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

M. van den Berg[1†], V. Giagos[2], C. Lee[3], W.Y. Brown[1], and G.N. Hinch[1]

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

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

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

4   fermentation horse's foraging preferences may be largely influenced by sensory input (e.g.

5   volatiles), it has been established that horses are able to differentiate and select familiar foods (e.g.

6   concentrates and hay) based on nutritional content. Yet it remains unclear how this translates to the

7   acceptance of nutritious novel foods (NF). Therefore, the influence of food cues and nutrient

8   composition on NF acceptance were examined in two experiments using 11 adult mares. In

9   experiment 1, we investigated the influence of a familiar odour (FO) on the acceptance of a

10   nutritious NF and in experiment 2, we determined if horses have the ability to select nutritious NF

11   based on the nutritional content, regardless of sensory preferences. In experiment 1 horses received

12   identical NF in a two-choice test with one of the choices being masked with a FO over a 9-day

13   period. In experiment 2 horses were offered a high or low protein option of an otherwise identical

14   NF in a two-choice test in which the NFs were paired with two unfamiliar flavours (odours). The

15   two-choice test lasted for 14 days and the flavour-protein pairing was switched after 7 days. NF

16   intakes were recorded over a 10 min test period on each test day and analysed using Bayesian

17   hierarchical models. The results of experiment 1 indicate that a FO had a strong positive influence

18   on the NF intake for the first 5 days (90-100% of total consumption and strong evidence for non-

19   zero temporal effects (Bayes factor $B_{12}=110$)). This was followed by a more even distribution of

20   intake for the remaining period. In experiment 2 horses had a greater intake of high protein NF

21   regardless of the flavour on days 4, 6 and 7 (80-87% of total consumption) and this continued after

22   the switch over (Day 9 to 14; 57-81% of total consumption). However, 4 out of 11 horses showed

23   neophobia throughout the testing period, which could have been associated with the novel odours as

24   horses scanned the buckets with little to no sampling. The results suggest that pre-ingestive cues

25   (e.g. smell, taste) of foods play an important role in diet selection and that a FO can increase the

26 acceptance of NF. This new knowledge could be applied by the horse industry to encourage the

27 consumption of new food or forages by horses.

28

29 **Keywords**

30 Diet Selection, Flavour, Food Neophobia, Horses, Odour, Novel Food

31

32 **1. Introduction**

33

34 The senses of taste and smell help animals to discriminate between foods and play an important role

35 in food preferences and food selection. Orosensory senses together with post-ingestive mechanisms

36 allow animals to make associations with pleasant or unpleasant experiences depending on whether

37 the effect on the internal environment of the animal is aversive or positive (gut-brain feedback)

38 (Provenza, 1995). For example foods can be rejected (aversion) as a direct result of sensory input

39 and its link to post-ingestive consequences, either from toxins or nutrients that are in excess or

40 deficient. Learned food aversions have been demonstrated in a number of animal species including

41 ruminants (Burritt and Provenza, 1991; Provenza, 1995; 1996), rats (Garcia et al., 1972) and horses

42 (Houpt et al., 1990; Pfister et al., 2002). Conversely, the liking of a food (preference) increases

43 when it contains adequate nutrients and provides a positive feedback.

44

45 Investigations of the ability of horses to differentiate foods based on the orosensory characteristics

46 and nutrient content are limited. There is some evidence that horses are able to detect

47 macronutrients in foods and can adapt to deficiencies by increasing intake or by changing food

48 choices (Laut et al., 1985; Cairns et al., 2002; Redgate et al., 2014). For example, Cairns et al.

49 (2002) showed that horses selected a higher energy concentrate over a lower energy one, regardless

50 of the preferred flavour (mint or garlic). However, it has been suggested that horses, due to hindgut

51 fermentation, may experience difficulties in associating the chosen food with its post-ingestive

52 consequences, particularly when several foods are presented simultaneously. Therefore, a more

53 recent study compared the effect of exposure to a single forage that was rich in either protein, lipids

54 or hydrolysable carbohydrates on the selection of three forages in a simultaneous choice session

55 (Redgate et al., 2014). The horses showed a greater preference for the forages that were rich in

56 protein or hydrolysable carbohydrates, which suggests that horses responded to the macronutrients

57 in the diets and that the dietary experience (single presentation) facilitated feedback mechanisms,

58 and hence affected dietary preferences.

59

60 However, other researchers have suggested that diet selection and intake are more influenced by the

61 organoleptic qualities of forages (e.g. taste, odour, ease of prehension, texture) and that nutrient

62 content appeared to be weak indicators (Dulphy et al., 1997; Cuddeford, 2005). Food selection

63 based on orosensory perception has been observed in a previous study (van den Berg et al., 2016)

64 that examined the effect of energy status (low and high) in horses on diet selection of familiar and

65 novel forages. These findings demonstrate that horses had a greater preference for familiar forages

66 and that, regardless of the energy intake, horses showed a strong neophobic response towards

67 unfamiliar nutritious forages, frequently scanning the buckets with little to no consumption. These

68 novel forages had comparable nutritional profiles to the familiar forages and therefore it seems that

69 volatiles and odour (olfaction) may be important factors in forage selection by horses, in particular

70 when dealing with a familiar-novel dichotomy. The influence of plant odours on herbivore feeding

71 behaviour and dietary preferences has been clearly demonstrated in sheep (Arnold et al., 1980). In

72 addition, odour profiling has been used to make predictions about the preferences (and links with

73 nutritional traits) for familiar forages (oat and lucerne hay) by horses and dairy cattle (Pain and

74 Revell, 2009).

75

76 While it has been established that horses are able to make associations with the nutritional content

77 of familiar foods, it is unclear how this applies to the selection of nutritious novel foods and how

78 volatiles may affect this selection. Therefore, the aim of the present study was to improve our

79 understanding of the acceptance of nutritious novel foods by horses by examining the sensory

80 behaviour and the ability of horses to learn about positive post-ingestive consequences of a novel

81 food in two experiments. The first experiment follows our previous study (van den Berg et al.,

82 2016) that demonstrated a strong neophobic response in horses towards novel forage volatiles and

83 focused on the question of whether odour influences diet selection of novel foods by horses. It was

84 hypothesised that horses would cautiously sample all foods on offer, but initially a greater intake

85 was expected for the novel choice with the familiar odour. The second experiment focused on the

86 question of whether horses make associations with the orosensory characteristics of an unfamiliar

87 nutritious food and their post-ingestive consequences. The hypothesis was that horses would

88 cautiously sample all foods available, but a greater intake for food with a superior nutrient profile

89 was expected regardless of the preferred flavours.

90

91 **2. Material and methods**

92

93 *2.1. Animals and feeding management*

94

95 A total of 11 healthy horses were used for the study. Horses were managed at a commercial horse

96 facility in the New England region (NSW, Australia). The mares were between the ages of 3 and 15

97 years, weighing 480-640 kg and were of Australian Stock Horse (n=8) and Thoroughbred (n=3)

98 breeds. Horses initially were grazing pasture and had a Henneke's body condition score between 4

99 and 8 (moderately thin to fat, Henneke et al., 1983). The management and feeding of horses was

100 based on the horse owner's usual practices and throughout the study period horses were managed

101 on pasture as one group and were only offered lucerne (*Medicago sativa*) hay *ad libitum* to

102 supplement poor winter pasture. The horses were not exercised, apart from one horse that was

103 ridden (light work) on three occasions during the experiment period (after the tests). This horse did

104   not receive any additional supplementation. The study was conducted between the months of July

105   and October 2014.

106

107   *2.2. Experimental design*

108

109   In the first experiment horses received identical novel forages in a two-choice test for 9 days with

110   one of the choices being masked with a familiar odour (Figure 1). The aim was to examine intake

111   patterns and the time required for horses to learn about the post-ingestive consequences of the novel

112   foods. We propose that a positive experience can be assumed when horses consumed 50% or more

113   of the total food offered and consumed equal portions of both choices.

114

115   In the second experiment horses were offered novel foods from a similar source in a two-choice test

116   that were high or low in protein paired with one of two unfamiliar flavours. The two-choice tests

117   were conducted over 14 days with the flavour-protein pairing switched after 7 days (Figure 1). The

118   objective was to examine intake patterns and the time taken for horses to increase consumption of

119   the high-protein food (post-ingestive feedback) and if horses would continue to make this selection

120   when flavour-protein pairing was switched.

121

122   *2.3. Feed collection and flavour preparation*

123

124   Golden bamboo (*Phyllostachys aurea*) was chosen as the test forage for experiment 1 based on the

125   novelty and literature describing the use as supplementary fodder in horse diets (Nelson, 1997;

126   Triebe et al., 2012; van den Berg et al., 2016). Bamboo was sourced from the nursery of the

127   University of New England, NSW. The browse was stripped to leaves and twigs, dried in a climate-

128   controlled room at $27^{\circ}C$ for 3-4 days and cut in pieces similar to the familiar chaff form (2-3 cm).

129

130 For the preparation of forage odour a similar procedure was used as described by Hinch et al.

131 (2004). Fresh lucerne was collected from a commercial lucerne grower in South East Queensland,

132 Australia. For the familiar odour solution 200 g fresh lucerne was shredded in a food blender with 1

133 L water to make up 20 L of total solution. This was allowed to stand overnight (12 h) in a cool

134 room at 4° C before straining through a cheese-cloth. The resultant extract was stored at -20° C in

135 airtight containers.

136

137 Commercially sourced non-nutritive human/animal food flavours (aniseed and citrus; Lucta, Spain)

138 were used in experiment 2. The aniseed flavour had a liquorice aroma and citrus had a sweet orange

139 aroma. Both flavours had no added taste and were novel to the horses. The flavour powder (5 g)

140 was diluted in 500 ml cold water to make a stock solution.

141

142 *2.4. Testing area*

143

144 Horses were individually tested in a holding yard (4 m x 8 m) that was familiar to them and within

145 view of other horses. Before testing (experiment 1) horses were adapted to the holding yard and

146 buckets for two days during which they were fed lucerne chaff (Figure 1). Two large feeding

147 buckets were mounted on the yard door (0.5 m apart and 1 m height) and two smaller labelled

148 feeding tubs were placed inside the larger mounted buckets. The position of the feeding tubs was

149 changed randomly for each testing day.

150

151 *2.5. Testing procedures*

152

153 2.5.1. Experiment 1: Familiar odour (FO)

154

155 Horses were offered two identical feeding buckets, each containing 200 g bamboo chaff (novel

156 forage) with and without a FO (lucerne). The forages were placed on mesh cloths that acted as the

157 floor of each feeding tub; the mesh size prevented the foods from falling through while allowing

158 any odour below to permeate the food. To deliver the FO, 200 ml of the lucerne extract was placed

159 3.5 cm below the feeder base. In addition, the mesh cloth of the bucket with the FO was drenched in

160 the lucerne solution and dried overnight. Each day new cloths were prepared for the feeding tubs.

161 The labelled feeding tubs were rinsed with water and dried with paper tissue between each horse

162 and test. Feeding tubs used with the FO were kept separate from the other feeding tubs.

163

164 2.5.2. Experiment 2: Nutrient composition (Post-ingestive feedback)

165

166 Horses were offered two novel foods (2 x 200 g; soybean hull pellets) that contrasted in crude

167 protein (CP) levels (13.9% *vs.* 22.3%). The two diets were paired with one of two unfamiliar

168 flavours (aniseed or citrus odours). Commercially sourced soybean hull pellets (SHP) with a CP

169 level of 13.9% and digestible energy content of 8.4 MJ/kg on a dry matter (DM) basis constituted

170 the low protein (LP) option. Soybean meal (65g; 47.5% CP) was added to the soybean hulls to

171 create a high protein (HP) option (22.3% CP) with minimal change to the fibre content and volume.

172 This meant that the total offered was 265 g for the high CP option, which resulted in a digestible

173 energy of 9.9 MJ/kg for HP diet. The flavour stock solutions (3 ml citrus or 5 ml aniseed) were

174 further diluted in water (300 ml) before adding to the novel foods to dampen the feed and create a

175 homogenous mixture. This concentration created an aromatic odour that was detectable by human

176 senses. Horses were randomly allocated to a flavour-protein pairing based on age and estimated

177 weight/ body condition score as this allowed for distribution of treatments across testing days and

178 eliminated the influences of potential weather conditions for one particular treatment. At the start of

179 the experiment 6 horses received HP-aniseed/LP-citrus and 5 horses HP-citrus/LP-aniseed. The

180 flavour-protein pairing was switched after 7 days.

181

2.6. Measurements

182

183

184 The two-choice tests (10 min) were conducted between 09:00 and 12:30 h each day of the testing

185 period. The intake of NF by each horse was determined by weighing the foods in each feeding

186 bucket before and after each test. For the SHP trial (experiment 2) the intake was adjusted for

187 moisture (added water) and calculated to a DM basis. On completion of testing horses were allowed

188 to return to their pasture paddock.

189

2.7. Statistical analysis

190

191

192 All the statistical analyses were performed with the R3.1.3 free software (R Core Team, 2015).

193 Additional information about the statistical analysis used in this study is covered in the

194 supplementary material supplied.

195

196 2.7.1. Experiment 1: Familiar odour

197

198 To determine if there was an effect of familiar odour on the intake of a novel forage and if horses

199 learn about the nutritional consequences of novel forages over time, the intake (g, DM) and

200 proportions (percentage) of bamboo without lucerne odour (BO⁻) and bamboo with lucerne odour

201 (BO⁺) over the 9 testing days were examined. We denoted the proportion of BO⁺ intake out of the

202 total intake of the i-th horse and the j-th day with p_{ij} and we chose a logistic link function with our

203 covariates. Our first covariate was an intercept β_0, which expressed a *main* effect and followed a

204 Normal distribution with mean zero and variance 1000^2. The zero mean indicates that overall the

205 horses had a balanced consumption (distribution of 50% from each bucket) whereas the large prior

206 variance allowed us to consider a wider range of common behaviours across all horses and days.

207

208   The experimental design suggested two consistent sources of variation: a *random* effect, which

209   could be attributed to each different horse and a *temporal* effect where each measurement depended

210   on the day of the observation. In previous ruminant studies (Launchbaugh et al., 1997; Van Tien et

211   al., 1999; Hinch et al., 2004) novel food acceptance (reduction in neophobia) was measured as a

212   transition from very limited consumption of a novel food to an incremental increase in intake that

213   could reach total offered amounts (plateau) depending on the post-ingestive feedback. We

214   hypothesised that this would be the same for horses and therefore the inclusion of the temporal

215   effects expresses this transition, which we assume is common for all horses. Whereas, the inclusion

216   of the random effects expresses the horse-specific variation (e.g. age, body condition score).

217

218   We addressed both sources of variation using a Bayesian hierarchical model. Each horse's foraging

219   behaviour was modeled with a random variable (β_h) which followed a Normal distribution with a

220   mean 0 and variance which in turn followed a Half-Cauchy prior (Gelman, 2006). The heavy-tailed

221   prior on the variance allowed us to explore a wide class of models for the foraging behaviour

222   (consistent *vs.* variable) of each horse. Similarly, for the temporal effect we have chosen a Normal-

223   distributed random variable (β_d) for each day with mean 0 and variance σ_d^2 with a Half-Cauchy

224   prior as well. The residual terms ϵ_{ij} followed a Normal distribution with mean 0 and variance σ_ϵ^2.

225   Other choices were considered (linear model, lineal change-point model, auto-regressive model) but

226   the aforementioned choice had the smallest deviance information criterion (DIC) (Spiegelhalter et

227   al., 2002) with the linear change-point (Day 6) model having a slightly increased DIC. Overall the

228   hierarchical model was:

229

230   $\beta_{hi} \sim$Normal $(0, \sigma_h^2)$ $\sigma_h^2 \sim$Half-Cauchy $(0,1)$ $\beta_{dj} \sim$Normal $(0, \sigma_d^2)$ $\sigma_d^2 \sim$Half-Cauchy $(0,1)$

231   $\beta_0 \sim$Normal $(0,1000^2)$ $\epsilon_{ij} \sim$Normal $(0, \sigma_\epsilon^2)$ $\sigma_\epsilon^2 \sim$Half-Cauchy $(0,1)$ logit $(p_{ij})= \beta_0+\beta_{dj}+\beta_{hi}+ \epsilon_{ij}$

232

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

234   (Plummer and Stukalov, 2014).

235

236   2.7.2. Experiment 2: Nutrient composition (Post-ingestive feedback)

237

238   To establish if horses make associations with the orosensory characteristics of a novel food and

239   their post-ingestive consequences, the intake (g, DM) and proportions (percentage) of SHP based on

240   protein levels and flavour over 14 testing days were examined. Only observations of seven horses

241   were used in this analysis, as 4 of the 11 horses did not have an intake of 25 g SHP or more after

242   Day 4 and remained neophobic throughout the testing period. Our measurements consisted of

243   intakes (g, DM) of SHP, which contained either HP, or LP and could have either a citrus or aniseed

244   flavour. Both the protein and the flavour have been treated as two-level factors. Our parameter of

245   interest is the proportion of HP-SHP out of the total intake. During the first 7 days 4 horses received

246   HP-aniseed/LP-citrus and 3 horses HP-citrus/LP-aniseed. The flavour-protein pairing was switched

247   after 7 days.

248

249   Similar to the previous model, we denoted the proportion of HP-SHP out of the total intake of the i-

250   th horse and the j-th day with q_{ij} and we used a logit link function as well. In this model we omitted

251   an intercept term but we considered a flavour coefficient α_f based on the citrus flavour to HP

252   proportion, which followed a Normal distribution with mean 0 and variance τ_f^2. The temporal

253   effects are modeled again as Normal-distributed random variables η_{dj} with mean 0 and variance

254   τ_d^2. Similarly, the random horse effects are Normal-distributed random variables η_{hi} with mean 0

255   and variance τ_h^2. The residual terms ξ_{ij} followed a Normal distribution with mean 0 and variance τ_ξ^2

256   and all the scale hyper-parameters τ_f^2, τ_d^2, τ_h^2, followed a Half-Cauchy (0,1) distribution. We also

257   introduced the indicator function $I(i,j)$ to denote if the HP-SHP had a citrus flavour, i.e. $I(i,j) =$

258   1 for the i-th horse on the j-th day or not ($I(i,j) = 0$). The hierarchical model was as follows:

259

260 $\alpha_f \sim$Normal $(0, \tau_f^2)$ $\tau_f^2 \sim$Half-Cauchy $(0,1)$ $\eta_{dj} \sim$Normal $(0, \tau_d^2)$ $\tau_d^2 \sim$Half-Cauchy $(0,1$

261 $\eta_{hi} \sim$Normal $(0, \tau_h^2)$ $\tau_h^2 \sim$Half-Cauchy $(0,1)$ $\xi_{ij} \sim$Normal $(0, \tau_\xi^2)$ $\tau_\xi^2 \sim$Half-Cauchy $(0,1)$

262 $$\text{logit } (q_{ij}) = \eta_{hi} + \eta_{dj} + \alpha_f I(i,j) + \xi_{ij}$$

263

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

265

266 **3. Results**

267

268 *3.1. Experiment 1: Familiar odour*

269

270 The total bamboo consumption (g, DM) is illustrated with boxplots in Figure 2. The model

271 described in Section 2.7.1 was positively evaluated (see for more details supplementary material)

272 and a greater BO^+ consumption was found for specific days. Since it is a Bayesian model, a

273 marginal posterior distribution is estimated for each model parameter instead of a point estimate

274 and it is used as the basis of the statistical analysis. The posterior distribution of bamboo

275 consumption (in percentage) at a particular day is summarised in Table 1; we emphasize that these

276 estimates include main, temporal and also random effects. The posterior percentages express the

277 posterior probability as a percentage, i.e. the BO^+ consumption out of the total intake per day. The

278 contribution of the temporal effects and the main effect to the BO+ consumption is shown in the

279 violin plots (Figure 3), where the random effects are omitted (see supplementary material for more

280 details). On the logistic scale of Figure 3 the posterior mass above zero expresses a higher BO^+

281 consumption. An equal distribution of consumption of BO^- and BO^+ is expressed when the posterior

282 percentage is close to 50% on Table 1 and a symmetric posterior density centered at zero on Figure

283 3.

284

285 In Table 1, the first 5 days the BO^+ accounts for 89.7–100% of the total consumption whereas at

286 Day 6 BO^- accounts for 83.3% of the total consumption. During Days 7 and 8 we start observing a

287 more balanced distribution between the two choices (the BO^+ consumption accounted for 65.7%

288 and 52.9% of the total intake for Days 7 and 8 respectively) and the BO^+ consumption increased

289 again on the last day (Day 9, 86.2%). Additionally, the posterior distribution of the temporal effects

290 for the intake of bamboo (Figure 3) showed a greater consumption for the BO^+ on Day 2, Day 4 and

291 Day 5 (the corresponding 95% creditable intervals in Figure 3 are above zero). There is also strong

292 evidence for non-zero temporal effects (Bayes Factor $B_{21}= 110$ in favor of non-zero effects; see

293 details in supplementary material) (Kass and Raftery, 1995).

294

295 *3.2. Experiment 2:Nutrient composition (Post-ingestive feedback)*

296

297 The total SHP consumption (g, DM) is illustrated with boxplots in Figure 4. The Bayesian model

298 (Section 2.7.2) was assessed in a similar fashion as described in the bamboo experiment, but it

299 received less support from the data (see for more details supplementary material). The distribution

300 of SHP consumption (in percentage) at a particular day is summarised in Table 2, again these

301 estimates include main, temporal and also random effects. Similar to the bamboo data presentation,

302 the posterior probability percentages in Table 2 express the HP-SHP consumption out of the total

303 intake. The contribution of the temporal effects and main effect to the HP-SHP consumptions is

304 shown in the violin plots (Figure 5). When the posterior mass is positioned above zero this indicates

305 a preference towards HP-SHP. When the consumption of HP-SHP and LP-SHP is balanced the

306 posterior percentage is close to 50% (Table 2) and a symmetric posterior density centered at zero on

307 Figure 5. In order to keep the bulk and fibre content of the two foods similar, we offered 265 g for

308 the HP-SHP option and 200 g for the LP-SHP. The added protein meal (65 g) did not contribute to

309 the expansion of the SHP, bonding to the fibrous structure, and we preserved the volume between

310   the two options by adding the same amounts of water. We observed total intakes greater than 400 g

311   for Horse 5 on Day 6 and 7 and for Horse 9 on Day 10 and 12 (outliers and whisker Figure 4), and

312   we acknowledge that this a limitation based on weight but not for volume.

313

314   With the exception of Horse 5, in Table 2, the random effects indicated a greater consumption of

315   the HP-SHP, which was, on average, between 1.56 to 2.45 times more than the LP-SHP intake. The

316   flavour effect was expressed by α_f, which was minimal (posterior mean -0.045 ± SD; 0.216; see

317   details in supplementary material). Initially (in the first week) a variable intake pattern emerged:

318   the first two days indicated a moderate intake for LP-SHP (~64%), which was followed by a greater

319   consumption of LP-SHP on Day 3 (~85%) and an equal intake was recorded on Day 5 (~49% HP).

320   For the remaining days 4, 6 and 7 a greater consumption of HP-SHP was noted (80-87%). In the

321   second week, immediately after the flavour switch-over, a higher consumption of LP-SHP was

322   recorded followed by a steady recovery on the remaining days (9-11) to a greater intake of HP-SHP

323   (57 to 81%). However, the posterior distribution of the temporal effects for the intake of SHP

324   (Figure 5) showed large variations and no clear distribution in favor of the HP-SHP was observed

325   (all 95% creditable intervals cross the zero line in Figure 5).

326

327   **4. Discussion**

328

329   *4.1 Nutrient composition*

330

331   Food acceptance and preference is a result of an interrelationship between the foods flavour (smell,

332   taste and texture) and its post-ingestive consequences (positive or negative) (Provenza, 1995). A

333   previous study in horses suggest that preferences for particular flavours can be overridden by post-

334   ingestive consequences of a familiar food (Cairns et al., 2002) and this study (second experiment)

335   hypothesised that horses would be similar when they are offered nutritious novel foods; having a

336 greater intake of high-protein novel food, regardless of the novel odour (aniseed or citrus). It was

337 also expected that horses would be able to recognise a flavour-protein pairing if the link between

338 the cue and post-ingestive feedback was altered. This study showed a greater posterior percentage

339 for the HP-SHP on days 4, 6 and 7 and after the switch over on days 9 to 14. This suggest that the

340 recognition of the post-ingestive associations (from CP and/or DE content) initially seemed to take

341 4 to 5 days which was also apparent in experiment 1 and is in accordance with the findings of

342 Cairns et al. (2002). However, the posterior distribution of the temporal effects (omitting the

343 random effects) for the intake of SHP showed large variations between days and was not supported

344 by the model. This lack of support could be attributed to the dropout in this study, as 4 out of 11

345 horses did not sample the novel SHP diet within the time frame of the test and were removed from

346 the analysis.

347

348 Horses, like ruminants, habitually eat only small quantities of food when it is presented for the first

349 time (Thorhallsdottir et al., 1987; Provenza, 1995; van den Berg et al., 2016). This cautious

350 sampling or even complete rejection of new foods is commonly referred to as neophobia (meaning

351 "the fear of new") and has been suggested as an innate herbivore survival mechanism for avoiding

352 the over-consumption of toxic plants in the wild (Provenza and Balph, 1987). The results from

353 experiment 2 would seem to affirm this pattern. This raises the question of individual variation in

354 the level of neophobia toward novel volatiles (in this case flavours citrus and aniseed) and/or the

355 texture of the SHP. Ott et al. (1979) reported a similar pattern with 6 of 8 mature horses reducing

356 intake of a grain concentrate when it was mixed with 30% dried citrus pulp consuming only 8.6%

357 of the feed offered. The strength of neophobia seems to vary considerably between individuals of

358 ruminant species (Launchbaugh et al., 1997; Nolan and Hinch, 1997; Hinch et al., 2004) and this

359 seems to be the case in horses as well.

360

361 *4.2 Food cues*

362

Food cues (flavour; smell, taste and texture) play an important role in an animal's ability to identify and remember foods with aversive post-ingestive consequences. This has been well documented in ruminants (Provenza et al., 1990; Launchbaugh and Provenza, 1993; Kyriazakis et al., 1997; Pfister et al., 2010) and ponies have been shown to learn to avoid relatively novel foods when apomorphine hydrochloride (APO) was injected intramuscularly to induce illness (Houpt et al., 1990). These authors also showed that ponies could form an aversion to a novel food, based on previous experience, even when it is ingested at the same time as another, more familiar, food. However, this pattern was not completely consistent with high-energy dense foods suggesting that ponies were more likely to develop aversions to less "palatable" foods.

4.2.1 Odour

Odour (volatiles) is an important food characteristic, which allows animals to discriminate amongst foods and link these to pleasant or unpleasant experiences. While it is likely that neophobia in experiment 2 was caused by the novel volatiles (aniseed and citrus), we can confirm that a familiar odour can have a positive effect on novel food acceptance. Our study (experiment 1) showed for the first 5 days a posterior percentage between 89.7 and 100% for the BO^+ when main, random and temporal effects were included. In addition the posterior distribution of the temporal effects showed that 3 out of 5 days had 95% creditable intervals that were above zero. There was also strong evidence for temporal effects as the support by the data is 110 times (Bayes factor) greater than the no effects model. This suggests that lucerne odour positively influenced the intake of bamboo during the first 5 days of exposure. After day 5 the intake shifted to an equal BO^+ and BO^- consumption distribution and horses consumed 50% or more of the bamboo offered which may suggest that horses had recognised the unfamiliar bamboo as an acceptable food (i.e. lack of a negative post-ingestive feedback).

388 It has been shown that animals can generalise preferences and a well-liked familiar flavour can

389 increase the acceptance of a novel food (even if it contain some toxins). Dohi and Yamada (1997)

390 demonstrated that sheep and goats had a greater preference for a less palatable hay when it was

391 sprayed with an extract of a well-liked high-grain concentrate and Van Tien et al. (1999) showed

392 that sheep increased their intake of a novel food (rice bran) more quickly when a familiar grass

393 odour and combination of odour and taste was added. The results of this study (experiment 1)

394 suggest a similar pattern for horses. Flavours have been effectively used to encourage intake of

395 water and (medicated) foods by horses. Mars et al. (1992) used apple- or clover-flavours to

396 encourage water acceptance and showed a significant preference for the apple flavour while Burton

397 et al. (1983) reported that apple, lucerne and caramel flavours but not anise-molasses were partially

398 effective in increasing acceptance of a diet containing levamisol or piperazine, and Goodwin et al.

399 (2005) demonstrated that well-liked flavours (fenugreek and banana) significantly reduced relative

400 consumption time of mineral pellets compared to unflavoured pellets. However, these studies have

401 not always clearly defined what type of flavouring (i.e. non-nutritive *vs.* nutritive) was used and if it

402 only affected the smell or also impacted the taste.

403

404 In our current study we only used odour as a food cue, which appeared to be strong enough to

405 encourage the acceptance of a nutritious novel food. This suggests that volatiles (pre-ingestive

406 feedback) can play an important role in diet choices by horses and supports the findings of our

407 previous study (van den Berg et al., 2016). The influence of volatiles on the preference of familiar

408 foods (oaten and lucerne) by horses has been previously demonstrated by Pain and Revell (2007;

409 2009). These authors showed that volatiles can be positively linked to nutritive and physical traits

410 of lucerne or oaten hay. However they also found volatiles that influenced the preference negatively

411 but were not related to any measurable nutritive or physical traits, possibility due to other plant

412 factors such as secondary compounds. This suggests that diet selection by horses cannot always be

413 explained by nutritive traits and that olfaction and gustation cues may also be important. This may

414 be more apparent when dealing with strong herbaceous volatiles such as from browse species (tree

415 and shrub leaves), which were used in our previous study (van den Berg et al., 2016).

416

417 *4.2.2 Taste*

418 Taste (gustation) could have also influenced the food consumption observed in our study. In

419 experiment 2 we added soybean meal, which may have contributed to a greater acceptance of the

420 HP-SHP option. It has been shown that horses can distinguish between 4 of the 5 taste sensations

421 (sweet, bitter, salty and sour) (Randall et al., 1978), but there may even be an indication that horses

422 could also have a taste for "umami". Umami is a Japanese loanword and can be translated as

423 "pleasant savoury taste". Umami perception occurs through the detection of the carboxylate anion

424 of glutamic acid, which is a naturally occurring amino acid common in meats, cheese, broth, stock

425 and other protein-heavy foods (Chandrashekar et al., 2006). While umami taste responses has been

426 mainly linked to a carnivorous or omnivorous diet, it seems that herbivores such as horses and cattle

427 express also the taste receptor genes involved with the umami taste sensation (Zhao et al., 2010). In

428 addition, Favreau et al. (2010) has demonstrated in sheep that an umami taste resulted in a greater

429 intake/preference compared to a bitter taste and Bach et al. (2012) showed that sheep form

430 preferences for umami-flavoured feeds, even when those feeds are novel and low in CP. This could

431 indicate that horses may also have the ability to detect foods with a "protein taste" which could

432 explain why horses seem to have a greater preference for HP-SHP diet. However, to our knowledge

433 no studies are known that have explicitly assessed this in horses, therefore in hindsight it could have

434 been noteworthy to examine how taste (sweet or umami) could have influenced the neophobia seen

435 in this study.

436

437 *4.2.3 Texture*

438 The texture and ease of prehension are also food characteristics that can influence the food

439 acceptance and intake by grazing animals. In experiment 2 we used soybean hulls, a by-product of

440 the soybean processing industry which contain 53-70% neutral detergent fibre (NDF) and less than

441 3% starch (NRC, 2007). It is considered to be a good alternative fibre-product for inclusion in

442 livestock feeds and has a growing use in commercial horse feeds due to its low glycaemic index

443 (GI) profile. While it is high in digestible fibre its initial palatability may be modest when it is fed

444 on its own which could have attributed to the texture or taste. When fed dry SHP can be dusty and

445 when dampened the fibres expand which result in a "cottony" texture. Overall texture and/or

446 palatability could have influenced the horse's foraging behaviour in our study.

447

448 **5. Conclusion**

449

450 The findings of the present study confirm that horses use organoleptic qualities of forages (e.g.

451 odour, taste, texture, ease of prehension etc.) when selecting foods. Horses in this study were able to

452 make an association with the nutritional content of a novel food, but neophobia significantly

453 impacted on initial testing of novel foods. We were also able to show that the acceptance of a novel

454 food can be enhanced by introducing a familiar odour, which highlights the important role of the

455 pre-ingestive signals in diet selection by horses. The present study was not able to examine the role

456 of taste in dietary preferences by horses, as non-nutritive flavours (odours) were used. Future

457 preference studies should incorporate odour, taste and texture to determine the relative importance

458 of these "cues" to horses making diet choices in grazing environments. Nevertheless, the present

459 study adds to the understanding of mechanisms involved in diet selection by horses and proposes

460 that odour cues can be successfully applied in equine feeding management when forages/foods are

461 scarce and new foods or forage batches are introduced or when horses are moved to different

462 environments.

463

464 *Ethical statement*

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).

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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 (i.e. day) and random (i.e. horse) effects.

Table 2: A Bayesian hierarchical model was used 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 (i.e. day) and random (i.e. horse) effects.

Figure 1. Timeline (day) of experiments.

Figure 2. Experiment 1: Boxplot of the total bamboo intake (g, DM) over the 9 testing days (n=11).

Figure 3. 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 (i.e. day) 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 4. Experiment 2: 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 5. A Bayesian hierarchical model was used 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 (i.e. day) 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.

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 (i.e. day) and random (i.e. horse) effects.

				Days				
1	2	3	4	5	6	7	8	9
94.9%	97.7%	89.7%	100.0%	99.9%	16.7%	65.7%	52.9%	86.2%

Table 2: A Bayesian hierarchical model was used 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 (i.e. day) and random (i.e. horse) effects. Flavour-protein pairing was switched over after week 1.

	Days						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Week 1	36.1%	36.4%	14.8%	79.6%	48.6%	82.5%	87.0%
	8	**9**	**10**	**11**	**12**	**13**	**14**
Week 2	18.2%	57.3%	69.8%	73.8%	80.5%	80.9%	77.9%

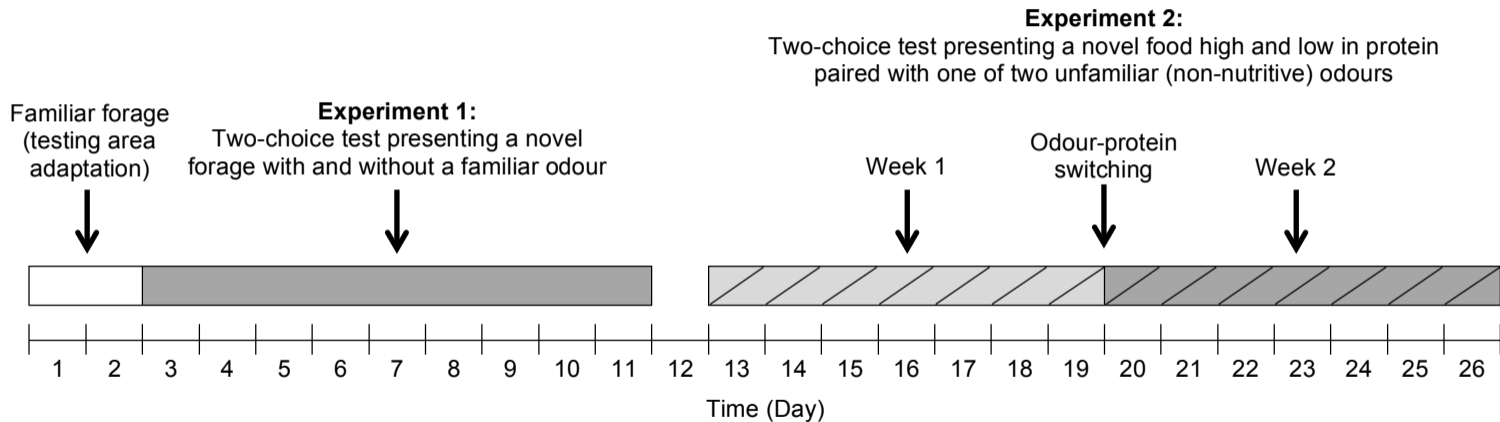**Figure 1: Timeline experiments**
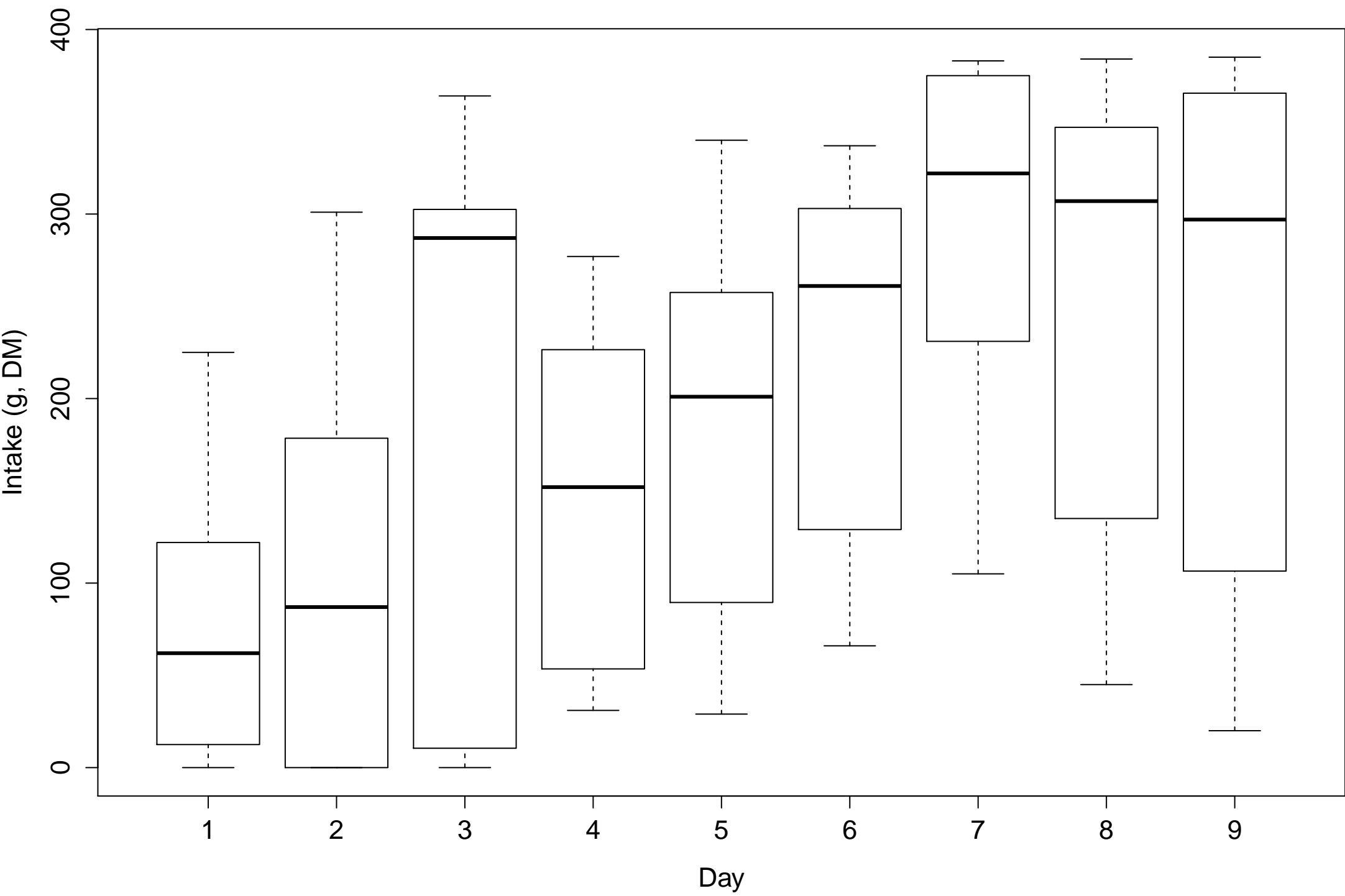
Figure 2: Boxplot of total bamboo intake

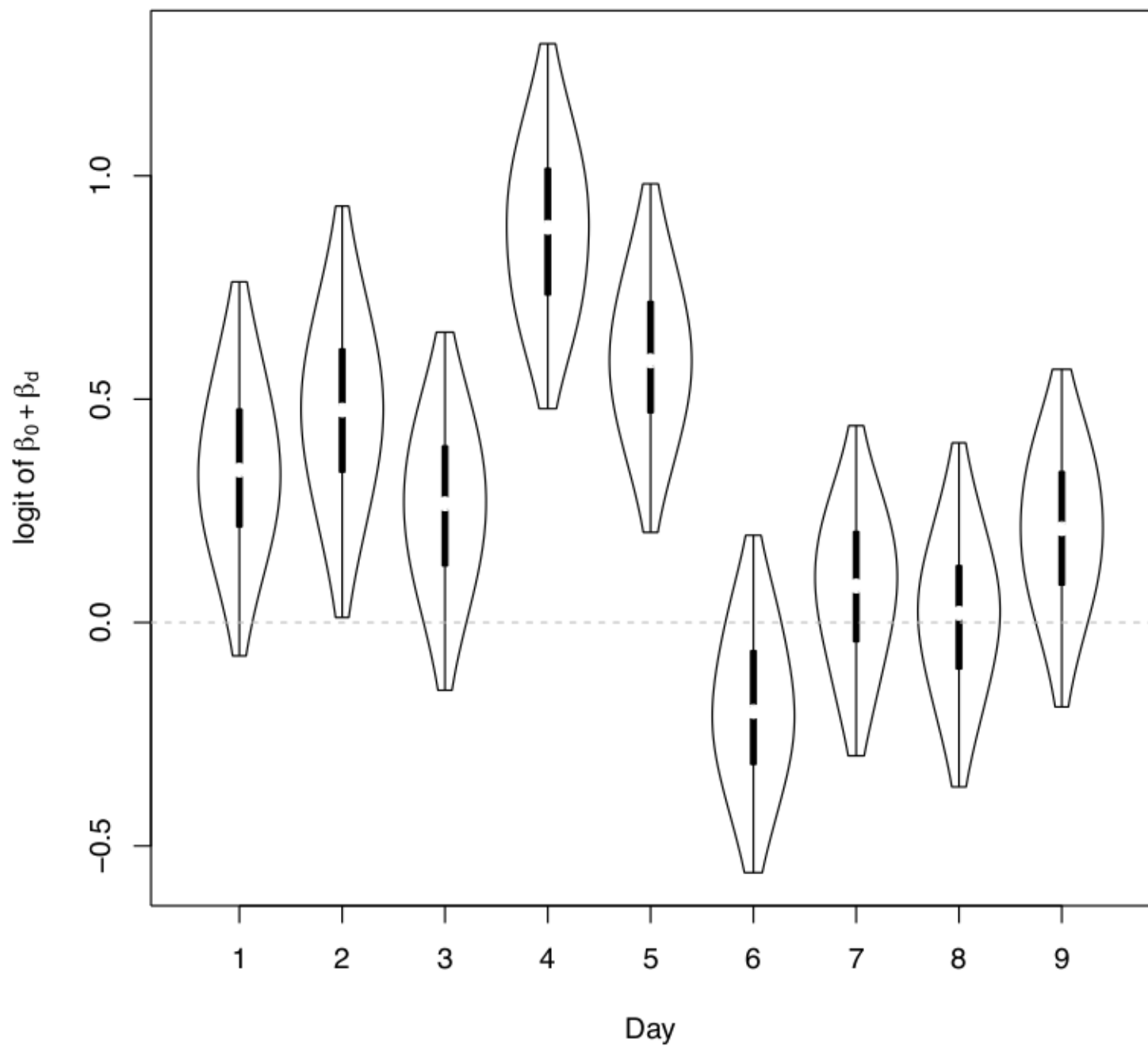**Figure 3: Violin plots of posterior distribution (Experiment 1)**
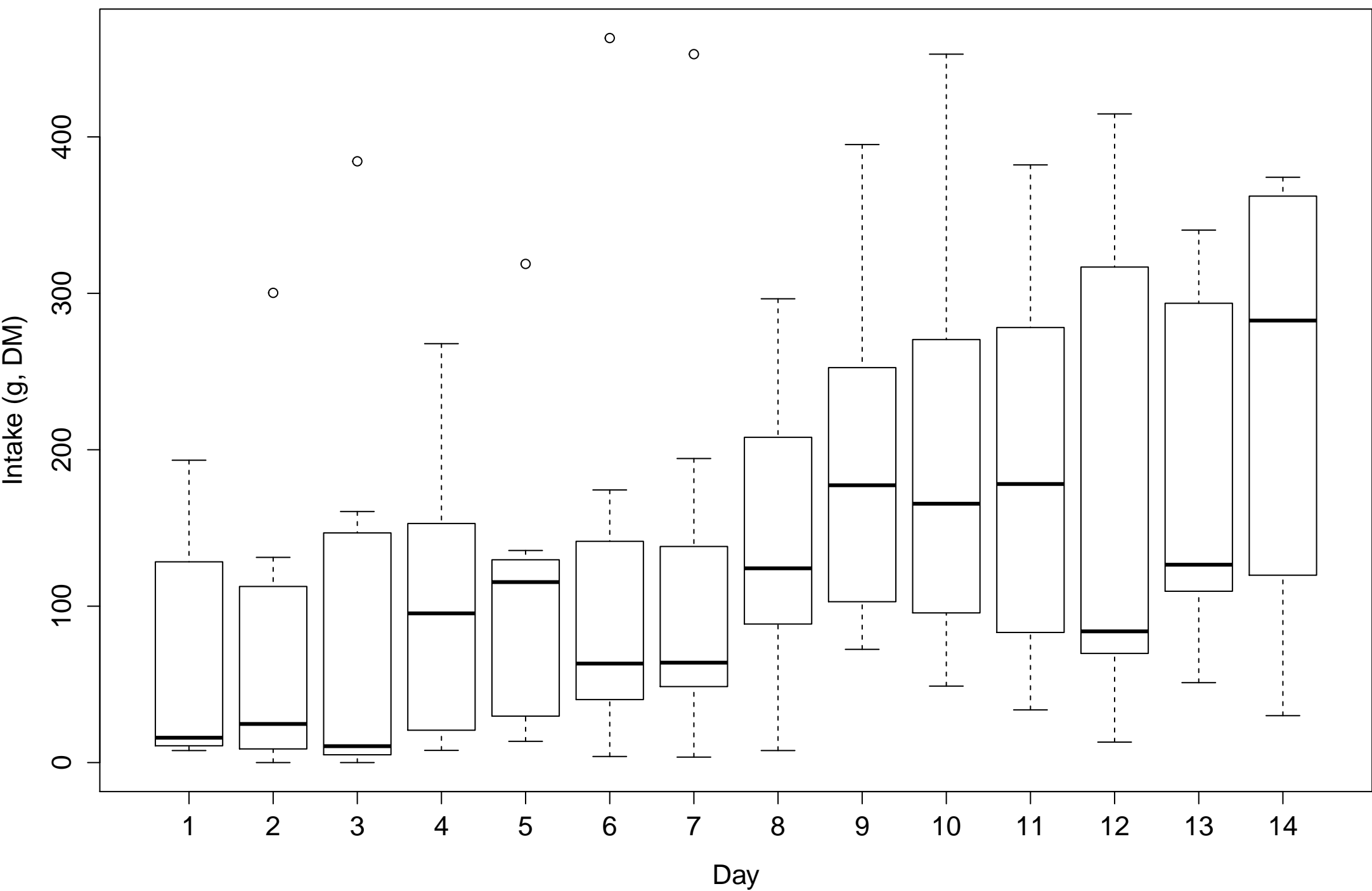
Figure 4: Boxplot of total SHP intake

Figure 5: Violin plots of posterior distribution (Experiment 2)