

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.**

17

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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

63 INTRODUCTION

64 Greenhouse gas emissions from livestock production systems, specifically ruminant, are a
65 major source of environmental concern. With normal bovine feeding practises, a large
66 percentage of dietary protein is inefficiently utilized leading to increased manure N outputs
67 resulting in environmental, health (Butler, 1998) and economic implications. Excess N
68 excretions from ruminants can be converted to many forms such as (i) ammonia, a major air
69 pollutant, (ii) N₂O, a greenhouse gas, and (iii) nitrate, a water pollutant. The considerable
70 variation in levels of N excretion in urine across a range of dietary treatments highlights the
71 potential for alleviation (Castillo et al., 2000). Grasslands are the most economical feedstuff
72 for dairy farmers in Northern and Western Europe (Peyraud and Delagarde, 2013). As
73 controlling forage nutrient composition can prove difficult, a feasible mitigation option for
74 improving nitrogen utilization efficiency (NUE) may be to reduce the CP content in
75 concentrate feeds. This may be possible in pasture-based systems as opposed to indoor systems
76 on silage based diets due to pasture often possessing a CP content in excess of or close
77 proximity to 20% on DM basis (Kavanagh et al., 2003), a value considerably greater than that
78 typically found in conserved forage. Hence, it is vital N partitioning is assessed in all commonly
79 used farming practices to reduce pollution and maintain herd health in a cost-effective manner
80 across the different dairy production systems. Previous studies have shown improved NUE in
81 particular reduced urinary N excretion via reduced concentrate CP level (Castillo et al., 2000;
82 Marini and Van Amburgh, 2005; Burke et al., 2008). However, whether improved NUE and N
83 partitioning in addition to production responses can be achieved using low CP concentrates in
84 a fresh grass based diet is yet to be determined.

85 There is also evidence of a genetic effect on N metabolism (Pareek et al., 2007; Beecher et al.,
86 2014), although to a lesser extent than dietary CP content (Huhtanen et al., 2015). It is well
87 documented that MUN is used as a tool to monitor feed management practice specifically
88 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 \times 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).

113 ***Experimental Design***

114 The current study was conducted during the 2014 grazing season at Agri-Food and Biosciences
115 Institute (Hillsborough, Northern Ireland, UK), using 6 pure Holstein and 6 crossbred (50:50
116 Holstein × Swedish Red) cows, fed fresh-cut grass and 3 differing concentrate feeds in a 3-
117 period (25 d/period) changeover design study. Cows within each genotype were blocked into
118 3 groups of 2 cows, based on MY, BW and lactation stage, and were then randomly allocated
119 to 3 dietary treatments. The mean MY, BW and DIM at the commencement of the trials were
120 26 ± 4.9 kg/d, 550 ± 39.9 kg and 119 ± 20.5 d, respectively. The diet treatments were a low CP
121 concentrate (LCP, 14.1%), a medium CP concentrate (MCP, 16.1%) and a high CP concentrate
122 (HCP, 18.1%) on a DM basis offered at 35% DMI in combination with fresh-cut perennial
123 ryegrass offered at 65% DMI. Each experimental period consisted of: (i) an initial 18-d feed
124 adaption phase where cows were housed as a single group with individual feed intake recorded,
125 (ii) a 1-d adaption phase in individual stalls, and (iii) a 6-d digestibility unit phase, with daily
126 recording of feed intake and total collection of feces, urine and milk outputs.

127 The LCP and HCP concentrates were formulated separately and both contained the same feed
128 ingredients and similar chemical composition (with the exception of CP content). Subsequently
129 the MCP concentrate was then produced by mixing LCP and HCP in a 1:1 (w/w) ratio. The
130 ingredient and chemical compositions of LCP and HCP concentrates are presented in Tables 1
131 and 2, respectively. Half of the daily concentrate rations were offered at morning milking
132 (0700) and half at afternoon milking (1500), while fresh-cut grass, harvested with a Haldrup
133 1500 from a single sward, was offered at 1000 each morning ad libitum. Herbage received
134 primary cut during April 2014 and was subsequently harvested at regrowth intervals according
135 to month (increasing from 22 to 30-d from June to September), generating grass of a similar
136 quality to that under commercial management. Grass in the sward consisted of a three year re-
137 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% H₂SO₄) 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 Kjeltac
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.

210 ***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 (N₂O 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 N₂O emissions
334 from dairy farms; it was estimated to cause a 7-fold improvement on mitigation efficiency
335 compared with alleviating N₂O 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 N₂O 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.

380 **CONCLUSION**

381 The current results suggest urine N excretion from grazing lactating dairy animals can be
382 alleviated by offering a concentrate with a CP level of 14.1% DM when good quality perennial
383 ryegrass is consumed. This practice can also reduce urine N as a proportion of total N excretion,
384 which is considered environmentally desirable as it decreases volatilization of nitrogenous
385 compounds including N₂O emissions. Feeding the low CP concentrate did not affect voluntary
386 grass intake, total intake or production traits, implying that the proposed mitigation strategy
387 should not compromise economic performance of the dairy farm, although sustainability of
388 production would have to be confirmed on a long-term study. The linear and multiple
389 relationships developed in the current study may assist in the estimation of urine N output from
390 animals fed fresh grass and concentrate diets, using readily available data at commercial level,
391 such as MUN data either in conjunction with feed chemical composition or not.

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543 **FIGURE CAPTIONS**

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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.

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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

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

²Trace 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.

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		

¹LCP = 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

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

²Crossbred 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

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

²Crossbred 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

¹Probability 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.

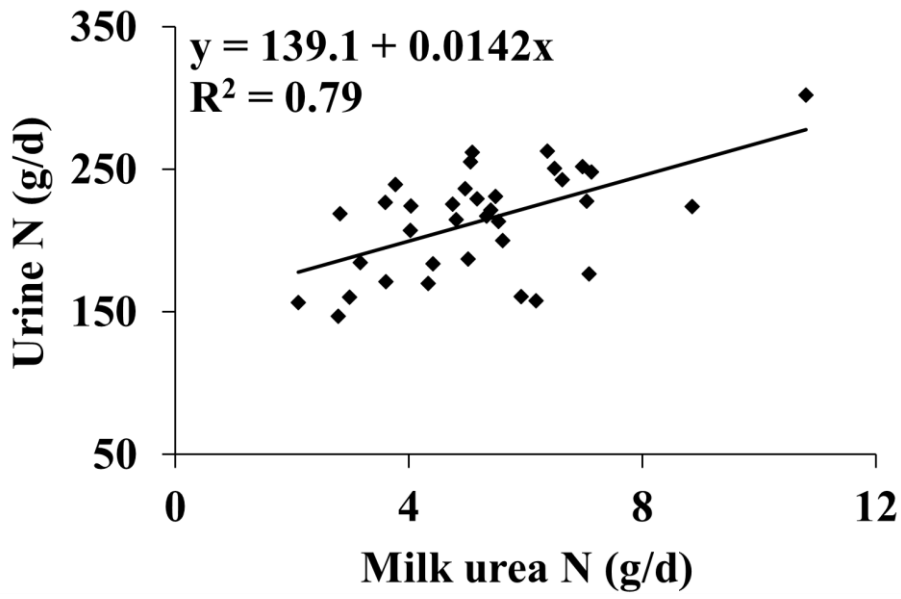
Equation no.	Equations ¹	R ²
1	MUN output, g/d = $-3.1_{(2.69)} + 0.015_{(0.0047)}$ N intake (g/d)	0.946
2	MUN content, mg/dL = $-31.3_{(8.64)} + 0.295_{(0.0486)}$ diet CP content (g/kg DM)	0.975
3	Urine N output, g/d = $139.1_{(18.07)} + 0.0142_{(0.00316)}$ MUN (mg/d)	0.792
4	Urine N output, g/d = $-144.4_{(72.32)}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

R² = 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

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5 Figure 1.