

Chapter 1

General introduction



1.1 Introduction

Research into foraging behaviour and diet selection in mammalian herbivores has largely focussed on wild and domestic ruminant species. This is mostly due to the economic value of domestic livestock in production systems and the impact of wild ruminant ungulates on ecosystem management. While ruminants play an important role in production systems and management of rangelands, horses also have an effect on many grassland ecosystems (Celaya et al., 2011; Fleurance et al., 2012) and have a considerable economic impact worldwide due to its use for sporting and leisure activities (e.g. racing, breeding and equestrian).

Compared to ruminants, little is known about how horses utilize forages, develop dietary preferences and make dietary selections to meet their nutritional requirements in various conditions. In a natural system horses forage on an almost continuous basis and are able to select from a variety of plant species to meet nutritional needs such as maintaining fibre, protein and energy intake (Hansen, 1976; Salter and Hudson, 1979; Putman et al., 1987; Celaya et al., 2011). For horses in stabled and pastured environments this can be different. In these housing systems horses largely rely on humans for the timing, selection and delivery of their food. Domestic horses are typically fed in ways that require minimal foraging; homogenous hay and/or foods that are milled contain low fibre and are energy-dense. Restricted quantities of roughage have been repeatedly correlated with increased risk of development of digestive disorders and abnormal behaviours in stabled horses (Harris, 1999; Nicol, 1999; Davidson, 2002; Cooper and McGreevy, 2007; Davidson and Harris, 2007; Harris, 2007; Ellis, 2012). In addition, a few studies have addressed the significance of variety in the diet of stabled horses and associated adaptive behaviours (McGreevy et al., 1995; Goodwin et al., 2002).

The high economic value of horses globally warrants increased scientific research into equine performance, nutrition and welfare. Research in equine nutrition has mainly focussed on the understanding of the physiological mechanisms of feed intake with little regard for behavioural mechanisms of food choice modulation in equines. Foraging is

an important but often overlooked aspect of welfare in equines, not only for the provision of adequate nutrients, but also for expression of normal behaviours related to searching and selection of foods. Therefore, this thesis sets out to provide a more in-depth understanding of factors that influence foraging behaviour and diet selection in horses, particularly how different food characteristics may modulate feed choices. This information is of relevance to the feeding management and welfare of horses in stabled and pastured conditions.

The following literature review outlines what is known regarding foraging behaviour and diet selection of free-roaming and domesticated horses. It then focuses on the factors that influence foraging behaviours by reviewing the nutritional basis for food selection in large herbivores. Specifically intrinsic and extrinsic constraints on diet selection and foraging behaviour will be examined. This leads to a more detailed discussion on the physical and metabolic regulation of feed intake by horses and their ability to discriminate and generalise amongst foods using their senses.

Chapter 2

Literature review



“The herbivore’s challenge is to acquire sufficient nutrients to evade starvation and produce viable offspring while avoiding the consumption of lethal doses of phytochemicals. The fact that herbivores generally succeed in walking this biological tightrope is credit to a highly sophisticated foraging process” - Launchbaugh et al. (1999)

2.1 Foraging behaviour and diet selection by horses

Foraging and feeding behaviour is driven by the need for nutrients (e.g. protein, carbohydrates, fats, minerals). There are a variety of means to obtain food - these being correlated to the diversity of foods and the numerous animal species and types (e.g. herbivores, carnivores, omnivores). Animals require a regular supply of nutrients for normal function of body cells and metabolism. In multicellular animals the body fluids surrounding each cell are the instant source of nutrients and the nutrient concentrations of these fluids are kept at a relatively constant level mainly by mobilisation of nutrients stored in the body (Sherwood et al., 2013). For example in vertebrates, glucose is stored in the liver and muscles, fats in the fat tissues and calcium in the bones. However, these reserves will become depleted unless the animal can access food nutrients through behavioural changes - an appetitive phase involving the seeking of food sources and a consumptive phase involving ingestion (Houpt, 2006). Animals can correct the lack of nutrients in their body by the intake of any of a variety of foods that provide carbohydrates, protein, fats, minerals or vitamins. Most food types will contain a combination of such components. Thus, nutrient deficiencies may be alleviated by increased responsiveness to food in general and ingested food feedback, which potentially occurs from the mouth, digestive tract, bloodstream and possibly storage sites. These feedback mechanisms and the factors that modulate feed intake and selection will be discussed in more detail later on. In the following section the findings of behavioural and observational studies that examined foraging behaviour in free-roaming and domesticated horses will be reviewed.

2.1.1 Foraging behaviour by free-roaming and feral horses

2.1.1.1 Feeding patterns

Horses, feed, lie, stand and travel, but the main activity is foraging which, in the case of free-roaming horses, can be grazing (e.g. consumption of graminoids and legumes) and/or browsing (e.g. consumption of forbs, shrubs and trees). Foraging involves walking, but horses don't move at a uniform rate. Horses typically display patch

foraging behaviour, which involves taking several bites of plants and then walking a step or two forward and/or sideways to the next feeding station. The time spent at each feeding station varies and will depend on a number of factors such as plant density, plant species richness, nutrients and secondary compounds (Bailey et al., 1996; Prache et al., 1998).

Horses usually graze and browse during both the day and night, with the time spent foraging being influenced by the season, weather condition, age, sex of the horse and quality and quantity of food available. Duncan (1980) investigated the 24 h time-budget of free-roaming horses in the Camargue region of France and reported that adult horses spent about 50-60% of their time grazing in the summer and 55-63% of their time grazing in the winter. New Forest ponies have been observed to devote most of their time grazing and browsing in the winter season (Tyler, 1972). As summer approached, ponies would spend less time foraging and more time resting. In particular between 0900 h and 1400 h (end of first grazing period of the day) no grazing would occur - in fact most of the grazing would take place during the night in the summer season. This is in accordance with the findings of Berger et al. (1999) who found similar patterns for day and night depending on the season in semi-feral Przewalsky horses (Nature park, Germany). Similar time budgets of 50-60% grazing and browsing were also reported in free-roaming and feral horses in the American West (Salter and Hudson, 1979; Berger, 1986), and the steppes of Mongolia (Boyd, 1998; Boyd and Bandi, 2002).

2.1.1.2 Selection of forage species in rangelands

Free-roaming and feral horses are known to preferentially select herbaceous vegetation, mostly grasses and legumes, but woody plants and other plant species can comprise up to 50% of the total diet of horses depending on the vegetation conditions and season (see Table 2.1). In addition, different parts of the plants can be eaten – stem, leaf, fruit or inflorescence (Negi et al., 1993). For example, New Forest ponies will consume a variety of grasses, rushes and small flowering plants with purple moor grass (*Molinia caerulea*) being the bulk of the diet throughout the summer months. It was also

observed that these ponies would wade into the water to consume emergent and submerged aquatic plants.

Browse species ingested by the ponies consisted mostly of gorse (*Ulex europaeus*), dwarf gorse (*Ulex minor*), brambles (*Rubus* spp.), oak (*Quercus* spp), beech (*Fagus* spp.) and holly (*Ilex aquifolium*). In particular the leaves of gorse and holly provided almost the sole constituents of their diet during the winter when snow covered the ground (Tyler, 1972). The seasonal selection of gorse as a supplement to grasses has also been recorded in Basque ponies, a free-roaming horse breed of Northern Spain (Aldezabal et al., 2012). However, browsing of gorse didn't occur at random. It appeared that sward height of grass patches was the best indicator for feeding site preference, and gorse patch edges surrounding the most popular grass patches were browsed with greater intensity. The authors suggest that these animals may adopt a feeding strategy that allows for a trade-off between maximising intake rate and nutritive benefits.

A high proportion of browse in the diet was also observed in feral horses in southern New Mexico (USA), which consumed around 40% grasses and grass like species, with other plants such as Russian thistle (*Salsola kali*), dropseed (*Sporobolus* spp.) and mesquite (*Prosopis juliflora*) forming the larger portion of the diet (Hansen, 1976). Feist and McCullough (1976) observed horses in the Pryor Mountains Wild Horse Range (Wyoming USA) where grasses, the preferred food, were scarce. The horses consumed marsh grasses, reeds and numerous forbs. Where these were not available, they ate new growth of woody plants, such as saltbushes (*Atriplex* spp.), greasewood (*Sarcobatus vermiculatus*), Rabbitbrushes (*Chrysothamnus* spp.) and sagebrushes (*Artemisia tridentata* and *nova*). They also pawed up the roots of Winterfat (*Eurotia lanata*), spiny milkvetch (*Astragalus kentrophyta*) and Plains milkvetch (*Astragalus gilviflorus*).

Table 2.1 Percentage botanical composition of free-roaming horse diets in summer (S) and winter (W).

Study	Breed	Location	Graminoids & legumes		Herbaceous plants (Forbs)		Woody plants (Browse)	
			S	W	S	W	S	W
Hansen (1976)	Feral	New Mexico, USA	58	37	13	42	28	21
Hubbard and Hansen (1976)	Feral	Colorado, USA	85 - 95		NA		5 - 12*	
Olsen and Hansen (1977)	Feral	Wyoming, USA	94		3		3	
Salter and Hudson (1979)	Feral	Western Alberta, Canada	98	89	<1	<1	2	6
Krysl et al. (1984)	Feral	Wyoming, USA	70-79	60-66	3	1	18-27	33-39
Putman et al. (1987)	New Forest Ponies	New Forest, England	80-90	50	<1	0	10-20	45-50
McInnis and Vavra (1987)	Feral	Oregon, USA	87-91	86-90	6-7	2-3	2-7	2-3
Berman (1991)	Feral	Central Australia	72 - 92		NA		8 - 28*	
Cran et al. (1997)	Feral	Wyoming, USA	92	67	NA		1*	23*
Smith et al. (1998)	Feral	New Mexico, USA	72-95	98	<1	<1	5-28	2
Cosyns et al. (2001)	Konik ponies	Belgium	94	81	4	18	2	1
Lamoot et al. (2005a)	Shetland ponies	Belgium	92		8		NA	
Pratt-Phillips et al. (2011)	Feral	North Carolina, USA	98	89	1.5	10	<1	1
Kaz'min et al. (2013)	Feral	Vodnyi Island, Russia	48	83	NA		38*	14*

* Include forbs

NA; Not applicable

2.1.1.3 Bark-chewing

Likewise in Australia free-roaming or feral horses have been reported to browse on various parts of forbs, shrubs and trees, in particular stripping bark of eucalypt species (Nadolny, 1983; Berman, 1991; Black, 2000; Ashton, 2005; Schott, 2005; Hampson et al., 2011). Bark-chewing or stripping by free-roaming horses has been primarily studied in relation to the damage of trees and the impact on the vegetation in national parks. Some reasons for this behaviour have been proposed such as to provide dietary supplementation or for medicinal purposes, however the reasons for bark chewing remains unclear and investigations into the digestibility and macronutrient content of bark are not conclusive. Ashton (2005) and Schott (2005) suggested that bark chewing could be linked to seeking nutrients such as sugars, minerals and proteins, however both studies did not analyse the bark for nutrient composition.

Bark chewing or stripping by horses has been observed in other parts of the world as well. Kuiters et al. (2006) investigated the incidence and intensity of bark-stripping of European beech (*Fagus sylvatica* L.) and other woody trees by horses in the Veluwezoom National Park (Netherlands). The results indicated that the macronutrients of bark (calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and phosphorus (P)) did not explain why horses stripped the beech. However, the authors suggested that it may have been linked to fibre and/or sugar intake. Another explanation that most of these studies propose is water/moisture intake. A higher incidence of bark chewing or stripping was observed in drought periods, in summer or near water locations (Ashton, 2005; Schott, 2005).

2.1.1.4 Diet selection and nutritional composition

There are only a few studies that report on the nutritional quality of different forages consumed by feral or free-roaming horses. Kaz'min et al. (2013) assessed the nutritional value of plant fodder, daily consumption, and digestibility of forages in the habitat of an isolated group of free-roaming horses on the Vodnyi Island in Russia during different seasons. For the greater part of the year, the diets of the horses consist mainly of

graminoid species (69-84%), which decreased to 48% when knotweed (*Polygonum patulum*) and sagebrush (*Artemisia austriaca*) flowered at the end of summer (August). They found that during all the recorded seasons that the horses of the Vodnyi Island have a total consumption of forage similar to the recorded amounts in domesticated horses (~2% of body weight (BW) and they appear to meet their energy and protein requirements throughout the seasons (Anonymous, 2007).

Another study reviewed the nutritional composition of the diets of free-roaming horses of the Shackleford Banks in North Carolina USA (Pratt-Phillips et al., 2011). The researchers collected faecal samples for microhistological examinations to estimate the plant types in the horse's diets. This information was used to select plants in the horse's habitat, which they analysed for the nutritional composition during a period of 4 seasons (2 years total). Grasses were the primary plant type found in the faeces of the horses, although forbs were also found, particularly in the winter and spring. The authors recorded that the horses met most of their requirements based on the recommended intake for domesticated horses (Anonymous, 2007), but that may be deficient in some minerals or trace minerals at certain times of the year. Interestingly Pratt-Phillips et al. (2011) reported that the plant nutrient content did not have a significant relationship with consumption. The authors suggest that plant availability and forage characteristics may have a greater effect on feed intake. This seemed also the case for the Basque ponies in Northern Spain, who consumed mainly grasses, but would commence browsing when resources reduced even though gorse had a better nutritional profile (e.g. crude protein) on average than the grasses throughout the year (Aldezabal et al., 2012).

2.1.2 Foraging behaviour by domestic horses at pasture

2.1.2.1 Selection of pasture species

Research into foraging behaviour and diet selection by horses has largely focussed on free-roaming and feral horses and the context of ecosystem management. Little work has been conducted with domestic, pastured horses. Like free roaming horses, pastured horses can graze and browse for up to 14 h/day (Edouard et al., 2009), selectively

foraging on a wide variety of species (Archer, 1971; 1973). Archer (1973; 1978) examined the preference of certain grasses, legumes and herbs and observed that horses would select well-liked with less-liked plant species. In the 1973 study it was reported that of the 26 plant species tested, wild white clover-rich pastures (*Trifolium repens* L.) were the most preferred followed by perennial ryegrasses (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and timothy (*Pheum pratense*). The least preferred species were brown top (*Agrostis tenuis*), red fescue (*Festuca rubra*) and meadow foxtail (*Alopecurus pratensis*). Conversely, in the 1978 study ranking 12 grass species it was observed that creeping red fescue and tall fescue (*Festuca arundinacea*) were the most preferred, and the least favourite species were meadow foxtail, timothy and perhaps surprisingly ryegrass. Hybrid ryegrass species (*Lolium italicum* x *Lolium perenne*) were more preferred than perennial ryegrass. In a New Zealand study on a Waikato Thoroughbred stud, Hunt and May (1990) observed seasonal differences in pasture preference by horses grazing 16 pasture species. Grasses, particularly prairie grass (*Bromus willdenowii* Kunth) and Italian ryegrass (*Lolium multi-florum*), were preferred in winter and spring. In warmer weather the preference for legumes and herbs were more noticeable. Another New Zealand study also found that (tetraploid) ryegrasses (*Lolium perenne* and *Lolium multi-florum*) were the most preferred grass species, and Yorkshire fog (*Holcus lanatus*) and cocksfoot (*Dactylis glomerata*) were the least preferred by the horses (Randall et al., 2014). The differences observed in these studies are most likely influenced by the diverse measurements of preference (e.g. time grazing, height of grass left after grazing or herbage yield), cultivars, seasonal effects and individual variations amongst animals (previous experiences).

2.1.2.2 Pasture intake

It has been estimated that the voluntary dry matter intake (VDMI) of grazing horses typically ranges between 1.5-3.1 % of BW (Marlow et al., 1983; Dulphy et al., 1997a; Fleurance et al., 2001; Grace et al., 2002a; b; Menard et al., 2002; Grace et al., 2003). The intake will depend on the class of the horse, for example the average daily dry matter (DM) intake was highest for lactating mares, which on average consumed 2.8% of BW, whereas the remaining classes of horses consumed about 2 % of their BW daily.

Clearly the intake will be related to the energy/nutrient expenditure and will increase when horses are growing, pregnant, lactating or exercised. The VDMI of pasture species and forages appears to be generally related to the rate of intake, digestibility and/or nutritional quality (van Wieren, 1996a; Dulphy et al., 1997a; Sneddon and Argenzio, 1998; Fleurance et al., 2001; Menard et al., 2002; Edouard et al., 2010). Studies that have investigated grazing patterns in domestic horses suggest that horses seem to adopt a strategy designed to maximise digestible nutrient intake (e.g. energy, protein) (Fleurance et al., 2001; Naujeck et al., 2005; Fleurance et al., 2009). For example, Edouard et al. (2009) showed that where sward quality was high and constant, horses offered different sward heights between 6 and 17 cm preferred the taller one. In another study, where the taller sward height was mature (crude protein (CP): 7%; DM, neutral detergent fibre (NDF): 62%), horses preferred the shorter grasses of higher quality (CP: 13.5%; DM, NDF: 55.5%), which they grazed for an average of 70% of daily foraging time (Edouard et al., 2010). The digestible dry matter and energy intake rates were higher for the taller sward and the authors suggested that digestible protein was possibly the best predictor of horses' selection patterns. However, the authors also indicated that the animals showed partial preference; as the shorter swards allowed for higher rates of digestible protein intake but lower net energy than the taller grasses. The horses actually may have been balancing their energy and protein intake by sampling from both swards (ie. patch foraging).

2.1.2.3 Browsing and bark-chewing

It is generally acknowledged that horses are predominantly grass feeders, but as highlighted in the previous paragraph horses are able to consume significant amounts of non-pasture vegetation (e.g. forbs, shrubs and trees). Anecdotally, domesticated horses have also been observed to consume a wide range of browse plants and chew bark of different tree species while being outdoors. To my knowledge there is only one study known that has reported the occurrence of bark-chewing in domesticated horses maintained on pastures in Australia (Keenan, 1986). This is most likely because Australian horses are typically managed on small acreage pastures (paddocks) all year round and are kept in variable climates including temperate, arid and tropical

environments. These horses can be exposed to a wide range of vegetation types including forest and shrub lands. Keenan (1986) observed that bark stripping increased when horses were grazing improved pasture compared to native pasture. The dry matter and fibre values were significantly lower in improved pasture suggesting that the lower fibre of the dominant diet may have induced a search for fibre by the horses resulting in increased bark chewing.

The limited information available about the time budget, selection of non-pasture species and the prevalence of bark chewing by pastured horse populations highlights a need for additional research particularly an examination of the motivation to chew bark. This has led to the conduct of a horse industry survey to investigate the horse owners observations of browse-related behaviours by Australian horses on pasture and secondly key aspects of husbandry and management practices (van den Berg et al., 2015). The results of this study are outlined in detail in Appendix I of this report. In brief, horse owners were invited to complete an online questionnaire. Information was collected on location, husbandry and feeding management of their horse(s), pasture condition and the occurrence of surveyed behaviours (e.g. bark-chewing, eating/licking dirt, pacing, crib-biting) and non-pasture species selection by their horse(s) at pasture. The survey had 497 respondents across Australia who owned a total of 3082 horses. The results indicated that the majority of horses (85%) had access to grazing areas 16-24 hours per day and that owners commonly (90%) observed one or more pasture problems on their property, and the mean number of problems was similar across grazing areas of <2, 2-10 and >10 ha. While the horses had access to pasture, there was a heavy reliance on supplementary feeding. Approximately 95% of the horses were fed concentrates and/or supplements on a daily basis, and 86% of the horses were offered conserved forage. Surveyed behaviours were observed by 74% of the respondents. Bark-chewing and licking/eating dirt were the most prevalent behaviours reported. Almost three-quarters of the horse owners indicated that their horses browse on parts of trees, shrubs or other non-pasture species. The study verifies that Australian horses consume a range of non-pasture species and demonstrate bark-chewing behaviour while at pasture. However it is currently unclear if the selections of browse and bark stripping observed in this study were a function of reduced pasture availability or vegetation diversity and diet choices.

2.1.3 Consumption of other feed stuffs by domesticated horses

Domesticated horses are mainly kept for sporting and recreation purposes and are commonly managed in stables and/or pasture areas that vary in size and quality. In both of these systems horses rely partially or completely upon humans for the selection and delivery of their diets. Conserved forages and feed (concentrates) typically originating from cereal grains are provided in meals to cover the energy demands of these horses.

2.1.3.1 Intake of conserved forages

There are a number of equine studies that have assessed the VDMI and preference of different conserved forages such as grass hay, cereal hay/chaff, legume hay/chaff and silage or haylage. Contrary to fresh pasture intake, greater VDMI was generally observed for lucerne hay compared with long-grass hay (Cymbaluk, 1990; Crozier et al., 1997; Dulphy et al., 1997b). The VDMI was on average 2 and 2.4% of BW for grass and lucerne hay, respectively. Higher intakes were also observed for grass or lucerne haylage/ silage, with the VDMI ranging between 1.47-2.2 percent (Moore-Colyer and Longland, 2000; Bergero et al., 2002; Müller and Udén, 2007). Cereal straws are the least preferred; the VDMI of horses fed cereal straw ad libitum was lower compared with grass/legume hay or haylages/silages. Pooled data from a number of studies showed that the intake of cereal straws average 54 and 64% of the intakes of grass and lucerne hays (Dulphy et al., 1997b). The scope of this review does not extend to the effect of particles size on VDMI and chewing activity of different types of forages, this information is described in more detail by Anonymous (2007) and Ellis (2010). The preference for the different conserved forages remains largely unexplained as research into the nutritional composition (e.g. DE, CP, crude fibre (CF), acid detergent fibre (ADF), NDF) and DM digestibility are inconclusive or even at times conflicting (Aiken et al., 1989; Martin-Rosset and Vermorel, 1991; Crozier et al., 1997; Dulphy et al., 1997b). This suggests that voluntary intake cannot always be explained by the nutritional profile and that other factors may play a more imperative role such as organoleptic (sensory) perceptions, which will be described in more detail later on (Ralston, 1984; Doreau et al., 1990; Dulphy et al., 1997a; b; Cuddeford, 2005).

2.1.3.2 Forage variety

It has been stated early that horses in a natural and pastured environment select from a diverse range of plant species. Even in a stabled environment this searching and selection seem to be an important adaptive behaviour. Goodwin et al. (2002) and Thorne et al. (2005) investigated foraging behaviour in stabled horses to determine if variety (i.e. different forage types and forms) and the total amount of forage available will affect the behaviour. Both studies showed that horses spent more time on foraging when offered a multiple choice and it appears that they consume preferred forages with less preferred forages. Goodwin et al. (2007) expanded these studies by offering horses a choice between a stable with a single forage and a stable with multiple forages. Horses inspected both stables but showed again a preference for the multiple forages. The studies emphasise that selecting a variety of forages reflects patch foraging strategies (Prache et al., 1998), which also has been observed in free-roaming (Putman et al., 1987) and pastured horses (Archer, 1973). This could potentially be an adaptive management practice to reduce abnormal behaviours in confined horses, however further investigation is needed to gain more information about the long-term effects of such practices as sensory-satiety may influence preferences over time.

2.1.3.3 Intake of concentrate based diets

There is little published information about the VDMI and preferences of concentrate-type feed stuffs for horses, in particular how concentrate diets may influence feeding behaviour. There is a range of concentrates that differ in nutritional profiles that can be fed to horses, such as cereal grains, legume seeds, oil seeds and fibrous by-products. These are typically offered as a loose mix, or in processed forms (pellets, cubes) that may have undergone heat treatment for improved digestibility of starches or proteins. Compared to forage these concentrate feeds have smaller particles sizes and therefore a higher rate of intake is generally observed. For example Argo et al. (2002) reported that when a loose mix of chaff and concentrates were fed the intake was only slightly higher than most forages, but when the same feed was pelleted the VDMI increased considerably over a 26 day period, before horses were changed over to a chaff form

diet. However, the rate of intake of concentrates may also be linked to the nutritional profile and/or sensory preferences (e.g taste, smell) (Ellis, 2010). This may be the case when horses are offered feeds containing molasses. Yet concentrate diets for horses are not frequently changed, mainly because of maintaining similar nutritional profiles to avoid digestive upset. It has been suggested that when monotonous diets are offered over longer term sensory specific satiety may influence the feeding behaviour and intake. Goodwin et al. (2005a) have demonstrated that sensory cues in concentrates impacts the feeding behaviour. The authors tested four low-energy concentrates and linked these to different flavours (mint, carrot, herbs and garlic). Horses were offered either a single concentrate or all four concentrates (multiple) in two identical stables. There were significant differences between the single and multiple concentrates in feeding and non-foraging behaviour. When multiple and single session behaviour was compared horses foraged more frequently and for longer in multiple sessions but foraging bouts were shorter suggesting a preference for diet mixing which relates to the natural patch foraging behaviour.

2.1.4 Factors that influence food intake and choice

We can conclude that horses, free-roaming and domesticated, select from a diverse range of foods (e.g, plant species, growth stages and plant parts) that will vary in nutritional composition. Even when horses are offered conserved forages and concentrates they will show patch foraging behaviour when possible. In natural systems many animals are faced with a habitat that provides a variety of foods some of which they are able and prepared to eat. However, most animals are selective feeders that are more or less euryphagic —i.e., they ingest a variety of different foods; and strict monophagy is uncommon. Even if animals select from a diverse range of food items, they do not include all the potential foods available or eat in proportion to the amount available. So we may question on what grounds are diets selected? In particular, herbivores are challenged on a daily basis by having to choose from a diverse range of plants that differ in nutrients and that have an array of defence mechanisms such as morphological (e.g. spines, hairs) and biological (e.g. secondary plant compounds; tannins, saponins, terpenes).

It has been suggested by researchers that herbivores choose from a heterogeneous resource to ingest the necessary nutrients to meet dietary needs and reduce the potential of over-ingestion of secondary plant compounds (Freeland and Janzen, 1974; Westoby, 1978; Hanley, 1982; Provenza, 1996). To be able to make sensible diet choices herbivores have to learn to associate the sensory properties of a food (e.g. visual appearance, odour and taste) with the metabolic consequences of eating that food (Forbes, 2007a). Previous dietary experiences through the process of trial and error and social facilitation of mother and/or peers has been shown to further influence preferences and aversions to certain foods (Villalba and Provenza, 2009). This highlights that there are a number of factors that can modulate foraging and feed intake, which can be attributed to physiological mechanism (e.g. central nerve system (CNS), peripheral nervous system (PNS), organs, metabolites, hormones) or behavioural mechanisms (e.g. environment, previous experience, learning, sensory food cues). However, both feedback systems don't work separately from each other and should be regarded as an integrated system that modifies the feeding behaviour, food intake and selection. In other words feeding behaviour is a result of both the internal environment and external environment of the animal.

Studies that investigated the influence of physiological and behavioural factors on food preferences and intake by ungulate herbivores have predominately focussed on ruminants. While only a few studies have investigated feeding choice modulation in equid species, it seems that these mechanisms can influence diet selection by horses in a similar way as demonstrated in ruminants. However, horses use hindgut fermentation to breakdown fibrous plant material, which may propose some different adaptations that are currently not well defined. Therefore the following section will examine the mechanisms that modulate food selection and food intake in ungulate herbivores and relevant theories that may relate to horses will be described.

2.2 The nutritional bases for food selection by large herbivores

Do herbivores eat a plant species faster because they prefer it (sensory stimuli) or do they prefer it because they can eat it faster (optimisation of behaviour)? - Illius et al. (1999)

2.2.1 Foraging hierarchies and scale

Diet selection by herbivores is of major importance within grazing systems because selective foraging is a reciprocal process that on one hand determines the nutritional welfare of the herbivore and on the other hand alters the dynamics of the plant community (Launchbaugh et al., 1999). The understanding of how animals make dietary decisions while foraging has long been of interest to ecologists and graziers for the management of animals and vegetation. Range scientists, population ecologists and landscape ecologists have demonstrated that large herbivores interact with forage resources at various spatial and temporal scales. Laca and Ortega (1995) and Bailey et al. (1996) identified that there are six spatial scales for large herbivores in a foraging hierarchy; bite, feeding stations or plants, patch, feeding site, camp and home range (Table 2.2). Each scale is defined on the basis of characteristic animal behaviours that occur at different rates and each level is associated with different foraging strategies of the herbivore.

Foraging decisions made at any scale can influence the other levels. For example, choices made at higher levels can limit decisions at lower levels. In a situation where the home range or pasture is small the decision as to where to commence grazing at the beginning of a bout has hardly any consequences because the entire area is readily available to the animal. However, if the home range or pasture is large this decision limits the potential number of smaller scale choices. The remote plants and patches are not directly available during a current bout because of the geographical separation; vegetation in distant locations are not visible and animals may have to use up energy to travel to these other feeding sites (Bailey et al., 1996).

Table 2.2 Attributes of spatial and temporal scales to describe large-herbivore foraging. Each level are units that large herbivores may select among (adapted from Bailey et al. 1996).

Spatial scale	Temporal level	Defining behaviours or characteristics	Some potential selection criteria	Potential mechanisms that may affect grazing distribution patterns
Bite	1-2 seconds	Jaw, tongue and neck movement	Nutrient concentrations, toxin concentration, secondary compounds, plant size	Intake rate, diet selection and post-ingestive consequences
Feeding station	5-100 seconds	Front feet placement	Forage abundance, forage quality, plant species, social interaction	Transit rate, intake rate, turning frequency
Patch	1-30 minutes	Animal reorientation to a new location. A break in the foraging sequence	Forage abundance, forage quality, plant species, social interactions, topography	Transit rate, intake rate, turning frequency, optimal foraging theory and other rules of thumb, frequency of selection (spatial memory)
Feeding site	1-4 hours	Feeding bout	Topography, distance to water, forage quality, forage abundance, phenology, predation	Frequency of selection (spatial memory) and rules of thumb
Camp	1-4 weeks	Central areas where animals drink, rest between foraging bouts	Water availability, forage abundance, phenology, cover, thermoregulation, competition	Transhumance, migration, frequency of selection (spatial memory)
Home range	1 month- 2 years	Dispersal or migration	Water availability, forage abundance, phenology, competition, thermoregulation	Migration, dispersal, transhumance

Bite: sequence of herbage yield, jaw and tongue movements, and severance by head movements; **Feeding station:** range of plants accessible to a herbivore without moving their feet; **Patch:** collection of feeding stations, which is separated from others by a break in the foraging sequence when an animal moves to a new location; **Feeding site:** a cluster of patches in adjacent spatial area that animals graze during a feeding bout; **Foraging bouts:** changes in behaviours from foraging to resting, ruminating or other behaviours not directly related to foraging; **Camp:** a central area where animals drink and rest between foraging bouts; **Home ranges:** a cluster of camps and are distinct by topographical barriers, fences, and extent of migration or transhumance.

Likewise at a smaller scale, selection of a feeding station will limit the potential number of plants that an animal may select. If the plants within the feeding station are rejected, animals may have to search and move to another station. The energy costs of moving from one feeding station to another are usually little and the consequences of selecting a feeding station are also small. Therefore, decisions at larger spatial and temporal scales have potentially greater impact on the herbivore since they occur infrequently and constrain decisions at lower levels (Senft et al., 1987).

Equally, the consequences of foraging behaviour at a lower-level may be used to predict choices at higher levels. When animals forage they select a feeding site, a patch in that site, within which numerous bites are selected. The memory necessary to remember each bite or feeding station would be excessive and therefore, scientists suggest that herbivores must integrate information of lower level behaviours (bite, patches and feeding stations) if they are to use those experiences to evaluate spatial alternatives at higher levels (feeding sites, camps and home range) (Bailey et al., 1996). Animals may use intake rate and post-ingestive consequences to assimilate information obtained through diet selection. For example, if the instantaneous intake rate remains above a certain threshold the herbivore may continue selecting bites within a patch, but if the rate drops below this threshold the animal may select a new patch (Charnov, 1976). If acceptable patches become inadequate, a new feeding station and feeding sites may be selected. These grazing processes enable foraging behaviours to be delineated in terms of hierarchy of scales where animals make choices and integrate these over different spatial and temporal levels (Prache et al., 1998).

2.2.2 Theoretical bases of foraging behaviour

Explanations of herbivore foraging decisions have followed two general theoretical pathways:

- (1) optimal foraging (i.e. synthetic or ultimate approach) and
- (2) foraging for nutritional requirements and dietary diversity (i.e. analytical or proximate approach). The first approach involves various theories of optimal foraging in an attempt to construct predictive mathematical models for food choice (Belovsky, 1984). The theory is based on the assumption that natural selection acts on phenotypic variations within a population with the result that greatest genetic fitness is extended to individuals which feed most efficiently (i.e. prefer an optimal diet) (Hanley, 1982). The optimal diet is defined by the maximisation of the net rate of energy intake, which can be ranked on the net value (i.e. ratio of food value to handling time), abundance of food types and total time foraging (Pyke et al., 1977). It has three key aspects:
 - (1) acceptance of a food item is independent of the abundance of that food type and depends only on the abundance of higher quality food types;
 - (2) as abundance of a

preferred food type increases, less preferred options will drop out of the optimal diet; and (3) any food type within the optimal diet is either eaten whenever encountered - unless differential nutrient constraints also are considered; e.g. Pulliam (1975) (Pyke et al., 1977).

The application of the optimal foraging theory to large herbivores is challenging, partly because the optimal foraging theory has been largely developed around data on food selection by carnivores, granivores and frugivores. Predators generally prey upon spatially scattered food items of nearly constant and high nutritional value (i.e. packages), whereas herbivores encounter a food supply that is low and highly variable in nutritive quality (Westoby, 1978). In addition different species of plants differ greatly in toxins and digestion inhibitors, so called secondary compounds. Therefore an alternative hypothesis to the optimal foraging theory is the principle of “satisficing”, which proposes that a behavioural option may be taken, not only when it is optimal, but when it is adequate to overcome some threshold requirements for the animal (Stephens and Krebs, 1986; Ward, 1992). This theory links to the second theoretical approach which implies that herbivores consume plants to balance nutritional needs and avoid over-ingestion of toxic plant secondary compounds (Freeland and Janzen, 1974). This analytical approach uses cause-effect contiguous associations to determine foraging behaviour, such as sensory stimuli, post-ingestive feedback from previous choices and dietary experiences. The synthetic-ultimate and analytical-proximate approaches should not be regarded as exclusive but rather complementary. For example, even though the optimal foraging theory emphasises the importance of natural selection in determinants of animal behaviour, it cannot rule out the significance of short-term dietary experiences (Prache et al., 1998).

The optimisation approach provides a functional synthesis (i.e. the integration of evolutionary and phylogenetic analysis with those of molecular biology, biochemistry and structural biology) of foraging behaviour and allows for quantitative predictions, but is most likely a simplified portrayal of reality (Prache et al., 1998). There are various behavioural options that have not been extensively studied or experimental testing which has led to inconclusive explanations, such as the necessity of a herbivore

to sample its environment to keep track of constant changing values of food items and adjust their net value ranking as a function of inconsistent phenologies and habitats (Hanley, 1982; Illius et al., 1999; WallisDeVries et al., 1999). Herbivores would need to distinguish the values of individual plants while consuming proximate multi-species meals. Therefore researchers suggest that it is not feasible to predict the “optimal diet” for herbivores, rather it would appear more probable to predict a general type diet, that a herbivore, given a choice, will select (Westoby, 1978; Hanley, 1982). In addition, the optimisation model is difficult to extrapolate from small to large spatial and temporal scales. The majority of the earlier models have been primarily developed to define short-term foraging behaviour. When the spatial and temporal scales become larger foraging behaviours become more intricate to define and comprehend because it needs to integrate trade-offs with other processes and behaviours (Prache et al., 1998).

2.2.3 Constraints on diet selection and foraging behaviour in large herbivores.

While synthetic-ultimate and analytical-proximate approaches can be used to explain foraging decisions in large herbivores, it is important to acknowledge the intrinsic and extrinsic constraints that limit an animal’s ability to recognise, select or utilise a particular food from amongst those apparently available to it. Extrinsic constraints originate largely from the nature of the food source, and interact with intrinsic constraints, which are commonly related to body size, but also include morpho-physiological (e.g. digestive system, mouth size and teeth) or behavioural constraints such as perception and discrimination (Illius and Gordon, 1993). By considering these constraints we will enhance our understanding of food resource partitioning in herbivore communities.

2.2.3.1 Food source and selection by herbivores

One of the greatest differences between herbivores is in the selection of different herbaceous forages, particular between grasses (monocots) and browse (herbaceous and woody dicots such as forbs, shrub leaves and stems) (Hofmann, 1973). These

differences are associated with variations in cell structure, plant chemistry, plant architecture and plant dispersion (Table 2.3).

Table 2.3 A relative composition of structural and chemical differences between grasses (monocots) and browses (herbaceous and woody dicots).

Characteristic	Grasses	Browse
Cell wall	<ul style="list-style-type: none"> • Thick • Greater portion is cellulose/hemicellulose 	<ul style="list-style-type: none"> • Thin • Greater portion of lignin
Plant defence compounds	<ul style="list-style-type: none"> • Silica • Phenolics 	<ul style="list-style-type: none"> • Phenolics - tannins • Terpenes • Alkaloids & other toxins
Plant architecture	<ul style="list-style-type: none"> • Fine-scaled heterogeneity in nutritional quality within a plant • New growth added at the base • Low growth form • 3-dimensional volume 	<ul style="list-style-type: none"> • Coarse-scale heterogeneity in nutritional quality within a plant • New growth added at the tips • Low to high growth form • Complex, diffuse branching architecture
Dispersion	<ul style="list-style-type: none"> • Uniform 	<ul style="list-style-type: none"> • Dispersed/discrete

Plant cell material can be divided into cell contents, which are highly digestible, and cell wall, which are the less digestible part of the plant. The cell wall acts as a fibrous “skeleton” for plants and is composed primarily of cellulose, hemicellulose and lignin. Cellulose is digested by microbes in the digestive system of herbivores, hemicellulose may or may not be digestible, depending on the type and lignin is generally considered indigestible (Hanley, 1982; Van Soest, 1994). Grasses generally have a thicker cell wall than browse and their cell wall consist mainly of slowly digestible plant carbohydrates such as cellulose. Conversely, browse (e.g. forbs, leaves and some woody stem) have a thinner cell wall and more cell content that contain highly digestible and rapidly fermentable sugars, proteins and lipids (Gordon and Illius, 1994). However, the cell wall of browse typically has higher values of indigestible fibre such as lignin. The structural carbohydrates composition of grasses and browse are depict in Figure 2.1.

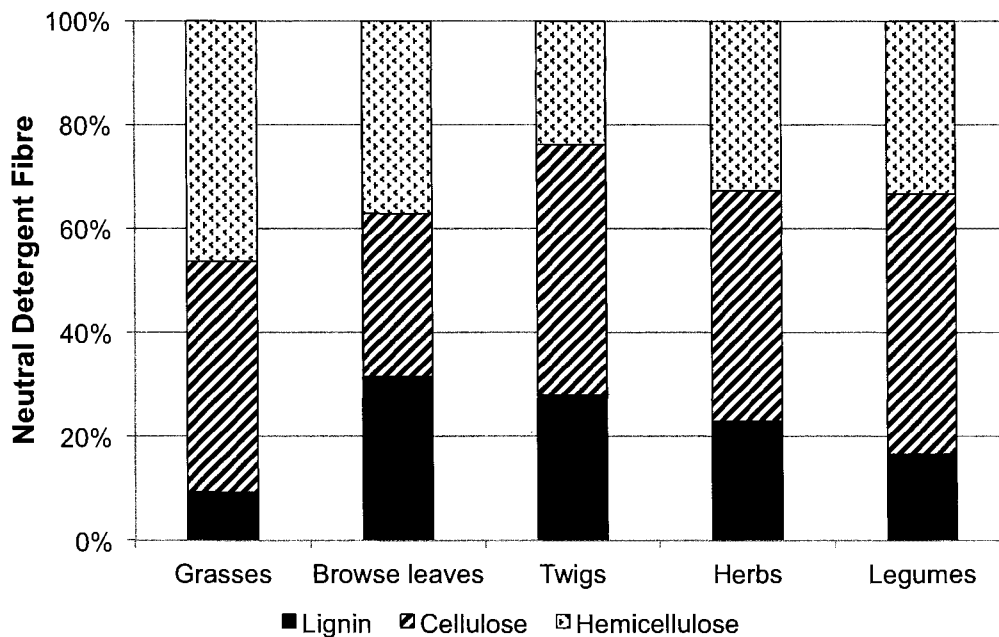


Figure 2.1 The structural carbohydrate compositions (neutral detergent fibre; hemicellulose, cellulose and lignin) of forages (adapted from Hummel et al. 2006).

The amount of cell carbohydrates and lignin varies between seasons and with the age of the plant. Levels of cellulose and hemicellulose increase with increasing plant maturity with a corresponding decrease in plant digestibility. In particular cellulose may become lignified to varying degrees. Lignin can become intimately associated with cell wall carbohydrates, which reduces the nutritive value of the forage. Generally rapidly growing plant tissue such as grasses and young foliage of shrubs and forbs are relatively high in cellular content and low in cell wall, whereas mature grasses and woody tissue generally have thick cell walls, composed of mainly cellulose in grasses and relatively high lignin in shrubs (Hanley, 1982; Duncan and Poppi, 2008).

Grasses and browse also differ in the plant secondary chemicals that influence forage quality. Browsers typically have a wider range of plant defence compounds such as phenolics (e.g. tannins) that reduce protein digestibility, terpenes that can reduce dry matter digestibility, and toxins such as alkaloids (Robbins et al., 1995). In contrast, grasses tend to have a higher silica concentration that can increase tooth wear and reduces the ability for herbivores to digest fibre (Janis, 2008; Mihlbachler et al., 2011).

However, grass species can contain similar secondary compounds as browse such as alkaloids and phenolics.

There are differences in the architectural arrangement of grasses and browse, which create unique challenges for herbivores when harvesting food (Shipley, 1999). Grasses consist of many leaves, stem, sheath and seed heads that have very small differences in form and quality, which make them a relatively homogenous food source. They also have a low growth form, with new tillers added at the base of the plant, which creates a rather constant 3-dimensional layer of vegetation with a relatively unvarying density (Jarman, 1974). Conversely, browse tends to have a more heterogeneous architecture with an array of plant parts that differ in quality and form, such as leaves, twigs, buds, flowers and woody materials. Browsers have a low to high growth form and new plant material is added at the tips, which creates a branching geometry that is scattered and irregular (Jarman, 1974). In addition, many browse species have spines, prickles or thorns that slow down the bites from herbivores. Browse plants are generally discrete in distribution.

Ecologists have observed that herbivores tend to focus on either grasses or browse components of habitat, which has become the basis of classifications for mammalian herbivores (Shipley, 1999). The most cited classification is from Hofmann and Stewart (1972) which is based on diet choices and related morphology and physiology of ruminants (e.g reticulo-rumen size, retention time rumen). They divided herbivores into: (1) bulk or roughage feeders or grazers that select a diet containing <25% browse; (2) concentrate selectors or browsers that select a diet containing at least 75% fruits, dicot foliage, and tree and shrub stems and foliage; or (3) intermediate or mixed feeders that select both grasses and browses, depending on the season and availability. Other researchers later expanded this to include non-ruminant herbivores (Shipley, 1999).

Hofmann's original classification and later work on ruminants suggest that morpho-physiological adaptations such as reticulo-rumen volume, retention time and passage rate, mean particle size escaping rumen, fibre digestion and fermentation rate in the rumen, absorption surface of rumen, rumen musculature and salivary glands could

provide predictions about the digestion between grazers and browsers (Hofmann and Stewart, 1972; Hofmann, 1973; 1989). However, only a few of these parameters have been tested quantitatively. Recent research suggests that characteristics of food, animal's body size and phylogeny are important in contributing to the variations in diet strategies (Gordon and Illius, 1994; Gordon and Illius, 1996; van Wieren, 1996b; Pérez-Barbería et al., 2001). There is an on-going debate in the research community as to whether ruminant grazers and browsers show marked morphological and physiological differences which might influence their digestive efficiency (e.g. fibre digestion) (Duncan and Poppi, 2008).

2.2.3.2 Morpho-physiological and digestive characteristics

2.2.3.2.1 Body size

The body size of mammalian herbivores has implications for the minimum quality of food the animal requires for survival and consequently determines the feeding niche and absolute size of important spatial and temporal scales (Illius and Gordon, 1993). The larger the animal the higher the requirements because there are more costs for maintenance and production but it appears that this does not follow a linear pattern. Bell (1970) and Jarman (1974) were the first to use allometric scaling of metabolic rate in ruminants and showed that larger animals require more food on a daily basis compared to smaller animals, but when comparing the relative requirements (per unit body weight) they are actually lower. Smaller animals require high quality (low fibre) food to satisfy their relative high metabolic rates, whereas larger animals can survive on larger quantities of poor quality forages with slower fermentation rate (Demment and Soest, 1985). However, a larger animal has less time per nutrient unit to spend selectively foraging than does a smaller herbivore, which suggest that energy costs for foraging is a direct function of time spent foraging (Osuji, 1974).

2.2.3.2.2 Digestive system

As previously discussed, plant cell material consists of cell contents and cell wall. The cellular content of plants is highly digestible, whereas structural cell wall can pose digestive difficulties for herbivores. Ungulates have two basic types of digestive

systems that can deal with structural plant carbohydrates, which are ruminant (fore-gut fermenters) and caecal (hind-gut fermenters) (Figure 2.2). The ruminant system is found in most species of the Artiodactyla (even-toed) order and the caecal system has evolved in all species of the Perissodactyla (odd-toed) order (Janis, 1976).

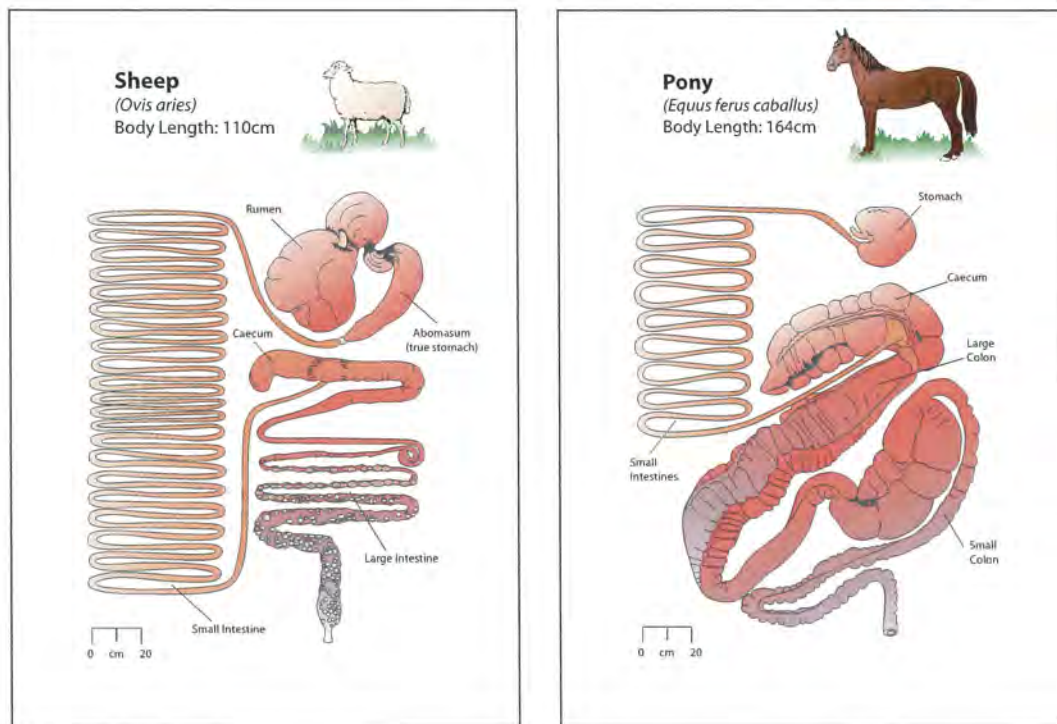


Figure 2.2 The structure of the gastrointestinal tract of a foregut fermenter (*Ovis aries*) and a hindgut fermenter (*Equus ferus caballus*) (adapted from Stevens and Hume 1995).

There appears to be little difference in the biochemistry of fermentation in ungulates whatever the affinities of the animal or the site of fermentation chamber (Janis, 1976; Van Soest, 1994). The taxonomic composition of micro-organisms in the digestive system of all animals is also apparently broadly similar. Comparative studies of ruminants (cattle and sheep) and the equids have been undertaken to understand how they differ in their ability to digest forages (Cymbaluk, 1990; Menard et al., 2002; Pearson et al., 2006). It was observed that digestibility of the feed components were generally greater in ruminants than the equids. This is probably related with the ability of ruminants to retain forages for a longer period of time in the rumen to extract the most feed components and energy out of the feed.

True digestibility of cell content of forages in ruminants is close to 100% (Van Soest, 1994) whereas the horse may have lower values as they are hind-gut fermenters. However estimated values by Pearson et al. (2006) for equids and ruminants (over 0.97) show that extraction of cell components by hindgut fermenters can be as effective as ruminants.

Overall it seems there is a consensus suggesting that the ruminant digestive system has two principal advantages over the caecal system. Ruminants are able to regurgitate the “cud” and can chew repeatedly to grind the food to smaller particles before it enters the rumen. This process is important because reduced particle size increases the surface for microbial action in the rumen (Van Soest, 1994). The other advantage is that the food is digested first by micro-organisms before it enters into the true stomach (abomasum). Plant material is broken down by bacteria and protozoa in the rumen and converted to microbial tissue and waste products (e.g. volatile fatty acids, ammonia, CO₂). Some of the microbial protein is broken down in the rumen and its nitrogen is recycled (i.e. taken up by microorganisms). These organisms pass through the abomasum and small intestine and their cell proteins are digested and absorbed by the ruminant (McDonalds et al., 2002). The disadvantage of the ruminant digestive system is that foods must be broken down to small particles before they can pass through the remaining parts of the digestive system. This means that very fibrous materials will limit the rate of food passage through the digestive tract of the ruminant and can restrict the consumption of additional forages (Van Soest, 1994).

Horses, in contrast, are not limited by the need to reduce the particle size of food for passage through the digestive system because they first digest enzymatically before fermentation takes place in the hindgut (Janis, 1976). Janis (1976) suggested that the efficient foregut digestion system allows cattle to extract more digestible dry matter per unit feed than horses from medium/low-quality grasses (defined by fibre content). The hindgut system allows horses to extract more digestible dry matter per unit time than cattle from grasses which are medium or low in quality (high fibre content). Bovids retain medium/low-quality forages for a longer period of time in the rumen to extract the most energy out of the feed. This decreases passage rate and digestibility of dry

matter per unit time. The passage rate of horses stays unaffected by fibre content of forages. Thus, horses may be less efficient at digesting the fibrous cell walls of plants but can pass much more plant material through its system daily. The ruminant must be a more selective forager than the caecal digester must be; but being a more efficient digester, it requires a lower absolute quantity of forage. It is therefore generalised that when forage *quantity* is limiting, a ruminant digestive system is beneficial and when forage *quality* is limiting a caecal digestive system is advantageous (Janis, 1976). However, Pearson et al. (2006) observed that donkeys digest straw diets nearly as effectively as ruminants, and regardless of the level of feeding, digested the fibre in low-quality forages more effectively than ponies. This suggests that some equids not only may be more efficient in extracting digestible dry matter per unit time from medium/low-quality forages but also may be closer to ruminants in their ability to extract a higher content of digestible dry matter from medium/low-quality forages per unit feed than thought.

2.2.3.2.3 Mouth size

The morphology of mouthparts is associated with body size and differences in feed selection between herbivores. Small animals tend to have smaller mouths that allow them to harvest small bites of browse or grass plants more efficiently (Shiple, 1999). In addition, this selectivity allows them to crop leaves and annual growth without harvesting the older material, which increases the relative value of forage (Hanley, 1982). In contrast, larger animals with larger mouths tend to be less selective and take larger bites, possibly because of the overall time-energy constraints on forage selectivity. However, the selectivity may also depend on the teeth morphology. Equines are large herbivores but unlike cattle they have two sets of incisors, which allows them to feed faster than cattle on short grasses, graze grasses too short for cattle and select different parts of plants (Menard et al., 2002).

2.2.4 Nutritional status and diet selection

Dietary preferences are influenced by palatability and both depend on the animal's current nutritional status. The animal is able to use its behaviour as a flexible means to deal with the internal milieu (physiological constraints) and external milieu and animals will learn from the consequences of their behaviours by positive or negative reinforcement (Provenza and Villalba, 2006). For example hungry or thirsty animals will search for nutritious food or water as a response to their body's needs (positive reinforcement), but they may also do so to obtain relief from adverse stimuli – lack of food and water (negative reinforcement). Punishment will decrease the likelihood of a behaviour reoccurring. Animals that experience toxicosis after eating a plant will stop eating (positive punishment) and negative punishment results from the removal of a positive enforcer. For example an animal eating a plant that was once nutritious but is no longer will decrease its intake because nutrients have been removed. This learning provides the feedback necessary to adapt to the internal and external changes. There are two feedback mechanism that have been shown experimentally in ruminants to be important for foraging; (1) learning from post-ingestive feedback and (2) learning from conspecifics, through social transmission of behaviours such as between a mother and her young (Provenza and Cincotta, 1993).

2.2.4.1 Post-ingestive feedback mechanisms

Diet choices in animals are dependent on the interrelationship between a food's flavour and its post-ingestive feedback (Figure 2.3; Provenza and Villalba, 2006). Flavour is a collective for odour, taste and texture and is associated with the post-ingestive feedback from cells and organs in response to concentration of nutrients and toxins in the food. The senses of smell, taste and sight (pre-ingestion or prospective) makes it possible for animals to discriminate between foods (cognitive process) and together with post-ingestive (or retrospective) mechanisms allows animals to make associations with pleasant or unpleasant experiences depending on whether the effect on the internal milieu is aversive or positive (affective process).

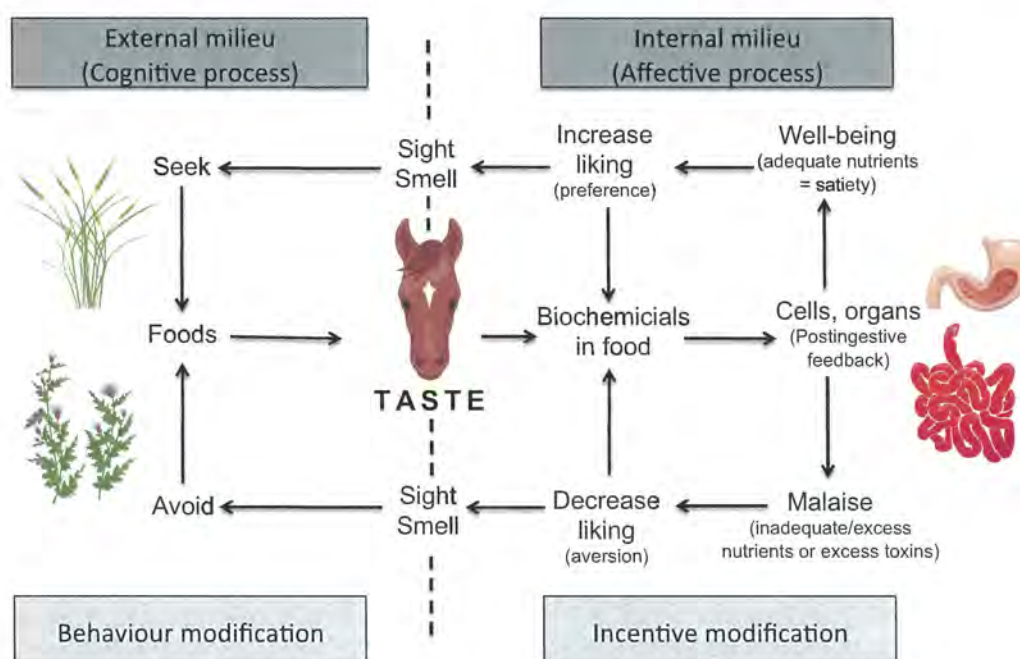


Figure 2.3 Food intake, food preferences and palatability all result from the interrelationship between food's flavour and its post-ingestive consequences. The integration of the internal (cells and organs) and external (foods) environments occurs through two interrelated systems: affective (implicit or associative) and cognitive (explicit or declarative). Taste plays an essential role in both systems, and receptors for taste are situated at the junction between the internal and external milieus. The affective and cognitive systems allow animals to be adaptable and modify their behaviours to ongoing changes in the internal and external milieus (adapted from Provenza and Villalba, 2006).

The affective processes are non-cognitive (involuntary) and the environmental variables that control these affective processes are comparable for ruminants and monogastric animals (Provenza et al., 1994a). However, there are no studies that have investigated the central nervous system control of feed intake in horses and ponies. We assume that like other mammals, the regulation of equine feed intake engages both homeostatic and hedonic mechanisms controlled by hunger and satiety cues that are regulated in both the peripheral and central nervous systems (Julliand et al., 2008).

Nutrients and toxins can influence organs and glands in the body and the central nervous system (CNS) along the vagal and splanchnic afferent nerves and then proceed back to the body along efferent nerves (e.g. gastrointestinal tract) to complete the

feedback loop (Garcia, 1989; Provenza, 1995). The interaction of the central and enteric systems facilitates self-organisation. When something changes in the body, the body assesses or appraises these changes and the animal will act accordingly (e.g. change food intake and preferences). As a result of this action a new signal will now interact with the receptors, which will lead to a new action. This feedback loop is particularly important in living organisms to achieve homeostasis. However, homeostasis is dynamic and set points, equilibrium states and biochemical processes change through time as the organisms develop and preserve their integrity (Rose, 1998). These dynamics can be experienced in short-term conditions (e.g. within a meal) or long-term conditions such as changes in dietary requirements due to pregnancy or lactation. For example Lamoot et al. (2005b) demonstrated that lactating donkeys and Shetland ponies selected regrowth of high quality more strongly than non lactating females. Lactating animals have higher energy and protein requirements, therefore it seems that these animals selected forages that allowed for greater digestible protein intake.

2.2.4.1.1 Learned food aversions and preferences

Herbivores exploit the heterogeneity of resources to increase the intake of nutrients and reduce the possibility of over-ingesting plant secondary compounds (Freeland and Janzen, 1974; Westoby, 1978; Hanley, 1982; Provenza, 1996). The post-ingestive feedback from toxins causes animals to quickly experience internal malaise and limit intake before toxicosis develops. Learned food aversions have been demonstrated in a number of animal species including ruminants (Burritt and Provenza, 1991; Provenza, 1995; 1996), rats (Garcia et al., 1972) and horses (Houpt et al., 1990). A study in sheep has shown that aversions to a food containing a toxin, lithium chloride, can be brought on within an hour (Provenza et al., 1993a). This has also been observed in goats eating twigs containing tannins (Provenza et al., 1994b). In horses strong aversions to novel foods has been reported following ingestion of the food, even when the novel food was given concurrently with a familiar food (Houpt et al., 1990). The aversion resulting from post-ingestive feedback reduced the intake or preference for foods, but the aversion to a food is reported to increase with the severity of the illness and decreases the longer the delay between the food ingested and illness (Garcia et al., 1972). The longer the illness is delayed the greater the potential positive feedback from the food

before any negative effects. Consequently satiety and malaise interact to influence outcomes of diet choices and intake rate. Herbivores are able to eat nutritious foods containing toxins because the improved nutrient status may increase the ability to deal with toxins and animals are able to limit intake to minimise toxicity. As herbivores take many bites of food, there are relatively few instances of toxicosis unless animals are exposed to unfamiliar environments, have limited resources and/or are forced to eat plant species that allow accumulation of toxins (Provenza, 1995).

When diets are deficient in nutrients or have excess nutrients the preference and intake may also be affected. Deficits or imbalances of energy, nitrogen and amino acids have been shown to cause animals to decrease intake of the food they were initially eating and readily sample alternatives. For example, sheep and goats fed energy and protein deficient diets learned to prefer lower quality foods such as straw when their intake is accompanied by a ruminal infusion of energy (Villalba and Provenza, 1996; 1997a; b) or protein (Villalba and Provenza, 1997c). At the same time animals will also respond to an excess of nutrients by decreasing their intake of foods. In one study, lambs avoided phosphorus (P) when P was fed in excess and increased their preference for P during periods of P deficiency (Villalba et al., 2006). In another study, sheep on a P deficient diet increased their intake of a P supplement when given a choice between a P or Calcium (Ca) supplement. Conversely, sheep eating a Ca-deficient diet ate more of a Ca supplement than sheep fed a diet high in Ca (Villalba et al., 2008). The balance or ratio between nutrients (e.g. energy and protein) in foods appears to also be important and can influence diet selection by herbivores. For example, lambs acquired a strong preference for foods when the energy and nitrogen fermented at similar rates and in similar amounts in the rumen. On the other hand, when there was an imbalance between the ratio of energy and protein animals tended to form aversions to the food (Kyriazakis and Oldham, 1997).

The ability of horses to differentiate diets based on their nutrient content has had limited study. Cairns et al. (2002) showed that horses are able to select a higher energy concentrate over a lower energy one, regardless of the preference for associated flavour (mint or garlic). However, it has been suggested that when foods are presented

simultaneously the development of learned associations between sensory cues and post-ingestive consequences may be challenging, particular in animals such as the horse with long gut transit time (Rose and Kyriazakis, 1991; Cairns et al., 2002). Therefore, a recent study investigated the effect of monadic phase (2 separate periods for 3 days each) to a single forage rich in either protein, lipids or hydrolysable carbohydrates on the choice of these forages when offered together (Redgate et al., 2014). The results showed horses had a greater preference for the forages rich in protein or hydrolysable carbohydrates compared to the lipid rich diet. The authors suggested that these horses responded to the macronutrients in the diets and that the dietary experience (single presentation) facilitated feedback mechanisms, and hence affected dietary preferences. Whilst, it has been established that horses can make associations with macronutrients, it is presently unclear if this is also apparent when horses are offered different minerals or trace-minerals. It seems that ponies do not develop preference for Ca supplements when fed a Ca-deficient diet (Schryver et al., 1978). However, they do have a liking for salt if Na deficient (Meyer et al., 1983).

Still, diet choices are not only influenced by the concentration of nutrients and toxins but can also be a result of a hedonic shift based on other food characteristics (e.g. flavour and texture). Studies in ruminants and horses have demonstrated that herbivores will choose a variety of forages or foods even when a nutritionally complete diet is presented and varied only in flavour (Scott and Provenza, 1998; Atwood et al., 2001; Goodwin et al., 2005a). Although, animals may have strong preferences for particular foods, due to the interactions of nutritional status, post-ingestive feedback mechanisms and sensory satiety animals may alter eating habits (Rolls, 1986).

2.2.4.2 Social experience

Dietary experiences, particular early in life, modulate foraging behaviour and diet selection (Provenza, 1995). The acceptance or avoidance of foods is initially acquired through social facilitation (e.g. mother or peers). In ruminants it has been demonstrated that when lambs were offered a choice, they avoid or prefer foods as a function of their mother's avoidance or preferences (Mirza and Provenza, 1990; 1994). In particular

when a mother's food selection behaviour to eat or avoid is subsequently reinforced by post-ingestive feedback from nutrients or toxins (positive or negative), the offspring responded strongly to eat or avoid a food (Provenza et al., 1993b). There are a limited amount of studies looking at the influence of mother and peers (social facilitation) on diet selection in horses. Houpt (1990) reported that there is a cultural transmission of food preferences from the mother to the foal and that social facilitation plays also a role in total VDMI. In foals the avoidance of plants high in toxins and the selection of nutritious grasses and herbaceous plants occurs simultaneous between the ages of 4 and 6 weeks when it is feeding with the mother (Marinier and Alexander, 1995). However the close association of the foal with the dam did not particular coincide with grazing behavioural changes. The study did report that coprophagy of the foal on maternal faeces does correspond chronologically with the foal learning to graze selectively. This highlights that more studies are required that gain a better understanding of this cultural transmission of preferences to foals.

It has been demonstrated that the maternal influences on diet choice start during the gestation phase. Flavours and other chemical cues are able to cross the placental barrier from the mother to the foetus and these prenatal chemosensory may be stored by the animal for some time after birth and influence their foraging behaviour in later life (Nolte and Mason, 1995). In addition, in rats it has been recorded that flavours in the diet of the mother can influence the milk. The nursing animal that is exposed to these flavours in the milk often show a preference for feeds with similar flavours post-weaning (Galef and Clark, 1972; Galef and Henderson, 1972). There is some evidence that this may also happen in ruminants but the extent to which this enhances preference for the feed depends on the flavour (Nolte and Provenza, 1992). Learning by observation from mother and peers plays an important role in diet selection in young animals, but it seems that individual experience of trial and error is required to continue learning efficiency and recognition for foods. In addition individual experience can override any influence that the mother or other adults have on diet selection. For example lambs that were exposed to elm leaves with their mothers and later received elm leaves paired with a dose of aversive LiCl avoided the leaves whether or not their mother ingested them (Provenza et al., 1993b).

2.2.4.3 Dietary experience

When animals mature they rely less on their mother for food and more on the consequences of the foods ingested. By trial and error animals will learn to associate the flavours of the foods with its post-ingestive consequences, which play an important role in the adaptation and the acquisition of appropriate dietary habits. Although this process is self-organising, there appears to be some limitations with this type of learning; for example why do herbivores sometimes over-ingest nutrients and/or toxins and how do herbivores know which plant made them ill if they have been grazing on 6 different species in a short time frame? It seems that there are number of factors that can affect the cognitive and/or affective system, in the case of over-ingestion, the emetic system (i.e. feeling of malaise) may not be activated. For example over-ingestion of lucerne can cause bloat in ruminants, which apparently works through the tension receptors in the rumen and reticulum and may stimulate short-term discomfort. However, bloat doesn't stimulate the emetic system. It can also be that some toxins (e.g. gallamine and naloxone) may not stimulate the emetic system but cause aversion to physical locations or other external stimuli (Lett, 1985). Post-ingestive feedback is usually delayed after ingestion and animals are more likely to be poisoned when the post-ingestive feedback from a toxin is not experienced for more than 12 hours after ingestion. For example it has been demonstrated in ruminants and horses grazing in North American range conditions, that the food ingested may have an immediate positive post-ingestive feedback but the aversive post-ingestive feedback may be delayed and animals may continue consuming the foods (e.g. locoweeds and larkspur) (Ralphs and Olsen, 1990; Ralphs et al., 1994; Pfister et al., 2002). However, it has been demonstrated that ruminants are able to make associations even over longer time intervals (Burritt and Provenza, 1991).

To reduce the risk of toxicosis animals use the familiar-novel dichotomy, which allows animals to discriminate and generalise foods based on their past experiences. Generalising aversions from past experiences reduces the risks associated with toxic novel foods or familiar foods whose flavours have changed. For example lambs that eat cinnamon flavoured rice and subsequently experienced toxicosis will reduce their

preference and intake of the rice. If these animals are subsequently offered wheat, which they like, but a cinnamon-flavour was added, the animals will refuse to eat the cinnamon-flavoured wheat. Thus, lambs generalise their aversion from rice to wheat based on the common flavour cinnamon (Launchbaugh and Provenza, 1993). Ruminants can also generalise preferences. For example, sheep that prefer grains with a high starch content (e.g. wheat, milo and barley) subsequently preferred novel feeds such as grape pomace infused with starch (Villalba and Provenza, 2000) and ate readily more novel forages such as rice bran when the feeds were sprayed with an extract from a familiar grass (Van Tien et al., 1999).

Ruminants discriminate among foods on the basis of the concentration of flavour and they typically avoid the flavour concentrations most different from what they have eaten in the past. For example if an animal is exposed to a familiar and novel feed and is subsequently made ill, the animal will associate illness with the novel feed. If all feeds are novel then it will associate illness with the most novel, which in turn appears to be based on the relative amount of novel feed ingested and the prominence of the flavour of these feeds (Provenza et al., 1994b). In the case of familiar foods, it has been shown in sheep that temporal contiguity (close association in time) between feed ingestion and toxicosis affects the acquisition of feed aversion (Provenza et al., 1993a). The concentrations of nutrients and toxins in plants vary on a daily basis and from season to season which means that herbivores typically shift between familiar and novel foods categories. Thus, herbivores require to sample plants on a continuous basis (Freeland and Janzen, 1974).

2.2.4.4 External environment

There is no doubt that social facilitation and past dietary experiences influence diet preferences and food intake in herbivores. Another factor that may also be important is the external environment (e.g. landscape and vegetation). When animals are moved to an unfamiliar environment they are likely to experience difficulties in differentiating nutritious from toxic foods because all foods may be novel (Provenza and Balph, 1987). For example lambs in the study by Burritt and Provenza (1997) ate less of a familiar-

aversive food in a familiar environment compared to an unfamiliar environment. Conversely, when the lambs were placed in an unfamiliar environment they ate less of the novel-harmless food than when in familiar surroundings. These results suggest that food neophobia is greater in unfamiliar environments than familiar environments, even if familiar foods contain toxins. Thus, animals should be exposed to novel foods in familiar environments to expedite intake of novel foods and provide familiar foods in unfamiliar environments to prevent reduction in intake. Even if there are familiar foods available in an unfamiliar environment, changes in the animals' environment may alter the physiology mechanisms (e.g. binding of toxins, metabolising and detoxifying). This effect has primarily been studied in rats and humans in the context of drug effects. For example, rats with or without previous experience of heroin were given a strong dose either in a familiar and unfamiliar environment. The results showed that the dose was lethal for 32% of the experienced rats in a familiar setting, 64% of the experienced rats in unfamiliar environment and 96% of the unexperienced rats in unfamiliar environment (Siegel et al., 1982). There may be large inter-individual variability in physiological mechanisms and how animals cope with environmental changes through previous experiences and adaptability. The significance of this variability in animals is not well known, but its consequence demonstrates the plasticity of foraging behaviours and the different trade-offs an animal can make between different behavioural options (Prache et al., 1998).

2.3 Mechanisms that modulate feed intake by horses

The interactions occurring between feeding behaviour and post-ingestive physiology provide some of the most important challenges in the study of nutrition - Simpson and Raubenheimer (1999)

Up until now we have mainly focused on the behavioural mechanism of food intake and selection. But as described earlier we cannot interpret this without understanding the physiological mechanisms. Most information on the control of feeding behaviour in vertebrates has come from studies of mammals, such as rats, humans, cattle, sheep, goats, and pigs (Rozin, 1976; Provenza, 1995; Galef, 1996; Forbes, 2007b). These

studies indicate that there are several organs (e.g. central nervous system (CNS), stomach, liver); metabolites (e.g. glucose, lipids) and hormones (e.g. insulin, leptin, ghrelin, cholecystokinin (CCK)) that can play a role in the control of feeding (Forbes, 2007b). In addition, it has become clear that the senses (i.e. taste and smell in particular) play a fundamental part in food intake and preference control (Garcia et al., 1972; Provenza, 1996). In horses most of these mechanisms also appear to play a similar role but hindgut fermentation may have disadvantages compared to ruminants when it comes to mechanisms controlling appetite and the ability to learn about the consequences of foods. In the following section the physical and metabolic mechanisms of feed intake by horses and roles of sensory stimuli on the recognition of foods by horses will be discussed.

2.3.1 Physiological basis of feed intake

It remains unclear whether control of intake is similar for different breed types (ponies and horses), sexes, and feedstuffs. Ralston (1992) reported that given the opportunity, non-working mares would exceed their energy needs, and as a result, become obese. Conversely, other studies have been able to show that there is some sensitivity to energy intake. Laut et al. (1985) offered concentrate diets of different caloric densities and showed that ponies can respond to decreasing density by increasing their intake. However, the ponies' response to the change in dietary density took between 2 and 14 days to stabilise which suggests that the feedback mechanism is not as precise as seen in other species such as rats and monkeys where compensation occurs in less than 24 h (Gibbs and Smith, 1978). However, it has been recorded that ponies were able to compensate intake of a pelleted diet within 24 h when they were given additional intra-gastric inputs of energy in the form of glucose, corn oil or α -cellulose (Ralston and Baile, 1983a; b). The authors reported that decreases in intake occurred at times which seems to reflect the post-absorptive state; 10 to 15 minutes for glucose and 4 to 6 h for cellulose. On the other hand, an intra-gastric load of corn oil immediately after a 4 h fast and before a meal appeared to have no influence on the ingestion of the meal but did moderate the subsequent intake by tripling the normal inter-meal interval.

Ralston et al. (1983) also studied VFA and its role in the large intestine for the control of feed intake. VFA solutions were given as a bolus 15 min before the ponies were allowed free access to a concentrate feed after a 4 h fast. An intra-gastric infusion of acetate (0.75 mmol/kg BW) significantly altered the feeding behaviour of the ponies by reducing the duration of the first inter-meal interval and increased feeding activity. Likewise an intra-caecal infusion of propionate at 0.4 mmol/kg BW significantly increased total feed intake by 7.5% relative to the control. Conversely, elevated levels of propionate (0.75 mmol/kg BW) and acetate (1.0-1.25 mmol/kg BW) significantly reduced feed intake by prolonging the first inter-meal interval, although the 24-h intakes did not differ from controls. In addition, when an infusion of 1 mmol propionate/kg BW was given it significantly reduced the first meal by 22% without affecting subsequent feeding behaviours. These results suggest that caecal VFA concentrations can generate cues that may influence meal size and frequency in ponies. However, all this work was conducted using complete pelleted diets fed *ad libitum* and the relevance of this research for the intake of a forage based diet remains to be elucidated.

The role of gut fill in feed intake remains unclear. Stomach fill by intragastric infusion of kaolin had no effect on VDMI of ponies compared to control animals (Ralston and Baile, 1982). In addition, the removal of caecal contents (1.2-1.5 L) had no influence on subsequent feeding behaviour of ponies (Ralston et al., 1983). However intra-gastric infusion of α - cellulose solution did significantly reduce VDMI after 3 to 18 h post-infusion, and a load of glucose solution decreased intakes 0 to 3 h post-treatment compared to the control treatment (water). Because of the duration it seems that this is a post-absorptive effect rather than a localised gastric “fill” response (Ralston and Baile, 1982). It has also been shown that NDF apparent digestibility and NDF content of feeds do not affect the VDMI in horses (Cymbaluk, 1990). This suggests that the physical capacity of the hindgut is unlikely to limit feed intake. The flow of digesta through the horses gastrointestinal tract (GIT) is protracted but is not limited by the particle size of foods in the same way as is seen in ruminants since there is no analogous structure to the reticulo-omasal orifice. This means that digesta is not retained in the horse’s GIT in the same way as in ruminants and therefore, there is less probability of “fill” negatively affecting intake.

Hormones such as the gut peptides CCK, pancreatic polypeptide, peptide YY, glucagon-like-peptide and in particular leptin and ghrelin have been linked to the regulation of hunger and satiety in many species (Morton et al., 2006). It has been shown that gut peptides have a dual role as both hormones and neurotransmitter, acting on the neurons in the hypothalamic and brainstem centres that control appetite. Leptin is a mediator of long-term regulation of energy balance, suppressing food intake. In horses it has been demonstrated that leptin serum concentrations increased with body condition score (Buff et al., 2002; Steelman et al., 2006) and feed restriction both short and long term resulted in a decline in serum leptin concentrations (McManus and Fitzgerald, 2000; Gentry et al., 2002). Leptin serum concentrations will fluctuate daily as a consequence of meal feeding and appears to be related to the subsequent insulin response. Steelman et al. (2006) demonstrated that in response to large carbohydrate meals serum leptin levels in horses increased in episodes over a 24hr period, whereas Cartmill et al. (2005) showed that horses that were not fed a grain meal did not display these diurnal variations in leptin. Ghrelin on the other hand is a fast-acting hormone, seemingly playing a role in meal initiation. In horses ghrelin serum levels were negatively correlated with body condition score and increased concentrations were linked with higher intakes (Gordon et al., 2007a). However, in horses the regulation of ghrelin was not associated with plasma insulin as seen in other mammalian species (Gordon and McKeever, 2005). Studies that investigated the effect of physical activity on leptin and ghrelin plasma concentrations have been inconclusive. It appears that there is some post-exercise response compared to non-exercise controls, but this actually may have been related to their decreased feed intake (Gordon et al., 2007b).

In summary, there is some evidence that shows that meal frequency and food intake in horses and ponies is regulated by the presence and/or absorption of products of digestion together with metabolic cues that may reflect body energy stores. However researchers conclude that even though that this may occur it seems that horses reply primarily on the oropharyngeal sensory and external stimuli to control the size of the and the duration of a meal which may be due to their digestive adaptation (Ralston, 1984; Cuddeford, 2005).

2.3.2 Identifying feeds and their consequences

As described earlier diet choices and food intake in animals are dependent on the interrelationship between a foods flavour (texture, odour, smell and taste) and its post-ingestive feedback (Provenza and Villalba, 2006). Animals use their senses to locate and discriminate between foods. Sight and odour (exteroceptive stimuli) enable animals to orient themselves within their feeding environment and taste (interoceptive stimulus) enables associations between the sensory characteristics of foods with its post-ingestive consequences. The chemical senses – smell and taste – appear to be important cues for herbivores to evaluate potential forages and foods because they can provide rapid and detailed information about the biochemical characteristics of feedstuffs. In addition, animals can use smell and taste to relay on information about foods (pre-ingestive) without actually ingesting it (Pain and Revell, 2007). This is particularly of importance when generalising aversions from past experiences, which reduces the risks associated with toxic novel foods, or familiar foods whose flavours have changed.

2.3.2.1 Equine Senses

The senses, autonomic nervous system (or visceral nerves), the brain stem, limbic system (thalamus, hypothalamus, amygdala, hippocampus) and higher cortical centres interact through neuronal fibres that can facilitate or inhibit behaviours (see Figure 2.4). Cranial (olfactory, optic and auditory, vagus nerve) and visceral afferent nerves that first synapse in the brain stem (involved with visceral, cardiac and respiratory function) proceeds to the limbic system (concerned with emotional memory). Feedback from the gut to the brain stem and limbic system causes changes in the preference for particular foods, which are non-cognitive and depend on the food's effect on the internal milieu. On that basis, higher cortical centres (involved with declarative memory) interact with the limbic system to facilitate changes in food selection behaviour (Provenza, 1995).

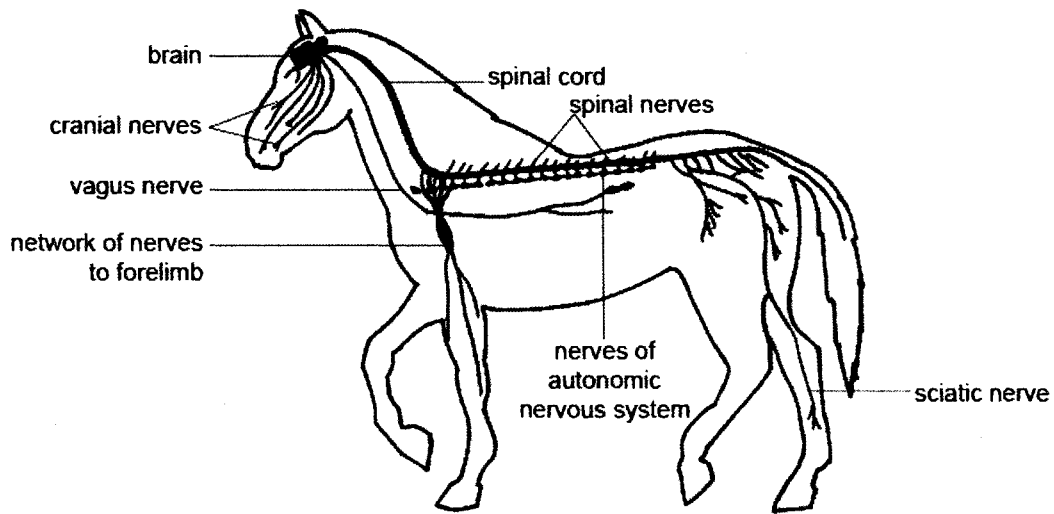


Figure 2.4 The nervous system of a horse (Wikibooks diagram by J. Ruth Lawson, Otago Polytechnic, Dunedin, New Zealand, 2008).

It appears that horses are able to detect macronutrients (carbohydrates, protein) in concentrates or (supplemented) forage diets and can adapt by increasing intake or change diet choices (Laut et al., 1985; Cairns et al., 2002; Redgate et al., 2014). However, other researchers reported that food selection and intake is largely based on the organoleptic qualities of forages/foods (taste, odour, ease of prehension, toughness, ease of sorting etc.), given that nutrient content of single foods/forages tend to be weak indicators of VDMI (Doreau et al., 1990; Dulphy et al., 1997a; b; Cuddeford, 2005). It seems that correlations between nutrient content and food intake and choice will largely depend on the constituents and the site and delay of absorption in the digestive system. Horses rely on hindgut fermentation to gain energy from plant fibrous materials and therefore potentially do not have the same “feedback” systems available to ruminants. As described in the previous section horses are less sensitive to cell wall content in forages than ruminants and this is most likely because horses do not have a reticulo-omasal orifice (Cymbaluk, 1990; Dulphy et al., 1997a). The reticulo-omasal orifice is highly sensitive to, and selectively retains, large forage particles for regurgitation and masticate foods until particles are small enough to move further down the digestive tract. Consequently it seems likely that the organoleptic qualities of forages may have a greater impact on diet selection and intake when there are limited post-ingestive signals from foregut and/or hindgut. However, there are no known studies that have examined

this specifically in horses and/or compared this with ruminants. In addition, there is limited information about the sensory responses of horses to the physical and chemical constituents found in forages and other feedstuffs and how this may affect diet choice and intake.

2.3.2.1.1 Sight, texture and odour

As a herbivorous flight animal, horses have a good distance vision, allowing it to scan broadly for danger and, despite being comparatively poor at eye accommodation, with a vertical field of 178°, is somewhat able to visualise the ground closely ahead while grazing (McGreevy, 2004). Thus, grazing herbivores primarily use sight to identify plants that are familiar and for orientation within their home range. It appears that sight only plays a minor role in determining plant parts eaten.

Plant texture such as thorns, spikes and hairs and the physical characteristics (i.e. dry matter content, particle size) of forages may potentially reduce the amount that is eaten or determine whether herbivores ingest it. Studies in sheep have shown that physical characteristics of grass plants such as dry matter content and particle size and resistance to fracture are known to effect ease of prehension and thus intake rate (Inoue et al., 1994). However, to my knowledge no studies have specifically investigated how texture or morphology of fresh grasses/legumes and browse effect food selection by horses. Naujeck et al. (2005) did report that horses in their study preferred grasses with tall sward heights over short mowed grass, which may have been due to the texture. An effect of texture has been reported when different forms of conserved forages were compared. In an earlier experiment it was demonstrated that loose, wafered and pelleted lucerne hay were consumed in different quantities (Haenlein et al., 1966), showing a greater intake for the pelleted and wafered hay forms. Even though the chemical composition was the same, the density and particle size differed. This in accordance with more recent studies showing that when lucerne-based forages are fed *ad libitum* the range for VDMIs (g DM per kg W^{0.75}) was lowest for long hay (75-122) (Haenlein et al., 1966; Cymbaluk, 1990; Crozier et al., 1997; Dulphy et al., 1997a; b), followed by dried wafers, cubes or pellets (88-139) (Haenlein et al., 1966; Cymbaluk, 1990; Todd et al., 1995), and short-chopped, high temperature dried materials, had the highest range (100-

155) (Pearson et al., 2001). This indicates that physical density of forages can encourage VDMI by horses. In addition, Moore-Colyer and Longland (2000) showed that the total VDMI decreased (39 g/kg BW^{0.75}) when horses were offered a wet clamp silage compared to dry hay-silage or hay (79 and 63 g DM/kg BW^{0.75}, respectively). Even over a continued period of time no adaption of intake rates took place. This clearly shows that texture of forages can considerably influence feed intake and diet selection by horses.

That orosensory cues play an important role in diet choice and intake by horses may also be evident in the morphology of the nose and size of the nasal epithelium. Horses have long noses and olfactory senses are likely to be well developed and used in combination with other signals to respond to their environment (Pain et al., 2005). The horse's olfactory receptors are located in the mucosa of the upper nasal cavity. Due to the length of the nasal cavity, there is a considerable area of these receptors, and the horse has a better ability to smell than a human. Moreover, the horse also has a vomeronasal organ (VNO), or Jacobson's Organ, which is in the hard palate, and is able to pick up pheromones and other scents when a horse exhibits the flehmen response. The animal will lift its head after finding an odorant, wrinkle its nose while lifting its lips, and cease to breathe momentarily. This flehmen response forces air through slits in the nasal cavity and into the VNO. However horses, unlike humans, do not have an incisive duct communication between the nasal and the oral cavity because they do not breathe through their mouths, instead, the VNOs connect to the nasal passages by the nasopalatine duct.

Odour profiling has been used to make predictions about the preferences for familiar forages such as oaten and lucerne hay by horses and dairy cattle (Pain and Revell, 2009). This study indicated that there were eight oaten hay and 15 lucerne hay volatiles related to the cow and/or horse preferences. They also showed that the volatiles that were positively related to preference were also positively related to the nutritive or physical traits, and negatively related to the traits that were negatively related to the preference. However, the authors did find an odour that influenced the preference negatively but was not related to the any measurable nutritive or physical trait. The authors suggested

that this volatile could have been associated with plant secondary compounds that can affect taste and gut fermentation. Horses differ from ruminants in their ability to detoxify plant secondary compounds due to the differences in digestive anatomy. Non-ruminants (e.g. pigs, poultry and horses) are usually more susceptible to plant toxins than ruminants, which have the capacity to denature toxins in the rumen. Therefore, pre-ingestive cues such as odours may play important role in reducing potentially toxicosis in horses.

2.3.2.1.2 Taste

Research has shown that horses respond to at least four of the five taste sensations that are found in human and primates; sweet, salty, sour and bitter solutions (Randall et al., 1978). Although there was a large individual variability among horses, they demonstrated that horses had a strong preference for sucrose concentrations of 1.25-10 g/ml (also noted by Danel and Merckies, 2009; Jankunis and Whishaw, 2013), but no preference for sour (hydrochloric acid), bitter (quinine) or salt (sodium chloride) tastes. Horses rejected salt solutions at concentrations greater than 0.63 g/100 ml, acetic acid at concentrations greater than 0.16 mg/100ml and quinine at concentrations of 20 mg/100 ml. Immature horses seemed to respond in a manner similar to sheep.

Diet preferences due to flavours have been studied to a limited extend in horses. However, its not well defined by researchers what type of flavouring was used. For example flavours can be divided in non-nutritive and nutritive, which are only aromatic and/or contain a taste that may have calories or not (sugar versus artificial or natural sweeteners). Flavours have been added to encourage the acceptance of unpalatable anthelmintic drugs or concentrate/supplement with different effects. Burton et al. (1983) used apple, lucerne, caramel and anise-molasses to mask levamisole or piperazine in an oats, corn and soybean basal diet and showed that only apple, lucerne and caramel were partially effective. On the other hand, Goodwin et al. (2005b) examined a large variety of flavours and showed that banana, carrot, cherry, cumin, fenugreek, oregano, peppermint and rosemary were well accepted by eight horses. Individual variation among the horses was highlighted, with one horse rejecting coriander completely and three horses rejecting or partially rejecting nutmeg and Echinacea. The accepted

flavours were tested in a two-choice preference design and the results showed the following ranking of preference; 1) Fenugreek, 2) Banana, 3) Cherry, 4) Rosemary, 5) Cumin, 6) Carrot, 7) Peppermint and 8) Oregano. The researchers then added the two most preferred flavours (fenugreek and banana) into an unpalatable mineral pellets, which led to a significant increase in the rate of intake, compared to the unflavoured option. The impact of flavours on feed intake will most likely be determined by previous experiences and therefore, the type of flavour/odour has to be considered when using it to encourage the acceptance of unpalatable foods. In addition, further research is warranted to clarify the effect on different flavour types (i.e. nutritive versus non-nutritive) to determine the role of odour and taste cues in diet selection by horses.

2.4 Conclusion

This review has allowed us to gain a better understanding of herbivore foraging behaviour patterns and mechanisms involved in diet selection. From observational studies it is well defined that equines select from a diverse range of foods and typically display patch foraging behaviour. The mechanisms that modulate this selection and intake are not straightforward and cannot be simply explained by nutritional content or hedonic effect alone. Researchers have now shown that feed intake, preference and palatability all result from an interrelationship between the food flavour (smell, taste, texture) and the nutritional consequences (nutrients and toxins) of ingesting the food. When we look at diet selection in this way we can clearly distinguish that we are dealing with integrated feedback mechanisms from the internal milieu (physiology) and the external milieu (behavioural).

Research into diet choice and intake modulation has primarily focused on ruminant herbivores. While, not extensively studied, it appears that most of these mechanisms are also relevant to large ungulate hindgut fermenters such as horses. However, due to their digestive design it seems that horses have a long gut transit time and their physical regulation of intake may be weaker compared to ruminants. Thus, some researchers have suggested that organoleptic qualities (odour, taste, texture) of foods may play a more imperative role in dietary decisions and control of feed intake by horses. A few

equine studies have been able to show that horses can discriminate between (identical) familiar foods (e.g. concentrates and conserved forages) based on their nutrient concentrations and flavours. Yet, there is a lack of knowledge regarding the behavioural mechanisms of olfaction, gustation and somatic sensory perception and its effect on diet selection by horses. In particular how this relates to natural or pastured environments where horses could be continuously exposed to a variety of plant patches, including familiar and novel species that may differ in flavour, nutrients and plant toxins from day to day and season to season.

2.5 Scope of thesis

The objective of this thesis was to further develop our understanding of behavioural mechanism of diet selection by horses, in particular how they identify and select amongst a variety of foods (familiar and/or novel) based on sensory perception and post-ingestive feedback. This was undertaken through a series of experiments examining:

1. The selection of familiar and novel forages in a checkerboard design to simulate patch foraging conditions. As changes in dietary preferences are largely influenced by the nutritional requirements of the animal and palatability of a food, it was of interest to investigate the effect of the nutritional status (energy intake) on novel forage selection by horses (Chapter 3);
2. The influence of orosensory (smell) stimuli and post-ingestive feedback in the recognition of novel foods by horses (Chapter 4). This was achieved by assessing the acceptance of a novel food with a familiar odour or with contrasting crude protein levels and;
3. The influence of different food characterises i.e. nutrients, odour and taste, on the voluntary intake and preferences of horses exhibited when a number of foods were offered simultaneously (i.e. Smörgåsbord) (Chapter 5).

Chapter 3

Does energy intake influence diet selection of novel forages by horses?



M. van den Berg^a, C. Lee^b, W.Y. Brown^a and G.N. Hinch^a

^a School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia

^b CSIRO Agriculture, Armidale NSW 2350, Australia

Paper formatted to *Livestock Science* 186: 6-15

(Special issue: *The impact of nutrition on metabolism in equines*)



This chapter has been removed as it was published as the following journal article:

van den Berg, M., Lee, C., Brown, W. Y. & Hinch, G. N. (2016). Does energy intake influence diet selection of novel forages by horses? *Livestock Science*, 186, 6-15

<http://dx.doi.org/10.1016/j.livsci.2015.07.022>

Chapter 4

Acceptance of novel food by horses: the influence of food cues and nutrient composition



M. van den Berg^a, V. Giagos^b, C. Lee^c, W.Y. Brown^a, and G.N. Hinch^a

^a School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia

^b School of Science and Technology, University of New England, Armidale NSW 2351, Australia

^c CSIRO Agriculture, Armidale NSW 2350, Australia

Paper formatted to *Applied Animal Behaviour Science* (Under review)



This chapter has been removed as it was published as the following journal article:

van den Berg, M., Giagos, V., Lee, C., Brown, W. Y. & Hinch, G. N. (2016). Acceptance of novel food by horses: The influence of food cues and nutrient composition. *Applied Animal Behaviour Science*, 183, 59-67

<http://dx.doi.org/10.1016/j.applanim.2016.07.005>

Chapter 5

Influence of odour, taste and nutrients on feeding behaviour and food preferences in horses



M. van den Berg^a, V. Giagos^b, C. Lee^c, W.Y. Brown^a, A.J. Cawdell-Smith^d and G.N. Hinch^a

^a School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia

^b School of Science and Technology, University of New England, Armidale NSW 2351, Australia

^c CSIRO Agriculture, Armidale NSW 2350, Australia

^d School of Agriculture and Food Science, University of Queensland, Gatton QLD 4343, Australia

Paper formatted to *Applied Animal Behaviour Science* (Under review)



This chapter has been removed as it was published as the following journal article:

van den Berg, M., Giagos, V., Lee, C., Brown, W. Y., Cawdell-Smith, A. J. & Hinch, G. N. (2016). The influence of odour, taste and nutrients on feeding behaviour and food preferences in horses. *Applied Animal Behaviour Science*, 184, 41-50

<http://dx.doi.org/10.1016/j.applanim.2016.08.015>

Chapter 6

General Discussion



6.1 Introduction

The literature review of this thesis highlighted that there is a lack of knowledge of the behavioural mechanisms of olfaction, gustation and somatic sensory perception and their effect on diet selection by horses. In particular there is a lack of knowledge about the natural or pastured environments where horses are continuously exposed to a wide variety of plant patches, that include both familiar and novel species that may differ in flavour, nutrients and plant toxins within species and throughout the year.

6.2 How sensory characteristics and post-ingestive feedback influence food preferences

Horses can choose from a diverse range of vegetation and have to evaluate the quality and quantity of forages they need consume to meet their dietary needs. Animals are able to assess foodstuffs by two pathways: pre-ingestive feedback; the food sensory characteristics perceived by the animal before ingesting and post-ingestive feedback; the metabolic consequences (positive or negative) experienced by the animal after consumption of the food. These two pathways are interrelated and both are used by the animal to evaluate a food's palatability (Garcia, 1989; Provenza, 1995). In grazing animals most of the evidence of these interrelationships have been demonstrated in ruminants and it seems that hindgut fermenters such as the horse have not been studied extensively in this context.

Cairns et al. (2002) showed that horses selected a higher energy concentrate (11.3 MJ/kg) over a lower energy (9.3 MJ/kg) one, regardless of the preferred flavour (mint or garlic), and that they formed associations between foods and their nutritional composition. The authors created the contrast of energy density by altering diet formulations, causing differences in protein (10.7% vs 12.4%), fat (3.3% vs. 7.4%) and NDF (36.1% vs 22.3%) as well. Consequently it is possible that the orosensory characteristics (taste and texture) and/or feedback from other macronutrient (e.g. protein) of the higher energy diet may have contributed to the palatability differences.

The experimental evidence presented in Chapter 3 revealed that energy intake was in fact not the main driver for diet mixing of novel forages with equal or even greater digestible energy content as familiar forages, even if horses had an energy intake of about 80% RDI. It was suggested that macronutrients such as CP or other plant factors (orosensory characteristics) may have a greater influence on diet choice. This seems to confirm the findings of Gordon (1989) who reported that diet selection of grazing ponies was influenced by the quality of food (plant based variable) rather than the rate of energy intake (animal process). In the present study (Chapter 3) horses on LE and HE diet had adequate CP and lysine intake based on the NRC recommendations, therefore it remains to be determined if a deficiency in protein could have been a factor influencing diet choice and acceptance of nutritious alternatives. Yet, Chapter 5 confirms that when horses are presented with pelleted diets equal in digestible energy but that differ in CP levels, they have a higher intake of the high CP diet. Redgate et al (2014) also found that when horses were offered forage-based diets rich in 1 of 3 macronutrients (protein, lipids and hydrolysable carbohydrates) in a 3-way choice that horses, after a period of experience, had a greater preference for the protein rich diet, then hydrolysable carbohydrates and lastly lipids. Such findings suggest that horses have a preference for deriving energy from protein and carbohydrate sources (over lipids) and may indicate that they use nutrient intake targets to regulate feed intake. While it is well known that in comparison to ruminants horses have higher protein requirements, as they are less efficient at utilising nitrogen (Cheeke and Dierenfeld, 2010), the implications for this on diet selection are far from clear. The results of Chapter 4 confirm the above reports and also demonstrated that when horses are presented with a choice of a novel food (soybean hulls) with contrasting CP levels (14 % vs. 22%) and linked with odour (citrus and aniseed), horses showed a greater preference for the novel food with the higher protein regardless of odour. In this study the added protein also altered digestible energy content, and therefore it is not completely clear if the preference was attributed to CP or to increased digestible energy content.

The experiments reported in Chapter 4 also showed that horses were able to make associations between the nutritional content of novel foods and its odour cues and it appeared that this process took around 5 days. Cairns et al. (2002) also observed that

recognition of high-energy concentrate diets seemed to be established within 4-5 days when horses were offered two meals per day. In both studies the diet and flavour links were switched after a certain period, and it was observed that the new associations between the nutrient content of foods and flavour cues (odour and/or taste) appeared much quicker after the switch over. While horses in the experiment of Cairns et al. (2002) were familiarised to the flavours and diets, this may suggest that when multiple foods are offered simultaneously herbivores may at first experience difficulties with these associations (Duncan and Young, 2002; Redgate et al., 2014). Hence the initial delay (4-5 days) observed could be attributed to increased sampling time needed to learn about these flavour-to-post-ingestive consequences. In addition, when dealing with a novel food as presented in Chapter 4, horses may spend more time sampling as a result of neophobia and making a “nutritional assessment”. However, in Chapter 4 it took only 1 day/meal for horses to differentiate between diets after a flavour change, this may indicate that associations become quicker once neophobia of the “new” had been overcome. In ruminants a similar period of 3-5 days has been observed for post-ingestive associations when new foods are presented (Van Tien et al., 1999; Hinch et al., 2004) but it has been demonstrated that delays between food ingestion and consequences can be up to 8 hr for toxins (Burritt and Provenza, 1991) and up to 1 hr for calories (Villalba et al., 1999). This suggests that feedback from nutrients or toxins can be rapid in ruminants, but when animals are offered a novel food this can delay acceptance due to neophobia and initial limited intake (i.e. sampling). Ruminants are very discriminating when selecting diets, via physical appetite-controlling mechanisms (Faverdin et al., 1995); showing classical relations between intake and the chemical composition. In contrast horses are not endowed with sensitive physical appetite-regulating mechanisms (hindgut fermentation) and therefore may have different gut-brain feed back mechanisms. While ponies can develop learned aversions to novel feeds closely associated with malaise in under 1 hr, they continued the consumption of palatable feeds, regardless of the consequences (Houpt et al., 1990). Some sensitivity to energy intake was observed by Laut et al. (1985) who offered four pony geldings ad lib a mixed grain diet either undiluted (14.2 MJ/kg of DE) or diluted with 25% sawdust (10.9 MJ/kg) or with 50% sawdust (7.1 MJ/kg) and showed that pony's responded to decreasing density, by increasing food intake, but there was a large variation in the

response time (2- 14 days). Ralston et al. (1983) studied the effect of intra-gastric and intra-caecal infusions of VFA in the stomach and large intestine on concentrate intake by horses and found that acetate and propionate influenced both meal size and feeding frequency, but the affect on diet choices was not determined. She later concluded that whilst horses are capable of utilising both enzymatic digestion in the small intestine and bacterial fermentation in the hindgut (ceacum and colon) as sources for metabolizable energy, it appears that horses rely predominantly upon oropharyngeal and external stimuli to control the size and duration of meals (Ralston, 1984). Other researchers have also reported that diet selection and intake by horses are largely influenced by the organoleptic qualities of foods such as odour, taste, ease of prehension and texture, and that nutrient content appeared to be weak indicators (Dulphy et al., 1997a; Cuddeford, 2005). At present it is unknown if different food types (concentrate vs. roughage) may induce different timing of feedback from the gut to the brain in horses. There are no known studies that have investigated the different pathways of feedback (foregut vs. hindgut) in horses when different foods types are fed (and compared this to ruminants). This highlights the opportunity for research that investigates post-ingestive feedback mechanisms, including pathology and morphology of the neurological system of signalling (i.e. vagus nerve) from the gut to the brain in horses.

The experiments in this thesis recognise the complexity of the post-ingestive regulatory system, and demonstrate the important role of pre-ingestive feedback on diet preference and intake. The influence of odour was demonstrated in Chapter 3 and Chapter 4 and this will be discussed later in relation to neophobia. Whilst there is some data on the role of odour on diet preference by horses, there is minimal research that has investigated the effects of taste. Taste responses in horses have been identified using solutions, that illustrate that horses can distinguish between salty, sour, bitter and sweet, and have a preferences for sweet (Randall et al., 1978; Jankunis and Whishaw, 2013; Merkies and Bogart, 2013). Goodwin and colleagues (2005a; b) assessed the effect of flavour on diet selection and intake by horses, but the type of flavours (non-caloric or caloric) were not always clearly defined. It seems that the diet choices observed in their studies were largely linked to odour, and that the role of taste was not really tested.

The findings presented in Chapter 5 are possibly the first to demonstrate the effect of a non-caloric sweetener on diet preference and intake by horses. Whilst nutrient (CP) was the main driver for diet choice and volume of intake seen in both preference tests, there were no differences in the total intake between the LP+ and HP diet which suggests that when a sweetener (taste) was added horses evaluated these two diets to be of “equal palatability”. Clearly taste influenced the food reward system, which was reflected in the significantly greater intake/ preference for the HP+ diet. However, these findings raise interesting questions about the potential feedback that horses may receive from the non-caloric sweetener. For example is the response seen in this study attributable to the hedonic experience of the sweetener; or does a non-caloric sweetener trigger pathways in the gut that may be related to changes in post-ingestive feedback?

In rats, pigs and ruminants it has been demonstrated that artificial sweeteners can act on the intestinal sweet taste receptors T1R2/T1R3 and its partner G-protein, gustducin, to activate pathways leading to Na⁺/glucose co-transporter 1 (SGLT1) up-regulation (Shirazi-Beechey, 1995; Moran et al., 2010; Moran et al., 2014). In horses this has been confirmed with hydrolysable carbohydrates (Daly et al., 2012), but presently it is unknown if sweeteners (artificial or natural) can induce the same response. In ruminants it has been suggested that dietary supplementation with an artificial sweetener (Sucram; consisting of saccharin and neohesperidin dihydrochalcone) can induce glucagon-like peptide-2 (GLP2), which is evoked by the enteroendocrine L-cells in response to the T1R2/T1R3 sensing, and could be used to enhance intestinal growth, maturity and glucose absorption leading to greater productivity. Further research would be valuable to confirm if similar responses occur in horses. A greater glucose absorption in the small intestine could be beneficial for horses, particular for performance horses to enhance energy uptake and could potentially assist in reducing digestive upset due to overloading of starch in the hindgut.

In human nutrition the use of non-caloric sweeteners such as aspartame and sucralose have increased to aid in reducing calorie intake and in response to increasing obesity. However, human research is now showing that these artificial sweeteners may actually contribute to weight gain due to overcompensation of energy intake. A sweet taste,

whether from a sweetener or glucose, enhances the human appetite (Yang, 2010) and it seems that a preload of glucose or sucrose decreases the energy intake when a test meal was offered, whether the artificial sweetener preload had no effect or increased subsequent energy intake in humans (Rogers et al., 1988; Black et al., 1993; Lavin et al., 1997; King et al., 1999). Similar responses were also observed in rodents, and rats that were conditioned with saccharin supplement had a significantly greater total energy intake and gained more weight compared to the controls conditioned with glucose (Swithers and Davidson, 2008). This suggests that there may be an arbitrary coupling between sweet taste and caloric content, which may lead to compensatory overeating and positive energy balance. In other words a lack of caloric contribution seems to eliminate the post-ingestive regulatory system. This has been demonstrated in rats, where they learned to prefer the food associated with regular feeding over the “sham feeding” in which the ingested food flowed out of the body through a gastric fistula (Sclafani and Ackroff, 2004). It has also been demonstrated in ruminants that when animals were fed energy- and protein deficient diets they were conditioned to prefer a lower quality food such as straw when their intake was accompanied by ruminal infusion of energy (Villalba and Provenza, 1996; 1997a; b; c). The findings of these studies seem to suggest that sweetness decoupled with calories offer partial, but not complete, activation of the food reward pathway (pre-ingestive). This suggests that sweetness is more related to the hedonic sensation which is supported by the findings in Chapter 5 showing that horses had a greater intake of diets linked to the non-caloric odours banana and coconut, which had a sweet aromatic sensation. Goodwin et al. (2005b) also found that banana was the most preferred of 15 flavours tested in their study.

This thesis has clearly demonstrated the influence of a sweet taste and odour on diet selection and intake. While it has been shown that horses can distinguish between 4 of the 5 taste sensations (sweet, bitter, salty and sour) (Randall et al., 1978), there may be an indication that horses could also have a taste for “umami”. Umami can be translated as “pleasant savory taste” and its perception occurs through the detection of the carboxylate anion of glutamic acid, which is a naturally occurring amino acid common in meats, cheese, broth, stock and other protein-heavy foods. The taste receptors T1R1

and T1R3 combined form a heterodimer for the umami taste (Chandrashekar et al., 2006). While umami taste responses have been mainly linked to carnivorous or omnivorous diets, it seems that herbivores such as horses and cattle express the T1R1 gene (Zhao et al., 2010). In addition, Favreau et al. (2010) has demonstrated in sheep that an umami taste (monosodium glutamate) resulted in a greater intake/preference for hay compared to a bitter taste (quinine). This suggests that horses may also have the ability to detect foods with a “protein taste” which could explain why horses seem to have a greater preference for CP (Chapter 4 and 5). However, there appears to be no research that has investigated “umami” taste responses in horses, or the expression of T1R1 and T1R3 and its potentially role in feed preference. Recent studies in rats suggest that not only T1R1 and T1R3 receptors, but also metabotropic glutamate receptors 1 and 4 (mGluR1 and mGluR4) may be involved with the umami sensation (Yasumatsu et al., 2015). Which receptors are involved with taste experiences are still to be defined and further studies are needed in various animal groups.

6.3 The influence of novelty, generalisation and post-ingestive feedback in the recognition of foods by horses

While it has been demonstrated that diet selection by horses can be influenced by nutritional factors, it is unclear how diet choices may be modulated when horses are faced with a familiar-novel dichotomy, commonly encountered at pasture or when new food stuffs are offered. The experimental evidence presented in Chapter 3 indicates that horses have a greater preference for familiar forages (oaten and lucerne), regardless of the energy intake, and that they show a neophobic response towards nutritious novel forages (willow, bamboo, saltbush, tagasaste), only ingesting small amounts. The novel forages had a comparable or greater energy or protein content, but horses initially rejected these forages based on smell, suggesting that diet selection cannot always be explained by nutritive traits which may be overridden by the sensory characteristics of foods. This observation is supported by the findings of Pain and Revell (2009) who used odour profiling to assess the preference for oaten and lucerne hay by horses.

Animals almost invariably consume only small quantities of food when it is presented for the first time. This cautious sampling of new food types is referred to as neophobia (“fear of new”) and has been described as an innate herbivore survival mechanism for avoiding the over-consumption of toxic plants in the wild (Provenza and Balph, 1988). In addition, because horses are monogastric they are usually more susceptible to plant toxins than ruminants which have the capacity to denature toxins in the rumen (Norton, 1998). Such differences may help to explain why horses rejected most of the novel forages based on smell without actually ingesting the food to avoid possible malaise. On the other hand, familiar orosensory characteristics could possibly be used to reverse the effects of neophobia. A familiar odour or taste can be used to generalise preferences and this has been previously demonstrated in ruminants (Launchbaugh et al., 1997; Van Tien et al., 1999) and for the first time in horses in Chapter 4. In horses the “learning time” appears to be 4-5 days before there is even a partial preference shown. This suggests that post-ingestive consequences need to be established before a new food is completely accepted.

In this thesis the influence of taste on the acceptance of new foodstuffs was not explicitly assessed, but if horses have a taste for umami it could be argued that the preference for a novel food higher in CP observed in Chapter 4 could have been contributed to the taste differences between the foods. Clearly flavours can have a positive effect on the dietary experience and therefore food flavours are commonly used by the commercial horse feed industry with the objective of overcoming feed neophobia. There are limited studies that have investigated the acceptance of (novel) flavours, although Goodwin et al. (2005a) has shown that in multiple choice trials (short term) horses can respond to sensory variety in concentrate diets that differ in flavours (odour and caloric taste) and/or formulations (nutrients), selecting from preferred and less preferred foods. It is unclear how horses respond to (non-nutritive) flavour changes over a longer term (when a single diet is presented). In Chapter 5 it was demonstrated that with each introduction of a new odour, an initially large variation in intake was recorded with some animals showing a neophobic response while others exhibited no apparent recognition of the odour being new. The variation was similar for all odours and significantly declined over time taking a minimum of 3-4 days adaptation to reduce

variability in response to a new odour. This was also shown in Chapter 4 and suggests that odour cues can have a strong influence on feed intake by horses. In addition, it also shows a large variability amongst horses and indicates that the impact of flavours/odours on feed intake is likely to be influenced by previous experience/exposure to the food or odour. Therefore the type of flavouring (odours/taste) should be considered when using it to encourage the acceptance of novel or unpalatable foods.

6.4 Multiple-choice test to simulate patch foraging behaviour

It is well recognised that horses select from a heterogeneous resource, mixing preferred with less preferred forages (Archer, 1971; 1973; Goodwin et al., 2002; Thorne et al., 2005; Goodwin et al., 2007) or feedstuffs (Goodwin et al., 2005a) and showing patch foraging behaviours. The pattern of foraging is similar in many herbivore ungulate species and is associated with changes in palatability and/or satiety for particular flavours, nutrients or secondary compounds (Freeland and Janzen, 1974; Westoby, 1974; Provenza, 1996). Patch foraging may also be an adaptive strategy by herbivores that play an important role in allowing vegetation to recover. In Chapter 3 and 5 patch conditions were simulated using a checkerboard test design, and the results confirm a similar pattern of foraging behaviour as observed in the above-mentioned equine studies. In both studies horses used trial and error sampling on a continuous basis to identify preferred feeds and appeared to use spatial cues minimally to locate their preferred zones/buckets. Such a pattern has also been observed in ruminants, and seems to be an adaption for dealing with a heterogeneous environment, where there are regular changes (species, flavours, nutrients, toxins) (Illius and Gordon, 1990; Hewitson et al., 2005). In grazing herbivores it also seems to be unnecessary to retain memory of each location of an individual plant, or patch, and it appears that they memorise larger spatial scales such as feeding site, camp and home range, and not detailed position (Senft et al., 1987; Bailey et al., 1996). These observations led to questions about short-term spatial memory in horses as tested in “forage tasks” of Chapter 3.

Preferences for diets or flavours are commonly tested with two-choice test protocols to provide information about ranking. This seems to be practical when familiar forages or concentrate diets are tested. However, when dealing with a familiar-novel dichotomy such tests may limit the ability to mix diets to avoid malaise and neophobia as observed in the first study (Chapter 3). Diet mixing and increased species richness has been shown to increase the intake of feed by ruminants and animals which switch between prefer and less preferred foods (Burritt and Provenza, 2000; Wang et al., 2010). In the final preference test (Chapter 3) it was found that horses increased the intake of some novel forages, but it is unclear if this was due to the provision of multiple options or due to the previous exposure (i.e. familiarisation).

One of the problems with simulating patch conditions and offering several different foods simultaneously is the difficulty of the increasing number of consequent associations that need to be made with each food (Duncan and Young, 2002). This dictates that a long-term exposure is required or diets need to be introduced individually to facilitate development of clear associations. A single presentation of diets before multiple choice testing was used by Redgate et al. (2014) and was also adopted in the last study of this thesis (Chapter 5). In both these studies horses sampled all foods, but showed clear preferences for the higher protein diet and/or hydrolysable carbohydrates. In addition, in the last study of this thesis a clearer difference between intakes of the four diets was observed in the multiple-choice testing compared to the two-choice contrast tests, where no significant differences in intake ratios were observed (Chapter 5). A ranking only appeared when the overall intake of all tests were combined suggesting that multiple-choice testing could be advantageous when ranking diets (nutrients) or flavours. However, further research is needed to assess if multiple-choice models (with a preceding monadic phase) could be adapted to natural or pasture conditions. While Archer (Archer, 1971; 1973; 1978) has conducted multiple-choice testing in pastured environment to determine the preference of grass, legume and herb species in horses, no adaptation phase was implemented and no links with organoleptic qualities and/or nutritional content were established. This highlights the opportunity for more research in pasture and natural environments using multiple-choice models.

This thesis highlights the difficulty of assessing food preferences and intake based on single food properties (e.g. energy content), and indicates the need for the integration of other factors such as nutrient “dimensions” and animal’s current nutritional state in relation to requirements. These concepts are integrated into the geometric framework for nutrition (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993), which defines the important components of animal nutrition (e.g. foods, nutrient requirements, body composition, nutrient utilisation) in a cartesian space, where each dimension represents a food constituent. This type of modelling enables the combined effect of various food constituents to be analysed, and the numerous levels of responses by the animal (e.g. food intake, nutrient absorption, performance, growth) to be integrated within this multi-dimensional context (Raubenheimer et al., 2015). Geometric models of nutrition have been extensively studied in various species of herbivorous insects, particular locusts, but over recent years have also been adapted to birds and mammals, including humans (Raubenheimer and Simpson, 1993; 1997; Simpson and Raubenheimer, 1997; 2001; 2012; Raubenheimer et al., 2015). At present there are no known equine studies but the present results highlight that such models could help to answer some of the complex questions on how horses regulate feed intake given a number of food choices.

The findings of studies in herbivores and omnivores suggest that patterns of diet intake cannot always be explained by the classical nutritional goals such as the maximisation of energy and protein or avoidance of secondary compounds. Instead it appears that animals work towards the attainment of a balanced diet, for example they maintain a relatively stable daily protein intake, but allow total energy intake (carbohydrates and fats) to vary as a function of the composition of the food available. This so-called protein-leverage effect has been observed in primates, humans and rats and has been shown to be particularly important in the development of obesity in humans (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 2005; Sørensen et al., 2008; Felton et al., 2009; Simpson and Raubenheimer, 2012).

While horses are herbivores and forage almost on a continuous basis, they are also monogastrics and seem to have many similar physiological adaptations to those seen in

humans, rats and pigs. The findings in this thesis seem to indicate that protein is more likely to be a determinant for diet choices and intake by horses than energy intake alone. Therefore, further investigation is warranted to determine if a similar protein-leverage effect is applicable to horses. Firstly, it would be beneficial to gain more insight into how horses use nutrient intake targets to regulate feed intake particular in natural or pasture environments. This could be followed up with controlled studies using modern husbandry practices (e.g supplementation of diets with different macronutrient ratios). This information could potential increase our understanding in the development of metabolic disorders in the domestic horse population and could assist with better feeding practices and improving welfare.

6.5 Conclusion

This thesis has provided insight into the behavioural mechanisms of diet selection by horses and has demonstrated the importance of oro-pharyngeal sensory and external stimuli on feeding behaviour and intake. While diet choice and intake appear to be driven primarily by nutrients, taste and odour cannot be ignored and are shown to play an integrated role in the food reward system. Odour and taste can enhance the feeding experience or reduce feed neophobia in horses, and it appears that there are close links between flavours that have a sweet or protein sensations, which may be an indicator for nutrient content of feeds.

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Appendix 1

Browse-related behaviors of pastured horses in Australia: A survey



M. van den Berg^a, W.Y. Brown^a, C. Lee^b and G.N. Hinch^a

^a School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia

^b CSIRO Agriculture Flagship, Armidale NSW 2350, Australia

Paper formatted to *Journal of Veterinary Behavior: Clinical Applications and Research*
10 (2015) 48-53



This chapter has been removed as it was published as the following journal article:

van den Berg, M., Brown, W. Y., Lee, C., & Hinch, G. N. (2015). Browse-related behaviors of pastured horses in Australia: A survey. *Journal of Veterinary Behavior: Clinical Applications and Research*, 10 (1), 48-53

<http://dx.doi.org/10.1016/j.jveb.2014.11.001>

Appendix 2

Supplementary material (Statistical analysis):

Acceptance of novel food by horses: the influence of food cues and nutrient composition



M. van den Berg^a, V. Giagos^b, C. Lee^c, W.Y. Brown^a, and G.N. Hinch^a

^a School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia

^b School of Science and Technology, University of New England, Armidale NSW 2351, Australia

^c CSIRO Agriculture, Armidale NSW 2350, Australia

Paper formatted to *Applied Animal Behaviour Science* (Under review)



This chapter has been removed as it has been submitted for publication elsewhere.