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

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ABSTRACT

Compared to ruminants little is known about how horses modulate food intake and learn about flavour-to-post-ingestive consequences. While it has been suggested that due to hindgut fermentation horse's foraging preferences may be largely influenced by sensory input (*e.g.* volatiles), it has been established that horses are able to differentiate and select familiar foods (*e.g.* concentrates and hay) based on nutritional content. Yet it remains unclear how this translates to the acceptance of nutritious novel foods (NF). Therefore, the influence of food cues and nutrient composition on NF acceptance were examined in two experiments using 11 adult mares. In experiment 1, we investigated the influence of a familiar odour (FO) on the acceptance of a nutritious NF and in experiment 2, we determined if horses have the ability to select nutritious NF based on the nutritional content, regardless of sensory preferences. In experiment 1 horses received identical NF in a two-choice test with one of the choices being masked with a FO over a 9-day period. In experiment 2 horses were offered a high or low protein option of an otherwise identical NF in a two-choice test in which the NFs were paired with two unfamiliar flavours (odours). The two-choice test lasted for 14 days and the flavour-protein pairing was switched after 7 days. NF intakes were recorded over a 10 min test period on each test day and analysed using Bayesian hierarchical models. The results of experiment 1 indicate that a FO had a strong positive influence on the NF intake for the first 5 days (90–100% of total consumption and strong evidence for non-zero temporal effects (Bayes factor $B_{12} = 110$)). This was followed by a more even distribution of intake for the remaining period. In experiment 2 horses had a greater intake of high protein NF regardless of the flavour on days 4, 6 and 7 (80–87% of total consumption) and this continued after the switch over (Day 9–14; 57–81% of total consumption). However, 4 out of 11 horses showed neophobia throughout the testing period, which could have been associated with the novel odours as horses scanned the buckets with little to no sampling. The results suggest that pre-ingestive cues (*e.g.* smell, taste) of foods play an important role in diet selection and that a FO can increase the acceptance of NF. This new knowledge could be applied by the horse industry to encourage the consumption of new food or forages by horses.

1. Introduction

The senses of taste and smell help animals to discriminate between foods and play an important role in food preferences and food selection. Orosensory senses together with post-ingestive mechanisms allow animals to make associations with pleasant or unpleasant experiences depending on whether the effect on the internal environment of the animal is aversive or positive (gut-brain feedback) (Provenza, 1995). For example foods can be rejected (aversion) as a direct result of sensory input and its link to post-ingestive consequences, either from toxins or nutrients that are in excess or deficient. 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; Pfister et al., 2002). Conversely, the liking of a food (preference) increases when it contains adequate nutrients and provides a positive feedback.

Investigations of the ability of horses to differentiate foods based on the orosensory characteristics and nutrient content are limited. There is some evidence that horses are able to detect macronutrients in foods and can adapt to deficiencies by increasing intake or by changing food choices (Laut et al., 1985; Cairns et al., 2002; Redgate et al., 2014). For example, Cairns et al. (2002) showed that horses selected a higher energy concentrate over a lower energy

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one, regardless of the preferred flavour (mint or garlic). However, it has been suggested that horses, due to hindgut fermentation, may experience difficulties in associating the chosen food with its post-ingestive consequences, particularly when several foods are presented simultaneously. Therefore, a more recent study compared the effect of exposure to a single forage that was rich in either protein, lipids or hydrolysable carbohydrates on the selection of three forages in a simultaneous choice session (Redgate et al., 2014). The horses showed a greater preference for the forages that were rich in protein or hydrolysable carbohydrates, which suggests that horses responded to the macronutrients in the diets and that the dietary experience (single presentation) facilitated feedback mechanisms, and hence affected dietary preferences.

However, other researchers have suggested that diet selection and intake are more influenced by the organoleptic qualities of forages (*e.g.* taste, odour, ease of prehension, texture) and that nutrient content appeared to be weak indicators (Dulphy et al., 1997; Cuddeford, 2005). Food selection based on orosensory perception has been observed in a previous study (van den Berg et al., 2016) that examined the effect of energy status (low and high) in horses on diet selection of familiar and novel forages. These findings demonstrated that horses had a greater preference for familiar forages and that, regardless of the energy intake, horses showed a strong neophobic response towards unfamiliar nutritious forages, frequently scanning the buckets with little to no consumption. These novel forages had comparable nutritional profiles to the familiar forages and therefore it seems that volatiles and odour (olfaction) may be important factors in forage selection by horses, in particular when dealing with a familiar-novel dichotomy. The influence of plant odours on herbivore feeding behaviour and dietary preferences has been clearly demonstrated in sheep (Arnold et al., 1980). In addition, odour profiling has been used to make predictions about the preferences (and links with nutritional traits) for familiar forages (oat and lucerne hay) by horses and dairy cattle (Pain and Revell, 2009).

While it has been established that horses are able to make associations with the nutritional content of familiar foods, it is unclear how this applies to the selection of nutritious novel foods and how volatiles may affect this selection. Therefore, the aim of the present study was to improve our understanding of the acceptance of nutritious novel foods by horses by examining the sensory behaviour and the ability of horses to learn about positive post-ingestive consequences of a novel food in two experiments. The first experiment follows our previous study (van den Berg et al., 2016) that demonstrated a strong neophobic response in horses towards novel forage volatiles and focused on the question of whether odour influences diet selection of novel foods by horses. It was hypothesised that horses would cautiously sample all foods on offer, but initially a greater intake was expected for the novel choice with the familiar odour. The second experiment focused on the question of whether horses make associations with the orosensory characteristics of an unfamiliar nutritious food and their post-ingestive consequences. The hypothesis was that horses would cautiously sample all foods available, but a greater intake for food with a superior nutrient profile was expected regardless of the preferred flavours.

2. Material and methods

2.1. Animals and feeding management

A total of 11 healthy horses were used for the study. Horses were managed at a commercial horse facility in the New England region (NSW, Australia). The mares were between the ages of 3 and 15 years, weighing 480–640 kg and were of Australian Stock Horse (n = 8) and Thoroughbred (n = 3) breeds. Horses initially were grazing pasture and had a Henneke's body condition score between 4 and 8 (moderately thin to fat, Henneke et al., 1983). The management and feeding of horses was based on the horse owner's usual practices and throughout the study period horses were managed on pasture as one group and were only offered lucerne (*Medicago sativa*) hay *ad libitum* to supplement poor winter pasture. The horses were not exercised, apart from one horse that was ridden (light work) on three occasions during the experiment period (after the tests). This horse did not receive any additional supplementation. The study was conducted between the months of July and October 2014.

2.2. Experimental design

In the first experiment horses received identical novel forages in a two-choice test for 9 days with one of the choices being masked with a familiar odour (Fig. 1). The aim was to examine intake patterns and the time required for horses to learn about the post-ingestive consequences of the novel foods. We propose that a positive experience can be assumed when horses consumed 50% or more of the total food offered and consumed equal portions of both choices.

In the second experiment horses were offered novel foods from a similar source in a two-choice test that were high or low in protein paired with one of two unfamiliar flavours. The two-choice tests were conducted over 14 days with the flavour-protein pairing switched after 7 days (Fig. 1). The objective was to examine intake patterns and the time taken for horses to increase consumption of the high-protein food (post-ingestive feedback) and if horses would continue to make this selection when flavour-protein pairing was switched.

2.3. Feed collection and flavour preparation

Golden bamboo (*Phyllostachys aurea*) was chosen as the test forage for experiment 1 based on the novelty and literature describing the use as supplementary fodder in horse diets (Nelson et al., 1997; Triebe et al., 2012; van den Berg et al., 2016). Bamboo was sourced from the nursery of the University of New England, NSW. The browse was stripped to leaves and twigs, dried in a climate-controlled room at 27 °C for 3–4 days and cut in pieces similar to the familiar chaff form (2–3 cm).

For the preparation of forage odour a similar procedure was used as described by Hinch et al. (2004). Fresh lucerne was collected from a commercial lucerne grower in South East Queensland, Australia. For the familiar odour solution 200 g fresh lucerne was shredded in a food blender with 1 l water to make up 20 l of total solution. This was allowed to stand overnight (12 h) in a cool room at 4°C before straining through a cheese-cloth. The resultant extract was stored at −20 °C in airtight containers.

Commercially sourced non-nutritive human/animal food flavours (aniseed and citrus; Lucta, Spain) were used in experiment 2. The aniseed flavour had a liquorice aroma and citrus had a sweet orange aroma. Both flavours had no added taste and were novel to the horses. The flavour powder (5 g) was diluted in 500 ml cold water to make a stock solution.

2.4. Testing area

Horses were individually tested in a holding yard (4 m × 8 m) that was familiar to them and within view of other horses. Before testing (experiment 1) horses were adapted to the holding yard and buckets for two days during which they were fed lucerne chaff (Fig. 1). Two large feeding buckets were mounted on the yard door (0.5 m apart and 1 m height) and two smaller labelled feeding tubs