

1 Title: Effect of varying the dose of corn syrup on the insulin and glucose response to the oral
2 sugar test

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15

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19

20 Authorship:

21 N. A. Jocelyn, N.J. Menzies-Gow and P.A. Harris contributed to the study design, preparation

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24

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50 **Summary**

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52 **Reasons for performing study:** The oral sugar test (OST) is used to identify equine insulin
53 dysregulation (ID); however only a dose of 0.15 ml/kg bwt corn syrup (Karo Light Corn
54 Syrup)^a has been evaluated.

55

56 **Objectives:** To determine the effect of varying the dose of corn syrup syrup on insulin and
57 glucose response to the OST and the test's ability to distinguish between ponies with (PL)
58 and without (NL) a history of laminitis.

59

60 **Study Design:** Randomised crossover experiment.

61

62 **Methods:** After an overnight fast, in a 3-way randomised crossover study with a 7-day
63 washout, 0.15 ml/kg bwt, 0.3 ml/kg bwt or 0.45 ml/kg bwt corn syrup (Karo Light Corn
64 Syrup)^a was administered orally to eight ponies (5PL, 3NL) and blood obtained between 0
65 and 120 min. Serum [insulin] and [glucose] were measured using previously validated
66 radioimmunoassay and colorimetric assays respectively. The repeatability of and the effect of
67 continued pasture access on the dose that best distinguished PL and NL ponies was then
68 assessed. The effect of dose, laminitis history and fasting on serum [insulin] and [glucose]
69 responses were assessed using mixed effects models.

70

71 **Results:** The serum [insulin] following 0.15 ml/kg bwt were not significantly different from
72 0.3 ml/kg bwt at any time point; whilst serum [insulin] following 0.45 ml/kg bwt
73 significantly ($p < 0.01$) differed from 0.15 ml/kg bwt and 0.3 ml/kg bwt at all time points apart
74 from 0 min. The serum [insulin] concentration significantly ($p < 0.01$) differed between NL
75 (mean 86 [95% CI 59, 113] $\mu\text{iu/ml}$) and PL (146 [95% CI 124, 167] $\mu\text{iu/ml}$) only following

76 0.45 ml/kg bwt at 60 min. Repeatability of serum [insulin] at 60 min following 0.45 ml/kg
77 bwt dose under fasted conditions was 0.51. Using AUC insulin improved repeatability to
78 0.83. There was no significant difference between the fasted and at pasture results.

79

80 **Main Limitations:** The OST was performed in small numbers of ponies on limited
81 occasions.

82

83 **Conclusions:** A dose of 0.45 ml/kg bwt corn syrup may be preferable to differentiate PL and
84 NL ponies.

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100 **Introduction**

101 Insulin dysregulation (ID) in the horse encompasses fasting hyperinsulinaemia, an excessive
102 insulin response to oral carbohydrates and tissue insulin resistance [1]. The importance of ID
103 and its association with an increased risk of laminitis [2] is clinically relevant to practitioners
104 and a practical reliable and repeatable test for ID diagnosis would therefore be advantageous
105 [3]. The oral sugar test (OST) has been advocated as simple field based dynamic test to
106 identify equids with an excessive insulin response following ingestion of carbohydrate [4]
107 and tissue insulin resistance [5]. Ease of administration of the sugar in syrup form and the
108 possibility of obtaining a single blood sample post-administration make the OST an attractive
109 option for use by practitioners.

110

111 Initial work suggested a good positive correlation between the OST and the intravenous
112 glucose tolerance test [4] and the OST and the oral glucose test [6]. However recent
113 publications found the OST to have poor sensitivity [7] and no significant relationship when
114 directly compared to other ID testing [8]. Both these studies [7,8] however, compare
115 intravenous tests, which are measures of tissue insulin resistance to the OST. The OST is a
116 test that explores ID characterised by an excessive response to oral carbohydrate and thus
117 evokes the enteroinsular axis [9]. As such, a direct association is not expected. Repeatability
118 has varied from acceptable [10] to poor [11].

119

120 A single dose of 0.15ml/kg bwt corn syrup (Karo Light Corn Syrup)^a for the OST has been
121 used [11] [12], but limited differing doses have been investigated [13]. Higher doses, which
122 provide amounts of sugar more similar to the oral glucose test, may afford improved
123 diagnostic abilities. Current advice is to perform OST after fasting [3], however a study
124 comparing horses at pasture and after fasting found no significant difference in OST

125 outcomes [8]. Another study [11] with ponies at pasture found significant differences
126 between fasting and fed state for area under curve insulin and insulin concentration at 60,75
127 and 90 minutes, however dichotomous interpretation for ID was similar using study identified
128 cut-off values.

129

130 The aim of this study was to evaluate the effect of varying the dose of corn syrup on 1) the
131 insulin and glucose response and 2) the ability of the OST to distinguish between ponies with
132 a history of laminitis (PL) and non-laminitic ponies (NL). Once an optimal dose was
133 identified, further aims of the study were to further explore this dose with respect to
134 repeatability, season and the effect of fasting.

135

136 **Materials and Methods**

137 *Animals*

138 Eight British native pony mares from a research herd kept at pasture were used in the study.
139 All were clinically healthy, aged between 12 and 23 years and weighing between 245-441kg
140 (Supplementary information 1); 5 had a known clinical history of laminitis but had no active
141 signs of laminitis in the 3 months prior to and during the entire study period; 3 had no history
142 of laminitis. All of the animals had been part of the herd for at least 10 years. None had
143 clinical signs of pituitary pars intermedia dysfunction and basal ACTH concentrations were
144 within the seasonally adjusted reference range.

145

146 *Study design*

147

148 *Dose Study*

149 The study was undertaken in December 2015. All eight ponies were brought into a bare dirt
150 paddock the night before each study day. Haylage was provided to last until midnight and
151 adlib water was provided throughout. The following morning, a 14 g jugular catheter
152 (Angiocath)^b was placed under local anaesthesia (Intra-Epicaine)^c and a baseline blood
153 sample obtained (T₀). In a randomised crossover design, animals were given either 0.15ml/kg
154 bwt, 0.3ml/kg bwt or 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)^a by oral syringe.
155 Further blood samples were collected at 30, 60, 75, 90 and 120 (T_{30–120}) min after oral
156 dosing. A 7-day washout period between doses was undertaken during which times the
157 animals were kept at pasture.

158

159 Analysis of the corn syrup (Karo Light Corn Syrup)^a using the following method (Longland
160 personal communication) found it to contain 356.3 mg/ml of maltose and glucose combined.
161 (Supplementary information 2) The doses used therefore equated to
162 53.4mg/106.9mg/160.3mg/kg bwt of maltose and glucose combined respectively. Samples
163 were diluted on a weight/volume basis (100mg/ml). Then 50 μ L of sample were added to 950
164 μ L of a buffer comprising 5 mM H₂SO₄ with a 5 mM crotonic acid internal standard. Samples
165 were analyzed using via high-performance liquid chromatography^d. Injection volume was 25
166 μ L. Sugars were separated on a Rezex ROA-Organic acid column^e and a mobile phase of 5
167 μ M H₂SO₄ at 0.6 ml min⁻¹. Sugars were detected with a refractive index detector and
168 identified by comparison with an internal library of standard compounds.

169

170 Blood for serum insulin concentration measurement was collected into plain tubes
171 (vacutainer)^a and allowed to clot at 37°C for at least 20 min. Blood for glucose concentration
172 measurement was collected into fluoride oxalate tubes (vacutainer)^a. All samples were

173 centrifuged (3000 x *g*) for 10 min at 4°C and the serum or plasma stored at -80°C before
174 analysis

175 *Repeatability Study*

176 The study was undertaken in June 2016. The OST was repeated with all eight ponies
177 receiving 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)^c on 2 occasions with a 7-day
178 washout period between.

179

180 *Fasting vs Fed Study*

181 Seven days after the repeatability study was completed, the OST was repeated for a third time
182 with all ponies receiving 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)^c, however the
183 ponies were not fasted and instead remained at pasture prior to and during the study.

184

185 *Sample analysis*

186 Serum insulin concentrations were measured using a radioimmunoassay (Insulin CT)^f and
187 serum glucose using a colorimetric assay (Glucose Colormetric Assay Kit)^g. All samples
188 were measured in duplicate and both assays had been previously validated for use in ponies
189 [11].

190

191 *Data analysis*

192 All analysis was performed using statistical (IBM SPSS Statistics 22)^h and graphic (Graphpad
193 Prism)ⁱ software. The area under the curve (AUC) was calculated for the full insulin response
194 (T0-120; AUC_i), insulin response at T0,60 and 90 (AUC_{insulin modified}; AUC_{iM}) and full glucose
195 response (AUC_g) for each test using the trapezoidal sum method with the x axis (y = 0) as the
196 baseline insulin or glucose concentration. Linear mixed effects models were generated to
197 investigate the differences between NL and PL. Sampling time (for single time points), dose,

198 NL/PL and their interactions were initially included as fixed variables and removed
199 according to statistical significance. Pony was included as a random variable. Insulin
200 concentration/ AUC_i/AUC_{iM} , maximal insulin concentration (C_{max_i}), time to maximal insulin
201 concentration ($T_{max_{iM}}$), maximal insulin concentration T0,60 and 90, glucose concentration/
202 AUC_g , maximal glucose concentration (C_{max_g}) and time to maximal glucose concentration,
203 (T_{max_g}) were the outcome variables examined and an auto- regressive covariance structure
204 (AR1) was used. Estimated marginal means were calculated from the final model and
205 pairwise post-hoc comparisons were performed (without adjustment of confidence intervals
206 for multiple comparisons/least significant difference). The normality of the distribution of the
207 residuals was assessed by histogram to ensure normality. Linear mixed effects model was
208 repeated using the 0.45ml/kg bwt dose and differences between NL and PL investigated.
209 Sampling time (for single time points), NL/PL, pasture, season and their interactions were
210 initially included as fixed variables and removed according to statistical significance.
211 Repeatability was assessed using repeated measures [14], briefly, using estimates of
212 covariance parameters the pony variance was divided by the sum of residual and pony
213 variance combined. Statistical significance was set at $P \leq 0.05$.

214

215 **Results**

216 No adverse effects were seen in any of the ponies throughout the two study periods. All
217 ponies tolerated the corn syrup administration very well and received the full dose on all
218 occasions.

219

220 *Insulin*

221 *Dose response*

222 The serum insulin concentration was significantly different for both 0.15 ml/kg bwt and 0.3
223 ml/kg bwt dose compared to the 0.45 ml/kg bwt at all time points apart from T₀ for all ponies
224 combined (P<0.001); whilst the 0.15 ml/kg bwt and 0.3 ml/kg bwt doses were not
225 significantly different at any time point (Figure 1). The C_{max_i} was significantly (P<0.001)
226 greater following 0.45ml/kg bwt dose (mean 174 [95% CI 141, 206] µiu/ml) compared to
227 either 0.15ml/kg bwt (72 [95% CI 39, 104] µiu/ml) or 0.3ml/kg bwt dose (87 [95% CI 54,
228 119] µiu/ml).

229

230 When the serum insulin concentrations were compared between the two groups of ponies
231 (NL and PL) at the 6 time points, the only significant (P=0.04) relationship between group
232 and dose was at 60 minutes for the 0.45ml/kg bwt dose (NL mean 86 [95% CI 59, 113] vs PL
233 146 [95% CI 124, 167] µiu/ml; Figure 2). The 0.45ml/kg bwt dose with an insulin
234 concentration cut off value of ≥ 110 µiu/ml at 60 min allowed for correct identification of all
235 5 PL and 3 NL ponies for the current data.

236

237 When the results from the two groups of ponies (NL and PL) were compared, there was a
238 significant interaction (P=0.05) between dose and group for AUC_i. There was a significant
239 (p=0.01) difference between NL and PL for AUC_i following 0.45ml/kg bwt, but not
240 following 0.15ml/kg bwt or 0.3 ml/kg bwt (Table 1). The 0.45ml/kg bwt dose with an AUC_i
241 cut off value of $\geq 10,000$ µiu/ml/min allowed for correct discrimination of all 5 PL and 3 NL
242 ponies. There was no significant interaction between dose and group for C_{max_i} and T_{max_i}
243 (Table 1).

244

245 *Repeatability*

246 When comparing the 0.45ml/kg bwt dose repeated under fasting conditions in the same
247 month (June), the repeatability for AUC_i was 0.83; whereas the repeatability of the serum
248 insulin concentration at the single significant time point of 60 minutes was 0.5. T_{max_i}
249 repeatability was low at 0.19 but C_{max_i} was 0.64.

250

251 *Season*

252 When comparing the fasted 0.45ml/kg bwt dose between December (Winter, northern
253 hemisphere) and June (Summer, northern hemisphere), there was a significant interaction
254 between season and group (NL and PL) when comparing both AUC_i ($P=0.04$) and insulin
255 concentration at 60 minutes ($P=0.03$) but no significant interaction for C_{max_i} and T_{max_i} .
256 There was a significant difference ($P=0.03$) between the AUC_i in winter and the summer in
257 PL but not NL (Table 1). Similarly, the serum insulin concentration at 60 minutes was
258 significantly ($P=0.01$) lower in winter (146, [95% CI 108, 184], $\mu\text{iu/ml}$) compared to summer
259 (204, [95% CI 172, 236], $\mu\text{iu/ml}$) in PL but not NL (winter 86 [95% CI 37, 136] $\mu\text{iu/ml}$ and
260 summer 71 [95% CI 30, 112] $\mu\text{iu/ml}$). There was no significant difference for C_{max_i}
261 ($P=0.53$) or T_{max_i} ($P=0.9$) between winter and summer. (Table 1)

262

263 *Fed vs Fasting*

264 When comparing the insulin response of all 8 ponies combined when fasted and at pasture
265 during the summer following 0.45ml/kg bwt dose, there was no significant interaction
266 between group (NL and PL) and feeding and no significant differences between fasted and at
267 pasture for the outcomes variables (Table 1).

268

269 *Modified $AUC_{insulin}$*

270 Further analysis of the AUC_i was explored to attempt to reduce the frequency of the blood
271 sampling requirements. Using data from only 3 time points, namely T0, 60 and 90, a
272 modified insulin AUC (AUC_{iM}) was calculated. There was a significant (p<0.001) difference
273 between AUC_{iM} for all the 8 ponies combined following the 0.45ml/kg bwt dose (8526, [95%
274 CI 7060, 9991] μ iu/ml/min) and both 0.15ml/kg bwt (4249, [95% CI 2784, 5715]
275 μ iu/ml/min) and 0.3ml/kg dose (4481, [95% CI 3016, 5947] μ iu/ml/min). However, there was
276 no significant difference between the 0.15ml/kg bwt dose and 0.3ml/kg bwt dose. This
277 relationship also held true for C_{max}_{iM} (data not shown).

278

279 A significant interaction was found between group (NL and PL) and dose for AUC_{iM}
280 (P=0.04) and C_{max}_{iM} (P=0.05) but not T_{max}_{iM} (P=0.96). There was a significant difference
281 for AUC_{iM} between NL and PL following 0.45ml/kg bwt (p=0.001) but not following
282 0.15ml/kg bwt or 0.3 ml/kg bwt dose (Table 1). A cut off value of ≥ 7500 μ iu/ml/min AUC_{iM}
283 distinguished between NL and PL ponies in the current data. The repeatability of the
284 modified AUC_i was 0.63.

285

286 *Glucose*

287 *Dose*

288 When the data from all 8 ponies was combined, the AUC_g was significantly greater following
289 the 0.45ml/kg bwt (P= 0.049) and 0.3ml/kg bwt (P=0.005) doses compared to the 0.15ml/kg
290 bwt dose. C_{max}_g was significantly greater following the 0.3ml/kg bwt (P=0.001) dose
291 compared to the 0.15ml/kg bwt dose and the 0.45ml/kg bwt (P=0.04). T_{max} was significantly
292 (P=0.05) later following 0.45ml/kg bwt dose compared to 0.15ml/kg bwt dose.(Table 2).
293 There was no significant interaction between dose and group (NL and PL) for AUC_g, C_{max}
294 or T_{max} (data not shown).

295

296 *Group, season and pasture*

297 When the data from all 8 ponies was combined, in summer AUC_g ($P=0.04$) and C_{max_g}
298 ($P=0.02$) were significantly greater whilst T_{max} was significantly ($p=0.004$) shorter
299 compared to winter (Table 2). AUC_g ($P= 0.02$) and C_{max_g} ($P=0.01$) were significantly greater
300 when the ponies were fasted compared to when they remained at pasture.

301

302 **Discussion**

303

304 In this small study population, only a dose of 0.45ml/kg bwt of corn syrup allowed NL and
305 PL ponies to be reliably distinguished compared to lower doses. The lower 0.15ml/kg bwt
306 dose was unable to distinguish between the two populations which contrasts to two previous
307 studies [4,13], but is consistent with a third [7]. Only one single time point in our study, a
308 blood sample at 60-minutes post corn syrup administration allowed for the test to correctly
309 assign all the individual ponies to their respective groups (NL and PL); time points either side
310 of this failed to provide certainty. This is in agreement with previously published data, in
311 which a blood sample obtained 60 minutes after corn syrup administration provided the
312 strongest correlation with the result obtained using the intravenous glucose tolerance test [4].

313

314 The poor repeatability of the serum insulin concentration at the 60-minute sampling point is
315 concerning. Individual animal variability has been similarly established in other studies
316 including healthy horses [15]. A previous study found within subject agreement for a single
317 sample to be moderate to fair, with the same animal having varying response at the same time
318 point [11]. A study using larger numbers of animals ($n=53$) found the agreement to be good
319 at 60 and 75 minutes [10]. AUC_i was more repeatable in the present study than a single time

320 point. Thus, taking multiple samples and calculating area under the curve may help
321 counteract individual variation and provide more repeatable results. The modified AUC_{iM}
322 reduced the number of sampling points to 3 and required only 90 minutes to complete.
323 However, the repeatability was reduced to 0.63, which is better than that of the single time
324 point of 0.5, but less repeatable than full AUC_i of 0.83.

325

326 The significant effect of season on the insulin response in only the PL ponies undergoing
327 OST is novel. However, other metabolic hormones have been observed to vary with season
328 [16]. Bailey et al [17] found that basal serum insulin concentrations were increased in
329 summer but not winter in a group of PL ponies compared to a group of NL ponies. There was
330 no effect of season, on serum insulin concentration at T75 following the 0.15ml/kg bwt dose
331 during two seasons in horses considered insulin sensitive [8]. Contrastingly, Borer et al[18]
332 found an increased insulin response in autumn (October –November) only in PL ponies
333 undergoing an oral glucose test compared to Spring (May-June). An exaggerated insulin
334 response to the greater pasture non-structural carbohydrates during growing season in those
335 ponies who may be insulin dysregulated would be consistent with the suggested
336 pathophysiology of endocrinopathic laminitis [9]. This includes alterations such an
337 exaggerated intestinal incretin response to the ingested carbohydrate [9], lower hepatic insulin
338 clearance [19], worse peripheral tissue insulin resistance [20] or altered insulin-like growth
339 factor signalling in lamellar tissue [21,22]. Further repeated testing over a 12-month period
340 would provide better grounding for an understanding of the seasonal changes and the
341 relationship with pasture alterations.

342

343 This study provides further evidence that allowing ponies to remain at pasture does not
344 significantly alter the diagnostic abilities of the OST compared to fasted animals [8]. A

345 previous study using the lower dose 0.15ml/kg bwt found a significant effect, but that the
346 results still allowed for a comparable diagnostic outcome with correct identification of ID vs
347 insulin sensitive animals. [11].

348

349 There was no relationship between previous laminitis and glucose response in these PL and
350 NL ponies. This is in contrast to previous studies [4] which found the glucose concentration
351 to be higher at all time points and AUC_g greater in a group considered to have equine
352 metabolic syndrome (EMS) compared to controls. The EMS group in this prior study [4] all
353 had a history of forelimb lameness, consistent with laminitis and were classified as EMS on
354 body condition score, adiposity and intravenous tests of insulin resistance. A further study
355 [13] using a modified OST with a dose of 0.2ml/kg bwt also found glucose at 120-180
356 minutes to be significantly different between EMS and healthy animals. Though testing was
357 undertaken up to 120 minutes in this current study, no further blood samples were taken
358 beyond that point, therefore it is not possible to state whether a difference would have been
359 seen at 180 mins. However, a more recently published study [10] with larger numbers, found
360 no significant difference in glucose response between insulin dysregulated and normal
361 animals at time 0-75 minutes on 2 occasions. It is surprising that the dose relationship in this
362 study was not incremental in that 0.45ml/kgBW dose did not lead to a significantly higher
363 glucose response than the 0.3ml/kgBW dose. However, it should be acknowledged that using
364 0.3ml/kgBW, 2 ponies, both PL, had very large, over double, glucose responses compared to
365 other PL. So the results from these two individual animals are potentially responsible for
366 absence of a dose relationship. The results may also reflect the variable bioavailability of oral
367 glucose seen in other studies [9].

368

369 This study used the radioimmunoassay (RIA) to measure insulin concentrations, whereas in
370 the UK a chemiluminescence immunoassay (CL) is widely used by commercial laboratories.
371 Previous studies have reported that the two techniques are inequivalent [23]. When two RIAs
372 were compared to the CL, all differed significantly with values from the CL being
373 significantly lower than those from the two RIAs [20]. When the CL was compared with the
374 now discontinued gold standard RIA^j, there was a strong positive correlation between results
375 but with fixed and proportional bias[24]. Both of these studies [20][24] found the greatest
376 relative differences to be observed at lower concentrations. Thus, the cut off values suggested
377 in this paper may not be applicable for values obtained with different assays and different
378 populations of animals. Previous studies [6]have found differing insulin responses in ponies
379 verses horses and between breeds [25].

380

381 No adverse effects were seen in any of the 4 occasions the highest dose was given and the
382 amount of oral sugar provided is still much lower than that administered in the oral glucose
383 test. Our analysis of the maltose and glucose content of the corn syrup (Karo Light Corn
384 Syrup)^a found it to contain lower digestible sugars than previously thought [4]. The dose of
385 sugar given using the 0.45mls/kg bwt dose equates to 160.3mg/kg bwt. This is 6 times lower
386 than the dose recommended for the oral glucose test of 1000mg/kg bwt. Further work at the
387 0.45ml/kg bwt dose should be undertaken in larger numbers but in this limited population it
388 would appear safe and there may be room to use a higher dose still.

389

390 The OST is a promising dynamic test, employing the enteroinsular axis for identification of
391 PL ponies, whom show an excessive insulin response to oral carbohydrate. Previous reports
392 of poor sensitivity and repeatability may be improved by the adoption of the higher
393 0.45ml/kg bwt dose and calculation of the insulin area under the curve from at least three

394 sampling time points. Further studies with larger numbers of both ponies and horses of
395 differing breeds, during all four seasons and using the various insulin assays are needed to
396 provide a frame work for reference ranges and better understanding of intra pony variability.

397 Tables

398 TABLE 1: Mean (95% confidence intervals) area under curve insulin (AUC_i), maximal insulin concentration (Cmax_i), time to maximal insulin
 399 concentration (Tmax_i), modified (T0-90) area under curve insulin (AUC_{iM}), modified maximal insulin concentration (Cmax_{iM}) and modified
 400 time to maximal insulin concentration (Tmax_{iM}) for the 3 doses of corn syrup (Karo Light Corn Syrup)^a in normal (NL; n=3) and previously
 401 laminitic ponies (PL; n=5). ^{a-r} Significant (P≤0.05) difference between values with the same letter superscript in both the horizontal and vertical
 402 direction.

Dose (ml/kg bwt)	AUC _i		Cmax _i		Tmax _i		AUC _{iM}		Cmax _{iM}		Tmax _{iM}	
	(μiu/ml/min)		(μiu/ml)		(min)		(μiu/ml/min)		(μiu/ml)		(min)	
	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
0.15	5409 ^a	5797 ^d	72 ^f	80 ^g	80	72	3948	4430	62	61	70	72
	(2282, 8535)	(3375, 8219)	(21-124)	(40-119)	(52-108)	(51-94)	(2229- 5669)	(3097- 5762)	(11-113)	(10-112)	(50-90)	(56-88)
0.3	4653 ^b	7434 ^e	61	111	80	93	3301	5189	98	78	80	78
	(1526, 7780)	(5012, 9857)	(10-112)	(71-150)	(52-108)	(71-115)	(1581- 5021)	(3857- 6522)	(47-149)	(39-118)	(60-100)	(62-94)

0.45	9681 ^{a,b,c}	14968 ^{c,d,e}	135 ^f	206 ^g	75	78	5722 ^q	10208 ^q	98 ^r	206 ^r	80	78
	(6554, 12808)	(12546, 17390)	(83-186)	(166-246)	(47-103)	(56-100)	(4001- 7442)	(8876- 11541)	(58-137)	(167-246)	(60-100)	(62-94)

403

404

405 TABLE 2: Mean (95% confidence intervals) area under curve insulin (AUC_i), maximal
406 insulin concentration (Cmax_i), time to maximal insulin concentration (Tmax_i) for season and
407 fasting following 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)^a. ^{a-i} Significant
408 (P≤0.05) difference between values with the same letter superscript in both the horizontal and
409 vertical direction.

410

411

Season	AUC _i		Cmax _i		Tmax _i	
	(μiu/ml/min)		(μiu/ml)		(min)	
	NL	PL	NL	PL	NL	PL
Summer	6304 ^a (614, 11993)	19032 ^{a,c} (14625, 23439)	87 ^d (10-164)	262 ^d (202-321)	55 (28-82)	86 (68-109)
Winter	9681 ^b (3551, 15812)	14968 ^{b,c} (10220, 19717)	135 ^e (48-221)	206 ^e (139-273)	75 (46-104)	78 (55-101)
Fasting						
Fasting	6304 ^f (-211-12819)	19032 ^f (13985-24079)	87 ^h (19-155)	262 ^h (209-315)	55 (32-78)	89 (32-78)

Pasture

7639 ^g	16397 ^g	107 ⁱ	245 ⁱ	40	75
(919-14359)	(11191-21602)	(24-190)	(181-309)	(12-68)	(53-106)

412

413 TABLE 3: Mean (95% confidence intervals) area under curve glucose (AUC_g), maximal
 414 glucose concentration (Cmax_g) and time to maximal glucose concentration (Tmax_g) for the 3
 415 doses of corn syrup (Karo Light Corn Syrup)^a in normal (NL; n=3) and previously laminitic
 416 ponies (PL; n=5). Effect of season and fasting on these variables following 0.45ml/kg bwt
 417 dose. ^{a-k} Significant (P≤0.05) difference between values with the same letter superscript in
 418 both the horizontal and vertical direction.

	AUC _g (μiu/ml/min)	Cmax _g (μiu/ml)	Tmax _g (min)
Dose (ml/kg bwt)			
0.15	507 ^{a,b} (297-717)	5.4 ^{c,d} (2.7-8.1)	76 ^f (61-90)
0.3	907 ^a (697-1117)	12.0 ^{e,d} (9.5-14.9)	89 (74-103)
0.45	759 ^b (550-969)	8.6 ^{c,e} (5.9-11.3)	96 ^f (82-111)
Disease state			
NL	756 (579-934)	9.0 (7.2-10.8)	55 (38-72)
PL	880 (742-1017)	10.7 (9.4-12.1)	71 (58-84)
Season			
Winter	742 ^g (590-894)	8.5 ⁱ (6.7-10.3)	94 ^k (77-112)
Summer	889 ^g (750-1027)	10.9 ⁱ (9.5-12.4)	61 ^k (48-73)
Fasting			
Fasting	874 ^h (759-989)	10.8 ^j (9.4-12.1)	60 (51-87)
Pasture	706 ^h (571-840)	8.1 ^j (6.4-9.7)	69 (47-73)

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421 Figure Legends

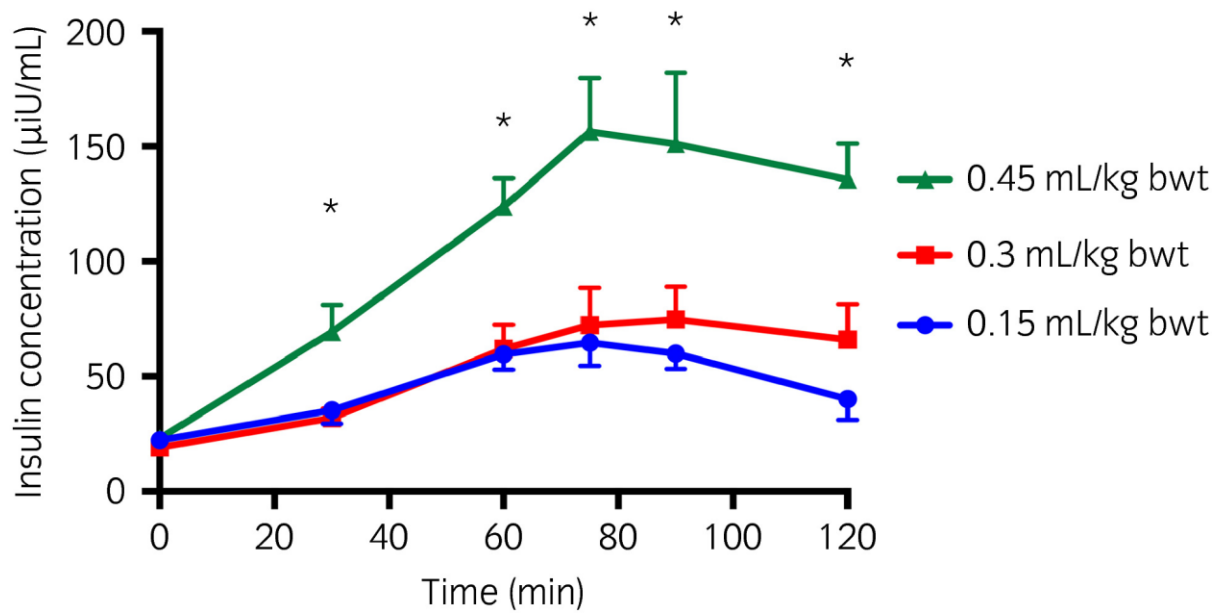
422

423 Figure 1: Estimated marginal mean (± 1.96 s.e.) serum insulin concentration at single time

424 points in response to 3 different doses of corn syrup (Karo Light Corn Syrup)^a. (n=8)

425 *Values that are significantly different ($P < 0.05$) from the equivalent values from a different

426 dose.

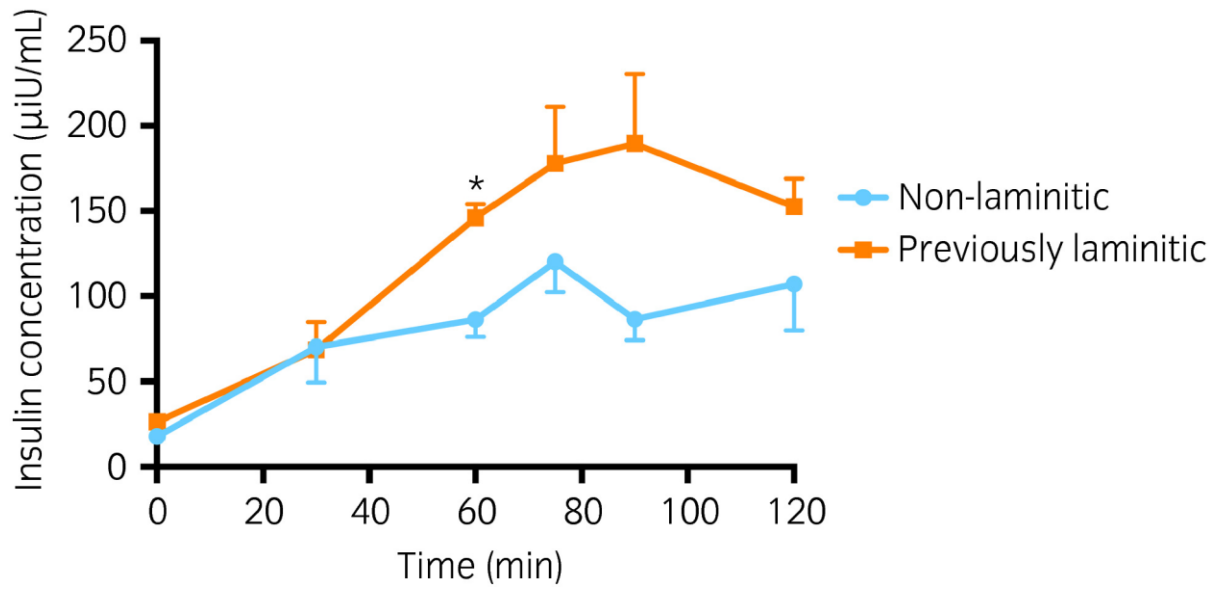


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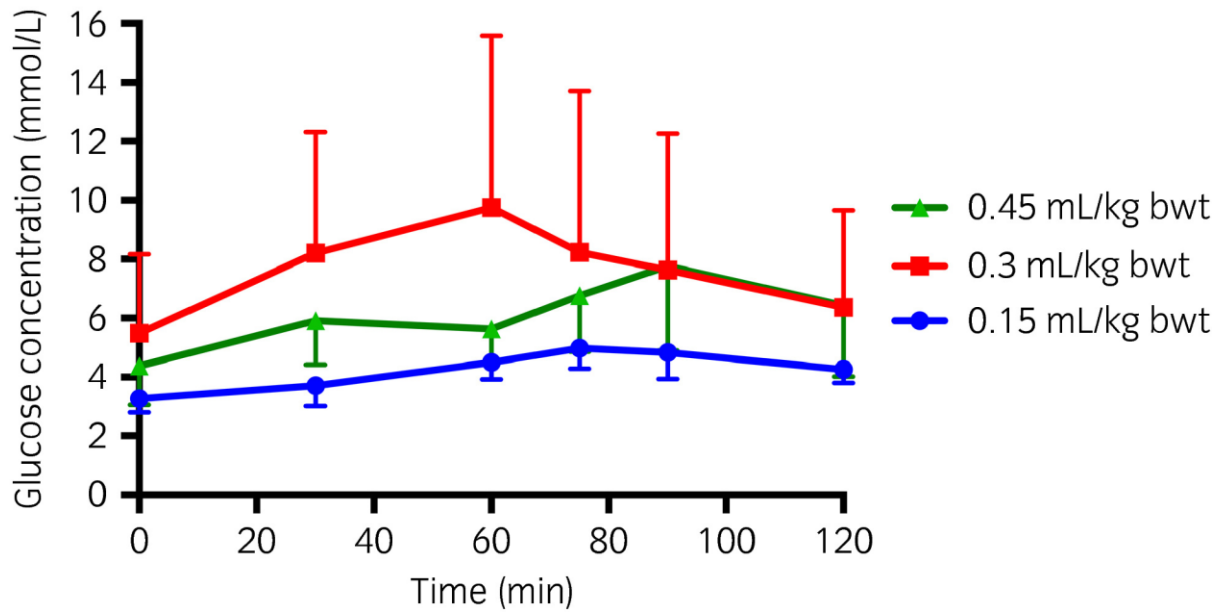
430 Figure 2: Estimated marginal mean (± 1.96 s.e.) insulin concentration at single time points for
431 NL (n=3) and PL (n=5) ponies when given a dose of 0.45ml/kg bwt corn syrup (Karo Light
432 Corn Syrup). *Values that are significantly different ($P < 0.05$) between groups (NL and PL).



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435 Figure 3. Estimated marginal mean (± 1.96 s.e.) serum glucose concentration at single time
436 points in response to 3 different doses of corn syrup (Karo Light Corn Syrup)^a. (n=8)
437 *Values that are significantly different ($P < 0.05$) from the equivalent values from a different
438 dose.



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442 Manufacturers' addresses

443 ^aACH Food Companies Inc, Cordova, Tennessee, USA.

444 ^bBecton Dickinson, Sandy, Utah, USA.

445 ^cDechra Veterinary Products, Shrewsbury, Shropshire, UK.

446 ^dJasco Ltd., Essex, UK

447 ^ePhenomenex, Torrance, California, USA

448 ^fMP Biomedical, Ilkirch, France.

449 ^gCayman chemical company, Michigan, USA

450 ^hIBM UK, Portsmouth, Hampshire, UK.

451 ⁱGraphpad Software, La Jolla, California, USA.

452 ^jCoat-A-Count, Siemens, Camberley, Surrey, UK.

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534 insulin sensitivity and insulinemic responses to oral glucose in horses and ponies of
535 moderate body condition score. *Domestic Animal Endocrinology* **47**, 101–107.

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538 Supplementary Information Items

539 1. Table – Signalment, laminitis history and weight from the pony subjects.

540 2. Table- Corn Syrup analysis

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