

**Please cite the Published Version**

van den Berg, M, Giagos, V, Lee, C, Brown, WY, Cawdell-Smith, AJ and Hinch, GN (2016) The influence of odour, taste and nutrients on feeding behaviour and food preferences in horses. *Applied Animal Behaviour Science*, 184. pp. 41-50. ISSN 0168-1591

**DOI:** <https://doi.org/10.1016/j.applanim.2016.08.015>

**Publisher:** Elsevier

**Version:** Accepted Version

**Downloaded from:** <https://e-space.mmu.ac.uk/617341/>

**Additional Information:** This is an accepted manuscript of a paper published in *Applied Animal Behaviour Science*, by Elsevier.

**Enquiries:**

If you have questions about this document, contact [openresearch@mmu.ac.uk](mailto:openresearch@mmu.ac.uk). Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

## **The influence of odour, taste and nutrients on feeding behaviour and food preferences in horses**

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

<sup>1</sup> *School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia*

<sup>2</sup> *School of Science and Technology, University of New England, Armidale NSW 2351, Australia*

<sup>3</sup> *CSIRO, Agriculture, Armidale NSW 2350, Australia*

<sup>4</sup> *School of Agriculture and Food Sciences, University of Queensland, Gatton QLD 4343, Australia*

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

## 1 **Abstract**

2 While it has been established that nutrients and flavours (odour, taste) play an important role  
3 in diet selection by horses, previous studies have not always clarified what type of flavouring  
4 (e.g. non-nutritive or nutritive) was used. Therefore, the objective of this study was to  
5 determine the influence of distinct food characteristics (odour, taste, nutrients) on the  
6 preference of horses using different preference testing protocols. This experiment consisted of  
7 three phases; adaptation (P1), two-choice testing (P2) and multiple-choice testing using a  
8 chequerboard design (P3). Four pelleted diets equal in digestible energy, but contrasted in  
9 crude protein (LP; 14% and HP; 27%) and added non-caloric (natural) sweetener (i.e. LP,  
10 LP+, HP, HP+) were consecutively fed to each of sixteen adult horses. The diets were paired  
11 with four non-nutritive odours (coconut, banana, cinnamon, spearmint), with a unique odour  
12 and diet combination allocated to each group of four horses. In P1, each diet was presented  
13 solely for five days to facilitate pre- and post-ingestive associations; in P2 a two-choice test  
14 was conducted with four diet combinations (contrasts) over three days; and in P3 the four  
15 diets were presented simultaneously in a checkerboard fashion over a 5-day period. Feed  
16 intake, bucket/zone visits and time spent foraging or moving were recorded. The key findings  
17 of this study were: (1) In P1 an initially large variation in intake was recorded with only some  
18 horses showing a neophobic response to a new odour/food, but variation declined within 2  
19 days with the majority of the horses consuming over 90% of the diets. (2) Nutrient (HP)  
20 content appeared to be the main driver for diet intake in P2 ( $P < 0.05$ ) and P3 ( $P < 0.001$ ). (3)  
21 Taste appeared to be the secondary determinant of preference and this was more evident with  
22 the LP diet. (4) Consumption of diets linked to sweet aromatic odours (banana and coconut)  
23 was greater in P3 ( $P < 0.001$ ). (5) The multiple-choice test, which was designed to promote  
24 patch foraging behaviour, showed more explicit differences in diet ranking compared to the  
25 two-choice test. These findings confirm previous studies that horses prioritise diets on

26 nutrients, but this is the first equine study that shows the positive influence of a non-caloric  
27 natural sweetener on diet choice. A non-nutritive sweet taste or odour appears to encourage  
28 diet intake by horses, but more research is needed that examines different sweeteners coupled  
29 with and without odour and/or dietary nutrients and its long-term effects on food intake.

30

### 31 **Key words**

32

33 Food intake, Horses, Multiple-choice Design, Natural Sweetener, Odour, Protein.

34

### 35 **Introduction**

36

37 Food choice is determined by a complex of factors that include food sensory characteristics  
38 (smell, taste and texture), as well as post-ingestive feedback (positive or negative) (Garcia,  
39 1989; Provenza, 1995). Typically nutritional consequences influence food preferences and  
40 sensory characteristics regulate the discrimination between various food items as  
41 demonstrated in humans (Stubbs and Whybrow, 2004), rats (Sclafani and Ackroff, 2004), and  
42 ruminants (Provenza and Villalba, 2006). However, pre-ingestive stimuli have been shown to  
43 override post-ingestive signals in some cases and sensory characteristics can induce  
44 preferences in the absence of any immediate post-ingestive feedback (Gherardi and Black,  
45 1991; Berthoud, 2004).

46

47 While the interactions between pre- and post-ingestive feedback on food intake and  
48 preferences have been extensively studied in ruminants (sheep, goats and cattle), less is  
49 known about hindgut fermenters such as horses. It has been established that horses can  
50 develop conditioned food aversions (Haupt et al., 1990) and preferences (Goodwin et al.,  
51 2005a; b) and also make associations based on the nutritional content of foods (Laut et al.,

52 1985; Cairns et al., 2002; Redgate et al., 2014), but other studies have reported that diet  
53 selection and intake are largely influenced by the organoleptic qualities of foods such as  
54 odour, taste, ease of prehension and texture and that nutrient content appeared to be a weak  
55 indicator (Dulphy et al., 1997; Cuddeford, 2005). These equivocal results may be associated  
56 with long gut transit time, which may results in different gut-brain feedback mechanisms  
57 and/or secondary plant compound detoxification compared to ruminants, but no studies have  
58 been done to evaluate this.

59

60 Odour profiling has been used to make predictions about horses' preferences for different  
61 hays based on positive correlations found between detectable volatiles and nutritive or  
62 physical traits (Pain and Revell, 2007; 2009). However, these reports also identified volatiles  
63 in the hay that negatively influenced the preference but were not linked to any measurable  
64 nutritive and physical traits. The authors suggest that this may be related to other plant  
65 characteristics such as plant secondary compounds that may affect the taste or gut  
66 fermentation. This is in accordance with our previous study, which showed that strong  
67 herbaceous volatiles from novel forages affected preference negatively, even though the food  
68 itself had a good nutritional profile (van den Berg et al., 2016a). This implies that diet  
69 selection cannot always be explained by nutrient composition and that orosensory cues may  
70 override choices based on nutrition.

71

72 While it has been recognised that olfaction plays an important role in diet selection by horses,  
73 less is known about the influence of taste. It appears that horses have a preference for sweet  
74 (sucrose) solutions over sour, bitter or salty (Randall et al., 1978; Danel and Merkies, 2009;  
75 Merkies and Bogart, 2013). However, the influence of taste on food intake of horses has not  
76 been clearly defined. Commercially used flavours can either be categorized as aromatic

77 (odour) and non-nutritive such as a non-caloric sweetener; or nutritive, which include a  
78 caloric sweetener. Goodwin et al. (2005a) showed that well-liked flavours can be used to  
79 encourage intake of an unpalatable supplement. However, it is unclear as to what type of  
80 flavouring was used and whether it only affected the smell or also impacted the taste. In  
81 another study Goodwin et al. (2005b) offered four concentrate diets simultaneously that  
82 contained a combination of odour cues (mint, carrot, herbs, garlic) and added taste cues  
83 (molasses and sweetened syrup), and demonstrated that horses mix diets, selecting from  
84 preferred and less preferred diets. However due to the combination of odours and tastes it is  
85 unclear which food cues were the main drivers for the choices observed. In addition, a  
86 combination of formulations with different mix of macronutrients was tested and so it was  
87 also not clear if there was an effect of nutritional content on the diet selection.

88

89 Therefore, to enhance our understanding of the roles of pre- and post-ingestive cues on food  
90 intake and preference by horses the following study was conducted to examine the influence  
91 of distinct food characteristics i.e. nutrients (post-ingestive feedback) and, non-caloric taste  
92 and odour on the voluntary intake and preferences by horses. Horses were first exposed to  
93 individual diets to learn about the characteristics and post-ingestive associations. This was  
94 followed by two different preference tests (two-choice and multiple choice) to investigate  
95 feeding behaviour and food preferences. The multiple-choice test was developed using a  
96 checkerboard design and we hypothesised that horses would display patch foraging behaviour  
97 selecting all available foods, and they would do this in a sequence ranking of food choices  
98 primarily based on nutrients, followed by taste and then odour.

99

## 100 **Materials and methods**

101

102 ***Animals & husbandry***

103 The study was conducted using 16 healthy horses; 10 mares and 6 geldings that had been  
104 managed as two groups on the same property at the University of Queensland (UQ Equine  
105 Unit). The horses were between the ages of 4 and 15 years (mean; 9), weighing 516-602 kg  
106 (mean; 559) and were of Australian Stock Horse, Standardbred or Thoroughbred breeds.  
107 Horses initially were grazing pasture and had a Henneke's body condition score between 4.5  
108 and 5.5 (moderately thin to moderately fleshy, Henneke et al., 1983). The management and  
109 feeding of horses was based on the UQ Equine Unit's usual practices and throughout the  
110 study period horses were managed on pasture with no additional supplementary feeding, other  
111 than the experimental test diets. The study was conducted between the months of April and  
112 May 2015.

113

114 ***Diets and flavours***

115 Four pelleted diets were formulated with similar digestible energy (DE) content (mean;  $12.6 \pm$   
116 SD; 0.22 Megajoule (MJ)) but differing in crude protein (CP) levels (Low CP (LP); 14% and  
117 High CP (HP); 27%) and added sweetener (included or absent). The chemical analysis of the  
118 diets is presented in Table 1. The pelleted diets were manufactured at the University of New  
119 England. The low energy/fibre pellets comprised of soybean hulls, beet pulp, black sunflower  
120 seeds and corn. To contrast the CP levels a proportion of corn was replaced with corn gluten  
121 in the HP diet. A commercially sourced human-grade non-caloric natural sweetener (blend of  
122 erythritol and stevia; Natures Flavors Inc, Orange, CA, USA) was added at 2.25% to one  
123 choice of the LP and HP diets. Erythritol is 60–70% as sweet as sucrose (table sugar) (de  
124 Cock, 2012) and Stevia is 300 times sweeter than table sugar (Goyal et al., 2010), yet both are  
125 almost non-caloric; the commercial blend had a 1:1 sensation with table sugar. To our  
126 knowledge no equine studies are known that have tested sweeteners in horse diets, therefore

127 the inclusion of 2.25% sweetener was based on an equal sugar sensation as 5% cane molasses  
128 inclusion, which is a standard rate used in sweet feeds by horse feed companies (Pratt-Phillips  
129 and Lawrence, 2014). Cane molasses is about 45-50% sugar (Najafpour and Poi Shan, 2003).

130

131 The four pelleted diets were paired with one of four odours (banana, coconut, cinnamon and  
132 spearmint) and the combination was randomised based on horse groups (Table 2).  
133 Commercially sourced human-grade (non-caloric) food flavour emulsions (coconut, banana,  
134 spearmint and cinnamon; Natures Flavors Inc, Orange, CA, USA) were used to make up  
135 odour solutions. Each odour was selected from a different odour class to aid the contrast i.e.  
136 fruit flavour (banana), nut flavour (coconut), herb flavour (spearmint) and spice flavour  
137 (cinnamon). Between 1 and 10 ml was diluted in 500 ml water to create a distinctive odour  
138 that was detectable by human senses and accepted by horses. The dilution ratio was based on  
139 a pilot study with four horses that were not part of this study. The diluted odour solutions  
140 were stored in four marked spraying bottles and 2-5 ml was misted (based on two enclosed  
141 hand squeezes of the spraying nozzle) onto the diets before they were offered to the horses.

142

### 143 ***Experimental design***

144 The study was conducted in three phases. Before commencing the experiment, 16 horses were  
145 allocated to one of the four groups (A, B, C, D) (Table 2). The grouping of horses was done to  
146 ensure that the experiment was able to test the hypothesis based on nutrient composition and  
147 avoid bias to one particular odour. Hence each of the four diets was linked to all possible  
148 odour combinations (Latin square 4 x 4). Each horse was paired with another of similar  
149 weight, age and sex before randomly allocating one horse from each pair to one of the four  
150 groups (Table 3). This resulted in 2 groups with 3 female horses and 1 male horse and 2

151 groups with 2 female horses and 2 male horses with an almost identical weight and age  
152 distribution.

153

154 During phase 1 (adaptation) all horses were offered four pelleted diets paired with one of the  
155 four odours according to their allocated group, over a period of 20 days. Each diet was  
156 presented solely for five consecutive days to allow horses to make an association between  
157 each of the four diets and its allocated odour. This monadic phase also ensured that all horses  
158 were primed by this dietary experience (regardless of previous experiences) and equalized  
159 diet acceptance (intake of 80% or more) over five days. In phase 2 a series of two-choice tests  
160 were conducted with four diet combinations (contrasts) over three consecutive days to  
161 determine preferences (Table 4). Finally, in phase 3 preferences were tested again using a  
162 multiple-choice model that utilised a chequerboard design over a period of five days. The  
163 timeline of the experiments is illustrated in Figure 1.

164

### 165 *Testing procedures*

166 For the duration of phases 1 and 2, horses were individually fed in a yard that was familiar to  
167 them with other horses in sight to prevent undesired behaviours. In phase 1, horses were  
168 presented their allocated diet (400 g) for 15 minutes on five consecutive days before  
169 switching to the next diet/odour pair. In phase 2, horses were presented with two food choices  
170 (2 x 200 g) simultaneously (5 min). All four contrast two-choice tests were conducted on the  
171 same day, and this was repeated over three consecutive days. Horses were tested in a  
172 sequential order and presented with two tests consecutive with a 10 minutes break between.  
173 After all horses were tested the remaining two tests were presented in a similar fashion. The  
174 combination of the consecutive tests was randomised daily. The diets were presented in  
175 feeding tubs of a similar colour that were labelled for each odour to avoid odour mixing.

176 These feeding tubs were placed in larger bins that were mounted on the yard railing and under  
177 a shelter. When two food choices were offered the buckets were 0.5 m apart and the position  
178 of the bucket changed randomly for each testing day. Horses had *ad libitum* access to water in  
179 their yards. On completion of testing horses were returned to pasture.

180

181 In phase 3 a barren testing area (12 m x 12 m) divided into 16 zones (2.5 m<sup>2</sup>) was used for the  
182 multiple-choice test. There were four zones allocated to each diet option in a chequerboard  
183 fashion, which was adapted from our previous study (van den Berg et al., 2016a) (Figure 2).  
184 Each zone contained 100 g of one of the diets, which was offered in feeding tubs of a similar  
185 colour and placed in rubber tyres. To avoid odour mixing each feeding tub was labelled for  
186 odour (4 x 4) and used throughout the testing period. In addition, the rubber tyres were  
187 labelled with coloured tape corresponding to the odour to facilitate randomisation to zones.  
188 Rubber matting 1 x 1 m was placed under the feeding tubs and rubber tyres. Horses were  
189 individually led into the testing area by a handler and allowed 7.5 min to forage the area  
190 uninhibited. A longer testing period was selected to allow for exploration and movement time  
191 between zones/buckets. On every testing day the diets were randomly allocated to a new zone.  
192 There were group yards with companion animals on both sides of the testing area. Before the  
193 start of the experiment, horses were familiarised with the test area and the routine of leading  
194 them separately into the testing area (Figure 1). On completion of testing horses were returned  
195 to pasture.

196

### 197 ***Feeding and measurements***

198 In phase 1, horses were fed the single diets in the morning between 08:30 to 09:30 h and the  
199 intake (g) recorded on each of the five days. In phase 2 the four two-choice contrast tests (5  
200 min each) were conducted in two parts; morning (08:00 – 12:00 h) and afternoon (13:00-

201 17:00 h) and in phase 3 the multiple-choice test (7.5 min) was conducted between 8:00-12:00  
202 h. Behaviours for phase 2 and 3 were recorded with two video recorders (Panasonic HC-  
203 V160, Panasonic Corporation, Kadoma, Osaka, Japan and GoPro Hero 3+, GoPro, San  
204 Mateo, CA, USA) and by a person sitting 10 m outside the testing arena (under a shelter  
205 construction). The number of visits to each bucket or zone (categorised as both front hooves  
206 being placed in a zone) and sequence to each zone/bucket were documented. In addition, the  
207 time spent foraging (labelled as standing and chewing) or moving to each zone/bucket  
208 (classified as walking towards a new zone/bucket) were recorded. The intake of foods by each  
209 horse was determined by weighing the foods in each feeding bucket before and after each test.  
210 The intake was adjusted for moisture and calculated to a dry matter (DM) basis.

211

### 212 *Statistical analysis*

213 Diet intake, bucket/zone visits and time spent foraging or moving were analysed in R Studio  
214 version 0.99.484 (Team, 2015) and all data were checked for normality (Q-Q plots and  
215 Shapiro-Wilk test) and transformed where necessary. For all tests the level of significance  
216 was set to 5%.

217

### 218 *Phase 1: Adaptation*

219 Feed intake of each diet over the four weeks was assessed to determine the acceptance of the  
220 diets and post-ingestive associations. We considered an intake of 80% (~ 300 g DM) as the  
221 threshold for diet acceptance, based on the identified plateau curve of feed intake. The intake  
222 of each diet (and week) was denoted as the proportion (%) consumed out of the total offered  
223 and were logit-transformed. However, due to the large variation between the animals in feed  
224 intake behaviour on the first and second day of the diet introduction none of the classical  
225 statistical models applied showed a correct fit. Therefore, descriptive analyses were

226 conducted and the variance between diets, odours, groups and days were examined using a  
227 Fligner-Killeen test of homogeneity of variances.

228

### 229 *Phase 2: Two-choice contrast tests*

230 To determine the diet preference of each two-choice test the intake ratio of lower (Bucket 1)  
231 to higher (Bucket 2) palatability contrast over a 3-day testing period was examined using a  
232 generalized linear model (GLM) with a binomial distribution. In the model day and group  
233 were included as factors; odour was left out of the model as it was coupled to the group.  
234 Similar GLM models were used for the ratios (Bucket 1: Bucket 2) of bucket visits and time  
235 spent foraging or moving towards the buckets. Additionally, the levels of the diets, odours  
236 and groups (independent variables-factors) for all tests and days of Phase 2 were ranked using  
237 three linear regression models having the intake (g, DM) as response variable.

238

### 239 *Phase 3: Multiple-choice test*

240 The intake (g, DM) of each diet over the 5-day testing period was examined using a linear  
241 regression model with diet, day, odour and group included as factors. A similar model was  
242 used for the time spent foraging. For the zone count a GLM model with a Poisson distribution  
243 was fitted with diet, day, odour and group as factors. For the time spent moving a similar  
244 GLM model was used with the same explanatory factors.

245

## 246 **Results**

247

### 248 *Phase 1: Adaptation*

249 The intake proportion (%) of the four diets consumed out of the total offered over five days is  
250 given in Figure 3. The Fligner-Killeen tests indicated a departure from homogeneity for the

251 population's variances of intake proportions between diets ( $P < 0.001$ ) and days ( $P < 0.001$ ). In  
252 week 1 (LP diet), a large variation in intake between horses was observed on Day 1 and 2  
253 (from 0% to 100% ingestion), which declined over time with 12 out of 16 horses consuming  
254 90% or more after Day 2 and by Day 5 all horses ingested 95-100% of the offered diet. In  
255 week 2 (LP+ diet) a greater variation was only observed during the first two days, with all  
256 horses consuming over 90% of the offered diet after Day 2. Similar patterns were observed  
257 for week 3 (HP diet), however one horse was below 90% intake on Day 4 only. In week 4  
258 (HP+ diet), horses showed a stable intake (95-100%) over all days, with only one horse below  
259 80% on Day 4 and one horse below 90% on Day 5. The decreasing pattern in variance over  
260 time was also observed when reviewing the intake proportions for each group and odour.  
261 However, the Fligner-Killeen tests indicated a departure from homogeneity for the  
262 population's variances of intake proportions for groups ( $P < 0.001$ ), whereas we cannot reject  
263 the null-hypothesis for odours ( $P = 0.08$ ); indicating an equality of variance. The plotted data  
264 of Group B and D showed a larger distribution of variance compared to Group A and C.

265

#### 266 *Phase 2: Two-choice contrast tests*

267 The fitted parameters of the GLM (binomial) model to ratios of intake, bucket visits and time  
268 spent foraging or moving of lower (Bucket 1) to higher (Bucket 2) palatability contrast for the  
269 four two-choice tests are given in Table 5. Data is presented as log-transformed ( $\pm$  SE) and  
270 expected back-transformed (multiplicative) ratios. Expected back-transformed ratios are used  
271 for the interpretation of the results for each test.

272

#### 273 *Test 1: LP vs. LP+*

274 Analysis of deviance using GLM models indicated a significant effect for days ( $P = 0.02$ ). The  
275 expected intake ratios were increased for Day 2 ( $\times 1.09$ ) and Day 3 ( $\times 1.11$ ) compared to the

276 initial ratio (0.93). Groups did not contribute to the model at the 5% significance level  
277 ( $P=0.051$ ). Similar results were found for the time spent foraging ratio, showing a significant  
278 contribution for day factor (deviance test;  $P<0.001$ ). In addition, a significant group effect  
279 was recorded (deviance test;  $P<0.001$ ). The expected ratio was decreased for Group B (x  
280 0.81), showing that more time was spent foraging on the LP+ diet, compared to the initial  
281 ratio (0.92). For both the bucket visit and time spent moving ratios the analysis of deviance  
282 did not suggest a contribution for days and groups.

283

#### 284 *Test 2: LP vs. HP*

285 For the intake ratios the day factor did not contribute to the model showing similar ratios  
286 across days. Only a significant contribution for groups (deviance test;  $P<0.001$ ) was observed.  
287 The expected intake ratio was decreased for Group B (x 0.9), showing a greater preference for  
288 the HP diet, compared to the initial ratio (0.93). This was linked to a significant odour effect  
289 (deviance test;  $P<0.001$ ), indicating a lower intake ratio for the diet linked to the cinnamon  
290 odour (i.e. LP diet for Group B). Comparable results for the time spent foraging were found,  
291 suggesting no effect for days. A significant contribution for groups (deviance test;  $P<0.001$ )  
292 was observed. The expected ratio was decreased for Group B (x 0.76) compared to the initial  
293 ratio (0.86), whereas the ratios for Group C (x 1.12) and D (x 1.05) were increased. Group A  
294 and Group B appeared to spend more time foraging on the HP diet. For both the time spent  
295 moving and bucket visit ratios the day and group factors did not contribute to the models.

296

#### 297 *Test 3: HP vs. HP+*

298 The GLM model does not suggest a significant contribution for days and groups for the intake  
299 ratio. However, for time spent foraging day factor (deviance test;  $P<0.001$ ) contributed to the  
300 model. The expected ratios were increased for Day 2 (x 1.28) and Day 3 (x 1.06) compared to

301 the initial ratio (0.9). In addition, a significant contribution for group factor (deviance test;  
302  $P < 0.001$ ) was observed. The expected time spent foraging ratios were increased for Group C  
303 ( $\times 1.15$ ) and Group D (1.09) compared to the initial ratio (0.9). For both bucket visit and time  
304 spent moving ratios the analysis of deviance did not suggest a contribution for days and  
305 groups.

306

#### 307 *Test 4: LP+ vs. HP+*

308 The analysis of deviance suggests that only the group factor ( $P = 0.003$ ) contributed to the  
309 model for the intake ratios. The expected intake ratio was decreased for Group B ( $\times 0.86$ ),  
310 showing a greater preference for the HP+ diet, compared to the initial ratio (0.99). This was  
311 linked to a significant odour effect (deviance test;  $P < 0.001$ ), indicating a lower intake ratio for  
312 the diet linked to the coconut odour (i.e. LP+ diet for Group B). The GLM model for the time  
313 spent foraging suggests a contribution for day ( $P < 0.001$ ). The expected ratio was decreased  
314 for Day 3 ( $\times 0.79$ ) compared to the initial ratio (1.19). There was also a significant group  
315 effect ( $P < 0.001$ ) recorded for the time spent foraging ratios. The expected ratios were  
316 decreased for Group B ( $\times 0.64$ ), Group C ( $\times 0.88$ ) and Group D ( $\times 0.87$ ), showing that more  
317 time was spent foraging on the HP+ diet, compared to the initial ratio (1.19). For both the  
318 bucket visits and time spent moving ratios the day and group factors did not contribute to the  
319 model.

320

#### 321 *Ranking*

322 The rankings of the diets, odours and groups were based on the mean intake (g, DM) of all  
323 tests and days combined. A significantly lower mean intake was recorded for the LP diet  
324 (163.9) compared to the other diets with the highest consumption for the HP+ diet (177.0)  
325 (SE;  $\pm 1.73$ ;  $P < 0.05$ ). Mean intake of HP (171.1) and LP+ (169.6) diets did not significantly

326 differ. No significant differences between odours were recorded, showing a similar mean  
327 intake for spearmint (172.5), banana (171.5), coconut (169.9) and cinnamon (167.6) (SE;  $\pm$   
328 1.78). The difference between cinnamon and spearmint approached significance ( $P=0.053$ ). A  
329 significantly greater consumption was recorded for Group C (179.8) and D (178.6) compared  
330 to Group A (167.9), with Group B (155.2) showing the lowest mean intake (SE;  $\pm$  1.47;  
331  $P<0.001$ ).

332

### 333 *Phase 3: Multiple-choice test*

334 The fitted parameters of the Linear regression and GLM (Poisson) models to intake, zone  
335 count and time spent foraging or moving are given in Table 6. The fitted parameters of the  
336 GLM models are presented as log-transformed ( $\pm$  SE) and expected back-transformed means.  
337 Expected back-transformed means (multiplicative) are used for the interpretation of the time  
338 spent moving and zone count results.

339

### 340 *Intake and time spent foraging*

341 The ANOVA using linear models indicated a significant effect for diet, odour and group  
342 ( $P<0.001$ ). The intercept of the model was  $109.3 \pm 15.0$  g and comprised LP diet, Day 1,  
343 Group A and banana odour. A significantly lower mean intake (g) was observed for the LP  
344 diet compared to the other diets with the highest consumption for the HP+ diet (increase of  
345  $73.6 \pm 11.3$  g) ( $P<0.001$ ). Mean diet intake increased with  $40.3 \pm 11.3$  g for the LP+ diet and  
346  $41.5 \pm 11.3$  g for the HP diet, which did not differ significantly. No differences in mean intake  
347 between the days ( $P=0.52$ ) were recorded but there was a significantly greater preference for  
348 banana odour compared to cinnamon ( $-34.7 \pm 11.3$  g) and spearmint odour ( $-55.0 \pm 11.3$  g)  
349 ( $P<0.001$ ). A group difference was observed, with Group D ( $50.9 \pm 11.3$  g) and Group C

350 (45.8 ± 11.3 g) having a significantly higher intake compared to group A (P<0.001), but  
351 Group A did not differ from Group B.

352

353 A strong linear correlation between the intake and time spent foraging (r=0.80) was observed.  
354 The linear models suggested a significant effect for diet and odour (ANOVA; P<0.001). The  
355 intercept of the model was 89.6 ± 11.2 sec and comprised LP diet, Day 1, Group A and  
356 banana odour. In accordance with the intake, significantly less time was spent foraging (sec)  
357 on the LP diet compared to the other diets (P<0.001), and the greatest time spent foraging was  
358 observed for the HP+ diet (increase of 44.6 ± 8.5 sec). More time was spent foraging on diets  
359 linked to the banana odour compared to the other odours (P<0.001). No differences in mean  
360 time spent foraging were observed for the different days and groups.

361

#### 362 *Time moving and zone count*

363 Whilst there was a high correlation between time spent moving and zone count (r=0.94),  
364 showing a very close agreement, we continued using the time spent moving and zone counts  
365 as dependent variables to the two GLM models. The analysis of deviance for time spent  
366 moving towards zones/buckets suggests a significant effect for diets (P=0.013), days  
367 (P=0.009), group (P<0.001) and odour (P<0.001). The expected mean for the intercept was  
368 8.8 sec and comprised LP diet, Day 1, Group A and banana odour. The model indicated that  
369 horses spent more time moving towards HP (x 1.16) and HP+ (x 1.13) diets compared to LP  
370 diet, which did not differ from LP+ diet (x 1.01). Horses spent more time moving on Day 5 (x  
371 1.18) compared to the other days. Group A spent more time moving towards zones/buckets  
372 compared to Group D (x 0.84) with the lowest time observed for Group B (x 0.61). In  
373 accordance with the intake and time spent foraging trends, less time was spent moving  
374 towards the diets with spearmint odour (x 0.77) compared to the other odours. The GLM

375 model suggests only a significant effect for groups on the zone count (deviance test;  
376  $P < 0.001$ ). The expected mean for the intercept was 2.7 and comprised LP diet, Day 1, Group  
377 A and banana odour. Group B ( $x = 0.62$ ) made fewer zone visits compared to the other groups.

378

## 379 **Discussion**

380 We hypothesised that horses would display more distinct patch foraging behaviour in the  
381 multiple-choice model selecting all available foods, and that horses would rank preferences  
382 based on nutritional content, followed by taste then odour. The key findings of this study  
383 were: (1) An initial large variation in diet intake was observed in the adaptation phase with  
384 some horses showing a neophobic response while others exhibited no apparent recognition of  
385 the odour/food being new, but variances declined within 2 days with majority of the horses  
386 consuming over 90% of the diets. (2) Nutrient (HP) content appeared to be the main driver for  
387 diet selection and feed intake in both preference tests. (3) Taste appeared to be the secondary  
388 determinant for preference by horses and this was more evident with the lower CP diet. (4) A  
389 greater intake of diets linked to sweet aromatic odours (banana and coconut) was observed.  
390 (5) The multiple-choice test promoted patch foraging behaviour and showed more explicit  
391 differences in diet selection compared to the two-choice test. (6) A significant group effect for  
392 diet preference and total feed intake was recorded.

393

### 394 *The influence of nutrients on diet selection*

395 After the monadic phase the preferences for the four diets were initially evaluated in four  
396 contrast tests using a two-choice test. None of the models were able to demonstrate that  
397 horses had an obvious preference for diets with a greater palatability, showing a close to 1:1  
398 intake ratio for most of the tests and days. Yet, some of the tests suggested that more time was  
399 spent foraging on the diets with enhanced palatability, showing a slight departure from a 1:1

400 ratio; which was not consistent for all test days. The discrepancy between the observations for  
401 intake and time spent foraging may be a result of the fact that a number of horses were able to  
402 empty both buckets before the 5 min time period had elapsed and subsequently continued  
403 visiting the buckets to try and obtain left-over pellets. Therefore some of the time spent  
404 foraging could have been searching rather than ingestive behaviour. In hindsight, the test time  
405 should have been 3.5-4 min. Nonetheless, the contrast test results and mean intake ranking of  
406 diets suggest that horses did discriminate based on the nutrient content and showed a  
407 preference for the higher CP diet. This difference was less evident when a sweetener was  
408 added to the diet, an observation supported by the mean intake measures showing a ranking  
409 based on protein content but there were no significant differences in intake for the LP+ and  
410 HP diets. A similar ranking was also recorded in the multiple-choice test and these findings  
411 are in accord with other studies that have reported that preferences and intake are linked to  
412 macronutrient content (Laut et al., 1985; Cairns et al., 2002; Goodwin et al., 2005a; Redgate  
413 et al., 2014; van den Berg et al., 2016b). Such studies demonstrate that horses can  
414 discriminate between diets based on both energy and CP content, even if foods are novel and  
415 regardless of flavour (odour) preferences.

416

#### 417 *The influence of sweetener and odour on diet selection*

418 Diet preferences due to flavours have not been widely examined in horses (Burton et al.,  
419 1983; Kennedy et al., 1999; Goodwin et al., 2005a; b) and in these studies it is not always  
420 clear what type of flavouring was used; for example non-nutritive vs nutritive, or aromatic vs  
421 taste that may have calories or not (sugar versus artificial or natural sweeteners). In the  
422 present study a non-caloric (natural) sweetener was used so that a taste effect could be  
423 assessed without interfering with the nutritional content. While nutrient content seems to be  
424 the primary determinant for diet selection, the results of the two-choice and multiple-choice

425 testing also suggest that an added taste enhances preference, with a partial preference for LP+  
426 and HP and the highest consumption for HP+.

427

428 A recent study has shown that horses express the taste receptor gene T1R2 in lingual  
429 epithelium (taste buds) and both T1R2 and T1R3 in intestinal endocrine cells, which play an  
430 important role in the sensing of sugars and other sweet compounds (Daly et al., 2012).  
431 However, to our knowledge there are no previous equine studies that have reported the use of  
432 non-caloric artificial or natural sweeteners in horse diets and that clearly show the positive  
433 effects on preferences of taste using non-caloric natural sweeteners. The inclusion of artificial  
434 or natural sweeteners to animal diets is a common practice in the swine industry (Munro et al.,  
435 2000; Sterk et al., 2008; Moran et al., 2010) where sweeteners are routinely included in piglet  
436 diets to enhance feed palatability and avoid a drop in feed intake post-weaning. However,  
437 there are somewhat variable results of the effect of sweetener on feed intake, feed conversion  
438 and daily weight gain in piglets; showing positive effects when an artificial sweetener  
439 (Sucram) was used (Sterk et al., 2008), whereas the natural sweetener Stevia did not appear to  
440 have detrimental effects on feed consumption and performance of piglets (Munro et al.,  
441 2000). It is well known that stevia can have a bitter aftertaste in humans (Goyal et al., 2010),  
442 which could explain why stevia may not be as useful in enhancing palatability. In our study  
443 we used a blend of erythritol and stevia (with erythritol being the bulk sweetener), which  
444 reduces the bitter aftertaste of stevia and provides an equal sugar (1:1) sensation (de Cock,  
445 2012). As a bulk sweetener, erythritol provides volume, texture and microbiological stability  
446 similar to sucrose. In addition, quantitative descriptive analysis shows that erythritol solutions  
447 taste similar to sucrose (de Cock, 2012) and therefore may be more effective in enhancing  
448 palatability. While this study showed the positive effect of a blend of erythritol and stevia on  
449 diet preference, further research is needed that tests the effect of different (pure and blended)

450 natural and artificial sweeteners on the food palatability and voluntary feed intake by horses.  
451 This could provide new insight in useful additives for the horse feed industry.

452

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

460

#### 461 *Multiple-choice test model to simulate patch foraging conditions*

462 In a natural or grazing environment horses select from a diverse range of resources, which  
463 suggests that multiple-choice tests may be advantageous when assessing preferences. In the  
464 present study a chequerboard ‘patch’ design was used, which clearly demonstrated that horses  
465 select from all foods but have ranked preferences associated with macronutrients, taste then  
466 odour. This ranking was also identified in the contrast tests based on the mean intake of the  
467 diets, but was less obvious when two diets were compared (contrasts). It seems that a patch  
468 design was the most appropriate for pasture field studies that reviewed the preference for  
469 short and tall sward heights (Naujeck et al., 2005; Edouard et al., 2009; Edouard et al., 2010).  
470 Other equine studies (Goodwin et al., 2002; Thorne et al., 2005; Goodwin et al., 2007) have  
471 used a multiple choice design to assess the intake and feeding behaviour of stabled horses and  
472 demonstrated that horses selected from preferred and less preferred forages, evidently mixing  
473 diets. Goodwin et al. (2007) also showed that horses moved between forage locations  
474 regardless of the palatability of the forages or horse’s preference for a particular forage

475 indicating that searching/ patch foraging behaviour is an important component in diet  
476 selection by horses.

477

478 In the present study, searching behaviour, i.e. time spent moving towards the buckets/ zones  
479 and the visits to each bucket/zone, was assessed in both the two-choice and multiple-choice  
480 test. No differences in the ratios for bucket visits and time spent moving between days and  
481 groups were recorded for the two-choice testing. In addition, the results showed a close to 1:1  
482 ratio for time spent moving and bucket visits for all tests. In the multiple-choice test horses  
483 did spend significantly more time moving towards the HP and HP+ diets compared to the LP  
484 and LP+ diets. However no differences in the mean zone count between diets were observed.  
485 The equal zone count suggests that horses displayed continuous sampling behaviour and  
486 possibly did not appear to use spatial cues to identify preferred patches/ zones. This confirms  
487 the findings of a previous study (van den Berg et al., 2016a). It has been suggested that  
488 grazing animals may rely more on visual or orosensory cues rather than on memory of spatial  
489 cues when faced with a heterogeneous environment (unpredictability) and depending on the  
490 spatial and temporal scale of the foraging hierarchy (Illius and Gordon, 1990; Hewitson et al.,  
491 2005). Hewitson et al. (2005) demonstrated that sheep can use spatial cues on the smaller  
492 spatial scales (feeding site or patch) to improve foraging efficiency where resource  
493 distribution was predictable, but when feed position became less predictable animals  
494 increased sampling behaviour, which suggests that grazing animals can switch between  
495 foraging tactics. In this study, where feed bucket positions were daily randomised, the  
496 motivation to move from one patch to another can therefore be related to sampling behaviour  
497 (trial and error), which allows animals to get information about the sensory characteristics that  
498 animal's link to the nutritional consequences of foods (olfactory memory).

499

500 *Group effect*

501 A strong group effect was observed for both the two-choice and multiple-choice tests with  
502 Group B showing a significantly greater preference for the diets with greater palatability  
503 (higher contrast) compared to the other groups in the two-choice contrast tests. This was  
504 linked with the lowest overall mean intake and was similar for both test protocols. This group  
505 also spent less time moving and had the lowest mean zone count, which makes this group of  
506 horses more selective in terms of feed choices. It is unclear why this group displayed such  
507 differences as the groups were randomly allocated based on age, weight and sex. The age of  
508 the group ranged from 4 to 14, showing a similar age distribution as Group A and C. Group D  
509 had a lower average age, however like Group B had 1 male horse and 3 female horses. In  
510 addition, during the adaptation phase both Group B and D showed similar variance in diet  
511 intake. Therefore these results may simply reflect individuality and highlight that there may  
512 be large variation between animals in how they regulate intake of nutrients to meet dietary  
513 needs. Further studies that integrate nutritional geometry models could gain more insight in  
514 these regulatory mechanisms of individuals. In a geometric framework for nutrition, the  
515 important components of animal nutrition (e.g. foods, nutrient requirements, nutrient  
516 utilisation) are defined in a Cartesian space, where each dimension represents a food  
517 constituent (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). While  
518 these frameworks have been extensively studied in various insect and vertebrate species, at  
519 present no studies have been conducted with horses (Raubenheimer and Simpson, 1997). This  
520 highlights the opportunity to integrate these geometric models to answer some of the more  
521 complex questions as to how (individual) horses use nutrient intake targets to regulate feed  
522 intake given a number of choices.

523

524 **Conclusion**

525 This study was able to show that horses sample all diets on offer but show clear preferences  
526 ranked on nutrients, followed by taste then odour. This ranking was more evident in the  
527 multiple-choice testing than the two-choice testing and suggests that a multiple-choice model  
528 such as a chequerboard design could be more informative when ranking preferences.  
529 However, an adaptation period is needed to allow for post-ingestive associations. Further  
530 research is required to assess the use of these types of preference models in natural or pasture  
531 environments. While our study is in accordance with other research showing that nutrients  
532 have a strong influence on diet selection, we should also acknowledge the importance of taste  
533 and odour on diet selection. To our knowledge this is the first study that has been able to  
534 show the positive effects of a non-caloric natural sweetener (erythirol and stevia blend) on  
535 diet intake and selection. This new knowledge could be useful for enhance palatability in  
536 equine diets, without affecting the glycaemic index. However, further studies are needed that  
537 evaluate different types of sweeteners coupled with and without odour and/or dietary nutrients  
538 and its long-term effects on food intake by horses.

539

#### 540 **Conflict of interest**

541 Funding for this project was kindly provided by the University of New England, New South  
542 Wales, Australia. We wish to confirm that there are no known conflicts of interest associated  
543 with this publication and there has been no additional financial support for this work that  
544 could have influenced its outcome.

545

#### 546 **Ethical statement**

547 The care and use of the animals followed the guidelines set by The University of New  
548 England Animal Ethics Committee, in accordance with section 25 of the Animal Research  
549 Act (1985).

550

551 **Acknowledgements**

552 The authors would like to thank the University of Queensland (UQ Equine Unit) for their kind  
553 assistance in providing horses and facilities. We also acknowledge Michael Raue for his help  
554 with the logistics of this research project. For assistance during the experiments and care of  
555 horses we would like to give special thanks to all the staff and students from UQ; Mitchell  
556 Coyle, Luke Gilbert, Paula Lever, Glenn Reisenleiter, Camille Hilliere, Charmaine Tan,  
557 Louise Cooper, Roxy Cameron, Jess Blockland and Courtney Windsor.

558 **References**

559

560 Berthoud, H-R., 2004. Neural control of appetite: cross-talk between homeostatic and non-  
561 homeostatic systems. *Appetite* 43, 315-317.

562 Burton, J.H., Price, D.J., Aspinal, J., 1983. The effect of feed flavour and feed consumption in  
563 horses. In: *Proceedings of the 8th Equine Nutrition and Physiology Symposium*, Lexington,  
564 Kentucky, USA, pp. 27-32.

565 Cairns, M.C., Cooper, J.J., Davidson, H.P.B., Mills, D.S., 2002. Association in horses of  
566 orosensory characteristics of foods with their post-ingestive consequences. *Anim. Sci.* 75,  
567 257-265.

568 Cuddeford, D., 2005. Voluntary food intake by equids. *Pferdeheilkunde* 21, 7-8.

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

573 Danel, J., Merkies, K., 2009. Discrimination and selection by horses of sucrose solution. *J.*  
574 *Equine Vet. Sci.* 29, 384-385.

575 de Cock, P., 2012. Erythritol, in: O'Donnell, K., Kearsley, M. (Eds.), *Sweeteners and sugar*  
576 *alternatives in food technology*. Wiley-Blackwell Publishers, West Sussex, UK, pp. 215-242.

577 Dulphy, J.P., Martin-Rosset, W., Dubroeuq, H., Ballet, J.M., Detour, A., Jailler, M., 1997.  
578 Compared feeding patterns in ad libitum intake of dry forages by horses and sheep. *Livest.*  
579 *Prod. Sci.* 52, 49-56.

580 Edouard, N., Duncan, P., Dumont, B., Baumont, R., Fleurance, G., 2010. Foraging in a  
581 heterogeneous environment - An experimental study of the trade-off between intake rate and  
582 diet quality. *Appl. Anim. Behav. Sci.* 126, 27-36.

583 Edouard, N., Fleurance, G., Dumont, B., Baumont, R., Duncan, P., 2009. Does sward height  
584 affect feeding patch choice and voluntary intake in horses? *Appl. Anim. Behav. Sci.* 119, 219-  
585 228.

586 Garcia, J., 1989. Food for Tolman: cognition and cathexis in concert, in: Archer, T., Nilsson,  
587 L-G. (Eds.), *Aversion, Avoidance and Anxiety*. Lawrence Erlbaum Associates, Hillsdale,  
588 New Jersey, UK, pp. 45-85.

589 Gherardi, S., Black, J., 1991. Effect of palatability on voluntary feed intake by sheep. I.  
590 Identification of chemicals that alter the palatability of a forage. *Aust. J. Agric. Res.* 42, 571-  
591 584.

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

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

- 596 Goodwin, D., Davidson, H.P.B., Harris, P., 2005b. Sensory varieties in concentrate diets for  
597 stabled horses: effects on behaviour and selection. *Appl. Anim. Behav. Sci.* 90, 337-349.
- 598 Goodwin, D., Davidson, H.P.B., Harris, P., 2007. Responses of horses offered a choice  
599 between stables containing single or multiple forages. *Vet. Rec.* 160, 548-551.
- 600 Goyal, S.K., Samsher, Goyal, R.K., 2010. Stevia (*Stevia rebaudiana*) a bio-sweetener: a  
601 review. *Int. J. Food Sci. Nutr.* 61, 1-10.
- 602 Henneke, D., Potter, G.D., Kreider, J.L., Yeated, B.F., 1983. Relationship between condition  
603 score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15, 371-372.
- 604 Hewitson, L., Dumont, B., Gordon, I.J., 2005. Response of foraging sheep to variability in the  
605 spatial distribution of resources. *Anim. Behav.* 69, 1069-1076.
- 606 Houpt, K.A., Zahorik, D.M., Swartzman-Andert, J.A., 1990. Taste aversion learning in  
607 horses. *J. Anim. Sci.* 68, 2340-2344.
- 608 Illius, A.W., Gordon, I.J., 1990. Constraints on diet selection and foraging behaviour in  
609 mammalian herbivores, in: Hughes, R.N. (Ed.), *NATO Advanced Research Workshop on  
610 Behavioural Mechanisms of Food Selection*. Springer-Verlag, Gregynog, Wales, UK, pp.  
611 369-393.
- 612 Kennedy, M.A.P., Currier, T., Glowaky, J., Pagan, J.D., 1999. The influence of fruit flavours  
613 in feed preference in Thoroughbred horses. In: *Proceedings of Kentucky Equine Research  
614 Nutrition Conference*, Lexington, Kentucky, USA, pp. 70-72.
- 615 Laut, J.E., Houpt, K.A., Hintz, H.F., Houpt, T.R., 1985. The effects of caloric dilution on  
616 meal patterns and food intake of ponies. *Physiol. Behav.* 35, 549-554.
- 617 Merkies, K., Bogart, E., 2013. Discrimination of sour and sweet solutions by mature horses. *J.*  
618 *Equine Vet. Sci.* 33, 330-331.
- 619 Moran, A.W., Al-Rammahi, M.A., Arora, D.K., Batchelor, D.J., Coulter, E.A., Daly, K.,  
620 Ionescu, C., Bravo, D., Shirazi-Beechey, S.P., 2010. Expression of Na<sup>+</sup>/glucose co-  
621 transporter 1 (SGLT1) is enhanced by supplementation of the diet of weaning piglets with  
622 artificial sweeteners. *Br. J. Nutr.* 104, 637-646.
- 623 Munro, P.J., Lirette, A., Anderson, D.M., Ju, H.Y., 2000. Effects of a new sweetener, Stevia,  
624 on performance of newly weaned pigs. *Can. J. Anim. Sci.* 80, 529-531.
- 625 Najafpour, G.D., Poi Shan, C., 2003. Enzymatic hydrolysis of molasses. *Bioresour. Technol.*  
626 86, 91-94.
- 627 Naujeck, A., Hill, J., Gibb, M.J., 2005. Influence of sward height on diet selection by horses.  
628 *Appl. Anim. Behav. Sci.* 90, 49-63.
- 629 Pain, S., Revell, D.K., 2007. Natural odorants can influence the preference of horses for  
630 lucerne hay. In: *Proceedings of Recent Advances in Animal Nutrition in Australia*, Armidale,  
631 NSW, Australia, pp. 27-33.

- 632 Pain, S., Revell, D.K., 2009. Fodder quality specifications: Identifying predictors of  
633 preference between hays. Rural Industries Research and Development Corporation,  
634 Publication no. 09/011. Kingston, ACT, Australia.
- 635 Pratt-Phillips, S.E., Lawrence, L.M., 2014. Nutrition of the performance horse, in: Hodgson,  
636 D.R., McKeever, K.H., McGowan, C.M. (Ed.), *The athletic horse: principles and practice of*  
637 *equine sports medicine*. Elsevier Saunders, St. Louis, Missouri, USA, pp. 34-55.
- 638 Provenza, F.D., 1995. Postingestive feedback as an elementary determinant of food  
639 preference and intake in ruminants. *J. Range Manage.* 48, 2-17.
- 640 Provenza, F.D., Villalba, J.J., 2006. Foraging in domesticated herbivores: linking the internal  
641 and external milieu., in: Bels, V. (Ed.), *Feeding in domesticated vertebrates: from structure to*  
642 *behaviour.*, CABI Publishing, Oxfordshire, UK, pp. 210-240.
- 643 Randall, R.P., Schurg, W.A., Church, D.C., 1978. Response of horses to sweet, salty, sour and  
644 bitter solutions. *J. Anim. Sci.* 47, 51-55.
- 645 Raubenheimer, D., Simpson, S.J., 1993. The geometry of compensatory feeding in the locust.  
646 *Anim. Behav.* 45, 953-964.
- 647 Raubenheimer, D., Simpson, S.J., 1997. Integrative models of nutrient balancing: application  
648 to insects and vertebrates. *Nutr. Res. Rev.* 10, 151-179.
- 649 Redgate, S.E., Cooper, J.J., Hall, S., Eady, P., Harris, P.A., 2014. Dietary experience modifies  
650 horses' feeding behavior and selection patterns of three macronutrient rich diets. *J. Anim. Sci.*  
651 92, 1524-1530.
- 652 Sclafani, A., Ackroff, K., 2004. The relationship between food reward and satiation revisited.  
653 *Physiol. Behav.* 82, 89-95.
- 654 Simpson, S.J., Raubenheimer, D., 1993. A multi-level analysis of feeding behaviour: The  
655 geometry of nutritional decisions. *Phil. Trans. R. Soc. B.* 342, 381-402.
- 656 Sterk, A., Schlegel, P., Mul, A.J., Ubbink-Blanksma, M., Bruininx, E.M.A.M., 2008. Effects  
657 of sweeteners on individual feed intake characteristics and performance in group-housed  
658 weanling pigs. *J. Anim. Sci.* 86, 2990-2997.
- 659 Stubbs, R.J., Whybrow, S., 2004. Energy density, diet composition and palatability:  
660 influences on overall food energy intake in humans. *Physiol. Behav.* 81, 755-764.
- 661 Team, R.D.C., 2015. R: A language and environment for statistical computing, R Foundation  
662 for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- 663 Thorne, J.B., Goodwin, D., Kennedy, M.J., Davidson, H.P.B., Harris, P., 2005. Foraging  
664 enrichment for individually housed horses: Practicality and effects on behaviour. *Appl. Anim.*  
665 *Behav. Sci.* 94, 149-164.
- 666 van den Berg, M., Lee, C., Brown, W.Y., Hinch, G.N., 2016a. Does energy intake influence  
667 diet selection of novel forages by horses? *Livest. Sc.* 186, 6-15.  
668

669 van den Berg, M., Lee, C., Brown, W.Y., Giagos, V., Hinch, G.N., 2016b. Acceptance of  
670 novel food by horses: the influence of food cues and nutrient composition. *Appl. Anim.*  
671 *Behav. Sci.* Under review.

**Table 1. Chemical composition<sup>a</sup> (g/kg dry matter (DM)) of the four 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.**

**Table 2. Four treatment diets and associated odours for each group of horses (n = 4) in a 4 x 4 Latin Square design.**

**Table 3. Sixteen adult horses were paired based on weight, age and sex (mare (M) and gelding (G)) and randomly allocated to one of the four treatment groups to create even animal group characteristics.**

**Table 4. Phase 2: Two-choice test. Diets were paired based on contrast to examine preferences and diet ranking. (LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener).**

**Table 5. GLM (binomial) parameters fitted to ratios of intake, bucket visits and time spent foraging or moving of lower (Bucket 1) to higher (Bucket 2) palatability contrast for the four two-choice tests (16 horses; n=4 per group). The fitted parameters ( $\pm$  SE) of the GLM model with the (back-transformed) expected ratios are presented.**

**Table 6. Linear regression and GLM (Poisson) parameters ( $\pm$  SE) fitted to intake, zone count and time spent foraging or moving for the multiple-choice test (16 horses; n=4 per group). Intake and time spent foraging are based on linear regression models. For time spent moving and zone count fitted parameters of the GLM models with the (back-transformed) expected means are presented.**

**Figure 1. Timeline of the experiments. Phase 1 was the adaptation phase to establish flavour-to-post-ingestive associations (LP; low protein diet, LP+; low protein diet + sweetener, HP; high protein diet and HP+; high protein diet + sweetener). Phase 2 was the two-choice contrast tests (LP v.s. LP+, LP v.s. HP, HP v.s. HP+ and LP+ v.s. HP+). Phase 3 was the multiple-choice test using a checkerboard design (Smörgåsbord).**

**Figure 2. Field and patch layout. A testing area (12 m x 12 m) divided into 16 zones (2.5 m<sup>2</sup>). There were 4 zones allocated to each odour/diet combination in a checkerboard fashion. On every testing day the diets were randomly allocated to a new zone. Horses (n=16) were individually led into the testing area and allowed 7.5 minutes to forage the area uninhibited, which was recorded with video recorders and by direct observation.**

**Figure 3. Feed intake of each diet over the four weeks (adaptation phase) was assessed to determine the acceptance of the diets and post-ingestive associations. For illustration purposes the proportion (%) and trends (line) of diet intake on the logit scale 0-100% (min; -15 to max; 15) over 5 test days was selected (n=16 horses). Logit of 1.4 is equal to 80% feed intake. LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener.**

**Table 1. Chemical composition<sup>a</sup> (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.**

<b>Constituent</b>	<b>LP</b>	<b>LP+</b>	<b>HP</b>	<b>HP+</b>
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

<sup>a</sup>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.

**Table 2. Four treatment diets and associated odours for each group of horses (n = 4) in a 4 x 4 Latin Square design.**

<b>Protein</b>	<b>Sweetener</b>		<b>Group A</b>	<b>Group B</b>	<b>Group C</b>	<b>Group D</b>
Low	-	LP	Coconut	Cinnamon	Spearmint	Banana
Low	+	LP+	Banana	Coconut	Cinnamon	Spearmint
High	-	HP	Spearmint	Banana	Coconut	Cinnamon
High	+	HP+	Cinnamon	Spearmint	Banana	Coconut

**Table 3. Sixteen adult horses were paired based on weight, age and sex (mare (M) and gelding (G)) and randomly allocated to one of the four treatment groups to create even animal group characteristics.**

	Group A			Group B			Group C			Group D		
	Weight	Age	Sex	Weight	Age	Sex	Weight	Age	Sex	Weight	Age	Sex
Horse 1	516	15	M	528	4	M	520	4	G	530	12	G
Horse 2	538	6	G	532	12	G	548	12	G	538	5	M
Horse 3	582	7	M	578	14	M	578	12	M	572	5	M
Horse 4	602	10	G	602	7	M	584	13	M	602	6	M
Mean $\pm$ SD	560 $\pm$ 39	10 $\pm$ 4		560 $\pm$ 36	9 $\pm$ 5		558 $\pm$ 30	10 $\pm$ 4		561 $\pm$ 33	7 $\pm$ 3	

**Table 4. Phase 2: Two-choice test. Diets were paired based on contrast to examine preferences and diet ranking.**

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)

**Table 5. GLM (binomial) parameters fitted to ratios of intake, bucket visits and time spent foraging or moving of lower (Bucket 1) to higher (Bucket 2) palatability contrast for the four two-choice tests (16 horses; n=4 per group). The fitted parameters ( $\pm$  SE) of the GLM model with the (back-transformed) expected ratios are presented.**

**a) Log-ratio Intake**

<b>Test</b>	<b>Intercept</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Group B</b>	<b>Group C</b>	<b>Group D</b>	<b>P (Day)</b>	<b>P (Group)</b>
<b>1: LP vs. LP+</b>	-0.068 $\pm$ 0.039 (0.93)	0.086 $\pm$ 0.039 ( $\times$ 1.09)	0.1 $\pm$ 0.039 ( $\times$ 1.11)	-0.098 $\pm$ 0.046 ( $\times$ 0.91)	0.009 $\pm$ 0.044 ( $\times$ 1.0)	0.009 $\pm$ 0.044 ( $\times$ 1.0)	0.02	0.051
<b>2: LP vs. HP</b>	-0.07 $\pm$ 0.039 (0.93)	-0.034 $\pm$ 0.039 ( $\times$ 0.97)	0.036 $\pm$ 0.039 ( $\times$ 1.04)	-0.11 $\pm$ 0.047 ( $\times$ 0.9)	0.044 $\pm$ 0.044 ( $\times$ 1.05)	0.059 $\pm$ 0.044 ( $\times$ 1.06)	NS	<0.001
<b>3: HP vs. HP+</b>	-0.043 $\pm$ 0.038 (0.96)	0.012 $\pm$ 0.039 ( $\times$ 1.01)	0.034 $\pm$ 0.038 ( $\times$ 1.04)	-0.073 $\pm$ 0.045 ( $\times$ 0.93)	0.023 $\pm$ 0.044 ( $\times$ 1.02)	0.014 $\pm$ 0.044 ( $\times$ 1.01)	NS	NS
<b>4: LP+ vs. HP+</b>	-0.015 $\pm$ 0.038 (0.99)	0.018 $\pm$ 0.038 ( $\times$ 1.02)	0.004 $\pm$ 0.038 ( $\times$ 1.0)	-0.149 $\pm$ 0.045 ( $\times$ 0.86)	-0.028 $\pm$ 0.043 ( $\times$ 0.97)	-0.012 $\pm$ 0.044 ( $\times$ 0.99)	NS	0.003

**b) Log-ratio Time spent foraging**

<b>Test</b>	<b>Intercept</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Group B</b>	<b>Group C</b>	<b>Group D</b>	<b>P (Day)</b>	<b>P (Group)</b>
<b>1: LP vs. LP+</b>	-0.082 $\pm$ 0.043 (0.92)	0.158 $\pm$ 0.045 ( $\times$ 1.17)	0.247 $\pm$ 0.044 ( $\times$ 1.28)	-0.217 $\pm$ 0.05 ( $\times$ 0.81)	-0.037 $\pm$ 0.05 ( $\times$ 0.96)	-0.041 $\pm$ 0.05 ( $\times$ 0.96)	<0.001	<0.001
<b>2: LP vs. HP</b>	-0.151 $\pm$ 0.042 (0.86)	-0.024 $\pm$ 0.043 ( $\times$ 0.98)	0.004 $\pm$ 0.043 ( $\times$ 1.0)	-0.273 $\pm$ 0.049 ( $\times$ 0.76)	0.111 $\pm$ 0.05 ( $\times$ 1.12)	0.053 $\pm$ 0.049 ( $\times$ 1.05)	NS	<0.001
<b>3: HP vs. HP+</b>	-0.105 $\pm$ 0.043 (0.9)	0.244 $\pm$ 0.044 ( $\times$ 1.28)	0.055 $\pm$ 0.043 ( $\times$ 1.06)	-0.1 $\pm$ 0.049 ( $\times$ 0.91)	0.138 $\pm$ 0.051 ( $\times$ 1.15)	0.089 $\pm$ 0.051 ( $\times$ 1.09)	<0.001	<0.001
<b>4: LP+ vs. HP+</b>	0.175 $\pm$ 0.043 (1.19)	0.045 $\pm$ 0.044 ( $\times$ 1.05)	-0.23 $\pm$ 0.044 ( $\times$ 0.79)	-0.449 $\pm$ 0.05 ( $\times$ 0.64)	-0.13 $\pm$ 0.051 ( $\times$ 0.88)	-0.137 $\pm$ 0.051 ( $\times$ 0.87)	<0.001	<0.001

**c) Log-ratio Time spent moving**

Test	Intercept	Day 2	Day 3	Group B	Group C	Group D	<i>P</i> (Day)	<i>P</i> (Group)
<b>1: LP vs. LP+</b>	-0.201 ± 0.185 (0.82)	0.005 ± 0.177 (× 1.01)	0.209 ± 0.185 (× 1.23)	0.149 ± 0.201 (× 1.16)	0.198 ± 0.184 (× 1.22)	0.062 ± 0.187 (× 1.06)	NS	NS
<b>2: LP vs. HP</b>	-0.162 ± 0.215 (1.18)	-0.119 ± 0.21 (× 0.89)	-0.052 ± 0.22 (× 0.95)	-0.356 ± 0.243 (× 0.7)	-0.257 ± 0.23 (× 0.77)	-0.234 ± 0.24 (× 0.79)	NS	NS
<b>3: HP vs. HP+</b>	0.192 ± 0.197 (1.21)	-0.252 ± 0.183 (× 0.78)	-0.079 ± 0.184 (× 0.92)	0.033 ± 0.22 (× 1.03)	-0.133 ± 0.205 (× 0.87)	-0.007 ± 0.209 (× 0.99)	NS	NS
<b>4: LP+ vs. HP+</b>	0.115 ± 0.221 (1.12)	-0.394 ± 0.203 (× 0.67)	0.033 ± 0.202 (× 1.03)	0.073 ± 0.25 (× 1.08)	0.075 ± 0.231 (× 1.08)	0.03 ± 0.25 (× 1.03)	0.059	NS

**d) Log-ratio Bucket visits**

Test	Intercept	Day 2	Day 3	Group B	Group C	Group D	<i>P</i> (Day)	<i>P</i> (Group)
<b>1: LP vs. LP+</b>	-0.035 ± 0.267 (0.97)	-0.103 ± 0.257 (× 0.9)	0.115 ± 0.272 (× 1.12)	0.118 ± 0.316 (× 1.13)	0.082 ± 0.285 (× 1.09)	0.102 ± 0.287 (× 1.11)	NS	NS
<b>2: LP vs. HP</b>	0.106 ± 0.324 (1.11)	-0.158 ± 0.315 (× 0.85)	0.07 ± 0.316 (× 1.07)	-0.243 ± 0.378 (× 0.78)	-0.081 ± 0.365 (× 0.92)	-0.104 ± 0.367 (× 0.9)	NS	NS
<b>3: HP vs. HP+</b>	0.12 ± 0.266 (1.13)	-0.081 ± 0.26 (× 0.92)	-0.062 ± 0.258 (× 0.94)	-0.067 ± 0.319 (× 0.94)	-0.09 ± 0.291 (× 0.91)	0.005 ± 0.297 (× 1.0)	NS	NS
<b>4: LP+ vs. HP+</b>	0.013 ± 0.304 (1.01)	-0.159 ± 0.295 (× 0.85)	0.095 ± 0.297 (× 1.1)	0.098 ± 0.385 (× 1.1)	0.072 ± 0.335 (× 1.07)	0.04 ± 0.355 (× 1.04)	NS	NS

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

NS: Not significant

All models had 48 observations (Residual df. 45 (Day) and 42 (Group)).

**Table 6. Linear regression and GLM (Poisson) parameters ( $\pm$  SE) fitted to intake, zone count and time spent foraging or moving for the multiple-choice test (16 horses; n=4 per group). Intake and time spent foraging are based on linear regression models. For time spent moving and zone count fitted parameters of the GLM models with the (back-transformed) expected means are presented.**

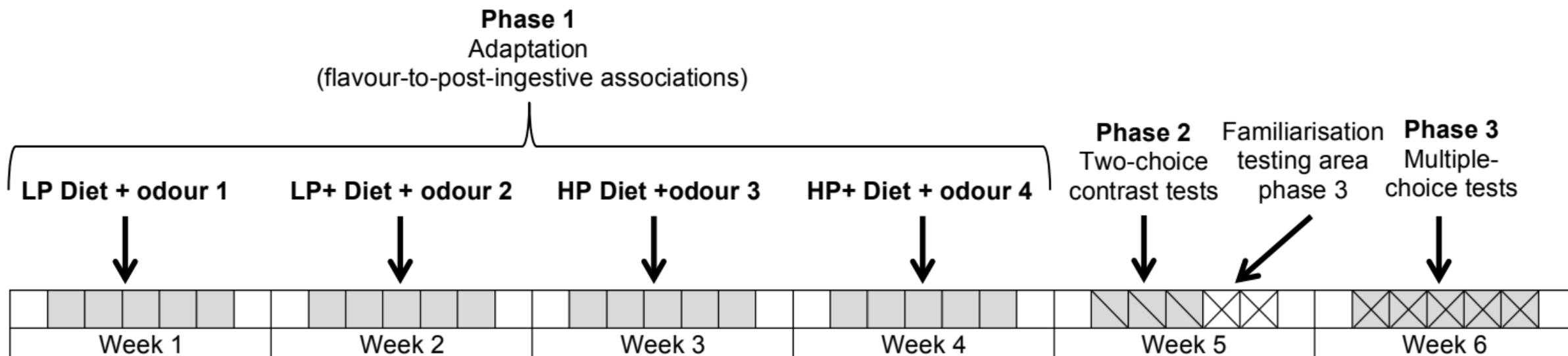
	<b>Intake</b> (g, DM)	<b>Time spent foraging</b> (sec)	<b>Time spent moving</b> (log-mean; (sec))	<b>Zone count</b> (log-mean; (count))
<b>Intercept</b>	109.3 $\pm$ 15	89.6 $\pm$ 11.2	2.2 $\pm$ 0.07 (8.8)	0.99 $\pm$ 0.13 (2.7)
<b>Diet LP+</b>	40.4 $\pm$ 11.3	22.5 $\pm$ 8.5	0.01 $\pm$ 0.06 ( $\times$ 1.01)	0.05 $\pm$ 0.1 ( $\times$ 1.05)
<b>Diet HP</b>	41.5 $\pm$ 11.3	29.6 $\pm$ 8.5	0.15 $\pm$ 0.06 ( $\times$ 1.16)	0.16 $\pm$ 0.1 ( $\times$ 1.18)
<b>Diet HP+</b>	73.6 $\pm$ 11.3	44.6 $\pm$ 8.5	0.12 $\pm$ 0.06 ( $\times$ 1.13)	0.14 $\pm$ 0.1 ( $\times$ 1.15)
<b>Day 2</b>	20.1 $\pm$ 12.6	10.7 $\pm$ 9.5	-0.04 $\pm$ 0.07 ( $\times$ 0.96)	0.09 $\pm$ 0.11 ( $\times$ 1.09)
<b>Day 3</b>	15.9 $\pm$ 12.6	9.1 $\pm$ 9.5	0.01 $\pm$ 0.07 ( $\times$ 1.01)	0.08 $\pm$ 0.11 ( $\times$ 1.08)
<b>Day 4</b>	11.4 $\pm$ 12.6	6.4 $\pm$ 9.5	0.01 $\pm$ 0.07 ( $\times$ 1.01)	0.03 $\pm$ 0.11 ( $\times$ 1.03)
<b>Day 5</b>	18.1 $\pm$ 12.6	8.1 $\pm$ 9.5	0.17 $\pm$ 0.06 ( $\times$ 1.18)	0.21 $\pm$ 0.11 ( $\times$ 1.23)
<b>Odour Cinnamon</b>	-34.7 $\pm$ 11.3	-35.2 $\pm$ 8.5	-0.06 $\pm$ 0.06 ( $\times$ 0.94)	-0.09 $\pm$ 0.1 ( $\times$ 0.91)
<b>Odour Coconut</b>	-20.6 $\pm$ 11.3	-18.8 $\pm$ 8.5	-0.03 $\pm$ 0.06 ( $\times$ 0.97)	-0.04 $\pm$ 0.1 ( $\times$ 0.96)
<b>Odour Spearmint</b>	-55.0 $\pm$ 11.3	-41.9 $\pm$ 8.5	-0.26 $\pm$ 0.06 ( $\times$ 0.77)	-0.21 $\pm$ 0.1 ( $\times$ 0.81)
<b>Group B</b>	-20.3 $\pm$ 11.3	5.9 $\pm$ 8.5	-0.49 $\pm$ 0.06 ( $\times$ 0.61)	-0.48 $\pm$ 0.11 ( $\times$ 0.62)
<b>Group C</b>	45.8 $\pm$ 11.3	4.4 $\pm$ 8.5	-0.02 $\pm$ 0.05 ( $\times$ 0.98)	0.01 $\pm$ 0.09 ( $\times$ 1.01)
<b>Group D</b>	50.9 $\pm$ 11.3	4.3 $\pm$ 8.5	-0.18 $\pm$ 0.06 ( $\times$ 0.84)	-0.07 $\pm$ 0.09 ( $\times$ 0.93)
<b>P (Diet)</b>	P<0.001	P<0.001	P=0.013	NS
<b>P (Day)</b>	NS	NS	P=0.009	NS
<b>P (Odour)</b>	P<0.001	P<0.001	P<0.001	NS
<b>P (Group)</b>	P<0.001	NS	P<0.001	P<0.001

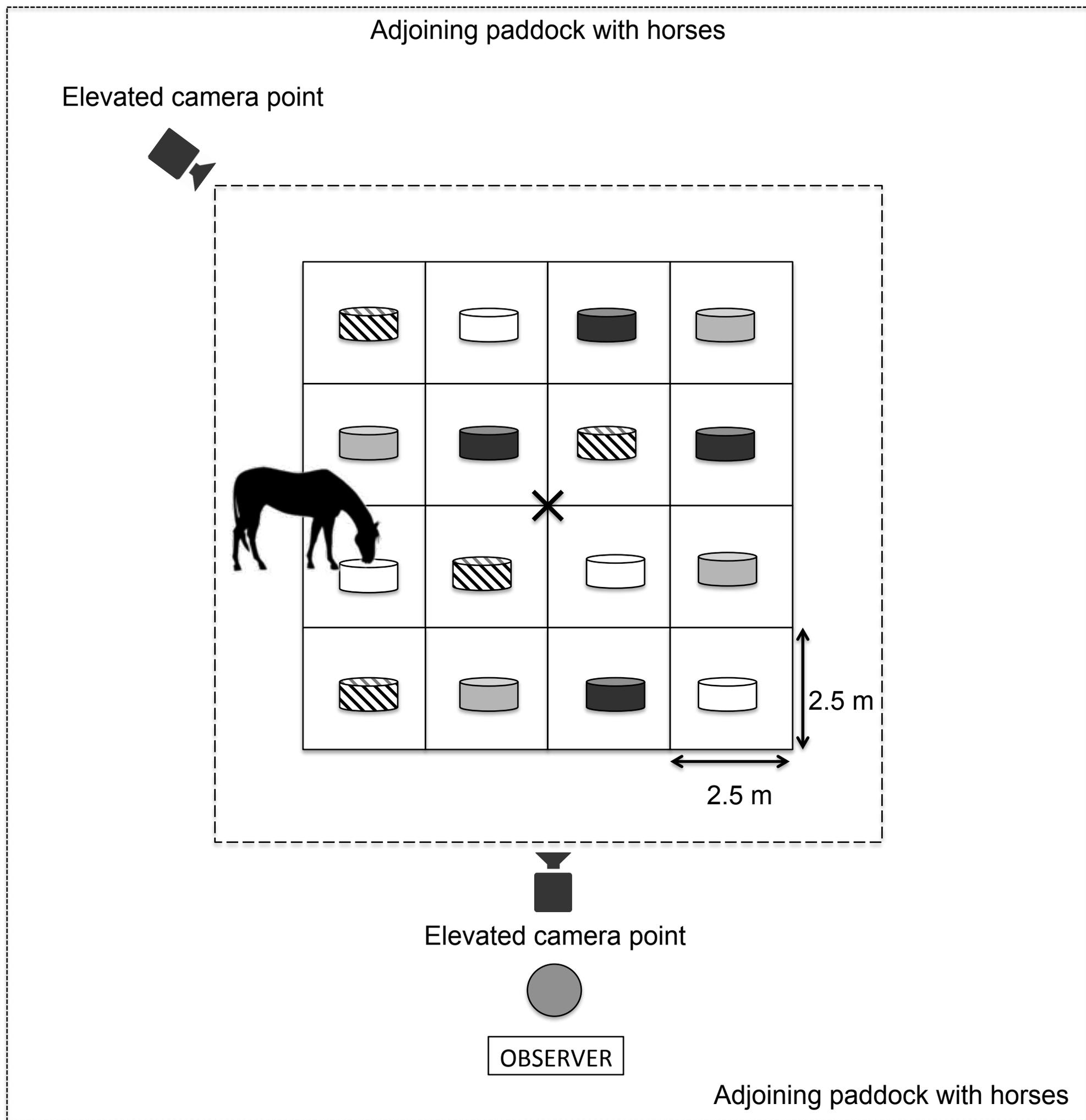
LP; low protein, LP+; low protein + sweetener, HP; high protein, and HP+; higher protein + sweetener

NS: Not significant

320 observations (Residual df. 316 (Diet), 312 (Day), 309 (Odour) and 306 (Group)).

**Figure 1-Timeline-experiments**





Odour 1



Odour 3



Odour 2



Odour 4



Steel frame yard



Electric fencing



Release area horse

Figure3-Phase1-Diets-xyplot

