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Efficacy and tolerance of oral versus parenteral cyanocobalamin supplement in hypcobalaminaemic dogs with chronic enteropathy: a controlled randomised open-label trial

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28 biochemical phenotypes, and should be considered as a suitable treatment option regardless
29 of disease severity.

30

31 **Keywords:** chronic enteropathy, dog, oral cyanocobalamin, vitamin B12, treatment tolerance

32

33 **Abbreviations**

34 BCS Body condition score

35 CI Confidence interval

36 CIBDAI Canine Inflammatory Bowel Disease Activity Index

37 EPI Exocrine Pancreatic Insufficiency

38 PLE Protein-Losing-Enteropathy

39 QOL Quality-of-life

40 TLI Trypsin-Like Immunoreactivity

41 UPCR Urine Protein:Creatinine Ratio

42

43

44 **Introduction**

45 Cobalamin, also referred to as vitamin B12, is a water-soluble vitamin derived from animal
46 products, especially red meat, dairy, and eggs (Antony 2003). Cobalamin is ingested bound to
47 animal protein and then released in the stomach under the action of activated pepsin and
48 gastric acid (Qureshi *et al.* 1994). Free gastric cobalamin binds to the R-protein before binding
49 to intrinsic factor. Intrinsic factor is a glycoprotein produced by gastric parietal cells and the
50 canine pancreas. In dogs, only a minor fraction of intrinsic factor is produced in the stomach
51 (Marcoullis & Rothenberg 1981, Batt & Horadagoda 1989). This allows for absorption within
52 the distal ileum (Marcoullis & Rothenberg 1981, Batt & Horadagoda 1989, Steiner 2016). The
53 cobalamin/intrinsic factor complex binds to specific cubam receptors localised within the brush
54 border of the ileal enterocytes. Approximately 1% of dietary cobalamin is absorbed via passive

55 diffusion across the entire length of the intestinal mucosal epithelium, in addition to the
56 receptor-mediated cobalamin uptake by the ileal enterocytes.

57
58 In hypocobalaminaemia, the enzymatic reactions where cobalamin is involved as a cofactor
59 are inhibited (e.g. conversion of L-methylmalonyl-CoA into succinyl-CoA), leading to
60 accumulation of methylmalonic acid which is excreted in the urine. Methylmalonic acid
61 concentrations can be measured either in serum (Vaden *et al.* 1992, Ruaux *et al.* 2009,
62 Berghoff *et al.* 2012) or urine (Fyfe *et al.* 1991, Lutz *et al.* 2012), where in people, its
63 concentration is up to 40-fold higher than in serum (Norman & Cronin 1996). Elevations in
64 methylmalonic acid can hence serve as a marker of cellular cobalamin deficiency (Savage *et*
65 *al.* 1994, Berghoff *et al.* 2012, Kather *et al.* 2020). Apart from cobalamin deficiency, increased
66 methylmalonic acid concentrations can occur in renal disease, plasma volume contraction and
67 primary abnormalities in hepatic methylmalonyl-CoA mutase activity (Carmel *et al.* 2003,
68 Ruaux *et al.* 2013), but this has never been demonstrated in dogs.

69
70 Hypocobalaminaemia has been reported in dogs with different medical conditions including
71 Imerslund-Gräsbeck syndrome, chronic enteropathies, canine parvovirus, alimentary or
72 multicentric lymphoma, and exocrine pancreatic insufficiency (EPI) (Kather *et al.* 2020,
73 Engelbrecht *et al.* 2022). In chronic enteropathy, the prevalence of hypocobalaminaemia
74 ranges from 19% to 38% (Heilmann *et al.* 2016a, Heilmann *et al.* 2016b, Volkmann *et al.* 2017,
75 Heilmann *et al.* 2018). Historically, parenteral cobalamin supplementation was recommended
76 over oral supplementation as the first line treatment in dogs with hypocobalaminaemia
77 secondary to chronic enteropathy (Hall & Day 2016), and it has been shown to result in
78 eucobalaminaemia and reduction in methylmalonic acid concentration (Ruaux *et al.* 2005,
79 Toresson *et al.* 2019). However, several studies comparing oral cobalamin supplementation
80 with parenteral cobalamin supplementation in people with hypocobalaminaemia have shown
81 equal efficacy (Kuzminski *et al.*, 1998; Bolaman *et al.*, 2003; Castelli *et al.*, 2011; Kim *et al.*,
82 2011). Moreover, studies have shown that oral cobalamin supplementation was non-inferior to

83 parenteral cobalamin supplementation in normalising serum cobalamin concentration in dogs
84 with chronic enteropathy (Toresson *et al.* 2018, Toresson *et al.* 2019, Chang *et al.* 2022) and
85 dogs with EPI (Toresson *et al.* 2021, Chang *et al.* 2022). More recently, the efficiency of oral
86 cobalamin supplementation at treating hypocobalaminaemic dogs with hereditary intestinal
87 cobalamin malabsorption was also demonstrated in a study published by Kook & Hersberger
88 (2019). Despite promising results, it remains unclear whether treatment efficiency and
89 tolerance is comparable between oral cobalamin supplementation and parenteral cobalamin
90 supplementation in dogs suffering with severe chronic enteropathy (severe clinical
91 presentation, severe hypocobalaminaemia or protein-losing enteropathy; PLE).

92

93 The primary aim of this study was to prospectively determine whether oral cobalamin
94 supplementation is as effective as parenteral cobalamin supplementation at restoring
95 eucobalaminaemia in dogs with PLE, severe chronic enteropathy (as defined by severity of
96 clinical signs) or severe hypocobalaminaemia secondary to chronic enteropathy. We
97 hypothesised that oral cobalamin supplementation is non-inferior to parenteral cobalamin
98 supplementation in each setting. The secondary aim of this study was to evaluate ease of
99 administration and tolerance for both types of administration by assessing pet owners' opinions
100 on the protocols used. We hypothesised that oral cobalamin supplementation protocols are
101 perceived as less stressful and better tolerated compared to parenteral cobalamin
102 supplementation protocols.

103

104

105 **Materials & Methods**

106 **Study design, cobalamin supplementation and inclusion/exclusion criteria:** This
107 controlled, randomised, multicentric, non-inferiority study was conducted in dogs with
108 hypocobalaminaemia secondary to chronic enteropathy. Three UK-based small animal referral
109 centres participated in the study.

110

111 Dogs with clinical signs of chronic enteropathy and hypocobalaminaemia (serum cobalamin
112 concentration <250 ng/L (reference interval: 240-590 ng/L) were recruited in a prospective
113 manner. Cases were enrolled from August 2018 to April 2020. Dogs with chronic enteropathy
114 were characterised by chronic persistent or recurrent clinical signs of gastrointestinal disease
115 (such as vomiting, diarrhoea, weight loss, or a combination of those) for at least three weeks.
116 Written informed consent was obtained from owners or authorised agents for each dog to
117 participate in the study. Owners received an information form to optimise their understanding
118 of the study protocols.

119

120 The study was approved by an ethics committee in March 2018 (reference number SN1702).
121 Local ethical approval was obtained by participating centres in accordance with their local
122 regulations.

123

124 At the time of presentation, each dog underwent a mandatory workup which included a serum
125 biochemistry, complete blood cell count, serum cobalamin concentration, trypsin-like
126 immunoreactivity (cTLI), and serum methylmalonic acid concentrations, all assessed by the
127 same commercial laboratory. The canine inflammatory bowel disease activity index (CIBDAI)
128 was also completed for each dog (Jergens *et al.* 2003). Additional investigations, clinical
129 management and treatment were determined at the discretion of the clinician in charge of each
130 respective case. Diet was not standardised prior to the study or during the study. Dogs were
131 fasted for at least 12 hours prior to blood sampling.

132

133 Using block randomisation (Excel®, Microsoft Office 2016), dogs were randomly assigned to
134 oral cobalamin supplementation (Cobalapex®, Protexin, UK) or parenteral cobalamin
135 supplementation (Vitbee 250®, Dechra, UK).

136

137 Dogs enrolled in the oral cobalamin supplementation group received cyanocobalamin based
138 on their weight orally once daily for 12 weeks as recommended by the manufacturer, which is
139 equivalent to a minimum dose of 25ug/kg (Table 1). Dogs enrolled in the parenteral cobalamin
140 supplementation group received a weekly subcutaneous cyanocobalamin injection for 6 weeks
141 and a seventh dose 4 weeks later, at a minimum dose of 25ug/kg of cyanocobalamin per
142 injection (Table 1).

143

144 In all dogs, physical examination, body weight, serum folate concentration, serum cobalamin
145 concentration and CIBDAI score were repeated at week 7. Physical examination, body weight,
146 body condition score, serum biochemistry, complete blood count, serum folate concentration,
147 serum cobalamin concentration, CIBDAI score, and serum methylmalonic acid concentration
148 were repeated at week 13. Treatment failure was defined by recurrence of
149 hypocobalaminaemia at week 13.

150

151 At the end of data collection, dogs were additionally grouped by several clinical characteristics
152 to allow statistical analysis: presumptive PLE chronic enteropathy (defined as dogs with
153 gastrointestinal signs and serum albumin below the reference interval, absence of azotaemia,
154 absence of significant proteinuria, and absence of hyperbilirubinaemia) compared to non-PLE
155 chronic enteropathy, moderate to severe hypocobalaminaemia (defined as a serum cobalamin
156 concentration of <200ng/L) compared to mild hypocobalaminaemia (defined as a serum
157 cobalamin between 200 and 250 ng/L), severe clinical disease based on CIBDAI score
158 categories (CIBDAI >9 compared to CIBDAI score ≤9).

159

160 Dogs were excluded from the study if they had received cobalamin administration in the 12
161 weeks preceding the study, or if there was a known hypersensitivity to active ingredients and/or
162 excipients of the oral or injectable cobalamin products. Enrolment to the study was terminated
163 if recruited dogs developed concomitant disease, if oral or injectable treatment was interrupted,
164 if cobalamin supplement dosing errors occurred, or upon owner's withdrawal of consent.

165

166 **Owner questionnaire design:**

167 Owners were asked to complete a Treatment Adherence & Satisfaction Questionnaire (Table
168 S1) and a Treatment Tolerance Questionnaire (Table S2 & S3) at week 13. The Treatment
169 Adherence & Satisfaction Questionnaire was identical for both groups, the Treatment
170 Tolerance Questionnaire was different as questions specific to the type, protocol and duration
171 of cobalamin were required (Table S2 & S3). An additional Treatment Palatability
172 Questionnaire was also completed by owners of dogs in the oral cobalamin supplementation
173 group (Table S4). In the absence of pre-existing validated scores, the questionnaires and the
174 scoring system used were designed for the purpose of this study. For each questionnaire
175 designed, a high score signified an excellent satisfaction/ treatment tolerance. Inversely, a low
176 score implied a poor satisfaction/ treatment tolerance.

177

178 The Treatment Adherence & Satisfaction Questionnaire included 8 questions divided into 3
179 categories aiming at assessing the ease of treatment administration (administration, treatment
180 planning, observance), any perceived stress caused by treatment administration (to the owner
181 and to the dog), and the owner's overall satisfaction. A score from 0 to 4 was allocated to each
182 answer, which provided a Treatment Adherence & Satisfaction Score rated out of 32 (Table
183 S1). A score of 32 was consistent with perceived perfect ease of administration. Additionally,
184 owners were asked whether they would choose the same treatment should their dog require
185 cobalamin supplementation in the future. By extracting replies to a subgroup of questions
186 within the Treatment Adherence & Satisfaction Questionnaire, an Owner Satisfaction Score
187 out of 8 was also calculated (Table S2).

188

189 The Treatment Tolerance Questionnaire and the Treatment Palatability Questionnaire for dogs
190 receiving oral cobalamin supplementation (Table S3 & S4) included 22 questions scored via a
191 5-point Likert scale, divided into 4 groups aimed at assessing tolerance of taking capsules or
192 tablets before the study (5 questions), assessing tolerance of taking cobalamin capsules during

193 the study (5 questions), assessing behavioural signs of stress while taking the cobalamin
194 capsules during the study (11 questions), and finally, 1 question regarding the technique
195 owners used to administer the cobalamin capsules. Following completion, a score ranging from
196 0 to 4 or from 0 to 8 was allocated to each answer. The Oral Cobalamin Supplementation
197 Tolerance Score was a mean of subscores from questions related to dog's response to taking
198 cobalamin capsules and behavioural changes when being given the cobalamin supplement,
199 providing a final score rating out of 72, the highest score indicating perfect tolerance to
200 treatment. To determine whether the cobalamin capsules were well tolerated compared to
201 other types of capsules/tablets administered prior to this trial, we compared the Oral Capsule
202 Tolerance Score Before Trial designed from the 5 questions related to tolerance taking
203 capsules or tablets before the study to the Oral Capsule Tolerance Score During Trial designed
204 from the 5 questions related to tolerance of taking Cobalplex® capsules during the study.
205 Both scores were calculated to obtain a final score rated out of 10, a score of 10 indicating
206 complete tolerance to treatment. The nature of the tablets or capsules administered prior to
207 this trial was not documented.

208

209 The Treatment Tolerance Questionnaire for dogs receiving parenteral cobalamin
210 supplementation (Table S5) included 23 questions divided into three categories as follows:
211 dog's response and tolerance to visiting the veterinarian before this trial (11 questions), dog's
212 response to visiting the veterinarian for the cobalamin injections (12 questions), including 11
213 questions about the dog's behaviour at the veterinarian before the injection and 1 question
214 about the dog's response to the cobalamin injections. A score ranging from 0 to 4 was allocated
215 to each answer. The Parenteral Cobalamin Supplementation Tolerance Score was a mean of
216 subscores from questions related to dog's response to visiting the veterinarian for the
217 cobalamin injections, which provided a total score rated out of 48, the highest score indicating
218 perfect tolerance to treatment. To determine tolerance of the visits at the veterinary clinic to
219 administer cobalamin injections, the Veterinarian Visit Tolerance Score Before Trial designed
220 from 11 questions, were compared to the Veterinarian Visit Tolerance Score During Trial

221 designed from 12 questions. Both scores were calculated to obtain a number out of 10 (Table
222 S5), a score of 10 indicating complete absence of anxiety during veterinary visits. The purpose
223 of previous visits at a veterinary clinic and/or the nature of injections undertaken during
224 consultations prior to this trial were not documented.

225

226 **Blood sample processing:** Routine bloods and serum collected for methylmalonic acid
227 assessment were sent to the same commercial laboratory by each participating centre within
228 48h of collection using priority delivery (Veterinary Pathology Group, Exeter, United-Kingdom).
229 Serum collected for methylmalonic acid assessment was refrigerated within 2 hours of
230 collection, frozen at -20°C on the same day upon arrival at this laboratory, and later sent as a
231 batch on dry ice, to a different branch for analysis (Synlab laboratory, Augsburg, Germany).

232

233 **Assays:** Serum cobalamin concentration was assessed using a chemiluminescent assay
234 (Immulite 2000 Vitamin B12, Siemens Healthcare Diagnostics) which has been validated in
235 dogs (Grutzner *et al.* 2016) and has proved good analytical performance (McLeish *et al.* 2019).
236 Methylmalonic acid was analysed using a liquid chromatography–mass spectrometry method.
237 The reference interval used for canine serum methylmalonic acid was 415 to 1193 nmol/L, as
238 previously determined (Berghoff *et al.* 2012).

239

240 **Data analysis:**

241 A commercially available statistical software (R 4.1.3, R Core Team, 2022) was used for all
242 data analyses. Continuous data were assessed for normality using the Shapiro Wilk test and
243 presented as mean \pm sd if normally distributed or median \pm range if not. Mann–Whitney U
244 test was used for non-normally distributed variables and t-test used for normally distributed
245 variables comparisons. The Wilcoxon rank sum test was used for age and weight comparison
246 and the Fisher's exact test was used for duration of symptoms comparison. Statistical
247 significance for all tests was set at $P < 0.05$. A Chi-squared test was used to assess gender
248 distribution.

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Multivariable mixed-effects linear models were run in the oral cobalamin supplementation and parenteral cobalamin supplementation dogs included in the study, and also in each dog category, for each of the seven following outcome variables: cobalamin, body weight, body condition score (BCS), CIBDAI score, serum folate concentration, serum methylmalonic acid concentration, and every scores from the different questionnaires (Oral Cobalamin Supplementation Tolerance Score, Oral Capsule Tolerance Score Before Trial, Oral Capsule Tolerance Score During Trial, Parenteral Cobalamin Supplementation Tolerance Score, Treatment Adherence & Satisfaction Questionnaire, Treatment Adherence & Satisfaction Score, Veterinarian Visit Tolerance Score Before Trial, Veterinarian Visit Tolerance Score During Trial). In addition, changes were assessed as absolute values for each outcome from baseline to week 7 (where available), and baseline to week 13.

277 **Results**

278 **Case recruitment**

279 Thirty-seven dogs were enrolled in the study and randomly assigned treatment as follows:
280 n=18 in the oral cobalamin supplementation group, and n=19 in the parenteral cobalamin
281 supplementation group. Nineteen dogs completed the study: n=11 in the oral cobalamin
282 supplementation group, and n=8 in the parenteral cobalamin supplementation group. From the
283 oral cobalamin supplementation group, seven dogs were excluded from analysis, due to lack
284 of follow-up (n=4), or euthanasia due to clinical deterioration (n=3). From the parenteral
285 cobalamin supplementation group, 11 dogs were excluded from analysis, due to lack of follow-
286 up (n=8), euthanasia due to clinical deterioration (n=2), or euthanasia due to a comorbidity
287 (n=1).

288

289 **Signalment, clinical signs, body weight, body condition score, CIBDAI score**

290 The 19 dogs completing the study represented 13 different breeds. Labrador retrievers (n=5),
291 Cairn Terriers (n=2), and mixed breed dogs (n=2) were the most commonly represented
292 breeds (Table 2). There were 9 female neutered dogs (47.4%), 2 entire male dogs (10.5%)
293 and 8 neutered male dogs (42.1%) (Table 2). There was no difference in gender distribution
294 amongst treatment groups (P=0.156).

295

296 The mean (+/-sd) age was 6.62 years (+/-3.55 years). Dogs in the oral cobalamin
297 supplementation group received a median daily cobalamin dose of 33.2 mg/kg (range: 28.1-
298 42.2 mg/kg), and dogs in the parenteral cobalamin supplementation group received a median
299 weekly cobalamin dose of 30.5 mg/kg (range: 22.7-43.9 mg/kg). There was no significant
300 difference of cobalamin dose per kg body weight per administration between the two groups
301 (P=0.3856).

302

303 The most common clinical signs were diarrhoea (n=18), vomiting (n=15), inappetence/anorexia
304 (n=13), weight loss (n=11) and lethargy (n=11) (Table 2). Other clinical signs included

305 abdominal distension (n=8), borborygmi (n=3), prayer stance (n=2), constipation (n=2) and
306 pica (n=1). Most dogs (11/19, 57.9%) had shown clinical signs of gastrointestinal disease for
307 one month to one year. However, a large proportion of dogs also presented signs for more
308 than three weeks but less than one month (7/19, 36.8%) (Table 2).

309

310 At inclusion, the median (range) body weight was 16.9 kg (6.1-44 kg), and the median (range)
311 body condition score was 4/9 (2/9-7/9). Body weight and body condition score were not
312 significantly different between dogs assigned to receive oral cobalamin supplementation and
313 dogs assigned to receive parenteral cobalamin supplementation (P=0.103 and P=0.933,
314 respectively).

315 At week 13, body condition score was not significantly different in the oral cobalamin
316 supplementation group (median 5 +/- 1.27) and in the parenteral cobalamin supplementation
317 group (median 5 +/- 1.11) (P=0.377). The change in body weight from baseline to week 13
318 was +2 kg in the oral cobalamin supplementation group (95% CI [0.62, 3.38]) and +0.51 kg in
319 the parenteral cobalamin supplementation group (95% CI [-1.28, 2.31]), and was not
320 significantly different (+1.49 kg, 95% CI [-0.63, 3.6], P=0.21). The change in body condition
321 score from baseline to week 13 was also not significantly different in the oral cobalamin
322 supplementation group (P=0.07), and in the parenteral cobalamin supplementation group
323 (P=0.511).

324 Both treatment groups included, dogs with a CIBDAI score >9 at inclusion gained significantly
325 more weight than dogs with a CIBDAI score ≤9 (+2.81 kg, 95% CI [1.35, 4.27], P=0.005), with
326 no significant difference between dogs with a CIBDAI score >9 in the oral cobalamin
327 supplementation and in the parenteral cobalamin supplementation groups (P=0.076). There
328 was no significant change in body condition score between baseline and week 13 in dogs with
329 a CIBDAI score >9 (P=0.139), and also in dogs with a CIBDAI score ≤9 (P=0.135).

330

331 At inclusion, the median CIBDAI score was 8 (range: 3–17) in the oral cobalamin
332 supplementation group and 10 (range: 5–17) in the parenteral cobalamin supplementation

333 group, and was not statistically different between the oral cobalamin supplementation and the
334 parenteral cobalamin supplementation groups (P=0.77). The change in CIBDAI score between
335 week 0 and week 7 was -7.27 (95% CI [-8.49, -6.06]) in the oral cobalamin supplementation
336 group and -7.38 (95% CI [-8.7, -6.01]) in the parenteral cobalamin supplementation group. The
337 change in CIBDAI score between week 0 and week 13 was -8.27 (95% CI [-9.49, -7.06]) in the
338 oral cobalamin supplementation group and -8.38 (95% CI [-9.74, -7.01]) in the parenteral
339 cobalamin supplementation group. There was no significant difference in reduction of CIBDAI
340 between the two treatment groups at week 7 (P=0.932) or week 13 (P=0.49). Eight dogs had
341 severe CIBDAI scores at the start of the study (CIBDAI>9), including 4 dogs in the oral
342 cobalamin supplementation group and 4 dogs in the parenteral cobalamin supplementation
343 group.

344

345 **Haematology, serum biochemistry, and other investigative procedures**

346 The most common haematological changes were neutrophilia (n=7) and leucocytosis (n=4).
347 Anaemia (n=2), eosinopenia (n=2), monocytosis (n=2), eosinophilia (n=1), thrombocytopenia
348 (n=1) and thrombocytosis (n=1) were also identified (Table S4). The most common
349 biochemical changes were panhypoproteinaemia (n=7/19, mean total protein concentration in
350 these 7 hypoproteinaemic dogs = 37.9 +/- 9.15 g/L, reference interval: [54-77 g/L]),
351 hypoalbuminaemia (n=7/19, mean albumin concentration in these 7 hypoalbuminaemic dogs
352 = 13.8 +/- 3.8 g/L, reference interval: [25-40 g/L]), hypocholesterolaemia (n=6, reference
353 interval: [3.8-7 mmol/L]), and increased ALT (n=6, reference interval: [5-66 U/L]). Among
354 hypoalbuminaemic dogs, serum albumin concentration was below 20g/L in all dogs (range: 9-
355 19 g/L). Increased ALP (n=3, reference interval: [0.1-150 U/L]) and azotaemia (n=2, creatinine
356 reference interval: [40-150 umol/L], urea reference interval: [3-9 mmol/L]) were also
357 documented (Table S6).

358 Two dogs had subnormal serum TLI concentrations of 3.2 and 4 ng/mL (reference interval: 5-
359 40 ng/mL). TLI concentration in these dogs was not reassessed during the study.
360 Hypofolataemia, suggestive of proximal small intestinal malabsorption, was more frequent

361 (n=7, mean 4.4 +/- 1.7 ug/L, reference interval: [7.2-23.8 ug/L]) than hyperfolataemia (n=1).
362 Basal cortisol and ACTH stimulation testing were conducted in 5 dogs and 1 dog respectively.
363 Results of faecal parasitology testing (n=2), ultrasonography (n=11), abdominal Computed
364 Tomography scan (n=1) and histopathology of endoscopy-guided intestinal biopsies (n =9)
365 were available in 13 dogs. Full urinalysis including urine protein:creatinine ratio (UPCR) was
366 available in 3 dogs, UPCR alone was available in 4 additional dogs, and a bile acid stimulation
367 test was performed in 1 dog. Based on these results, 7 dogs were diagnosed with presumptive
368 PLE chronic enteropathy, including 3 dogs in the oral cobalamin supplementation group and 4
369 dogs in the parenteral cobalamin supplementation group. Among the remaining non-PLE
370 chronic enteropathy dogs, 8 dogs received oral cobalamin supplementation and 4 dogs
371 received parenteral cobalamin supplementation.

372

373 **Definitive diagnosis and concurrent diseases**

374 A definitive diagnosis of inflammatory chronic enteropathy was achieved with endoscopy-
375 guided intestinal biopsies in 9 dogs. Concurrent diseases included chronic kidney disease
376 (CKD) (n=3), pulmonary carcinoma (n=1), primary hyperadrenocorticism (n=1), immune-
377 mediated haemolytic anaemia (n=1), stump pyometra (n=1), and idiopathic hyperlipidaemia
378 (n=1).

379

380 **Concurrent treatments**

381 Details of concurrent treatments are available for 17/19 dogs, and information on dietary
382 recommendations is available for 15/19 dogs (Table S7).

383

384 **Serum cobalamin concentration**

385 At inclusion, mean serum cobalamin concentration was 188 ng/L (sd +/- 33) in the oral
386 cobalamin supplementation group and 204 ng/L (sd +/- 30) in the parenteral cobalamin
387 supplementation group, and there was no significant difference between the two treatment
388 groups (CI 95% [-3.78, 4.22], P=0.919). Twelve dogs had severe hypocobalaminaemia at

389 inclusion (cobalamin \leq 200 ng/mL), including 7 dogs in the oral cobalamin supplementation
390 group and 5 dogs in the parenteral cobalamin supplementation group.

391 At week 7, serum cobalamin concentrations were significantly higher in the oral cobalamin
392 supplementation group (mean 1931 ng/L; sd +/- 167) compared to the parenteral cobalamin
393 supplementation group (mean 914 ng/L; sd +/- 427) ($P < 0.001$).

394 At week 13, serum cobalamin concentrations were also significantly higher in the oral
395 cobalamin supplementation group (mean 1750 ng/L; sd +/- 517) compared to the parenteral
396 cobalamin supplementation group (mean 515 ng/L; sd +/- 227) ($P < 0.001$) (Figure 1).

397 In the parenteral cobalamin supplementation group, the mean increase in serum cobalamin
398 concentration was 644 ng/L (95% CI [410.81, 878.14]) between week 0 and week 7, and 372
399 ng/L (95% CI [138.23, 605.56]) between week 0 and week 13. In the oral cobalamin
400 supplementation group, the mean increase in serum cobalamin concentration was 1791 ng/L
401 (95% CI [1583.52, 1998.15]) between week 0 and week 7, and 1518 ng/L (95% CI [1310.94,
402 1725.57]) between week 0 and week 13. When comparing both groups, the mean increase in
403 cobalamin between week 0 and week 7 was significantly higher in the oral cobalamin
404 supplementation group compared to the parenteral cobalamin supplementation groups
405 ($P < 0.001$) (Figure 1). The mean increase in cobalamin between week 0 and week 13 was
406 significantly higher in the oral cobalamin supplementation group compared to the parenteral
407 cobalamin supplementation group ($P < 0.001$) (Figure 1).

408 Treatment failure was identified in one dog in the parenteral cobalamin supplementation group,
409 despite reaching eucobalaminaemia at week 7.

410

411 **Methylmalonic acid concentrations**

412 On baseline, 10/11 samples were available for serum methylmalonic acid assessment in the
413 oral cobalamin supplementation group and 6/8 in the parenteral cobalamin supplementation
414 group. At week 13, only 9/11 samples were available in the oral cobalamin supplementation
415 group and 5/8 in the parenteral cobalamin supplementation group.

416 On admission, methylmalonic acid concentration was increased in 3/6 dogs in the parenteral
417 cobalamin supplementation group and 4/10 in the oral cobalamin supplementation group. At
418 week 13, methylmalonic acid concentration was persistently increased in 1/5 dogs in the
419 parenteral cobalamin supplementation group and 1/9 in the oral cobalamin supplementation
420 group (Figure 2).

421 From baseline to week 13, dogs receiving oral cobalamin supplementation experienced a
422 decrease in methylmalonic acid concentration of 801.9 nmol/L (95% CI [-1065.7, -539.3]), and
423 dogs receiving parenteral cobalamin supplementation a decrease of 632.8 nmol/L (95% CI [-
424 1032.7, -232.7]). This was not significantly different between treatment groups (-169.9 nmol/L,
425 95% CI [-655.5, 309.1], P=0.454).

426

427 **Questionnaires**

428 Treatment Adherence & Satisfaction Questionnaires, Treatment Tolerance Questionnaires
429 and Treatment Palatability Questionnaire were completed by owners in 16/19 dogs (oral
430 cobalamin supplementation group n = 9, parenteral cobalamin supplementation group n = 7)
431 at week 13.

432

433 *Treatment Adherence & Satisfaction Score and Owner Satisfaction Score:* At week 13, dogs
434 in the oral cobalamin supplementation group had a median Treatment Adherence &
435 Satisfaction Score of 32/32 (range: 27-32) compared to a median of 31/32 (range: 25-32) for
436 dogs in the parenteral cobalamin supplementation group, which was not significantly different
437 (2.22, 95% CI [-0.42, 4.86], P=0.093). At week 13, the Treatment Adherence & Satisfaction
438 Score from dogs with severe hypcobalaminaemia at inclusion (<200 ng/L) (median 32/32,
439 range 25-32) was compared to the Treatment Adherence & Satisfaction Score from dogs with
440 mild to moderate hypcobalaminaemia (median 27/32, range 25-31), regardless of treatment
441 group. The former rated 2.37/32 points higher which was statistically significant (95% CI [0.22-
442 4.52], P=0.033). The Treatment Adherence & Satisfaction Score from dogs with severe
443 hypcobalaminaemia at inclusion (<200 ng/L) in the oral cobalamin supplementation group

444 was significantly higher than in the parenteral cobalamin supplementation group (+3.11/32
445 points, 95% CI [0.81-5.41, P=0.012]). There was no statistical difference of the Owner
446 Satisfaction Score between supplementation groups (P=0.55). Only one owner notified that
447 they would have preferred the “other treatment modality”, should another cobalamin
448 supplementation protocol be necessary. This dog belonged to the parenteral cobalamin
449 supplementation group.

450

451 *Oral Cobalamin Supplementation Tolerance Score, Oral Capsule Tolerance Score Before*
452 *Trial, Oral Capsule Tolerance Score During Trial:* Dogs treated with oral cobalamin had a
453 median Oral Cobalamin Supplementation Tolerance Score of 8.6/10 (range: 4.4-10) compared
454 to a median Parenteral Cobalamin Supplementation Tolerance Score of 7.7/10 (range: 5-9.2)
455 in dogs treated with parenteral cobalamin, which was not significantly different (P=0.22). Dogs
456 in the oral cobalamin supplementation group treated with oral tablets and/or capsules before
457 this trial, had a median Oral Capsule Tolerance Score Before Trial of 6.4/10 (range: 0-10). The
458 same dogs given oral cobalamin capsules at home had a median Oral Capsule Tolerance
459 Score During Trial of 8.6/10 (range: 0-10). There was no significant difference between the
460 Oral Capsule Tolerance Score Before Trial and the Oral Capsule Tolerance Score During Trial
461 (P=0.44). Owners reported the technique they used to administer the “Cobalaplex®” capsules
462 in 9 dogs. One capsule administration technique was used in 6 dogs, and 2 different techniques
463 were used in 3 dogs, as follows: capsule unopened (entire) hidden in the dog’s regular food
464 (n=5), the entire unopened capsule given alone (n=3), wrapped in a treat (n=3), capsule
465 opened and sprinkled on food (n=1). Although reduced appetite was documented in most dogs
466 at inclusion (13/19), major difficulties at administering the cobalamin capsules were reported
467 in only 1 dog (1/11) in the oral cobalamin supplementation group. In this dog, the Oral Capsule
468 Tolerance Score Before Trial (4/28) was however similar to the Oral Cobalamin Tolerance
469 Score During Trial (4/28), its serum cobalamin concentration normalised at week 13, and its
470 owner satisfaction was excellent (32/32).

471

472 *Parenteral Cobalamin Supplementation Tolerance Score, Veterinarian Visit Tolerance Score*
473 *Before Trial, and Veterinarian Visit Tolerance Score During Trial:* Dogs in the parenteral
474 cobalamin supplementation group who had already attended visits and injections at a
475 veterinary clinic before the trial, had a median Veterinarian Visit Tolerance Score Before Trial
476 of 6.4 (range: 5.5-8.6). The same dogs given VitBee 250 injections at the veterinary practice
477 had a median Veterinarian Visit Tolerance Score During Trial of 7.7 (range: 5-9.2). There was
478 no significant difference between the Veterinarian Visit Tolerance Score Before Trial and the
479 Veterinarian Visit Tolerance Score During Trial (P=0.60).

480

481 **Comparison of clinical phenotypes by severity**

482 *Presumptive PLE chronic enteropathy versus non-PLE chronic enteropathy dogs at inclusion:*
483 At baseline, in dogs with PLE, serum cobalamin concentrations were not statistically different
484 between dogs assigned to the oral cobalamin supplementation group (median 170 ng/L (range
485 150-242)) compared to dogs assigned to the parenteral cobalamin supplementation group
486 (median 199 ng/L (range 197-252)) (P=0.372). At week 13, all dogs with PLE achieved
487 eucobalaminaemia, regardless of treatment group. In dogs with PLE at week 13, the median
488 cobalamin concentration was significantly higher in the oral cobalamin supplementation group
489 (median 2000 ng/L (range: 2000-2000)), compared to the parenteral cobalamin
490 supplementation group (median 614 ng/L (range: 505-786)) (P=0.043).

491 Regardless of treatment modality, the decrease in methylmalonic acid from baseline to week
492 13 was significantly lower in non-PLE dogs compared to PLE dogs (-70.56 mg/L, 95% CI
493 [13.89, 127.24], P =0.020). The number of available results was insufficient to compare serum
494 methylmalonic acid concentration in PLE dogs between oral cobalamin supplementation and
495 parenteral cobalamin supplementation groups at baseline and week 13.

496

497 *Severe versus moderate hypcobalaminaemia at inclusion:* At week 13, all dogs with severe
498 hypcobalaminaemia at inclusion achieved eucobalaminaemia, regardless of treatment group.
499 At that time point, serum cobalamin concentration was significantly higher in dogs with severe

500 hypocobalaminaemia at inclusion in the oral cobalamin supplementation group (median 2000
501 ng/L (range: 412-2000)) compared to the parenteral cobalamin supplementation group
502 (median 402 ng/L (range: 320-786)) (P=0.009). At week 13, there was no significant difference
503 in serum methylmalonic acid concentration between oral cobalamin supplementation (median:
504 88.2 mg/L (range: 58.4-216)) and parenteral cobalamin supplementation (median: 79.1 mg/L
505 (range: 58.4-82.6)) groups in dogs with severe hypocobalaminaemia at inclusion (P=0.45).

506

507 *CIBDAI score ≤9 versus >9 at inclusion:* In dogs with CIBDAI score >9, there was no difference
508 in serum cobalamin concentration at baseline (P=0.885). The oral cobalamin supplementation
509 group had a median of 201.5 ng/L (range: 159-242), and the parenteral cobalamin
510 supplementation group a median of 212 ng/L (range: 150-252). At week 13, dogs with a
511 CIBDAI score >9 within the oral cobalamin supplementation group (median 2000 ng/L (range:
512 2000-2000)) had a significantly higher serum cobalamin concentration than dogs in the
513 parenteral cobalamin supplementation group (median 562.5 ng/L (range: 348-805)) (P=0.021).
514 At week 13, there was no significant difference in serum methylmalonic acid concentration
515 between oral cobalamin supplementation (median: 110.1 mg/L (range: 86.8-216)) and
516 parenteral cobalamin supplementation (median: 112.7 mg/L (range: 58.4-167)) groups in dogs
517 with CIBDAI >9 at inclusion (P=0.8).

518

519

520

521

522 **Discussion**

523 This study established non-inferiority of oral cobalamin supplementation compared to
524 parenteral cobalamin supplementation, at restoring eucobalaminaemia and for treatment
525 tolerance, when administered in hypocobalaminaemic dogs with chronic enteropathy. In
526 particular, this included the subpopulation of dogs with severe clinical and/or biochemical
527 presentation (“severe biochemical presentation” meaning “severe hypocobalaminaemia”).

528 However, we failed to demonstrate superiority at normalising serum cobalamin in the oral
529 cobalamin supplementation group in this study. Although comparable performance of oral
530 cobalamin supplementation and parenteral cobalamin supplementation at normalising serum
531 cobalamin concentration had already been reported in previous studies (Toresson *et al.* 2018,
532 Toresson *et al.* 2019), treatment tolerance and efficacy had never been specifically examined
533 in dogs with high CIDBAI scores (>9), severe hypcobalaminaemia (<200 ng/L) or PLE. As
534 reported in Toresson *et al.* publication (2019), our study also demonstrated efficacy and non-
535 inferiority of oral cobalamin supplementation compared to parenteral cobalamin
536 supplementation at improving cobalamin deficiency at a cellular level. We showed a significant
537 decrease in serum methylmalonic acid concentration from baseline to week 13 in oral
538 cobalamin supplementation and parenteral cobalamin supplementation groups, with no
539 significant difference between treatment groups overall, including dogs with severe
540 hypcobalaminaemia or high CIDBAI scores.

541

542 Dogs' and owners' QOL and owners' satisfaction during treatment were not significantly
543 different between treatment groups, indicating that both are equally acceptable from a welfare
544 perspective. Only one owner in the parenteral cobalamin supplementation group notified that
545 they would have preferred the "other treatment modality", should another cobalamin
546 supplementation protocol be necessary.

547 In both treatment groups, dogs with severe hypcobalaminaemia (<200 ng/L) had a
548 significantly higher Treatment Adherence & Satisfaction Score compared to dogs with
549 moderate hypcobalaminaemia. However, cobalamin supplementation was not the only
550 treatment change undertaken during the study period, as diet and/or medications were also
551 adjusted alongside based on clinicians' judgment. Therefore, it remains unclear whether
552 cobalamin supplementation alone was the reason for higher dogs' and owners' QOL in dogs
553 with severe hypcobalaminaemia. Nevertheless, the randomised design of the study would
554 mitigate for this confounding factor.

555

556 This study also demonstrates that dogs' tolerance to oral cobalamin capsules was similar to
557 their tolerance to other oral medication. Although poor oral treatment compliance was initially
558 feared, as reduced appetite was documented in most dogs at inclusion, only 1 dog was
559 described to show resistance to administering cobalamin capsules. Regardless of this, serum
560 cobalamin concentration normalised in this dog at week 13 and owner satisfaction was
561 excellent.

562

563 Although the mean increase in cobalamin between week 0 and week 13 was significantly
564 higher in the oral cobalamin supplementation group compared to the parenteral cobalamin
565 supplementation group, the decrease in serum methylmalonic acid was not significantly
566 different between treatment groups. The decrease in serum methylmalonic acid was lower in
567 the oral cobalamin supplementation group compared to the parenteral cobalamin
568 supplementation group, however, this later difference did not reach statistical significance. We
569 hypothesised that the low number of cases might have led to this result. Moreover, oral
570 cobalamin supplementation delivered a large surplus of cobalamin. This could explain the
571 significantly greater increase in cobalamin in the oral cobalamin supplementation group at
572 week 7 and at week 13 compared to the parenteral cobalamin supplementation group.
573 Interestingly, in a similar study conducted by Toresson *et al.* (2018), the increase in serum
574 cobalamin concentration was significantly higher in the parenteral group than the oral group
575 after 4 weeks, while the increase in cobalaminaemia was significantly lower in the parenteral
576 cobalamin supplementation group than the oral cobalamin supplementation group at week 12.
577 The reason for this different outcome halfway through the study remains unclear. As the first
578 serum cobalamin reassessment was performed at week 4 in this study, compared to week 7
579 in our study, we hypothesize that serum cobalamin levels may be slower to increase with oral
580 cobalamin compared to parenteral supplementation.

581

582 Although all dogs were hypocobalaminaemic at inclusion, only around half had evidence for
583 cobalamin deficiency on a cellular level. The presence of dogs with normal serum

584 methylmalonic acid concentration at inclusion could be explained by a lack of genuine cellular
585 cobalamin deficiency (either because of mild or short duration cobalamin deficiency), a true
586 cellular cobalamin deficiency with an inadequate reference range of serum methylmalonic acid
587 concentration at determining an abnormal result, or because cobalamin metabolism in dogs
588 differs from that of humans, such that elevation in methylmalonic acid is not indicative of a
589 cellular cobalamin deficiency.

590

591 Serum cobalamin concentration was above the normal range in 8/11 dogs in the oral cobalamin
592 supplementation group at week 7 and 13, while they were above the reference in 0/8 dogs in
593 the parenteral cobalamin supplementation group at weeks 7 and 13. These results suggest
594 that a lower oral cobalamin dosage might be effective as demonstrated in people with Crohn's
595 disease (Gomollon *et al.* 2017). As the optimal dose of oral cyanocobalamin in
596 hypcobalaminaemic dogs with chronic enteropathy has not yet been fully determined, future
597 studies of possible dosing ranges are warranted.

598

599 Limitations of this study included small sample size, presumptive rather than definitive
600 diagnosis of CE, and non-controlled diets or adjunctive treatments.

601 Gastrointestinal biopsies and histopathology were lacking in 10 dogs and faecal parasitology
602 was lacking in 17 dogs. Similarly, a definitive diagnosis of PLE was hampered by incomplete
603 availability of diagnostic tests to rule out hepatopathies and protein-losing nephropathy in most
604 dogs. The multicentric nature of the study, and the permission for adjunctive treatments to be
605 non-standardised resulted in variations in case management. Despite the prospective nature
606 of the study, numerous data points were missing which reduced the number of dogs included
607 in the final analysis.

608

609 Although cobalamin supplementation, either oral or parenteral, is suspected to remain the main
610 parameter contributing to changes in serum cobalamin concentration during the study period,

611 other factors such as dietary trials or concurrent medication may also have affected
612 cobalaminaemia.

613 Mean serum cobalamin concentration was 163 pg/mL higher in dogs fed a standard dry
614 commercial diet than dogs fed a standard raw diet in one prospective study. This highlights the
615 possibility that diet at inclusion could have affected cobalaminemia and also that a dietary
616 change undertaken during the study period might have contributed to the changes in serum
617 cobalamin concentration (Anturaniemi *et al.* 2020). As the diet provided before the start of the
618 trial was only documented in a few dogs, dietary cobalamin deficiency could not be fully
619 excluded as a cause of hypcobalaminaemia. However, most commercial foods and non-
620 vegetarian/non-vegan home-made foods are not restricted in cobalamin which makes dietary
621 cobalamin deficiency unlikely.

622 As adjunctive treatments were not controlled at inclusion and during the study period, they
623 could have had a potential effect on cobalaminaemia. Some of them, such as proton pump
624 inhibitors or probiotics could have contributed to a decrease in serum cobalamin concentration,
625 as shown in people (Lam *et al.* 2013) and dogs (Lucena *et al.* 2018) respectively. Antibiotics
626 could also have interfered with serum cobalamin concentration by altering intestinal microbiota
627 resulting in intestinal dysbiosis (Suchodolski 2016). We also hypothesise that steroids could
628 affect cobalamin intestinal absorption by reducing ileal inflammation.

629

630 As well as containing cobalamin, Cobalaplex[®] capsules contain folate and a prebiotic (fructo-
631 oligosaccharide) which could have provided an additional clinical benefit to the oral cobalamin
632 supplementation group.

633 In the absence of pre-existing validated scores to assess owners' satisfaction and treatment
634 palatability, the questionnaires and the scoring system used were designed for the purpose of
635 this study, and these scores have not been validated in clinical studies. Reliability on subjective
636 owners' assessment is also a significant limitation of these questionnaires, partly as owners
637 were not blinded to treatment group.

638

639 Methylmalonic acid concentration was assessed by Synlab laboratory (Augsburg, Germany)
640 which did not establish its own reference interval. Instead, we used the reference interval
641 established from 43 healthy dogs ([414.7–1192.5nmol/L]), published by *Berghoff et al.* (2012),
642 referenced in *Toresson et al.* (2019) publication. Both laboratories use the same assay
643 (chromatography - mass spectrometry method). The only other methylmalonic acid reference
644 interval published was established from 48 healthy dogs ([393-1,476nmol/L]), referenced in
645 *Kook et al.* publication (2019). A mild difference in methylmalonic acid reference interval exists
646 between laboratories. ~~At the present time, the Gastrointestinal laboratory provides results from~~
647 ~~the largest healthy canine population (43 healthy dogs).~~

648 Lastly, the lack of long-term follow-up did not allow assessment of the sustainability of
649 treatment efficacy.

650

651

652

653 **Conclusion**

654 This study has demonstrated that oral cobalamin supplementation was well tolerated and non-
655 inferior to parenteral cobalamin supplementation at normalising serum cobalamin
656 concentration and decreasing serum methylmalonic acid concentration in dogs with
657 hypcobalaminaemia due to chronic enteropathy, including subgroups with severe clinical or
658 biochemical abnormalities. Oral cobalamin supplementation and parenteral cobalamin
659 supplementation yielded similar tolerance and owners' satisfaction scores, even in severely
660 affected dogs. This emphasises that the severity of chronic enteropathy should not preclude
661 the use of oral cobalamin supplementation in these dogs.

662

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