

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

This is the Accepted Version of the following article:

Fracassi, F., Linari, G., Del Baldo, F., Di Cunzolo, A., D'Angelo, S., Malerba, E., Carotenuto, G., Bonfanti, U., Corradini, S.

(2018) Comparison of lente insulin and NPH insulin therapy for the treatment of newly diagnosed diabetic dogs: a randomised study

Veterinary Record 183, 262.

© British Veterinary Association 2018 doi: <http://dx.doi.org/10.1136/vr.104818>

20 **COMPARISON OF LENTE INSULIN AND NPH INSULIN THERAPY FOR THE TREATMENT OF NEWLY**
21 **DIAGNOSED DIABETIC DOGS: A RANDOMISED STUDY**

22
23 F. Fracassi DVM PhD DECVIM, G. Linari DVM, F. Del Baldo DVM, A. Di Cunzolo DVM, S. D'Angelo DVM, E.
24 Malerba DVM, G. Carotenuto DVM, U. Bonfanti DVM DECVCP, S. Corradini. DVM PhD.

25 *From the Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy (Fracassi,*
26 *Linari, Del Baldo, D'Angelo, Malerba, Carotenuto and Corradini), Clinica Veterinaria Vetlan, Battipaglia, Italy (Di*
27 *Cunzolo) and Laboratorio analisi La Vallonea, Passirana di Rho, Italy (Bonfanti).*

28

29 *Presented in abstract form at the 26th Annual European Society of Veterinary Internal Medicine Companion Animals*
30 *Congress, Goteborg, Sweden, September 2016.*

31

32 Corresponding author e-mail address (Dr. Federico Fracassi): federico.fracassi@unibo.it

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58 **Abstract**

59

60 Clinical studies that compare Lente insulin and Neutral Protamine Hagedorn (NPH) insulin in diabetic dogs are lacking.
61 This is a prospective, randomised, controlled clinical study aimed to compare the efficacy and safety of lente insulin
62 and NPH insulin in diabetic dogs. Thirty client-owned, newly diagnosed diabetic dogs were included. Animals were
63 randomised into two groups and received lente insulin or NPH insulin administered q12h. Follow-up re-evaluations
64 were done at 1, 2, 4, 6, 8, and 12 weeks. At each re-evaluation, a physical exam, blood glucose curve (BGC), and serum
65 fructosamine concentrations were performed. At the end of the study, the median insulin dose per injection was 0.61
66 U/kg (range, 0.34 to 0.92 U/kg) and 0.49 U/kg (range, 0.23 to 0.68 U/kg) in the lente and NPH groups, respectively.
67 There was a significant improvement of polyuria and polydipsia and glucose concentrations in both groups. At the end
68 of the study, the glycaemic control was considered good in 9/15 (60%) and 11/15 (73%) in the lente and NPH group,
69 respectively. These differences were not significant. Lente insulin and NPH insulin were similarly effective in the
70 treatment of dogs with DM.

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98 **Introduction**

99 Various types of insulin are used to treat diabetes mellitus (DM) long-term.¹⁻⁸ Based on duration of action and potency,
100 they include intermediate-acting (i.e. lente, Neutral Protamine Hagedorn [NPH]) and long-acting insulins (i.e.
101 protamine zinc insulin [PZI], insulin glargine, and insulin detemir). Current guidelines for dogs with newly diagnosed
102 DM recommend the use of insulin preparations with an intermediate duration of action.⁹
103 Lente is a porcine-origin zinc 40 U/mL insulin that consists of 35% short-acting amorphous insulin and 65% long-
104 acting, microcrystalline insulin. Lente is approved by the Food and Drug Administration (FDA) for use in dogs and
105 allows a good glycaemic control in most treated diabetic dogs.³ NPH (100 U/mL) is recombinant human insulin, usually
106 administered q12h. Some studies have demonstrated a good efficacy of this insulin in the treatment of canine DM.^{1,4}
107 One study observed that with NPH insulin, postprandial hyperglycaemia could occur in some well-regulated dogs.⁴
108 Several clinical studies evaluated single insulin products for the treatment of dogs with DM but clinical articles
109 comparing the efficacy and safety of different insulin preparations are uncommonly reported in the veterinary literature.
110 The aim of the present study is to compare the efficacy and safety of lente insulin and NPH insulin in newly diagnosed
111 diabetic dogs.

112

113 **Materials and Methods**

114 ***Dogs***

115 Thirty client-owned newly diagnosed diabetic dogs were prospectively enrolled in the study between November 2014
116 and September 2016. Dogs were recruited through the Teaching Hospital of the University of Bologna and three Italian
117 private practices. Authors provided written instructions about diagnosis, treatment and monitoring of the disease to the
118 practitioners involved in the study. Only one veterinarian in each recruiting center was responsible for the management
119 of the dogs. DM was diagnosed based on clinical signs such as polyuria, polydipsia (pu/pd), weakness, weight loss,
120 blood glucose concentration > 11 mmol/l after food had been withheld for at least 10 h, glucosuria, and serum
121 fructosamine concentration > 340 µmol/L. To identify any concurrent disorders, complete blood count (CBC), serum
122 biochemical profile, and complete urinalysis were performed at the time of enrollment in the study. Additional testing
123 were done if clinically indicated. Dogs with a relevant concurrent disease (e.g., renal insufficiency, neoplasia,
124 hypothyroidism, or hypercortisolism), that received insulin for > 7 days before admission and dogs that had received
125 glucocorticoids or progestagens within the previous 60 days were not enrolled. Dogs with diabetic ketoacidosis (DKA)
126 requiring aggressive management were used if their condition had been stabilized by medical treatment, including
127 regular insulin therapy.

128 The recruitment of dogs in the study was voluntary and the only cost for the owner was the purchase of insulin, also the
129 food was provided for free. The protocol and informed consent forms were approved by the Scientific Ethics
130 Committee of the University of Bologna. All owners signed the written informed consent before enrollment in the
131 study.

132 **Study design**

133 The trial was designed as a prospective, randomised, and controlled 3-month clinical study. Before treatment (day 0),
134 anamnesis and physical examination were obtained as well as a CBC, serum biochemical profile (that included
135 measurement of serum fructosamine concentration), and urinalysis were performed. At the time of diagnosis each dog
136 was randomly assigned to receive lente insulin (Caninsulin, MSD, Boxmeer, The Netherlands) or NPH insulin
137 (Humulin I, Eli Lilly Italia S.p.A., Sesto Fiorentino (FI), Italy). The randomisation was performed using a computer-
138 generated randomisation program based on the Fisher–Yates shuffle algorithm. All dogs received the same prescription
139 diet (Diabetic Royal Canin, © Royal Canin SAS, Milano, Italy), which was low in simple carbohydrates and high in

140 protein content. The diet was dry, canned, or a mixture of both based on the preferences of the dog. The diet and the
 141 formulation (dry/canned) were maintained for the entire duration of the study. The prescription diet was introduced as
 142 the dog's only food with a transition of 2–3 days from the dog's previous diet at the time of enrollment. Owners were
 143 instructed to feed dogs at the same time of insulin administration. The initial insulin dose for both products was 0.25–
 144 0.5 U/kg administered SC every 12 h. Six follow-up re-evaluations were performed 1, 2, 4, 6, 8, and 12 weeks after the
 145 initial evaluation. These evaluations included an assessment of clinical signs and determination of serum fructosamine
 146 concentration and BGCs. During each re-evaluation, food and insulin were given at home and blood glucose
 147 concentrations were measured after the dog arrived at the clinic (≤ 1 hour after insulin administration). To generate the
 148 blood glucose curves, blood capillary glucose was obtained from the pinna and was measured after 1, 2, 4, 6, 8, 10, and
 149 12 h from insulin injection. The insulin dose was adjusted by 0.5 - 2U/dose at each evaluation, as required; the aim was
 150 to maintain blood glucose concentrations between 5 and 15 mmol/l. Insulin dosage adjustments were made by the
 151 attending veterinarian and were based on the owner's perception of clinical signs in response to treatment (including
 152 evidence of hypoglycaemic episodes, body weight, and physical examination results), BGC, and serum fructosamine
 153 concentration. Hypoglycaemia was defined as blood glucose concentration < 4.4 mmol/l.

154 **Analytical Methods**

155 Blood glucose concentrations were measured in capillary blood obtained from the inner surface of the pinna using a
 156 hand-held glucometer produced for the dog (Glucocalea Wellionvet, Isomedic srl, (LO), Italy)¹⁰. Detectable blood
 157 glucose concentrations ranged from 1.1 to 33 mmol/l. When blood glucose concentrations were < 1.1 mmol/l and > 33
 158 mmol/l, registered as "LO" and "HI" on the glucometer were arbitrarily given a value of 1.1 mmol/l and 33 mmol/l,
 159 respectively. Fructosamine analyses were performed using a colorimetric nitroblue tetrazolium reduction method
 160 (Fructosamine, Olympus, Milano, Italy). The intra- and interassay coefficient of variation (CV) for serum fructosamine
 161 were 4.1% and 2.5%, respectively. The sensitivity of the assay was 5 $\mu\text{mol/L}$. CBC (Advia 2120 Hematology System,
 162 Siemens Healthcare Diagnostics. Tarrytown, NY), serum biochemical profiles (AU2700 Beckman-Coulter/Olympus,
 163 O'Callaghan's Mills, Ireland) including lipase (1,2-diglyceride enzymatic/colorimetric assay)(Lipase, OSR 6130,
 164 Olympus/Beckman Coulter, Lismeehan O'Callaghan's Mills, Co. Clare Ireland) and urinalyses were performed by
 165 standard laboratory methods in a reference laboratory (Mylav Laboratorio Lavallonea, Alessano, Italy).

166 **Assessment of Efficacy**

167 In order to objectively evaluate the glycaemic control, the following parameters were used: body weight, presence of
 168 polyuria/polydipsia, median glucose of the BGC, blood glucose nadir of the BGC, overall evaluation of the BGC, and
 169 serum fructosamine concentration. For each parameter, a score was arbitrarily assigned: 2 = good, 1 = moderate, and 0
 170 = poor. Maintaining or increases of body weight was considered good (score = 2), conversely a decrease ($> 5\%$) of the
 171 body weight was judged as poor (score = 0). In obese dogs, the weight loss needed to obtain an optimal BCS was not
 172 considered as negative in the scoring system. In such dogs, even if they were losing weight a score of 2 was given.
 173 Absent, improved, and present/unchanged-present/worsen pu/pd was considered good (score = 2), moderate (score = 1),
 174 and poor (score = 0), respectively. Median glucose of the BGC < 12.7 mmol/l, between 12.7–16.6 mmol/l, and > 16.6
 175 mmol/l was considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively. Glucose nadir of the
 176 BGC was considered good (score = 2), moderate (score = 1), and poor (score = 0), if it was < 10 mmol/l, between 10.0–
 177 13.9 mmol/l, and > 13.9 mmol/l, respectively. The overall evaluation of the BGC was considered good (score = 2) if
 178 $\geq 50\%$ of blood glucose measurements were between 4.4–15.0 mmol/l or poor (score = 0) if $< 50\%$ of the glucose
 179 measurements were between 4.4–15 mmol/l. Serum fructosamine concentration < 450 $\mu\text{mol/L}$, between 450–550
 180 $\mu\text{mol/L}$, and > 550 $\mu\text{mol/L}$ were considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively.

181 A total clinical score between 0 and 12 was obtained adding all the scores. A total clinical score between 8–12, 4–7, and
 182 0–3 points was suggestive of good, moderate, and poor glycaemic control, respectively.

183

184 **Data Analysis**

185 Statistical analysis was performed with commercially available software (Prism version 5.0d, GraphPad software Inc,
 186 San Diego, Calif.).

187 The distribution of data was assessed by using the D'Agostino and Pearson tests. The parameters normally distributed
 188 were expressed as mean \pm SD, while the data without a normal distribution were expressed as median (minimum and
 189 maximum value). Proportions and percentages were used to describe categorical variables. Parametric and non-
 190 parametric tests were used to analyze data based on the distribution. Categorical variables were compared using the
 191 Fisher's exact test. Differences between groups for age, body weight, laboratory results, and insulin dose, recorded at
 192 admission and body weight, laboratory results, and insulin dose over the 3 months study period were analyzed using the
 193 Mann-Whitney U-test or t-test. Within each group, differences in body weight, insulin dose, blood glucose, and
 194 fructosamine concentrations between baseline or first re-evaluation and the end of the study were evaluated using the
 195 Wilcoxon signed rank test or paired t-test. Differences were considered significant at $P < .05$.

196

197 **Results**

198 Thirty dogs were enrolled in this study. Fifteen dogs were treated with lente insulin and 15 with NPH insulin. Mean age
 199 was 9.6 years (SD, \pm 1.9 years). There were 17 mixed-breed dogs, 5 English Setters, 3 Labrador Retrievers, 2 Yorkshire
 200 Terriers, 1 Maltese, 1 Cocker Spaniel, and 1 Yugoslavian Shepherd Dog. Thirteen were spayed females, 3 intact
 201 females, 5 neutered males, and 9 intact males. All 3 intact female dogs were spayed within 4 weeks after inclusion in
 202 the study. Median body weight was 17.8 kg (range, 4.2 to 59.8 kg). At the time of the enrollment no significant
 203 differences between dogs assigned to lente or NPH group considering age, sex, or body weight were observed (Table 1).
 204 Six dogs were enrolled after resolution of DKA, 3 were in the lente insulin group, and 3 in the NPH insulin group. No
 205 differences considering serum glucose and fructosamine concentrations at the time of enrollment in the study between
 206 the two groups were detected (Table 1). All dogs accepted the new diet and in all subjects it was maintained throughout
 207 the study. Of the expected 180 follow-up re-evaluations (30 dogs for 6 follow up re-evaluations) only 170 were
 208 performed. 10 re-evaluations were lost because owners did not come to the clinic, i.e. skipped the appointment. Two
 209 dogs lost 2 re-evaluations and 6 dogs lost one re-evaluation. The last re-evaluation (at the 3rd month) was performed on
 210 all animals. Mean insulin dosages per injection at the beginning and at the end of the study were 0.36 ± 0.08 U/kg and
 211 0.6 ± 0.14 U/kg in lente group and 0.32 ± 0.07 U/kg and 0.47 ± 0.14 U/kg in dogs treated with NPH insulin. The increase
 212 of the insulin dose throughout the study was significant in both groups and at the end of the study the insulin dose was
 213 significantly lower in the NPH group when compared to the lente group ($P = 0.0206$).

214 Blood glucose concentrations < 4.4 mmol/l were identified in 3/86 (3.5%) and 6/84 (7.1%) of total BGCs performed by
 215 dogs treated with lente and NPH insulin, respectively. Such difference was not significant. Symptomatic hypoglycemia
 216 was not recorded in both groups and no reactions at the site of insulin administration were reported.

217 Evaluating all the BGCs, the glucose nadir was observed more commonly 4-6 h and 2-4 h after insulin injection in the
 218 lente group and in the NPH group, respectively (Figure 1).

219 Throughout the study, body weight did not change significantly either in the lente group ($P = 0.85$) nor in the NPH
 220 group ($P = 0.95$). Median blood glucose concentrations of the BGCs at the end of the study, compared with the first re-
 221 evaluation (1 week), were significantly decreased in both groups: from 23.0 mmol/l (range, 9.6–29.6) to 13.9 mmol/l

222 (range, 5.0–22.6) in the lente group ($P=0.009$); and from 19.8 mmol/l (7.8–28.1) to 11.7 mmol/l (4.6–23.2) in the NPH
223 group ($P = 0.04$). Serum fructosamine concentrations at the end of the study were significantly decreased compared
224 with the evaluation before treatment only in the group treated with NPH insulin: from 607 $\mu\text{mol/L}$ (288–880) to 418
225 $\mu\text{mol/L}$ (292–848) in the NPH group ($P = 0.005$); and from 455 $\mu\text{mol/L}$ (224–849) to 457 $\mu\text{mol/L}$ (329–749) in the
226 lente group (0.854).

227 Table 2 reports the assessment of the glycaemic control in the 2 groups at the end of the study and considers body
228 weight, polyuria-polydipsia, median blood glucose concentration of the BGCs, blood glucose nadir, overall evaluation
229 of the BGCs, and the serum fructosamine concentrations.

230 At the end of the study, the glycaemic control as evaluated using the total clinical score was classified as good in 9/15
231 (60%), moderate in 3/15 (20%), and poor in 3/15 (20%) dogs treated with lente insulin. In the group treated with NPH
232 insulin, the glycaemic control was classified as good in 11/15 (73%), moderate in 4/15 (27%), and poor in 0/15 (0%) of
233 dogs. Such differences between the two groups were not statistically significant. In the 4 dogs treated with NPH insulin
234 that at the end of the study had a moderate glycaemic control this was apparently not due to short insulin duration but
235 rather to insufficient glycaemic suppression (nadir >13.9 mmol/l).

236 The 3 dogs included after the resolution of DKA in the lente group at the end of the study were classified with good
237 ($n=2$) or moderate ($n=1$) glycaemic control, respectively. The 3 dogs included after the resolution of DKA in the NPH
238 group at the end of the study were all classified with moderate glycaemic control. None of the dogs included in the
239 study showed clinical signs (e.g. vomiting, painful abdomen at the physical examination, diarrhea) consistent with
240 pancreatitis. In the group treated with lente insulin serum lipase activity resulted above the reference range in 4/15 dogs
241 at T0 and in 4/15 dogs at T12. In the group treated with NPH insulin 7/15 dogs at T0 and 4/15 dogs at T12 had serum
242 lipase activity above the reference range. In the group treated with lente insulin the 3 dogs classified at the end of the
243 study with moderate glycaemic control had lipase activity above the reference range in 2/7, 1/7 and 0/7 re-evaluations,
244 respectively and the 3 dogs classified with poor glycaemic control had lipase activity above reference range in 5/7, 1/7
245 and 5/7 re-evaluations, respectively. In the group treated with NPH insulin the 4 dogs classified with moderate
246 glycaemic control had lipase above reference range in 3/7, 0/7, 1/7 and 1/6 re-evaluations, respectively.

247

248 Discussion

249 The results of this study indicate that both lente and NPH insulin are safe and efficacious as treatment for dogs with
250 newly diagnosed DM.

251 Starting insulin dosage in the lente and NPH groups, according to the treatment protocol, were commonly reported in
252 the veterinary literature.¹¹ At the end of the study, insulin dosage observed in both groups was similar to what was
253 obtained in previous studies that evaluated lente and NPH insulin in dogs.^{3,1} Mean insulin dose after three months of
254 treatment was significantly different between the lente and NPH groups, this is likely related to the greater potency of
255 NPH insulin. Median blood glucose concentrations were significantly reduced after three months of insulin treatment in
256 both groups; whereas the median fructosamine concentration was significantly reduced only in dogs treated with NPH
257 insulin. This finding must be interpreted with caution because at time of enrollment, despite not significant ($P = 0.08$),
258 the median fructosamine concentration was higher in the NPH group (607 $\mu\text{mol/L}$) than in the lente group (455
259 $\mu\text{mol/L}$), which was already a value closer to the normal reference range. Blood glucose nadir was identified mostly at
260 2 and 4 h from the insulin administration in dogs treated with NPH insulin and at 4 and 6 h in dogs treated with lente
261 insulin. These results are similar to those obtained in other studies where time to nadir in dogs treated with NPH insulin
262 resulted at 2, 5, and 4.9 h^{12,13,4} and from 4–8 h in dogs treated with lente insulin.¹⁴

263 In the NPH group, three of the four dogs classified as having a moderate glycaemic control at the end of the study, were
264 enrolled after resolution of DKA. There is no evidence to support that dogs after the resolution of DKA are more
265 difficult to control as diabetic patients. However, it is possible that, despite the complete diagnostic work-up before
266 enrolment, such dogs had an insulin resistance for not clarified reasons (e.g. undiagnosed disease such as subclinical
267 pancreatitis). In both groups no clinical signs consistent with acute pancreatitis were observed; however, the presence of
268 mild acute pancreatitis or chronic pancreatitis cannot be excluded. The diagnosis of chronic pancreatitis can be very
269 challenging because of the nonspecific and often low-grade nature of the clinical signs and the relatively low sensitivity
270 of non-invasive diagnostic tests.¹⁵ At the time of diagnosis no differences in terms of serum lipase activity between the
271 two groups was observed. Two of 3 dogs treated with lente insulin and classified with poor glycaemic control showed
272 serum lipase activity above the reference range in most of the re-evaluations. In these dogs a chronic pancreatitis as a
273 cause of insulin resistance cannot be excluded. A limitation of the present study was that serum lipase activity was
274 determined using the 1,2diglyceride enzymatic/colorimetric assay and not the canine pancreatic lipase immunoreactivity
275 that seems to have higher sensitivity in detecting chronic pancreatitis.¹⁶ However, the present study is focused on the
276 comparison of the efficacy and safety of two different insulin products, rather than evaluating the possible causes of
277 insulin resistance.

278 In terms of hypoglycaemic events, this study obtained better results in comparison with other studies evaluating lente
279 and NPH insulin. In a study performed on 53 dogs treated with lente insulin, clinical hypoglycaemic events were
280 reported in 38.6% of patients with total of 24 events (15%) with glucose concentration < 3.3 mmol/l on 159 BGCs. One
281 possible reason for the high incidence of hypoglycemia was a starting insulin dosage that was high, > 1 U/kg every 24
282 h; in such a study, 41% of dogs enrolled had necessity for insulin dose reduction.³ Another study performed on dogs
283 treated with NPH insulin showed clinical hypoglycemia in 4 dogs of 57 (7%).¹ The low incidence of hypoglycaemic
284 episodes observed in the present study is probably related to low starting insulin doses (0.25–0.5 U/kg twice daily),
285 frequent re-evaluations, and consequent frequent dosage adjustments.

286 All dogs have been fed with the same diet for the entire length of the study, which minimized food dependent glycaemic
287 variability. In contrast to similar studies¹⁻⁸, only newly diagnosed diabetic dogs were enrolled. This is in accordance
288 with the study's aim to compare two insulin treatment options to investigate if one of the two was more effective and/or
289 safe as a first-line treatment. In this study, the residual endogenous insulin secretion has not been tested. Likely, some of
290 the included dogs had some insulin production, i.e. the so-called "honeymoon period", and this could have partially
291 influenced the results of this study.

292 The main limitation of the present study, similarly to other clinical veterinary studies on DM, was the small number of
293 dogs included. This has been a limit in terms of reaching significance when comparing results between the two groups.
294 For example, at the end of the study, glycaemic control was classified as good in 11/15 (73%) dogs treated with NPH
295 insulin and in 9/15 (60%) dogs treated with lente insulin; however, such differences were not significant. According to
296 the calculation of statistical power and sample size and assuming the same percentages of glycaemic control, 136
297 instead of 15 dogs in each group would have been necessary to achieve statistical significance.

298 Some authors consider NPH insulin a second choice in comparison with lente insulin¹⁷; this is due to a study performed
299 on 10 diabetic dogs in which duration of insulin action was too short.⁴ That study evaluated the serum insulin and
300 glucose concentrations for a period of 10 h from insulin administration. In four dogs, the insulin duration of action at
301 the end of the study was 5.5 h; in another four dogs the duration was longer than 10 h; and in the remaining two dogs, it
302 was not possible to evaluate insulin duration of action because there was not enough blood glucose concentration
303 reduction to assess the duration of action. The authors of that study concluded that more investigations are needed to

304 assess the real duration of action for NPH insulin.⁴ This study did not to evaluate NPH insulin's duration of action;
305 however, we observed for dogs in the NPH group that moderate glycaemic control was not related to the short duration
306 of insulin action.

307 Both lente and NPH insulin have demonstrated safety and efficacy in the treatment of dogs with uncomplicated DM. In
308 general, dogs with NPH insulin obtained a higher percentage of better glycaemic control; although these differences
309 were mostly not significant. The low incidences of hypoglycaemic events were likely obtained because of low insulin
310 starting doses that were gradually increased and frequent re-evaluations. According to this study, NPH and lente insulin
311 can be considered similarly effective for the treatment of uncomplicated DM in dogs.

312

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334 **Acknowledgments**

335 The authors acknowledge and thank Dr Paola Palagiano and Dr Nadia Leoni for assistance during this study.

336

337

338 **References**

- 339 1 LORENZEN, F.H. (1992) The use of isophane insulin for the control of diabetes mellitus in dogs. *Acta Vet Scand*
340 33, 219-227
- 341
- 342 2 DAVISON, L.J., HERRTAGE, M.E., CATCHPOLE, B. (2005) Study of 253 dogs in the United Kingdom with
343 diabetes mellitus. *Vet Rec* 156, 467-471
- 344
- 345 3 MONROE, W.E., LAXTON, D., FALLIN, E.A., *et al.* (2005) Efficacy and safety of a purified porcine insulin
346 zinc suspension for managing diabetes mellitus in dogs. *J Vet Intern Med* 19, 675-682
- 347
- 348 4 PALM, C.A., BOSTON, R.C., REFSAL, K.R., *et al.* (2009) An investigation of the action of neutral protamine
349 Hagedorn human analogue insulin in dogs with naturally occurring diabetes mellitus. *J Vet Intern Med* 23, 50-55

- 350
351 5 DELLA MAGGIORE, A.D., NELSON, R.W., DENNIS, J., *et al.* (2012) Efficacy of protamine zinc recombinant
352 human insulin for controlling hyperglycemia in dogs with diabetes mellitus. *J Vet Intern Med* 26, 109-115
353
- 354 6 FRACASSI, F., BORETTI, F.S., SIEBER-RUCKSTUHL, N.S., *et al.* (2012) Use of insulin glargine in dogs with
355 diabetes mellitus. *Vet Rec* 170, 52-55
356
- 357 7 HESS, R.S., DROBATZ, K.J. (2013) Glargine insulin for treatment of naturally occurring diabetes mellitus
358 in dogs. *J Am Vet Med Assoc* 243, 1154-1161
359
- 360 8 FRACASSI, F., CORRADINI, S., HAFNER, M., *et al.* (2015) Detemir Insulin for the Treatment of Diabetes
361 Mellitus in Dogs. *J Am Vet Med Assoc* 247, 73-78
362
- 363 9 FRACASSI, F. (2017) Canine Diabetes Mellitus. In Textbook of Veterinary Internal Medicine, 8th ed. Eds S. J.
364 Ettinger, E. C. Feldman, E. Cotè. St. Louis, MO, Elsevier. pp 1767-1781
365
- 366 10 DEL BALDO, F., MALERBA, E., CORRADINI, S., ROVATTI, I., *et al.* (2017) Evaluation of one portable
367 blood glucose meter and one portable glucose-ketones meter in dogs. Research Communications of the ^{27th}
368 ECVIM-CA Congress. Malta, September 14 to 16, 2017. *J Vet Intern Med* 32, 525-609
369
- 370 11 RUCINSKY, R., COOK, A., HALEY, S., *et al.* (2010) AAHA diabetes management guidelines. *J Am Anim*
371 *Hosp Assoc* 46, 215-224
372
- 373 12 GOEDERS, L.A., ESPOSITO, L.A., PETERSON, M.E. (1987) Absorption kinetics of regular and isophane
374 (NPH) insulin in the normal dog. *Domest Anim Endocrinol* 4, 43-50
375
- 376 13 MORI, A., SAKO, T., LEE, P., *et al.* (2008) Comparison of time-action profiles of insulin glargine and NPH
377 insulin in normal and diabetic dogs. *Vet Res Commun* 32, 563-573
378
- 379 14 FLEEMAN, L.M., RAND, J.S., MORTON, J.M. (2009) Pharmacokinetics and pharmacodynamics of porcine
380 insulin zinc suspension in eight diabetic dogs. *Vet Rec* 164, 232-237
381
- 382 15 WATSON, P. (2012) Chronic pancreatitis in dogs. *Top Companion Anim Med* 27, 133-139
383
- 384 16 WATSON, P.J., ARCHER, J., ROULOIS, A.J., SCASE, T.J., *et al.* (2010) Observational study of 14 cases of
385 chronic pancreatitis in dogs. *Vet Rec* 167, 968-976
386
- 387 17 NELSON, R.W. (2015) Canine Diabetes Mellitus. In Canine and Feline Endocrinology. 4th ed. E. C. Feldman,
388 R. W. Nelson, C. E. Reusch, J. C. R. Scott-Moncrieff, E. Behrend. St. Louis, MO, Elsevier. pp 213-257
389
390
391
- 392
- 393
- 394
- 395
- 396
- 397
- 398

399

400 Figure 1. Histograms indicate the number of blood glucose curves (%) from dogs with diabetes mellitus treated by
 401 administration of lente insulin (n = 15) or Neutral Protamine Hagedorn (NPH) insulin (n = 15) twice daily for 3 months
 402 where the glucose nadir was observed at 1 or 2, 4, 6, 8, 10, and 12 h after insulin injection, respectively

403

404

405 Table 1 Baseline characteristics of 30 dogs included in the study

Variable	Lente Group	NPH Group	P Value
N° of Dogs	15	15	
Female	8 (1 intact, 7 spayed)	8 (2 intact, 6 spayed)	1
Male	7 (4 intact, 3 neutered)	7 (5 intact, 2 neutered)	1
Age (years)	9 (6–12)	10 (7–13)	0.35
Body weight (Kg)	12.5 (4.2–59.8)	16.0 (4.4–50.0)	0.60
Serum glucose (mmol/l)	22.2 (3.6–34)	21.3 (7.3–45.8)	0.69
Serum fructosamine (µmol/L)	455 (224–849)	607 (288–880)	0.08
Serum lipase activity (IU/l)	373 (179-2795)	410 (107-1343)	0.79

406

407 Table 2. Assessment of the glycaemic control using different parameters in 30 diabetic dogs treated for 3 months

408 (results at the end of the study) with lente insulin (n = 15) or NPH insulin (n = 15). BCG = blood glucose curve

Method of assessment	Score	Lente Group	NPH Group	P value
Body weight	Good	13/15 (87%)	15/15 (100%)	0.80
	Poor	2/15 (13%)	0/15 (0%)	0.49
Polyuria-polydipsia	Good	10/15 (67%)	13/15 (87%)	0.78
	Moderate	1/15 (7%)	2/15 (13%)	1.00
	Poor	4/15 (27%)	0/15 (0%)	0.11
Median blood glucose concentration (BGC)	Good	7/15 (47%)	9/15 (60%)	0.76
	Moderate	3/15 (20%)	3/15 (20%)	1.00
	Poor	5/15 (33%)	3/15 (20%)	0.70
Glucose nadir (BGC)	Good	8/15 (53%)	10/15 (67%)	0.77
	Moderate	3/15 (20%)	3/15 (20%)	1.00
	Poor	4/15 (27%)	2/15 (13%)	0.66
Overall evaluation of the blood glucose curve	Good	9/15 (60%)	11/15 (73%)	0.78
	Poor	6/15 (40%)	4/15 (27%)	0.72
Serum fructosamine concentration	Good	6/14 (43%)	10/15 (67%)	0.54
	Moderate	4/14 (29%)	3/15 (20%)	1.00
	Poor	4/14 (29%)	2/15 (13%)	0.66

409

410