van den Berg, M., Giagos, V., Lee, C., Brown, W., Cawdell-Smith, A., & Hinch, G. (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

The influence of odour, taste and nutrients on foraging behaviour and food preferences in horses

M. van den Berg[1†], V. Giagos[2], C. Lee[3], W.Y. Brown[1], A.J. Cawdell-Smith[4] and G.N. Hinch[1]

[1] *School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia*

[2] *School of Science and Technology, University of New England, Armidale NSW 2351, Australia*

[3] *CSIRO, Agriculture Flagship, Armidale NSW 2350, Australia*

[4] *School of Agriculture and Food Sciences, University of Queensland, Gatton QLD 4343, Australia*

[†] Corresponding author: Mariette van den Berg. Email: info@mbequineservices.com

Highlights

- Effect of different food cues (odour, taste, nutrients) on diet intake by horses was examined
- Two-choice and multiple-choice models were used to evaluate feeding behaviour
- Nutrient content appeared to be the main drive for diet choices in both preference-testing models
- Taste appear second to nutrients in determining the diet selected and this was more evident with the lower protein diet
- The multiple-choice model promoted patch foraging behaviour and was able to more clearly differentiate in diet selection.

Abstract

While it has been established that nutrients and flavours (odour, taste) play an important role in diet selection and mixing of supplementary feedstuffs by horses, it is not always well defined what type of flavouring (e.g. non-nutritive or nutritive) has been used by previous equine studies. Therefore, the objective of this study was to determine the influence of distinct food characteristics (odour, taste, nutrients) on the voluntary intake and preference by horses using different preference testing models. This experiment consisted of 3 phases; adaptation (P1), two-choice testing (P2) and multiple-choice testing (P3) using a chequerboard design. Four experimental pelleted diets equal in digestible energy, but contrasted in crude protein (LP; 14% and HP; 27%) and added non-caloric (natural) sweetener (i.e. LP, LP+, HP, HP+) were consecutively fed to each of sixteen adult horses. The diets were paired with 4 non-nutritive odours (coconut, banana, cinnamon and spearmint), with a unique odour and diet combination allocated to each group of four horses. In P1, each diet was presented solely for 5 days to facilitate associations; in P2 a two-choice test was conducted with 4 diet combinations (contrasts) over 3 days; and in P3 the 4 diets were presented simultaneously over a 5-day period. In P1 only feed intake was measured and for P2 and P3 feed intake, bucket/zone visits and time spent foraging or moving were recorded. The preference ranking of diets was more evident in the multiple-choice testing compared to the two-choice testing. In P3 the intake (g) of the LP diet (114) was the lowest and HP+ (188) had the highest consumption, with a partial preference for the LP+ (154) and HP (156) diet (± s.e.; 8.9). A clear group effect was recorded for both P2 and P3, with Group B showing a greater preference for the higher diet contrast (P2 all 4 tests) compared to the other groups, and this group had overall the lowest feed intake in both tests. In addition, a preference for diets linked to banana odour was observed in both tests. The results showed that horses displayed patch foraging behaviour sampling from all foods on offer, but they clearly rank these based on nutrients, followed by taste and odour. We propose that a multiple-choice model such a chequerboard design could be successfully adapted for preference testing of heterogeneous resources. However, more research is needed that assesses the potential for adopting these types of models to natural or pasture environments.

Key words

Food intake, flavour, nutrients, patch foraging behaviour, multiple-choice design

Introduction

In a natural or pastured environment horses are faced with a habitat that offers a variety of plant species some of which they are able and prepared to eat. They typically show patch foraging behaviour, taking several bites of plants and then walking a step or two forward or sideways to the next feeding station (Prache et al., 1998). Even if horses select from a heterogeneous resource they do not include the entire range of foods available or eat them in the proportions to the amounts available, evidently showing partial preferences (Archer, 1973; Goodwin et al., 2002; Thorne et al., 2005). Therefore, on what basis do horses make diet choices? The biological value and ecological causes of diet mixing/switching by large generalist herbivores have been mainly attributed to nutritional balance (Westoby, 1974), detoxification of plant secondary compounds limitations (Freeland and Janzen, 1974) and transient food aversions (Provenza, 1996).

Conditioned aversions and preferences appear to have evolved as important mechanisms by which large generalist herbivores can optimise diet selection by learning to associate the sensory properties (taste, smell, texture) of food plants with the nutritional consequences (negative and positive) (Provenza, 1996). The mechanisms of food aversions and preferences have been predominately demonstrated in ruminants (sheep, goats and cattle) and typically show that animals will either alter intake or selection when malaise occurs after ingestion (induced by a toxin) or when foods are deficient or have excess nutrients. Also the flavour (smell, taste and texture) of a food may modulate foraging behaviour. Thus transient aversions are attributed to satiety of flavour, nutrients or plant toxins, which alter food intake patterns and lead to the consumption of a varied diet (Burritt and Provenza, 1989; du Toit et al., 1991; Kyriazakis et al., 1998).

While it has been established that horses can develop conditioned food aversions (Houpt et al., 1990) and preferences (Goodwin et al., 2005a; b), the role of transient satiety in diet mixing remains unclear. Horses can make associations based on the nutritional content of foods and either increase intake or change preferences (Laut et al., 1985; Cairns et al., 2002; Redgate et al., 2014). However, horses rely on hindgut fermentation to gain energy from fibrous plant materials and therefore potentially do not have the same “feedback” systems available to ruminants. It has been suggested that due to these digestive “constraints” the horse has a weak regulation of food intake/ feedback and may rely more on the organoleptic qualities of forages (e.g. odour, taste, ease of prehension, texture etc.) when making diet choices (Ralston, 1984; Doreau et al., 1990; van den Berg et al., 2015a).

Odour profiling has been used to make predictions about horses’ preferences for different hays based on positive correlations found between detectable volatiles and nutritive or physical traits (Pain and Revell, 2009). However, the research also identified volatiles that negatively influenced preference but were not linked to any measurable nutritive and physical traits. The authors suggest that this may be related to other plant characteristics such as plant secondary compounds that may affect the taste or gut fermentation. This is in accordance with our previous study, which showed that strong herbaceous volatiles from novel forages affected preference negatively, even though the food itself had a good nutritional profile (van den Berg et al., 2015a). This implies that diet selection cannot always be explained by nutrient composition and that olfaction and gustation cues may override choices based on nutrition. Conversely, a familiar forage odour can be used to increase the acceptance of a nutritious unfamiliar forage, thereby reducing neophobia (van den Berg et al., 2015b).

While it has been established that olfaction plays an important role in diet selection by horses, less is known about the influence of taste. Randall et al. (1978) demonstrated that horses respond to at least 4 of the 5 taste sensations that are found in humans and primates: sweet, salty, sour and bitter solutions. Although there was 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 Merkies, 2009; Jankunis and Whishaw, 2013), but no preference for sour (hydrochloric acid), bitter (quinine) or salt (sodium chloride) tastes. Yet, the influence of taste in relation to the selection of feedstuffs by horses has not been clearly defined. Commercially used flavours can either be categorized as aromatic (odour) and non-nutritive (non-caloric sweetener); or nutritive, which include a (caloric) sweetener. Goodwin and colleagues examined the influence of 15 commercial food flavours on concentrate selection by stabled horses (Goodwin et al., 2005a). The authors ranked the flavours and added the two most liked flavours, fenugreek and banana, to less palatable mineral pellets and showed that the flavours significantly reduced the consumption time. However, it is ambiguous as to what type of flavours (non-nutritive vs nutritive) were used and if it had only an affect on the smell or also impacted the taste. Goodwin et al. (2005b) also examined the effect of sensory variety, using a combination of odour cues (mint, carrot, herbs, garlic) and added taste cues (molasses and sweetened syrup), on foraging behaviour of horses when presented with a single concentrate diet or four concentrate diets at once. The horses foraged more frequently and for longer in the multiple choice sessions compare to the single choice, but foraging bouts were shorter. The authors suggest that this may resemble natural patch foraging behaviour, which is in accordance with their previous studies investigating different types of roughages (Goodwin et al., 2002; Thorne et al., 2005). However, it is unclear from their study if this patch foraging behaviour is attributed to satiety (i.e. transient aversions). In this study horses were familiar with at least one of the concentrates/flavours (fed before the study), which was the most preferred when formulations were constant but flavours varied. Hence there may have been some neophobic responses towards the other flavours of the concentrate diets. In addition, they also tested a combination of formulations with different compositions of energy, protein, fat and fibre and so it is also not clear if there may have been an effect of nutritional content on the diet selection by horses. Finally the role of taste on patch foraging behaviour observed seems to be equivocal in this study model.

To gain a better understanding of the mechanisms of diet selection in horses and the influence of transient aversions (satiety) on patch foraging behaviour we suggest that a multiple choice model should be developed that clearly distinguishes the different levels of food characteristics i.e. nutrients (postingestive feedback), odour and taste. Consequently the major objective of this study was to examine the influence of different food characterises on the voluntary intake and preferences by horses in a multiple choice model using a checkerboard design. We hypothesised that horses will display patch foraging behaviour selecting all available foods, and they will do this in a sequence ranking of food choices primarily based on nutrients, followed by taste and then odour. We also expected that there would be individual variation based on preferences for different odours.

Materials and methods

Animals & husbandry

A total of 16 healthy horses were used for the study. The care and use of the animals followed the guidelines set by The University of New England Animal Ethics Committee, in accordance with section 25 of the Animal Research Act (1985) and the University of Queensland Animal Ethics Committee, in compliances with the Queensland Animal Care and Protection Act 2001. Horses were housed at the Equine Unit of the University of Queensland (QLD, Australia). The study was conducted using 10 mares and 6 geldings that had been managed as two groups on the same property. The horses were between the ages of 4 and 15 years (mean; 9), weighing 516-602 kg (mean; 559) and were of Australian Stock Horse, Standardbred or Thoroughbred breeds. Horses initially were grazing pasture and had a Henneke's body condition score between 4.5 and 5.5 (moderately thin to moderately fleshy, (Henneke et al., 1983)). The management and feeding of horses was based on the UQ Equine Unit's usual practices and throughout the study period horses were managed on pasture with no additional supplementary feeding, other than the experimental test diets. The study was conducted between the months of April and May 2015.

Experimental design

The study was conducted in three phases. Before commencing the experiment, 16 horses were allocated to one of the four groups (A, B, C, D). Each horse was paired with another of similar weight and sex before randomly allocating one horse from each pair to one of the four groups. This resulted in 2 groups with 3 female horses and 1 male horse and 2 groups with 2 female horses and 2 male horses. During phase 1 (adaptation) all horses were offered four pelleted diets paired with four different odours (coconut, banana, spearmint and cinnamon) according to their allocated group, over a period of 20 days. Each diet was presented solely for five consecutive days to allow horses to make an association between each of the four diets and its allocated odour. The four diets had equal digestible energy content, but contrasted in crude protein levels (CP; Low and High) and added sweetener (non-caloric) (Table 1).

Table 1. Diets and associated odours for each group of horses (n = 4).

Protein	**Sweetener**		**Group A**	**Group B**	**Group C**	**Group D**
Low	-	LP	Coconut	Cinnamon	Spearmint	Banana
Low	+	LP+	Banana	Coconut	Cinnamon	Spearmint
High	-	HP	Spearmint	Banana	Coconut	Cinnamon
High	+	HP+	Cinnamon	Spearmint	Banana	Coconut

In phase 2 a series of two-choice tests were conducted with four diet combinations (contrasts) over three consecutive days to determine preferences (Table 2). Finally, in phase 3 preferences were tested again using a multiple-choice model that utilised a chequerboard design.

Table 2. Phase 2: Two-choice test

Test	Choice 1	Choice 2
1	LP	LP+
2	LP	HP
3	HP	HP+
4	LP +	HP+

(LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener)

Diets and flavours

Four pelleted diets were formulated with similar digestible energy (DE) content (12.5 ± 0.4) but that differed in CP levels (LP; 14% and HP; 27%) and added sweetener (Table 1). The pelleted diets were manufactured at the University of New England. The low energy/fibre pellets comprised of soybean hulls, beet pulp, black sunflower seeds and corn. To contrast the CP levels a proportion of corn was replaced with corn gluten. A commercially sourced human-grade non-caloric natural sweetener (blend of erythritol and stevia; Natures Flavors Inc, USA) was added at 2.25% to one choice of the LP and HP diets. This amount equalled a similar sugar sensation as 5% molasses inclusion, which is a standard rate used by horse feed companies. The sugar sensation was based on the assumption that Erythritol and stevia seem to have equal sensation to sugar and that cane molasses is about 45-50% sugar. The chemical analysis of the diets is presented in Table 3.

Commercially sourced human-grade (non-caloric) food flavour emulsions (coconut, banana, spearmint and cinnamon; Natures Flavors Inc, USA) were used to make up odour solutions. Each odour was selected from a different odour class to aid the contrast i.e. fruit flavour, nut flavour, herb flavour and spice flavour. Between 1 and 10 ml was diluted in 500 ml water to create a distinctive odour that was detectable by human senses. The diluted odour solution (2-5 ml) was sprayed onto the diets before offering to the horses.

Table 3. Chemical composition[a] (g/kg dry matter (DM)) of the diets (LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener) offered to horses (n=16) during the feeding trial.

Constituent	LP	LP+	HP	HP+
Dry Matter	903	902	920	925
Digestible Energy (MJ/kg DM)	12.7	12.9	12.4	12.5
Crude Protein	140	141	266	270
NDF	334	312	325	306
ADF	212	209	219	203
NFC	431	451	314	327
Starch	277	249	145	144
WSC	58	58	50	48
ESC	43	33	25	31
Calcium	3.5	3.6	4.1	3.6
Phosphorus	2.3	2.7	2.7	3.0
Magnesium	1.7	1.8	1.5	1.5
Potassium	6.7	6.8	6.4	5.9

[a] NDF, neutral detergent fibre; ADF, acid detergent fibre; NFC, non-fibre carbohydrates, WSC; water soluble carbohydrates, ESC; ethanol soluble carbohydrates. Units are g/kg DM, unless otherwise stated.

Testing procedures

For the duration of phases 1 and 2, horses were individually fed in a yard that was familiar to them with other horses in sight to prevent undesired behaviours. In phase 1, horses were presented their allocated diet (400 g) for 15 minutes on five consecutive days before switching to the next diet/odour pair. In phase 2, horses were presented with two food choices (2 x 200 g) simultaneously (5 min). All four contrast two-choice tests were conducted on the same day, and this was repeated over three consecutive days. The diets were presented in feeding tubs that were colour-coded for odour to avoid odour mixing. These tubs were placed in larger bins that were mounted on the yard railing and under a shelter. When two food choices were offered the buckets were 0.5 m apart and the position of the bucket changed randomly for each testing day. Horses had *ad libitum* access to water in their yards. On completion of testing horses were returned to pasture.

In phase 3 a barren testing area (12 m x 12 m) divided into 16 zones (2.5 m^2) was used for the multiple-choice test. There were four zones allocated to each diet option in a chequerboard fashion, which was adapted from our previous study (van den Berg et al., 2015a) (Figure 1). Each zone contained 100 g of one of the diets, which was offered in feeding tubs that were placed in rubber tyres. Rubber matting 1 m x 1 m was placed under the feeding tubs. To avoid odour mixing (in buckets) each feeding tub was colour coded to an odour (4 x 4) and used throughout the testing period. Horses were individually led into the testing area by a handler and allowed 7.5 min to forage the area uninhibited. On every testing day the diets were randomly allocated to a new zone. There were group yards with companion animals on both sides of the testing area. Before the start of the experiment, horses were familiarised with the test area and the routine of leading them separately into the testing area. On completion of testing horses were returned to pasture.

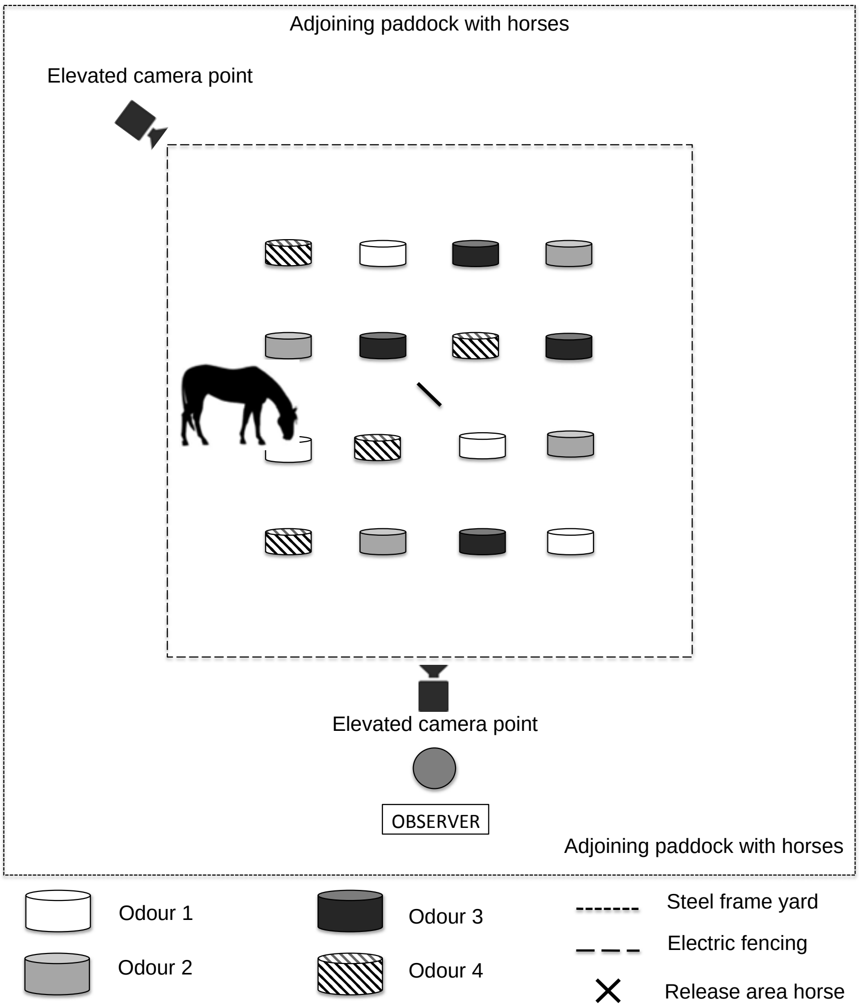

Figure 1. Field and patch layout. A testing area (12 m x 12 m) divided into 16 zones (2.5 m^2).

Feeding and measurements

In phase 1, horses were fed the single diets in the morning between 8:30-9:30 h and the intake (g) recorded on each of the five days. In phase 2 the four two-choice contrast tests (5 min each) were conducted in two parts; morning (8:00 – 12:00 h) and afternoon (13:00-17:00 h) and in phase 3 the multiple-choice test (7.5 min) was conducted between 8:00-12:00 h. Behaviours for phase 2 and 3 were recorded with two video recorders (Panasonic, Japan and GoPro Hero 3+, USA) and by a person sitting 10 m outside the testing arena (under a shelter construction). The number of visits to each bucket or zone (categorised as both front hooves being placed in a zone) and sequence to each zone/bucket were documented. In addition, the time spent foraging (labelled as standing and chewing) or moving to each zone/bucket (classified as walking towards a new zone/bucket) were recorded. The intake of foods by each horse was determined by weighing the foods in each feeding bucket before and after each test. The intake was adjusted for moisture and calculated to a DM basis.

Statistical analysis

Data are presented as mean ± s.e., unless stated otherwise. Diet intake, bucket/zone visits and time spent foraging or moving were analysed in R Studio version 0.99.484 (Team, 2015) and all data were checked for normality and transformed were necessary. For illustration of data (i.e. tables) the Least-squares means predictions (lsmeans package (Lenth and Hervé, 2015)) were computed to compare factors. The predictions were back-transformed and standard error estimated using the Delta method (Weiss, 2006). For all tests the level of significance was set to 5%.

Phase 1; adaptation

Feed intake of each diet over the four weeks was assessed to determine the acceptance of the diets and post-ingestive associations (learning). We considered an intake of 80% (~ 300 g DM) as the threshold for diet association/ acceptance, based on the identified plateau curve of feed intake. The intake of each diet (and week) was denoted as the proportion (%) consumed out of the total offered and was logit-transformed. However, due to the large discrepancy between the animals in intake behaviour on the first and second day of the diet introduction none of the classical statistical models applied showed a correct fit. Therefore, descriptive analyses were conducted and the variance between diets, odours, groups and days were examined using a Fligner-Killeen test of homogeneity of variances.

Phase 2; two-choice contrast tests

To determine the diet preference of each two-choice test the intake ratio of lower (Bucket 1) to higher (Bucket 2) palatability contrast over a 3-day testing period was examined using a generalized linear model (GLM) with a binomial distribution. In the model day and group were included as factors; odour was left out of the model as it was coupled to the group. The ranking of intake (g, DM) for diets, odours and groups was assessed using a linear regression model without the intercept. Similar GLM models were used for the ratios of bucket visits and time spent foraging or moving towards the buckets.

Phase 3; multiple choice test

The intake (g, DM) of each diet over the 5-day testing period was examined using a linear regression model with diet, day, odour and group included as factors. A similar model was used for the time spent foraging. For illustration of the data (i.e. tables) the intake and time spent foraging for diets, odours and groups were assessed separately using a linear regression model without the intercept. For the zone count a GLM model with a Poisson distribution was fitted with diet, day, odour and group as factors. For the time spent moving a logistic regression was used with the same explanatory factors.

Results

Phase 1: Adaptation

The intake proportion (%) of the four diets consumed out of the total offered over five days (0 to 4) is given in Figure 2. The Fligner-Killeen tests indicated a departure from homogeneity for the population’s variances of intake proportions between diets (P<0.001) and days

(P<0.001). In week 1 (LP diet) a large variation in intake between horses was observed on Day 0 and 1 (from 0% to 100% ingestion), which declined over time with horses consuming 80% or more after Day 2. In week 2 (LP+ diet) horses consumed over 90% of the offered diet on all days, although a greater variation was observed on Day 0 and 1. Similar patterns where observed for week 3 (HP diet). In week 4 (HP+ diet) horses showed a stable intake (95-100%) over all days. This decreasing pattern in variance over time was also observed when reviewing the intake proportions for each group and odour. However, the Fligner-Killeen tests indicated a departure from homogeneity for the population's variances of intake proportions for groups (P<0.001), whereas we cannot reject the null-hypothesis for odours (P=0.08); indicating an equality of variance. The plotted data of Group B and D showed a larger distribution of variance compared to Group A and C.

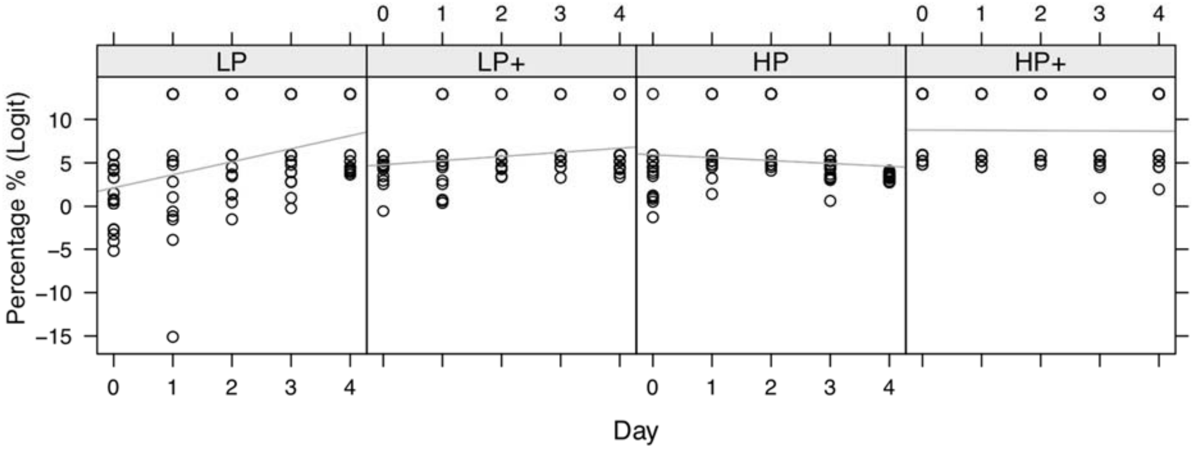

Figure 2. Proportion (%) and trends (line) of diet intake on the logit scale 0-100% (min; -15 to max; 15) over 5 test days was selected for illustration purposes (n=16 horses). LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener.

Phase 2: Two-choice contrast tests

The predicted mean intake, bucket visits and time spent foraging or moving ratios of lower (Bucket 1) to higher (Bucket 2) palatability contrast for the four tests on each day is given in Table 4. In Table 5 the predicted ratio data for the four tests of each group is presented.

Test 1: LP vs. LP+

Analysis of deviance using GLM models indicated a significant effect for days (P=0.02), but the Wald test did not show clear differences in the ratios between days. While the contribution of the groups was not significant at the 5% level (P=0.051) for the analysis of deviance, the Wald test showed that Group B had a significantly (P=0.03) greater preference for the LP+ diet compared to the other groups. This was linked to a significant (P=0.02) lower intake ratio for the cinnamon odour that was paired to the LP diet for Group B. Similar results were found for the time spent foraging ratio, showing a significant contribution for both day and group factors (deviance test; P<0.001). In addition, the Wald tests showed identical patterns for days and groups as the intake ratios. For both the bucket visit and time spent moving ratios the analysis of deviance did not suggested a contribution for days and groups.

Test 2: LP vs. HP

For the intake ratios the day factor did not contribute to the model showing similar ratios across days. Only a significant contribution for groups (deviance test; P<0.001) was observed, with the Wald test recording for Group B a significantly (P=0.02) greater preference for the HP diet compared to the other groups. This was again linked to a significant (P<0.001) lower intake ratio for the diet linked to the cinnamon odour (i.e. LP diet for Group B). Comparable results for the time spent foraging were found, suggesting no effect for days. A significant contribution for groups (deviance test; P<0.001) was observed with the Wald test showing that Group B significantly (P<0.001) spent more time foraging on the HP diet compared to the other groups, but Group A and D did not differ and Group C significantly (P=0.02) spent less time foraging on the HP diet. For both the time spent moving and bucket visit ratios the day and group factors did not contribute to the models.

Test 3: HP vs. HP+

The GLM model does not suggest a significant contribution for days and groups for the intake ratio, but does suggest this for the time spent foraging (deviance test; P<0.001). The Wald test indicated that more time was spent foraging on the HP+ diet on Day 1 (P=0.01) compared to Day 2, but Day 1 did not differ from Day 3. A significant group effect was suggested with Group B (P=0.04) spending more time foraging on the HP+ diet compared to Group A. While Group A had a significantly (P=0.006) lower ratio compared to Group C, this group did not differ from Group D. For both bucket visit and time spent moving ratios the analysis of deviance did not suggested a contribution for days and groups.

Test 4: LP+ vs. HP+

The analysis of deviance suggests that only the group factor (P=0.003) contributed to the model for the intake ratios and, in particular, the Wald test shows that Group B had a significantly (P<0.001) greater preference for the HP+ diet compared to the other groups. This was linked to a significant (P<0.001) lower intake ratio for the diet linked to the coconut odour (i.e. LP+ diet for Group B). The GLM model for the time spent foraging suggests that both day and group are contributing factors (deviance test; P<0.001). The Wald test shows that horses spent more time foraging on the HP+ diet on Day 3 (P<0.001) compared to the other days. There was a group effect recorded with Group B spending significantly (P<0.001) more time foraging on the HP+ diet compared to Group A. Group A also significantly differed from the Group C (P=0.01) and Group D (P=0.007). For both the bucket visits and time spent moving ratios the day and group factors did not contribute to the model.

Table 4. Predicted mean (± s.e.) intake, bucket visit and time spent foraging or moving ratios of lower (Bucket 1) to higher (Bucket 2) palatability contrast for the four tests over a 3-day testing period (n=16 horses). The data is presented as back-transformed log-values.

	Test 1 LP : LP+			Test 2 LP : HP			Test 3 HP : HP+			Test 4 LP+ : HP+		
Day	1	2	3	1	2	3	1	2	3	1	2	3
Intake	0.92 ± 0.025	1.0 ± 0.027	1.02 ± 0.028	0.93 ± 0.026	0.90 ± 0.025	0.96 ± 0.026	0.95 ± 0.025	0.96 ± 0.026	0.98 ± 0.026	0.94 ± 0.025	0.96 ± 0.026	0.94 ± 0.025
Foraging	0.86 ± 0.027	1.0 ± 0.032	1.1 ± 0.034	0.84 ± 0.025	0.82 ± 0.025	0.84 ± 0.025	0.93 ± 0.028	1.06 ± 0.03	0.98 ± 0.03	1.0 ± 0.03	1.04 ± 0.033	0.79 ± 0.025
Moving	0.91 ± 0.13	0.91 ± 0.09	1.12 ± 0.13	0.95 ± 0.16	0.85 ± 0.11	0.9 ± 0.13	1.18 ± 0.16	0.92 ± 0.11	1.09 ± 0.13	1.17 ± 0.17	0.79 ± 0.11	1.21 ± 0.17
Bucket visits	1.04 ± 0.21	0.94 ± 0.15	1.17 ± 0.22	1.0 ± 0.23	0.85 ± 0.18	1.07 ± 0.23	1.09 ± 0.2	1.0 ± 0.18	1.02 ± 0.18	1.07 ± 0.22	0.91 ± 0.19	1.17 ± 0.25

LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener.

Table 5. Predicted mean (± s.e.) intake, bucket visit and time spent foraging or moving ratios of lower (Bucket 1) to higher (Bucket 2) palatability contrast for each group over a 3-day testing period (n=4 horses per group). The data is presented as back-transformed log-values.

	Test 1				Test 2				Test 3				Test 4			
	LP : LP+				LP : HP				HP : HP+				LP+ : HP+			
Group	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Intake	0.99 ± 0.032	0.90 ± 0.03	1.0 ± 0.031	1.0 ± 0.031	0.93 ± 0.03	0.84 ± 0.028	0.98 ± 0.03	0.99 ± 0.03	0.97 ± 0.031	0.91 ± 0.029	1.0 ± 0.03	0.99 ± 0.03	0.99 ± 0.031	0.85 ± 0.028	0.96 ± 0.029	0.98 ± 0.03
Foraging	1.05 ± 0.037	0.85 ± 0.03	1.02 ± 0.038	1.01 ± 0.038	0.85 ± 0.03	0.65 ± 0.022	0.95 ± 0.034	0.9 ± 0.032	0.99 ± 0.035	0.9 ± 0.031	1.14 ± 0.041	1.09 ± 0.04	1.12 ± 0.039	0.72 ± 0.025	0.98 ± 0.036	0.98 ± 0.036
Moving	0.88 ± 0.12	1.02 ± 0.16	1.07 ± 0.14	0.94 ± 0.13	1.11 ± 0.19	0.78 ± 0.14	0.86 ± 0.14	0.88 ± 0.15	1.09 ± 0.17	1.12 ± 0.18	0.95 ± 0.13	1.08 ± 0.15	1.0 ± 0.18	1.07 ± 0.18	1.07 ± 0.15	1.03 ± 0.17
Bucket visits	0.97 ± 0.2	1.09 ± 0.26	1.05 ± 0.21	1.07 ± 0.22	1.08 ± 0.29	0.85 ± 0.22	1.0 ± 0.24	0.97 ± 0.24	1.08 ± 0.23	1.0 ± 0.23	0.98 ± 0.19	1.08 ± 0.22	0.99 ± 0.26	1.09 ± 0.31	1.07 ± 0.22	1.03 ± 0.25

LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener.

Ranking

The rankings of the diets, odours and groups were based on the mean intake (g, DM) of all tests and days combined. The highest mean intake was for the HP+ diet (177.0), followed by HP (171.1) and LP+ (169.6) that did not significantly differ, and LP (163.9) had the lowest consumption (163.86) (± s.e.; 1.73). A similar mean intake was observed for spearmint (172.5) and banana (171.5), followed by coconut (169.9) and the lowest intake was recorded for cinnamon (167.6) (± s.e.; 1.78). Group C (179.8) and D (178.6) showed a comparable consumption, followed by Group A (167.9) and Group B (155.2) consumed the least (± s.e.; 1.47).

Phase 3: Multiple-choice test

The predicted mean intake, zone count and time spent foraging or moving for each diet, group and odour over a 5-day testing period is given in Table 6 a-c.

Intake and time spent foraging

The ANOVA using linear models indicted a significant effect for diet, odour and group (P<0.001). A significantly lower mean intake (g) was observed for the LP diet compared to the other diets (P<0.001) with the highest consumption for the HP+ diet. No differences in mean intake between the days were recorded but there was a significantly greater preference for banana odour compared to cinnamon (P=0.002) and spearmint odour (P<0.001). A group difference was observed, with Group D having a significantly higher intake compared to Group A and B (P<0.001).

A strong linear correlation between the intake and time spent foraging (r=0.80) was observed. The linear models suggested a significant effect for diet and odour (ANOVA; P<0.001). In accordance with the intake, significantly less time was spent foraging on the LP+ diet compared to the other diets (P<0.001), and the greatest time spent foraging was observed for the HP+ diet. More time was spent foraging on diets linked to the banana odour compared to the other odours (P<0.05). No differences in mean time spent foraging were observed for the different days and groups.

Time moving and zone count

Whilst there was a high correlation between time spent moving and zone count (r=0.94), showing a very close agreement, we continued using the time spent moving and zone counts as dependent variables to the two GLM models. The analysis of deviance for time spent moving towards zones/buckets suggests a significant effect for diets (P=0.01), days (P=0.009), group (P<0.001) and odour (P<0.001). The Wald test showed that horses spent significantly more time moving towards the HP (P=0.01) and HP+ (P=0.04) diets compared to the LP and LP+ diets. Horses spent more time moving on Day 5 (P=0.007) compared to the other days. Group A and C spent more time moving towards zones/buckets compared to Group D (P=0.002) with the lowest time observed for Group B (P<0.001). In accordance with the intake and time spent foraging trends, less time was spent moving towards the diets with spearmint odour (P<0.001) compared to the other odours. The GLM model suggests only a significant effect for group on the zone count (deviance test; P<0.001). The Wald test showed that Group B made significantly fewer zone visits compared to Group A (P<0.001), but Group A did not differ from the other groups. The Wald test also showed that significantly fewer visits were made to the zones that contained diets linked with spearmint odour (P=0.03) compared to the banana odour, but banana odour did not differ from the other odours.

Table 6. Predicted mean (± s.e.) intake, zone count and time spent foraging or moving for each diet, group and odour over 5-day testing period (n=16 horses). Intake and time spent foraging values are based on linear regression model without the intercept for each factor. Time spent moving and zone count are presented as back-transformed log-values.

a) Diets

	LP	LP+	HP	HP+
Intake (g, DM)	114.0 ± 8.9	154.3 ± 8.9	155.5 ± 8.9	187.6 ± 8.9
Time spent foraging (sec)	76.2 ± 6.2	98.7 ± 6.2	105.8 ± 6.2	120.7 ± 6.2
Time spent moving (sec)	7.0 ± 0.3	7.0 ± 0.3	8.1 ± 0.32	7.9 ± 0.31
Zone counts	2.4 ± 0.17	2.5 ± 0.18	2.8 ± 0.19	2.7 ± 0.18

b) Groups

	A	B	C	D
Intake (g, DM)	133.7 ± 8.7	113.4 ± 8.7	179.5 ± 8.7	184.6 ± 8.7
Time spent foraging (sec)	96.7 ± 6.5	102.6 ± 6.5	101.1 ± 6.5	101.0 ± 6.5
Time spent moving (sec)	8.9 ± 0.33	5.4 ± 0.26	8.7 ± 0.33	7.4 ± 0.3
Zone counts	2.9 ± 0.19	1.8 ± 0.15	3.0 ± 0.19	2.7 ± 0.18

c) Odours

	Coconut	Banana	Cinnamon	Spearmint
Intake (g, DM)	159.8 ± 9.1	180.4 ± 9.1	145.7 ± 9.1	125.4 ± 9.1
Time spent foraging (sec)	105.6 ± 6.2	124.3 ± 6.2	89.1 ± 6.2	82.5 ± 6.2
Time spent moving (sec)	7.9 ± 0.31	8.2 ± 0.32	7.7 ± 0.31	6.3 ± 0.28
Zone counts	2.7 ± 0.18	2.8 ± 0.19	2.5 ± 0.18	2.6 ± 0.17

Discussion

The objective of this study was to examine the influence of different food characterises (nutrients, odours and taste) on the voluntary intake and the preferences by horses using initially a two-choice model, followed by a multiple-choice model to simulate a natural feeding patch environment. We hypothesised that horses would display more distinct patch foraging behaviour in the multiple-choice model selecting all available foods, but that horses would rank preferences based on nutritional content, followed by taste then odour. The key findings of this study were (1) a large variation in intake was observed in the adaptation phase with some horses showing neophobic behaviour while other animals exhibited a more neophilic response but differences declined over time. (2) Nutrient content appeared to be the main driver for diet selection and feed intake in both preference tests. (3) Taste appeared to be the second determinant for preference by horses and was more evident with the lower CP diet (4) A greater intake of diets linked to sweet aromatic odours (banana and coconut) was observed. (5) The multiple-choice test model promoted patch foraging behaviour and showed more explicit differences in diet selection. (6) An obvious group effect for diet preference and total feed intake was recorded.

Association of diets with post-ingestive consequences

In the adaptation phase all horses ingested over 80% of the experimental diet (~300 g, DM/day) over the course of 5 days of testing and this level of consumption could be assumed to have been adequate to facilitate post-ingestive associations. Redgate et al. (2014) adopted a similar adaptation period, but horses were exposed to a single diet in two separate 3-day monadic sessions, and showed that a single presentation of diets during choice studies facilitated expressions of dietary preferences in horses. In our study, the intake was only below 80% for some horses on the first and second day of the study (LP diet), which is likely to be associated with neophobia of food or surroundings when animals are exposed for the first time. With time/exposure and the routine introduction of new diets (i.e. food cues) this variance declined and by week 4 (HP+ diet) a stable intake pattern (95-100%) was observed. Yearsley et al. (2006) modelled food type with their post-ingestive consequences and suggested that the rate of learning about new food types (i.e. the rate of information transfer) is maximised when food types occur with an incremental frequency in the diet, which is related to the time delays in associated post-ingestive consequences. In this study with each introduction of a new odour, an initially large variation in intake was recorded with some animals showing a more neophobic response while others showing neophilic behaviour. This variance was similar for all odours and reduced over time, suggesting a minimum of 4 to 5-day adaptation before post-ingestive association occurs. These finding suggest that orosensory cues have a strong influence on feed intake by horses and that individual variation in response to such cues need to be considered when introducing new foods and flavours to a diet. This confirms previous reports showing that feed neophobia can be reversed using a familiar odour over the course of five days (van den Berg et al., 2015b).

The influence of nutrients on diet selection

The preferences for the diets were initially evaluated in four contrast tests using a two-choice test. None of the models were able to show that horses had an obvious preference for diets with a greater palatability, showing a close to 1:1 intake ratio for most of the tests and days. Yet, some of the test models suggested that more time was spent foraging on the diets with enhanced palatability, showing a slight departure from a 1:1 ratio; but this was not consistent for all test days. The discrepancy between the observations for intake and time spent foraging may be a result of the fact that a number of horses were able to empty both buckets before the 5 min time period had elapsed and subsequently continued visiting the buckets to try and obtain left-over pellets. Therefore some of the time spent foraging could have been searching rather than ingestive behaviour. In hindsight, the test time should have been 3.5-4 min. Nonetheless, the contrast test results and mean intake ranking of diets suggest that horses did discriminate based on the nutrient content and showed a preference for the higher CP diet.

This difference was less evident when a sweetener was added to the diet, an observation supported by the mean intake measures showing a ranking based on protein content but there were no significant differences in intake for the LP+ and HP diets. A similar ranking was also recorded in the multiple-choice test and these findings are in accord with other studies that have reported that preferences and intake is linked to macronutrient content (Laut et al., 1985; Cairns et al., 2002; Goodwin et al., 2005a; Redgate et al., 2014; van den Berg et al., 2015b). Such studies demonstrate that horses can discriminate between diets based on both energy and CP content, even if foods are novel and regardless of flavour (odour) preferences.

The influence of sweetener and odour on diet selection

Diet preferences due to flavours have not been widely examined in horses (Burton et al., 1983; Goodwin et al., 2005a; b) and in these studies it is not always clear what type of flavouring was used; for example non-nutritive vs nutritive, or aromatic vs taste that may have calories or not (sugar versus artificial or natural sweeteners). In the present study a non-caloric (natural) sweetener was used so that a taste effect could be assessed without interfering with the nutritional content. While nutrient content seem to be the primary determinant for diet selection, the results of the two-choice and multiple-choice testing also suggest that an added taste enhances preference, with a partial preference for LP+ and HP and the highest consumption for HP+.

To our knowledge there are no previous equine studies that have reported the use of non-caloric artificial or natural sweeteners in horse diets and clearly show the positive effects on preferences of taste using non-caloric natural sweeteners. The inclusion of artificial or natural sweeteners to animal diets is a common practice in the swine industry (Munro et al., 2000; Sterk et al., 2008; Moran et al., 2010) where sweeteners are routinely included in piglets' diet to enhance feed palatability and avoid a drop in feed intake post-weaning. However, there are somewhat variable results of the effect of sweetener on feed intake, feed conversion and daily weight gain in piglets. The use of the artificial sweetener such as Sucram seems to have a positive effect on both feed intake and performance (Sterk et al., 2008) although the authors indicate that weanling pigs required a certain period of time (7 days) before clear effects of dietary sweeteners on individual feed intake characteristics and pig performance was observed. This may suggest that some adaptation is needed before acceptance of the sweetness and it is what might be expected of a neophobic reponse. On the other hand the use of Stevia in diets did not appear to have detrimental effects on feed consumption and performance of piglets (Munro et al., 2000). Similar results were found in a study using Stevia leaves or stevioside in diets for broiler chickens (Atteh et al., 2008). It is well known that stevia can have a bitter aftertaste in humans (Goyal et al., 2010), which could explain why stevia may not be as useful in enhancing palatability. In our study we used a blend of erythritol and stevia, which reduces the bitter aftertaste of stevia and provides an equal sugar (1:1) sensation. Whilst we only demonstrated palatability of sweeteners in this study, we also would like to point out that sweeteners could potentially alter intestinal glucose absorption, which may have some beneficial implications for the equine industry. In rodents, pigs and ruminants it has been demonstrated that when sweeteners are included in the diet, they enhance the expression of Na^+/glucose co-transporter (SGLT1) and the capacity of the gut to absorb glucose (Shirazi-Beechey, 1995; Moran et al., 2010; Moran et al., 2014). Artificial sweeteners are known to act on the intestinal sweet taste receptor T1R2/T1R3 and its partner G-protein, gustducin, to activate pathways leading to SGLT1 up-regulation (Moran et al., 2010). In horses a similar mechanism has been identified, showing that diets containing increasing levels of hydrolysable carbohydrates induce a significant increase in SGLT1 expression over time in the small intestine (Dyer et al., 2009; Daly et al., 2012). However, at present it is not clear if sweeteners would trigger the same pathways as seen in other animals. Therefore, further research could provide more insight in the use of artificial or natural sweeteners in horse diets and its effect on glucose absorption. This may particular be advantageous for performance horses (energy uptake) and could potentially reduce digestive upset due to overloading of starch (cereal diets) in the hindgut.

While nutrients and taste seem to have a greater influence on diet intake, our study was also able to show that an aromatic flavour (odour) can affect intake. When assessing both preference tests, a greater intake was recorded for diets linked to the banana odours followed by coconut. This pattern is in accordance with the results of Goodwin et al. (2005a), who also ranked banana flavouring (of the 15 flavours) as most preferred. These findings suggest that horses have a preference for odours that can be described as a sweet aromatic sensation, even when not linked to nutritive characteristics.

Multiple-choice test model to simulate patch foraging conditions

In a natural or grazing environment horses select from a diverse range of resources, which suggests that multiple-choice test may be advantageous when assessing preferences or ranking of preferences. In the present study a chequerboard ‘patch’ design was used, which clearly demonstrated that horses select from all foods but have ranked preferences associated with macronutrients, taste then odour. This ranking was also identified in the contrast tests based on the mean intake of the diets, but was less obvious when two diets were compared (contrasts). It seems that a patch design was the most appropriate for pasture field studies that reviewed the preference for short and tall sward heights (Naujeck et al., 2005). Other equine studies (Goodwin et al., 2002; Thorne et al., 2005; Goodwin et al., 2007) have used a multiple choice design to assess the intake and feeding behaviour of stabled horses that were offered a single and multiple choice of conserved forages. The studies confirm that in the multiple-choice test horses selected preferred and less preferred forages, evidently mixing diets, which was reflected in greater time spent searching and foraging. Goodwin et al. (2007) also showed that horses moved between forage locations regardless of the palatability of the forages or horse's preference for a particular forage indicating that searching behaviour is an important component in diet selection by horses and therefore should be measured when choices between diets/ forages are being assessed.

In the present study, searching behaviour, i.e. time spent moving towards the buckets/ zones and the visits to each bucket/zone, was assessed in both the two-choice and multiple-choice test. No differences in the ratios for bucket visits and time spent moving between days and groups were recorded for the two-choice testing. In addition, in the multiple-choice test no differences in the mean zone count between diets were observed. The equal zone count suggests that horses displayed continuous sampling behaviour and possibly do not use short-term spatial memory to identify preferred patches/ zones. This confirms the findings of a previous study (van den Berg et al., 2015a). It has been suggested that grazing animals may rely more on visual or orosensory cues rather than on memory of spatial cues (Illius and Gordon, 1990). When ungulates forage they select a feeding site, a patch in the site, within which numerous bites are selected. Researchers have suggested that the memory necessary to remember each bite or feeding station would be excessive (Bailey et al., 1996; Senft et al., 1987). The motivation to move form one patch to another can therefore be related to dissatisfaction or explorative behaviour (trial and error). This behaviour was also observed in the study of Naujeck et al. (2005), where horses would move between patches of short and longer sward heights. Patch foraging may be an adapted strategy by herbivores that play an imperative role in allowing vegetation to recover. In addition, this sampling may also allow animals to get an important picture of the environment. However, this transfer of information seems to be more related to a larger scale spatial scale and temporal level in the foraging hierarchy such as feeding site, camp and home range (Bailey et al., 1989). But more research is required to confirm this in horses.

Group effect

A strong group effect was observed for both the two-choice and multiple-choice tests with Group B showing a significantly greater preference for the diets with greater palatability (higher contrast) compared to the other groups in the two-choice contrast tests. This was linked with the lowest overall mean intake and was similar for both test models. This group also spent less time moving and had the lowest mean zone count, which makes this group of horses more selective in terms of feed choices. It is unclear why this group displayed such differences as the groups were randomly allocated based on weight and sex. The age of the group ranged from 4 to 14, showing a similar age distribution the other groups. Therefore the results may have been attributed to some individuals and highlights that there may be a large variation between animals in how they regulate intake of nutrients to meet dietary needs. Further studies that integrate nutritional geometry models could gain more insight in these regulatory mechanisms of individuals. In a geometric framework for nutrition, the important components of animal nutrition (e.g. foods, nutrient requirements, nutrient utilisation) are defined in a Cartesian space, where each dimension represents a food constituent (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). This type of modelling enables the combined effect of various food constituents (e.g. protein, carbohydrate’s, fats) to be analysed, and the different levels of animals responses (e.g. food intake, nutrient absorption) to be incorporated within this multi-dimensional framework (Raubenheimer et al., 2015). These frameworks 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; Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 1997; Felton et al., 2009; Simpson and Raubenheimer, 2012). However, at present no studies have been conducted with horses and highlight the opportunity to integrate these geometric models to answer some of the more complex questions as to how (individual) horses use nutrient intake targets to regulate feed intake given a number of choices.

Conclusion

This study was able to show that horses sample all diets on offer but show clear preferences ranked on nutrients, followed by taste than odour. This ranking was more evident in the multiple-choice testing than the two-choice testing and suggests that a multiple-choice model such as a chequerboard design could be more advantageous when ranking preferences. However, an adaptation period is needed to allow for post-ingestive associations. Further research is required to assess the use of these types of models in natural or pasture environments. Nutritional geometry models could be integrated to advance our understanding of diet selection patterns in horses and how individual animal regulate their intake of multiple nutrients to meet dietary requirements. While our study is in accordance with other research showing that nutrients have a strong influence diet selection, we should also acknowledge the importance of taste and odour on diet selection. In addition, this study was able to show the positive effects of a non-caloric sweetener on diet intake and selection.

Conflict of interest

Funding for this project was kindly provided by the University of New England, New South Wales, Australia. We wish to confirm that there are no known conflicts of interest associated

with this publication and there has been no additional financial support for this work that could have influenced its outcome.

Acknowledgements

The authors would like the University of Queensland (UQ Equine Unit) for their kind assistance in providing horses and facilities. We also acknowledge Micheal Raue for his help with the logistics of this research project. Special thanks go to all the staff and students from UQ; Mitchell Coyle, Luke Gilbert, Paula Lever, Camille Hilliere, Charmaine Tang, Louise Cooper, Roxy Cameron, Jess Blockland and Courtney Windsor.

References

Archer, M., 1973. The species preferences of grazing horses. J. Br. Grassl. Soc. 28, 123-128.

Atteh, J.O., Onagbesan, O.M., Tona, K., Decuypere, E., Geuns, J.M.C., Buyse, J., 2008. Evaluation of supplementary stevia (*Stevia rebaudiana*, *bertoni*) leaves and stevioside in broiler diets: effects on feed intake, nutrient metabolism, blood parameters and growth performance. J. Anim. Physiol. Anim. Nutr. 92, 640-649.

Bailey, D.W., Rittenhouse, L.R., Hart, R.H., Richards, R.W., 1989. Characteristics of spatial memory in cattle. Appl. Anim. Behav. Sci. 23, 331-340.

Burritt, E.A., Provenza, F.D., 1989. Food aversion learning: Ability of lambs to distinguish safe from harmful foods. J. Anim. Sci. 67, 1732-1739.

Burton, J.H., Price, D.J., Aspinal, J., 1983. The effect of feed flavour and feed consumption in horses, Eight Equine Nutrition and Physiology Symposium Lexington, KY.

Cairns, M.C., Cooper, J.J., Davidson, H.P.B., Mills, D.S., 2002. Association in horses of orosensory characteristics of foods with their post-ingestive consequences. Anim. Sci. 75, 257-265.

Daly, K., Al-Rammahi, M., Arora, D.K., Moran, A.W., Proudman, C.J., Ninomiya, Y., Shirazi-Beechey, S.P., 2012. Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport. Am. J. Physiol. Regul. Integr. Comp. Physiol. 303, R199-208.

Doreau, M., Moretti, C., Martin-Rosset, W., 1990. Effect of quality of hay given to mares around foaling on their voluntary intake and foal growth. Ann. Zootech. 39, 125-131.

du Toit, J.T., Provenza, F.D., Nastis, A., 1991. Conditioned taste aversions: How sick must a ruminant get before it learns about toxicity in foods? Appl. Anim. Behav. Sci. 30, 35-46.

Dyer, J., Al-Rammahi, M., Waterfall, L., Salmon, K.S., Geor, R.J., Boure, L., Edwards, G.B., Proudman, C.J., Shirazi-Beechey, S.P., 2009. Adaptive response of equine intestinal Na+/glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. Pflugers Arch 458, 419-430.

Felton, A.M., Felton, A., Raubenheimer, D., Simpson, S.J., Foley, W.J., Wood, J.T., Wallis, I.R., Lindenmayer, D.B., 2009. Protein content of diets dictates the daily energy intake of a free-ranging primate. Behav. Ecol. 20, 685-690.

Freeland, W.J., Janzen, D.H., 1974. Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. Am. Nat. 108, 269-289.

Goodwin, D., Davidson, H.P.B., Harris, P., 2002. Foraging enrichment for stabled horses: effects on behaviour and selection. Equine Vet. J. 34, 686-691.

Goodwin, D., Davidson, H.P.B., Harris, P., 2005a. Selection and acceptance of flavours in concentrate diets for stabled horses. Appl. Anim. Behav. Sci. 95, 223-232.

Goodwin, D., Davidson, H.P.B., Harris, P., 2005b. Sensory varieties in concentrate diets for stabled horses: effects on behaviour and selection. Appl. Anim. Behav. Sci. 90, 337-349.

Goodwin, D., Davidson, H.P.B., Harris, P., 2007. Responses of horses offered a choice between stables containing single or multiple forages. Vet. Rec. 160, 548-551.

Goyal, S.K., Samsher, Goyal, R.K., 2010. Stevia (*Stevia rebaudiana*) a bio-sweetener: a review. Int J Food Sci Nutr 61, 1-10.

Henneke, D., Potter, G.D., Kreider, J.L., Yeated, B.F., 1983. Relationship between condition score, physical measurments and body fat percentage in mares. Equine Vet. J. 15, 371-372.

Houpt, K.A., Zahorik, D.M., Swartzman-Andert, J.A., 1990. Taste aversion learning in horses. J. Anim. Sci. 68, 2340-2344.

Illius, A.W., Gordon, I.J., 1990. Constraints on diet selection and foraging behaviour in mammalian herbivores, In: Hughes, R.N. (Ed.), NATO Advanced Research Workshop on Behavioural Mechanisms of Food Selection, Springer-Verslag, Gregynog, Wales, UK, pp. 369-393.

Kyriazakis, I., Anderson, D.H., Duncan, A.J., 1998. Conditioned flavour aversions in sheep: The relationship between the dose rate of a secondary plant compound and the acquisition and persistence of aversions. Br. J. Nutr. 79, 55-62.

Laut, J.E., Houpt, K.A., Hintz, H.F., Houpt, T.R., 1985. The effects of caloric dilution on meal patterns and food intake of ponies. Physiol. Behav. 35, 549-554.

Lenth, R.V., Hervé, M., 2015. lsmeans: Least-Squares Means.

Moran, A.W., Al-Rammahi, M.A., Arora, D.K., Batchelor, D.J., Coulter, E.A., Daly, K., Ionescu, C., Bravo, D., Shirazi-Beechey, S.P., 2010. Expression of Na+/glucose co-transporter 1 (SGLT1) is enhanced by supplementation of the diet of weaning piglets with artificial sweeteners. Br. J. Nutr. 104, 637-646.

Moran, A.W., Al-Rammahi, M., Zhang, C., Bravo, D., Calsamiglia, S., Shirazi-Beechey, S.P., 2014. Sweet taste receptor expression in ruminant intestine and its activation by artificial sweeteners to regulate glucose absorption. J. Dairy Sci. 97, 4955-4972.

Munro, P.J., Lirette, A., Anderson, D.M., Ju, H.Y., 2000. Effects of a new sweetener, Stevia, on performance of newly weaned pigs. Can. J. Anim. Sci. 80, 529-531.

Naujeck, A., Hill, J., Gibb, M.J., 2005. Influence of sward height on diet selection by horses. Appl. Anim. Behav. Sci. 90, 49-63.

Pain, S., Revell, D.K., 2009. Fodder quality specifications: Identifying predictors of preference between hays, Rural Industries Research and Development Corporation, Kingston, ACT, Australia.

Prache, S., Gordon, I.J., Rook, A.J., 1998. Foraging behaviour and diet selection in domestic herbivores. Ann. Zootech. 47, 335-345.

Provenza, F.D., 1996. Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J. Anim. Sci. 74, 2010-2020.

Ralston, S.L., 1984. Controls of feeding in horses. J. Anim. Sci. 59, 1354-1361.

Randall, R.P., Schurg, W.A., Church, D.C., 1978. Response of horses to sweet, salty, sour and bitter solutions. J. Anim. Sci. 47, 51-55.

Raubenheimer, D., Simpson, S.J., 1993. The geometry of compensatory feeding in the locust. Anim. Behav. 45, 953-964.

Raubenheimer, D., Simpson, S.J., 1997. Integrative models of nutrient balancing: application to insects and vertebrates. Nutr. Res. Rev. 10, 151-179.

Raubenheimer, D., Machovsky-Capuska, G.E., Chapman, C.A., Rothman, J.M., 2015. Geometry of nutrition in field studies: an illustration using wild primates. Oecologia 177, 223-234.

Redgate, S.E., Cooper, J.J., Hall, S., Eady, P., Harris, P.A., 2014. Dietary experience modifies horses' feeding behavior and selection patterns of three macronutrient rich diets. J. Anim. Sci. 92, 1524-1530.

Shirazi-Beechey, S.P., 1995. Molecular biology of intestinal glucose transport. Nutr. Res. Rev. 8, 27-41.

Simpson, S., Raubenheimer, D., 1997. Geometric analysis of macronutrient selection in the rat. Appetite 28, 201-213.

Simpson, S.J., Raubenheimer, D., 1993. A Multi-Level Analysis of Feeding Behaviour: The Geometry of Nutritional Decisions. Phil. Trans. R. Soc. B. 342, 381-402.

Simpson, S.J., Raubenheimer, D., 2012. The nature of nutrition: a unifying framework from animal adaptation to human obesity. Princeton University Press, Princeton, NJ.

Sterk, A., Schlegel, P., Mul, A.J., Ubbink-Blanksma, M., Bruininx, E.M.A.M., 2008. Effects of sweeteners on individual feed intake characteristics and performance in group-housed weanling pigs. J. Anim. Sci. 86, 2990-2997.

Team, R.D.C., 2015. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.

Thorne, J.B., Goodwin, D., Kennedy, M.J., Davidson, H.P.B., Harris, P., 2005. Foraging enrichment for individually housed horses: Practicality and effects on behaviour. Appl. Anim. Behav. Sci. 94, 149-164.

van den Berg, M., Lee, C., Brown, W.Y., Hinch, G.N., 2015a. Does energy intake influence diet selection of novel forages by horses? Livest. Sc., In press.

van den Berg, M., Lee, C., Brown, W.Y., Giagos, V., Hinch, G.N., 2015b. The roles of orosensory stimuli and post-ingestive feedback in the recognition of novel foods. Appl. Anim. Behav. Sci. In Press.

Weiss, R.E., 2006. Modeling Longitudinal Data. Springer New York, USA.

Westoby, M., 1974. An Analysis of Diet Selection by Large Generalist Herbivores. Am. Nat. 108, 290-304.

Yearsley, J.M., Villalba, J.J., Gordon, I.J., Kyriazakis, I., Speakman, J.R., Tolkamp, B.J., Illius, A.W., Duncan, A.J., 2006. A theory of associating food types with their postingestive consequences. Am. Nat. 167, 705-716.