Effects of crude protein levels in concentrate supplements on animal performance and nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass

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1   **INTERPRETIVE SUMMARY**

2   Effects of crude protein level in concentrate supplements on animal performance and nitrogen

3   utilization of lactating dairy cows fed fresh-cut perennial grass. By Hynes et al.

4   Manure nitrogen from dairy herds is a major source of pollution of air and ground water. The

5   aim of this study was to reduce nitrogen output in dairy cows' manure, while sustaining milk

6   production, by feeding low protein concentrates. When good quality grass was fed, reducing

7   concentrates crude protein level from 18.1 to 14.1% (dry matter basis) had no adverse effect

8   on milk production, but decreased urine nitrogen outputs. This may mitigate nitrogen pollution

9   from grazing dairy herds, without comprising production efficiency. Linear and multiple

10   relationships estimating urinary nitrogen, to be used at farm, research and policy-making levels,

11   were produced.

12

13   **RUNNING HEAD:** URINARY NITROGEN ALLEVIATION

14

15   **Effects of crude protein level in concentrate supplements on animal performance and**

16   **nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass.**

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31 **ABSTRACT**

32 There are increased concerns regarding N pollution of air and ground water from grazing cattle.

33 Although a number of studies have investigated mitigation strategies for N output from dairy

34 cows fed conserved forages and concentrates, similar research on fresh-cut grass in addition to

35 production parameters is limited. Therefore the current study, using 3 dietary treatments and

36 incorporating 2 genotypes, was designed to evaluate the effects of concentrate crude protein

37 (**CP**) level on animal production and N utilization efficiency (**NUE**) of lactating dairy cows.

38 Twelve multiparous cows (6 Holstein and 6 Holstein × Swedish Red) were used in a change-

39 over study with three 25-d periods and 3 diet treatments; low, medium and high CP concentrate

40 (14.1, 16.1 and 18.1% respectively, dry matter (**DM**) basis) fed at 32.8% DM intake in

41 combination with good quality zero-grazed perennial ryegrass (18.2% CP, DM basis). Each

42 period consisted of an adaption phase (18-d) housed as a single group, 1-d adaption in

43 individual stalls and a 6-d measurement phase with feed intake and feces, urine and milk output

44 recorded. There was no significant interaction between cow genotype and concentrate CP level

45 on any animal performance or NUE parameters. Total DM intake, milk yield and composition

46 and NUE were not affected by dietary treatment. However, increasing concentrate CP level

47 increased (i) N intake by 42 g/d and excretion in urine and manure, by 38 and 40 g/d,

48 respectively, and (ii) the ratio of urine N over manure N. Feeding high CP, rather than low CP

49 concentrate, increased milk urea N (**MUN**) content by 3.6 mg/dL and total MUN output by

50 1.08 g/d. Crossbred cows had lower grass DM intake, total DM intake, total N intake and

51 consequently energy-corrected milk yield. However, cow genotype had no significant effect

52 on NUE or MUN parameters. Equations have been developed to predict urine N excretion

53 using MUN output as sole predictor or in combination with dietary CP level. The present study

54 indicated that when grazing cows are fed on good quality pasture, feeding concentrates with a

55 protein content as low as 14.1% may not negatively affect productivity. In addition, reducing

56 concentrate CP concentration may be a successful method of reducing urinary N excretion of

57 lactating dairy cattle on pasture-based systems, but further research is needed to investigate

58 long-term effects of supplementary concentrate CP content on milk production.

59

60 Key words: dairy cow, concentrate protein content, fresh grass, milk production, nitrogen

61 utilization

62

INTRODUCTION

Greenhouse gas emissions from livestock production systems, specifically ruminant, are a major source of environmental concern. With normal bovine feeding practises, a large percentage of dietary protein is inefficiently utilized leading to increased manure N outputs resulting in environmental, health (Butler, 1998) and economic implications. Excess N excretions from ruminants can be converted to many forms such as (i) ammonia, a major air pollutant, (ii) N_2O, a greenhouse gas, and (iii) nitrate, a water pollutant. The considerable variation in levels of N excretion in urine across a range of dietary treatments highlights the potential for alleviation (Castillo et al., 2000). Grasslands are the most economical feedstuff for dairy farmers in Northern and Western Europe (Peyraud and Delagarde, 2013). As controlling forage nutrient composition can prove difficult, a feasible mitigation option for improving nitrogen utilization efficiency (**NUE**) may be to reduce the CP content in concentrate feeds. This may be possible in pasture-based systems as opposed to indoor systems on silage based diets due to pasture often possessing a CP content in excess of or close proximity to 20% on DM basis (Kavanagh et al., 2003), a value considerably greater than that typically found in conserved forage. Hence, it is vital N partitioning is assessed in all commonly used farming practices to reduce pollution and maintain herd health in a cost-effective manner across the different dairy production systems. Previous studies have shown improved NUE in particular reduced urinary N excretion via reduced concentrate CP level (Castillo et al., 2000; Marini and Van Amburgh, 2005; Burke et al., 2008). However, whether improved NUE and N partitioning in addition to production responses can be achieved using low CP concentrates in a fresh grass based diet is yet to be determined.

There is also evidence of a genetic effect on N metabolism (Pareek et al., 2007; Beecher et al., 2014), although to a lesser extent than dietary CP content (Huhtanen et al., 2015). It is well documented that MUN is used as a tool to monitor feed management practice specifically excess dietary CP and has been suggested as an indicator for urinary N excretion (Jonker et al.,

89 1998; Kauffman and St-Pierre, 2001). Previous literature has found the relationship between

90 urinary N and MUN concentration may be subject to genetic influence (Kauffman and St-

91 Pierre, 2001) with significant differences found between Holstein and Jersey animals. It has

92 been speculated some of the variation may be explained by milk yield (MY) and BW (Huhtanen

93 et al., 2015) or as a result of genetic variation in urea transporters located in the kidney and

94 across the rumen epithelium, with different alleles resulting in increased or reduced activity

95 (Aguilar et al., 2012). Conversely, some trials found no evidence of a genetic effect on N

96 utilization (Zou et al., 2016) or MUN concentration (Carlsson et al., 1995). Swedish Red is a

97 high-producing breed in common use in Northern Europe which has been crossed with

98 Holsteins to improve fertility, udder health and longevity (Heins and Hansen, 2012) resulting

99 in greater projected lifetime profit and profit per cow-day than Holstein breed (Heins et al.,

100 2012). As Holstein and Swedish red represent important bovine breeds for MY and solids

101 output, a comparison between Holstein and Holstein × Swedish red crossbreds would be

102 suitable to examine the genetic and physiological effects on variation of N partitioning in dairy

103 cattle.

104 Therefore, the objective of the present study was to (i) investigate the effects of animal genetics

105 and varying concentrate CP content on production levels in combination with NUE and N

106 partitioning parameters and (ii) develop linear and multiple relationships to estimate MUN and

107 urinary N outputs for lactating dairy cows on similar diets to those offered in the present study

108 using readily available data at farm-level.

109 **MATERIALS AND METHODS**

110 All animal procedures in the present study were conducted under experimental license from

111 the Department of Health, Social Services and Public Safety of Northern Ireland in accordance

112 with the Animal (Scientific Procedures) Act (Home Office, 1986).

Experimental Design

The current study was conducted during the 2014 grazing season at Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK), using 6 pure Holstein and 6 crossbred (50:50 Holstein × Swedish Red) cows, fed fresh-cut grass and 3 differing concentrate feeds in a 3-period (25 d/period) changeover design study. Cows within each genotype were blocked into 3 groups of 2 cows, based on MY, BW and lactation stage, and were then randomly allocated to 3 dietary treatments. The mean MY, BW and DIM at the commencement of the trials were 26 ± 4.9 kg/d, 550 ± 39.9 kg and 119 ± 20.5 d, respectively. The diet treatments were a low CP concentrate (LCP, 14.1%), a medium CP concentrate (MCP, 16.1%) and a high CP concentrate (HCP, 18.1%) on a DM basis offered at 35% DMI in combination with fresh-cut perennial ryegrass offered at 65% DMI. Each experimental period consisted of: (i) an initial 18-d feed adaption phase where cows were housed as a single group with individual feed intake recorded, (ii) a 1-d adaption phase in individual stalls, and (iii) a 6-d digestibility unit phase, with daily recording of feed intake and total collection of feces, urine and milk outputs.

The LCP and HCP concentrates were formulated separately and both contained the same feed ingredients and similar chemical composition (with the exception of CP content). Subsequently the MCP concentrate was then produced by mixing LCP and HCP in a 1:1 (w/w) ratio. The ingredient and chemical compositions of LCP and HCP concentrates are presented in Tables 1 and 2, respectively. Half of the daily concentrate rations were offered at morning milking (0700) and half at afternoon milking (1500), while fresh-cut grass, harvested with a Haldrup 1500 from a single sward, was offered at 1000 each morning ad libitum. Herbage received primary cut during April 2014 and was subsequently harvested at regrowth intervals according to month (increasing from 22 to 30-d from June to September), generating grass of a similar quality to that under commercial management. Grass in the sward consisted of a three year re-seed of Aberstar, Aberzest and Alice varieties, sown in ratio of 8:5:1 respectively and paddocks

138 had not been grazed since the end of the previous grazing season (November 2013). Post-

139 harvesting fertilisation was implemented within 3-d at 35 kg N/ha. Temperature of fresh-cut

140 grass was monitored throughout the study to minimise risk of nutrient degradation by plant

141 proteases (Callis, 1995). Animals had free access to water throughout the experiment.

142 Concentrate offered was calculated for individual animals as 35% total DMI using the previous

143 7-d running average of ad libitum forage intake.

144 *Measurements*

145 Bodyweight was recorded before and after the digestibility unit phase. Daily herbage intakes

146 and refusals were recorded, sampled and analyzed for oven DM at 85°C during the 6-d

147 measurement phase at the end of each period. Fresh herbage samples were dried in an oven at

148 60°C for 72 h (Ruiz et al., 2001; Jiao et al., 2014), milled through a 0.8 mm screen and analyzed

149 for ADF, NDF, ash, gross energy (**GE**), N and water-soluble carbohydrates (**WSC**) contents

150 on a daily basis. Concentrate samples (200 g) were taken 4 times per week and dried for 48 h

151 at 100°C according to AOAC (1980; Official method 14.063). Samples were then composited,

152 milled through a 0.8 mm screen and analyzed for weekly determination of DM, ADF, NDF,

153 ash, GE, starch and N concentrations. Feces and urine outputs were weighed, recorded and

154 sampled separately as a percentage (5%) of total fecal output (by weight) and urine output (by

155 volume) for the 6-d collection phase in the digestibility units. Daily urine and fecal samples

156 were stored at 4°C after collection and 3-d samples were pooled for analysis. Samples were

157 thoroughly mixed and a representative sample was obtained for fresh analysis of N content for

158 feces and urine, according to method in Jiao et al. (2013). A sub-sample of the bulked 3-d feces

159 samples were dried at 85°C for subsequent DM, ADF, NDF and ash analysis, as described by

160 Cushnahan and Gordon (1995). To prevent ammonia volatilization from urine samples during

161 the 24 h collection, sulphuric acid solution (50% H_2SO_4) was added to the urine canisters prior

162 to collection to achieve a pH between 2.0 and 4.0 (Freudenberger et al., 1994). Milk samples

163 of 2% volume were collected twice daily, bulked for 3-d phases and frozen (-20°C) until

164 analysis. Milk samples were analyzed by Milkoscan (Foss Electric, Hilleröd, Denmark) for fat,

165 protein and lactose. Contents of MUN were measured by the QuantiChrom urea assay kit

166 (DIUR-500) after a de-proteination step (BioAssay Systems, Hayward, USA). Analysis of milk

167 GE was performed according to the method described by Jiao et al. (2013). Determination of

168 GE, N (grass and concentrate only) and ash were performed as described previously by

169 Cushnahan and Gordon (1995). For the analysis of grass, concentrate and milk concentrations

170 of GE a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Illinois, USA) was

171 used. Total N content was determined on a DM basis for grass and concentrate, and on a fresh

172 basis for feces and urine, using a Vario Max CN (Elementar, Hanau, Germany) and a Kjeltec

173 2400 analyzer (Foss Tecator AB, Höganäs, Sweden) respectively. Ash in grass, concentrate

174 and feces was determined by incineration in a muffle furnace (Vecstar, Derbyshire, UK) at

175 550°C for approximately 10 h (AOAC, 1990). Ash-corrected concentrations of ADF and NDF

176 were determined sequentially using Fibretec fiber analyzer (Foss, Denmark).The NDF was

177 assayed with a method using sodium sulphite and α-amylase, as described by Van Soest et al.

178 (1991). Total starch content of concentrate was measured using total starch assay kit

179 (Megazyme International Ireland Ltd., Wicklow, Ireland; McCleary et al., 1994). The WSC

180 content of grass was determined spectrophotometrically using anthrone in sulfuric acid

181 utilizing the Technicon Autoanalyzer (Technicon Corp., New York, NY; Thomas, 1977).

182 *Statistical Analysis*

183 Energy-corrected MY (**ECMY**) was calculated as milk energy output (MY multiplied by

184 measured milk energy concentration) divided by milk energy content in one kg of standard

185 milk (40 g/kg fat, 32 g/kg protein and 48 g/kg lactose) using the equation of Tyrrell and Reid

186 (1965). Experimental data were analyzed using Genstat statistical package (VSN International,

187 2013). All variables were analyzed using the linear mixed model methodology with REML

188 estimation (Gilmour et al., 1995). In the analysis, which was based on individual animal data,

189 cow and date (of entry to collection phase) were fitted as random effects, and genotype and

190 treatment as fixed effects. Orthogonal polynomial contrasts (linear and quadratic) were used to

191 examine treatment effect on response variables. The significance of fixed effects was assessed

192 by comparing a F Statistic against a F-distribution. Residuals showed no deviation from

193 normality. The differences between treatments, genotypes and interactions were assessed and

194 declared as non-significant, at $P > 0.05$ and significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$. A

195 REML analysis was also performed to develop a range of linear and multiple relationships to

196 estimate MUN and urine N outputs, using the method previously described by Stergiadis et al.

197 (2015). In brief, linear regression relationships were developed where the responses were MUN

198 output, MUN concentrations and urine N output and the explanatory variables were N intake,

199 dietary CP content and MUN output, respectively. A multiple linear regression was also

200 developed for the prediction of urine N output using MUN and dietary CP content as

201 explanatory variables. The potential random effects of cow and date of entry were removed in

202 all equations. The Wald statistic was used to evaluate the significance of the fixed terms. For

203 all equations, a pseudo-R^2 which describes the squared correlation of the response and the fitted

204 values, to represent the amount of variability explained was also generated.

205 **RESULTS**

206 The effect of the main factors was significant on a number of feed/nutrient intake, production

207 and NUE parameters investigated, but there was no significant interaction between cow

208 genotype and dietary treatment. Hence focus in the results and discussion sections will

209 primarily be on main treatment effects.

Diet Composition

211   The chemical composition of individual dietary components is given in Table 2. Grass NDF

212   and ADF contents both decreased and WSC contents increased from July through to

213   September; but no seasonal variation was observed for ash, CP and GE contents of grass. The

214   perennial ryegrass offered during the present experiment contained on average DM of 154 g/kg,

215   GE of 18.6 MJ/kg DM, CP of 18.2% DM and 95.4, 456, 231 and 167 g/kg DM for ash, NDF,

216   ADF and WSC respectively. Chemical composition of the 3 concentrates was very similar,

217   except for the CP content which resulted in total dietary CP levels for the LCP, MCP and HCP

218   diets of 16.9, 17.6 and 18.3% DM respectively.

219   *Feed Intake and Milk Production*

220   The effects of concentrate CP contents and cow genotype on feed intake and animal

221   characteristics and production parameters are displayed in Table 3. On average, animal diets

222   were composed of (DM basis) 67.2% fresh grass and 32.8% concentrate feed. Concentrate CP

223   level had no significant effect on voluntary feed intake and milk production and composition.

224   In contrast, cow genotype had significant effect on feed intake, animal characteristics and milk

225   production and composition parameters. We found Holstein cows had significantly higher

226   grass intake (+6.7%) and DMI (+5.4%) than crossbred cows. Holstein cows produced

227   significantly higher yields of ECM (3.6 kg/d or + 14.1%) and had significantly higher milk

228   lactose contents (+3.4%) but lower milk protein contents (-10.5%).

229   *Nitrogen Partitioning and Utilization*

230   The effects of concentrate CP contents and cow genotype on N intake, outputs and utilization

231   variables are displayed in Table 4. We observed intakes of total and digestible N increased

232   linearly with increasing concentrate CP content. Cows fed HCP diet consumed 42 g/d (total N)

233   and 37 g/d (digestible N) more than those fed LCP diets. Feeding LCP concentrates

234   significantly and linearly reduced urine N excretion compared to feeding HCP concentrates (-

235   38 g/d). We found excretion of manure N increased linearly with increasing concentrate CP

236   content (+ 40 g/d for cows offered the HCP diet in comparison to those fed the LCP diet).

237   Dietary treatment exerted no significant effect on N outputs in feces and milk, retained N and

238   a number of NUE parameters (proportion of N intake excreted in feces, urine, manure, milk,

239   and the ratio of retained to digested N). On the contrary, we observed a shift in N excretion

240   from urine to feces when expressed relative to manure N, with proportion of urine N

241   significantly decreased and proportion of feces N significantly increased when the LCP diet

242   was fed, in comparison to the HCP.

243   When compared with crossbred cows, Holstein cows had significantly higher intakes of total

244   N (+25 g/d) and digestible N (+19 g/d), while genotype had no significant effect on any NUE

245   variable.

246   *MUN Output*

247   Milk urea N output values are shown in Table 5. We observed MUN output linearly increased

248   with increasing concentrate CP content, resulting in MUN values of cows fed HCP diet being

249   on average 1.08 g/d higher than cows offered LCP diet. We also found MUN concentrations

250   declined linearly with decreasing concentrate CP content (-1.6 and -3.6 mg/dL for cows offered

251   MCP and LCP in comparison to HCP diets respectively). However, concentrate CP level had

252   no significant effect on MUN output when expressed as a proportion of total N intake or

253   digestible N intake. The effect of cow genotype on MUN excretion, concentrations or

254   proportion to total N or digestible N intakes was not significant.

255   *Estimation of MUN and Urine Nitrogen Output*

256   When linear and multiple relationships for estimating urine N output and MUN output and

257   concentration were developed, the explained variation was higher for the predictions of MUN

258   parameters (Table 6). The effect of (i) N intake and dietary CP content for the prediction of

259   MUN output and MUN concentrations respectively, and (ii) MUN and dietary CP content for

260   the prediction of urine N output, were significant according to the Wald statistic, and all

261   relations were positive. Figure 1 displays the positive relationship between urine N output (g/d)

262   and MUN output (mg/d), as shown in Eq. 3 in Table 6.

263   **DISCUSSION**

264

265   The manipulation of concentrate CP concentration is commonly used to optimize rumen

266   microbial activity and consequently milk production for grazing and confined dairy production

267   systems. Responses in NUE have been extensively evaluated in confined dairy cows offered

268   grass silage, but such information may not be accurate for grazing cows as the ensiling process

269   can considerably alter nutritive value of forage. Increases in the CP fraction A (NPN) at the

270   expense of CP fraction B (true protein), rate of proteolysis and VFA concentrations and

271   reductions in carbohydrate content occur during ensiling. In addition daily deviations in pasture

272   CP content are more pronounced in comparison to conserved forage which may also affect the

273   ruminal protein-energy balance. The present study was thus designed to evaluate the effects of

274   manipulation of concentrate CP concentration on milk production and NUE of dairy cows

275   offered fresh grass.

276

277   *Diet Composition*

278   Ryegrass utilized in the present study would be considered typical for good quality ryegrass

279   (Ministry of Agriculture, Fisheries and Food, 1992). Water-soluble carbohydrates content of

280   fresh-cut grass increased between July and September, which is possibly due to longer grass

281   regrowth intervals towards the end of the grazing season (Owens et al., 2008). Throughout the

282   present experiment, good quality ryegrass averaging 18.2% CP, 461 g/kg NDF and 162 g/kg

283   WSC, was offered. Consequently animals consumed higher than the expected levels of fresh

284   grass in the measurement periods leading to a marginally higher dietary forage proportion than

285   the designed level (67.2% vs. 65% DM basis). These two factors in combination may reduce

286   the extent of the responses between treatments for some of the parameters.

287   *Production Performance*

288   Although concentrate feed was designed to be 35% DMI, the actual concentrate intake was

289   32.8% of total DMI due to the higher grass DMI (14.0 kg/d) in the digestibility units than in

290   the housing cubicles (12.6 kg/d). The concentrate feed proportion was chosen to be

291   representative of commercial practice in the UK (Ferris, 2007) and to be of sufficient level to

292   achieve significant differences in total dietary CP content across treatments. The results from

293   the present study implied that feeding a concentrate of 14.1% CP when good quality perennial

294   ryegrass is grazed may sustain MY and milk quality in pasture-based systems. Previous studies

295   found that offering concentrate of 15% CP to supplement grazing was associated with a

296   decrease in MY of 2.9 kg/d when compared to feeding a 19% CP concentrate (Whelan et al.,

297   2012), while low-protein diets (14-16% CP) also decreased production and tended to decrease

298   milk protein content in corn and grass-clover silage based diets (Alstrup et al., 2014). More

299   recent studies have shown that concentrates with CP content as low as 14% might be fed to

300   dairy cows without negative implications on milk production (Sinclair et al., 2014). There is a

301   range of diet and animal factors which could influence the effect of concentrate CP levels on

302   milk production of grazing cows, such as milk production potential, stage of lactation and

303   forage quality (de Oliveira et al., 2010; Moran, 2005). In the present study, high milk protein

304   content observed across all treatments is generally considered indicative of a high energy diet

305   (Broderick, 2003), which may have been a result of the quality of grass offered. The results of

306   the present study indicate that dairy cows grazing good quality pasture can be offered low CP

307   concentrates resulting in a total dietary CP content of 16.9% DM with no negative effect on

308   feed intake or milk production.

309   *Nitrogen Partitioning and Utilization*

310   In the present study we observed that increasing concentrate CP levels in a predominantly fresh

311   ryegrass diet supplemented with concentrate increased total intakes of N and digestible N.

312   Feces N values were less variable (144-246 g/d) than urine N values (112-302 g/d) and this

313   result is similar to those observed in previous literature (Ruiz et al., 2001; Lee et al., 2009;

314   Kebreab et al., 2010). In the present study, the non-significant effect of concentrate CP

315   concentration on feces N excretion was partially due to similar DMI, an influential factor in

316   fecal N output, between treatments. It may also indicate that the ammonia-N supply from the

317   LCP diet was enough to meet the requirement of rumen microbial growth, and the excess

318   supply of degradable N in the MCP or HCP diet was excreted in urine as urea. Indeed, we

319   found that urine N outputs were significantly higher on the HCP diet. In comparison to the LCP

320   diet, the additional N intake in the HCP diet (42 g/d) was almost entirely excreted in urine (38

321   g/d), which displays the sensitivity of the correlation between urinary N excretion and

322   supplementary concentrate N. Broderick and Reynal (2009) observed an increase of 96 (g/d)

323   in urine N excretion associated with an increase in dietary CP intake from 15.1 to 18.4% which

324   was attributed mostly to an increase of urinary urea N. Furthermore, findings from a meta-

325   analysis on growing cattle offered CP supplement indicates that up to 90% of incremental N

326   intake, which exceeds the requirement of rumen microbial activity, is partitioned into urine

327   (Huuskonen et al., 2014). This is in agreement with results from the present study, in which 38

328   (g/d) out of the 42 (g/d) incremental CP was excreted as urinary N, a figure which is close to

329   the predicted value of 37.8 (g/d). Our results showed that feeding low protein concentrates

330   (14.1% CP) may serve as a mitigation strategy to reduce urine N output for cows consuming

331   fresh-cut grass and concentrate diets, thus reducing environmental footprint (N_2O emissions,

332 nitrate and ammonia pollution) from pasture-based systems. Reducing CP concentration of

333 ruminant diets has been recommended to be the most effective method to reduce N_2O emissions

334 from dairy farms; it was estimated to cause a 7-fold improvement on mitigation efficiency

335 compared with alleviating N_2O emissions through manure storage and management (Marini

336 and Van Amburgh, 2005).

337 Our work showed that feeding low CP concentrates in a fresh-cut grass based diet could shift

338 N excretion from urine to feces when expressed as a proportion of manure N output. Regarding

339 environmental concerns associated with grazing livestock, the shift of N excretion is considered

340 desirable because N in feces is less volatile than in urine and may be converted to ammonia

341 and N_2O at a slower rate (van der Weerden et al., 2011). This is due to fecal N being for the

342 most part organically bound N composing mainly of microbial and endogenous N with some

343 undigested feed N (Ellis et al., 2011), which must first undergo mineralization whereas urinary

344 N is primarily in the form of urea, which is rapidly hydrolyzed to ammonium (Beukes et al.,

345 2011).

346 Pure and crossbred Holstein cows showed similar NUE, thus being in line with Huhtanen et al.

347 (2008), who suggested dietary components may have a greater influence on milk protein N

348 efficiency than level of production, though it too plays a role.

349

350 *Development of Regression Equations Estimating Urine Nitrogen Excretion for Grazing*

351 *Dairy Cows*

352 Previous work has shown MUN concentration and urine N output are positively associated

353 with dietary CP level, which is most likely a result of increased BUN (Jonker and Kohn, 2001;

354 Huhtanen et al., 2015); therefore MUN has been suggested as a non-invasive indicator for urine

355 N excretion (Jonker and Kohn, 2001). MUN concentration is highly related to dietary CP

356 content and measurement is common practice in the dairy industry. However differences exist

357   between regression equations presented in the current study (Eq. 2, table 6) and in previous

358   studies in which prediction equations were developed with animals fed diets based on

359   conserved forage (Nousiainen et al., 2004; Spek et al., 2013). These differences may be a result

360   of a combination of factors such as animal diets, stage of lactation, genetic merit and analytical

361   techniques. Regression equations developed in the current study showed that urinary N output

362   is positively related to MUN output and dietary CP content, which can be used as readily

363   available predictors in practice. Positive relations between urine N output and MUN

364   concentration have been found previously and explained by the small neutral nature of a urea

365   molecule allowing MUN to equilibrate with BUN via diffusion into and out of the mammary

366   gland (Jonker and Kohn, 2001). Spek et al. (2013) also found urine N outputs' best sole

367   predictors were feed CP content and MUN content. The fact that addition of dietary CP content

368   to MUN content as predictors of urine N output only slightly improved R^2 in the present study,

369   implies that in practice the use of dietary CP content can be omitted without substantial

370   compromise on the prediction accuracy, when only routinely collected at farm-level MUN

371   content data is available. This allows for readily available, relatively reliable and non-

372   expensive estimations of urine N excretions in pasture-based systems. The model we developed

373   predicts urine N excretion to increase by 14.2 g/d with an increase of 1 g in MUN secreted,

374   within the range of MUN values measured in the current study.

375   Mitigating NUE in dairy cattle requires reducing urinary N output but without compromising,

376   and preferably increasing, milk protein N yields. As the majority of milk N is presented as

377   protein and protein yields are dependent on energy supplies, optimising dietary energy supply

378   while offering minimal levels of dietary CP, without reducing productivity and milk solid

379   concentrations, would show high potential to mitigate N outputs in pasture-based systems.

CONCLUSION

The current results suggest urine N excretion from grazing lactating dairy animals can be alleviated by offering a concentrate with a CP level of 14.1% DM when good quality perennial ryegrass is consumed. This practice can also reduce urine N as a proportion of total N excretion, which is considered environmentally desirable as it decreases volatilization of nitrogenous compounds including N_2O emissions. Feeding the low CP concentrate did not affect voluntary grass intake, total intake or production traits, implying that the proposed mitigation strategy should not compromise economic performance of the dairy farm, although sustainability of production would have to be confirmed on a long-term study. The linear and multiple relationships developed in the current study may assist in the estimation of urine N output from animals fed fresh grass and concentrate diets, using readily available data at commercial level, such as MUN data either in conjunction with feed chemical composition or not.

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FIGURE CAPTIONS

Figure 1. Relationship between MUN and urine N output for lactating dairy cows on diets of 2:1 fresh grass:concentrate ratio, as presented in Eq. 3 in Table 6.

546   **TABLES**

Table 1. Concentrate ingredient composition (g/kg DM)

	LCP[1]	HCP[1]
Corn	246	220
Wheat feed	140	135
Corn gluten	140	135
Soya hulls	140	135
Palm kernel exp.	110	110
Sugar beet pulp	45	0
Sunflower kernel	66	60
Soyabean meal	0	80
Rapeseed extract	0	27
Molaferm	70	50
Pure palm oil	7	7
Limestone flour	14	19
Salt	8.5	9.4
Calcined magnesite	8.8	8.6
Trace elements and vitamins[2]	4.0	4.0

[1]LCP = low CP concentrate (14.1%, DM basis); HCP = high CP concentrate (18.1%, DM basis).

[2]Trace elements and vitamins consisted of: 25 IU / kg of vitamin E, 5 mg / kg of I, 0.6 mg / kg of Se, 30 mg / kg of Cu, 50 mg / kg of Mn, and 100 mg / kg of Zn. 9,000 IU / kg vitamin A, 2,000 IU / kg vitamin D3.

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Table 2. Chemical composition (g/kg DM, unless otherwise stated) of dietary components used in the present experiment

	Grass			Concentrate	
	July	August	September	LCP[1]	HCP[1]
DM (g/kg)	154	147	161	898	898
Ash	100	94	94	89	91
CP	18.8	17.8	18.3	14.1	18.1
Gross energy (MJ/kg DM)	18.7	18.7	18.4	18.0	18.1
NDF	490	454	440	369	369
ADF	239	234	222	189	187
Starch				232	211
Water-soluble carbohydrates	130	171	184		

[1]LCP = low CP concentrate (14.1%, DM basis); HCP = high CP concentrate (18.1%, DM basis).

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Table 3. Effect of concentrate CP level and cow genotype on animal, feed intake and production parameters

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred[2]	SEM	P-value
Animal characteristics										
BCS	2.37	2.30	2.34	0.038	0.46	0.22	2.29	2.39	0.044	0.12
Bodyweight, kg	579	582	571	15.2	0.32	0.43	583	573	20.8	0.74
Feed intake, kg DM/d										
Grass intake	13.8	14.1	14.1	0.37	0.30	0.24	14.4	13.5	0.32	0.009
Concentrate intake	7.0	7.0	6.9	0.16	0.61	0.40	7.0	6.9	0.16	0.30
Total DM intake	20.7	21.0	21.0	0.47	0.57	0.21	21.5	20.4	0.43	0.019
Production										
Milk yield, kg/d	25.8	26.5	26.7	1.36	0.55	0.93	28.5	24.2	1.49	0.070
Energy corrected milk yield, kg/d	27.1	27.1	27.6	1.00	0.56	0.62	29.1	25.5	0.81	0.007
Milk fat content, g/kg	42.0	41.5	41.8	1.48	0.86	0.36	40.5	43.0	2.01	0.39
Milk protein content, g/kg	36.1	36.2	36.4	1.09	0.99	0.88	34.2	38.2	1.18	0.030
Milk lactose content, g/kg	44.7	45.0	45.0	0.39	0.091	0.82	45.7	44.2	0.38	0.016

[1]Probability of a linear (L) or quadratic (Q) effect of concentrate CP level in the diet.
[2]Crossbred cows were crosses between Holstein and Swedish Red.

Table 4. Effect of concentrate CP level and cow genotype on N intake and output and N utilization efficiency parameters

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred[2]	SEM	P-value
N intake/output, g/d										
Total dietary N intake	543	572	585	16.6	<0.001	0.17	579	554	12.6	0.039
Digestible N intake	358	382	395	13.7	<0.001	0.038	388	369	12.7	0.044
Feces N	187	187	188	6.4	0.86	0.81	190	185	6.0	0.55
Urine N	193	208	231	10.8	0.004	0.63	220	202	10.6	0.25
Manure N	380	394	420	12.8	0.017	0.65	409	387	12.5	0.24
Milk total N	149	154	156	5.5	0.41	0.89	157	149	6.2	0.42
Milk protein N	144	149	150	5.2	0.54	0.91	151	145	5.6	0.074
Retained N	15.4	22.3	7.9	15.54	0.61	0.17	14	17	15.6	0.86
N utilization, g/g										
Feces N /N intake	0.345	0.332	0.327	0.0118	0.088	0.67	0.334	0.336	0.0120	0.87
Urine N /N intake	0.356	0.363	0.402	0.0210	0.054	0.46	0.380	0.367	0.0214	0.65
Manure N /N intake	0.701	0.694	0.727	0.0253	0.29	0.35	0.711	0.703	0.0270	0.81
Milk total N /N intake	0.274	0.271	0.270	0.0085	0.63	0.88	0.272	0.271	0.0076	0.88
Milk protein N /N intake	0.265	0.262	0.260	0.0080	0.51	0.90	0.265	0.262	0.0074	0.78
Retained N /N intake	0.024	0.036	0.004	0.0288	0.43	0.12	0.017	0.026	0.0289	0.80
Feces N /Manure N	0.497	0.478	0.452	0.0157	0.007	0.77	0.469	0.481	0.0158	0.54
Urine N /Manure N	0.503	0.522	0.548	0.0157	0.007	0.77	0.531	0.519	0.0158	0.54

[1]Probability of a linear (L) or quadratic (Q) effect of concentrate CP level in the diet.
[2] Crossbred cows were crosses between Holstein and Swedish Red.

Table 5. Effect of concentrate CP level and cow genotype on MUN contents, excretion and ratios to N intake

| | Concentrate CP level | | | | P-value[1] | | Cow genotype | | | |
	Low	Medium	High	SEM	L	Q	Holstein	Crossbred[2]	SEM	P-value
MUN, g/d	4.85	5.35	5.93	0.476	0.016	0.86	5.82	4.89	0.473	0.13
MUN content, mg/dL	18.9	20.9	22.5	1.23	<0.001	0.75	20.7	20.7	1.26	0.96
MUN /N intake	0.0090	0.0094	0.0103	0.00087	0.093	0.41	0.0101	0.0090	0.00087	0.20
MUN /Digestible N intake	0.0141	0.0141	0.0157	0.00155	0.29	0.23	0.0155	0.0137	0.00156	0.26

[1]Probability of a linear (L) or quadratic (Q) effect of concentrate CP level in the diet.

[2] Crossbred cows were crosses between Holstein and Swedish Red.

Table 6. Regression models for the prediction of MUN and urine N excreta from lactating dairy cows.

Equation no.		Equations[1]	R^2
1	MUN output, g/d	$= -3.1_{(2.69)} + 0.015_{(0.0047)}$ N intake (g/d)	0.946
2	MUN content, mg/dL	$= -31.3_{(8.64)} + 0.295_{(0.0486)}$ diet CP content (g/kg DM)	0.975
3	Urine N output, g/d	$= 139.1_{(18.07)} + 0.0142_{(0.00316)}$ MUN (mg/d)	0.792
4	Urine N output, g/d	$= -144.4_{(72.32)} + 0.010_{(0.0028)}$ MUN (mg/d) $+ 1.74_{(0.432)}$ diet CP content (g/kg)	0.802

R^2 = pseudo correlation coefficient.

[1] Values in subscript parentheses represent standard errors. The effects of all explanatory variables were significant according to the Wald statistic (Fpr < 0.05). The potential random effects of cow and date were removed for all predicted variables.
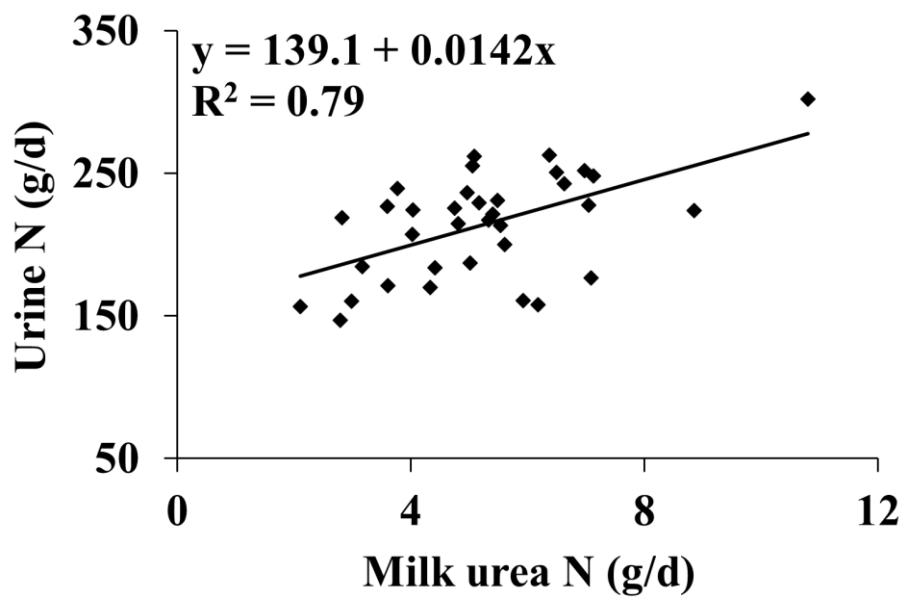
1   Figures
2

$y = 139.1 + 0.0142x$

$R^2 = 0.79$

Urine N (g/d)

Milk urea N (g/d)

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4
5   **Figure 1.**