



This is the pre-peer reviewed version of the following article:

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

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

Downloaded from e-publications@UNE the institutional research repository of the University of New England at Armidale, NSW Australia.

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

M. van den Berg^{1†}, C. Lee², W.Y. Brown¹, V. Giagos³ and G.N. Hinch¹

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

² *CSIRO, Agriculture, Armidale NSW 2350, Australia*

³ *School of Science and Technology, University of New England, Armidale NSW 2351, Australia*

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

1 **Abstract**

2 Compared to ruminants little is known about how horses modulate food intake and learn about
3 flavour-to-post-ingestive consequences. While it has been suggested that due to hindgut fermentation
4 horse's foraging preferences may be largely influenced by sensory input (e.g. volatiles), it has been
5 established that horses are able to differentiate and select familiar foods (e.g. concentrates and hay)
6 based on nutritional content. Yet it remains unclear how this translates to the acceptance of nutritious
7 novel foods (NF). Therefore, the influence of food cues and nutrient composition on NF acceptance
8 were examined in two experiments using 11 adult mares. In experiment 1, we investigated the
9 influence of a familiar odour (FO) on the acceptance of a nutritious NF and in experiment 2, we
10 determined if horses have the ability to select nutritious NF based on the nutritional content,
11 regardless of sensory preferences. In experiment 1 horses received identical NF in a two-choice test
12 with one of the choices being masked with a FO over a 9-day period. In experiment 2 horses were
13 offered a high or low protein option of an otherwise identical NF in a two-choice test in which the
14 NFs were paired with two unfamiliar flavours. The two-choice test lasted for 14 days and the flavour-
15 protein pairing was switched after 7 days. NF intakes were recorded over a 10 min test period on
16 each test day and analysed using Bayesian hierarchical models. The results of experiment 1 indicate
17 that a FO had a strong positive influence on the NF intake for the first 5 days (89.7-100% of total
18 consumption). This was followed by a more even distribution of intake for the remaining period. In
19 the 2nd experiment horses had a greater intake of high protein NF regardless of the flavour on days 4,
20 6 and 7 (80-86% of total consumption) and this continued after the switch over (Day 9 to 14; 60-80%
21 of total consumption). However, 4 out of 11 horses in experiment 2 showed neophobia throughout
22 the testing period, which could have been associated with the novel odours as horses scanned the
23 buckets with little to no sampling. The results suggest that preingestive cues (smell and/or taste) of
24 foods play an important role in diet selection and that a FO can increase the acceptance of NF. This
25 new knowledge could be applied by the horse industry to encourage the consumption of new food or
26 forages by horses.

27

28 **Keywords**

29 Diet Selection, Flavour, Food Recognition, Horses, Odour, Novel Food

30

31 **1. Introduction**

32

33 The senses of taste and smell help animals to discriminate between foods and play an important role
34 in food preferences and food selection. Orosensory senses together with post-ingestive mechanisms
35 allow animals to make associations with pleasant or unpleasant experiences depending on whether
36 the effect on the internal environment of the animal is aversive or positive (gut-brain feedback)
37 (Provenza, 1995). For example foods can be rejected (aversion) as a direct result of sensory input and
38 its link to post-ingestive consequences, either from toxins or nutrients that are in excess or deficient.
39 Learned food aversions have been demonstrated in a number of animal species including ruminants
40 (Burritt and Provenza, 1991; Provenza, 1995; 1996), rats (Garcia et al., 1972) and horses (Houpt et
41 al., 1990; Pfister et al., 2002). Conversely, the liking of a food (preference) increases when it contains
42 adequate nutrients and provides a positive feedback.

43

44 Investigations of the ability of horses to differentiate foods based on the orosensory characteristics
45 and nutrient content are limited. There is some evidence that horses are able to detect macronutrients
46 in foods and can adapt to deficiencies by increasing intake or by changing food choices (Laut et al.,
47 1985; Cairns et al., 2002; Redgate et al., 2014). For example, Cairns et al. (2002) showed that horses
48 selected a higher energy concentrate over a lower energy one, regardless of the preferred flavour
49 (mint or garlic). However, it has been suggested that horses, due to hindgut fermentation, may
50 experience difficulties in associating the chosen food with its postingestive consequences,
51 particularly when several foods are presented simultaneously. Therefore, a more recent study
52 compared the effect of exposure to a single forage that was rich in either protein, lipids or

53 hydrolysable carbohydrates on the selection of three forages in a simultaneous choice session
54 (Redgate et al., 2014). The horses showed a greater preference for the forages that were rich in protein
55 or hydrolysable carbohydrates, which suggests that horses responded to the macronutrients in the
56 diets and that the dietary experience (single presentation) facilitated feedback mechanisms, and hence
57 affected dietary preferences.

58

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

73

74 While it has been established that horses are able to make associations with the nutritional content of
75 familiar foods, it is unclear how this applies to the selection of nutritious novel foods and how
76 volatiles may affect this selection. Therefore, the aim of the present study was to improve our
77 understanding of the acceptance of nutritious novel foods by horses by examining the sensory
78 behaviour and the ability of horses to learn about positive postingestive consequences of a novel food

in two experiments. The first experiment follows our previous study (van den Berg et al., 2016) that demonstrated a strong neophobic response in horses towards novel forage volatiles and focused on the question of whether odour influences diet selection of novel foods by horses. It was hypothesised that horses would cautiously sample all foods, but initially a greater intake was expected for the novel choice with the familiar odour. The second experiment focused on the question of whether horses make associations with the orosensory characteristics of an unfamiliar nutritious food and their postingestive consequences. The hypothesis was that horses would cautiously sample all foods, but a greater intake for food with a greater nutrient profile was expected regardless of the preferred flavours.

2. Material and methods

2.1. Animals and feeding management

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

105

106 2.2. *Experimental design*

107

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

113

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

120

121 2.3. *Feed collection and flavour preparation*

122

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

128

129 For the preparation of forage odour a similar procedure was used as described by Hinch et al. (2004).

130 Fresh lucerne was collected from a commercial lucerne grower in South East Queensland, Australia.

131 For the familiar odour solution 200 g fresh lucerne was shredded in a food blender with 1 L water to
132 make up 20 L of total solution. This was allowed to stand overnight (12 h) in a cool room at 4° C
133 before straining through a cheese-cloth. The resultant extract was stored at -20° C in airtight
134 containers.

135

136 Commercially sourced non-nutritive human/animal food flavours (aniseed and citrus; Lucta, Spain)
137 were used in experiment 2. The aniseed flavour had a liquorice aroma and citrus had a sweet orange
138 aroma. Both flavours were novel to the horses. The flavour powder (5 g) was diluted in 500 ml cold
139 water to make a stock solution.

140

141 2.4. Testing area

142

143 Horses were individually tested in a holding yard (4 m x 8 m) that was familiar to them and within
144 view of other horses. Before testing (experiment 1) horses were adapted to the holding yard and
145 buckets for two days during which they were fed lucerne chaff. Two large feeding buckets were
146 mounted on the yard door (0.5 m apart and 1 m height) and two smaller labelled feeding tubs were
147 placed inside the larger mounted buckets. The position of the feeding tubs was changed randomly for
148 each testing day.

149

150 2.5. Testing procedures

151

152 2.5.1. Experiment 1: Familiar odour (FO)

153

154 Horses were offered two identical feeding buckets, each containing 200 g bamboo chaff (novel
155 forage) with and without a FO (lucerne). The forages were placed on mesh cloths that acted as the
156 floor of each feeding tub; the mesh size prevented the foods from falling through while allowing any

157 odour below to permeate the food. To deliver the FO, 200 ml of the lucerne extract was placed 3.5
158 cm below the feeder base. In addition, the mesh cloth of the bucket with the FO was drenched in the
159 lucerne solution and dried overnight. Each day new cloths were prepared for the feeding tubs. The
160 labelled feeding tubs were rinsed with water and dried with paper tissue between each horse and test.
161 Feeding tubs used with the FO were kept separate from the other feeding tubs.

162

163 2.5.2. Experiment 2: Nutrient composition (Postingestive feedback)

164

165 Horses were offered two novel foods (2 x 200 g; soybean hull pellets) that contrasted in crude protein
166 (CP) levels (13.9% vs 22.3%). The two diets were paired with one of two unfamiliar flavours (aniseed
167 or citrus). Commercially sourced soybean hull pellets (SHP) with a CP level of 13.9% and digestible
168 energy content of 8.4 MJ/kg on a dry matter basis constituted the low protein (LP) option. Soybean
169 meal (65g; 47.5% CP) was added to the soybean hulls to create a high protein (HP) option (22.3%
170 CP) with minimal change to the fibre content and volume. This meant that the total offered was 265
171 g for the high CP option, which resulted in a digestible energy of 9.9 MJ/kg for HP diet. The flavour
172 stock solutions (3 ml citrus or 5 ml aniseed) were further diluted in water (300 ml) before adding to
173 the novel foods to dampen the feed and create a homogenous mixture. This concentration created an
174 aromatic odour that was detectable by human senses. Horses were randomly allocated to a flavour-
175 protein pairing based on age and estimated weight/ body condition score as this allowed for
176 distribution of treatments across testing days and eliminated the influences of potential weather
177 conditions for one particular treatment. At the start of the experiment 6 horses received HP-
178 aniseed/LP-citrus and 5 horses HP-citrus/LP-aniseed. The flavour-protein pairing was switched after
179 7 days.

180

181 2.6. Measurements

182

183 The two-choice tests (10 min) were conducted between 09:00 and 12:30 h each day of the testing
184 period. The intake of NF by each horse was determined by weighing the foods in each feeding bucket
185 before and after each test. For the SHP trial (experiment 2) the intake was adjusted for moisture
186 (added water) and calculated to a DM basis. On completion of testing horses were allowed to return
187 to their pasture paddock.

188

189 2.7. Statistical analysis

190

191 All the statistical analyses were performed with the R3.1.3 free software (Team, 2015). Where
192 appropriate, the level of significance was set to $P < 0.05$. Additional information about the statistical
193 analysis used in this study is covered in the supplementary material supplied.

194

195 2.7.1. Experiment 1: Familiar odour

196

197 To determine if there was an effect of familiar odour on the intake of a novel forage and if horses
198 learn about the nutritional consequences of novel forages over time, the intake (g) and proportions
199 (percentage) of bamboo without lucerne odour (BO^-) and bamboo with lucerne odour (BO^+) over the
200 9 testing days were examined. We denoted the proportion of BO^+ intake out of the total intake of the
201 i -th horse and the j -th day with p_{ij} and we chose a logistic link function with our covariates. Our first
202 covariate was an intercept β_0 , which expressed a *main* effect and followed a Normal distribution with
203 mean zero and variance 1000^2 . The zero mean indicates that overall the horses had a balanced
204 consumption (distribution of 50% from each bucket) whereas the large prior variance allowed us to
205 consider a wider range of common behaviours across all horses and days.

206

207 The experimental design suggested two consistent sources of variation: a *random* effect, which could
208 be attributed to each different horse and a *temporal* effect where each measurement depended on the

day of the observation. In previous ruminant studies (Launchbaugh et al., 1997; Van Tien et al., 1999; Hinch et al., 2004) novel food acceptance (reduction in neophobia) was measured as a transition from very limited consumption of a novel food to an incremental increase in intake that could reach total offered amounts (plateau) depending on the postingestive feedback. We hypothesised that this would be the same for horses and therefore the inclusion of the temporal effects expresses this transition, which we assume is common for all horses. Whereas, the inclusion of the random effects expresses the horse-specific variation (e.g. age, body condition score).

We addressed both sources of variation using a Bayesian hierarchical model. Each horse's foraging behaviour was modeled with a random variable (β_h) which followed a Normal distribution with a mean 0 and variance which in turn followed a Half-Cauchy prior (Gelman, 2006). The heavy-tailed prior on the variance allowed us to explore a wide class of models for the foraging behaviour (consistent v.s. variable) of each horse. Similarly, for the temporal effect we have chosen a Normal-distributed random variable (β_d) for each day with mean 0 and variance σ_d^2 with a Half-Cauchy prior as well. The residual terms $\epsilon_{i,j}$ followed a Normal distribution with mean 0 and variance σ_ϵ^2 . Other choices were considered (linear model, lineal change-point model, auto-regressive model) but the aforementioned choice had the smallest DIC (Spiegelhalter et al., 2002) with the linear change-point (Day 6) model having a slightly increased DIC. Overall the hierarchical model was:

$$\begin{aligned} \beta_{h,i} &\sim \text{Normal}(0, \sigma_h^2) & \sigma_h^2 &\sim \text{Half-Cauchy}(0,1) & \beta_{d,j} &\sim \text{Normal}(0, \sigma_d^2) & \sigma_d^2 &\sim \text{Half-Cauchy}(0,1) \\ \beta_0 &\sim \text{Normal}(0, 1000^2) & \epsilon_{ij} &\sim \text{Normal}(0, \sigma_\epsilon^2) & \sigma_\epsilon^2 &\sim \text{Half-Cauchy}(0,1) & \text{logit}(p_{ij}) &= \beta_0 + \beta_{d,i} + \beta_{h,j} + \epsilon_{ij} \end{aligned}$$

We implemented this model in JAGS (Plummer, 2003) and processed the output in R using rJAGS (Plummer and Stukalov, 2014).

2.7.2. Experiment 2: Nutrient composition (Postingestive feedback)

235

236 To establish if horses make associations with the orosensory characteristics of a novel food and their
237 postingestive consequences, the intake (g) and proportions (percentage) of SHP based on protein
238 levels and flavour over 14 testing days were examined. Only observations of seven horses were used
239 in this analysis, as 4 of the 11 horses did not have an intake of 25 g SHP or more after Day 4 and
240 remained neophobic throughout the testing period. Our measurements consisted of intakes (g) of
241 SHP, which contained either HP, or LP and could have either a citrus or aniseed flavour. Both the
242 protein and the flavour have been treated as two-level factors. Our parameter of interest is the
243 proportion of HP-SHP out of the total intake. During the first 7 days 4 horses received HP-aniseed/LP-
244 citrus and 3 horses HP-citrus/LP-aniseed. The flavour-protein pairing was switched after 7 days.

245

246 Similar to the previous model, we denoted the proportion of HP-SHP out of the total intake of the i -
247 th horse and the j -th day with q_{ij} and we used a logit link function as well. In this model we omitted
248 an intercept term but we considered a flavour coefficient α_f based on the citrus flavour to HP
249 proportion, which followed a Normal distribution with mean 0 and variance τ_f^2 . The temporal effects
250 are modeled again as Normal-distributed random variables $\eta_{d,j}$ with mean 0 and variance τ_d^2 .
251 Similarly, the random horse effects are Normal-distributed random variables $\eta_{h,i}$ with mean 0 and
252 variance τ_h^2 . The residual terms $\xi_{i,j}$ followed a Normal distribution with mean 0 and variance τ_ξ^2 and
253 all the scale hyper-parameters τ_f^2 , τ_d^2 , τ_h^2 , followed a Half-Cauchy(0,1) distribution. We also
254 introduced the indicator function $I(i, j)$ to denote if the HP-SHP had a citrus flavour, i.e. $I(i, j)=1$ for
255 the i -th horse on the j -th day or not ($I(i, j)=0$). The hierarchical model was as follows:

256

$$257 \quad \alpha_f \sim \text{Normal}(0, \tau_f^2) \quad \tau_f^2 \sim \text{Half-Cauchy}(0,1) \quad \eta_{d,j} \sim \text{Normal}(0, \tau_d^2) \quad \tau_d^2 \sim \text{Half-Cauchy}(0,1)$$

$$258 \quad \eta_{h,i} \sim \text{Normal}(0, \tau_h^2) \quad \tau_h^2 \sim \text{Half-Cauchy}(0,1) \quad \xi_{i,j} \sim \text{Normal}(0, \tau_\xi^2) \quad \tau_\xi^2 \sim \text{Half-Cauchy}(0,1)$$

$$259 \quad \text{logit}(q_{ij}) = \eta_{d,i} + \eta_{h,j} + \alpha_f I(i, j) + \xi_{i,j}.$$

260

261 For the implementation of the model a similar approach was used to that described in experiment 1.

262

263 **3. Results**

264

265 *3.1. Experiment 1: Familiar odour*

266

267 The total bamboo consumption is illustrated with boxplots in Figure 1. The model described in
268 Section 2.7.1 was positively evaluated (see for more details supplementary material) and a greater
269 BO^+ consumption was found for specific days. Since it is a Bayesian model, a marginal posterior
270 distribution is estimated for each model parameter instead of a point estimate and it is used as the
271 basis of the statistical analysis. The posterior distribution of bamboo consumption (in percentage) at
272 a particular day is summarised in Table 1; we emphasize that these estimates include main, temporal
273 and also random effects. The posterior percentages express the posterior probability as a percentage,
274 i.e. the BO^+ consumption out of the total intake per day. The contribution of the temporal effects and
275 the main effect to the BO^+ consumption is shown in the violin plots (Figure 2), where the random
276 effects are omitted (see supplementary material for more details). On the logistic scale of Figure 2
277 the posterior mass above zero expresses a higher BO^+ consumption. An equal distribution of
278 consumption of BO^- and BO^+ is expressed when the posterior percentage is close to 50% on Table 1
279 and a symmetric posterior density centered at zero on Figure 2.

280

281 In Table 1, the first 5 days the BO^+ accounts for 89.7–100% of the total consumption whereas at Day
282 6 BO^- accounts for 83.3% of the total consumption. During Days 7 and 8 we start observing a more
283 balanced distribution between the two choices (the BO^+ consumption accounted for 65.7% and 52.9%
284 of the total intake for Days 7 and 8 respectively) and the BO^+ consumption increased again on the last
285 day (Day 9, 86.2%). Additionally, the posterior distribution of the temporal effects for the intake of

286 bamboo (Figure 2) showed a greater consumption for the BO⁺ on Day 2, Day 4 and Day 5 (the
287 corresponding 95% credible intervals in Figure 2 are above zero). There is also strong evidence for
288 non-zero temporal effects (Bayes Factor B₂₁= 110 in favor of non-zero effects see details in
289 supplementary material).

290

291 3.2. Experiment 2: Nutrient composition (Postingestive feedback)

292

293 The total SHP consumption is illustrated with boxplots in Figure 3. The Bayesian model (Section
294 2.7.2) was assessed in a similar fashion as described in the bamboo experiment, but it received less
295 support from the data (see for more details supplementary material). The distribution of SHP
296 consumption (in percentage) at a particular day is summarised in Table 2, again these estimates
297 include main, temporal and also random effects. Similar to the bamboo data presentation, the
298 posterior probability percentages in Table 2 express the HP-SHP consumption out of the total intake.
299 The contribution of the temporal effects and main effect to the HP-SHP consumptions is shown in
300 the violin plots (Figure 4). When the posterior mass is positioned above zero this indicates a
301 preference towards HP-SHP. When the consumption of HP-SHP and LP-SHP is balanced the
302 posterior percentage is close to 50% (Table 2) and a symmetric posterior density centered at zero on
303 Figure 4. In order to keep the bulk and fibre content of the two foods similar, we offered 265 g for
304 the HP-SHP option and 200 g for the LP-SHP. The added protein meal (65 g) did not contribute to
305 the expansion of the SHP, bonding to the fibrous structure, and we preserved the volume between the
306 two options by adding the same amounts of water. We observed total intakes greater than 400 g for
307 Horse 5 on Day 6 and 7 and for Horse 9 on Day 10 and 12 (outliers and whisker Figure 3), and we
308 acknowledge that this a limitation based on weight but not for volume.

309

310 With the exception of Horse 5, in Table 2, the random effects indicated a greater consumption of the
311 HP-SHP, which was, on average, between 1.56 to 2.45 times more than the LP-SHP intake. The

312 flavour effect was expressed by α_f , which was minimal (posterior mean -0.045, sd 0.216). Initially
313 (in the first week) a variable intake pattern emerged: the first two days indicated a moderate intake
314 for LP-SHP (~64%), which was followed by a greater consumption of LP-SHP on Day 3 (~85%) and
315 an equal intake was recorded on Day 5 (~49% HP). For the remaining days 4, 6 and 7 a greater
316 consumption of HP-SHP was noted (80-86%). In the second week, immediately after the flavour
317 switch-over, a higher consumption of LP-SHP was recorded followed by a steady recovery on the
318 remaining days (9-11) to a greater intake of HP-SHP (60 to 80%). However, the posterior distribution
319 of the temporal effects for the intake of SHP (Figure 4) showed large variations and no clear
320 distribution in favor of the HP-SHP was observed (all 95% creditable intervals cross the zero line in
321 Figure 4).

322

323 **4. Discussion**

324

325 *4.2 Nutrient composition*

326

327 Food acceptance and preference is a result of an interrelationship between the foods flavour (smell,
328 taste and texture) and its postingestive consequences (positive or negative) (Provenza, 1995). A
329 previous study in horses suggest that preferences for particular flavours can be overridden by
330 postingestive consequences of a familiar food (Cairns et al., 2002) and this study (second experiment)
331 hypothesised that horses would be similar when they are offered nutritious novel foods; having a
332 greater intake of high-protein novel food, regardless of the novel odour (aniseed or citrus). It was also
333 expected that horses would be able to recognise a flavour-protein pairing if the link between the cue
334 and postingestive feedback was altered. This study showed a greater posterior percentage for the HP-
335 SHP on days 4, 6 and 7 and after the switch over on days 9 to 14. This suggest that the recognition of
336 the post-ingestive associations (from CP and/or DE content) initially seemed to take 4 to 5 days which
337 was also apparent in experiment 1 and is in accordance with the findings of Cairns et al. (2002).

338 However, the posterior distribution of the temporal effects (omitting the random effects) for the intake
339 of SHP showed large variations between days and was not supported by the model. This lack of
340 support could be attributed to the dropout in this study, as 4 out of 11 horses did not sample the novel
341 SHP diet within the time frame of the test and were removed from the analysis.

342

343 Horses, like ruminants, habitually eat only small quantities of food when it is presented for the first
344 time (Thorhallsdottir et al., 1987; Provenza, 1995; van den Berg et al., 2016). This cautious sampling
345 or even complete rejection of new foods is commonly referred to as neophobia (meaning “the fear of
346 new”) and has been suggested as an innate herbivore survival mechanism for avoiding the over-
347 consumption of toxic plants in the wild (Provenza and Balph, 1987). The results from experiment 2
348 would seem to affirm this pattern. This raises the question of individual variation in the level of
349 neophobia toward novel volatiles (in this case flavours citrus and aniseed) and/or the texture of the
350 SHP. Ott et al. (1979) reported a similar pattern with 6 of 8 mature horses reducing intake of a grain
351 concentrate when it was mixed with 30% dried citrus pulp consuming only 8.6% of the feed offered.
352 The strength of neophobia seems to vary considerably between individuals of ruminant species
353 (Launchbaugh et al., 1997; Nolan and Hinch, 1997; Hinch et al., 2004) and this seems to be the case
354 in horses as well.

355

356 4.2 Food cues

357

358 Food cues (flavour; smell, taste and texture) play an important role in an animal’s ability to identify
359 and remember foods with aversive postingestive consequences. This has been well documented in
360 ruminants (Provenza et al., 1990; Launchbaugh and Provenza, 1993; Kyriazakis et al., 1997; Pfister
361 et al., 2010) and ponies have been shown to learn to avoid relatively novel foods when apomorphine
362 hydrochloride (APO) was injected intramuscularly to induce illness (Houpt et al., 1990). These
363 authors also showed that ponies could form an aversion to a novel food, based on previous experience,

364 even when it is ingested at the same time as another, more familiar, food. However, this pattern was
365 not completely consistent with high-energy dense foods suggesting that ponies were more likely to
366 develop aversions to less “palatable” foods.

367

368 *Odour*

369 Odour (volatiles) is an important food characteristic, which allows animals to discriminate amongst
370 foods and link these to pleasant or unpleasant experiences. While it is likely that neophobia in
371 experiment 2 was caused by the novel volatiles (aniseed and citrus), we can confirm that a familiar
372 odour can have a positive effect on novel food acceptance. Our study (experiment 1) showed for the
373 first 5 days a posterior percentage between 89.7 and 100% for the BO^+ when main, random and
374 temporal effects were included. In addition the posterior distribution of the temporal effects showed
375 that 3 out of 5 days had 95% creditable intervals that were above zero. This suggests that lucerne
376 odour positively influenced the intake of bamboo during the first 5 days of exposure. After day 5 the
377 intake shifted to an equal BO^+ and BO^- consumption distribution and horses consumed 50% or more
378 of the bamboo offered which may suggest that horses had recognised the unfamiliar bamboo as an
379 acceptable food (i.e. lack of a negative postingestive feedback).

380

381 It has been shown that animals can generalise preferences and a well-liked familiar flavour can
382 increase the acceptance of a novel food (even if it contain some toxins). Dohi and Yamada (1997)
383 demonstrated that sheep and goats had a greater preference for a less palatable hay when it was
384 sprayed with an extract of a well-liked high-grain concentrate and Van Tien et al. (1999) showed that
385 sheep increased their intake of a novel food (rice bran) more quickly when a familiar grass odour and
386 combination of odour and taste was added. The results of this study (experiment 1) suggest a similar
387 pattern for horses.

388

389 In our current study we only used odour as a food cue, which appeared to be strong enough to
390 encourage the acceptance of a nutritious novel food. This suggests that volatiles (pre-ingestive
391 feedback) can play an important role in diet choices by horses and supports the findings of our
392 previous study (van den Berg et al., 2016). The influence of volatiles on the preference of familiar
393 foods (oaten and lucerne) by horses have been previously demonstrated by Pain and Revell (2009).
394 These authors showed that volatiles can be positively linked to nutritive and physical traits of lucerne
395 or oaten hay. However they also found volatiles that influenced the preference negatively but were
396 not related to any measurable nutritive or physical traits, possibility due to other plant factors such as
397 secondary compounds. This suggests that diet selection by horses cannot always be explained by
398 nutritive traits and that olfaction and gustation cues may also be important. This may be more apparent
399 when dealing with strong herbaceous volatiles such as from browse species (tree and shrub leaves),
400 which were used in our previous study (van den Berg et al., 2016).

401

402 *Taste*

403 Taste (gustation) could have also influenced the food consumption observed in our study. In
404 experiment 2 we added soybean meal, which may have contributed to a greater acceptance of the HP-
405 SHP option. It has been shown that horses can distinguish between 4 of the 5 taste sensations (sweet,
406 bitter, salty and sour) (Randall et al., 1978), but there may even be an indication that horses could
407 also have a taste for “umami”. Umami is a Japanese loanword and can be translated as "pleasant
408 savory taste”. Umami perception occurs through the detection of the carboxylate anion of glutamic
409 acid, which is a naturally occurring amino acid common in meats, cheese, broth, stock and other
410 protein-heavy foods (Chandrashekar et al., 2006). While umami taste responses has been mainly
411 linked to a carnivorous or omnivorous diet, it seems that herbivores such as horses and cattle express
412 also the taste receptor genes involved with the umami taste sensation (Zhao et al., 2010). In addition,
413 Favreau et al. (2010) has demonstrated in sheep that an umami taste resulted in a greater
414 intake/preference compared to a bitter taste. This could indicate that horses may also have the ability

415 to detect foods with a “protein taste” which could explain why horses seem to have a greater
416 preference for HP-SHP diet. However, to our knowledge no studies are known that have explicitly
417 assessed this in horses, therefore in hindsight it could have been noteworthy to examine how taste
418 (sweet or umami) could have influenced the neophobia seen in this study.

419

420 *Texture*

421 The texture and ease of prehension are also food characteristics that can influence the food acceptance
422 and intake by grazing animals. In experiment 2 we used soybean hulls, a by-product of the soybean
423 processing industry which contain 53-70% neutral detergent fibre (NDF) and less than 3% starch
424 (NRC, 2007). It is considered to be a good alternative fibre-product for inclusion in livestock feeds
425 and has a growing use in commercial horse feeds due to its low glycaemic index (GI) profile. While
426 it is high in digestible fibre its initial palatability may be modest when it is fed on its own which could
427 have attributed to the texture or taste. When fed dry SHP can be dusty and when dampened the fibres
428 expand which result in a “cottony” texture. Overall texture and/or palatability could have influenced
429 the horse’s foraging behaviour in our study.

430

431 **5. Conclusion**

432

433 The findings of the present study confirm that horses use organoleptic qualities of forages (e.g. odour,
434 taste, texture, ease of prehension etc.) when selecting foods. Horses in this study were able to make
435 an association with the nutritional content of a novel food, but neophobia significantly impacted on
436 initial testing of novel foods. We were also able to show that the acceptance of a novel food can be
437 enhanced by introducing a familiar odour, which highlights the important role of the pre-ingestive
438 signals in diet selection by horses. The present study was not able to examine the role of taste in
439 dietary preferences by horses, as non-nutritive flavours were used. Future preference studies should
440 incorporate odour, taste and texture to determine the relative importance of these “cues” to horses

441 making diet choices in grazing environments. Nevertheless, the present study adds to the
442 understanding of mechanisms involved in diet selection by horses and proposes that odour cues can
443 be successfully applied in equine feeding management when forages/foods are scarce and new foods
444 or forage batches are introduced or when horses are moved to different environments.

445

446 **Acknowledgements**

447 Funding for this project was kindly provided by the University of New England. The authors thank
448 the Todd Family for their kind assistance in providing horses and facilities. The authors
449 also acknowledge Karina McLoughlan, Kelly McLean, Brittany McVicar and Amber Alsford for
450 assisting during the experimental work, and Michael Raue for technical support. Special thanks go to
451 our sponsors; BEC Feed Solutions and Energreen Nutrition Australia.

452

453 **References**

- 454 Arnold, G.W., Boer, E.S., Boundy, C.A.P., 1980. The influence of odour and taste on the food
455 preferences and food intake of sheep. *Aust. J. Agric. Res.* 31, 571-587.
- 456 Burritt, E.A., Provenza, F.D., 1991. Ability of lambs to learn with a delay between food ingestion
457 and consequences given meals containing novel and familiar foods. *Appl. Anim. Behav. Sci.* 32, 179-
458 189.
- 459 Burton, J.H., Price, D.J., Aspinall, J., 1983. The effect of feed flavour and feed consumption in horses,
460 *Eight Equine Nutrition and Physiology Symposium Lexington, KY, USA.*
- 461 Cairns, M.C., Cooper, J.J., Davidson, H.P.B., Mills, D.S., 2002. Association in horses of orosensory
462 characteristics of foods with their post-ingestive consequences. *Anim. Sci.* 75, 257-265.
- 463 Chandrashekar, J., Hoon, M.A., Ryba, N.J.P., Zuker, C.S., 2006. The receptors and cells for
464 mammalian taste. *Nature* 444, 288-294.
- 465 Cuddeford, D., 2005. Voluntary food intake by horses. *Pferdeheilkunde*, 7-8.
- 466 Dohi, H., Yamada, A., 1997. Preference of sheep and goats for extracts from high-grain concentrate.
467 *J. Anim. Sci.* 75, 2073-2077.
- 468 Dulphy, J.P., Martin-Rosset, W., Dubroeuq, H., Ballet, J.M., Detour, A., Jailler, M., 1997. Compared
469 feeding patterns in ad libitum intake of dry forages by horses and sheep. *Livest. Prod. Sci.* 52, 49-56.
- 470 Favreau, A., Baumont, R., Ferreira, G., Dumont, B., Ginane, C., 2010. Do sheep use umami and bitter
471 tastes as cues of post-ingestive consequences when selecting their diet? *Appl. Anim. Behav. Sci.* 125,
472 115-123.

473 Garcia, J., McKowan, B.K., Green, K.F., 1972. A theory of taste-aversion learning - biological
474 constraints on conditioning., in: Seligman, M.E.P., Hager, J.L. (Eds.), *Biological boundaries of*
475 *learning*, Meredith Corporation, New York.

476 Gelman, A., 2006. Prior distributions for variance parameters in hierarchical models (comment on
477 article by Browne and Draper). *Bayesian Anal.* , 515-534.

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

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

482 Hinch, G.N., Nolan, J.V., Lynch, J.J., Hill, J., 2004. Familiar odour and flavour cues reduce feed
483 neophobia in sheep. *Anim. Prod. Aus.* 25, 97-99.

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

486 Kyriazakis, I., Papachristou, T.G., Duncan, A.J., Gordon, I.J., 1997. Mild conditioned food aversions
487 developed by sheep towards flavors associated with plant secondary compounds. *J. Chem. Ecol.* 23,
488 727-746.

489 Launchbaugh, K.L., Provenza, F.D., 1993. Can plants practice mimicry to avoid grazing by
490 mammalian herbivores? *Oikos* 66, 501-504.

491 Launchbaugh, K.L., Provenza, F.D., Werkmeister, M.J., 1997. Overcoming food neophobia in
492 domestic ruminants through addition of a familiar flavor and repeated exposure to novel foods. *Appl.*
493 *Anim. Behav. Sci.* 54, 327-334.

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

496 Mars, L.A., Kiesling, H.E., Ross, T.T., Armstrong, J.B., Murray, L., 1992. Water acceptance and
497 intake in horses under shipping stress. *J. Equine Vet. Sci.* 12, 17-20.

498 Nelson, G., Keller M, Cheeke, P.R., 1997. Evaluation of temperate bamboo species as forage for
499 livestock., *Pacific Northwest Bamboo Agroforestry Workshop* Port Townsend, Washington, USA,
500 pp. 7-1 to 7-8.

501 Nolan, J.V., Hinch, G.N., 1997. Factors that determine whether grazing animals will accept
502 supplements, *Recent Adv. Anim. Nutr. Aust.*, University of New England, Armidale, NSW,
503 Australia, pp. 55-62.

504 NRC, 2007. *Nutrient requirements of horses*. 6th edition ed. The National Academies Press,
505 Washington D.C., USA.

506 Ott, E.A., Feaster, J.P., Lieb, S., 1979. Acceptability and digestibility of dried citrus pulp by horses.
507 *J. Anim. Sci.* 49, 983-987.

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

510 Pfister, J.A., Gardner, D.R., Cheney, C.C., Panter, K.E., Hall, J.O., 2010. The capability of several
511 toxic plants to condition taste aversions in sheep. *Small Ruminant Res.* 90, 114-119.

512 Pfister, J.A., Stegelmeier, B.L., Cheney, C.D., Ralphs, M.H., Gardner, D.R., 2002. Conditioning taste
513 aversions to locoweed (*Oxytropis sericea*) in horses. *J. Anim. Sci.* 80, 79-83.

514 Plummer, M., 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs
515 sampling, *Proceedings of the 3rd International Workshop on Distributed Statistical Computing*,
516 Vienna, Austria.

517 Plummer, M., Stukalov, A., 2014. rJAGS-package: Bayesian graphical models using MCMC, p.
518 Interface to the JAGS MCMC library.

519 Provenza, F.D., 1995. Postingestive feedback as an elementary determinant of food preference and
520 intake in ruminants. *J. Range Manage.* 48, 2-17.

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

523 Provenza, F.D., Balph, D.F., 1987. Diet learning by domestic ruminants: Theory, evidence and
524 practical implications. *Appl. Anim. Behav. Sci.* 18, 211-232.

525 Provenza, F.D., Burritt, E.A., Clausen, T.P., Bryant, J.P., Reichardt, P.B., Distel, R.A., 1990.
526 Conditioned Flavor Aversion: A Mechanism for Goats to Avoid Condensed Tannins in Blackbrush.
527 *Am. Nat.* 136, 810-828.

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

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

532 Spiegelhalter, D.J., B., N.G., , Carlin, B.P., Van Der Linde, A., 2002. Bayesian measures of model
533 complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* 64.

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

536 Thorhallsdottir, A.G., Provenza, F.D., Balph, D.F., 1987. Food aversion learning in lambs with or
537 without a mother: Discrimination, novelty and persistence. *Appl. Anim. Behav. Sci.* 18, 327-340.

538 Triebe, C., Van den Berg, M., Brown, W.Y., 2012. Tagasaste versus Golden Bamboo: Which do
539 horses prefer?, *Proceedings of the Australasian Equine Science Symposium*, Gold Coast, Australia,
540 p. 41.

541 van den Berg, M., Lee, C., Brown, W.Y., Hinch, G.N., 2016. Does energy intake influence diet
542 selection of novel forages by horses? *Livest. Sc.* 186, 6-15.

543 Van Tien, D., Lynch, J.J., Hinch, G.N., Nolan, J.V., 1999. Grass odor and flavor overcome feed
544 neophobia in sheep. *Small Ruminant Res.* 32, 223-229.

545 Zhao, H., Yang, J.-R., Xu, H., Zhang, J., 2010. Pseudogenization of the umami taste receptor gene
546 *Tas1r1* in the giant panda coincided with its dietary switch to bamboo. *Mol Biol Evol* 27, 2669-2673.

547

Table 1: A Bayesian hierarchical model was used to determine the effect of a familiar odour (lucerne) on the intake of novel forages (bamboo). In this table the estimated posterior percentage of bamboo with lucerne odour consumption out of the total intake are presented. These estimates include main, temporal and random effects.

Table 2: A Bayesian hierarchical model was used to determine to establish if horses make associations with the orosensory characteristics of a novel food (soybean hull pellets) and their post-ingestive consequences (protein levels). In this table the estimated posterior percentage of high-protein soybean hull pellets consumption out of the total intake are presented. These estimates include main, temporal and random effects.

Figure 1. Boxplot of the total bamboo intake (g, DM) over the 9 testing days (n=11).

Figure 2. A Bayesian hierarchical model was used to determine the effect of a familiar odour (lucerne) on the intake of novel forages (bamboo). In this figure the violin plots of the posterior distribution of 95% credible intervals in logistic scale of the temporal effects (Days) for the intake of bamboo with lucerne odour (BO⁺) are presented (n=11). When the posterior mass is positioned above zero this expresses a higher BO⁺ consumption.

Figure 3. Boxplot of the total soybean hull pellets intake over 14 testing days (flavour-diet combination was switch at the end of week 1) (n=7).

Figure 4. A Bayesian hierarchical model was used to determine to establish if horses make associations with the orosensory characteristics of a novel food (soybean hull

pellets) and their post-ingestive consequences (protein levels). In this figure the violin plots of the posterior distribution of 95% credible intervals in logistic scale of the temporal effects for the intake of high-protein soybean hull pellets (HP-SHP) are presented (n=7). When the posterior mass is positioned above zero this indicates a preference towards HP-SHP.