1   **Ileal and total-tract digestibility and nitrogen utilisation in blue foxes (*Vulpes***

2   ***lagopus*) fed low-protein diets supplemented with DL-methionine and L-**

3   **histidine**

4

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11

12   **Abstract**

13   A lower dietary protein supply for adult blue foxes has been suggested. To formulate a low-protein

14   diet with sufficient amounts of amino acids (AAs), AA digestibility and the AA requirement of the

15   animals are crucial information. Therefore, a digestibility and nitrogen (N) balance trial was

16   conducted with 20 blue foxes to determine the macronutrient and AA digestibility and N utilisation

17   in low-protein diets supplemented with DL-methionine (Met) and L-histidine (His). In addition,

18   plasma urea and plasma AAs were measured. The diets were designated as P24 (control), P20,

19   P20M, P16M and P16MH and contained digestible crude protein (DCP) amounting to 24%, 20% or

20   16% of metabolizable energy (ME). The 20% protein level was fed with or without Met and the

21   16% protein level was fed with Met and with or without His. The apparent total-tract digestibility

22   (ATTD) of crude protein linearly decreased with decreasing dietary protein level. The ATTD of dry

23   matter, organic matter and crude carbohydrates increased when wheat starch was added as a

24   replacement for protein. The apparent ileal digestibility (AID) and ATTD methods were compared

25   to determine the AA digestibility. The decreasing dietary protein supply decreased the ATTD of

26   most of the AAs: threonine, tryptophan (Trp), valine, alanine (Ala), aspartic acid (Asp), glutamic

27   acid, glycine (Gly), proline (Pro), serine (Ser) and total AAs. The AID of the AAs was constant

28   between diets. Diverging AAs showed higher or lower digestibility when determined in the AID or

29   ATTD methods. Isoleucine, lysine, Met, Ala and tyrosine showed higher levels of AID. Arginine,

30   His, cysteine (Cys), Trp, Asp, Gly, Pro and Ser showed higher levels of ATTD, which may reflect

31   the net loss of these AAs in the large intestine. Met and His supplementation improved the ATTD

32   and AID of the AAs in question, respectively, but did not affect the other variables examined. N

33   retention did not differ between diets and renal N excretion decreased with decreasing protein level;

34   thus N utilisation improved. We concluded that the protein supply and AA composition in low-

35   protein diets with supplemented Met were adequate for adult blue foxes, since the lower protein

36 supply improved N utilisation and did not affect N retention. However, His supplementation failed

37 to reach the designed level and therefore showed no clear results.

38

39 key words: amino acid; digestibility; blue fox; carnivore; nitrogen utilisation

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41

1. Introduction

Reduced dietary protein for more efficient nitrogen (N) utilisation, lower ammonia emissions and the feasibility of using low-cost or alternative feed ingredients are targets of both ecological and economical animal production. Feed formulation based on the animal's amino-acid (AA) requirements and digestible AAs is one of the key factors contributing to these targets. Farmed blue fox (*Vulpes lagopus*), as a carnivore species used for fur production, is traditionally fed with high protein diets. As high quality fur is the main product of this fur production, AA content and especially sulphur-containing AAs contributing markedly to fur growth, is one of the main interests when developing feeds for fur animal species. The current recommendations for protein feeding for blue foxes are based on the proportion of digestible crude protein (DCP) of metabolizable energy (ME). The protein evaluation system takes into account the calculated AA composition of the feed, but the protein and AA requirement of blue foxes at the late growing-furring period, when the winter-fur in priming are not completely know. However, taking account the AA requirement of the animal at different life stages might bring both economic and environmental benefits and allow the use of alternative dietary protein sources, as has been shown in other species such as poultry and swine (Williams 1995, Perttilä et al. 2002, Lemme et al. 2004, Aarnink & Verstegen 2007).

The current protein recommendations for fast-growing blue foxes in the late growing-furring period (from early September until pelting) is in Finland min. 22 % of metabolizable ME (Profur 2019). Previous recommendation has been established by Lassén et al. (2012), when it was 24% of ME. However, lower protein levels, even as low as 15 % of ME, has been suggested (Dahlman 2003, Ylinen et al. 2018). Particularly the lower protein levels could be used in the latter part of the late growing-furring period. However, when the dietary protein supply is limited, the AA composition should be balanced to meet the animal's AA requirements, since no excessive protein or AAs are present. Research is needed to increase our understanding of the limiting AAs and AA digestibility

67 in blue foxes. AA digestibility in blue foxes varies, depending on the protein sources (Skrede et al.

68 1980). Protein sources with low digestibility vary more widely in digestibility between individual

69 AAs than highly digestible protein sources (Skrede et al. 1980). Use of low-digestible protein

70 sources, such as meat-and-bone meal or alternative feed ingredients to replace more traditional

71 ingredients, such as fish, is growing in the fur feed industry. To ensure sufficient AA supply, it is

72 crucial to determine the AA digestibility coefficients and formulate the feed based on these data.

73

74 In the present study, AA digestibility was determined using both the apparent total-tract

75 digestibility (ATTD) and apparent ileal digestibility (AID) methods. Reliable evidence suggests that

76 in other monogastric species the AID rather than the ATTD is considered the preferred method of

77 determining AA digestibility, because the microbial fermentation in the large intestine affects both

78 the quantity and composition of faecal protein and AAs (Mosenthin et al. 2000, Sauer et al. 2000).

79 Most of the data concerning the differences between AID and ATTD have been carried out in

80 swine, but studies have also been conducted in minks, dogs and blue foxes (Szymeczko 2001,

81 Hendriks & Sritharan 2002, Vhile et al. 2005, Tjernsbekk et al. 2014). These predominantly

82 carnivore species have short colons with limited microbial fermentation capacity, and it was

83 therefore expected that the hindgut effect would not be significant. In dogs, the AID method has

84 been considered more accurate (Hendriks & Sritharan 2002, Hendriks et al. 2012, Tjernsbekk et al.

85 2014), while in blue foxes, the ATTD method has been primarily used (Dahlman 2003) and N

86 disappearance from the large intestine is minimal (Hendriks et al. 2012). However, in blue foxes,

87 the AID method has sometimes resulted in lower apparent AA digestibility, which may reflect the

88 net loss of AA in the large intestine and overestimation of AA digestibility carried out in the ATTD

89 method (Szymeczko 2001, Vhile et al. 2005). We determined AA digestibility using ATTD and

90 AID to compare results, expecting to see higher digestibility values in ATTD than AID. Our general

91 aim was to increase knowledge of the true digestible AA requirement of blue foxes. As ATTD is

92   less invasive method than AID and possible to conduct with intact animals, it would be beneficial to

93   be able to calculate the true AA digestibility from results gained using ATTD method. Therefore,

94   our objective was to compare AID and ATTD AA digestibility in blue foxes to determine

95   differences for single AAs that can be applied for estimating AID values from ATTD values.

96

97   In addition to the digestibility trial, an N balance trial was conducted to detect the effect of the

98   experimental diets on N retention. Our hypothesis was that lowering the protein supply improves N

99   utilisation and can thus decrease N excretion, as has been shown in various monogastric species,

100   including blue foxes (Dahlman et al. 2002a, Otto et al. 2003, Belloir et al. 2017). Our aim was to

101   used N balance to show, that can the dietary protein levels be reduced without affecting N retention

102   and if dietary supplementation of Met and His to low protein diets will increase N retention.

103

104   We determined the nutrient and AA digestibility and N utilisation in adult blue foxes fed low-

105   protein diets with or without DL-methionine (Met) and L-histidine (His) supplementation. The

106   study was conducted in two separate terms in late growing-furring period to define more closely the

107   protein and AA requirement of the blue fox, and thus elaborate on the results of Dahlman et al.

108   (2002a) which covered the entire growing-furring period. Met is the first limiting AA in fur animals

109   and, accordingly, was selected to be supplemented in the low-protein diets in this study. Based on

110   the Dahlmans (2002a) study, we hypothesised that the protein level 16% of ME needs to be

111   supplemented with Met but wanted to see the effect of supplementation at protein level 20% of ME.

112   His was selected as the second experimental supplemental AA as it was the third limiting AA in the

113   study of ideal protein for blue foxes (Dahlman et al. 2004). In the present study, the AID and ATTD

114   methods were compared to determine differences for single AAs that can be applied for estimating

115   AID values from ATTD values. The study aims for improved feed formulation, based on digestible

116 AAs and the animal's AA requirements, which could result in economic and environmental benefits

117 for the production sector.

118

119 **2. Materials and methods**

120

121 **2.1 Experimental diets and feeding**

122 The digestibility and N balance trial was conducted at the Kannus Research Farm Luova Oy,

123 Kannus, Finland. Three dietary protein levels were studied as a part of the performance trial carried

124 out with the feeds in question (Ylinen et al. 2018). Two lowest protein levels were studied with or

125 without Met and His supplementation. The control diet and four experimental diets were designated

126 as P24, P20, P20M, P16M and P16MH. The control diet (P24) contained DCP amounting to 24% of

127 ME. The experimental diets contained DCP amounting to 20% of ME with and without Met

128 supplement (P20 and P20M) and 16% of ME with Met and with Met and His supplements (P16M

129 and P16MH). The Met and His supplementation was calculated to bring the AA in question to the

130 same level as in group P24. Celite® (SiO_2), was added at 0.6% as a marker. The feed ingredients

131 are shown in Table 1. The daily feeding ratio was on a dry matter (DM) basis 298 g DM, fed once

132 daily at noon. Drinking water was freely available.

133

134 **2.2 Animals and experimental design**

135 The trial was conducted in autumn 2016 with 20 blue foxes males of 23 weeks of age..The average

136 body weight was (± standard error of the means SEM) 15.5 (±0.2) kg. The animals were in the hall

137 environment housed individually in wire-mesh metabolism cages for controlled feeding and

138 quantitative collection of faeces and urine. The animals were randomly distributed on five groups

139 giving four replicates for each diet. Before the experiment, the animals were fed a basic farm diet.

140 The trial period comprised four days of adjustment and three days of total collection of faeces and

141 urine. The daily feed consumption was recorded and potential residual feeds collected, weighed and

142 analysed. The chemical composition of the feeds was analysed in pooled samples collected daily

143 throughout the collecting period. Total faeces were collected daily prior to daily feeding. Total

144 urine was collected into containers added four ml of 10 N sulphuric acid to prevent ammonia

145 evaporation. Samples of the feed, faeces and urine were kept frozen (–20°C) pending analysis.

146 Digestibility of DM, organic matter (OM), crude protein (CP), crude fat (CF), crude carbohydrates

147 (CCH) and ash was determined using acid-insoluble ash (AIA) as an inert marker. To determine

148 AA digestibility, both the ATTD and AID methods were used, using AIA as a marker. To

149 determine AID, the animals were euthanized after the total collection period. Engine euthanasia was

150 performed with filtered exhaust gases (combination of CO, CO2, HC, O2); CO concentration in the

151 euthanasia chamber being 4%. Euthanasia was performed individually, 3.5 h after the last feeding.

152 After euthanasia, the digestive tract was exposed and the ileum dissected and divided into three

153 equal segments. The digesta of the terminal third of the ileum were gently squeezed out of the gut

154 and immediately frozen at –20°C. In addition, urine urea, urine N, plasma urea and plasma AAs

155 were recorded. For blood samples, the venous blood was collected once on the last total collection

156 day. Blood was sampled from the cephalic vein 24 h after last feeding.

157

158 **2.3 Calculations**

159 The apparent digestibility of the macronutrients and AA was calculated as follows:

160 ATTD or AID (%) = (1 – (AIA in nutrient/AIA in faeces or ileal digesta) * (nutrient in faeces or

161 ileal digesta/nutrient in feed)) * 100.

162

163 The N balance parameters were calculated as follows:

164 N absorbed = N intake – Faecal N,

165 N retained = N intake – (Faecal N + Urinary N),

166 In addition, the N retained was calculated as the percentage of intake and absorption and as grams

167 per kilogram live weight.

168

169 The ME (MJ/kg) content of the feeds was calculated, using the chemical composition and

170 digestibility coefficients carried out with the feed in question and ME (MJ/kg) values: protein (N x

171 6.25) 18.8; fat 39.8; carbohydrates 17.6 (Lassén et al. 2012). The energy distribution was calculated

172 according to Lassén et al. (2012).

173

174 **2.4 Chemical analysis**

175 The samples were pooled within diet (feeds) or animal (faeces and urine) or analysed as single

176 samples (ileal digesta, blood). The faecal samples were homogenized, using a hand-held electric

177 mixer. The analyses were conducted in the Laboratory of Agricultural Sciences, University of

178 Helsinki, Finland, except the CF, which was analysed in the Laboratory of Fin Furlab Oy/Ab ,

179 Vaasa, Finland and plasma urea, which was analysed in the Laboratory of Veterinary Medicine,

180 University of Helsinki. The chemical composition of the feed and faeces was analysed by standard

181 methods according to the AOAC International (1995). Dry matter was determined by oven drying at

182 103°C for 24 h and crude ash was determined at 600°C for 24 h. The feed and faecal samples were

183 dried using lyophilization to prevent protein breakdown. The hair was sifted, and the dry samples

184 were ground in a porcelain mortar in preparation for analysis. The CP was determined by the

185 Kjeldahl method (AOAC International, 1995) with a Tecator Auto Digestion unit and a Kjeltec

186 Auto 2300 Analyser (Foss A/S, Hillerød, Denmark). The CF was determined by solvent extraction

187 according to the Weibull-Stoldt technique (BÜCHI Hydrolysis Unit B-411 and BÜCHI Extraction

188 Unit B-811; BÜCHI Labortechnik AG, Flawil, Switzerland). The CCH were calculated as the

189 difference obtained by subtracting the ash, CP and CF from the DM. Urinary urea was determined

190 with a HUMAN Liquicolour Complete Test Kit and spectrophotometer (Shimadzu ultraviolet (UV)

191 mini 1240; Shimadzu Corp., Kyoto, Japan). AA were determined by ultraperformance liquid

192 chromatography (UPLC) method as described in Puhakka et al. (2016) was used. Prior to AA

193 analysis, the samples were hydrolysed according to Directive 98/64/EC (European Commission,

194 1998). Venous blood collected in tubes containing ethylenediaminetetraacetic acid (EDTA) was

195 centrifuged at 1000–-1300 G for 10 min. Following centrifugation, the plasma was removed and

196 frozen at –20 °C pending analysis. For AA analysis, the plasma samples were precipitated with

197 10% sulphosalicylic acid and further analysed by UPLC, as described above for the hydrolysed feed

198 samples. Plasma urea was analysed using a Konelab 60i analyser (Thermo Fisher Scientific,

199 Waltham, MA, USA).

200

201 **2.5 Statistical analysis**

202 Statistical analysis of the data was performed with the general linear model (GLM) procedure of

203 SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The data were tested for normal distribution, using

204 the Shapiro-Wilk test and the homogeneity of variance with Levene's test. The model used in

205 analysis was:

206 $$Y_{ij} = \mu + d_i + \varepsilon_{ij},$$

207 where Y_{ij} = the observation, μ = the general mean , d_i = the effect of diet (I = 1, …, 5) and ε_{ij} = a

208 random effect. The diet effect was tested, using four orthogonal contrasts: C1 tested the effect of the

209 control diet (P24) against the experimental diets and C2 the effect of the protein level between

210 groups P20 and P16. C3 tested the effect of Met supplementation between groups P20 and P20M,

211 and C4 the effect of His supplementation between groups P16M and P16MH. In the analysis of the

212 method effect, the average ATTD and AID across diets was used. The effect of the method and

213 interaction between the diet and method were tested, using the GLM-procedure of SAS 9.4.

214

215 **3. Results**

216 All animals were healthy, with normal faecal consistency throughout the experiment. The feeds

217 were eagerly consumed, and no feed residuals were detected. One animal in group P20M and one in

218 group P16MH showed extremely low protein digestibility values (< 2 SD), and their results were

219 excluded from the macronutrient digestibility and N balance analysis as outliers. However, the

220 digestibility values of individual AAs were within the normal range in these two animals, and all

221 AA results were included in the analysis.

222

223 **3.1 Feeds**

224 The experimental diets followed the designed levels, with some differences (Table 2). The total CP

225 content (g/kg DM) of the diets followed the levels designed, except in group P16MH, where the

226 total CP was lower than that designed. The DCP as a percentage of ME was lower than that

227 designed in all groups. On average, the DCP content was 21%, 17% and 15% of the ME in diets

228 P24, P20 and P16, respectively. Energy supply was $5.4 - 5.8$ MJ ME per day per animal. Since the

229 energy distribution of the macronutrients was calculated, using the digestibility values obtained

230 from this study, the low protein digestibility may have affected the low DCP content as a

231 percentage of the ME.

232

233 The CF content (g/kg DM) was lowest in groups P24 and P16MH. The AA content of the diets

234 (g/kg DM) is presented in Table 3. The low CP content reflected the low AA content of diet

235 P16MH (total and individual AAs). The Met content in groups P16M and P16MH exceeded that of

236 group P24. As planned, the lowest Met content was in the diet not supplemented with Met (P20). In

237 contrast, the His supplemented in group P16MH did not reach the level of the group P24, but was

238 higher than in the nonsupplemented groups.

239

240 **3.2 Apparent macronutrient and amino-acid digestibility**

11

241   Foxes fed the P24 showed higher ATTD of CP than with all other diets ($p = 0.01$, Table 4). In

242   group P16MH, protein digestibility was especially low. The ATTD of CCH, DM and OM was

243   higher in groups P16 than in groups P20 ($p < 0.0001$, $p = 0.0003$ and $p = 0.005$, respectively). The

244   ATTD of CF was high (over 94%) in all groups, but lower in group P24 ($p = 0.01$) than in all other

245   groups. Met and His supplementation did not affect the macronutrient digestibility coefficients.

246

247   The AID of Met increased by Met supplementation ($p = 0.0007$, Table 5) and His supplementation

248   positively affected the AID of His ($p = 0.04$). The AID of Met was lower in group P24 than in the

249   Met-supplemented groups ($p = 0.04$) and higher in group P16 than P20 ($p = 0.003$). All other AAs

250   and total AAs showed no differences in AID among diets. The ATTD of the AAs decreased with

251   decreasing protein level for the following AAs and total AAs: threonine (Thr), tryptophan (Trp),

252   valine (Val), alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), proline (Pro) and

253   serine (Ser) ($p \leq 0.05$, Table 6). The ATTD of Met increased with Met supplementation ($p = 0.001$).

254   His supplementation did not affect the ATTD of His between P16M and P16MH, but the ATTDs of

255   Met and His were higher in group P16 than in group P20 ($p = 0.001$).

256

257   **3.3 Apparent total tract and apparent ileal digestibility amino acid values**

258   The average ATTD and AID of AAs throughout the diets are shown in Table 7. The diverging AAs

259   showed higher or lower digestibility when determined in the AID or ATTD methods. For arginine

260   (Arg), cysteine (Cys), His, Trp, Asp, Gly, Pro and Ser, the ATTD method resulted in higher

261   digestibility coefficients ($p \leq 0.05$). For isoleucine (Ile), lysine (Lys), Met, Ala and tyrosine (Tyr),

262   the AID method resulted in higher digestibility coefficients ($p \leq 0.05$). No diet method interaction

263   was found.

264

265   **3.4 Nitrogen utilisation and plasma amino acids**

266 N intake, urinary N excretion, urea N (g/d) and absorbed N decreased with decreasing dietary

267 protein level ($p < 0.0001$, Table 8). Urea N as a percentage of total urinary N or daily retention of N

268 (as grams per day, grams per live weight, percentage of intake or percentage of absorption) did not

269 differ between groups. No differences between diets were found in plasma urea.

270 The content of the following AAs in plasma decreased with decreasing protein level ($p \leq 0.05$): Ile,

271 leucine (Leu), phenylalanine (Phe), Thr, Trp, Val, Asp and Tyr (Table 9). The content of plasma

272 Met and His was constant among the diets.

273

274 **4. Discussion**

275 In general, composition of the diet and related factors, such as feed intake, affect the digestibility of

276 main nutrients and AAs (McDonald et al. 2011). In developing feeds or optimizing diets, changes in

277 the diet and therefore in digestibility may markedly affect nutrient availability. In blue foxes,

278 changes in protein composition are especially important, since the quality of the pelt is dependent

279 on the protein level and AA composition of the diet (Dahlman et al. 2002a, 2002b). At the same

280 time, economic and environmental issues compel the reduction in protein use in fur animal feeds.

281 In the present study, CP ATTD linearly decreased with decreasing CP of the diets. In a previous

282 digestibility study in adult blue foxes fed low-protein diets, the protein digestibility has

283 correspondingly decreased with decreasing dietary protein levels, from 83% to 78% and from 86%

284 to 79% (Dahlman et al. 2002a). The low-protein levels used in that study were 22.5 and 15% of ME

285 and control protein level 30% of ME (Dahlman et al. 2002a). Opposite to our study, the

286 supplemented Met improved the digestibility of the DM, ether extract and CCH in the low-protein

287 diets in the study of Dahlman et al. (2002a).

288

289 In both studies, digestibility was determined, as apparent, and thus endogenous and microbial

290 protein was not taken into account. Studies in swine have shown that apparent digestibility values

291 are dependent on the dietary protein level, since the relative proportion of basal endogenous and

292 microbial protein in the digestive tract is higher when the dietary protein supply is lower

293 (Mosenthin et al. 2000). The effect is quadratic and greater when the protein supply is minimal (Fan

294 & Sauer 1997). Similarly, it have been obtained in blue foxes and minks that lower dietary protein

295 decreases apparent protein digestibility, and probably the effect of basal endogenous and microbial

296 protein has diluting effect on digestibility values (Skrede et al. 1980, Szymeczko & Skrede 1990,

297 Dahlman et al. 2002a). In our study, the effect of endogenous protein may have been pronounced in

298 the lowest protein group, where the protein supply was lower than that designed and the

299 digestibility value was low.

300

301 Higher ATTD of DM, OM and CCH was observed in group P16 than in P20. In P16, the proportion

302 of cooked wheat starch, the main replacement for the decreased protein, was higher than in P20.

303 Cooked wheat starch has a high digestibility of carbohydrates (87%) (LUKE 2006). The higher

304 proportion of starch likely increased the ATTD of CCH and further influenced the ATTD of DM

305 and OM. In the previous digestibility study in blue foxes (Dahlman et al. 2002a), the ATTD of

306 CCH, DM and CF decreased with decreasing dietary protein. In their study, the ingredients

307 replacing the decreasing protein were wheat bran and cooked wheat starch (in 1:2 ratio,

308 respectively). Fibre is known to decrease the digestibility of almost all nutrients and energy by

309 increasing passage rate and withholding the nutrient absorption by adsorption of nutrients into fibre

310 (Sauer et al. 1991, Wenk 2001). In addition, especially fermentable fibres tend to increase microbial

311 fermentation in the large intestine and thus microbial protein in the faeces, reducing the ATTD

312 values (Sauer et al. 1991, Silvio et al. 2000). In our study, the crude fibre content was not analysed,

313 but since the addition of wheat bran was low and the proportion equal between diets, we assumed

314 that it did not markedly interfere with the digestibility values between diets.

315

316   The ATTD of AAs showed that the digestibility of nonessential AAs (Ala, Asp, Glu, Gly, Pro and

317   Ser), some essential AAs (Thr, Trp and Val) and total AAs decreased. Met and His supplementation

318   increased the ATTD of the AAs in question, respectively. The results accorded with previous

319   studies, in which the supplemented AAs were thoroughly and rapidly digested (Wang & Fuller

320   1989, Dahlman et al. 2002a). In the study of Dahlman et al. (2002a), decreasing AA digestibility

321   along with decreasing protein levels were found for all AAs except Cys. In addition, Met and Lys

322   supplementation increased Met and Lys digestibility, respectively. In both studies, AA digestibility

323   was determined as apparent in lieu of true digestibility. As in macronutrient digestibility, we

324   presumed that the faecal endogenous protein affected the decreasing ATTD of the AAs with

325   decreasing protein level in the ATTD method. However, the diluting effect of endogenous protein

326   to digestibility values was not seen in the AID values, even though the ileal digesta contains

327   endogenous proteins such as bile, digestive enzymes and cells sloughed off in the mucus. As shown

328   in the study of Szymeczko & Skrede (1990) in mink, endogenous secretions already affect the AA

329   composition of the digesta in the first section of the small intestine.

330

331   The AID of the AAs did not differ between diets, with the exception of Met and His, since the

332   supplementation of the AAs increased the AID of the AA in question. In addition, Gugołek et al.

333   (2017) reported improved protein digestibility and N retention with abundant Met supplementation.

334   In our study, Met supplementation was slightly lower than in the study of Dahlman et al. (2002a)

335   and considerably lower than in Gugołek et al. (2017). However, in Gugolek et al. (2017) the aim

336   was to study the effects of extremely high Met supplementation and in our study we pursued the

337   lowest possible Met supplementation. In our study, the digestible Met was in low protein diets 0.32

338   g/MJ ME. Dahlman et al. (2003) concluded that adequate digestible Met should be 0.40 g/MJ ME

339   in blue foxes in late growing-furring season. In the present study, His supplementation was,

340 unfortunately, inadequate, since the His content of the supplemented diet did not reach the level of

341 the control diet (P24), making it impossible for us to conclude on the effect of His supplementation.

342

343 In our study, the variation in AA digestibility results was greater in the AID than in the ATTD

344 methods. Muir et al. (1996) and Murray et al. (1997) obtained correspondingly greater variation,

345 using the AID method in ileal-cannulated (simple T-type cannulas) dogs. Muir et al. (1996)

346 concluded that a possible source of error could have been the marker (chromic oxide) used in the

347 AID determination instead of the total collection method used in faecal determination. In our study,

348 AIA was used as a marker in both methods. The possible source of error and cause of variation in

349 the AID determination may have been the sampling technique, i.e. manually squeezing the digesta

350 out of the terminal ileum, which may have sucked in sloughed intestinal epithelial cells and other

351 endogenous material in varying amounts. In addition, the AID method resulted in only one sample

352 per animal, whereas in the ATTD method the sample was collected over 3 days, which may have

353 decreased the accuracy of the AID samples.

354

355 Microbial fermentation in the large intestine converts both quantity and quality of the protein and

356 AAs in the faeces. However, carnivores are considered to have low microbial fermentation

357 capacity, because their digestive tract is short, the digesta passage rate is rapid and the colon is

358 unsacculated (Szymeczko & Skrede 1990, Ahlstrøm & Skrede 1995, Tjernsbekk et al. 2014).

359 Previous studies in carnivores have shown that low fermentation capacity is most pronounced in

360 mink (Tjernsbekk et al. 2014, Gugołek et al. 2015). Blue foxes and dogs tend to have higher

361 microbial activity (Hendriks et al. 2013, Tjernsbekk et al. 2014, Gugołek et al. 2015). In our study,

362 the ATTD and AID methods resulted in diverging digestibility values among AAs. Arg, His, Cys,

363 Asp, Gly, Pro, Ser and total AAs showed higher ATTD than AID coefficients, while Ile, Lys, Met,

364 Ala and Tyr showed higher AID than ATTD coefficients. In previous studies, similar patterns of

365   AAs have shown higher or lower digestibility values using ATTD or AID measuring indicating

366   AAs undergoing similar microbial fermentation in the large intestine of dogs and blue foxes

367   (Szymeczko 2001, Hendriks & Sritharan 2002, Vhile et al. 2005, Tjernsbekk et al. 2014). In

368   addition, net synthesis of Met in the large intestine has been demonstrated in swine and dog, which

369   may lead to underestimation of Met digestibility (Mosenthin et al. 2000, Hendriks et al. 2012).

370

371   In the present study, more than half of the AAs showed lower AID than ATTD of AAs, which

372   reflects a net loss of these AAs (Arg, Cys, His, Asp, Gly, Pro, Ser and total AAs) in the large

373   intestine. Similar results were found in the study of Vhile et al. (2005) for Thr, Asp, Gly, Pro and

374   Ser and for all AAs in the study of Szymeczko (2001). AAs disappearing from the large intestine

375   are not considered to have nutritional value, since the fate of these AAs is to undergo microbial

376   fermentation, and the possible absorption of nonprotein N does not contribute markedly to the

377   protein metabolism of the animal. Thus, overestimation of the ATTD of these AAs is likely and

378   must be taken into account when applying ATTD method to determine AA digestibility in blue

379   foxes.

380

381   In dogs, the ATTD method has resulted in both over- and underestimation of digestibility in DM,

382   protein and AAs (Muir et al. 1996, Murray et al. 1997, Hendriks & Sritharan 2002), and the AID

383   method has been considered more accurate for the real AA availability, whereas in blue foxes the

384   findings have been more unresolved. In blue foxes, previous authors have concluded that the

385   difference between the AID and ATTD methods is numerically small, and the ATTD method may

386   therefore be acceptable (Szymeczko 2001, Vhile et al. 2005, Tjernsbekk et al. 2014). Results

387   obtained from our study support this approach. However, overestimation in the digestibility of Arg,

388   Cys, His, Asp, Gly, Pro, Ser and total AAs and underestimation in digestibility, especially of Met,

389   Ile, Lys, Ala and Tyr must be taken into account. In addition, endogenous and microbial proteins

390   may affect the ATTD values and show ostensible reduction in the ATTD of AAs.

391

392   N excretion decreased with decreasing protein level, as was expected. The reduction in N excretion

393   with reduced protein supply has been clearly demonstrated in various species, including blue foxes

394   (Canh et al. 1998, Noblet et al. 2001, Dahlman et al. 2002a, Otto et al. 2003, Carpenter et al. 2004.

395   Belloir et al. 2017). In addition, N retention remained constant and, therefore, N utilisation

396   improved in low-protein diets. In a previous study in blue foxes, a similar effect was found

397   (Dahlman et al. 2002a). Corresponding to the study by Dahlman et al. (2002a), neither Met nor His

398   improved N utilisation, indicating that there was no deficiency of these AAs. However, the lowest

399   protein level was not tested without Met supplementation in our study.

400

401   The plasma urea (or plasma urea nitrogen, PUN) concentration is a rapid method for estimating AA

402   requirements and is affected by the quality and quantity of dietary protein and AA composition

403   (Coma 1995, Pedersen & Boisen 2001). The quality of the protein inversely affects the PUN, and

404   when the AA requirement of the animal is fulfilled, the need for cycling excess N to urea is

405   minimal, and the PUN reaches a minimum plateau (Coma 1995). However, in addition to quality,

406   the quantity of the dietary protein affects the PUN values directly. In addition, factors not related to

407   dietary protein, such as renal function, water intake and body protein catabolism under acute

408   challenges to the immune system, affect PUN values (Coma 1995, Kiarie et al. 2009). In studies in

409   swine, N retention and PUN values have shown similar responses in determining the AA

410   requirement of the animal (Coma et al. 1995, Pedersen et al. 2003). Correspondingly, the absolute

411   plasma urea in our study was lowest in group P16M, which showed the highest N retention. This

412   supports the conclusions obtained from the N retention results that excess N is mainly excreted in

413   the urine. Still, plasma urea did not differ between diets. Varying protein contents of the diets may

414   have affected to plasma urea values. In previous studies, plasma urea reference value for healthy

415   blue foxes, fed normal diets based on recommendations, has been 6.4 mmol/l, while for breeding

416   blue fox females, the PUN has ranged from 6.18 to 6.67 mmol/l (Korhonen & Huuki 2014,

417   Sepponen et al. 2014). Our result for plasma urea from 4.92 to 5.96 mmol/l was lower, probably

418   due to lower dietary protein level than in normal farm diets. However, PUN was determined in

419   fasted state, which decreases the effect of

420

421   Despite the fairly constant digestibility coefficients and improved N utilisation results, decreased

422   plasma AAs of some AAs were measured in low-protein diets. The decreased plasma AAs may

423   indicate that the low-protein diets did not provide adequate AA to cover to requirement, especially

424   if the feeding period would have been extended. In dogs, acute protein deficiency results in

425   decreasing plasma AAs in all AAs. In long-term protein deficiency, however, only essential AAs in

426   plasma decrease, while nonessential tend to increase (NRC 2006). Our study lasted only 7 days, but

427   it may be noteworthy that essential AAs in plasma decreased with decreasing protein level. Met and

428   His supplementation probably influenced the concentration of these AAs in plasma, since the Met

429   and His plasma concentrations were constant between the control and in the supplemented low-

430   protein groups. In previous studies in pigs, supplemented AAs have markedly increased the plasma

431   content of the supplemented AAs in question (e.g. Figueroa et al. 2003). The plasma AA profile of

432   blue foxes has not been determined. In comparison to the plasma AA content of food-deprived (24

433   h) dogs, most of the AA concentrations in our study were similar or higher, except Cys and Thr,

434   which were lower and may reflect the importance of these AAs for blue foxes (Delaney et al. 2001).

435

436   **5. Conclusions**

437   In the present study, the ATTD of CP decreased with decreasing dietary protein. The effect of

438   greater proportions of endogenous protein in low-protein diets supposedly affected the decreasing

439   apparent digestibility. In addition, we concluded that the wheat starch added in the low-protein diets

440   contributed the increased ATTD of DM, OM and CCH. The supplemented Met and His were well

441   digested and increased the Met and His digestibility, respectively, but did not improve the overall

442   digestibility nor affected any of the other variables examined.

443

444   The ATTD method showed the decreasing digestibility of the nonessential and some essential AAs

445   with decreasing protein level. The AID method varied more between individuals and failed to show

446   the differences between the diets. However, the ATTD method may have resulted in overestimation

447   of the digestibility of Arg, Cys, His, Asp, Gly, Pro, Ser and total AAs and underestimation of the

448   digestibility of Ile, Lys, Met, Ala and Tyr. According to this study, ATTD can be applied in

449   determining AAs digestibility in blue fox as it is less invasive and more convenient method.

450   However, the differences in single AAs must be taken into account when estimating the true AA

451   digestibility.

452

453   N retention did not differ among the diets. N excretion decreased and N utilisation improved with

454   lower dietary protein supplies. Therefore, we concluded that the protein supply and AA

455   composition in the low-protein diet with supplemented Met was adequate for blue foxes in the late

456   growing-furring season. According to this study, digestible Met level of 0.32g/MJ ME. However,

457   His supplementation was inadequate compared with the design, and it is therefore impossible to

458   draw conclusions of the importance of His supplementation.

459

468   **Conflict of interest**

469   Vappu Ylinen received a personal research grant from the Helve Foundation for conducting the

470   study. At the time the research was done, Päivi Pylkkö worked for Kannus Research Farm Luova

471   Oy. Jussi Peura is the Research Director in Finnish Fur Breeders' Association. The above-

472   mentioned authors declare no financial interest or benefit from the direct applications of the

473   research. Jarmo Valaja declares that he has no conflict of interest, financial interest or benefit from

474   the direct applications of the research.

475

476   **Use of animals**

477   All institutional and national guidelines for the care and use of experimental animals were followed.

478   All the experimental procedures were approved by the National Animal Experiment Board in

479   Finland with the guidelines established by the European Union Directive 2010/63/EU and current

480   Finnish legislation on animal experimentation (Act on the Protection of Animals Used for Scientific

481   or Educational Purposes 497/2013).

482

483   **Data availability**

484   The data that support the findings of this study are available from the corresponding author [V. Y.]

485   upon reasonable request.

486

487   Table 1. Composition of the experimental diets (g/kg) and planned chemical composition.

	P24	P20	P20M	P16M	P16MH
Baltic herring, autumn	170	140	140	108	108
Slaughter by-products, pork	30	25	25	19	19
Slaughter by-products, broiler	260	213	213	165	165
Precooked barley	170	139	139	107	107
Animal protein, meal	21	15	15	11	11
Fish meal	36	29	29	23	23
Molasses from sugar beet pulp	20	25	25	26	26
Wheat bran	20	25	25	26	26
Lard	56	58	58	75	75
Mineral mixture[a]	2	2	2	2	2
Cooked wheat starch		54	54	78	78
DL-methionine			0.8	1.3	1.3
L-histidine					0.9
Celite	6	6	6	6	6
Water	209	269	268	353	352
Planned in DM (g/kg)					
Protein	291	248	249	212	214
Fat	277	258	257	285	284
Carbohydrates	363	453	453	479	478
Ash	68.3	58.2	58.1	49.9	49.8
ME (MJ ME/kg DM)	18.7	18.8	18.8	19.8	19.8
Energy distribution					
Protein	24	20	20.1	16	16.2
Fat	55	50.7	50.7	53.3	53.1
Carbohydrates	21	29.3	29.3	30.7	30.6
F:C	2.6	1.7	1.7	1.7	1.7

488   P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M =

489   crude protein 20% of ME + Met supplement, P16M = crude protein 16% of ME

490   + Met supplement, P16MH = crude protein 16% of ME + Met and His supplements. ME =

491   metabolizable energy, Met = DL-methionine, His = L-histidine.

492   [a] Containing 100 g1 mixture—Ca: 16 g; P: 11 g; Mg: 4 g; Fe: 650 mg; Zn: 600 mg; Mn: 300 mg;

493   Cu: 15 mg; Co: 4 mg; Se: 0.1 mg.

494

495   Table 2. Analysed chemical composition of the diets.

	P24	P20	P20M	P16M	P16MH
DM g/kg	398	371	361	371	371
In DM (g/kg DM)					
Protein	278	246	242	229	207
Fat	256	300	321	274	254
Carbohydrates	384	375	358	423	469
Ash	82	79	79	74	70
ME (MJ/kg DM)	18.2	18.4	19.5	19.0	18.4
Energy distribution*					
Protein	21.3	17.1	16.4	15.9	14.1
Fat	52.8	60.3	62.6	55.1	51.6
Carbohydrates	25.9	22.5	21.0	29.0	34.3
F:C	2.0	2.7	3.0	1.9	1.5

496   P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M

497   = crude protein 20% of ME + Met supplement, P16M = crude protein

498   16% of ME + Met supplement, P16MH = crude protein 16% of ME +

499   Met and His supplements. DM = dry matter, ME = metabolizable energy,

500   F:C = fat:carbohydrate ratio, Met = DL-methionine, His = L-histidine.

501   * Energy distribution as % of ME, calculated using chemical composition

502   and digestibility coefficients carried out with the feed in question.

503

504   Table 3. Analysed amino-acid composition of the diets (g/kg DM).

	P24	P20	P20M	P16M	P16MH
Essential AA + cysteine					
Arginine	16.9	14.6	15.1	14.2	12.6
Cysteine	3.8	2.7	3.3	3.6	2.7
Histidine	6.3	5.2	5.3	5.2	5.6
Isoleucine	10.2	9.5	9.8	9.0	8.2
Leucine	20.6	17.8	18.0	17.4	15.1
Lysine	15.3	14.2	14.1	14.0	11.8
Methionine	5.1	4.0	6.4	7.1	6.9
Phenylalanine	11.9	10.0	10.3	9.7	8.7
Threonine	10.8	9.7	9.8	9.4	8.3
Tryptophan	1.9	1.7	1.7	1.5	1.5
Valine	14.7	12.8	12.9	12.5	10.8
Nonessential AA					
Alanine	16.4	14.2	14.3	14.0	11.9
Aspartic acid	22.4	20.1	20.2	19.8	16.9
Glutamic acid	38.2	34.5	34.3	32.9	28.7
Glycine	20.3	16.9	17.2	16.6	14.5
Proline	17.2	14.6	14.5	14.0	12.0
Serine	13.4	11.7	11.9	11.4	10.0
Tyrosine	7.9	6.8	7.3	6.7	6.1
Σ Essential[a]	117	102	107	104	92
Σ Nonessential[a]	136	119	120	115	100
Σ Total[b]	253	221	226	219	192

505   [a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential.

506   P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude

507   protein 20% of ME + Met supplement, P16M = crude protein 16% of ME

508   + Met supplement, P16MH = crude protein 16% of ME + Met and His supplements. AA = amino

509   acid, ME = metabolizable energy, Met = DL-methionine, His = L-histidine.

510   Table 4. Apparent total-tract digestibility of macronutrients.

	P24	P20	P20M	P16M	P16MH	SEM[1]	c1	c2	c3	c4
DM	72.2	70.3	71.3	73.7	74.4	0.748	ns	**	ns	ns
CP	74.0	70.1	71.5	70.8	68.1	1.136	*	ns	ns	ns
OM	77.8	76.0	77.4	79.5	79.7	0.774	ns	**	ns	ns
CCH	69.7	64.5	65.1	73.6	76.8	1.439	ns	***	ns	ns
CF	94.1	95.3	95.6	95.8	94.3	0.360	*	ns	ns	*
Ash	9.08	3.48	0.79	1.75	4.92	1.327	***	ns	ns	ns

511   P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of

512   ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude

513   protein 16% of ME + Met and His supplements. DM = dry matter, CP = crude protein, OM =

514   organic matter, CCH = crude carbohydrates, CF = crude fat. SEM = standard error of the means.

515   SEM[1] is for diets P24, P20 and P16M. SEM for diets P20M and P16MH is proportionately 1.154

516   of the reported value. ns = nonsignificant, * = significance at $p < 0.05$, ** = significance at $p <$

517   0.01, *** = significance at $p < 0.001$. ME = metabolizable energy, Met = DL-methionine, His =

518   L-histidine. Contrasts: C1 = P24 vs. others, C2 = protein level (P20 and P20M vs. P16M and

519   P16MH), C3 = MET-supplementation (P20 vs. P20M), C4 = HIS-supplementation (P16M vs.

520   P16MH).

521   Table 5. Apparent ileal digestibility of amino acids.

	P24	P20	P20M	P16M	P16MH	SEM	c1	c2	c3	c4
Essential AA + cysteine										
Arginine	77.7	77.1	77.7	77.9	77.6	1.096	ns	ns	ns	ns
Cysteine	37.2	14.6	32.4	42.0	23.8	4.844	ns	ns	*	*
Histidine	77.0	77.6	78.5	77.9	82.2	1.391	ns	ns	ns	*
Isoleucine	75.9	75.2	75.5	73.3	75.6	1.375	ns	ns	ns	ns
Leucine	76.1	76.4	77.0	76.0	76.7	1.278	ns	ns	ns	ns
Lysine	81.5	83.7	84.9	84.7	83.8	1.162	ns	ns	ns	ns
Methionine	86.6	84.2	90.4	90.3	91.6	1.020	*	**	***	ns
Phenylalanine	75.5	74.6	76.3	75.2	75.8	1.416	ns	ns	ns	ns
Threonine	67.6	65.4	66.1	63.2	63.8	2.067	ns	ns	ns	ns
Tryptophan	63.7	60.3	61.5	54.5	57.9	2.346	ns	ns	ns	ns
Valine	72.4	72.0	72.1	71.0	71.8	1.508	ns	ns	ns	ns
Nonessential AA[a]										
Alanine	76.8	77.5	77.4	77.4	76.0	1.103	ns	ns	ns	ns
Aspartic acid	63.8	63.9	64.9	63.1	65.3	2.212	ns	ns	ns	ns
Glutamic acid	77.7	77.3	77.1	76.7	77.2	1.355	ns	ns	ns	ns
Glycine	68.8	67.4	67.7	67.9	67.1	1.522	ns	ns	ns	ns
Proline	66.6	64.2	63.6	63.2	61.2	1.768	ns	ns	ns	ns
Serine	62.6	60.1	60.2	57.4	58.9	2.214	ns	ns	ns	ns
Tyrosine	75.7	73.3	75.2	74.7	77.2	1.650	ns	ns	ns	ns
Total[b]	73.6	73.0	73.8	73.0	73.6	1.419	ns	ns	ns	ns

522   [a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential. P24 = crude protein 24% of ME, P20

523   = crude protein 20% of ME, P20M = crude protein 20% of ME + Met supplement, P16M = crude

524   protein 16% of ME + Met supplement, P16MH = crude protein 16% of ME + Met and His

525   supplements. SEM = standard error of the means. ns = nonsignificant, * = significance at $p < 0.05$,

526   ** = significance at $p < 0.01$, *** = significance at $p < 0.001$. AA = amino acid, ME =

527   metabolizable energy, Met = DL-methionine, His = L-histidine. Contrasts: C1 = P24 vs. others, C2

528   = protein level (P20 and P20M vs. P16M and P16MH), C3 = MET-supplementation (P20 vs.

529   P20M), C4 = HIS-supplementation (P16M vs. P16MH).

530

Table 6. Apparent total-tract digestibility of amino acids.

	P24	P20	P20M	P16M	P16MH	SEM	c1	c2	c3	c4
Essential AA + cysteine										
Arginine	81.7	79.5	80.1	82.5	79.8	0.789	ns	ns	ns	*
Cysteine	50.8	32.0	48.7	52.1	43.2	4.113	ns	ns	*	ns
Histidine	81.8	79.5	81.2	83.3	84.3	0.853	ns	**	ns	ns
Isoleucine	73.2	70.5	72.9	73.4	70.7	0.991	ns	ns	ns	ns
Leucine	76.3	74.0	75.7	77.5	74.0	0.766	ns	ns	ns	**
Lysine	81.2	81.3	81.9	84.6	79.5	0.988	ns	ns	ns	**
Methionine	78.1	73.7	84.8	86.5	87.3	1.964	*	**	**	ns
Phenylalanine	75.7	72.0	74.1	76.8	73.2	1.114	ns	ns	ns	*
Threonine	70.8	65.0	67.6	68.6	64.4	1.180	**	ns	ns	*
Tryptophan	68.2	61.8	60.7	60.6	60.9	1.943	**	ns	ns	ns
Valine	72.6	69.2	71.0	73.0	68.9	0.875	*	ns	ns	**
Nonessential AA[a]										
Alanine	75.7	71.7	72.7	74.1	69.5	1.035	**	ns	ns	**
Aspartic acid	72.3	68.8	68.7	69.0	64.0	0.826	***	*	ns	***
Glutamic acid	79.3	76.4	76.4	76.6	73.3	0.639	***	*	ns	**
Glycine	79.4	75.0	75.6	78.9	76.0	1.141	*	ns	ns	ns
Proline	75.3	70.7	70.8	74.2	69.9	1.125	**	ns	ns	*
Serine	69.3	63.7	64.8	68.1	63.3	1.158	**	ns	ns	*
Tyrosine	71.9	67.6	70.9	72.5	68.3	1.432	ns	ns	ns	ns
Total[b]	77.2	73.9	75.2	77.0	73.5	0.751	*	ns	ns	**

[a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential. P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude protein 16% of ME + Met and His supplements. SEM = standard error of the means. ns = nonsignificant, * = significance at $p < 0.05$, ** = significance at $p < 0.01$, *** = significance at $p < 0.001$. AA = amino acid, ME = metabolizable energy, Met = DL-methionine, His = L-histidine. Contrasts: C1 = P24 vs. others, C2

538   = protein level (P20 and P20M vs. P16M and P16MH), C3 = MET-supplementation (P20 vs.

539   P20M), C4 = HIS-supplementation (P16M vs. P16MH).

540

541   Table 7. Average apparent ileal and total-tract digestibility of amino acids

	Ileal	Total-tract	SEM	Method	Diet*method
Essential AA + cystine					
Arginine	77.6	80.7	0.426	***	ns
Cysteine	30.0	45.4	2.666	***	ns
Histidine	78.6	82.0	0.613	***	ns
Isoleucine	75.1	72.2	0.540	***	ns
Leucine	76.4	75.5	0.479	ns	ns
Lysine	83.7	81.7	0.542	*	ns
Methionine	88.6	82.1	1.150	***	ns
Phenylalanine	75.5	74.4	0.585	ns	ns
Threonine	65.2	67.3	0.812	ns	ns
Tryptophan	59.6	62.4	1.105	ns	ns
Valine	71.8	70.9	0.567	ns	ns
Nonessential AA[a]					
Alanine	77.0	72.7	0.554	***	ns
Aspartic acid	64.2	68.6	0.800	***	ns
Glutamic acid	77.2	76.4	0.526	ns	ns
Glycine	67.8	77.0	0.619	***	ns
Proline	63.8	72.2	0.741	***	ns
Serine	59.8	65.9	0.850	***	ns
Tyrosine	75.2	70.2	0.722	***	ns
Total[b]	73.4	75.4	0.517	*	ns

542   [a]As listed in the table, [b]Total = Σ Essential + Σ Nonessential.

543   ns = nonsignificant, * = significance at $p < 0.05$,** = significance at $p < 0.01$,

544   *** = significance at $p < 0.001$. SEM = standard error of the means, AA =

545   amino acid.

546

547
548

549   Table 8. Nitrogen (N) metabolism.

	P24	P20	P20M	P16M	P16MH	SEM[1]	c1	c2	c3	c4
N intake	13.2	11.7	11.5	10.9	9.8					
Faecal N g/d	3.2	3.1	3.0	3.1	2.9	0.137	ns	ns	ns	ns
UrinaryN g/d	8.4	7.4	7.0	6.2	5.6	0.291	***	***	ns	ns
Urea g/d	16.1	12.9	13.0	12.0	10.5	0.564	***	*	ns	ns
Urea N g/d	7.5	6.0	6.4	5.6	4.9	0.263	***	*	ns	ns
Urea N as % of total urinary N	89.0	81.6	86.4	91.2	87.4	2.464	ns	ns	ns	ns
N absorbed g/d	10.0	8.6	8.5	7.8	6.9	0.137	***	***	ns	**
N retained										
g per day/animal	1.5	1.2	1.3	1.6	1.3	0.313	ns	ns	ns	ns
% of intake	11.5	10.5	11.7	14.7	13.7	2.977	ns	ns	ns	ns
g kg live weight	0.1	0.1	0.1	0.1	0.1	0.020	ns	ns	ns	ns
% of absorption	15.2	14.4	15.8	20.3	19.0	4.100	ns	ns	ns	ns
Plasma urea mmol/l	5.96	5.52	4.98	4.92	5.54	0.424	ns	ns	ns	ns

550   P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of

551   ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude

552   protein 16% of ME + Met and His supplements. PUN = plasma urea nitrogen. SEM = standard

553   error of the means. SEM[1] is for diets P24, P20 and P16M. SEM for diets P20M and P16MH is

554   proportionately 1.154 of the reported value. ns = nonsignificant, * = significance at $p < 0.05$, ** =

555   significance at $p < 0.01$, *** = significance at $p < 0.001$. ME = metabolizable energy, Met = DL-

556   methionine, His = L-histidine. Contrasts: C1 = P24 vs. others, C2 = protein level (P20 and P20M

557   vs. P16M and P16MH), C3 = MET-supplementation (P20 vs. P20M), C4 = HIS-supplementation

558   (P16M vs. P16MH).

559

560

561   Table 9. Plasma amino acids µM/l.

	P24	P20	P20M	P16M	P16MH	SEM	c1	c2	c3	c4
Essential AA + cystine										
Arginine	148.0	148.7	153.8	148.6	157.0	12.329	ns	ns	ns	ns
Cysteine	34.63	36.03	37.20	37.88	39.95	3.5803	ns	ns	ns	ns
Histidine	76.14	70.33	72.06	76.65	71.42	2.6257	ns	ns	ns	ns
Isoleucine	79.92	72.69	75.63	65.54	65.43	4.0402	*	*	ns	ns
Leucine	161.1	150.1	150.5	134.8	135.1	6.4887	*	*	ns	ns
Lysine	185.4	175.2	190.1	190.1	168.8	11.386	ns	ns	ns	ns
Methionine	44.06	42.57	42.35	39.75	42.64	2.4933	ns	ns	ns	ns
Phenylalanine	94.54	85.09	87.47	85.90	79.72	3.8300	*	ns	ns	ns
Threonine	120.6	109.5	104.4	95.28	90.39	5.3046	**	*	ns	ns
Tryptophan	51.71	45.36	39.84	41.35	39.35	2.6993	**	ns	ns	ns
Valine	196.0	181.2	182.7	168.0	165.4	7.8989	*	ns	ns	ns
Nonessential AA										
Alanine	415.3	449.1	469.2	535.7	471.2	38.956	ns	ns	ns	ns
Aspartic acid	73.04	64.90	67.44	62.25	60.67	2.6611	**	ns	ns	ns
Glutamic acid	850.0	733.8	703.1	606.2	667.3	78.765	ns	ns	ns	ns
Glycine	305.3	261.6	294.3	282.2	284.7	13.553	ns	ns	ns	ns
Proline	135.8	133.7	135.0	142.9	139.1	8.1806	ns	ns	ns	ns
Serine	234.0	241.5	232.6	212.9	208.4	12.809	ns	ns	ns	ns
Tyrosine	60.74	54.32	53.37	52.51	47.11	3.6975	*	ns	ns	ns

562   P24 = crude protein 24% of ME, P20 = crude protein 20% of ME, P20M = crude protein 20% of

563   ME + Met supplement, P16M = crude protein 16% of ME + Met supplement, P16MH = crude

564   protein 16% of ME + Met and His supplements, SEM = standard error of the means.

565   ns = nonsignificant, * = significance at $p < 0.05$, ** = significance at $p < 0.01$, *** = significance at

566    $p < 0.001$. AA = amino acid, ME = metabolizable energy, Met = DL-methionine, His = L-histidine.

567   Contrasts: C1 = P24 vs. others, C2 = protein level (P20 and P20M vs. P16M and P16MH), C3 =

568   MET-supplementation (P20 vs. P20M), C4 = HIS-supplementation (P16M vs. P16MH).

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