drafted into groups of three animals and placed in the appropriate holding yard without feed for one hour. The same cows made up the groups of three each day. Until required, the afternoon cows remained in a small holding paddock at the rear of the cattle yards with access to cereal hay and water.

The preference value measurements involved offering the cows 1.5 kg of the allocated trial hay and one of four standard hays (A, BC, D, E) simultaneously in two adjacent feed bins, for a period of 5 minutes. After this time any uneaten hay was removed and weighed in order to establish the relative amounts of each eaten. When each cow entered its allocated hut, a timer was started to record the 5 minutes. The timing of the cows entering the feeding huts was staggered so that they could be entered and removed sequentially. After 5 minutes the cows were removed and returned to their group of three in the holding pen. The uneaten hay was then collected from the feed bins and weighed, and the amount of hay consumed calculated. The next randomly allocated combination was placed in the feed bins and the second cow from each group of three was drafted and led into the feeding hut, and similarly for the third cow. In each replicate, the position (left or right) of the two hays was alternated. This sequence was repeated twice to complete three replications with each animal. The time between each repetition for each cow was no less than 30 minutes. Once the morning (AM) animals had finished their testing session, they were released into the resting paddock at the rear of the cattle yards and the afternoon (PM) cows drafted into their groups of three to begin the afternoon testing session. The PM cows were left for 1 hour without feed prior to commencement of the testing.

6.4.1.3 Intake rate of oaten hay with lactating Holstein Friesian cows

The same procedures of moving animals, allocation to groups and testing in AM and PM sessions used for the preference testing were used again for the measurements of intake rate. In order to measure intake rate, each cow was offered 2kg of its allocated trial hay, split into two adjacent feed bins in each of the feeding huts, for a period of 5 minutes. The "intake rate" for all hays was calculated as the amount of hay (grams) consumed per minute.

6.4.2 Thoroughbred horse component

6.4.2.1 Experimental animals

Thoroughbred horses were leased from various sources: Cheltenham Tafe Horse Skills Training Centre, Adelaide; Veterinary Research Synergies, Roseworthy; Ron Oldfield, Balaklava; and various privately owned animals. Each day a maximum of eight horses, ranging in age from 3 to 25 years old, were used. A total of 22 horses were used during the entire trial period (Figure 14). The horses were allocated individual stalls in a stable block where the testing took place (Figure 13). At all other times the horses were paddocked in groups of two to five animals approximately 200 metres from the stable.

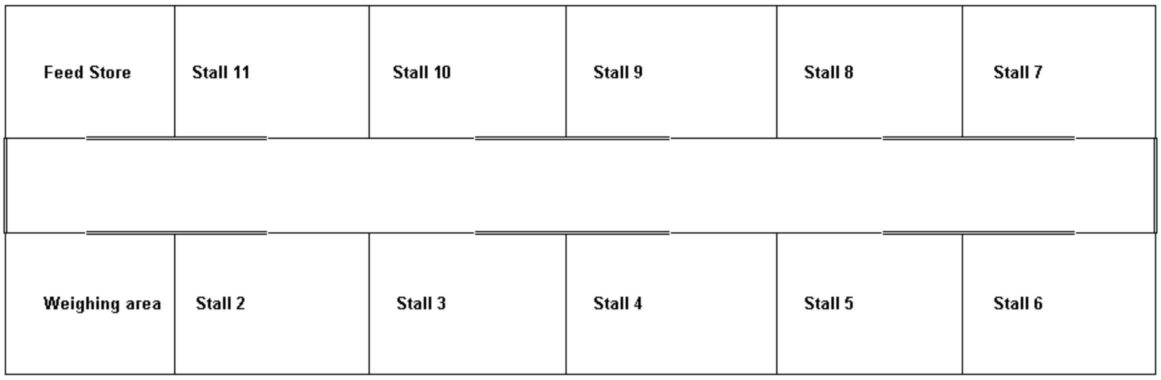


Figure 13. Layout of stables where intake rate and preference testing took place

Upon arrival to the Roseworthy Campus the horses were allowed a 7-day adaptation period in the paddocks to become familiar with their surroundings. The horses then underwent a 5-day training period where they were brought into the stables and fed to familiarise the animals with the walk to and from the stable block, the individual stalls themselves and the testing procedure. Water was available *ad libitum* for the duration of the trial, both in the stalls and in the paddocks. Feed (cereal hay and pasture) was available *ad libitum* in the paddocks. Any farrier or veterinary treatments were administered as required and animals temporarily removed from the experiment if necessary.

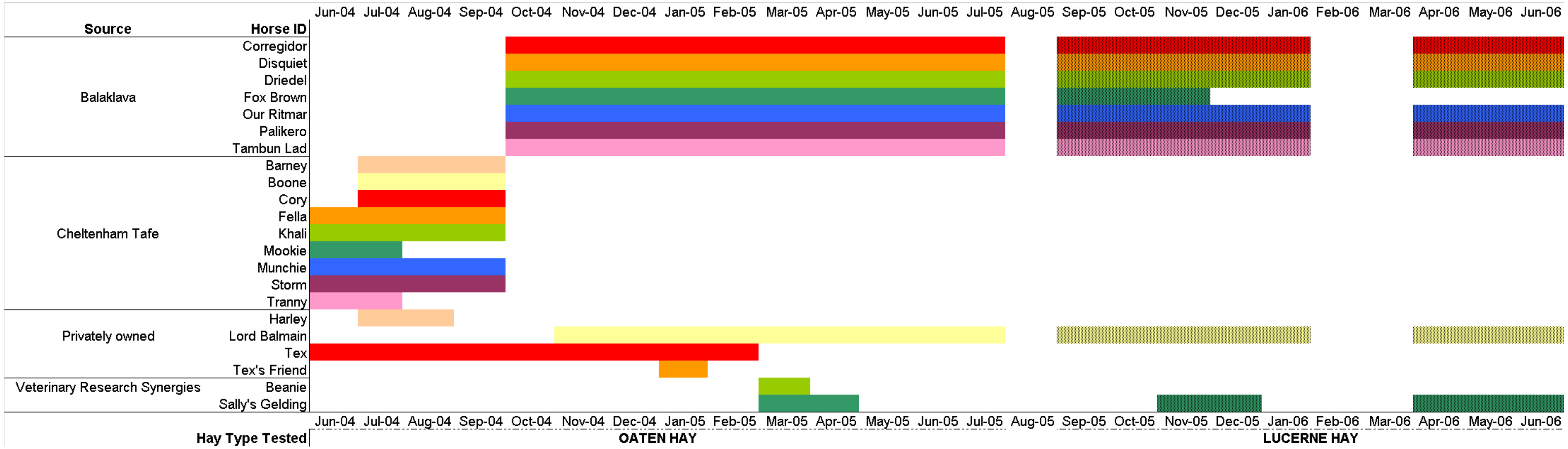


Figure 14. Feeding trial period and timing of horse usage for the oaten hay intake and preference tests

6.4.2.2 *Preference of oaten and lucerne hay with Thoroughbred horses*

The horses were haltered and led from their paddocks to the stable block each morning and placed in their allocated individual stalls. Testing days were divided into ‘morning’ (AM) and ‘afternoon’ (PM) sessions. The same horses were used for the both the AM and PM sessions. All animals were left for a minimum of 1 hour without feed on offer before each testing session (AM or PM). At the beginning of each AM and PM session, bags of pre-weighed test hay were transferred from the weighing station to the stables and placed in front of each appropriate stall.

Initially the same feed bins that were used for the cattle component of the trial were used for the horses but due to the excessive spillage from these bins by the horses, enclosed feed bags were purchased and used instead (Figure 15). All data reported were obtained with the use of the feed bags only. The preference value measurements involved offering the horses 1 kg of the allocated trial hay and 1 kg of one of four standard hays (oaten hay standards A, BC, D, E and lucerne hay standards LB, LM, LP and LC) simultaneously in two adjacent feed bags, for a period of 10 minutes. After this time any uneaten hay was removed and weighed in order to establish the relative amounts of each eaten.

Figure 15. Feed bins were used initially **(a)** but were replaced with feed bags **(b)** to reduce excess spillage.

The 1kg of trial hay and 1kg of standard hay were put into individual feed bags and placed in each stall. A timer was started to record ten minutes. The timing of feed bags going into each stall was staggered so that they could be placed and removed sequentially. After 10 minutes the feed bags were removed and the uneaten hay was weighed and the amount of hay consumed calculated. This sequence was repeated to complete three replications with each animal. As with the dairy cows, the positioning of the hay in each replicate of the preference test was alternated (left versus right). The first replication was completed with all the horses before the second replication commenced. The time between beginning the testing with the first horse and completing the testing with the final horse ensured that the time between each repetition for each horse was no less than 30 minutes.

6.4.2.3 Intake rate of oaten and lucerne hay with Thoroughbred horses

The same procedures of moving animals, allocation to stables and testing in AM and PM sessions used for the preference testing were used again for the measurements of intake rate testing for both oaten and lucerne hay with Thoroughbred horses. The intake rate measurements involved offering each horse 1kg of each test hay (oaten or lucerne) for a period of ten minutes. The intake rate for all the hays was calculated as the amount of hay (grams) consumed per minute.

6.5 Chemical and physical characterisation of the oaten and lucerne hay

Grab samples of all the oaten and lucerne trial and standard hays were taken throughout each of the testing periods and stored in clear plastic zip lock bags for both physical characterisation and nutritive value analyses. The analysis of physical characteristics for both oaten and lucerne hay involved measurements of green colour, relative percentages of leaf and stem, stem diameter and shear energy. The nutritive value analyses involved determination of: 1) dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and gross energy (GE) measurements by laboratory procedures (subsequently referred to as wet chemistry), and 2) DM, NDF, ADF, water soluble carbohydrate (WSC), in vitro dry matter digestibility (IVD) and metabolisable energy (ME) predicted by near infrared reflectance (NIR) spectroscopy. Hemicellulose content (Hem) was estimated by subtracting the value of ADF from that of NDF, under the assumption that a measure of NDF encompasses plant cellulose, hemicellulose and lignin whilst ADF includes only measures of cellulose and lignin, although it includes some protein attached to the plant cell walls. This calculation resulted in two additional nutritive value traits, those being a wet chemistry estimate of hemicellulose (wcHem) and an NIRS estimate of hemicellulose (nirHem). Determination of nutritive value for oaten hay was performed using both wet chemistry and NIRS whilst lucerne hay was only analysed using NIRS. An additional sample of each oaten and lucerne hay was taken in an airtight glass jar and Solid Phase Micro-Extraction coupled with Gas Chromatography and Mass Spectrometry (SPME GCMS) analysis was used to identify any volatile compounds present.

6.5.1 Wet chemistry nutritive value measurements of oaten hay

Wet chemistry measurements of dry matter (wcDM), crude protein (wcCP), neutral detergent fibre (wcNDF), acid detergent fibre (wcADF), hemicellulose (wcHem as calculated by wcNDF minus wcADF) and gross energy (wcGE) were performed at the South Australian Research and Development Institute (SARDI), Pig and Poultry Production Institute (PPPI) Nutrition Research Laboratory at the Roseworthy Campus of the University of Adelaide, in accordance with their established laboratory procedures which are summarised in the subsections below. The wet chemistry estimates of nutritive value for the oaten standard hays and the range covered by the oaten trial hays are summarised in Table 4. The values for each individual oaten hay are detailed in Appendix A.

Table 4. Wet chemistry measurements of dry matter (wcDM), crude protein (wcCP), neutral detergent fibre (wcNDF), acid detergent fibre (wcADF), hemicellulose (wcHem) and gross energy (wcGE) of standard oaten hays and the range covered by trial oaten hays

		Oaten Standard hays				Oaten Trial hays			
		A	BC	D	E	Min	Max	Mean	± SE
wcDM	%	92.5	92.8	93.0	92.7	88.3	94.5	93.0	0.13
wcCP	%DM	6.3	5.9	6.6	6.4	2.1	12.3	5.7	0.22
wcNDF	%DM	52.1	51.4	50.6	49.7	39.8	65.2	50.9	0.63
wcADF	%DM	26.1	25.6	25.3	25.0	20.3	37.6	26.9	0.43
wcHem	%DM	26.1	25.7	25.3	24.6	16.8	30.9	23.9	0.30
wcGE	MJ/kgDM	17.0	17.3	17.6	17.0	16.0	17.7	16.8	0.03

6.5.1.1 Dry matter content

Dry matter content was determined by the method described by the AOAC (1980). Weighed samples were heated at 105ºC for a minimum of 6 hours (maximum overnight) and then left for an additional 2 hours in a desiccator prior to re-weighing.

6.5.1.2 Neutral and acid detergent fibre content

Neutral detergent fibre (NDF) was measured using an Ankom Fibre Analyser in accordance with the operating instructions for the instrument. Samples of hay were ground to pass though a 1mm sieve and digested in the analyser using a neutral detergent solution for 60 minutes. NDF is a measure of both the digestible and indigestible fibre fractions; cellulose, hemicellulose and lignin.

Acid detergent fibre (ADF) was measured using an Ankom Fibre Analyser in accordance with the operating instructions for the instrument. Residue from the NDF procedure was digested in the analyser using an acid detergent solution for 60 minutes. ADF is a measure of the indigestible fibre fractions; cellulose and lignin.

6.5.1.3 *Crude protein content*

The crude protein (CP) content of the hay samples was determined using the Kjeldahl method described by Harris (1970).

6.5.1.4 *Gross energy content*

The gross energy (GE) content of the hay was determined using bomb calorimetry as described by Miller and Payne (1959) and Harris (1970).

6.5.2 NIRS predicted nutritive value measurements of oaten and lucerne hay

Near Infrared Reflectance Spectroscopy (NIRS) was carried out in accordance with Australian Fodder Industry Association (AFIA) specifications, by the South Australian Research and Development Institute (SARDI), Pig and Poultry Production Institute (PPPI) Nutrition Research Laboratory at the Roseworthy Campus of the University of Adelaide. The NIRS scans were performed on ground oaten hay samples (0.5 mm) to obtain reflectance spectra from 400 to 2500 nm at 2nm intervals using a FOSS NIRSystems Model 6500 instrument. All reflectance spectra were converted to pseudo-absorbance spectra using a Log(1/Reflectance) transformation (Figure 16). The spectra were trimmed as necessary and sent to FEEDTEST®, Hamilton, Victoria for spectral analysis. Established hay calibrations used by FEEDTEST® were used to predict dry matter (nirDM), crude protein (nirCP), neutral detergent fibre (nirNDF), acid detergent fibre (nirADF), water soluble carbohydrate (nirWSC) and digestibility (nirIVD). The hemicellulose (nirHem) content was calculated by subtracting the value of nirADF from the values obtained for nirNDF). The NIRS predictions of nutritive value for the oaten standard hays and the range covered by the oaten trial hays are summarised in Table 5. The values for each individual oaten and lucerne hay are detailed in Appendix B and D respectively.

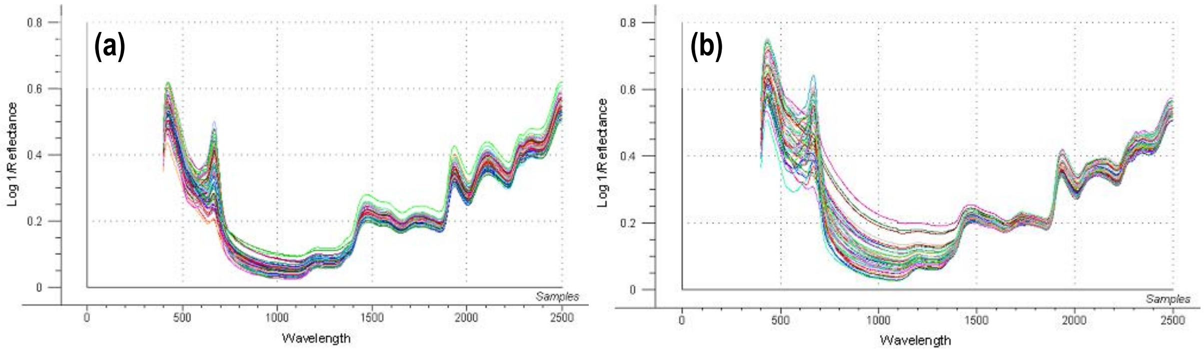


Figure 16. NIR spectra for each trial and standard **(a)** oaten hay and **(b)** lucerne hay

Table 5. NIRS predictions of dry matter (nirDM), crude protein (nirCP), neutral detergent fibre (nirNDF), acid detergent fibre (nirADF), hemicellulose (nirHem), water soluble carbohydrate (nirWSC) and digestibility (nirIVD) of standard oaten and lucerne hays and the range covered by trial oaten and lucerne hays (maximum, minimum and mean ± standard error)

		Oaten Standard hays				Oaten Trial hays			
		A	BC	D	E	Min	Max	Mean	± SE
nirDM	%	91.0	91.3	91.3	92.4	89.3	92.4	91.1	0.08
nirCP	%DM	7.1	8.3	5.8	6.3	2.3	13.5	6.6	0.23
nirNDF	%DM	56.3	53.0	51.3	49.2	40.3	62.2	51.5	0.52
nirADF	%DM	34.1	30.0	29.9	27.5	22.3	40.3	31.0	0.44
nirHem	%DM	22.2	23.0	21.5	21.7	15.0	25.3	20.8	0.27
nirWSC	%DM	12.9	19.1	23.9	32.2	8.9	43.0	26.5	0.78
nirIVD	%DM	62.4	66.2	67.0	71.1	56.9	78.6	66.5	0.50

		Lucerne Standard hays				Lucerne Trial hays			
		LA	LB	LC	LP	Min	Max	Mean	± SE
nirDM	%	92.0	92.6	93.0	91.3	88.8	95.6	92.0	0.23
nirCP	%DM	12.5	14.9	16.8	21.0	6.0	27.8	17.9	0.62
nirNDF	%DM	50.9	50.8	49.2	41.7	34.7	67.9	46.7	1.05
nirADF	%DM	32.9	38.7	38.0	29.5	24.4	50.9	35.1	0.78
nirHem	%DM	18.0	12.1	11.2	12.2	2.6	23.2	11.6	0.57
nirWSC	%DM	12.0	2.6	3.0	4.9	0.9	20.1	5.6	0.44
nirIVD	%DM	60.0	52.3	52.6	64.5	42.4	73.0	61.3	0.85

6.5.3 Physical characterisation of oaten and lucerne hay

The colour score (ranked from 1-60 for oaten hay and 1-5 for lucerne hay), stem diameter (minimum, maximum and average) and leaf percent of each oaten and lucerne trial hay were measured by Agrilink Agricultural Consultants Pty Ltd, South Australia. The shear energy of each oaten and lucerne trial hay was calculated by D.B. Purser, Western Australia, using a prediction equation based on the NIR spectra of the hays. The measurements of physical traits for the oaten and lucerne standard hays and the range covered by the oaten and lucerne trial hays are summarised in Table 6. The measurements of physical traits for each individual oaten and lucerne hay are detailed in Appendix C and E respectively.

Table 6. Measurements of the colour (scored out of 1-60 for oaten hay and 1-5 for lucerne hay), stem diameter (minimum, maximum and average) and leaf percent for the of standard oaten and lucerne hays and the range covered by trial oaten and lucerne hays (maximum, minimum and mean ± standard error)

	Oaten Standard hays				Oaten Trial hays			
	A	B	D	E	Min	Max	Mean	± SE
Colour (/60)	28	22	29	22	22	55	31.3	0.82
Ave Stem Diameter (mm)	6.1	5.8	5.6	4.7	4.6	6.8	5.6	0.06
Min Stem Diameter (mm)	3.8	4.9	3.7	3.1	2.5	5.0	3.7	0.06
Max Stem Diameter (mm)	9.0	8.4	7.2	6.2	5.7	11.0	7.8	0.12
Leaf %	31.0	34.0	21.0	35.0	11.0	58.0	33.4	1.12
Shear (KJ/m^2)	9.7	10.1	10.8	10.2	8.6	12.8	10.8	0.10

	Lucerne Standard hays				Lucerne Trial hays			
	LA	LB	LC	LP	Min	Max	Mean	± SE
Colour (/5)	4	4	5	4	2	5	3.6	0.12
Ave Stem Diameter (mm)	4.3	2.3	1.9	1.8	1.3	4.2	2.0	0.07
Min Stem Diameter (mm)	2.0	1.5	1.2	0.8	0.3	2.4	1.0	0.06
Max Stem Diameter (mm)	7.1	3.3	2.9	2.6	2.0	6.9	3.2	0.11
Leaf %	23.0	12.0	26.0	26.0	0.0	57.0	28.0	1.62
Shear (KJ/m^2)	11.6	12.9	12.2	10.4	8.9	14.4	11.0	0.13

6.5.3.1 Stem diameter measurements of oaten and lucerne hay

The minimum, maximum and average stem diameter (SD) of each oaten and lucerne trial hay was calculated by measurement of 10 stems measured at random from a grab sample, using vernier calipers to the nearest 0.1 mm.

6.5.3.2 Relative percentage of leaf and stem in oaten and lucerne hay

Each hay sample was placed in a 28L plastic box and any fines allowed to sift through to the bottom. An overhead transparency slide marked with an 18 x 18 cm grid, comprising of 2 x 2cm boxes (thus giving 100 grid cross points), was then placed on top of the sample in the centre of the box. The relative amount of leaf and stem that occur beneath each of the 100 grid crosses was counted and recorded. Florets, glumes, etc. were recorded as stem tissue in the oaten hay samples.

6.5.3.3 Colour measurement of oaten hay

Oaten hay colour was assessed at Balco Australia Pty Ltd, South Australia. A TrueGrade scanner and software was used to give each oaten hay sample a colour score ranging from 1 to 60. TrueGrade is Canadian-based software originally calibrated for Timothy hay. Balco Australia Pty Ltd have calibrated this software for oaten hay to assess "greenness". Prior to scanning any of the oaten hay samples the machine is first calibrated using an ideal colour standard (colour score of 60) and then allowed to run empty for the first two scans to warm up the globe and ensure consistent scanning once it has reached normal operating temperature. The hay sample was removed from the sample bag and placed within the scanning box (a container that has a transparent perspex bottom to allow observation and scanning of the sample on a flat surface). The sample was spread evenly over the base of the container to minimise air pockets against the plastic, as these pockets scan darker and give inaccurate measures of colour. Oaten hays were scanned and the TrueGrade software assessed each sample's colour relative to the "ideal" oaten colour standard, and calculated a colour score for each hay within the range of 1 to 60 (i.e., the further the number from 60, the 'poorer' the hay colour). Each sample was scanned 5 times and averaged. The sample was then removed from the scanning box and the box cleaned before running any further samples.

6.5.3.4 Colour measurement of lucerne hay

It was not possible to use the method previously described for colour measurement of lucerne hay because the lucerne contained a higher proportion of fibrous stalks, and the leaf portion was very fragile and susceptible to crushing and the excessive handling required in transferring the hay from its sample bag, to the scanning box and back again. Consequently the scanning plate became covered with fines and the scan was not an accurate representation of the total sample. Therefore a colour grading system was designed to score the colour of the lucerne samples by direct comparison with a prepared lucerne colour chart (Figure 17), which ranged from a colour score of 1 (representing an excellent colour, as perceived by professional hay exporters at Balco Australia and experts at Agrilink Agricultural Consultants Pty Ltd) through to a score of 5 (a dark grey/green colour associated with mouldy poor quality hay). The colour chart was formulated using the Windows "Paint' program via adjusting the relative amounts of red, green and blue colour when making a custom colour (Table 7). All other variables such as hue, saturation, and luminescence, were left as the default. Once the lucerne colour chart was created it was printed in high quality and a direct comparison made with each lucerne sample.

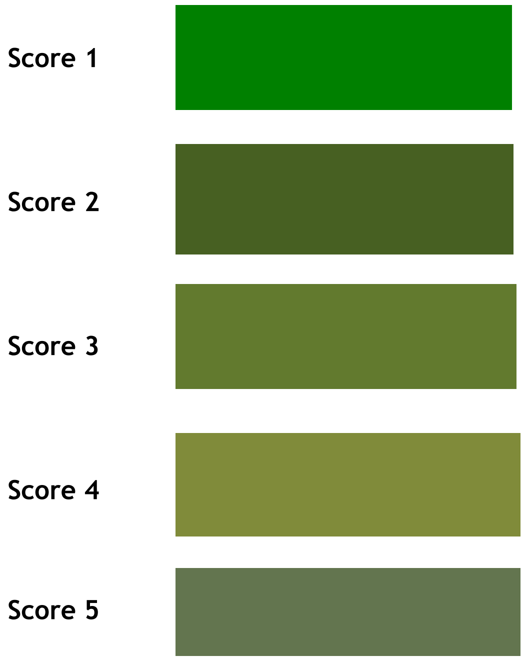


Figure 17. Green colour chart used to score the colour of the lucerne trial hays from 1 to 5.

Table 7. Relative amounts of red, green and blue used in the Windows 'Paint' program to generate each custom colour used in the lucerne colour chart.

Colour score	Red	Green	Blue
1	35	82	27
2	71	96	34
3	98	122	46
4	128	139	58
5	99	117	79

6.5.3.5 Shear energy of oaten and lucerne hay as predicted by NIRS

A shear prediction calibration was developed by D.B. Purser using 259 wavelengths that included both the visible and near infrared regions of the spectra (408 – 1092 and 1108 - 2492nm respectively, at 8nm intervals) of the oaten trial hays and the global equation development feature of WinISI (Infrasoft International). The NIR spectra used were those prepared by SARDI PPPI Nutrition Research Laboratories, South Australia, for determining of nutritive value traits using a FOSS NIRSystems Model 6500 instrument.

6.5.4 Solid Phase Micro Extraction coupled with Gas Chromatography Mass Spectroscopy (SPME-GCMS) of oaten and lucerne hay

The oaten and lucerne hay aroma was analysed by CSIRO Livestock Industries Plant Toxin Research Group in Geelong, Victoria, using solid phase microextraction (SPME) (Figure 18) coupled with gas chromatography (GC) and mass spectroscopy (MS). All analyses were performed using a Finnigan GCQ, an ion trap mass spectrometer coupled to a GC. The volatiles were separated on a Zebron ZB-1, MS certified low bleed capillary column (100% dimethylpolysiloxane).

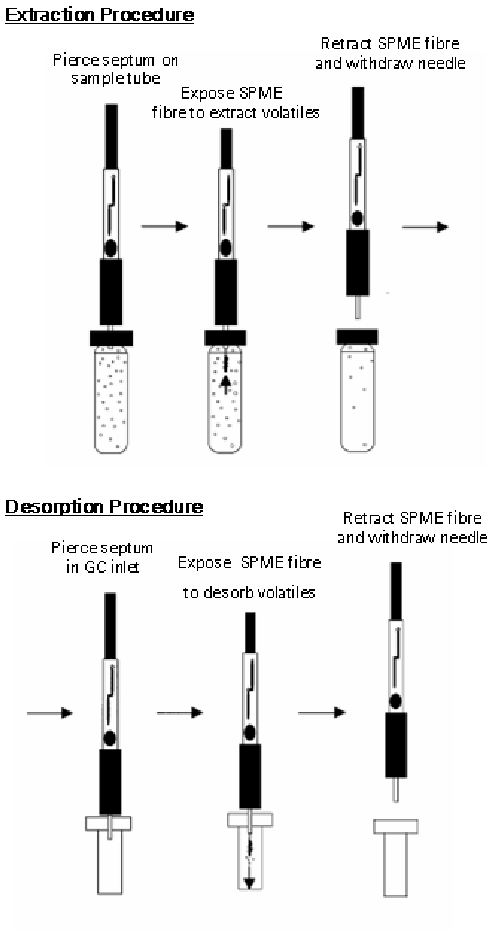


Figure 18. Solid phase microextraction adsorption/desorption procedure (adapted from Sigma-Aldrich 1998)

Grab samples of whole hay were taken and stored in airtight glass jars prior to being ground (Tecator mill, 1 mm sieve). One gram of the ground hay was placed in a 16 x 100mm round bottomed glass culture tube. The culture tube was capped using an open screw top and septa. All analyses were performed using a polydimethylsiloxane (PDMS) fibre with a film coating of 100µm. All PDMS fibres were conditioned before use by insertion into the GC injector port for half an hour at 250°C. A PDMS fibre was inserted into the sample tube and exposed to the hay headspace for 20 min at 90°C. The fibre was then inserted in the GC injector port and held for 15 min, to ensure complete desorption of the adsorped volatiles from the hay headspace. The program used had an initial temperature of 60°C (kept 1 min) ramping up to 230°C (kept 5 min), at a rate of 15°C/min, creating a total run time of 17.33 minutes. The splitless injector temperature was 250°C, with a close time of -0.2 minutes and an open time of 5 minutes. The carrier gas used was helium, at a constant flow rate of 0.3 ml/min. The mass spectrophotometer operated with a source temperature of 200°C, a scan rate of 3 scans/sec and a mass range of m/z 50-650. At random time points throughout the GCMS analysis, the SPME fibres being used were reanalysed after the desorption of sample volatiles to ensure complete elution of the volatiles into the GCMS during the sample analysis procedure. Empty culture tube standards prepared in the same manner as the hay sample tubes, with the exception of the addition of a ground hay sample, were also analysed by SPME GCMS to establish the background contaminant levels. A number of oaten and lucerne hays, selected at random, were also analysed in triplicate to ensure consistency in the GC method being used. Figure 19 and 20, respectively; show the triplicate analysis of an oaten hay sample and a lucerne hay sample and the near identical scans for each replicate.

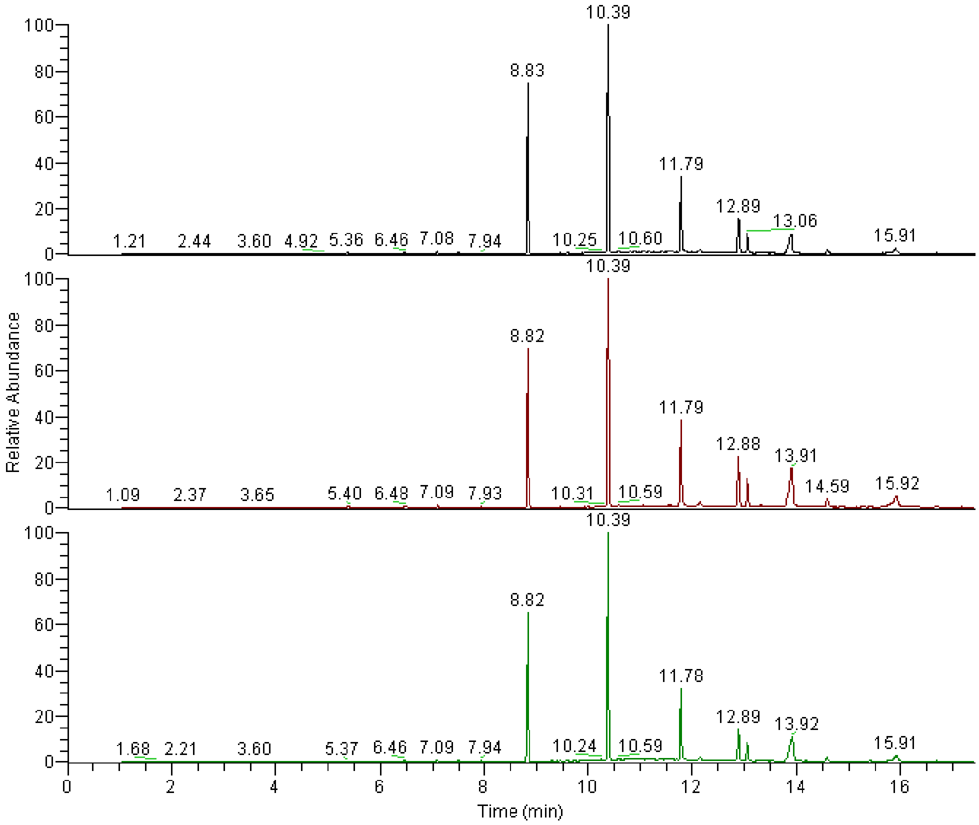


Figure 19. Typical gas chromatographs resulting from triplicate GC analysis of oaten hay 26.

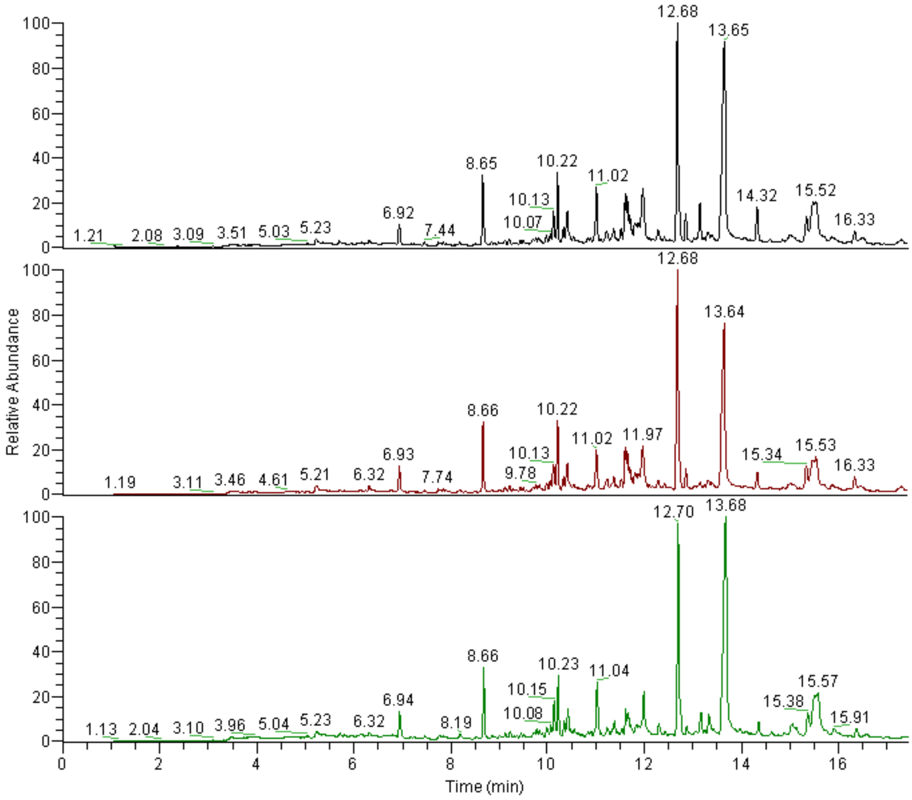


Figure 20. Typical gas chromatographs resulting from triplicate GC analysis of lucerne standard LA.

Oaten hay samples were analysed first and methodological improvements were identified and implemented for the analysis on the lucerne hay. A TFE/Silicone septa (Adelab, Australia) was used for the analysis of the oaten hay sample but due to excess septa bleeds of siloxanes, this was replaced with a low bleed La-Pha-Pack septa (Pacific Laboratory Products) for the analysis of the lucerne hay sample. The low bleed septa, the sample tubes, caps and septa were also pre-heated in a 90°C oven for 72hrs in an effort to remove the majority of contaminants prior to adding 1 gram of each ground lucerne hay sample to individual tubes and performing the SPME GCMS as described above.

The absolute area (A) under the curve of oaten and lucerne hay GC peaks were extracted from the gas chromatograph (Figure 21) using a combination of handpicked selection and the Genesis peak detection algorithm of the QualBrowser feature in the GCMS software XcaliberTM (Finnigan Corporation 1998-2000). The Genesis peak detection settings used in Xcaliber are detailed in Table 8. The area under the curve of major peaks common to each of the hay’s chromatographs were used to identify particular peaks and particular combinations of peaks whose presence or absence influenced the dietary preferences exhibited by dairy cows and horses. Oaten hay peak areas and lucerne hay peak areas obtained from the GC analysis were assigned a unique number identifier.

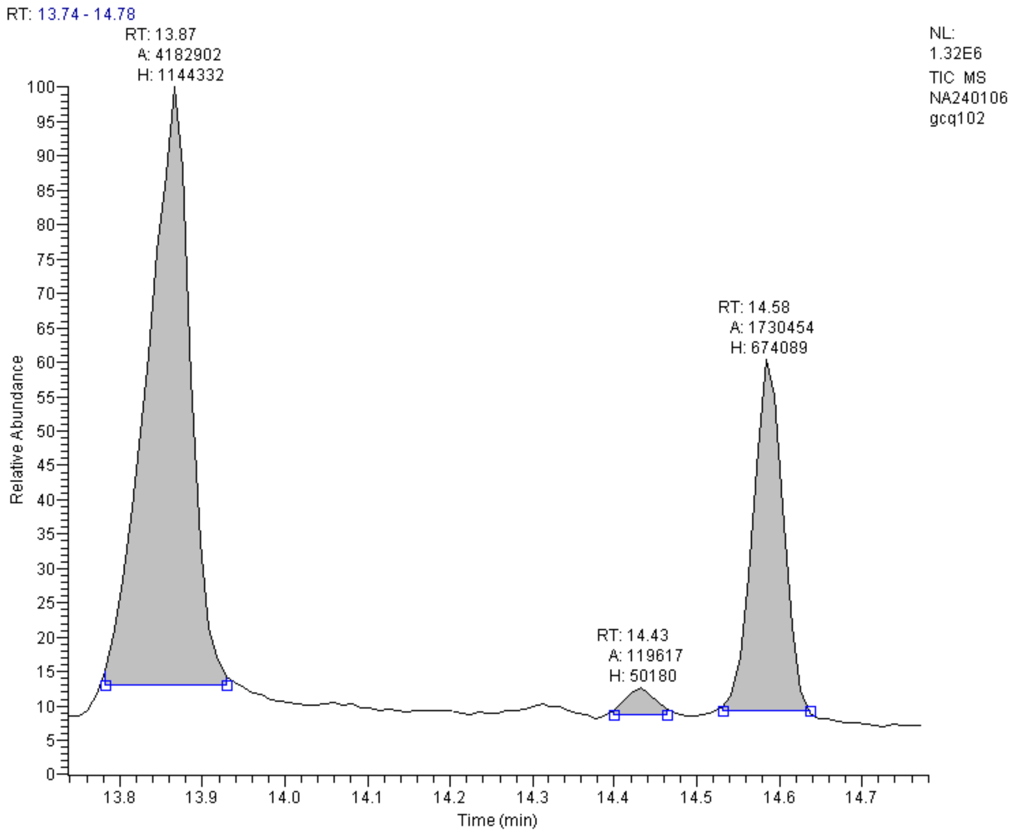


Figure 21. Magnified section of an oaten hay 37 chromatograph, section 13.74 to 14.78 minutes, showing peak area (A) of three GC peaks at retention times (RT), 13.87, 14.43 and 14.58 minutes.

Table 8. Genesis detection algorithm and settings used to extract oaten and lucerne hay gas chromatograph peaks

Peak Parameters	**Value assigned**	**Advanced**	**Value assigned**
Percent of Highest Peak	10	Baseline Noise Tolerance	10
Minimum Peak Height (S/N)	1	Min Number Of Scans In Baseline	16
S/N Threshold	0.5	Baseline Noise Rejection Factor	2
Valley Detection Enabled:		Peak S/N Cutoff	200
Expected Width	0.5	Rise Percentage	10
Constrain Peak Width:		Valley S/N	1
Peak Height	5	Background Recomputation Interval	5
Tailing Factor	1	Number Of Scans In Background	5
		Injection volume (µl)	10

6.6 Statistical analysis

6.6.1 Overview

Statistical analysis was performed in consultation with BiometricsSA at the University of Adelaide and the statistical consulting group within the School of Mathematics and Statistics at the University of Western Australia. Firstly, all the observed oaten hay intake rate (IR) and preference (Pref) values for dairy cows and horses and the lucerne hay IR and Pref values for horses were corrected for variation introduced by the use of different animals, different feeding stations, different dates of testing and the influence of the four different standard hays used (as described in section 6.6.2). Sets of "corrected" IR and Pref values were established and used in all subsequent analyses. Equations to predict the preferences for hays by cows and horses were then developed using the variables collected for each hay type, including intake rate, nutritive value traits, physical characteristics and volatile profile. The preference predictive equations were structured to allow the inclusion of all appropriate variables and the products of up to two (but not more) variables:

$$\text{Predicted Preference} = \alpha + \beta_1 V_a + \beta_2 V_b + \ldots\ldots + \beta_{1,2} V_a . V_b \ldots\ldots$$

Where α = constant, $\beta_{(1-n)}$ = coefficient and V_x = explanatory variable

Analyses relating to oaten hay intake and preference by cows and horses were conducted using the statistical program; GenStat 8th Edition (Service Pack 1) GenStat Procedure Library Release PL16, whilst analyses for lucerne hay intake and preference by horses were conducted using the statistical program; SAS, SAS Institute Inc. The volatile peaks obtained from the SPME GCMS of both the oaten and the lucerne hays, and their relationship to preference was investigated using the statistical program; SAS, SAS Institute. In addition to the development of predictive equations based on measured nutritive, physical and volatile traits of the hay, the potential to develop a NIRS calibration to predict preference directly from the NIR spectra was also investigated. Oaten and lucerne hay preferences as predicted directly from a NIRS calibration was investigated using the FOSS NIRSystems, Infrasoft International software, WinISI.

6.6.2 Modelling oaten and lucerne hay intake rates and preference values to remove experimental variation

A linear mixed model approach was fitted to the original measurements of intake rate (IR) and preference (Pref) of oaten hay for dairy cows and oaten and lucerne hay for horses, to correct for experimental variation introduced by the use of different animals, the different hut/stall the tests took place in, the different dates of testing, the time of day (AM or PM) in which the test were performed, the hay itself and the two different years over which the standard hays were used and any possible interactions between these effects. A 'corrected' set of values for intake rate and preference were developed and used in all subsequent analysis. Those random and fixed effects found to have a significant impact on cow oaten and horse oaten and lucerne intake rate are shown in Table 9, whilst those random and fixed effects found to be have a significant impact on cow oaten and horse oaten and lucerne preference values are shown in Table 10. Outliers were removed from each model where necessary. The 'corrected' IR and Pref variables obtained from this initial analysis were then used in all subsequent analyses.

Table 9. Terms used in the models fitted to cow oaten and horse oaten and lucerne intake rate

Model	Terms included	Fitted as Fixed (F) or Random (R) effect
Cow Oaten Hay Intake Rate	Cow	R
	Date	R
	Hay*	F
	Year	F
	Hay.Year**	F
Horse Oaten Hay Intake Rate	Horse	R
	Stall	R
	Date	R
	Time (AM vs PM)	R
	Horse.Date	R
	Horse.Time (AM vs PM)	R
	Hay*	F
	Year	F
	Hay.Year**	F
Horse Lucerne Hay Intake Rate	Horse	R
	Date	R
	Horse.Date	R
	Time (AM vs PM)	R
	Hay*	F

* the Hay effect includes both the trial hays and the standard hays

** the Hay.Year interaction effects apply for the four standard hays only

Table 10. Terms used in the models fitted to cow oaten and horse oaten and lucerne preference values

Model	Terms included	Fitted as Fixed (F) or Random (R) effect
Cow Oaten Hay Preference	Trial hay	F
	Standard hay	F
Horse Oaten Hay Preference	Horse	R
	Trial hay	F
	Standard hay	F
Horse Lucerne Hay Preference	Trial hay	F
	Standard hay	F
	Trial hay.Standard hay	F

Upon investigating the pattern of preference values observed when the trial hays were fed against each of the four standards it was recognized that it was necessary to determine specific preference prediction equations for cow oaten hay preferences when fed against each of the oaten hay standards A, BC, D and E and as well as a preference prediction equation for the average cow oaten hay preference. The cow's preference of the trial oaten hays was significantly different depending upon which oaten standard hay it was tested against, thus it is necessary to investigate those factors influencing preference in each situation (Figure 22).

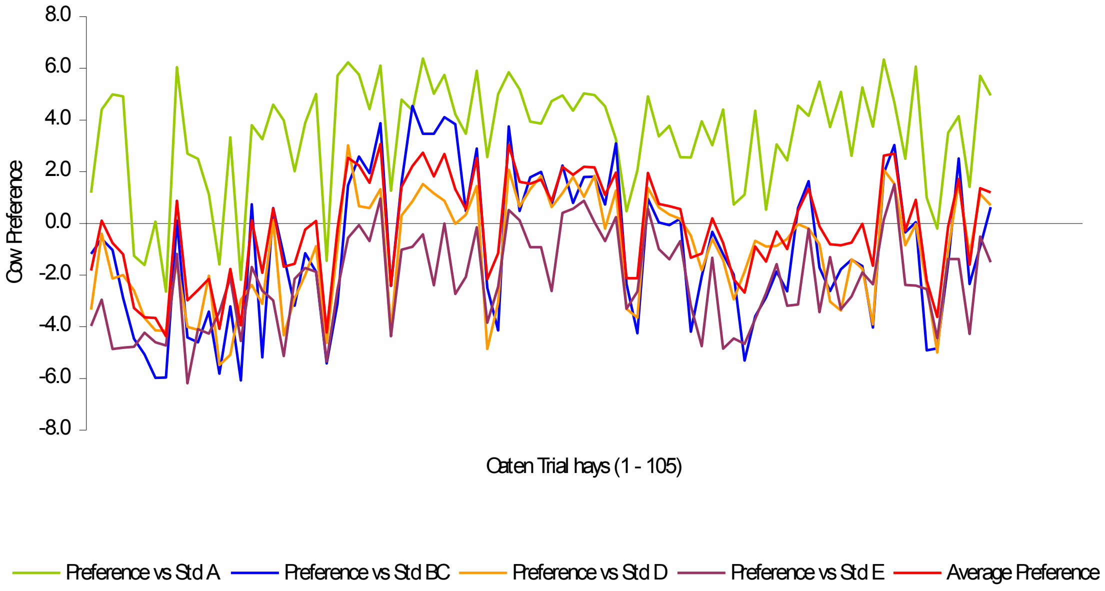


Figure 22. Average cow preference for the trial oaten hay and their preference against each of the individual oaten standard hays (A, BC, D and E). Each point along the x-axis represents one trial oaten hay, ranked numerically for their identification number from 1 to 105.

The oaten trial hay preferences of horses, however, whilst being significantly different depending upon what standard it was fed against, varied on a constant scale for each standard, in that the preferences against each oaten hay standard were parallel with each other (Figure 23), thus making it necessary to investigate only the average horse preference and then add the appropriate correction factor in order to scale the predicted preference values to that of those when fed against each of the oaten hay standards A, BC, D and E (Table 11).

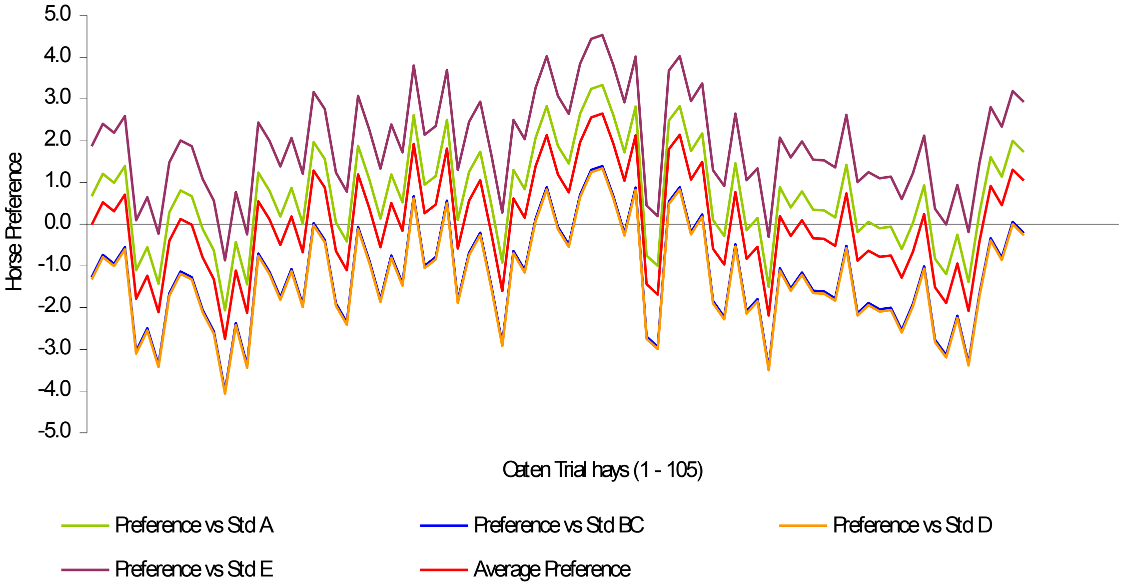


Figure 23. Average horse preference for the trial oaten hay and their preference against each of the individual oaten standard hays (A, BC, D and E), illustrating the parallel relationship between the preference values. Each point along the x-axis represents one trial oaten hay, ranked numerically for their identification number from 1 to 105

Table 11. Correction factors required to scale predicted average horse oaten hay preference values to that of predicted oaten trial hay preference values for horses when fed to each of the four oaten hays standards, A, BC, D and E

	HPrefA	HPrefBC	HPrefD	HPrefE
Constant	0.665	-1.243	-1.301	1.880

Examination of horse lucerne hay preference values showed it was necessary to determine specific preference prediction equations for lucerne hay preferences when fed against each of the lucerne standards LA, LB, LC and LP and as well as a preference prediction equation for the average lucerne hay preference as horse preference of the trial lucerne hays was significantly different depending upon which lucerne standard hay it was tested against. Thus as for the cow oaten hay preference it is necessary to investigate those factors influencing preference in each situation (Figure 24)

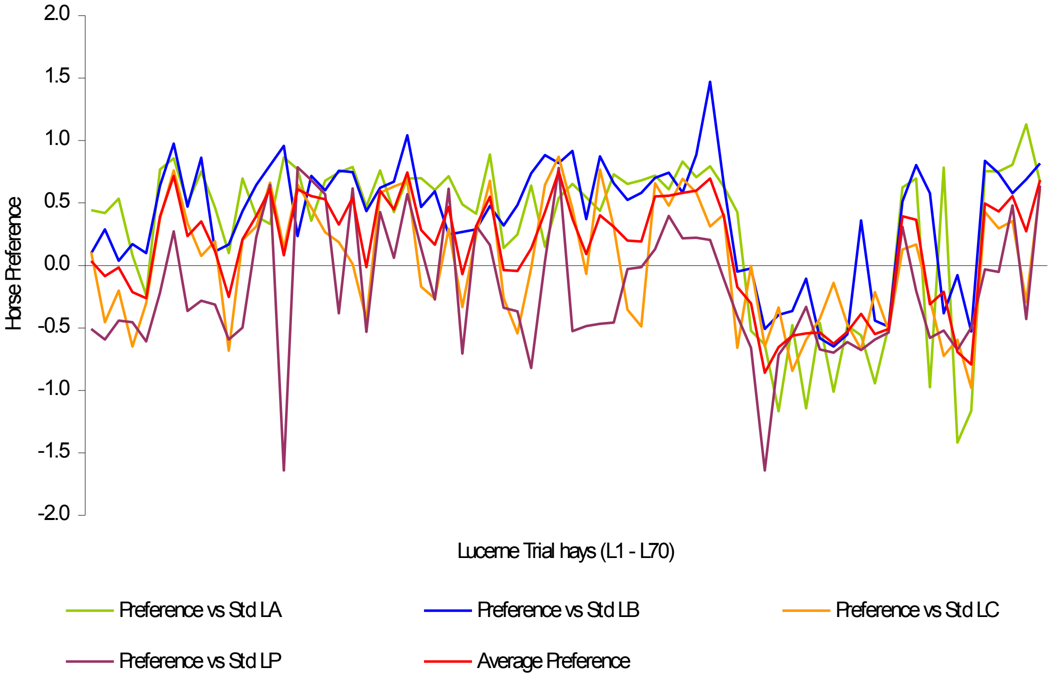


Figure 24. Average horse preference for the trial lucerne hay and their preference against each of the individual lucerne standard hays (LA, LB, LC and LP). Each point along the x-axis represents one trial lucerne hay, ranked numerically for their identification number from L1 to L70

6.6.3 Examination of oaten hay nutritive value and physical variables

In an attempt to maximise the number of unrelated variables included in each predictive model and reduce the incidence of confounding factors, all explanatory variables (nutritive value, as measured by both WC and NIR; and physical characteristics) were examined for colinearity and only one of any interrelated traits were included in subsequent analyses.

The explanatory variables were separated into three distinct groups: 1) nutritive value as measured by wet chemistry (variables henceforth denoted ‘wc’); 2) nutritive value as measured by NIRS (variables henceforth denoted ‘nir’); and, 3) physical traits (Table 12). The nutritive value traits measured by wet chemistry included acid detergent fibre (wcADF), neutral detergent fibre (wcNDF), hemicellulose (wcHem), crude protein (wcCP) and gross energy (wcGE). The nutritive value traits measured by NIRS included acid detergent fibre (nirADF), neutral detergent fibre (nirNDF), hemicellulose (nirHem), crude protein (nirCP), water soluble carbohydrates (nirWSC), in vitro digestibility (nirIVD) and estimated metabolisable energy (nirME). The physical characteristics measured included colour (Colour), average stem diameter (SD), a measure of the variation in stem diameter (LMM, the natural logarithmic transformation of the maximum stem diameter divided by the minimum stem diameter), leaf percentage (Leaf%) and NIR predicted shear energy (nirSE).

Table 12. Explanatory variables available from measurement of oaten hay nutritive value, by wet chemistry and near infrared spectroscopy (NIR), and physical characteristics, indicating those removed from subsequent modelling and those that ultimately remained

Variable group	Variable description	Units	Abbreviation
Wet chemistry measured nutritive value traits	Acid detergent fibre	%DM	wcADF
	Neutral detergent fibre *	%DM	wcNDF
	Hemicellulose	%DM	wcHem
	Crude protein	%DM	wcCP
	Gross energy	MJ/kgDM	wcGE
NIRS measured nutritive value traits	Acid detergent fibre	%DM	nirADF
	Neutral detergent fibre *	%DM	nirNDF
	Hemicellulose	%DM	nirHem
	Crude protein	%DM	nirCP
	Metabolisable energy *	MJ/kgDM	nirME
	In vitro Digestibility **	%DM	nirIVD
	Water soluble carbohydrate	%DM	nirWSC
Physical Characteristics	Colour	/60	Colour
	Average stem diameter	mm	SD
	Variation in stem diameter	log(maxSD/minSD)	LMM
	Amount of leaf	%	Leaf%
	NIR predicted shear energy	KJ/m2	nirSE

* variables ultimately excluded from subsequent analyses

** separate analyses were performed with nirsIVD as a single expanatory variable

When the wet chemistry nutritive value traits were examined, large correlations between wcNDF and wcADF, wcHem were evident. A similar linear relationship between nirNDF and nirADF, nirHem was seen when the NIR variables were examined. As such both the single variables wcNDF and nirNDF were excluded from subsequent modelling, whilst the two less correlated variables of ADF and Hem, remained for both the wet chemistry modelling and NIR modelling of preference. Additional NIR variables discarded from subsequent modelling were nirME and nirIVD. The variable nirME was excluded as it has a direct relationship with nirIVD and was also closely related to both nirNDF and nirADF. The variable nirIVD was excluded from the multi-variable modelling due to its close relationship with several other NIR measured traits. However, it was not completely discarded from the analyses and the relationship of preference to nirIVD was examined in a separate single variable model. There were no interrelated variables found amongst the physical characteristics and as such, all the variables were left in the analysis.

There was also interest in examining the relationship of oaten hay preference to a combination of nutritive and physical traits, specifically the NIR derived measures of IVD, CP, WSC and SE. The scatterplot matrix (Figure 25) of nirIVD, nirCP, nirWSC and nirSE shows there is a strong negative linear relationship between nirIVD and nirSE. However, all four variables were still included in a preference predictive model, despite the somewhat confounded influence of nirIVD and nirSE.

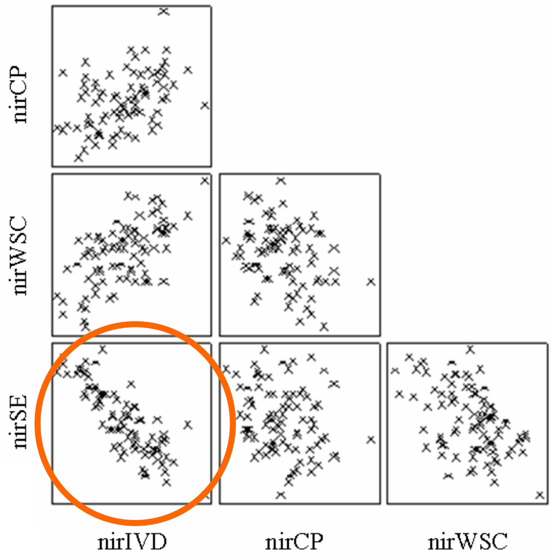


Figure 25. Scatterplot matrix illustrating the relationships between the near infrared derived traits, in vitro digestibility (nirIVD), crude protein (nirCP), water soluble carbohydrate (nirWSC) and shear energy (nirSE), whilst the circle highlights the strong negative linear relationships between nirIVD and nirSE.

6.6.4 Examination of lucerne hay nutritive value and physical variables

The nutritive and physical characteristics measured in lucerne hay were the same as those measured in oaten hay, with the only exception being the absence of nutritive value measurement made by wet chemistry (Table 13). Lucerne nutritive value was only measured using NIRS and in the same manner as for oaten hay variables, lucerne hay variables (nutritive value and physical characteristics) were examined for colinearity and only one of any interrelated traits were included in subsequent analyses.

Table 13. Explanatory variables available from measurement of lucerne hay nutritive value by near infrared spectroscopy (NIR), and physical characteristics, indicating those removed from subsequent modelling and those that ultimately remained.

Variable group	Variable description	Units	Abbreviation
NIRS measured nutritive value traits	Acid detergent fibre[2]	%DM	nirADF
	Neutral detergent fibre *	%DM	nirNDF
	Hemicellulose[1, 2]	%DM	nirHem
	Crude protein[1]	%DM	nirCP
	Metabolisable energy *	MJ/kgDM	nirME
	In vitro Digestibility **	%DM	nirIVD
	Water soluble carbohydrate[1, 2]	%DM	nirWSC
Physical Characteristics	Colour	/5	Colour
	Average stem diameter	mm	SD
	Variation in stem diameter	log(maxSD/minSD)	LMM
	Amount of leaf	%	Leaf%
	NIR predicted shear energy	KJ/m2	SE

* variables ultimately excluded from subsequent analyses

** separate analyses were performed with nirsIVD as a single expanatory variable

[1] indicates those traits included Model 1

[2] indicates those traits included in Model 2

Similar to the oaten hay, a strong linear relationship was evident between nirNDF and nirADF, nirHem. As such the single variable, nirNDF, was excluded from subsequent modelling, whilst the two less correlated variables of nirADF and nirHem remained for NIR modelling of preference. Also as for the oaten hay analyses, nirME and nirIVD were discarded from subsequent modelling. However, nirIVD was examined in a separate single variable model. In contrast to the oaten hay, there was also a strong correlation evident between nirCP and the fibre traits, nirADF and nirNDF, however, given the interest in lucerne as a high protein roughage, rather than removing from the analysis the only measure of protein content (nirCP) or conversely the remaining measure of fibre, nirADF, it was decided to generate two models to investigate the potential to predict preference from multiple lucerne nutritive value traits (Model 1: nirCP, nirHem, nirWSC; and Model 2: nirADF, nirHem, nir WSC; Table 13 above). There were no interrelated variables found amongst the physical characteristics and as such, all the variables were left in the analysis.

6.6.5 Predicting preference of oaten hay by cows and horses and lucerne hays by horses based on nutritive value and physical characteristics

To develop preference prediction equations, when only one explanatory variable was included in the model, simple linear regression was used. When more than one explanatory variable was included, stepwise multiple regression was used: stepwise from a full model (all covariates and all products); backwards elimination for the full model; forward selection beginning a basic model containing the individual covariates but no products; and stepwise regression from this basic model. In this manner any non-significant individual explanatory variable can be considered for removal from or inclusion in the final model generated if necessary due to an interaction or product term involving that explanatory variable being present in the model.

The influence of intake rate, IR (cow oaten hay IR, CIR; horse oaten hay IR, HIR; and horse lucerne hay IR, HLIR) and oaten and lucerne nirIVD, were examined as single explanatory variables in simple linear regression model with the response being cow and horse oaten hay preference and horse lucerne hay preference respectively. Additional modelling was performed to relate multiple nutritive value traits, multiple physical traits and a combination of nutritive and physical traits of the respective hays to cow and horse oaten hay preference and horse lucerne hay preference where appropriate (Table 14).

Each model was used to examine cow preference for oaten trial hay when fed against each of the four standards, A, BC, D and E (CPrefA, CPrefBC, CPrefD and CPrefE respectively) and also cow preference for oaten hay average across all four standards (CPrefave). Similarly, horse trial oaten hay preference was examined when fed against each of the four standards, A, BC, D and E (HPrefA, HPrefBC, HPrefD and HPrefE respectively) and also when averaged across all four standards (HPrefave). Horse lucerne trial hay preference was also examined when fed against each of the four standards, LA, LB, LP and LC (HPrefLA, HPrefLB, HPrefLP and HPrefLC respectively) and when averaged across all four standards (HPrefLave).

Table 14. Models fitted and their included terms, to predict preference of oaten hay by dairy cows and horses and lucerne hay by horses

Response	Explanatory variables fitted
Cow preference for oaten hay	CIR
	nirIVD
	wcADF, wcHem, wcCP, wcGE
	nirADF, nirHem, nirCP, nirWSC
	Colour, SD, LMM, Leaf%, nirSE
	nirIVD, nirCP, nirWSC, nirSE
Horse preference for oaten hay	HIR
	nirIVD
	wcADF, wcHem, wcCP, wcGE
	nirADF, nirHem, nirCP, nirWSC
	Colour, SD, LMM, Leaf%, nirSE
	nirIVD, nirCP, nirWSC, nirSE
Horse preference for lucerne hay	HLIR
	nirIVD
	nirCP, nirHem, nirWSC
	nirADF, nirHem, nirWSC
	Colour, SD, LMM, Leaf%, nirSE

Preference prediction models were developed using standardised values of the explanatory variables, creating variable co-efficients within the predictive equations that were more directly comparable as each of the standardised variable values were on a similar scale. Standardised explanatory variables were generated by subtracting the mean of all the actual values from individual actual value and dividing that by the standard deviation of the actual values. This calculation gave variables with a mean of zero and unit variance that ranged on average between -2 and +2, and was as follows:

Standardised variable = (individual **V** – population mean **V**)/standard deviation of **V** population

where **V** = explanatory variable

The models reported were the simplest models possible that gave the highest R^2. An adjusted R^2 is reported for each final model, and is defined as;

$$\text{adjusted R2} = 1 - \text{residual MS} / \text{total MS}$$

where MS = mean square

The R^2 is expressed as a percentage, and is the proportion of the total variance that is explained by the model. An adjusted R^2 allows for the fact that adding another variable automatically gives the possibility of extra explanatory power but at the cost of a less simple model.

6.6.6 Potential to predict oaten hay preference and intake rate of dairy cows and horses and lucerne hay preference and intake rate of horses directly from NIR spectra

The potential to predict preference and intake rate directly from near infrared reflectance spectroscopy (NIRS) was determined by regression of the measured data against the respective spectral profiles of the oaten hay produced by SARDI PPPI Nutrition Research Laboratories, South Australia. The calibration was developed, using the FOSS NIRSystems Infrasoft International software, WinISI, and a combination of Principal Component Analysis (PCA) and modified Partial Least Squares (MPLS) approach. Different segments of the total spectrum were used to develop preference and intake rate predictive calibrations for oaten hay with cows and horses and lucerne hay with horses; Model 1 included the full spectrum, visible and near infrared (NIR) regions; 259 wavelengths; 400 - 1098 and 1100 - 2498 nm respectively, at 8nm intervals; and Model 2 used only the NIR region; 173 wavelengths; 1100 - 2498 nm, at 8 nm intervals.

6.6.7 Examination of the relationship between cow and horse preference and oaten and lucerne hay volatiles

The influence of hay volatiles on cow and horse preference for oaten hay and horse preference for lucerne was examined to by fitting simple linear regression models to non-zero volatile peak area data with the response being either cow preference for oaten hay (CPrefA, CPrefBC, CPrefD, CPrefE and CPrefave) horse preference for oaten hay or horse preference for lucerne hay (HPrefLA, HPrefLB, HPrefLP, HPrefLC, HPrefLave), whilst the predictors were, individually, each of the individual volatile peak areas. As a very simple screening process, for each model an r^2 value was calculated. Those that had an r^2 greater than 0.2 and were models based on more than 10 observations were deemed to be of significant influence. In this manner volatiles that were consistently producing a high r^2 in the relationships with each of the different responses could be identified.

6.6.8 Examination of the relationship between nutritive and physical traits of oaten and lucerne hay and those volatiles related to preference

In order to investigate the potential role of volatiles as diet selection cues for animals regarding the nutritive value of feeds on offer, the relationship between oaten hay nutritive properties and those oaten hay volatiles that had a significant correlation with cow and horse preferences were examined by fitting simple linear regression (SLR) models to each significant peak with the response being each of the oaten hay nutritive traits. The relationship between lucerne hay nutritive properties and those lucerne hay volatiles that had a significant correlation with horse preferences was examined in the same manner as for the oaten hay. Only the nutritive values derived from NIRS were examined and for each SLR model an r^2 was calculated and deemed significant if greater than or equal to 0.2.

7 Results

7.1 Oaten and lucerne hay preference values and intake rates

The intake rates and preference values for oaten hay with dairy cows and horses, and for lucerne hay with horses, are summarised below in Table 15. The preference values for individual hays are detailed in Appendices F, G and H. Both the oaten and lucerne standard hays spanned a range of preference values. The average cow preference values of the oaten hay standards A, BC, D and E was -1.95, -0.59, -0.07 and 0.66 whilst the average horse preference values for the same oaten hay standards was -0.31, 0.33, 0.38 and -0.41. The oaten standard hay with the lowest preference for cows (Std A) was also a low ranked standard with horses, however the most preferred oaten standard hay for cows (Std E) was the least preferred by horses.

Table 15. A summary of intake rates and preference values of oaten hays offered to dairy cows and horses and lucerne hays offered to horses. Values presented are for each of the standard hays and the maximum, minimum and mean of the trial hays offered with each of the standard hays and also the average values pooled across the four standard hays (± standard error)

	Oaten Standard hays				Oaten Trial hays			
	A	**B**	**D**	**E**	**Min**	**Max**	**Mean**	**± SE**
OIR[1]	112	131	163	170	52	214	152	3.6
OPrefA[2]	0.00	1.95	2.11	1.78	-2.78	6.49	3.41	0.235
OPrefBC[3]	-1.95	0.00	1.04	0.73	-5.96	4.35	-0.95	0.308
OPrefD[4]	-2.11	-1.04	0.00	1.25	-5.85	3.00	-1.10	0.232
OPrefE[5]	-1.78	-0.73	-1.25	0.00	-5.96	1.36	-2.32	0.198
Oprefave[6]	-1.95	-0.59	-0.07	0.66	-4.60	2.95	-0.24	0.219
HIR[7]	36	44	47	24	15	60	40	1.0
HPrefA[8]	0.00	0.48	0.70	0.05	-2.52	4.32	0.71	0.157
HPrefBC[9]	-0.48	0.00	0.14	-0.99	-4.71	2.26	-1.20	0.171
HPrefD[10]	-0.70	-0.14	0.00	-0.69	-4.45	1.93	-1.26	0.170
HPrefE[11]	-0.05	0.99	0.69	0.00	-2.03	4.75	1.92	0.144
Hprefave[12]	-0.31	0.33	0.38	-0.41	-2.81	2.76	0.04	0.136

	Lucerne Standard hays				Lucerne Trial hays			
	LA	**LB**	**LC**	**LP**	**Min**	**Max**	**Mean**	**± SE**
HLIR[13]	40	37	46	62	2	70	45	2.4
HPrefLA[14]	0.00	-0.37	0.45	0.78	-1.42	1.12	0.16	0.099
HPrefLB[15]	0.37	0.00	0.23	0.55	-0.66	1.47	0.32	0.073
HPreLC[16]	-0.45	-0.23	0.00	0.45	-0.99	0.87	-0.04	0.070
HPrefLP[17]	-0.78	-0.55	-0.45	0.00	-1.65	0.78	-0.27	0.070
HPrefLave[18]	-0.21	-0.29	0.06	0.44	-0.86	0.75	0.05	0.065

[1] Lactating Holstein Friesian cow oaten hay intake rate (g/min)

[2] Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard A (log ratio)

[3] Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard BC (log ratio)

[4] Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard D (log ratio)

[5] Lactating Holstein Friesian cow preference when fed in combination with oaten hay standard E (log ratio)

[6] Average of OPrefA, OPrefBC, OPrefD and OPrefE

[7] Thoroughbred horse oaten hay intake rate (g/min)

[8] Thoroughbred horse preference when fed in combination with oaten hay standard A (log ratio)

[9] Thoroughbred horse preference when fed in combination with oaten hay standard BC (log ratio)

[10] Thoroughbred horse preference when fed in combination with oaten hay standard D (log ratio)

[11] Thoroughbred horse preference when fed in combination with oaten hay standard E (log ratio)

[12] Average of HPrefA, HPrefBC, HPrefD and HPrefE

[13] Thoroughbred horse lucerne hay intake rate (g/min)

[14] Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio)

[15] Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio)

[16] Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio)

[17] Thoroughbred horse preference when fed in combination with lucerne hay standard LA (log ratio)

[18] Average of HPrefLA, HPrefLB, HPrefLC and HPrefLP

Average preference values of the oaten trial hays offered to dairy cows ranged from -4.60 to 2.94, whilst the average preference values for the same oaten trial hays offered to horses was much narrower, ranging from -2.81 to 2.76. There was only a moderate relationship between the preference values obtained with dairy cows and that obtained with horses, $r^2 = 0.45$ (Figure 26).

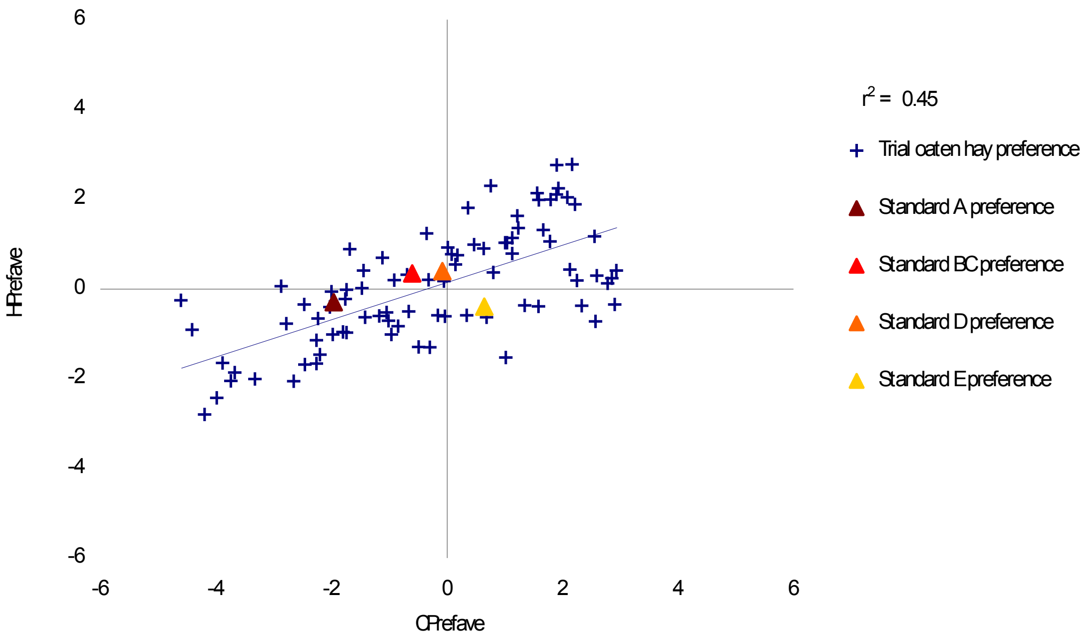


Figure 26. Scatterplot illustrating the moderate relationship ($r^2 = 0.45$) between average preference of oaten hay trial hays by dairy cows (CPrefave) and horses (HPrefave) and the relative positioning of each of the oaten hay standards A ▲, BC ▲, D ▲ and E ▲.

The average preference values of lucerne standard hays, LA, LB, LC and LP offered to horses covered a relatively narrow range of -0.21, -0.29, -0.06 and 0.44, respectively, similar in magnitude to the preference values for the standard oaten hays.

The intake rate of oaten hays by cows ranged from 52 g/min to 214 g/min, whilst the intake rate of oaten hay by horses only ranged from 15 to 60 g/min. The intake rate of lucerne hays by horses ranged from 1.7 g/min to 70 g/min.

7.2 Oaten and lucerne hay preference prediction equations for cows and horses

7.2.1 Relationship between intake rate and preference

The intake rate (IR) of oaten hay by dairy cows predicted oaten hay preferences by cows with an r^2 of 0.66. The equivalent prediction for horses was considerably poorer, with intake rate resulting in an r^2 of only 0.15 (Table 16). Intake rate provided a better prediction of preference when trial hays were compared against oaten hay standards BC or D than with A or E (0.63 and 0.61 versus 0.41 and 0.43, respectively).

The intake rate of lucerne hay by horses predicted the average preference of horses with an r^2 of 0.63 (Table 17), considerably higher than the correlation co-efficient with horses and oaten hay. However the use of intake rate to predict the preferences against each of the individual standards, LA, LB, LC and LP resulted in lower r^2 values (0.39, 0.53, 0.45 and 0.41, respectively).

Table 16. Equations to predict preference values of oaten hays offered to cows or horses from the intake rate (IR; being either CIR and HIR where appropriate) of the hays. Values presented are the equation constant, variable co-efficient and r^2 for the average preference values and preferences when compared against standards A, BC, D and E.

		Co-efficient	
	Constant	IR	r^{2*}
CPrefave	-7.7	0.05	0.66
s.e.	*0.59*	*0.004*	
P value	*< 0.001*	*< 0.001*	
CPrefA	-3.0	0.04	0.41
s.e.	*0.85*	*0.005*	
P value	*< 0.001*	*< 0.001*	
CPrefBC	-11.4	0.06	0.63
s.e.	*0.89*	*0.006*	
P value	*< 0.001*	*< 0.001*	
CPrefD	-8.7	0.05	0.61
s.e.	*0.68*	*0.004*	
P value	*< 0.001*	*< 0.001*	
CPrefE	-7.7	0.0	0.43
s.e.	*0.70*	*0.005*	
P value	*< 0.001*	*< 0.001*	
HPrefave	-2.1	0.05	0.15
s.e.	*0.54*	*0.013*	
P value	*< 0.001*	*< 0.001*	

[*] all r^2 are adjusted r^2

Table 17. Equations to predict preference values of lucerne hays offered horses from the intake rate (HLIR) of the hays. Values presented are the equation constant, variable co-efficient and r^2 for the average preference values and preferences when compared against standards LA, LB, LC and LP.

	Constant	Co-efficient HLIR	r^{2*}
HPrefLave	-0.9	0.02	0.63
s.e.	*0.10*	*0.002*	
P value	*< 0.001*	*< 0.001*	
HPrefLA	-0.6	0.02	0.39
s.e.	*0.15*	*0.003*	
P value	*< 0.001*	*< 0.001*	
HPrefLB	-0.7	0.02	0.53
s.e.	*0.13*	*0.003*	
P value	*< 0.001*	*< 0.001*	
HPrefLC	-1.0	0.02	0.45
s.e.	*0.14*	*0.003*	
P value	*< 0.001*	*< 0.001*	
HPrefLP	-1.1	0.02	0.41
s.e.	*0.15*	*0.003*	
P value	*< 0.001*	*< 0.001*	

* all r^2 are adjusted r^2

7.2.2 Relationship between *in vitro* digestibility and preference

Using NIRS derived *in vitro* digestibility (nirIVD) to predict preference values resulted in similar correlation co-efficients as that with intake rate. On average the nirIVD of oaten hay predicted cow oaten hay preference with an r^2 of 0.66. The relationship was not as strong for horses, with intake rate resulting in an r^2 of only 0.33 (Table 18). A better prediction of preference is generated using nirIVD when preference is expressed relative to standards BC or D, than A or E (0.62 and 0.61 versus 0.34 and 0.53, respectively).

Table 18. Equations to predict preference values of oaten hays offered to cows or horses from the *in vitro* digestibility (nirIVD) of the hays. Values presented are the equation constant, variable co-efficient and r^2 for average preference values and preferences when compared against standards A, BC, D and E.

		Co-efficient	
	Constant	**nirIVD**	**r^{2*}**
CPrefave	-23.8	0.35	0.66
s.e.	*1.84*	*0.028*	
P value	*< 0.001*	*< 0.001*	
CPrefA	-14.9	0.28	0.34
s.e.	*2.78*	*0.042*	
P value	*< 0.001*	*< 0.001*	
CPrefBC	-33.6	0.49	0.62
s.e.	*2.80*	*0.042*	
P value	*< 0.001*	*< 0.001*	
CPrefD	-25.2	0.36	0.61
s.e.	*2.09*	*0.031*	
P value	*< 0.001*	*< 0.001*	
CPrefE	-21.4	0.29	0.53
s.e.	*1.97*	*0.030*	
P value	*< 0.001*	*< 0.001*	
HPrefave	-10.3	0.15	0.33
s.e.	*1.59*	*0.024*	
P value	*< 0.001*	*< 0.001*	

* all r^2 are adjusted r^2

The NIRS derived *in vitro* digestibility (nirIVD) of lucerne hay predicted the average lucerne hay preference of horses with an r^2 of 0.63 (Table 19), whilst the use of nirIVD to predict preference when fed against each of the individual standards, LA, LB, LC and LP resulted in lower r^2 values (0.41, 0.54, 0.42 and 0.40, respectively).

Table 19. Equations to predict preference values of lucerne hays offered horses from the *in vitro* digestibility (nirIVD) of the hays. Values presented are the equation constant, variable co-efficient and r^2 for average preference values and preferences when compared against standards LA, LB, LC and LP.

		Co-efficient	
	Constant	nirIVD	r^{2*}
HPrefLave	-2.5	0.04	0.63
s.e.	*0.25*	*0.004*	
P value	*< 0.001*	*< 0.001*	
HPrefLA	-2.2	0.04	0.41
s.e.	*0.38*	*0.006*	
P value	*< 0.001*	*< 0.001*	
HPrefLB	-2.4	0.05	0.54
s.e.	*0.32*	*0.005*	
P value	*< 0.001*	*< 0.001*	
HPrefLC	-2.5	0.04	0.42
s.e.	*0.37*	*0.006*	
P value	*< 0.001*	*< 0.001*	
HPrefLP	-2.6	0.04	0.40
s.e.	*0.36*	*0.006*	
P value	*< 0.001*	*< 0.001*	

* all r^2 are adjusted r^2

7.2.3 Relationship between nutritive value and preference

Using a combination of wet chemistry-derived nutritive value traits allowed for prediction of cow oaten hay preferences with an r^2 of 0.74, and an r^2 of 0.57 when used to predict horse oaten hay preferences (Table 20). The wet chemistry-derived nutritive value traits that were significantly related to average cow preference were acid detergent fibre (wcADF), hemicellulose (wcHem) and crude protein (wcCP), whilst those significantly related to average horse preference were wcADF, wcHem, wcCP and gross energy (wcGE). There was a negative relationship between both wcADF and wcHem to oaten hay preferences of dairy cows and horses, and a positive relationship between wcCP and wcGE.

Table 20. Dairy cow and horse oaten hay preference prediction equation using wet chemistry-derived nutritive values measurements of acid detergent fibre (wcADF), hemicellulose (wcHem), crude protein (wcCP) and gross energy (wcGE), detailing the constant, variable co-efficient and resulting r² for prediction of average cow preference and cow preference values when compared against standards A, BC, D and E, as well as average horse preference values.

		Co-efficients								
	Constant	wcADF	wcHem	wcCP	wcGE	wcADF. wcHem	wcADF. wcCP	wcHem. wcCP	wcADF. wcGE	r^{2*}
CPrefave	-0.16	-1.40	-0.08	0.47				-0.23		0.74
s.e.	*0.111*	*0.152*	*0.145*	*0.131*				*0.118*		
P value	*0.143*	*< 0.001*	*0.579*	*< 0.001*	*ns*	*ns*	*ns*	*0.051*	*ns*	
CPrefA	3.58	-1.08		0.56			0.36			0.48
s.e.	*0.180*	*0.184*		*0.179*			*0.155*			
P value	*< 0.001*	*< 0.001*	*ns*	*0.002*	*ns*	*ns*	*0.024*	*ns*	*ns*	
CPrefBC	-0.93	-1.87		0.82						0.69
s.e.	*0.171*	*0.183*		*0.180*						
P value	*< 0.001*	*< 0.001*	*ns*	*< 0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	
CPrefD	-1.03	-1.44	-0.29	0.27				-0.30		0.67
s.e.	*0.134*	*0.183*	*0.173*	*0.158*				*0.142*		
P value	*< 0.001*	*< 0.001*	*0.101*	*0.970*	*ns*	*ns*	*ns*	*0.040*	*ns*	
CPrefE	-2.15	-0.94	-0.26	0.46				-0.41		0.65
s.e.	*0.122*	*0.167*	*0.160*	*0.145*				*0.130*		
P value	*< 0.001*	*< 0.001*	*0.108*	*0.002*	*ns*	*ns*	*ns*	*0.002*	*ns*	
HPrefave	0.10	-0.67	-0.24	0.25	0.52				-0.22	0.57
s.e.	*0.089*	*0.134*	*0.141*	*0.105*	*0.120*				*0.120*	
P value	*0.280*	*< 0.001*	*0.093*	*0.021*	*< 0.001*	*ns*	*ns*	*ns*	*0.075*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

Using a combination of NIR-derived nutritive value traits improved prediction of oaten hay preferences, with an r² of 0.79 for cows, and 0.61for horses (Table 21), compared to a smaller number of wet chemistry-derived values. The NIR-derived nutritive value traits significantly related to both average cow and horse preferences were acid detergent fibre (nirADF), hemicellulose (nirHem), crude protein (nirCP) and water soluble carbohydrates (nirWSC). There was a negative relationship between both nirADF and nirHem and oaten hay preferences for dairy cows and horses. Whilst dairy cow preference was positively related to both nirCP and nirWSC, horse preference was positively related to nirCP but negatively associated with nirWSC.

Table 21. Dairy cow and horse oaten hay preference prediction equation using NIR-derived nutritive values measurements of acid detergent fibre (nirADF), hemicellulose (nirHem), crude protein (nirCP) and water soluble carbohydrate (nirWSC), detailing the constant, variable co-efficient and resulting r^2 for prediction of average cow preference and cow preference values when compared against standards A, BC, D and E, as well as average horse preference values.

		Co-efficients									
	Constant	**nirADF**	**nirHem**	**nirCP**	**nirWSC**	**nirADF. nirHem**	**nirADF. nirCP**	**nirHem. nirCP**	**nirADF. nirWSC**	**nirHem. nirWSC**	**r^{2*}**
CPrefave	-0.11	-0.85	0.02	0.91	0.65			-0.25			0.79
s.e.	*0.111*	*0.212*	*0.154*	*0.173*	*0.243*			*0.097*			
P value	*0.341*	*< 0.001*	*0.881*	*< 0.001*	*0.009*	*ns*	*ns*	*0.012*	*ns*	*ns*	
CPrefA	3.67	-1.13		0.48			0.46				0.51
s.e.	*0.181*	*0.182*		*0.181*			*0.155*				
P value	*< 0.001*	*< 0.001*	*ns*	*0.010*	*ns*	*ns*	*0.004*	*ns*	*ns*	*ns*	
CPrefBC	-1.24	-1.41	0.21	1.13	0.75	-0.68			-0.57		0.76
s.e.	*0.183*	*0.320*	*0.234*	*0.263*	*0.370*	*0.226*			*0.198*		
P value	*< 0.001*	*< 0.001*	*0.364*	*< 0.001*	*0.046*	*0.004*	*ns*	*ns*	*0.005*	*ns*	
CPrefD	-0.90	-0.75	-0.35	0.99	0.66	-0.31		-0.41			0.70
s.e.	*0.143*	*0.269*	*0.195*	*0.222*	*0.307*	*0.150*		*0.132*			
P value	*< 0.001*	*0.007*	*0.075*	*< 0.001*	*0.036*	*0.043*	*ns*	*0.002*	*ns*	*ns*	
CPrefE	-2.05	-0.92	-0.38	0.64				-0.43			0.64
s.e.	*0.131*	*0.142*	*0.143*	*0.160*				*0.115*			
P value	*< 0.001*	*< 0.001*	*0.009*	*< 0.001*	*ns*	*ns*	*ns*	*< 0.001*	*ns*	*ns*	
HPrefave	0.31	-0.64	-0.36	0.43	-0.32			-0.55	0.39	-0.31	0.61
s.e.	*0.107*	*0.179*	*0.129*	*0.147*	*0.205*			*0.108*	*0.099*	*0.108*	
P value	*0.005*	*< 0.001*	*0.006*	*0.004*	*0.126*	*ns*	*ns*	*< 0.001*	*< 0.001*	*0.006*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

When examining the relationship between nutritive value and lucerne hay preferences of horses, two models were used: Model 1: nirCP, nirHem, nirWSC; and Model 2: nirADF, nirHem, nir WSC, as detailed in Table 13 in the Statistical Analysis section of this report. Both models resulted in very similar predictions of horse lucerne hay preference, both generating an r^2 of 0.74 for average horse preference (Table 22 and 23). The NIR-derived nutritive value traits significantly related to horse preferences were nirADF, nirHem, nirCP and nirWSC. There was a negative relationship between both nirADF and nirHem and lucerne hay preferences of horses, and a positive relationship between nirCP and preference. There was no consistent relationship of nirWSC with preference, at times being negatively associated with preference (vs std LA and std LB) and at other times positively associated (vs std E and with average horse preference). Based on the magnitude of the co-efficient for each standardised variable, nirWSC had little effect on preference, whilst nirADF and nirCP tended to have the biggest effects.

Table 22. Prediction equations for lucerne hay preferences of horse using NIR-derived nutritive value measurements of crude protein (nirCP), water soluble carbohydrate (nirWSC) and hemicellulose (nirHem), detailing the constant, variable co-efficient and resulting r^2 for prediction of average horse trial hay preferences and horse trial hay preference values when compared against standards LA, LB, LC and LP.

		Co-efficients						
	Constant	nirCP	nirWSC	nirHem	nirCP. nirWSC	nirCP. nirHem	nirsWSC.nir Hem	r^{2*}
HPrefLave	-0.56	0.06	0.09	-0.05	-0.003			0.74
s.e.	*0.159*	*0.008*	*0.019*	*0.007*	*0.001*			
P value	*0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.022*	*ns*	*ns*	
HPrefLA	1.72	-0.02	0.06	-0.24	-0.01	0.01	0.01	0.70
s.e.	*0.627*	*0.029*	*0.073*	*0.044*	*0.002*	*0.002*	*0.003*	
P value	*0.008*	*0.545*	*0.418*	*< 0.001*	*0.007*	*< 0.001*	*0.015*	
HPrefLB	0.48	0.01	0.13	-0.13	-0.005	0.005		0.60
s.e.	*0.430*	*0.026*	*0.029*	*0.032*	*0.002*	*0.002*		
P value	*0.271*	*0.735*	*< 0.001*	*< 0.001*	*0.010*	*0.009*	*ns*	
HPrefLC	-1.16	0.08	0.02	0.004	0.0005	-0.003	0.0006	0.48
s.e.	*0.800*	*0.037*	*0.094*	*0.056*	*0.003*	*0.002*	*0.004*	
P value	*0.151*	*0.032*	*0.829*	*0.941*	*0.879*	*0.312*	*0.892*	
HPrefLP	-0.59	0.04	0.05	-0.05				0.52
s.e.	*0.241*	*0.009*	*0.011*	*0.009*				
P value	*0.018*	*< 0.001*	*< 0.001*	*< 0.001*	*ns*	*ns*	*ns*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

Table 23. Prediction equations for lucerne hay preferences of horse using NIR-derived nutritive values measurements of acid detergent fibre (nirADF), water soluble carbohydrate (nirWSC) and hemicellulose (nirHem), detailing the constant, variable co-efficient and resulting r^2 for prediction of average horse trial hay preferences and horse trial hay preference values when compared against standards LA, LB, LC and LP.

		Co-efficients						
	Constant	nirADF	nirWSC	nirHem	nirADF. nirWSC	nirADF. nirHem	nirWSC. nirHem	r^{2*}
HPrefLave	1.70	-0.03	0.02	-0.05				0.74
s.e.	*0.141*	*0.004*	*0.007*	*0.005*				
P value	*< 0.001*	*< 0.001*	*0.002*	*< 0.001*	*ns*	*ns*	*ns*	
HPrefLA	0.31	0.03	-0.07	0.09		-0.005	0.01	0.64
s.e.	*0.774*	*0.019*	*0.037*	*0.056*		*0.001*	*0.002*	
P value	*0.691*	*0.106*	*0.054*	*0.102*	*ns*	*0.001*	*0.001*	
HPrefLB	0.79	0.001	-0.11	0.11	0.004	-0.004		0.66
s.e.	*0.688*	*0.018*	*0.057*	*0.048*	*0.002*	*0.001*		
P value	*0.254*	*0.951*	*0.058*	*0.032*	*0.026*	*0.002*	*ns*	
HPrefLC	1.71	-0.03		-0.04				0.47
s.e.	*0.230*	*0.006*		*0.009*				
P value	*< 0.001*	*< 0.001*	*ns*	*< 0.001*	*ns*	*ns*	*ns*	
HPrefLP	2.59	-0.06	0.02	-0.16		0.003		0.54
s.e.	*0.745*	*0.020*	*0.011*	*0.057*		*0.002*		
P value	*0.001*	*0.003*	*0.068*	*0.006*	*ns*	*0.059*	*ns*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

7.2.4 Relationship between physical traits and preference

When examining the relationship between physical characteristics of the fodder and oaten hay preferences of dairy cows and horses, the prediction models were poorer than those based on nutritive values or intake rate. The prediction of average cow preferences gave an r^2 of 0.51 whilst the model for horses had an r^2 of 0.44 (Table 24). Those physical traits significantly related to cow and horse preference were shear energy (nirSE), average stem diameter (SD) and the variation in stem diameter (LMM), calculated as a log ratio of the maximum and minimum stem diameters. These three physical traits were negatively related to preference. Cow preference was also significantly and positively related to the proportion of leaf material in the hay (Leaf%). Based on the magnitude of the co-efficient for each standardised variable, shear energy had the strongest relationship to hay preference values, followed by stem diameter and the variation in stem diameter. Colour score had a small relationship with preference values with cows but only when hays were compared with oaten hay standards A or E.

Table 24. Dairy cow and horse oaten hay preference prediction equation using measurements of physical characteristics including shear energy (nirSE), average stem diameter (SD), a measure of the variation in stem diameter (LMM), colour (/60) and the percentage of leaf within the hay (Leaf%), detailing the constant, variable co-efficient and resulting r^2 for prediction of average cow trial hay preferences and cow trial hay preference values when compared against standards A, BC, D and E, as well as average horse preference.

		Co-efficients								
	Constant	nirSE	SD	LMM	Colour	Leaf%	nirSE. SD	Colour. LMM	SD. Leaf%	r^{2*}
CPrefave	-0.21	-1.15	-0.28	-0.46		0.33			0.29	0.51
s.e.	*0.154*	*0.159*	*0.156*	*0.160*		*0.157*			*0.143*	
P value	*0.188*	*< 0.001*	*0.079*	*0.005*	*ns*	*0.038*	*ns*	*ns*	*0.048*	
CPrefA	3.36	-0.96	-0.57	-0.24	0.28		-0.46	0.54		0.31
s.e.	*0.200*	*0.217*	*0.211*	*0.215*	*0.210*		*0.220*	*0.273*		
P value	*< 0.001*	*< 0.001*	*0.008*	*0.279*	*0.188*	*ns*	*0.040*	*0.050*	*ns*	
CPrefBC	-0.99	-1.52	-0.57	-0.73		0.54				0.47
s.e.	*0.231*	*0.250*	*0.243*	*0.245*		*0.242*				
P value	*< 0.001*	*< 0.001*	*0.022*	*0.004*	*ns*	*0.030*	*ns*	*ns*	*ns*	
CPrefD	-1.09	-1.22		-0.57		0.35				0.46
s.e.	*0.175*	*0.189*		*0.185*		*0.179*				
P value	*< 0.001*	*< 0.001*	*ns*	*0.003*	*ns*	*0.056*	*ns*	*ns*	*ns*	
CPrefE	-2.32	-1.01	-0.44	-0.22	0.07			0.40		0.38
s.e.	*0.161*	*0.175*	*0.170*	*0.173*	*0.169*			*0.220*		
P value	*< 0.001*	*< 0.001*	*0.011*	*0.212*	*0.703*	*ns*	*ns*	*0.073*	*ns*	
HPrefave	0.06	-0.75	-0.31	-0.24						0.44
s.e.	*0.105*	*0.114*	*0.109*	*0.112*						
P value	*0.545*	*< 0.001*	*0.005*	*0.035*	*ns*	*ns*	*ns*	*ns*	*ns*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

The prediction models based on physical characteristics for lucerne hay preferences of horses again resulted in poorer predictions than those based on nutritive values or intake rate. The prediction of average horse preference gave an r^2 of 0.54 (Table 25). The physical traits significantly related to horse preference were nirSE, LMM and Leaf%. All three of these physical traits were negatively related to lucerne hay preferences of horses, although the effect of Leaf% was minimal. Shear energy was most strongly related to preference, but horses were less sensitive to changes in shear energy than dairy cows (as evidenced by the smaller co-efficient for nirSE in both the oaten and lucerne hay predictive equations for horses).

Table 25. Horse lucerne hay preference prediction equation using measurements of physical characteristics including shear energy (nirSE), average stem diameter (SD), a measure of the variation in stem diameter (LMM), colour (/5) and the percentage of leaf within the hay (Leaf%), detailing the constant, variable co-efficient and resulting r^2 for prediction of average horse trial hay preferences and horse trial hay preference values when compared against standards LA, LB, LC and LP.

		Co-efficient						
	Constant	**nirSE**	**SD**	**LMM**	**Leaf%**	**Colour**	**Leaf%. nirSE**	**r^{2*}**
HPrefLave	3.62	-0.32		-0.17	-0.05		0.005	0.54
s.e.	*0.776*	*0.067*		*0.076*	*0.025*		*0.002*	
P value	*< 0.001*	*< 0.001*	*ns*	*0.028*	*0.071*	*ns*	*0.027*	
HPrefLA	4.78	-0.42			-0.10		0.01	0.39
s.e.	*1.076*	*0.094*			*0.035*		*0.003*	
P value	*< 0.001*	*< 0.001*	*ns*	*ns*	*0.005*	*ns*	*0.002*	
HPrefLB	4.83	-0.40			-0.10	-0.08	0.01	0.40
s.e.	*1.064*	*0.094*			*0.034*	*0.047*	*0.003*	
P value	*< 0.001*	*< 0.001*	*ns*	*ns*	*0.005*	*0.101*	*0.002*	
HPrefLC	2.35	-0.21		-0.20	0.01			0.38
s.e.	*0.560*	*0.044*		*0.103*	*0.003*			
P value	*< 0.001*	*< 0.001*	*ns*	*0.062*	*0.020*	*ns*	*ns*	
HPrefLP	2.30	-0.18		-0.22	0.01	-0.09		0.40
s.e.	*0.550*	*0.043*		*0.099*	*0.003*	*0.045*		
P value	*< 0.001*	*< 0.001*	*ns*	*0.026*	*0.068*	*0.047*	*ns*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

7.2.5 Relationship between combined nutritive and physical traits and oaten hay preference of cows and horses

A prediction model based on a combination of nutritive and physical traits: *in vitro* digestibility; (nirIVD); NIR derived crude protein (nirCP); water soluble carbohydrate (nirWSC); and shear energy (nirSE), was investigated for cow and horse oaten hay preferences. The prediction of average cow preference included only the nutritive value terms, nirIVD, nirCP and nirWSC as nirSE was not significantly related, and gave an r^2 of 0.76 (Table 26). This was similar to the best predictive equation based on nutritive value traits, and an improvement on the predictions based solely on physical traits. All three of these traits (nirIVD, nirCP and nirWSC) were positively related to oaten hay preferences of dairy cows with crude protein having the most consistent and largest effect.

The prediction of average horse preference included all four terms (nirIVD, nirCP, nirWSC and nirSE), and gave an r^2 of 0.54 (Table 26), which again, is similar to the predictive equation based on nutritive value traits alone and an improvement on the predictions based solely on physical traits.

Table 26. Dairy cow and horse oaten hay preference prediction equation using a combination of physical and nutritive value traits including in vitro digestibility (nirIVD), NIR-derived crude protein (nirCP), water soluble carbohydrate (nirWSC) and shear energy (nirSE), detailing the constant, variable co-efficient and results r^2 for prediction of average cow trial hay preferences and cow trial hay preference values when compared against standards A, BC, D and E, as well as average horse preference.

		Co-efficients						
	Constant	**nirIVD**	**nirCP**	**nirWSC**	**nirSE**	**nirIVD. nirCP**	**nirCP. nirWSC**	r^{2*}
CPrefave	-0.23	0.73	1.15	0.71				0.76
s.e.	*0.109*	*0.253*	*0.211*	*0.250*				
P value	*0.035*	*0.005*	*< 0.001*	*0.006*	*ns*	*ns*	*ns*	
CPrefA	3.62	1.04	0.69			-0.42		0.47
s.e.	*0.188*	*0.188*	*0.197*			*0.183*		
P value	*< 0.001*	*< 0.001*	*< 0.001*	*ns*	*ns*	*0.026*	*ns*	
CPrefBC	-1.00	0.99	1.52	0.81				0.70
s.e.	*0.168*	*0.411*	*0.336*	*0.385*				
P value	*< 0.001*	*0.018*	*< 0.001*	*0.040*	*ns*	*ns*	*ns*	
CPrefD	-1.13		1.25	1.17	-0.54			0.75
s.e.	*0.116*		*0.148*	*0.162*	*0.158*			
P value	*< 0.001*	*ns*	*< 0.001*	*< 0.001*	*0.001*	*ns*	*ns*	
CPrefE	-2.25		1.29	0.82	-0.38			0.71
s.e.	*0.106*		*0.130*	*0.147*	*0.140*			
P value	*< 0.001*	*ns*	*< 0.001*	*< 0.001*	*0.008*	*ns*	*ns*	
HPrefave	0.08	-0.34	0.78	0.40	-0.69		0.18	0.54
s.e.	*0.101*	*0.352*	*0.208*	*0.240*	*0.194*		*0.109*	
P value	*0.450*	*0.341*	*< 0.001*	*0.103*	*< 0.001*	*ns*	*0.113*	

* all r^2 are adjusted r^2

ns indicates terms that were not significant

7.3 Prediction of preferences and intake rates of dairy cows for oaten hay and preferences and intake rates of horses for oaten and lucerne hay obtained directly from visible and near infrared spectra

The best prediction of oaten hay preference and intake rate by both cows and horses was model 1 (Table 27), generated using the full spectrum (visible and NIR regions) and resulted in an adjusted r^2 of 0.75 for average cow oaten hay preference (Figure 27) and 0.76 for cow oaten hay intake rate and an adjusted r^2 of 0.65 for average horse oaten hay preference (Figure 28) and 0.40 for horse oaten hay intake rate.

The best prediction of lucerne hay preference by horses was also model 1 (Table 28), generated using the full spectrum (visible and NIR regions) and resulted in an adjusted r^2 of 0.84 for average horse lucerne hay preference (Figure 29). Both models provided similar predictions of lucerne hay intake rate of horses (r^2 of 0.66 - 0.67).

Table 27. WinISI calibration models used to investigate the potential to predict oaten hay preference and intake rate by dairy cows and horses, directly from the near infrared and visible spectrums.

WinISI model	Response variabl	n	r^2	adjusted r^2
	CPrefA	84	0.66	0.54
	CPrefBC	84	0.74	0.70
	CPrefD	84	0.68	0.62
Calibration model 1: *	**CPrefE**	82	0.67	0.62
Modified PLS analysis	**CPrefave**	84	0.83	0.75
Full spectrum:	**CIR**	83	0.86	0.76
(259 wavelengths; 400-2498nm at 8nm intervals	**HPrefA**	84	0.77	0.65
1,4,4,1 SNV & Detrend	**HPrefBC**	84	0.77	0.65
Included validation file	**HPrefD**	84	0.77	0.65
	HPrefE	84	0.77	0.65
	HPrefave	84	0.77	0.65
	HIR	84	0.52	0.40
	CPrefA	85	0.47	0.37
	CPrefBC	84	0.76	0.68
Calibration model 2:	**CPrefD**	83	0.66	0.61
Modified PLS analysis	**CPrefE**	81	0.66	0.62
NIR spectrum only:	**CPrefave**	84	0.80	0.74
(173 wavelengths; 1100-2498nm at 8nm interva	**CIR**	85	0.80	0.70
1,4,4,1 SNV & Detrend	**HPrefA**	84	0.67	0.61
Included validation file	**HPrefBC**	84	0.67	0.61
	HPrefD	84	0.67	0.61
	HPrefE	84	0.67	0.61
	HPrefave	84	0.67	0.61
	HIR	83	0.61	0.43

* model 1 gave the best predictions of oaten hay preference and intake rate of cows and horses

Table 28. WinISI calibration models used to investigate the potential to predict lucerne hay preference and intake rate by horses, directly from the near infrared and visible spectrums.

WinISI model	Response variable	n	r^2	adjusted r^2
Calibration model 1: *	**HPrefLA**	70	0.80	0.67
Modified PLS analysis	**HPrefLB**	67	0.73	0.72
Full spectrum:	**HPrefLC**	70	0.46	0.45
(259 wavelengths; 400-2498nm at 8nm intervals)	**HPrefLP**	68	0.67	0.57
1,4,4,1 SNV & Detrend	**HPrefLave**	69	0.89	0.84
Included validation file	**HLIR**	69	0.76	0.66
Calibration model 2:	**HPrefLA**	65	0.72	0.68
Modified PLS analysis	**HPrefLB**	68	0.69	0.66
NIR spectrum only:	**HPrefLC**	70	0.56	0.44
(173 wavelengths; 1100-2498nm at 8nm intervals)	**HPrefLP**	67	0.69	0.60
1,4,4,1 SNV & Detrend	**HPrefLave**	69	0.86	0.81
Included validation file	**HLIR**	70	0.80	0.67

* model 1 gave the best overall predictions of lucerne hay preference and intake rate of horses

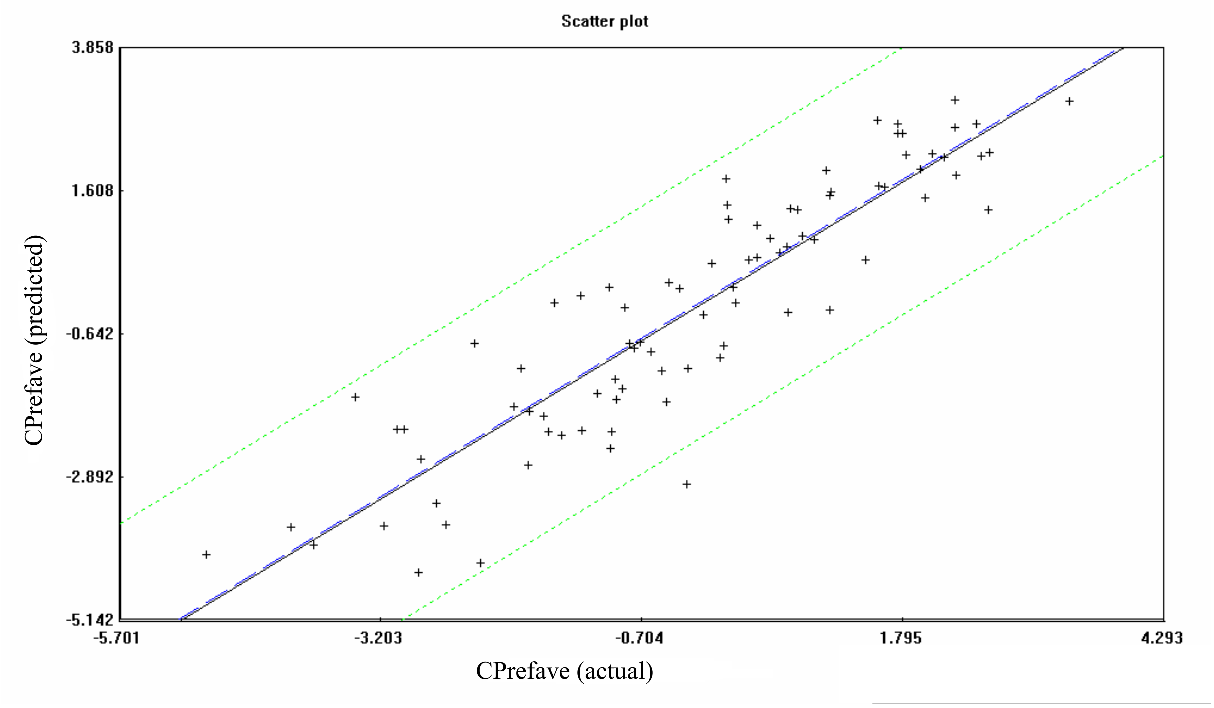


Figure 27. WinISI calibration model 1 (Full NIR and visible spectrum) showing predicted preference values in relation to actual preference values; oaten hay preference of dairy cows (slope = 1.00, r^2 = 0.83)

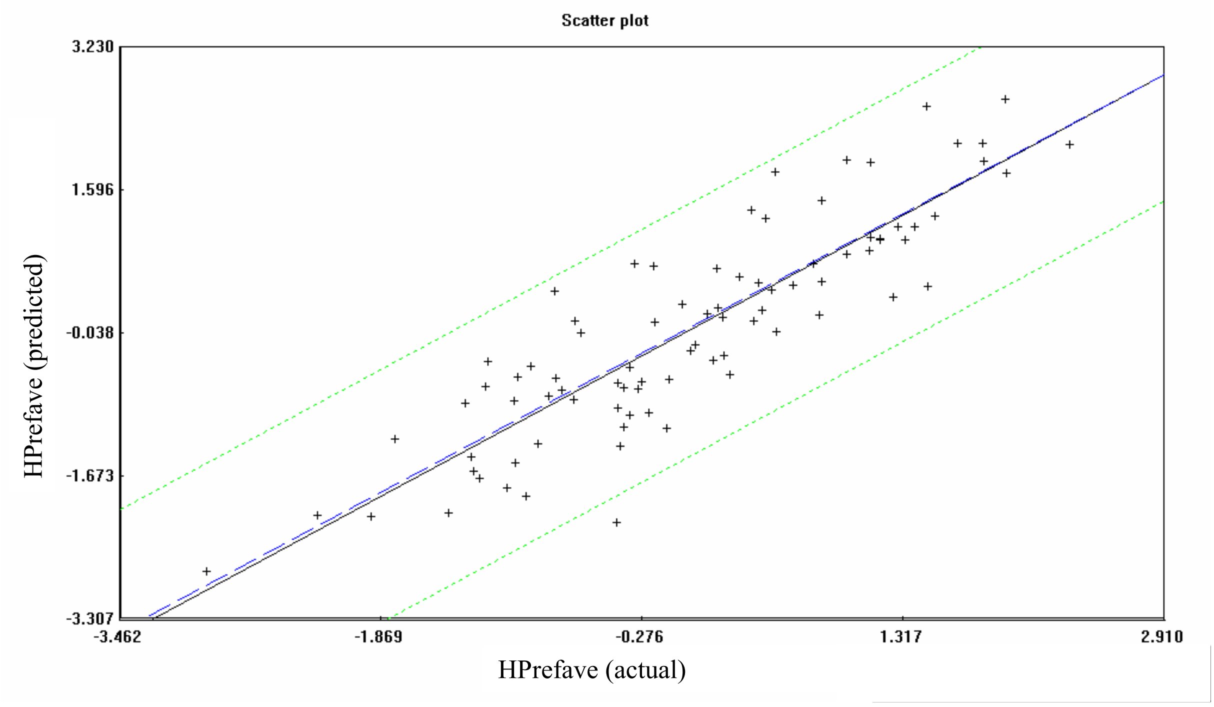


Figure 28. WinISI calibration model 1 (Full NIR and visible spectrum) showing predicted preference values in relation to actual preference values; oaten hay preference of horses (slope = 1.00, r^2 = 0.77)

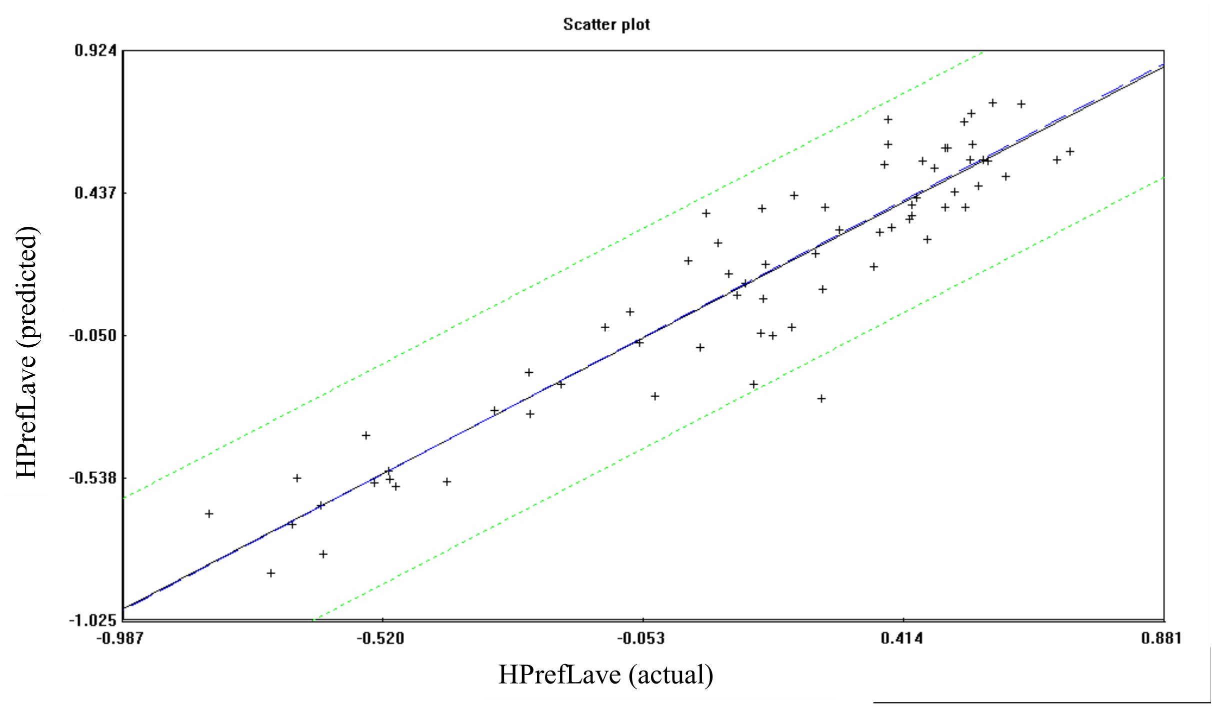


Figure 29. WinISI calibration model 1 (Full NIR and visible spectrum) showing predicted preference values in relation to actual preference values; lucerne hay preference of horses (slope = 1.00, r^2 = 0.89)

7.4 Relationship between oaten and lucerne hay volatiles and preference

A total of 120 oaten volatile compounds were found from the gas chromatographs. On average, each oaten hay possessed 64 volatile compounds (SE ± 0.79), although it ranged from 37 to 77 compounds per hay sample. Of the total of 120, six oaten hay volatiles had some relationship with cow preferences, either with each of the four standards (A, BC, D and E) and/or the average preference. Six oaten hay volatiles were also related to horse preferences (Table 29 and 30; respectively). Of the oaten hay volatiles significantly related to preference, four were consistently positively related to cow preference whilst two were consistently negatively related to cow preference. There were also four volatiles consistently positively related to horse preference and two consistently negatively related. Of the eight oaten hay volatiles found to influence the preferences of dairy cows and horses, four were common to both animal species (oaten volatile peaks 27 and 60, negatively related compounds in both species and 52 and 110, positively related compounds in both species). The remaining four peaks, whilst all positively related to preference were unique to either species; dairy cow preference was related positively to oaten hay peaks 50 and 109, whilst horse preference was related positively to oaten hay peaks 62 and 80.

A total of 147 lucerne volatile compounds were found from the gas chromatographs. On average, each lucerne hay possessed 82 volatile compounds (SE ± 2.14), although that ranged from 49 to 107 compounds per hay sample. Of the total 147, 15 of lucerne hay volatiles had some relationship with horse preference either with each of the four standards (LA, LB, LP and LC) and/or the average preference (Table 31), of which nine were consistently positively related to horse preference whilst six were consistently negatively related.

Table 29. Oaten hay volatiles significantly related to oaten hay preferences by dairy cows

Oaten volatile Peak #	n	Average r^2	OPrefave		OPrefA		OPrefBC		OPrefD		OPrefE	
			r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
27	17	0.59	0.69	Negative	0.67	Negative	0.59	Negative	0.60	Negative	0.40	Negative
50	84	0.20	.	.	.	.	.	.	.	.	0.20	Positive
52	84	0.40	0.47	Positive	0.24	Positive	0.43	Positive	0.42	Positive	0.46	Positive
60	11	0.37	0.38	Negative	0.24	Negative	0.43	Negative	0.43	Negative	.	.
109	83	0.22	0.22	Positive	.	.	0.22	Positive	.	.	.	.
110	33	0.23	.	.	.	.	.	.	0.23	Positive	.	.

. indicates models that although contained $n > 10$, had $r^2 < 0.2$

Table 30. Oaten hay volatiles significantly related to oaten hay preferences by horses

Oaten volatile Peak #	n	Average r^2	HPrefave		HPrefA		HPrefBC		HPrefD		HPrefE	
			r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
27	17	0.35	0.39	Negative	.	.	0.40	Negative	0.38	Negative	0.22	Negative
52	84	0.22	0.22	Positive	.	.	.	.	.	.	.	.
60	11	0.32	0.28	Negative	0.20	Negative	0.28	Negative	.	.	0.51	Negative
62	18	0.34	0.34	Positive	.	.	.	.	0.31	Positive	0.35	Positive
80	39	0.23	.	.	.	.	0.23	Positive	.	.	.	.
110	33	0.24	0.27	Positive	.	.	0.25	Positive	0.20	Positive	.	.

. indicates models that although contained $n > 10$, had $r^2 < 0.2$

Table 31. Lucerne hay volatiles significantly related to lucerne hay preferences by horses

Lucerne volatile Peak #	n	Average r^2	HPrefLav		HPrefLA		HPrefLB		HPrefLC		HPrefLP	
			r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
14	20	0.32	0.23	Positive	.	.	0.41	Positive	.	.	.	.
24	11	0.24	.	.	.	.	0.27	Negative	.	.	.	.
28	11	0.36	.	.	0.44	Negative	.	.	.	.	0.28	Negative
36	40	0.28	0.25	Positive	.	.	.	.	.	.	0.31	Positive
37	35	0.23	.	.	.	.	0.25	Negative	.	.	.	.
45	20	0.26	0.35	Negative	0.20	Negative	.	.	0.23	Negative	0.28	Negative
46	14	0.32	0.30	Positive	.	.	.	.	0.35	Positive	.	.
57	20	0.37	0.44	Positive	0.35	Positive	.	.	0.23	Positive	0.46	Positive
59	23	0.43	0.38	Positive	.	.	.	.	.	.	0.49	Positive
60	25	0.25	0.21	Positive	.	.	.	.	.	.	0.30	Positive
72	45	0.28	0.31	Positive	.	.	.	.	0.25	Positive	.	.
73	13	0.21	.	.	.	.	0.27	Positive	0.14	Positive	.	.
76	46	0.29	0.39	Positive	.	.	0.25	Positive	0.27	Positive	0.26	Positive
117	19	0.41	0.39	Negative	0.29	Negative	0.56	Negative	0.39	Negative	.	.
119	26	0.27	.	.	.	.	.	.	0.27	Negative	.	.

. indicates models that although contained n> 10, had $r^2 < 0.2$

7.5 Relationship between the nutritive and physical properties of the hays and oaten and lucerne volatiles

Of the eight oaten hay volatiles related to preferences of dairy cows and/or horses, all had some relationship with nutritive value or physical traits (Table 32 and 33). The relationship between the volatiles and nutritive quality was, in the main, associated with the fibre (nirNDF and nir ADF) and protein (nirCP) components of the hay. The relationship of the volatiles with the physical characteristics were generally strongest with shear energy and stem measures, which reflect the fibre content of the hay, and with the leaf percentage, a trait correlated to the protein content of the hay. There was some relationship with the soluble carbohydrate fraction of the hay, with half of the volatiles showing an association with nirWSC. Of the total of eight volatiles, only one showed a moderate positive relationship with hemicellulose (nirHem) for both cows and horses. Volatiles positively related to preference were also positively related to those nutritive and physical traits positively related to preference and, similarly, negatively related to those traits negatively related to preference.

Of the 15 lucence hay volatiles related to horse preference, 14 had some relationship with nutritive value or physical traits, whilst lucerne volatile peak 37 did not show any relationship with either nutritive value or physical traits (Table 34 and 35). Unlike the oaten volatiles, lucerne hay volatiles were not strongly related to nirADF, being instead highly related to nirHem and somewhat to nirNDF. They were also less related to nirCP than the oaten volatiles. Consequently the lucerne volatiles were also less related to those physical characteristics associated with structural fibre and protein such as shear energy, stem measures and leaf percentage. Lucerne hay volatiles showed no consistent pattern with WSC, reflecting the inconsistent relationship of nirWSC with preference. As for oaten hay, lucerne volatiles positively related to preference were positively related to nutritive and physical traits positively related to preference, and negatively related to traits negatively related to preference.

Table 32. Relationship between oaten hay nutritive value and volatiles related to oaten hay preferences of dairy cows and horses

Oaten volatile Peak #	n	Direction of linear relationship with Cow and Horse Preference	Horse Preference average r^2	Cow Preference average r^2	nirNDF		nirADF		nirHem		nirCP		nirWSC		nirIVD	
					r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
27	17	Negative	0.35	0.59	0.58	Positive	0.80	Positive	.	.	0.50	Negative	0.31	Negative	0.71	Negative
50	84	Positive	*n/a*	0.20	.	.	0.27	Negative	.	.	0.47	Positive	.	.	0.27	Positive
52	84	Positive	0.22	0.40	0.51	Negative	0.56	Negative	.	.	0.52	Positive	0.29	Positive	0.57	Positive
60	11	Negative	0.32	0.37	0.69	Positive	0.69	Negative	0.27	Positive	0.35	Positive	0.46	Negative	0.64	Negative
62	18	Positive	0.34	*n/a*	0.22	Negative	.	.	.	.	0.23	Positive	.	.	.	.
80	39	Positive	0.23	*n/a*	0.39	Negative	0.34	Negative	.	.	0.32	Positive	.	.	0.38	Positive
109	83	Positive	*n/a*	0.22	0.38	Negative	0.40	Negative	.	.	0.47	Positive	0.24	Positive	0.46	Positive
110	33	Positive	0.24	0.23	0.25	Negative	0.27	Negative	.	.	.	.	.	.	0.27	Positive

. indicates models that although contained n> 10, had r^2< 0.2

n/a indicates those votiles within species, that did not have a significant influence on preference

Table 33. Relationship between oaten hay physical characteristics and volatiles related to oaten hay preferences of dairy cows and horses

					nirSE		SD		LMM		Leaf%		Colour	
Oaten volatile Peak #	n	Direction of linear relationship with Cow and Horse Preference	Horse Preference average r^2	Cow Preference average r^2	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
27	17	Negative	0.35	0.59	0.43	Positive	0.32	Positive	0.38	Positive	0.24	Negative	0.24	Negative
50	84	Positive	*n/a*	0.20	.	.	0.26	Negative	.	.	0.31	Positive	.	.
52	84	Positive	0.22	0.40	0.31	Negative	0.25	Negative	0.20	Negative	0.28	Positive	.	.
60	11	Negative	0.32	0.37	0.59	Positive	0.38	Positive	0.57	Positive	0.37	Negative	.	.
62	18	Positive	0.34	*n/a*	.	.	0.52	Negative	0.34	Negative	.	.	0.24	Positive
80	39	Positive	0.23	*n/a*	0.52	Negative	.	.	.	.	0.26	Positive	0.23	Positive
109	83	Positive	*n/a*	0.22	0.28	Negative	.	.	.	.	0.21	Positive	0.35	Positive
110	33	Positive	0.24	0.23	0.40	Negative	.	.	.	.	0.30	Negative	.	.

. indicates models that although contained n> 10, had r^2< 0.2

n/a indicates those votiles within species, that did not have a significant influence on preference

Table 34. Relationship between lucerne hay nutritive value and volatiles related to lucerne hay preferences of horses

			nirNDF		nirADF		nirHem		nirCP		nirWSC		nirIVD	
Lucerne volatile Peak #	Horse Preference average r^2	Direction of linear relationship with preference	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
14	0.32	Positive	.	.	.	.	.	.	.	.	.	.	.	.
24	0.24	Positive	0.47	Negative	.	.	0.41	Negative	0.24	Positive	0.28	Negative	0.39	Positive
28	0.36	Negative	.	.	0.24	Negative	0.47	Positive	0.24	Positive	0.34	Negative	.	.
36	0.28	Positive	0.43	Negative	.	.	0.47	Negative	.	.	.	.	0.45	Positive
37	0.23	Negative	.	.	.	.	.	.	.	.	.	.	.	.
45	0.26	Negative	.	.	.	.	0.39	Positive	0.31	Positive	0.57	Negative	0.22	Negative
46	0.32	Positive	.	.	.	.	.	.	.	.	0.30	Positive	.	.
57	0.37	Positive	0.28	Negative	.	.	0.38	Negative	.	.	.	.	.	.
59	0.43	Positive	0.30	Negative	.	.	0.38	Negative	.	.	0.47	Positive	0.31	Positive
60	0.25	Positive	0.23	Negative	.	.	0.23	Negative	.	.	.	.	0.23	Positive
72	0.28	Positive	0.54	Negative	.	.	0.53	Negative	0.30	Positive	.	.	0.49	Positive
73	0.21	Positive	0.49	Negative	0.51	Negative	.	.	0.63	Positive	0.30	Negative	0.36	Positive
76	0.29	Positive	0.60	Negative	.	.	0.61	Negative	0.34	Positive	.	.	0.53	Positive
117	0.41	Negative	0.38	Positive	0.21	Positive	0.21	Positive	0.29	Negative	.	.	0.37	Negative
119	0.27	Negative	.	.	.	.	.	.	.	.	.	.	0.22	Negative

. indicates models that although contained n> 10, had r^2< 0.2

Table 35. Relationship between lucerne hay physical characteristics and volatiles related to lucerne hay preferences of horses

Lucerne volatile Peak #	Horse Preference average r^2	Direction of linear relationship with preference	nirSE		SD		LMM		Leaf%		Colour	
			r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship	r^2	Direction of linear relationship
14	0.32	Positive	.	.	.	.	.	.	0.20	Positive	.	.
24	0.24	Positive	0.39	Negative	0.25	Positive	.	.	0.37	Positive	0.64	Negative
28	0.36	Negative	.	.	.	.	.	.	.	.	0.47	Positive
36	0.28	Positive	0.22	Negative	.	.	.	.	.	.	.	.
37	0.23	Negative	.	.	.	.	.	.	.	.	.	.
45	0.26	Negative	.	.	0.29	Negative	.	.	.	.	0.56	Positive
46	0.32	Positive	.	.	.	.	.	.	.	.	.	.
57	0.37	Positive	0.22	Negative	.	.	0.33	Negative	.	.	0.38	Negative
59	0.43	Positive	.	.	.	.	.	.	0.28	Positive	0.25	Negative
60	0.25	Positive	.	.	.	.	0.39	Negative	.	.	.	.
72	0.28	Positive	0.41	Negative	0.24	Negative	.	.	0.20	Positive	0.25	Negative
73	0.21	Positive	0.44	Negative	0.46	Negative	0.46	Negative	0.40	Positive	.	.
76	0.29	Positive	0.37	Negative	0.36	Negative	.	.	0.31	Positive	.	.
117	0.41	Negative	.	.	0.32	Positive	0.29	Negative	0.53	Negative	.	.
119	0.27	Negative	0.21	Positive	.	.	0.34	Negative	.	.	.	.

. indicates models that although contained n> 10, had $r^2 < 0.2$

8 General Discussion

This project was designed to provide significant scale and replication (across time, animals and hays) to develop robust predictions of preference values for oaten and lucerne hays. It also was designed to use the animal species most relevant to end-users of the information and develop species-specific predictions, as it was recognised from previous work that the factors that influence animal preferences are not necessarily consistent across animal species (Flinn et al. 2005). Hence, I have been able to provide new information on the main factors that influence the preference of oaten hays by lactating Holstein Friesian cows and Thoroughbred horses, and the factors that influence the preference of lucerne hay by Thoroughbred horses. The analyses presented in this report used standardised variables, which allowed us to directly compare the effects of different variables, within and across the prediction equations produced, on preference values. That is, the magnitude of the co-efficient for a given variable is proportional to its scale of effect on the preference value. To my knowledge, this project is the largest of its kind undertaken and, for that reason, provides the first opportunity to confidently identify the main drivers of short-term preferences by the 'target' animals, and incorporate this information into quality assessment programs currently in use by the fodder industry.

8.1 Oaten hay and lactating dairy cows

Maintaining a high level of voluntary feed intake in high-producing dairy cows is a key issue in the industry, both in Australia and overseas. Maintenance of consistent roughage intake is also important because of its importance in maintaining a functional and healthy profile of rumen fermentation, and also because of its role in providing a precursor for milk fat synthesis (Pond et al. 2005). Short-term fluctuations in feed intake are often translated into fluctuations in milk output, and hence there is considerable interest to avoid periods when animals reduce voluntary feed intake in response to undesirable characteristics of the feed. The problems are exacerbated if a batch of hay, purchased as premium grade based on subjective assessment and/or nutritive value traits, is not readily consumed by the animals.

There was a strong correlation (r^2 =0.66) between the average preference value of oaten hays with lactating dairy cows (averaged across tests with all standard hays), and both the intake rate as a single factor and *in vitro* digestibility (IVD) as a single factor. This suggests that lactating dairy cows select against hays that are high in plant structural compounds that take more energy to chew and are less readily fermentable. Indeed, in the multi-factorial prediction equations based on nutritive value traits, both ADF and hemicelluloses, were significantly and negatively related to preference values. ADF had a particularly strong influence on hay preference, which is consistent with the notion that hays high in the less digestible structural components – lignin and cellulose – are less preferred by lactating dairy cows. Similarly, in the multi-factorial prediction equation based on the physical traits of hays, shear energy had the strongest influence on preference values, followed by descriptions of stem thickness.

Plant structural components are not the only drivers of oaten hay preference, because the 'best' equation, based on having the highest correlation co-efficient, to predict preferences by dairy cows was one that incorporated ADF, hemicellulose, crude protein and water soluble carbohydrates. In fact, the effect of a change in crude protein content was similar to the effect of a change in ADF content. So, in effect, dairy cows were putting approximately equal weighting, although in opposing directions, on ADF (negative weighting) and crude protein (positive weighting) in making their 'decisions' on what hay to eat during the preference tests, emphasising the importance of energy to protein ratios for ruminants (Villalba and Provenza 1999).

Besides shear energy and stem thickness, cows preferred for hays with a higher leaf percentage, but were not influenced by hay colour. This is an important finding, as hay is commonly assessed subjectively by people on the basis of colour, but for the hays tested in this work, colour was not related to preference. The reason people use colour to assess hay is a belief that colour (greenness) is an indicator of "freshness" and quality (eg, a green hay is associated with a higher protein content, a higher WSC content or a higher percentage of leaf material). Cows are able to distinguish between different hues of colour (Riol et al. 1989, Uetake 1991), which would indicate that they possess the ability to use colour to discriminate between hays. This would be useful if hay colour was, as people generally believe, associated with better quality nutritional value, allowing the animal to use colour as a dietary cue in their selection process. However, our data indicate the dairy cows assess oaten hays on other traits over and above colour and that colour is not associated with any of the other nutritive or physical traits measured in this particular trial. The lack of reliance on colour discrimination may be because vision is typically a sense used to make choices from a distance based more on a visual assessment of spatial arrangement than colour, and that when presented at close quarters with a hay in a feed bucket, other traits are more 'meaningful' to them. It is also possible that they are not able to sufficiently distinguish between the subtle differences in hues of oaten hay as their eyes are more suited to distinguishing more dramatic visual contrasts, or differences in colour brightness (Bazely 1988, Bazely and Ensor 1989).

The combination of four traits – IVD, crude protein, water soluble carbohydrates and shear energy - predicted by NIRS and commonly used by processors in the export hay industry as quality markers, accounted for approximately three-quarters of the variation in average preference. The high correlation with most preference values (average, vs hay A, vs Hay D and vs Hay E) suggest that NIR predictions of these four traits of hays can be used to provide useful predictions of hay preference across a range of conditions. That these traits are already commonly measured by industry is encouraging, as they could quite quickly be used to develop a 'predicted preference score' for oaten hays. IVD, CP and WSC were all positively related to preference and each had a similar weighting in the prediction equations. Shear energy was only significantly (and negatively) related when preference was expressed relative to the two higher quality standard hays, Hays D and E.

The studies reported here are the first to show that NIR spectra can be used to predict hay preferences. The variation in preference values accounted for was only marginally lower than that obtained with the four traits described above. A direct NIR prediction of preference represents a major opportunity for the oaten hay industry to rapidly and cheaply estimate preference, without the need to first determine other nutritive value or physical traits. As demonstrated in this study an animal's response to a particular feedstuff does not depend wholly on a single chemical entity, but rather the incorporation and balance of a number of feed characteristics. The ability of NIRS to give a complete profile of the chemical makeup of a sample would allow a prediction of preference that takes into account, simultaneously, all those individual influencing feed characteristics.

8.2 Oaten hay and horses

In general, less of the variation in oaten hay preferences of horses was accounted for than it was for dairy cows ($r^2 = 0.45$). Faverdin et al. (1995) similarly demonstrated that horses showed little variation in preference among forages when compared to ruminants. This means horses were either making selections based on traits that we did not quantify, or they were less selective. There is currently limited research on how horses develop dietary preferences and make dietary selections. Anecdotally, horses are recognised as 'fussy eaters', however, this work appears to contradict this with horses showing a lesser degree of preference variation. It is difficult to see that there could be any advantage for an animal to be fickle about food choice without a nutritional or physiological basis to it. Differences in selectivity (i.e., seeking diversity 'versus' seeking particular components from feeds) between cows and horses could be reflected in morphological differences in their mouth and different foraging methods (McBane and McCarthy 1991). Horses, like sheep use their lips to gather food and avoid ingesting foreign objects (weeds, stones, unpalatable plants or parts of plants) and are therefore able to more selectively seek out different components of the feed. Dulphy et al. (1997) concluded it was unreliable to use classical forage characteristics, such as crude protein (CP), crude fibre (CF) or neutral detergent fibre (NDF), to predict voluntary dry matter intake in horses, as they reacted less strongly to these forage characteristics due to their caecal fermentation, than do fore-gut fermenting sheep. Similarly in this study, the hays various nutritive and physical characteristics were much less influential in relation to horse preferences than they were for cattle, making preference predictions for horses largely unsuccessful.

Intake rate or IVD alone accounted for 33-40% of the variation in feed preferences of horses offered oaten hays. This was improved to about 60% when a range of nutritive value traits were incorporated into the prediction equation. The nutritive value traits identified as significantly related to preference were the same as identified with dairy cows; i.e., ADF, hemicellulose cruide protein and water soluble carbohydrates. As for cows, the major influence could be attributed to ADF (negative relationship with preference), although this influence was not as critical for horses. Given that ADF is associated with the structural components of the plant it is not surprising that when the relationships between physical traits of oaten hay and preference values for horses were examined, shear energy had the largest (negative) effect, followed by stem thickness. This was similar to the findings with the dairy cows, but again to a lesser extent. Neither colour nor the leafiness of hays was related to horse preferences. This is noteworthy because as with hay for dairy cows, many people attempt to assess hay quality based on colour and leafiness, but the animals themselves use other traits to ‘assess’ the hays, at least in the short-term tests used in this work.

Similar to this study, Cymbaluk (1990) found that horses were less sensitive to the cell-wall content of forages than ruminants. This is most likely due to differences in the mechanisms controlling appetite between ruminants and horses. Horses appear to be less sensitive to physical appetite controlling mechanisms (Ralston and Baile 1982, Laut et al. 1985, Faverdin et al. 1995, Dulphy et al. 1997). Horses lack the reticulo-omasal orifice found in ruminants, which is highly sensitive to, and selectively retains, large forage particles for regurgitation, repeated mastication and rumination until small enough to move further down the digestive tract. In light of evidence suggesting that physical regulation of intake in horses may be weak, organoleptic qualities of forages (taste, odor, ease of prehension, toughness, ease of sorting, etc.), may be of greater importance for their diet selection (Doreau et al.1990).

Two main differences between horses and dairy cows were found: (i) the gross energy content of the hay accounted for a significant portion of the variation in horse preferences when it did not for dairy cows; and (ii) water soluble carbohydrates were negatively related to horse preferences whilst they were positively related to cow preferences. The negative influence of water soluble carbohydrates is contrary to the findings of Randall et al. (1978) who concluded that horses showed a preference for sweet foods generally associated with higher energy contents. It could be argued that the horses in the study described in this thesis selected high-energy feeds but avoided those with very high levels of readily soluble carbohydrates that may increase the risk of intestinal problems, such as diarrhoea or the effect of certain VFAs in the caecum (Dietschke and Baker 1979, Martin-Rosset and Dulphy 1987).

For oaten hay to be offered to horses, the most promising approach to predict preference values is directly from NIR spectra. This approach yielded a correlation coefficient of 0.61 to 0.65 depending on the model used, which was equal to or greater than that obtained with any of the other prediction equations. This is very encouraging for the fodder industry – processors, retailers and purchasers – as it suggests that a quick, cheap and reliable method of estimating preference is possible.

8.3 Lucerne hay and horses

The prediction equations for lucerne hay generally yielded higher correlation coefficients than the equivalent equations for oaten hays. For example, the r^2 value for the relationship between intake rate and (average) preference for oaten hays was only 0.15, but it was 0.63 with lucerne hays. In general, horses did not consume lucerne hays any faster than oaten hays (both typically about 40 g/min), but the variation in intake rate was larger for lucerne hays than oaten hays (2 to 70 g/min versus 15 to 60 g/min respectively), with more preferred lucerne hays consumed at a greater rate than preferred oaten hays and less preferred lucerne hays consumed at a slower rate than the less preferred oaten hays. Similarly, IVD was more strongly related to horse preferences with lucerne hays than oaten hays. One possible explanation for this is the wider variety in the characteristics of the lucerne hays tested relative to the oaten hays, thus allowing a more profound effect to be observed for intake and IVD due to the wider range of fibre content, shear energy and stem diameter in the population of trial lucerne hays.

Prediction equations using a combination of nutritive value traits were able to describe a considerable portion of the variation in horse preferences, with r^2 values up to 0.74 using either crude protein, water soluble carbohydrates and hemicellulose, or using ADF, water soluble carbohydrates and hemicellulose. Interestingly, all of the coefficients for these variables were small (close to zero), with hemicellulose tending to have the largest effect on preference. This means that quite large differences in the content of these nutrients were required to have a large effect on preference values, but nevertheless, they collectively still accounted for much of the variation in preference values.

Less of the variation in preference values was accounted for by physical traits than nutritive traits. As with oaten hays, the main contributor to preference amongst the physical traits was shear energy. For lucerne hays, stem diameter was not related to preference, but the variability in stem diameter was negatively related to preference. This implies horses tended to avoid hays that were highly variable in stem characteristics rather than necessarily avoiding hays with a high average stem diameter. The underlying reason for this is not clear.

As for oaten hays offered to dairy cows or horses, there are excellent prospects for industry to use NIR spectra to estimate the preference value of lucerne hays offered to horses. Using the 'average' preference value of lucerne hays, a calibration between NIR spectra (using both the visible and NIR regions of the spectra) accounted for 80-84% of the variation in preferences. Considering that most of the other prediction equations developed were less satisfactory for horses than for cows, this is a particularly encouraging result. The NIR predictions of preferences when hays were offered with particular standard hays (ie, vs standards LA, LB, LC and LP) was not quite so encouraging, but nevertheless the strength of the predictive power using NIR spectra (r^2 values of 0.45-0.71) was comparable to the predictions obtained from nutritive value traits (r^2 values of 0.47-0.74) and superior to predictions based on physical traits (r^2 values of 0.38-0.54).

8.4 Standard hays and their role in observed hay preferences

In general, lower correlation coefficients were found for the predictions of preference compared against a single standard hay than for the 'average' preference across all four standard hays. There are two likely explanations for this. First, the 'average' preference value was obtained from a larger number of preference tests than the preference value when offered with any single standard hay. Thus, with more data points contributing to the development of the regression equation, a higher r^2 value was obtained. Second, preference is, by definition, a measure of a feed's acceptance when offered with an alternative. Therefore, the characteristics and the animal's preference of the alternative (in this case, the 'standard hay') will influence the preference for any given feed (in this case, any given 'trial hay').

The Australian fodder industry quality guidelines, as set out by the Australian Fodder Industry Association (AFIA), indicate that based on the nutritive profile, the ranked quality of the standard hays, oaten and lucerne, from an industry and marketing point of view, were as follows; A, BC, D then E and LA, LB LC and LP. However, the observed preferences for the standard hays by the animals did not always reflect this industry ranking. The relative preference of each standard hay (oaten and lucerne) by each of the animal species (dairy cow and horse) was established during my experimentation by conducting a small scale preference trial where each standard was offered to animals with every other standard. The preference ranking of the standard oaten hays for the dairy cows was consistent with the industry ranking; lowest preference to highest preference was A, BC, D then E (-1.95, -0.59, -0.07 then 0.66, respectively). However, the preference ranking of the standard oaten hays for the horses differed markedly. The ranking from lowest preference to highest preference was E, A, BC then D (-0.41, -0.31, 0.33 then 0.38, respectively). The preference ranking of the standard lucerne hays for the horses, from lowest preference to highest preference, was LB, LA, LC then LP (-0.29, -0.21, 0.06 then 0.44, respectively), which approximated the industry ranking. The differences seen in these rankings reinforce the value of using animal responses (actual or predicted) as a guide to hay quality rather than using estimates based solely on laboratory measures or human perceptions. The influence of particular standard hays on trial hay preferences are discussed in the following sub-sections.

8.4.1 Effect of particular standard hays on oaten hay preferences of dairy cows

Of the four standard oaten hays (A, BC, D and E), lower correlation co-efficients for the prediction equations were generally observed when oaten hay preferences of dairy cows were calculated for the trial hays offered with the standard hays at either end of the quality spectrum (A being the lowest quality and E the highest). Unlike tests conducted with the medium-quality standards (BC and D), the majority of trial hays would have differed distinctly from standards A or E for a broad variety of different reasons. Consequently the criteria available for use by the animal to aid its decision making was equally broadly varied. Thus, when the alternative was standard A or E it was harder to attribute the animal's preferences to any one (or small number of) hay characteristic(s) across the population of trial hays.

Acid detergent fibre (ADF) had a similar and negative influence on trial hay preference across all the standards (A, BC, D and E), although there was a slight tendency to be more influential when the standards themselves had a high content of ADF (namely standards A and BC). This may be because, when one hay (ie, standard hay) is high in ADF, the ADF content of the alternative (ie, trial hay) is likely to have a strong influence on the animal's selection process. If the ADF content of both the standard hay and the trial hay were similar, then a diet selection choice could not be based on differential ADF content. Rather, other hay attributes would have to be relied on more strongly. Fisher et al. (1999) demonstrated that high ADF content was associated with low preferences in sheep, goats and cattle.

Hemicellulose, a relatively digestible fibre, acted as a deterrent when the alternative was a highly digestible hay (ie, standard D and E). This was not evident when offered with A or BC (alternatives with low digestibility), where there was nil, or a slightly positive relationship, to hemicellulose. It is possible that in situations were the alternative is a readily digestible hay with high levels of water soluble carbohydrates, preference may have been driven by attempts to ensure an adequate consumption of effective fibre to maintain healthy rumen conditions.

Crude protein (CP) content (always an attractant) showed a relatively consistent influence across all of the standard alternatives. It had a slightly weaker influence on preference when the alternative was low CP (A) or high CP (E). This is mostly likely because in situations where the alternative was distinctly different from the trial hay, other factors, especially those influencing the ease of eating of a palatable hay, namely its fibre content or shear energy, became more important and slightly outweighed CP as more highly influential determinants of selection. Tolkamp et al. (1998) showed that dairy cows offered a choice between two feeds that differed in protein content selected a consistent combination of high protein feed and low protein feed that yielded a more than adequate amount of metabolisable protein, suggesting that the cows differentiated between low and high protein contents and selected their diet accordingly to achieve an optimum. Fisher et al. (1999) demonstrated that low CP content was associated with low preferences in sheep, goats and cattle.

Data from this study showed that water soluble carbohydrate (WSC) content positively influenced the preference of trial hays by cows. However, this was only the case when the alternative was by and large of a similar quality to that of the trial hay population (eg, medium quality standards BC and D). This suggests that WSC was “sugar on top” and that the cows used it as a selection determinant only when all other things, such as ADF and CP, were effectively equal. When a trial hay was offered with low quality (A) or high quality (E) standard the cows appeared to be influenced more strongly by fundamental traits such as ADF and shear energy. High WSC content has been associated with increased intake of pasture by sheep (Jones and Roberts 1991) and ryegrass by housed dairy cows (Moorby et al. 2001). Sheep, goats and cattle prefer hay cut in the afternoon when compared to hay cut in the morning (Fisher et al. 2002). Afternoon-cut hay contains a higher content of total nonstructural carbohydrates (sugars). Fisher et al. (1999 and 2002) demonstrated that low WSC content was associated with low preferences in sheep, goats and cattle.

8.4.2 Effect of particular standard hays on oaten and lucerne hay preferences of horses

Horses displayed a distinctly different pattern of preference for the standard oaten hays when compared to the dairy cows. Their preferences for the standard hays did not vary as widely as that for the dairy cows, nor was it as evenly spread, with two standards (E and A) having definite negative preferences and the remaining standards (BC and D) having definite, and similar, positive preferences.

Whilst the particular standard offered significantly affected preference for the trial hay, the main drivers of preference did not differ. That is, all the variables in the models effectively influenced preference in the same relative combination and intensity, irrespective of the standard being offered. This was a phenomenon observed only with preferences of horses for oaten hay and was not seen with lucerne hay.

Generally the co-efficients of the nutritive value traits used within the prediction models for lucerne hay preferences of horses were all very close to zero. Acid detergent fibre (ADF) acted as a slight deterrent when the alternative was of high quality (LC and LP) and very slight attractant when the alternative was of low quality (LA and LB), however, the reasons for this are unclear. Water soluble carbohydrates (WSC), as with the oaten hays, was a deterrent for horses when the alternative had a high WSC content, again suggesting that horses chose to avoid consuming “too much” soluble carbohydrate thereby reducing the risks associated with consuming large quantities of highly fermentable fodder (ie, laminitis). Hemicellulose was sought after when the alternative had a high content of neutral detergent fibre (NDF). This may reflect not only the need of horses for digestible carbohydrates, but a desire to avoid the rapidly fermentable sugars. Crude protein (CP) content was positively related to preference, with its strongest influence when the alternative was of high quality. This may be because CP exerts a stronger influence over preference as structural traits such as ADF become non-limiting.

8.5 Hay volatiles and their role in observed hay preferences

There were 8 oaten hay volatiles and 15 lucerne hay volatiles related to cow and/or horse preference. The fact that only a small proportion of the volatiles (approximately 7% of oaten hay volatiles and 10% of lucerne hay volatiles), showed a significant association with preference is not surprising given that current research suggests only a small fraction of the complex mixture of volatiles present in a food create the characteristic odour associated with that food (Grosch 1993). Most of the identified volatiles, in both oaten and lucerne hays, that were significantly related to preference were positively related, with only approximately two-fifths being negatively associated with preference. These data support evidence that various volatiles can act as “attractants” (having a positive relationship to preference) or “repellents” (having a negative relationship to preference) (Arnold et al. 1980).

In the main, the volatiles positively related to preference were also positively related to the nutritive and physical traits that were positively related to preference and, similarly, negatively related to the traits negatively related to preference. This provides additional evidence that animals may be using odour as a cue to help discriminate between difference feedstuffs in order to consume those feeds which best meet particular nutritional needs (e.g. fibre, protein and water soluble carbohydrates). Thus the dairy cows and horses may be associating particular volatiles with nutrient ‘rewards’ such as low fibre/high digestibility and high sugar and protein contents, and other volatiles with less desirable characteristics. There was a single lucerne volatile (peak 37) that, although related negatively to preference, did not show any relationship with either nutritive value or physical traits. This volatile may be associated with plant secondary compounds that can affect taste and gut fermentation patterns (i.e., influence gut microbes).

Volatiles that influence preference but are not related to any measurable nutritive or physical trait may provide information relating to hay preferences that are unexplained (or poorly predicted) (Pain and Revell 2007). This may be particularly useful to identify prior to sale hays animals are likely to find less acceptable than would otherwise be predicted using NV traits, thereby avoiding hays being sold as ‘premium’ quality only to find they are (at least initially) rejected by animals.

Further work is warranted to explore the potential impact of volatile compounds on digestive physiology of dairy cows and horses. In order to investigate the effects of these key volatiles further they would need to be chemically identified. This is possible using the mass spectral data collected during the course of the GCMS analysis but was beyond the scope of this particular project. The influence of combinations of volatiles should also be investigated as this is more representative of what an animal encounters in a natural setting where plants on offer have multiple volatiles, each at various concentrations. For most volatile compounds there are likely to be threshold levels of effectiveness that play a role in perception and response. Given the number of volatiles associated with positive feed preferences there is also scope to develop various additives that incorporate these chemicals to manipulate hay preference/acceptability (i.e., “palatants”), which could be applied to make certain feedstuffs more appetising. This would be useful during a transition from one feed to another (eg, one batch of hay to another) to minimise short-term decreases in feed intake due to the sudden presentation of an unfamiliar feedstuff or to help ensure a 'premium' grade hay is, in fact, highly acceptable to the animals. This requires the chemical identification of the odorants in order to exploit them as feed additives. However, it should be noted that post-ingestive feedback could negate the influence of a palatant if the feed was nutritionally inadequate for the animal, as feed preferences are a dynamic learning process involving complex interrelationships between a food's flavour and its post-ingestive effects (Provenza 1995 and 1996). Whilst foraging behaviour of livestock is influenced by perceived cues, such as fragrance, those behaviours are only maintained when the amount of reward received, such as energy and nutritive value, is sufficient to meet current physiological demands. Feed preference and diet selection is influenced by multiple signals, both internal and external to the animal, and requires extensive integration from numerous senses and as such presents a complex area of science with many possibilities.

9 Concluding remarks and broader implications

In summary, the study has shown that commonly measured traits of hays, such as nutritive value and physical measures, can be used as predictors of hay preference, so there is a good opportunity for members of the hay industry, particularly processors, to develop and use prediction equations such as these to better predict the acceptability or '*preference value'* of hays sold as feed for dairy cows or horses.

The data reported here suggest that a predicted preference value can be generated using commonly measured quality traits, to improve the confidence of animals responding favourably when offered a hay. More specifically, for an average preference value, the most reliable prediction equation was based on solely nutritive value traits: the content of ADF, hemicellulose, crude protein and water soluble carbohydrate in the hays. Alternatively, a combination of nutritive and physical traits can be used: the *in vitro* digestibility, crude protein content, water soluble carbohydrate content and shear energy of the hay were the most useful traits to predict preference.

The analyses presented in this thesis used standardised variables, to directly compare the effects of different variables, within and across the prediction equations, on preference values. That is, the magnitude of the co-efficient for a given variable is proportional to its scale of effect on the preference value. In order for these predictive equations to be used immediately by fodder producers and others in the industry to predict hay preference values, the relevant traits of the hays being assessed would have to be standardised for inclusion in the predictive equation. This is a simple procedure of relating the value of any given variable for a particular hay to a broader population of hays as explained in the Methods section of this thesis. The mean values of each variable for hays used in this study, as detailed in the Appendices, would provide users with a population mean with which to standardise any nutritive and physical measures of unknown oaten or lucerne hays in order to predict preference. Additionally the prediction equations reported here could be adapted for use with non-standardised variables in order for this information to be used directly by industry. The visible and near infrared spectra obtained by NIRS was also a promising method of prediction and, given the speed and affordability of NIRS, this technology should be further refined and used for routine measurement of predicted hay preference values

Particular volatile compounds were related to preference and this may offer opportunities to manipulate or select for a particular 'odour profile' of hays and modify short-term intake behaviour. The volatile compounds shown to influence preference values of the hay should be identified and further research undertaken to investigate novel approaches to manipulate the short-term preference of hays.

Caution should be taken when attempting to use these prediction equations on a single specific hay in a single specific situation. The predictions and relationships investigated in this thesis are based on populations of hays and the preferences of a group of animals kept under particular conditions. Some care should be taken when applying them to a particular situation with difference circumstances. Other factors that can influence feed preferences include an animal's feeding experiences, basal diet and diet history and various environmental factors unaccounted for here.

Appendix A: Nutritive value of individual oaten hays as measured by wet chemistry

Oaten hay #	wcDM %	wcCP %DM	wcNDF %DM	wcADF %DM	wcHem %DM	wcGE MJ/kgDM
1	92.0	7.4	60.6	30.1	30.5	17.2
2	93.4	5.2	49.4	23.9	25.5	17.5
3	92.9	4.5	54.4	29.4	25.0	17.1
4	93.1	5.9	58.2	29.1	29.1	17.2
5	93.0	4.9	57.5	31.7	25.8	16.8
6	93.2	3.0	56.8	30.5	26.3	16.6
8	93.5	2.7	60.0	33.9	26.1	16.7
9	93.7	3.8	56.6	31.7	24.8	17.0
12	93.6	5.2	49.6	26.6	23.0	16.9
13	92.8	8.2	54.5	27.8	26.6	17.4
14	93.6	2.5	53.7	28.6	25.1	16.7
15	92.9	5.2	55.9	29.5	26.3	17.0
16	93.4	3.6	65.2	37.6	27.6	16.6
17	93.9	4.4	54.4	29.1	25.4	17.1
18	93.8	2.1	61.4	36.5	24.8	16.7
19	92.9	7.2	50.1	26.1	24.0	17.2
20	92.8	4.3	55.5	29.5	26.0	17.0
21	93.1	6.1	54.6	28.3	26.2	17.2
22	93.0	7.1	60.0	29.1	30.9	17.7
23	93.0	6.2	59.8	29.9	29.8	17.0
24	92.8	6.8	51.0	25.5	25.6	17.0
25	92.9	7.0	57.9	28.6	29.3	17.4
26	93.3	3.8	53.5	28.9	24.6	17.0
27	93.8	5.4	57.8	30.5	27.3	16.8
28	93.5	8.4	49.6	23.9	25.8	17.2
29	92.3	7.9	46.2	20.9	25.3	16.9
30	92.5	5.8	48.5	23.4	25.1	16.6
31	92.2	9.9	51.8	26.3	25.5	16.9
34	92.6	7.5	55.9	30.2	25.8	17.0
35	92.8	6.8	50.9	24.9	25.9	17.4
37	91.4	8.4	46.1	20.9	25.2	16.6
38	92.5	6.7	47.6	22.4	25.2	16.9
40	93.4	5.4	49.6	24.0	25.6	17.2
41	92.4	6.7	48.2	22.3	25.8	16.7
43	92.9	12.3	46.8	22.0	24.8	16.7
44	93.0	8.6	48.9	23.8	25.0	16.9
45	93.2	7.1	40.7	20.3	20.4	16.7
46	94.2	4.7	57.8	33.7	24.2	16.4
48	93.7	6.4	54.5	28.6	25.9	17.2
50	93.0	6.5	-	-	-	16.5
51	93.0	8.3	48.8	26.0	22.8	16.6
52	92.8	8.9	48.0	25.4	22.7	16.6
53	93.8	4.2	45.5	23.2	22.4	16.6
54	94.0	4.0	45.3	22.9	22.4	16.7
55	93.7	5.1	43.7	21.6	22.1	16.7
56	93.7	7.2	44.3	21.2	23.1	16.8
57	93.5	7.8	45.4	23.3	22.1	16.9
58	93.8	7.3	43.1	22.6	20.5	16.8
59	94.1	5.0	49.1	26.4	22.8	16.8
60	92.8	7.2	39.8	21.2	18.6	16.8

Appendix A (continued): Nutritive value of individual oaten hays as measured by wet chemistry

Oaten hay #	wcDM %	wcCP %DM	wcNDF %DM	wcADF %DM	wcHem %DM	wcGE MJ/kgDM
61	94.3	3.6	60.0	34.8	25.2	17.0
62	93.8	3.2	57.9	31.2	26.7	17.0
63	93.7	6.2	46.2	23.0	23.2	17.3
64	93.8	6.0	45.3	22.4	22.9	17.3
65	93.7	5.0	45.5	24.7	20.8	16.8
66	93.9	4.5	46.2	24.5	21.7	16.7
67	94.1	4.9	50.2	27.0	23.2	16.8
68	94.1	3.0	52.2	29.6	22.6	16.6
69	94.3	4.8	47.4	25.7	21.8	16.8
70	94.5	3.4	52.3	29.4	22.9	16.6
71	94.4	3.4	47.5	26.9	20.7	16.7
72	94.3	3.3	48.3	27.6	20.6	16.6
73	94.2	5.0	48.3	26.5	21.9	16.9
74	94.1	4.2	49.8	28.7	21.2	16.8
75	94.2	2.8	43.9	24.7	19.2	16.6
76	93.9	2.8	42.3	25.5	16.8	16.6
77	90.4	4.4	47.7	26.6	21.2	16.3
78	90.5	4.0	43.1	22.8	20.3	16.0
79	92.2	4.6	46.5	25.4	21.1	16.4
80	92.2	4.1	46.0	25.1	20.9	16.4
81	91.4	6.3	56.6	31.5	25.1	16.9
82	91.1	7.2	54.2	30.1	24.2	16.7
83	90.1	5.9	55.4	29.9	25.5	16.6
84	89.8	8.3	57.1	31.7	25.5	16.6
89	88.3	7.4	40.2	20.8	19.4	16.5
90	88.5	9.3	42.3	21.7	20.6	16.6
91	91.5	3.1	47.3	24.3	23.0	16.3
92	91.9	3.5	44.0	22.9	21.1	16.4
99	94.0	3.2	50.2	27.3	21.2	16.6
100	93.7	8.0	59.3	32.9	20.3	16.6
101	93.7	5.2	55.2	30.6	21.1	16.8
102	93.6	6.8	45.5	25.4	20.9	16.8
103	94.0	3.8	60.2	32.5	25.1	16.7
104	93.6	7.3	46.5	24.3	24.2	16.8
105	93.7	7.7	51.0	27.0	25.5	16.5
A	92.5	6.3	52.1	26.1	26.1	17.0
BC	92.8	5.9	51.4	25.6	25.7	17.3
D	93.0	6.6	50.6	25.3	25.3	17.6
E	92.7	6.4	49.7	25.0	24.6	17.0
Min	*88.3*	*2.1*	*39.8*	*20.3*	*16.8*	*16.0*
Max	*94.5*	*12.3*	*65.2*	*37.6*	*30.9*	*17.7*
Mean	**93.0**	**5.7**	**50.9**	**26.9**	**23.9**	**16.8**
± SE	**0.13**	**0.22**	**0.63**	**0.43**	**0.30**	**0.03**

Appendix B: Nutritive value of individual oaten hays as measured by NIRS

Oaten hay #	nirDM	nirCP	nirNDF	nirADF	nirHem	nirWSC	nirIVD	nirME
	%	%DM	%DM	%DM	%DM	%DM	%DM	MJ/kgDM
1	90.4	8.0	59.9	36.5	23.4	10.2	60.6	8.7
2	90.9	5.4	50.8	29.7	21.1	20.3	66.0	9.5
3	91.0	4.9	55.2	34.4	20.8	27.0	61.5	8.9
4	90.8	6.9	57.7	33.0	24.7	22.2	63.0	9.1
5	90.8	5.5	55.8	34.7	21.1	20.4	61.3	8.8
6	91.1	3.3	56.0	36.2	19.8	25.4	62.5	9.0
8	91.5	3.0	57.4	37.6	19.8	25.7	60.5	8.7
9	91.6	4.0	54.6	35.1	19.5	22.5	63.0	9.0
12	91.4	5.7	49.9	30.2	19.7	32.2	67.3	9.7
13	90.3	8.8	55.8	31.2	24.6	15.5	64.7	9.3
14	91.4	3.3	51.9	33.3	18.6	27.6	65.0	9.4
15	91.3	5.6	56.8	33.6	23.2	27.6	63.8	9.2
16	91.5	4.4	62.2	40.3	21.9	15.1	57.6	8.2
17	91.5	4.4	50.0	30.4	19.6	23.5	66.4	9.6
18	91.9	2.3	56.1	37.9	18.2	29.0	59.9	8.6
19	90.9	7.8	52.7	29.9	22.8	22.7	67.8	9.8
20	91.0	5.0	57.7	35.5	22.2	26.1	61.4	8.8
21	91.3	6.7	50.4	30.0	20.4	30.2	67.5	9.8
22	91.2	7.7	55.7	31.1	24.6	16.5	62.3	8.9
23	91.2	7.1	56.8	31.9	24.9	22.9	65.7	9.5
24	91.5	7.0	51.4	29.7	21.7	31.5	68.4	9.9
25	90.4	7.0	55.6	30.3	25.3	16.3	65.4	9.4
26	91.5	3.8	52.5	32.2	20.3	23.4	65.0	9.4
27	91.3	6.1	53.5	32.0	21.5	28.8	66.7	9.6
28	91.1	8.8	49.8	28.2	21.6	24.0	69.2	10.0
29	89.9	8.7	46.6	24.1	22.5	27.6	71.1	10.3
30	90.3	6.7	50.8	28.5	22.3	31.4	68.2	9.9
31	90.3	10.6	52.0	29.2	22.8	22.1	67.8	9.8
34	91.0	8.1	57.0	34.7	22.3	18.4	62.4	9.0
35	90.5	7.2	48.2	26.8	21.4	21.4	70.0	10.2
37	89.4	9.6	47.4	24.9	22.5	30.6	72.8	10.6
38	90.0	7.5	47.5	24.9	22.6	29.5	69.7	10.1
40	91.0	6.0	52.2	27.1	25.1	28.4	68.4	9.9
41	90.1	7.8	46.3	24.9	21.4	33.6	72.5	10.6
43	90.6	13.5	51.5	26.5	25.0	19.4	72.5	10.6
44	90.6	9.2	49.1	27.5	21.6	19.8	70.3	10.2
45	91.8	7.0	46.6	26.6	20.0	32.3	73.1	10.7
46	91.4	5.2	53.0	35.0	18.0	26.7	63.6	9.2
48	91.1	7.3	55.3	33.0	22.3	19.8	63.6	9.1
50	91.4	6.5	40.3	22.3	18.0	43.0	78.6	11.5
51	90.8	10.0	52.0	31.0	21.0	19.4	68.5	9.9
52	90.5	10.7	48.4	28.5	19.9	19.8	69.8	10.1
53	91.5	5.8	45.5	27.0	18.5	36.1	71.5	10.4
54	91.6	5.3	46.5	27.5	19.0	35.4	70.9	10.3
55	91.5	6.7	44.0	26.0	18.0	37.9	72.3	10.5
56	91.3	8.8	43.7	24.6	19.1	31.8	72.6	10.6
57	91.1	9.4	44.9	25.1	19.8	30.8	72.4	10.6
58	91.2	8.9	44.9	26.5	18.4	32.1	71.8	10.5
59	91.8	6.1	50.3	30.8	19.5	32.0	66.7	9.7
60	90.2	9.1	44.1	25.0	19.1	35.0	76.1	11.1

Appendix B (continued): Nutritive value of individual oaten hays as measured by NIRS

Oaten hay #	nirDM	nirCP	nirNDF	nirADF	nirHem	nirWSC	nirIVD	nirME
	%	%DM	%DM	%DM	%DM	%DM	%DM	MJ/kgDM
61	92.3	4.6	57.9	38.0	19.9	23.5	59.7	8.5
62	91.9	4.8	59.6	37.8	21.8	22.8	58.3	8.3
63	91.2	7.7	48.2	27.6	20.6	27.5	69.6	10.1
64	91.4	7.3	47.6	27.4	20.2	26.9	70.0	10.2
65	91.0	6.4	47.8	29.4	18.4	29.8	68.6	9.9
66	91.5	5.8	46.8	29.5	17.3	35.0	69.0	10.0
67	91.9	5.9	50.5	32.3	18.2	27.2	66.0	9.5
68	92.0	3.9	52.8	35.9	16.9	30.1	61.3	8.8
69	92.2	6.0	48.6	30.8	17.8	31.5	68.6	9.9
70	92.3	3.9	51.6	35.0	16.6	30.9	65.0	9.4
71	92.4	4.0	49.0	32.9	16.1	33.5	65.5	9.5
72	92.0	4.2	51.8	35.8	16.0	30.1	62.6	9.0
73	91.7	6.3	50.7	32.5	18.2	27.6	66.8	9.7
74	91.7	5.3	51.4	32.9	18.5	29.0	63.4	9.1
75	92.0	4.0	45.0	30.0	15.0	39.5	67.3	9.7
76	91.6	3.9	46.4	30.5	15.9	39.4	68.4	9.9
77	89.3	5.9	53.4	31.1	22.3	27.7	65.3	9.6
78	89.6	5.6	50.8	28.0	22.8	31.8	67.4	10.0
79	90.6	6.5	49.7	30.0	19.7	29.0	66.2	9.8
80	90.6	5.9	48.9	29.0	19.9	31.5	68.0	10.1
81	90.2	7.4	59.8	35.1	24.7	15.6	61.1	8.9
82	89.8	8.5	59.3	35.2	24.1	13.4	61.1	8.9
83	89.8	7.0	58.4	34.3	24.1	13.9	60.4	8.9
84	89.9	9.7	60.7	35.9	24.8	8.9	61.0	8.9
89	89.3	8.0	46.5	24.8	21.7	32.8	74.2	11.2
90	89.8	10.6	46.7	25.2	21.5	27.4	72.9	10.9
91	90.9	4.5	46.2	28.4	17.8	38.5	71.0	10.6
92	91.1	4.8	45.1	27.2	17.9	39.2	71.6	10.7
99	91.8	4.1	52.5	33.6	22.3	29.8	62.7	9.0
100	91.7	8.9	60.1	38.0	22.8	12.2	57.5	8.2
101	91.4	6.6	54.9	35.4	19.7	22.3	62.3	8.9
102	91.2	8.1	46.0	27.4	19.9	31.4	71.5	10.4
103	91.9	4.7	58.5	38.2	24.7	16.2	56.9	8.1
104	91.3	9.0	50.5	30.2	24.1	25.4	68.2	9.9
105	91.5	9.4	52.4	33.0	24.1	20.1	67.0	9.7
A	91.0	7.1	56.3	34.1	22.2	12.9	62.4	9.0
BC	91.3	8.3	53.0	30.0	23.0	19.1	66.2	9.6
D	91.3	5.8	51.3	29.9	21.5	23.9	67.0	9.7
E	92.4	6.3	49.2	27.5	21.7	32.2	71.1	10.4
Min	*89.3*	*2.3*	*40.3*	*22.3*	*15.0*	*8.9*	*56.9*	*8.1*
Max	*92.4*	*13.5*	*62.2*	*40.3*	*25.3*	*43.0*	*78.6*	*11.5*
Mean	**91.1**	**6.6**	**51.5**	**31.0**	**20.8**	**26.5**	**66.5**	**9.7**
± SE	**0.08**	**0.23**	**0.52**	**0.44**	**0.27**	**0.78**	**0.50**	**0.08**

Appendix C: Physical characteristics of the individual oaten hays

Oaten hay #	Colour (/60)	Ave Stem Diameter (mm)	Min Stem Diameter (mm)	Max Stem Diameter (mm)	Leaf %	Shear (KJ/m2)
1	28	5.2	2.9	7.3	41.0	11.5
2	31	5.4	3.4	7.6	20.0	10.1
3	30	5.6	3.9	8.3	36.0	11.8
4	24	5.4	3.4	8.5	26.0	11.5
5	26	5.5	3.0	9.0	23.0	12.0
6	29	5.5	3.8	7.4	26.0	11.7
8	31	5.6	3.4	8.5	22.0	12.1
9	29	6.3	4.0	10.0	16.0	11.3
12	31	4.8	3.8	6.4	23.0	10.9
13	27	5.5	3.1	8.9	34.0	10.6
14	30	6.8	5.0	9.0	22.0	11.0
15	28	6.2	4.9	7.6	26.0	11.5
16	26	6.0	3.5	9.0	27.0	12.4
17	27	6.3	3.5	10.3	20.0	9.8
18	28	6.7	4.9	8.0	16.0	12.3
19	23	5.1	3.5	7.8	32.0	10.4
20	28	5.2	3.7	6.8	24.0	12.0
21	33	5.2	3.9	7.2	24.0	11.0
22	28	6.1	4.1	7.9	33.6	11.2
23	22	5.4	3.4	8.7	26.0	11.4
24	29	5.1	3.4	7.9	37.0	11.2
25	32	6.3	4.8	8.0	19.0	10.0
26	23	5.0	2.8	6.6	30.0	10.5
27	31	6.1	4.6	10.8	14.0	11.0
28	31	5.0	2.7	6.7	32.0	10.8
29	24	5.4	4.0	8.2	32.0	9.9
30	24	5.7	3.8	7.7	41.0	11.2
31	32	5.4	3.9	8.0	41.0	11.8
34	27	4.9	2.5	6.7	39.0	11.7
35	23	6.4	3.5	10.0	42.0	9.2
37	31	5.0	3.3	6.2	42.0	10.2
38	22	5.5	4.2	7.2	41.0	9.9
40	25	5.6	4.2	8.2	31.0	9.9
41	24	5.4	3.9	7.0	41.0	10.4
43	29	5.7	4.2	6.9	38.0	10.3
44	24	4.9	3.2	6.0	58.0	10.1
45	24	5.0	3.9	5.7	42.0	9.0
46	23	4.8	3.5	6.8	41.0	12.8
48	34	5.2	3.0	7.8	11.0	11.6
50	28	5.8	4.3	8.8	25.0	8.6
51	26	4.9	3.3	6.9	46.0	10.3
52	30	5.1	3.2	8.4	51.0	9.8
53	27	6.7	4.8	8.8	39.0	10.0
54	29	5.2	3.6	7.5	20.0	9.8
55	28	6.1	4.4	8.8	22.0	10.2
56	31	5.8	4.4	7.2	27.0	9.3
57	42	5.4	3.4	7.0	48.9	9.5
58	29	5.2	3.7	6.5	45.0	9.7
59	55	4.6	3.0	6.1	35.0	9.9
60	41	5.2	3.6	6.7	28.0	10.7

Appendix C (continued): Physical characteristics of the individual oaten hays

Oaten hay #	Colour (/60)	Ave Stem Diameter (mm)	Min Stem Diameter (mm)	Max Stem Diameter (mm)	Leaf %	Shear (KJ/m2)
61	24	5.4	3.7	7.4	43.0	12.1
62	30	5.0	3.6	7.4	31.0	12.2
63	27	4.6	2.9	5.9	21.0	9.4
64	22	5.9	4.3	7.6	32.4	9.3
65	31	6.4	4.0	9.1	39.0	10.4
66	30	5.9	3.9	8.3	32.0	10.8
67	39	5.6	3.8	7.6	46.0	10.5
68	29	5.7	3.4	7.7	32.8	11.7
69	46	4.8	3.6	6.4	48.0	10.2
70	35	5.8	3.5	7.5	43.0	10.9
71	28	5.5	3.6	7.3	40.0	10.6
72	28	5.9	2.7	7.5	39.0	11.3
73	52	6.0	4.0	7.7	25.0	10.6
74	30	5.8	3.7	8.5	27.0	10.7
75	43	6.0	4.1	7.7	26.0	10.3
76	53	5.0	3.5	6.5	25.0	10.4
77	41	6.0	3.8	7.5	38.0	11.5
78	43	5.1	3.4	8.4	29.0	10.9
79	37	4.8	3.2	7.4	24.3	10.8
80	47	6.0	4.3	9.7	49.0	10.1
81	48	6.3	3.8	8.6	47.0	12.5
82	29	5.7	4.0	7.5	26.0	11.5
83	31	5.4	3.1	7.2	41.0	12.3
84	31	5.8	3.0	9.0	37.0	12.2
89	29	6.1	3.8	8.0	51.0	9.6
90	26	5.4	3.0	6.9	36.0	9.7
91	24	5.7	4.2	7.0	52.0	10.3
92	45	6.3	4.0	10.0	31.0	9.9
99	45	6.3	4.3	11.0	45.0	11.4
100	37	5.4	3.5	7.7	31.0	12.6
101	29	5.8	3.3	7.7	26.0	12.5
102	36	5.8	4.0	6.9	29.0	10.8
103	30	5.9	4.4	7.5	44.0	12.2
104	31	5.1	4.1	7.6	54.0	11.2
105	39	5.2	4.1	6.5	19.0	11.2
A	28	6.1	3.8	9.0	31.0	9.7
BC	22	5.8	4.9	8.4	34.0	10.1
D	29	5.6	3.7	7.2	21.0	10.8
E	22	4.7	3.1	6.2	35.0	10.2
Min	*22*	*4.6*	*2.5*	*5.7*	*11.0*	*8.6*
Max	*55*	*6.8*	*5.0*	*11.0*	*58.0*	*12.8*
Mean	**31.3**	**5.6**	**3.7**	**7.8**	**33.4**	**10.8**
± SE	**0.82**	**0.06**	**0.06**	**0.12**	**1.12**	**0.10**

Appendix D: Nutritive value of individual lucerne hays as measured by NIRS

Lucerne hay #	nirDM	nirCP	nirNDF	nirADF	nirHem	nirWSC	nirIVD	nirME
	%	%DM	%DM	%DM	%DM	%DM	%DM	MJ/kgDM
L1	90.6	6.7	60.2	37.0	23.2	15.7	58.3	8.4
L2	90.5	6.7	57.0	35.4	21.6	20.1	60.4	8.8
L3	89.3	16.5	45.4	36.8	8.6	5.8	60.3	8.8
L4	88.8	20.5	41.9	29.0	12.9	5.1	64.0	9.4
L5	88.8	18.3	42.4	33.2	9.2	6.0	63.1	9.2
L6	90.7	18.5	44.7	33.1	11.6	5.2	63.4	9.3
L7	90.3	19.9	39.1	31.5	7.6	7.6	67.0	9.9
L8	89.3	21.4	40.8	27.7	13.1	5.2	64.6	9.5
L9	89.9	19.7	41.6	39.0	2.6	4.9	64.1	9.4
L10	89.9	24.5	38.5	27.0	11.5	4.5	67.8	10.0
L11	89.4	24.5	46.7	28.7	18.0	1.0	60.4	8.8
L12	90.2	23.8	45.8	28.3	17.5	0.9	62.0	9.1
L13	90.5	17.7	44.9	34.9	10.0	5.4	63.1	9.2
L14	88.9	22.5	38.8	28.2	10.6	7.8	66.0	9.7
L15	90.1	23.7	45.7	29.0	16.7	1.5	62.8	9.2
L16	89.3	21.3	37.8	27.3	10.5	10.5	68.4	10.1
L17	89.6	21.4	39.3	27.9	11.4	9.3	67.3	10.0
L18	94.2	18.0	40.1	32.7	7.4	5.9	67.7	10.0
L19	94.9	19.7	41.5	34.2	7.3	3.5	67.2	9.9
L20	94.5	19.0	41.5	33.2	8.3	3.7	64.5	9.5
L21	95.3	15.9	52.3	40.5	11.8	< 1.0	54.9	7.8
L22	95.0	24.0	38.4	27.7	10.7	2.2	69.6	10.4
L23	94.8	21.3	41.8	33.0	8.8	2.9	63.4	9.3
L24	93.4	21.3	35.5	29.7	5.8	6.3	72.8	10.9
L25	94.8	24.2	35.5	25.5	10.0	5.4	70.5	10.5
L26	94.4	15.1	45.2	35.9	9.3	5.0	60.4	8.8
L27	94.5	23.5	35.4	27.5	7.9	3.6	68.4	10.2
L28	95.6	8.2	58.8	49.9	8.9	2.6	46.1	6.3
L29	95.3	20.2	39.9	36.1	3.8	2.1	63.4	9.3
L30	94.2	19.3	40.6	34.5	6.1	4.2	67.4	10.0
L31	95.4	19.5	49.7	33.0	16.7	2.3	61.1	8.9
L32	94.6	13.4	46.1	37.1	9.0	6.7	59.9	8.7
L33	95.1	17.4	43.3	34.5	8.8	3.8	61.7	9.0
L34	94.8	19.4	41.5	31.2	10.3	5.6	67.8	10.0
L35	94.4	16.5	43.6	34.9	8.7	5.2	60.4	8.8
L36	92.0	23.0	46.2	31.5	14.7	1.6	59.1	8.5
L37	91.5	20.6	45.6	33.9	11.7	3.4	59.8	8.7
L38	91.3	27.8	37.2	24.4	12.8	3.8	70.1	10.4
L39	91.7	20.4	44.4	34.3	10.1	5.1	63.3	9.3
L40	89.5	20.8	40.7	30.6	10.1	5.9	66.6	9.8
L41	92.3	16.8	46.1	38.4	7.7	6.2	61.3	8.9
L42	91.6	19.0	41.8	35.5	6.3	6.1	62.7	9.2
L43	90.7	22.1	34.7	28.7	6.0	8.6	70.2	10.5
L44	91.5	22.5	38.6	30.9	7.7	6.3	66.5	9.8
L45	92.0	20.3	43.6	34.6	9.0	5.1	63.4	9.3
L46	92.8	11.9	49.9	44.5	5.4	7.8	55.2	7.9
L47	91.6	18.3	41.1	36.4	4.7	7.2	63.8	9.4
L48	90.8	13.1	53.6	34.6	19.0	9.7	62.1	9.1
L49	91.5	12.4	53.6	35.1	18.5	11.0	62.1	9.1
L50	92.5	16.0	59.5	40.4	19.1	3.8	48.9	6.8

Appendix D (continued): Nutritive value of individual lucerne hays as measured by NIRS

Lucerne hay #	nirDM %	nirCP %DM	nirNDF %DM	nirADF %DM	nirHem %DM	nirWSC %DM	nirIVD %DM	nirME MJ/kgDM
L51	93.1	14.9	60.2	45.3	14.9	1.0	45.0	6.1
L52	92.0	14.6	58.1	45.6	12.5	1.5	49.7	6.9
L53	92.0	15.6	56.5	43.7	12.8	1.9	51.3	7.2
L54	93.8	6.0	67.9	47.1	20.8	10.8	50.4	7.1
L55	93.4	6.7	65.7	44.1	21.6	9.3	51.2	7.2
L56	92.2	14.7	60.7	45.2	15.5	1.7	48.8	6.8
L57	92.2	9.8	63.4	43.1	20.3	7.6	53.8	7.6
L58	93.0	12.7	60.2	49.1	11.1	1.0	45.5	6.2
L59	92.7	11.0	62.7	50.9	11.8	1.0	42.4	5.7
L60	92.0	19.1	41.2	32.7	8.5	7.7	67.6	10.0
L61	91.9	15.8	44.7	39.0	5.7	7.2	62.0	9.1
L62	90.5	12.6	52.9	35.2	17.7	9.4	61.7	9.0
L63	91.6	7.1	60.5	41.5	19.0	14.3	56.8	8.2
L64	91.1	15.3	56.9	42.6	14.3	0.9	51.7	7.3
L65	92.2	11.8	60.2	46.0	14.2	2.6	49.9	7.0
L66	90.1	23.1	38.3	29.0	9.3	5.8	67.7	10.0
L67	90.1	26.7	41.7	26.1	15.6	2.0	64.0	9.4
L68	91.3	20.3	38.4	30.5	7.9	8.4	69.6	10.4
L69	92.2	22.9	46.3	31.1	15.2	1.0	60.5	8.8
L70	89.8	23.7	34.7	28.3	6.4	9.1	73.0	10.6
LA	92.0	12.5	50.9	32.9	18.0	12.0	60.0	8.7
LB	92.6	14.9	50.8	38.7	12.1	2.6	52.3	7.4
LP	91.3	21.0	41.7	29.5	12.2	4.9	64.5	9.5
LC	93.0	16.8	49.2	38.0	11.2	3.0	52.6	7.5
Min	*88.8*	*6.0*	*34.7*	*24.4*	*2.6*	*0.9*	*42.4*	*5.7*
Max	*95.6*	*27.8*	*67.9*	*50.9*	*23.2*	*20.1*	*73.0*	*10.9*
Mean	**92.0**	**17.9**	**46.7**	**35.1**	**11.6**	**5.6**	**61.3**	**8.9**
± SE	**0.23**	**0.62**	**1.05**	**0.78**	**0.57**	**0.44**	**0.85**	**0.15**

Appendix E: Physical characteristics of the individual lucerne hays

Lucerne hay #	Colour (/5)	Ave Stem Diameter (mm)	Min Stem Diameter (mm)	Max Stem Diameter (mm)	Leaf %	Shear (KJ/m2)
L1	4	4.2	1.7	6.4	18.0	12.0
L2	4	3.6	1.9	6.9	21.0	11.9
L3	3	1.9	0.9	3.0	39.0	11.8
L4	2	2.2	0.9	3.2	25.0	10.7
L5	4	2.4	1.5	3.5	11.0	11.0
L6	4	2.3	1.0	3.8	47.0	11.4
L7	2	1.5	0.8	2.1	26.0	9.9
L8	4	1.9	1.0	3.0	55.0	10.1
L9	2	1.7	1.3	2.3	31.0	11.0
L10	5	2.2	1.4	2.6	36.0	9.6
L11	5	1.6	0.9	2.7	30.0	10.9
L12	4	1.9	1.1	2.9	29.0	10.5
L13	2	2.1	1.0	3.7	37.0	10.9
L14	5	1.7	0.7	2.3	22.0	10.2
L15	4	1.9	1.4	2.8	20.0	10.9
L16	5	2.0	1.2	2.9	31.0	9.6
L17	3	1.4	0.9	2.4	35.0	9.9
L18	5	3.4	2.4	4.4	36.0	10.8
L19	2	2.7	1.3	4.4	26.0	10.5
L20	2	2.5	1.8	3.5	38.0	10.7
L21	4	2.3	0.9	3.2	34.0	12.3
L22	3	1.7	0.9	3.1	17.0	10.0
L23	3	1.9	1.1	3.0	31.0	10.9
L24	3	1.9	0.8	3.4	49.0	10.1
L25	5	2.6	1.4	3.8	21.0	9.5
L26	3	1.7	0.4	2.7	32.0	11.1
L27	2	2.2	1.5	2.7	24.0	9.4
L28	4	2.5	1.4	3.8	32.0	14.4
L29	2	2.0	1.2	3.1	32.0	10.3
L30	3	2.0	1.6	2.4	34.0	11.7
L31	3	2.1	1.0	3.5	35.0	12.1
L32	4	2.7	1.3	4.2	23.0	12.4
L33	3	2.2	1.1	3.6	24.0	10.6
L34	4	2.3	1.3	3.4	26.0	10.5
L35	3	2.0	1.0	3.2	38.0	11.6
L36	3	1.5	1.0	2.0	28.0	10.7
L37	3	1.5	0.6	3.2	25.0	10.8
L38	5	1.6	0.5	2.7	41.0	9.9
L39	3	1.5	0.5	2.8	14.0	10.7
L40	4	2.0	0.7	3.7	35.0	10.9
L41	4	2.1	1.2	2.8	43.0	11.7
L42	3	1.5	0.7	2.1	57.0	11.3
L43	2	1.5	0.4	3.3	51.0	9.4
L44	4	1.5	0.5	2.0	27.0	10.1
L45	3	1.5	0.6	2.3	20.0	10.8
L46	3	1.4	0.7	2.8	19.0	11.7
L47	2	1.6	0.3	2.7	56.0	9.9
L48	4	1.9	0.8	2.9	27.0	10.4
L49	4	2.3	0.5	4.1	18.0	10.6
L50	5	1.6	0.4	2.6	22.0	11.7

Appendix E (continued): Physical characteristics of the individual lucerne hays

Lucerne hay #	Colour (/5)	Ave Stem Diameter (mm)	Min Stem Diameter (mm)	Max Stem Diameter (mm)	Leaf %	Shear (KJ/m2)
L51	5	1.9	0.9	3.3	0.0	12.3
L52	4	1.8	0.5	3.0	16.0	12.0
L53	5	1.7	0.9	3.7	4.0	11.7
L54	4	2.2	0.9	3.8	20.0	13.2
L55	4	2.3	1.6	3.1	17.0	12.3
L56	5	1.9	0.5	3.2	17.0	12.1
L57	4	2.1	1.3	2.9	23.0	11.8
L58	5	3.1	2.3	3.7	5.0	12.9
L59	4	2.6	1.2	4.8	12.0	13.3
L60	3	1.4	0.5	2.4	14.0	10.3
L61	4	2.0	0.9	3.4	12.0	10.8
L62	4	1.5	0.3	2.5	19.0	10.2
L63	4	2.2	0.8	4.1	12.0	11.6
L64	4	3.2	2.0	5.8	4.0	12.0
L65	5	2.3	1.0	3.6	4.0	12.3
L66	3	1.3	0.4	2.1	40.0	10.3
L67	5	1.7	0.5	2.9	51.0	10.7
L68	2	1.4	0.7	2.1	48.0	9.6
L69	5	1.6	1.0	2.6	49.0	10.9
L70	3	1.6	0.6	2.9	48.0	8.9
LA	4	4.3	2.0	7.1	23.0	11.6
LB	4	2.3	1.5	3.3	12.0	12.9
LC	5	1.9	1.2	2.9	26.0	12.2
LP	4	1.8	0.8	2.6	26.0	10.4
Min	*2*	*1.3*	*0.3*	*2.0*	*0.0*	*8.9*
Max	*5*	*4.2*	*2.4*	*6.9*	*57.0*	*14.4*
Mean	**3.6**	**2.0**	**1.0**	**3.2**	**28.0**	**11.0**
± SE	**0.12**	**0.07**	**0.06**	**0.11**	**1.62**	**0.13**

Appendix F: Intake rates and preference values of oaten hay for dairy cows

Oaten hay #	CIR (g/min)	CPrefA	CPrefBC	CPrefD	CPrefE	CPrefave
1	99.87	1.071	-1.072	-3.243	-3.751	-1.749
2	169.11	4.316	-0.340	-1.016	-3.121	-0.040
3	150.74	5.050	-1.208	-1.965	-4.593	-0.679
4	136.88	4.294	-3.027	-2.316	-4.681	-1.433
5	142.46	-1.322	-4.494	-2.461	-4.987	-3.316
6	132.95	-1.812	-5.212	-4.079	-4.407	-3.877
8	112.08	0.125	-5.844	-4.124	-5.088	-3.733
9	74.38	-2.780	-5.883	-4.533	-5.183	-4.595
12	167.56	5.901	-0.053	-1.474	-1.131	0.811
13	106.17	2.459	-4.206	-3.745	-5.955	-2.862
14	138.59	2.529	-4.834	-4.389	-4.394	-2.772
15	150.18	1.088	-3.479	-2.281	-4.317	-2.247
16	52.08	-1.800	-5.814	-5.525	-3.597	-4.184
17	150.24	3.393	-3.018	-5.854	-2.396	-1.969
18	121.81	-1.878	-5.961	-3.376	-4.685	-3.975
19	153.51	4.277	0.439	-2.313	-1.778	0.156
20	140.99	3.406	-4.950	-3.312	-3.099	-1.988
21	148.65	4.640	0.341	0.132	-2.318	0.699
22	153.47	3.805	-1.550	-4.041	-5.124	-1.727
23	150.30	2.039	-3.418	-2.864	-2.917	-1.790
24	152.53	3.442	-1.364	-2.124	-1.316	-0.340
25	100.52	4.909	-1.868	-0.716	-1.950	0.094
26	113.86	-1.753	-5.500	-5.064	-5.286	-4.400
27	150.11	5.439	-3.076	-1.275	-3.016	-0.482
28	209.92	6.229	1.565	2.995	-0.521	2.567
29	206.44	5.604	2.473	0.513	-0.046	2.136
30	165.73	4.337	1.899	0.626	-0.492	1.593
31	201.53	6.132	3.901	1.133	0.304	2.867
34	115.27	1.084	-2.697	-3.952	-4.283	-2.462
35	163.18	4.977	1.373	0.104	-1.542	1.228
37	199.90	4.706	4.353	0.869	-0.882	2.262
38	198.00	6.490	3.550	1.633	0.106	2.945
40	187.85	4.745	3.554	0.927	-2.001	1.806
41	198.23	5.702	4.028	1.726	0.216	2.918
43	205.45	4.305	3.697	-0.096	-3.347	1.140
44	209.36	3.701	0.558	0.062	-2.398	0.481
45	202.70	5.960	2.942	1.246	-0.766	2.345
46	134.21	2.258	-2.713	-5.016	-4.317	-2.447
48	166.41	4.877	-4.196	-3.131	-1.978	-1.107
50	213.55	5.455	3.727	1.904	0.085	2.793
51	153.80	5.146	0.382	0.652	0.219	1.600
52	179.12	4.064	1.698	1.342	-0.815	1.572
53	176.29	3.685	2.076	1.899	-0.956	1.676
54	184.21	4.847	0.605	0.870	-2.243	1.020
55	200.79	5.196	2.381	1.057	0.285	2.230
56	187.99	4.463	0.717	1.501	0.954	1.909
57	197.01	5.141	1.800	1.216	0.543	2.175
58	178.17	5.028	1.783	1.618	-0.054	2.093
59	160.13	4.241	0.836	-0.055	-0.819	1.051
60	190.72	3.419	3.081	1.078	0.176	1.938

Appendix F (continued): Intake rates and preference values of oaten hay for dairy cows

Oaten hay #	CIR (g/min)	CPrefA	CPrefBC	CPrefD	CPrefE	CPrefave
61	120.21	0.364	-2.213	-3.276	-3.632	-2.189
62	116.56	2.159	-4.315	-3.490	-3.350	-2.249
63	159.29	4.878	0.952	1.116	0.682	1.907
64	151.98	3.496	0.074	0.393	-0.895	0.767
65	155.86	3.914	-0.127	0.413	-1.611	0.647
66	163.52	2.344	-0.012	-0.129	-0.709	0.374
67	126.55	2.595	-4.218	-0.460	-3.539	-1.406
68	139.41	3.692	-1.906	-1.828	-4.596	-1.160
69	140.88	2.947	-0.292	-0.361	-1.528	0.191
70	141.27	4.092	-1.159	-1.249	-5.502	-0.954
71	126.81	0.744	-2.005	-2.904	-3.908	-2.018
72	144.63	0.847	-5.297	-1.638	-4.497	-2.646
73	132.00	4.291	-3.725	-0.525	-3.637	-0.899
74	142.23	0.577	-2.786	-0.859	-2.775	-1.461
75	161.96	3.041	-1.949	-0.801	-1.518	-0.307
76	167.50	2.231	-2.490	-0.645	-3.227	-1.033
77	144.26	4.488	0.483	-0.199	-3.357	0.354
78	172.35	4.373	1.561	0.020	-0.536	1.354
79	155.11	5.617	-1.740	-0.461	-3.312	0.026
80	135.56	3.868	-2.304	-2.642	-1.520	-0.650
81	124.42	4.836	-1.667	-3.289	-3.882	-1.000
82	119.92	2.510	-1.303	-1.875	-2.665	-0.833
83	114.26	5.355	-1.723	-1.627	-2.104	-0.025
84	100.92	3.432	-3.879	-3.788	-2.681	-1.729
89	190.91	6.479	1.963	1.880	0.004	2.582
90	168.92	4.599	2.988	1.474	1.362	2.606
91	134.62	2.639	-0.465	-0.863	-2.463	-0.288
92	139.76	6.184	0.171	-0.008	-2.236	1.028
99	140.43	1.053	-5.067	-2.440	-2.426	-2.220
100	80.63	-0.227	-4.874	-4.934	-4.621	-3.664
101	121.69	3.525	-1.415	-1.196	-1.499	-0.146
102	171.49	4.195	2.351	1.739	-1.110	1.794
103	131.68	1.361	-2.444	-1.194	-4.405	-1.671
104	151.15	5.482	-0.725	0.891	-0.664	1.246
105	168.10	4.957	0.526	0.661	-1.592	1.138
A	112.00	0.000	-1.954	-2.111	-1.780	-1.948
BC	131.44	1.954	0.000	-1.042	-0.725	-0.589
D	162.67	2.111	1.042	0.000	-1.245	-0.068
E	170.22	1.780	0.725	1.245	0.000	0.657
Min	*52.1*	*-2.8*	*-6.0*	*-5.9*	*-6.0*	*-4.6*
Max	*213.5*	*6.5*	*4.4*	*3.0*	*1.4*	*2.9*
Mean	**151.9**	**3.4**	**-1.0**	**-1.1**	**-2.3**	**-0.2**
±SE	**3.58**	**0.24**	**0.31**	**0.23**	**0.20**	**0.22**

Appendix G: Intake rates and preference values of oaten hay for horses

Oaten hay #	HIR (g/min)	HPrefA	HPrefBC	HPrefD	HPrefE	HPrefave
1	31.94	0.370	-0.960	-1.247	0.895	-0.236
2	44.79	1.505	-0.816	-1.323	1.266	0.158
3	49.82	0.761	-1.030	-2.670	4.154	0.304
4	45.81	2.547	-1.025	-0.820	0.895	0.399
5	32.31	-1.985	-4.714	-1.723	0.335	-2.022
6	34.14	-0.420	-2.984	-3.231	-0.014	-1.662
8	39.24	-1.840	-3.747	-3.185	0.526	-2.062
9	38.13	0.379	-2.349	-1.445	2.350	-0.266
12	55.74	-0.465	-0.792	-1.085	3.768	0.356
13	48.05	0.236	-1.283	0.165	1.060	0.045
14	34.11	0.012	-3.403	-0.790	1.040	-0.785
15	38.68	-0.534	-3.175	-1.241	0.312	-1.159
16	14.86	-1.336	-4.556	-3.328	-2.034	-2.814
17	41.60	-0.177	-3.581	-3.054	2.684	-1.032
18	37.91	-1.213	-4.117	-3.590	-0.847	-2.442
19	52.33	1.899	-0.630	-0.826	1.682	0.531
20	41.26	1.456	-2.224	-2.164	2.642	-0.073
21	44.02	0.186	-2.409	-1.986	1.624	-0.646
22	46.69	0.208	-0.821	-1.348	1.839	-0.031
23	43.06	0.300	-1.767	-2.306	-0.121	-0.973
24	53.05	1.811	0.062	0.522	2.505	1.225
25	49.59	1.205	0.112	0.090	1.623	0.758
26	30.56	-0.010	-1.877	-3.089	1.298	-0.920
27	47.76	-1.287	-1.742	-3.005	0.797	-1.309
28	40.21	1.446	0.371	1.445	1.387	1.162
29	59.90	0.312	-0.136	-0.795	2.282	0.416
30	54.45	-2.515	-0.457	-0.571	1.940	-0.401
31	43.29	0.989	-0.545	-1.392	1.848	0.225
34	35.04	0.761	-1.765	-2.558	2.134	-0.357
35	46.61	1.734	0.434	1.224	3.055	1.612
37	59.70	-0.416	0.080	-0.286	1.316	0.173
38	57.28	1.274	-0.841	-0.073	1.214	0.393
40	55.73	4.318	0.679	0.013	2.909	1.980
41	50.40	-1.124	-1.836	-1.747	3.266	-0.360
43	43.77	1.076	0.237	-1.111	2.884	0.771
44	46.43	1.766	-0.303	0.771	1.662	0.974
45	30.54	0.744	-1.959	-1.633	1.296	-0.388
46	41.57	-0.113	-3.664	-3.878	0.864	-1.698
48	43.51	0.749	0.371	-0.504	2.109	0.681
50	35.55	-0.067	0.486	-0.700	0.721	0.110
51	37.78	3.407	0.935	-0.110	3.641	1.968
52	42.96	2.363	1.255	0.123	4.746	2.122
53	57.02	2.221	-0.692	-0.350	4.023	1.300
54	39.61	1.278	-0.345	-0.161	3.279	1.013
55	50.08	2.781	-0.002	1.933	2.781	1.873
56	48.89	3.678	1.606	1.720	3.994	2.750
57	42.65	4.295	1.431	1.044	4.283	2.763
58	52.10	2.673	1.936	0.034	3.483	2.032
59	42.06	2.129	-1.424	-0.100	3.473	1.019
60	34.65	2.783	0.548	1.363	4.228	2.231

Appendix G (continued): Intake rates and preference values of oaten hay for horses

Oaten hay #	**HIR (g/min)**	**HPrefA**	**HPrefBC**	**HPrefD**	**HPrefE**	**HPrefave**
61	36.72	-1.803	-2.460	-2.925	1.264	-1.481
62	26.49	-0.462	-4.042	-3.105	0.902	-1.677
63	44.92	1.804	2.188	0.880	3.498	2.093
64	38.08	1.618	2.262	1.053	4.211	2.286
65	33.60	2.145	-0.757	-0.623	2.798	0.891
66	42.96	2.284	-0.158	1.476	3.565	1.792
67	31.39	0.284	-2.689	-1.799	1.631	-0.643
68	32.68	-0.933	-1.435	-1.519	1.426	-0.615
69	35.20	1.231	-0.342	-0.617	2.690	0.740
70	35.20	1.208	-3.325	-2.863	0.874	-1.027
71	33.40	1.115	-1.522	-2.947	1.694	-0.415
72	42.23	-1.792	-2.987	-4.452	0.960	-2.068
73	37.07	1.593	-0.836	-1.013	0.998	0.185
74	33.82	1.264	-0.698	-2.187	1.643	0.006
75	28.61	-0.769	-1.405	-0.687	3.611	0.187
76	37.12	-0.976	-2.620	-0.217	1.660	-0.538
77	26.41	-0.431	-1.763	-2.105	1.904	-0.599
78	36.34	0.454	-0.683	-3.699	2.394	-0.383
79	41.10	1.681	-0.251	-0.497	2.708	0.910
80	45.45	-0.255	-2.090	-1.167	1.439	-0.518
81	25.61	1.073	-1.777	-3.179	0.995	-0.722
82	34.97	2.362	-2.673	-3.309	0.228	-0.848
83	30.00	0.979	-1.686	-2.317	0.516	-0.627
84	30.09	0.665	-2.138	-2.774	0.286	-0.990
89	15.28	-1.208	-0.317	-1.530	0.114	-0.735
90	33.67	1.807	-1.497	-1.711	2.530	0.282
91	52.28	-1.582	-0.885	-3.356	0.547	-1.319
92	47.49	-0.523	-3.230	-3.236	0.816	-1.543
99	40.13	2.158	-2.316	-3.554	1.003	-0.677
100	17.76	-0.858	-3.737	-3.308	0.378	-1.881
101	33.42	0.112	-2.519	-2.985	2.986	-0.602
102	45.25	0.882	0.676	0.628	2.003	1.047
103	40.93	0.555	0.080	0.250	2.594	0.870
104	40.67	1.626	-1.023	1.220	3.566	1.347
105	26.68	0.613	-0.032	0.092	3.807	1.120
A	36.11	0.000	-0.480	-0.703	-0.053	-0.309
BC	44.33	0.480	0.000	-0.136	0.988	0.333
D	47.22	0.703	0.136	0.000	0.691	0.383
E	23.92	0.053	-0.988	-0.691	0.000	-0.407
Min	*14.86*	*-2.5*	*-4.7*	*-4.5*	*-2.0*	*-2.8*
Max	*59.90*	*4.3*	*2.3*	*1.9*	*4.7*	*2.8*
Mean	**40.2**	**0.7**	**-1.2**	**-1.3**	**1.9**	**0.0**
±SE	**1.022**	**0.157**	**0.171**	**0.170**	**0.144**	**0.136**

Appendix H: Intake rates and preference values of lucerne hay for horses

Lucerne hay #	HLIR (g/min)	HPrefLA	HPrefLB	HPrefLC	HPrefLP	HPrefLave
L1	16.44	0.437	0.092	0.102	-0.513	0.029
L2	39.56	0.414	0.283	-0.459	-0.599	-0.090
L3	42.22	0.528	0.033	-0.206	-0.447	-0.023
L4	46.44	0.076	0.166	-0.655	-0.460	-0.218
L5	47.78	-0.242	0.092	-0.307	-0.613	-0.267
L6	55.11	0.762	0.632	0.370	-0.225	0.385
L7	60.44	0.852	0.970	0.756	0.267	0.711
L8	51.78	0.491	0.465	0.331	-0.370	0.229
L9	52.22	0.747	0.856	0.070	-0.289	0.346
L10	54.67	0.459	0.106	0.188	-0.318	0.109
L11	40.22	0.094	0.163	-0.688	-0.598	-0.257
L12	53.56	0.692	0.428	0.199	-0.503	0.204
L13	46.44	0.388	0.639	0.306	0.225	0.390
L14	66.44	0.327	0.797	0.656	0.639	0.605
L15	56.89	0.860	0.952	0.132	-1.645	0.075
L16	64.22	0.765	0.230	0.643	0.781	0.605
L17	62.00	0.352	0.712	0.457	0.678	0.550
L18	49.11	0.673	0.596	0.260	0.565	0.524
L19	42.22	0.738	0.751	0.181	-0.389	0.320
L20	39.33	0.784	0.742	0.007	0.611	0.536
L21	47.78	0.465	0.430	-0.436	-0.538	-0.020
L22	41.56	0.755	0.615	0.576	0.422	0.592
L23	49.33	0.421	0.664	0.627	0.056	0.442
L24	63.56	0.692	1.035	0.667	0.566	0.740
L25	48.00	0.693	0.463	-0.176	0.131	0.278
L26	49.56	0.599	0.588	-0.271	-0.278	0.159
L27	55.11	0.708	0.242	0.287	0.609	0.462
L28	37.11	0.483	0.263	-0.340	-0.711	-0.076
L29	45.11	0.408	0.284	0.237	0.312	0.310
L30	58.00	0.882	0.471	0.672	0.157	0.545
L31	32.44	0.134	0.314	-0.269	-0.344	-0.041
L32	40.44	0.242	0.477	-0.550	-0.374	-0.051
L33	47.56	0.635	0.730	-0.027	-0.825	0.128
L34	63.11	0.145	0.877	0.638	0.027	0.422
L35	64.22	0.535	0.813	0.865	0.774	0.747
L36	59.78	0.646	0.912	0.446	-0.532	0.368
L37	51.11	0.540	0.366	-0.073	-0.490	0.086
L38	63.56	0.431	0.867	0.762	-0.474	0.397
L39	65.11	0.725	0.653	0.302	-0.462	0.304
L40	57.78	0.648	0.518	-0.359	-0.034	0.193
L41	56.67	0.675	0.574	-0.492	-0.018	0.185
L42	62.00	0.713	0.695	0.652	0.123	0.546
L43	62.00	0.604	0.738	0.473	0.390	0.551
L44	66.44	0.825	0.579	0.689	0.212	0.577
L45	51.11	0.700	0.879	0.579	0.215	0.593
L46	58.89	0.789	1.466	0.307	0.199	0.690
L47	59.11	0.624	0.643	0.404	-0.113	0.389
L48	40.22	0.422	-0.055	-0.666	-0.412	-0.178
L49	50.67	-0.529	-0.030	-0.009	-0.666	-0.308
L50	35.56	-0.639	-0.514	-0.655	-1.647	-0.864

Appendix H (continued): Intake rates and preference values of lucerne hay for horses

Lucerne hay #	HLIR (g/min)	HPrefLA	HPrefLB	HPrefLC	HPrefLP	HPrefLave
L51	23.78	-1.171	-0.401	-0.343	-0.722	-0.659
L52	45.56	-0.482	-0.371	-0.849	-0.568	-0.568
L53	44.22	-1.150	-0.112	-0.600	-0.336	-0.550
L54	24.00	-0.465	-0.587	-0.432	-0.679	-0.541
L55	20.22	-1.017	-0.656	-0.146	-0.704	-0.631
L56	34.11	-0.488	-0.557	-0.478	-0.619	-0.536
L57	28.67	-0.565	0.356	-0.677	-0.683	-0.392
L58	9.89	-0.950	-0.446	-0.220	-0.598	-0.554
L59	10.22	-0.492	-0.496	-0.526	-0.541	-0.514
L60	46.44	0.618	0.506	0.122	0.305	0.388
L61	56.89	0.690	0.799	0.164	-0.209	0.361
L62	35.11	-0.981	0.572	-0.275	-0.586	-0.317
L63	30.22	0.777	-0.387	-0.729	-0.526	-0.216
L64	19.78	-1.424	-0.084	-0.602	-0.681	-0.698
L65	20.67	-1.170	-0.535	-0.985	-0.504	-0.799
L66	62.67	0.750	0.832	0.421	-0.037	0.492
L67	70.00	0.746	0.733	0.290	-0.058	0.428
L68	64.44	0.800	0.573	0.349	0.475	0.549
L69	47.78	1.123	0.682	-0.299	-0.434	0.268
L70	58.22	0.662	0.811	0.608	0.634	0.679
LA	39.89	0.00	0.37	-0.45	-0.78	-0.21
LB	37.44	-0.37	0.00	-0.23	-0.55	-0.29
LC	45.78	0.45	0.23	0.00	-0.45	0.06
LP	61.67	0.78	0.55	0.45	0.00	0.44
Min	*9.89*	*-1.424*	*-0.656*	*-0.985*	*-1.647*	*-0.864*
Max	*70.00*	*1.123*	*1.466*	*0.865*	*0.781*	*0.747*
Mean	**47.47**	**0.300**	**0.393**	**0.029**	**-0.200**	**0.130**
±SE	**1.729**	**0.074**	**0.056**	**0.058**	**0.062**	**0.052**

References

Abrams, S.M., Shenk, J.S., Westerhaus, M.O. and Barton, F.E. (1987). Determination of forage quality by near infrared reflectance spectroscopy: efficacy of broad-based calibration equations. Journal of Dairy Science 70:806-813.

Albright, J.L. (1993). Nutrition, feeding, and calves. Feeding behaviour of dairy cattle. Journal of Dairy Science 76:485.

AOAC (1980). Official Methods of Analysis of the Association of Official Analytical Chemists. Sections 7.009, 14.003 and 14.006, 13th Ed. AOAC, Washington DC.

Arnold, G.W., de Boer, E.S. and Boundy, C.A.P. (1980). The influence of odour and taste on the food preferences and food intake of sheep. Australian Journal of Agricultural Research 31, 571-587.

Balch, C.C. and Campling, R.C. (1962). Regulation of voluntary food intake in ruminants. Nutrition Abstracts and Reviews 32:669-86

Bazely, D.R. (1988). Foraging behaviour of sheep (*Ovis aries* L.) grazing on swards of perennial ryegrass (*Lolium perenne* L.), PhD Thesis. Oxford University UK.

Bazely, D.R. and Ensor, C.V. (1989). Discrimination learning in sheep with cues varying in brightness and hue. Applied Animal Behaviour Science, 23:293-300.

Bennet, D.K. (1980). Stripes do not a zebra make; Part 1: a cladistic analysis of Equus. Systematic Zoology 29:272-287.

Blummel, M. and Orskov, E.R. (1993). Comparison on in vitro gas production and nylon bag degradability of roughages in predicting food intake in cattle. Animal Feed Science and Technology 40:109-119.

Blundell, J.E. and Halford, J.C.G (1994). Regulation of nutrient supply: The brain and appetite control. Proceedings of the Nutrition Society 53(2):407-418

Brobeck, J.R. (1948). Food intake as a mechanism of temperature regulation. The Yale Journal of Biology and Medicine 20: 545-552

Bryant, J.P., Reichardt, P.B., Clausen, T.P., Provenza, F.D. and Kuropat, P.J. (1992). Woody Plant-Mammal Interactions in 'Herbivores, their Interactions with Secondary Plant Metabolites' (eds. G.A. Rosenthal and M.R. Berenbaum) pp343-370. Academic Press Inc., San Diego.

Bullard, R.W., Leiker, T.J., Peterson, J.E., and Kilburn, S.R. (1978). Volatile components of fermented egg, an animal attractant and repellent. Journal of Agricultural and Food Chemistry26, 155-159.

Cahn, M.A. and Harper, J.L., (1976). The biology of the leaf mark polymorphism in Trifolium repens L. II. Evidence for the selection of leaf marks in rumen fistulated sheep. Heredity 37:327-333.

Campion, D.P. and Leek, B.F. (1997). Investigation of a "fibre appetite" in sheep fed a "long fibre-free" diet. Applied Animal Behaviour Science 52(1):79-86.

Ciavarella, T.A., Dove, H., Leury, B.J. and Simpson, R.J. (2000). Diet selection by sheep grazing *Phalaris aquatica L.* pastures of differing water soluble carbohydrate content. Australian Journal of Agricultural Research 51:757-764.

Cooper, S.D, Kyriazakis, I. and Oldham, J.D. (1996). The effects of physical form of feed, carbohydrate source, and inclusion of sodium bicarbonate on the diet selections of sheep. Journal of Animal Science 74(6):1240-1251.

Cox, A. (2004). The effects of dry matter digestibility (DMD) and water soluble carbohydrates (WSC) on the short and long term preference and intake of export quality oaten hay by horses. Honours thesis, The University of Adelaide.

Cymbaluk, N.F. (1990). Comparison of forage digestion by cattle and horses. Canadian Journal of Animal Science 70:601-610.

Dalley, D.E., Roche, J.R., Grainger, C. and Moate, P.J. (1999). Dry matter intake, nutrient selection, and milk production of dairy cows grazing rainfed perennial pastures at different herbage allowances in spring. Australian Journal of Experimental Agriculture 39:923-931.

Dietschke, A.E. and Baker, J.P. (1979). A study of appetite control in the equine. Proceedings of the 6th Equine Nutrition and Physiology Symposium, Texas, USA, pp. 63-64.

Distel, H. and Hudson, R. (1985). The contribution of the olfactory and tactile modalities to the performance of nipple-search behaviour in newborn rabbits. Journal of Comparative Physiology 157:599-605.

Distel, R.A., Laca, E.A., Griggs, T.C., Demment, M.W. (1995). Patch selection by cattle: maximization of intake rate in horizontally heterogeneous pastures. Applied Animal Behavioural Science 45:11-21

Doreau, M., Moretti, C. and Martin-Rosset, W. (1990). Effect of quality of hay given to mares around foaling on their voluntary intake and foal growth. Annales de Zootechnie 39:125-131.

Doty, R.L. and Commetto-Muiz, J.E. (2003). Trigeminal Chemoreception. Handbook of Olfaction and Gustation, 2nd edition. Doty R.L. (Ed.). Dekker, New York, pp.981-999.

du Toit, J.T., Provenza, F.D. and Nastis, A. (1991). Conditioned taste aversions - how sick must a ruminant get before it learns about toxicity in foods. Applied Animal Behaviour 30:35-46.

Dulphy, J. P., Martin-Rosset, W., Dubroeucq, H. and Jailler. M. (1997). Evaluation of voluntary intake of forage trough-fed to light horses. Comparison with sheep. Factors of variation and prediction. Livestock Production Science 52(2):97-104.

Dulphy, J.P., Remond, B. and Theriez, M. (1980). Ingestive behavior and related activities in ruminants in 'Digestive physiology and metabolism in ruminants' pp103-122. MTP Press Ltd., Falcon House Ltd.

Emmans, G.C. (1991). Diet selection by animals: theory and experimental design. Proceedings of the Nutrition Society 50, 59–64.

Engku Azahan, E.A. and Forbes, J.M. (1992). Effects of intraruminal infusions of sodium salts on selection of hay and concentrate foods by sheep. Appetite 18:143.

Estell, R.E., Fredrickson, E.L., Tellez, M.R., Havstad, K.M., Shupe, W.L., Anderson, D.M. and Remmenga, M.D. (1998). Effects of Volatile Compounds on Consumption of Alfalfa Pellets by Sheep. Journal of Animal Science 76:228-233.

Faverdin, P., Baumont, R. and Ingvartsen, K.L. (1995). Control and prediction of feed intake in ruminants in 'Recent Developments in the Nutrition of Herbivores' pp95-120. Proceedings of the Fourth International Symposium on the Nutrition of Herbivores.

Fisher, D.S., Mayland, H.F. and Burns, J.C. (1999). Variation in ruminant's preference for tall fescue hays cut at sundown or sunup. Journal of Animal Science 77, 762-768.

Fisher, D.S., Mayland, H.F., and Burns, J.C. (2002). Variation in ruminant preference for alfalfa hays cut at sunup and sundown. Crop Science 42:231-237.

Flinn, P., Knott, S., Cummins, L. and Dalley, D. (2005). Objective Measurement of Fodder quality Across animal Species, RIRDC Publication No 05/088, RIRDC Project No DAV 187A. Rural Industries Research and Development Corporation, ACT, Australia.

Flinn, P.C. and Heazlewood, P.G. (2000). A uniform objective system for quality description of Australian fodder products, RIRDC Publication No 00/134, RIRDC Project No DAV-104A. Rural Industries Research and Development Corporation, ACT, Australia.

Forbes, J.M. (1995). Voluntary food intake and diet selection in farm animals. CAB International, Wallingford, UK.

Forbes, J.M. and Provenza, F.D. (2000). Integration of Learning and Metabolic Signals into a Theory of Dietary Choice and Food Intake in 'Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction' (eds P.B. Cronje) pp3-19. CABI Publishing, Oxon UK.

Freeland, W.J. and Janzen, D.H. (1974). Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. The American Naturalist 108:269-289.

Gallagher, J.R. and Hughes, T.P. (1993). Grazing behaviour of race horses on perennial ryegrass. Proceedings of the 7th Australian Agronomy Conference, pp 442.

Gherardi, S.C., Black, J.L. and Colebrook, W.F. (1991). Effect of palatability on voluntary intake by sheep. II. The effect of altering the palatability of wheaten hay on long-term and preference. Australian Journal of Agricultural Research 42:585-598.

Ginane, C., Duncan, A.J., Young, S.A., Elston, D.A. and Gordon, I.J. (2005). Herbivore diet selection in response to simulated variation in nutrient rewards and plant secondary compounds. Animal Behaviour 69(3):541-550.

Glusman, G., Yanai, I., Rubin, I. and Lancet, D. (2001). The complete human olfactory subgenome. Genome Research 11:685-702.

Goatcher W.D. and Church D.C. (1970). Review of some nutritional aspects of the sense of taste. Journal of Animal Science 31:973-981.

Grosch, W. (1993). Detection of potent odorants in foods by aroma extract dilution analysis. Trends in Food Science and Technology 4:68-73.

Harris, L.E. (1970). Nutrition Research Techniques for Domestic and Wild Animals. Volume 1.

Heinrichs, S. C., Deutsch, J.A. and Moore, B.O. (1990). Olfactory self-selection of protein-containing foods. Physiology and Behavior 47:409-413.

Hills, J., Kyriazakis, I., Nolan, J.V., Hinch, G.N. and Lynch, J. J. (1999). Conditioned feeding responses in sheep to flavoured foods associated with sulphur doses. Journal of Animal Science 69:313-325.

Holechek, J.L., Vavra, M., and Arthun, D. (1982). Relationships between performance, intake, diet nutritive quality and fecal nutritive quality of cattle on mountain range. Journal of Range Management 35:741-744.

Hovell, F.D.D., Ngambi, J.W.W., Barber, W.P. and Kyle, D.J. (1986). The voluntary intake of hay by sheep in relation to its degradability in the rumen as measured in nylon bags. Animal Production 42:111-118.

Illius, A.W. and Gordon, I.J. (1993). ‘Diet selection in mammalian herbivores: constraints and tactics’ in ‘Diet Selection: An Interdisciplinary Approach to Foraging Behaviour’ (eds R.N. Hughes) pp157-180. Blackwell, Oxford UK.

Illius, A.W., Jessop, N.S. and Gill, M. (2000). Mathematical Models of Food Intake and Metabolism in Ruminants in ‘Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction’ (eds P.B. Cronje) pp21-39. CABI Publishing, Oxon UK.

Ingvartsen, K.L. (1994). Models of voluntary food intake in cattle. Livestock Production Science 39:19-38.

Jones, E.L., Roberts, J.E. (1991). A note on the relationship between palatability and water-soluble carbohydrates content in perennial ryegrass. Irish Journal of Agricultural Research 30:163-167.

Kennedy, G.C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. Proceedings of the Royal Society of Biological Sciences 139:578-592.

Kern, D. and Bond, J. (1972). Eating patterns of ponies fed diets ad libitum. Journal of Animal Science 35:285.

Ketelaars, J.J.M.H. and Tolkamp, B.J. (1992). Toward a new theory of feed intake regulation in ruminants 1. Causes of differences in voluntary feed intake: critique of current views. Livestock Production Science 30(4):269-296.

Kyriazakis, I. and Oldham, J.D. (1993). Diet selection in sheep: the ability of growing lambs to select a diet that meets their crude protein (nitrogen×6.25) requirements. British Journal of Nutrition 69:617-629.

Kyriazakis, I. and Oldham, J.D. (1997). Food intake and diet selection of sheep: the effect of manipulating the rates of digestion of carbohydrates and protein of the foods offered as a choice. British Journal of Nutrition 77:243-254.

Laut, J.E., Houpt, K.A., Hintz, H.F. and Houpt, T. (1985). The effect of caloric dilution on meal patterns and food intake of ponies. Physiology and Behaviour 35:549-554.

Lawson, R.E., Redfern, E.J. and Forbes, J.M. (2000). Choices by lactating cows between concentrates high or low in digestible undegraded protein. Journal of Animal Science 70:515-525.

Leek, B.F. and Harding, R.H. (1975). Sensory nervous receptors in the ruminant stomach and reflex control of reticulo-ruminal motility in 'Digestion and Metabolism in the Ruminant' (eds I.W. McDonald and A.C.I. Warner) pp60-76. University of New England, Armidale, Australia.

Leffingwell, J.C. (2002). Olfaction - Update No. 5. Leffingwell Reports Vol 2 (No. 1). [Online, accessed 11 February 2005] http://www.leffingwell.com/download/Olfaction5.pdf

Lindström, T. (2000). Feeding behaviour in Dairy Cows; Motivational Aspects. Doctoral Thesis, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala

Mader, D.R. and Price, E.O. (1980). Discrimination learning in horses: effects of breed, age and social dominance. Journal of Animal Science, 56:962-965.

Martin-Rosset, W. and Dulphy, J.P. (1987). Interactions between Forages and Concentrates in Horses: Influence of Feeding Level - Comparison with Sheep. Livestock Production Science 17:263-276.

Mayer, J. (1953). Genetic, traumatic and environmental factors in the etiology of obesity. Physiological Reviews 33(4):472-508.

Mayland, H.F., Flath, R.A. and Shewmaker, G.E. (1997). Volatiles from fresh and air-dried vegetative tissues of tall fescue (Festuca arundinacea Schreb.): Relationship to cattle preference. Journal of Agricultural and Food Chemistry 45:2204-2210.

Mayland, H.F., Shewmaker, G.E., Harrison, P.A. and Chatterton, N.J. (2000). Nonstructural carbohydrates in tall fescue cultivars: Relationship to animal preference. Agronomy Journal 92:1203-1206.

McBane, S. and McCarthy, G. (1991). The Competition Horse: Breeding, Production, and Management. John Wiley and Sons Inc., Hoboken, New Jersey.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan C.A. (1995). Animal Nutrition 5th Edition. Longman Scientific and Technical, England.

Mertens, D.R. (1973). Application of theoretical mathematical models to cell wall digestion and forage intake in ruminants, Ph.D. dissertation. Cornell University, Ithaca, NY.

Miller, D.S. and Payne, P.R. (1959). A ballistic bomb calorimeter. British Journal of Nutrition 13(4):501-508.

Moorby, J.M., Miller, L.A., Evans, R.T., Scollan, N.D., Theodorou, M.K. and MacRae, J.C. (2001). Milk production and N partitioning in early lactation dairy cows offered perennial ryegrass containing a high concentration of water soluble carbohydrates. Proceedings of the British Society of Animal Science, p. 6.

Muller, L.D. (1993). Limitations of pastures for high production by dairy cows - a US perspective in ‘Improving the Quality and Intake of Pasture-based Diets for Lactating Dairy Cows’ (eds N.J. Edwards and W.J. Parker) pp33-58. Department of Agricultural and Horticultural Systems Management, Massey University, Palmerston North New Zealand.

Murray, I. (1993). Forage analysis by near infrared reflectance spectroscopy in ‘Sward Management Handbook’ (eds A. Davies, R.D. Baker, S.A. Grant, and A.S. Laidlaw) pp285-312. British Grassland Society: Reading, UK.

Natale, D., Mattiacci, L., Pasqualini, E. and Dorn, S. (2003). Response of female Cydia molesta (Lepidoptera: Torticidae) to plant derived volatiles. Bulletin of Entomological Research 93:335-342.

Newman, J.A., Parsons, A.J. and Harvey, A. (1992). Not all sheep prefer clover: diet selection revisited. Journal of Agricultural Science 119:275-283.

Nolte, D.L., Mason, J.R., Epple, G., Aronov, E. and Campbell, D.L. (1994). Why are predator urines aversive to prey? Journal of Chemical Ecology 20:1505-1516.

Norris, K.H. and Hart J.R. (1965). Direct spectrophotometric determination of moisture content of grain and seeds. Principles and Methods of measuring Moisture Content in Liquids and Solids 4:19-25.

Norris, K.H., Barnes, R.F., Moore, J.E. and Shenk, J.S. (1976). Predicting forage quality by near infrared reflectance spectroscopy. Journal of Animal Scence. 43:889-897.

Orr, R.J., Penning, P.D., Harvey, A., Champion, R.A. (1997). Diurnal patterns of intake rate by sheep grazing monocultures of ryegrass or white clover. Applied Animal Behaviour Science 52:65-77.

Ott, E.A., Feaster, J.P. and Leib, S. (1979). Acceptability and digestibility of dried citrus pulp by horses. Journal of Animal Science 49:983.

Pain S.J., Franklin-McEvoy J., Cox A., Revell D.K. (2005). The odour profile of feedstuffs as modulators of feed preference in herbivores. Recent Advances in Animal Nutrition in Australia 15:229-236.

Pain, S.J. and Revell, D.K. (2007). Natural odorants can influence the preferences for lucerne hay exhibited by horses. Recent Advances in Animal Nutrition in Australia 16:27-33.

Pfister, J.A., Stegelmeier, B.L., Cheney, C.D., Ralphs, M.H. and Gardner, D.R. (2002). Conditioning taste aversions to locoweed (*Oxytropis sericea*) in horses. Journal of Animal Science 80:79-83.

Phy, T.S. and Provenza, F.D. (1998). Sheep fed grain prefer foods and solutions that attenuate acidosis. Journal of Animal Science 76(4):954-960.

Piggins, D. (1992). Visual perception in ‘Farm Animals and the Environment’ (eds C. Phillips and D. Piggins) pp131-158. C.A.B. International, Wallingford,

Pond, W.G., Church, D.C., Pond, K.R. and Schoknecht, P.A. (2005). Basic Animal Nutrition and Feeding, 5th Edition, John Wiley and Sons Inc. USA.

Provenza, F.D. (1995). Post-ingestive feedback as an elementary determinant of food preference and intake in ruminants. Journal of Range Management 48:2-17.

Provenza, F.D. (1996). Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. Journal of Animal Science 74:2010-2020.

Provenza, F.D. and Balph, D.F. (1987). Diet learning by domestic ruminants: theory, evidence and practical implications. Applied Animal Behavioural Science 18:221.

Provenza, F.D., Kimball, B.A., and Villalba, J.J. (2000). Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. Oikos 88:424-432.

Provenza, F.D., Lynch, J.J. and Nolan, J.V. (1993). The relative importance of mother and toxicosis in the selection of foods by lambs. Journal of Chemical Ecology, 19(2).

Provenza, F.D., Scott, C.B., Phy, T.S. and Lynch, J.J. (1996). Preference of sheep for foods varying in flavours and nutrients. Journal of Animal Science 74:2355-2361.

Provenza, F.D., Villalba, J.J., Cheney, C.D. and Werner, S.J. (1998). Self-organization of foraging behaviour: From simplicity to complexity without goals. Nutrition Research Reviews 11:199-222.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B. and Banner, R.E. (2003). Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Research 49:257-274.

Ralston, S.L. (1983). Controls of Feeding in Horses. Journal of Animal Science 59:1354-1361.

Ralston, S.L. and Baile, C.A. (1982). Plasma Glucose and Insulin Concentrations and Feeding Behaviour in Ponies. Journal of Animal Science 54:1132-1137.

Randall, R.P., Schurg, W.A. and Church, D.C. (1978). Responses of horses to sweet, salty, sour and bitter solutions. Journal of Animal Science 47:51.

Rewot, P.I., Ogwu, D., Oyedipe, E.O. and Sekoni, V.O. (2001). The role of pheromones and biostimulation in animal reproduction. Animal Reproduction Science 65:157-170.

Riol, J.A., Sanchez, J.M., Eguren, V.G., Gaudioso, V.R., (1989). Colour perception in fighting cattle. Applied Animal Behavioural Science 23:199-206.

Romney, D.L. and Gill, M. (2000). Intake of Forages in ‘Forage evaluation in Ruminant Nutrition’ (eds. D.I. Givens, E. Owen, R.F.E. Axford, H.M. Omed). CABI Publishing, Wallingford, UK.

Rouquier, S., Gaviaux, S, Trask, B.J., Grand-Arpon, V., van den Engh, G., Dernaille, J. and Giorgi, D. (1998). Distribution of olfactory receptor genes in the human genome. Nature Genetics 18:243-250.

Sherwood, L. (1997). Human Physiology: from cells to systems, 3rd Edition pp194-195. Wadsworth Publishing Company, USA.

Shewmaker, G.E., Maryland, H.F. and Hansen, S.B. (1997). Cattle grazing preference among eight endophyte-free tall fescue cultivars. Agronomy Journal 89:695-701.

Silver, W.L. and Finger, T.E. (1991). Smell and Taste in Health and Disease in ‘The Trigeminal System’ (eds. T.V. Getchell, R.L. Doty, M. Bartoshuk, and J.B. Snow Jr.) pp97-108. Raven Press, New York.

Stubbs, A.K. (2000). Atlas of the Australian Fodder Industry, RIRDC Publication No 00/122, RIRDC Project No PTP-14A. Rural Industries Research and Development Corporation, ACT, Australia.

Susmel, P., Spanghero, M., Stefanon, B., Mills, C.R. and Cargnelutti, C. (1991). Effect of NDF concentration and physical form of fescue hay on rumen degradability, intake and rumen turnover of cows. Animal Production 54:473A.

Tixier, H., Maizeret, C. Duncan, P., Bertrand, R., Poirel, C. and Roger, M. (1998). Development of feeding selectivity in roe deer. Behavioural Processes 43:33-42.

Tolkamp, B.J., Dewhurst. R.J., Friggens, N.C., Kyriazakis, I., Veerkamp, R.F. and Oldham, J.D. (1998). Diet Choice by Dairy Cows; 1. Selection of Feed Protein Content During the First Half of Lactation. Journal of Dairy Science 81(10):2657-2669.

Uetake, K. (1991). Behavioral tests for color blindness in cattle. Japanese Journal of Livestock Management 27(2):57-63.

Ulyatt, M.J., and Waghorn, G.C. (1993). Limitations to high levels of dairy production from New Zealand pastures in 'Improving the Quality and Intake of Pasture-based Diets for Lactating Dairy Cows' (eds N.J. Edwards and W.J. Parker) pp11-32. Department of Agricultural and Horticultural Systems Management, Massey University, Palmerston North New Zealand.

Villalba, J.J. and Provenza, F.D. (1997a). Preference for wheat straw by lambs conditioned with intraruminal infusions of starch. British Journal of Nutrition, 77:287-297.

Villalba, J.J. and Provenza, F.D. (1997b). Preference for flavoured foods by lambs conditioned with intraruminal administration of nitrogen. British Journal of Nutrition, 78:545-561.

Villalba, J.J. and Provenza, F.D. (1997c). Preference of flavoured wheat straw by lambs conditioned with intraruminal infusions of acetate and propionate. Journal of Animal Science, 75:2905-2914.

Villalba, J.J. and Provenza, F.D. (1999). Nutrient-Specific Preferences by Lambs Conditioned with Intraruminal Infusions of Starch, Casein, and Water. Journal of Animal Science 77:378-387.

Villalba, J.J. and Provenza, F.D. (2000). Roles of novelty, generalization, and post-ingestive feedback in the recognition of foods by lambs. Journal of Animal Science 78:3060-3069.

Villalba, J.J., Provenza, F.D. and Bryant, J.P. (2002). Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants. Oikos 97, 282-292.

Villalba, J.J., Provenza, F.D. and Rogosic, J. (1999). Preference for Flavoured Wheat Straw by Lambs Conditioned with Intraruminal Infusions of Starch Administered at Different Times After Straw Ingestion. Journal of Animal Science, 77:3185-3190.

Villalba, J.J., Provenza, F.D., Hall, J.O. and Peterson, C. (2006). Phosphorus appetite in sheep: Dissociating taste from postingestive effects. Journal of Animal Science 84:2213-2223.

Villalba, J.J., Provenza, F.D., Hall, J.O. and Peterson, C. (2008). Learned appetites for calcium, phosphorous and sodium in sheep. Journal of Animal Science 86:738-747.

Ward, R.G., Smith, G.S., Wallace, J.D., Urquhart, N.S. and Shenk, J.S. (1982). Estimates of intake and quality of grazed range forage by near infrared reflectance spectroscopy. Journal of Animal Science 54:399.

Werner, S.A., Kimball, B.A. and Provenza, F.D. (2008). Food color, flavour, and conditioned avoidance among red-winged blackbirds. Physiology and Behavior 93:110-117.

Williams, P.C. and Norris, K.H. (1982). Effect of mutual interactions on the estimation of protein and moisture in wheat. Cereal Chemistry 60:202-207.

Zahorik, D.M. and Houpt K.A. (1981). Species differences in feeding strategies, food hazards and the ability to learn food aversions in ‘Foraging Behaviour’ (eds A.C. Kamil) pp289. Garland Press, New York.

Zwer, P. and Faulkner, M. (2006). Producing Quality Oaten Hay. Rural Industries Research and Development Corporation (RIRDC) Publication number 06/002.