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1 **Eutrophication risk arising from dairy cattle intensive rearing systems and assessment of the**
2 **potential effect of mitigation strategies**

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7

8 **ABSTRACT**

9 Nitrogen (N) and phosphorus (P) are fundamental nutrients in dairy cattle nutrition, but the majority
10 of N and P fed to dairy cattle are excreted in urine and faeces, which may cause water
11 eutrophication. The aim of the current study was to assess the eutrophication risk of dairy
12 production in order to evaluate potential mitigation strategies. A group of 16 dairy farms, following
13 an intensive rearing system and located in the northwest of Italy, was involved in the study. Data
14 were collected for general farm characteristics, diet compositions, feed samples, livestock
15 productive and reproductive performances as well as information on management to evaluate the
16 diet N and P feed content, manure N and P content and apparent N and P utilization efficiency at
17 herd level and for each category of reared animal (*i.e.*, lactating cows, dry cows, heifers, and
18 calves). In order to properly assess the environmental risk, the N release losses into the atmosphere,
19 prevalently as ammonia, were considered and the different forms of released N and P were
20 converted into phosphate equivalent (PO₄). On the basis of the characteristics of each farm, some
21 common dietary manipulation mitigation strategies, as adoption of precision feeding and reduction
22 of proteins content of the diet, were proposed to evaluate their potential reduction in excreted
23 nutrients. Feed analysis results showed interesting and notable differences from previously reported
24 N and P contents in diets for dairy cows. The N balance conducted for these farms showed
25 relatively good fitness of the diet for lactating cows, with only a slight deficit in crude protein
26 supplementation (-2%), while diets for dry cows were slightly more unbalanced for N (+17%). The

27 P balance calculations revealed high excess of P in the diets of the farms in this study, especially for
28 heifers (+106%). The highest and the lowest levels of N- and P-use efficiency were found for cows
29 (20 and 28%, respectively) and for young heifers and calves (0-12 months; 7 and 11% N- and P-use
30 efficiency respectively). It is possible to reduce N and P losses by levelling out this unbalance and
31 in addition, in particular for N, by generally reducing the diet crude protein content by at least 1%.
32 The estimated reduction in PO₄ by dietary manipulation was assumed to decrease the environmental
33 eutrophication risk by 17%. On the basis of these results, and considering the number of the dairy
34 cattle reared in the northern Italy, it is possible to estimate that the potential reduction in N and P
35 excretion by dietary manipulation could be equivalent to 5693.5 metric T y⁻¹ or 1 metric T (km²)⁻¹
36 of Utilized Agricultural Area per year of PO₄.

37

38 **Key words:** nitrogen; ammonia; phosphorus; nutrient excretion; phosphate equivalent

39

40

41 **1. Introduction**

42 Water eutrophication is a complex phenomenon that involves several physiochemical and biological
43 factors, but which begins from anthropogenic nutrient over-enrichment, particularly of nitrate and
44 phosphate (Shen *et al.*, 2013). Nutrient over-loading is not a risk in itself, *i.e.* nitrogen (N) and
45 phosphorus (P) are not toxic for the environment, but it over-stimulates primary production of
46 microbes and plant species in water, which changes the ecology of the system, and creates a cascade
47 of events that present environmental risks to ecosystems and water quality. Soluble and particulate-
48 associated P in runoff largely determines the degree eutrophication in lakes, streams and other
49 freshwater systems (Marchetti, 1994; Withers and Hodgkinson, 2009). In estuaries and marine
50 coastal ecosystems, N is most often the primary cause of eutrophication (Marchetti, 1994). In some
51 cases, the main problems are associated with organic fertilizers that are produced in intensive
52 livestock production systems (Eckolm *et al.*, 2005), a phenomenon which has been the subject of

53 European and North American legislation and control for several years (Ongley, 1996). The
54 European Union, after the regulation on N environmental release (Directive 91/676 EEC), in the
55 next years will rule on other pollutants, especially those, such as P, which have effects on the
56 eutrophication of rivers and seas and which can also have consequences on the public health
57 (Meschy and Ramirez-Perez, 2005). Nutrients recycling and efficiency (N and P) are regulated by
58 other European environmental policies, such as the Water Framework Directive (2000/60 EC) and,
59 indirectly, by other EU level strategic policy frameworks, such as the Raw Materials Initiative (EU
60 Council, 2017).

61 In Italy, the problem of water quality is particularly acute in intensive livestock production areas,
62 such as in the northern part of the country where the production of slurries and manure often
63 exceeds the capacity of the land to assimilate these wastes. Among the reared species, dairy cattle
64 represent one of the main livestock sectors, above all in some areas such as the North-West part of
65 Italy (Piedmont region). Here, approximately 10% of the national livestock was raised, equivalent
66 to about 1 million LUs (livestock units), and census data report the presence of approximately
67 580,000 LUs cattle, about 300,000 LUs pigs, about 100,000 LUs poultry and about 20,000 LUs of
68 others species, especially sheep (ISTAT, 2010). Because of the high presence of livestock heads,
69 and hence of the high number of animals per farm surface unit, and because of the kind of manure
70 produced (slurry), these cattle herds could represent an environmental problem and an
71 eutrophication risk, and could also affect agricultural water resources. Presently 54% of the
72 Piedmont plain agricultural area is designated as vulnerable to nitrate and since 2011 the EU
73 Commission (Decision 2011/721/EU) allows the farms to get derogations to go beyond 170 kg of
74 nitrogen limit, under strict conditions. At that time, among Piedmonts livestock, the dairy cattle
75 farms were the most problematic one in this area, with 266 farms exceeding the limit of 170 kg N
76 ha⁻¹, including 88 exceeding the limit of 250 kg N ha⁻¹ admitted with the derogation (Anagrafe
77 Agricola Regione Piemonte, 2011). Italy, with regard to Lombardia and Piemonte Region, have
78 again request derogation to EU Commission, granted with Decision 2016/1040/EU.

79 For these reasons, a study was carried out on dairy cattle farms in a homogeneous area (western part
80 of the Po Plain: between $44^{\circ}08'33''$ and $46^{\circ}08'13''$ N, $6^{\circ}70'42''$ $8^{\circ}23'02''$ E; surface: 13,722 km²)
81 to collect data from a representative sample of herds. Holstein breed is prevalent in the considered
82 area followed by Brown Swiss. Typically the regional farms adopt intensive rearing systems, with
83 cows housed in free-standing cubicle without grazing. The herd size is usually over 200 heads,
84 including both lactating cows (usually over 100), dry cows and replacing animals (AIA, 2016) and
85 diets are based on corn silage, leguminous and cereals meals and other concentrated feed. The
86 collected data pertain to the general characteristics of the farms, the livestock productive and
87 reproductive performances and the managerial choices so that the real N and P feed content, the
88 actual excretion level and apparent utilization efficiency of these nutrients could be evaluated at a
89 herd level and for different animal categories. In fact, the aim of this work has been to evaluate the
90 nutrient excretion and utilization efficiency not only of cows but also of the other most important
91 cattle categories reared on dairy farms, such heifers and young females, which are often not
92 considered in evaluations of this kind.

93 The objectives of this study were: a) to evaluate the nutrients surplus and to suggest the best
94 practise among diet manipulation and management solutions that best fit the farms characteristics,
95 that could be adopted in the future to reduce N and P release into the environment; b) to estimate
96 the potential effect that these strategies could have, at a regional level, on the reduction of the
97 eutrophication risk.

98

99 **2. Materials and methods**

100 The survey involved 16 commercial dairy farms, visited twice during spring (March-June), that rear
101 Italian Holstein Friesian or Brown Swiss cattle (3,484 animals and 1,722.5 metric T of live weight)
102 spread over a homogeneous area (North-West plain of Italy, the Cuneo and Torino provinces, as
103 previously detailed), which were selected on the basis of their representativeness, for breed, rearing
104 system, number of lactating cows and mean milk production per cow of the prevalent herds in the

105 area. In all farms, cows and heifers were kept in permanent confinement without pasture, the
106 replacement animals were reared in group in pen and all the diets unchanged throughout the year.
107 The number of total cows (TC), lactating cows (LC), dry cows (DC), heifers (H; 12 month-age to
108 first parity), young heifers (YH; 6-12 months) and calves (CA; 0-6 months) were recorded for each
109 of the 16 farms. The diet composition of each animal group (*i.e.*, LC, DC, H, YH and CA) for each
110 herd, deriving from management choice, were also recorded, as well as the productive and
111 reproductive indices. The considered productive indices were: feed consumption (kg d^{-1}), mean live
112 weight (LW; kg) and production of the animals: weight gains (kg y^{-1}), number ($n \text{ y}^{-1}$) and weight
113 (kg) of born calves, milk production (kg d^{-1} and kg y^{-1}) and its qualitative parameters (fat, %;
114 protein, %; casein, %; urea, mg L^{-1} ; somatic cells, $n \text{ mL}^{-1}$; bacterial count, CFU mL^{-1}). Specifically,
115 mean live weight was the weight used to prepare the diet on the basis of animals' requirements. The
116 weight gains, that affect the N and P balance less than 1%, were calculated as LW gain per year (kg
117 y^{-1}) obtained by dividing the difference between the real LW at the end of the productive period (the
118 cows were weighted when sold at the end of their career) and the estimated LW at the beginning of
119 the first lactation by the productive period. The collected reproductive indices were: calving interval
120 (d), calving-conception period (d), number of services per conception (n), age at first calving
121 (months), number of calving per year ($n \text{ y}^{-1}$) and fertility rate (%). The productive and reproductive
122 indices were useful to evaluate the possible effects of dietary N and P consumption on cattle
123 performance and statistical correlations were studied between the N and P concentrations in the diet
124 and the productive and reproductive parameters.

125 In each farm, all feeds were individually sampled two times during the trial to determine their
126 nutrient contents. To evaluate the true nutrients provision with the TMR diet, samples were also
127 collected from the feed bunk to determine N and P content used as input in the mass balance. All
128 samples were analysed in the laboratory of the Department of Agriculture, Forestry and Food
129 Science of the University of Torino to determine the proximate composition of the diets according
130 to AOAC International (2006): preparation of analytical sample (method 950.02); dry matter (DM)

131 content (method 934.01); total ash (method 942.05); crude protein (CP) content (method 984.13);
132 ether extract (EE) content (method 2003.05); neutral detergent fibre (NDF) content (method
133 2002.04); acid detergent fibre (ADF); acid detergent lignin (ADL) content (method 973.18) and P
134 content (method 965.17).

135 Mean total N and P feed contents of the feed samples bulked across farms were compared with
136 literature data collected from several bibliographic references (INRA, NRC and other authors) by
137 Cevolani (2005) to establish the possible advantage (avoiding excesses or deficit) derivable from
138 the correct knowledge of the feed characteristics.

139 The measured total N and P contents in the diets of each farm were compared to those of the animal
140 requirements determined according to the National Research Council (2001) recommendations,
141 based on animal empty body weight, daily body gain, equivalent shrunk body weight, dry matter
142 consumption, milk production, protein milk content and weight of the conceptus, in order to
143 evaluate their potential deficit or surplus in the diets.

144 In order to determine N and P excretion in faeces and urine in addition to the single categories of
145 animals, the estimation also referred to a herd level, *i.e.* calculated on the basis of replacement units
146 (RU), which considers how many calves and heifers per cow are reared on average on each farm.

147 Considering the difficulty to collect representative samples of faeces and urine in commercial farms
148 to analyse for total N and P content, nutrient excretion was calculated by means of the balance
149 method, following the procedure adopted by the EU Commission to estimate N excretion
150 (ERM/AB-DLO, 2002) that is: nutrient intake minus nutrient retention in animal products (milk,
151 meat, calves). The method does not require to calculate the digestibility of each single nutrient (*i.e.*,
152 N and P). Moreover, the mass balance method for N has been chosen because it is used by public
153 authorities at local scale to verify the N excretions and surpluses at farm level and because it is not
154 affected by digestibility of N source.

155 The actual total N and P intakes were calculated on the basis of the feed analysis, whereas the
156 retention, excretion and utilization efficiency were calculated on the basis of productive and

157 reproductive indices. In other words, according to the ERM/AB-DLO (2002) recommendations, the
158 retention per animal weight gain and calf production were estimated to calculate N excretion
159 considering an N content of 25.0 and 29.5 g kg⁻¹ of LW respectively. Instead, the N retention in
160 milk production was determined on the basis of the actual CP content of the milk divided by 6.38.
161 The P retention in weight gain, calf and milk was estimated considering a P content of 7.5, 6.0 and
162 0.95 g kg⁻¹, respectively, of LW or milk production according to the Food Standard Agency (FSA,
163 2002) recommendations.

164 Nutrient utilization efficiency was calculated as the ratio between N and P retention and intake. In
165 order to evaluate annual *per capita* nutrient use efficiency, the LC and DC group data were
166 processed to obtain the annual mean values of TC. Further clarifications are necessary to establish
167 the nutrient utilization, since N and P absorption efficiency indicates the retention as a fraction of
168 the sum of N and P entering the digestive tract (N or P absorption / [N or P intake + N or P
169 secretion]) where N and P absorption and secretion are calculated from the quota of exogenous and
170 endogenous faecal N and P (Pfeffer *et al.*, 2005). These quotas can be extremely variable for P, as
171 reported by Kleiber *et al.* (1951) who found a wide range of endogenous faecal P (43-70%).
172 Considering that, in this study, the faeces were not collected or analysed for their N and P contents,
173 but the absorbed N and P were calculated on the basis of the cattle productions (increase in LW,
174 milk and calf production for TC, increase in LW and calf production for H and increase in LW for
175 YH and CA) and of their mean N and P concentration, calculating the nutrient efficiency as (N or P
176 retention on N or P intake), it was decided to name this ratio “apparent efficiency”.

177 The excreted nutrients will be spread on fields , and, in order to evaluate their supplies, N and P
178 contents in the manure were converted to nitrate (NO₃) and phosphorus pentoxide (P₂O₅),
179 respectively, on the basis of their stoichiometric coefficient of conversion (4.427 and 2.2915) to the
180 respective molecular weight.

181 In order to properly assess the environmental risk related to the level of N and P with potential to
182 contaminate soil and water, the N losses due to volatilization into the atmosphere during the waste

183 removal, storage and spreading phases, prevalently as ammonia (NH₃), were considered as
184 proposed by ERM/AB-DLO (2002), and adopted for the specific Italian waste management systems
185 as equal to 28% of excreted N , as stated by the Committee of the Interregional project on N
186 excretion from livestock for the waste management systems in Italy (Xiccato *et al.*, 2005). It was
187 decided not to consider the oxidation of NH₃ to N₂O or NO_x, the latter having a different
188 eutrophication potential (*e.g.* NO_x has an eutrophication potential of 37% of that of NH₃), because
189 they are difficult to quantify exactly and are negligible in quantity (*e.g.* N₂O is about 0.01% of the
190 NH₃ produced, as deduced from IPPC reports; IPPC, 2014). The estimated N, NH₃ and P losses
191 were converted to phosphate equivalent (PO₄; whose eutrophication potential is 1) using the
192 equivalence factors suggested in the CML method (proposed by the Centre of Environmental
193 Sciences, University of Leiden in 1992, and updated in 2000), which is generally used in Life Cycle
194 Assessments (LCA). The reduction in the PO₄ potential eutrophication obtainable with more correct
195 livestock management strategies was assumed as a potential reduction of the environmental
196 eutrophication risk. Where necessary, literature data were taken into account to evaluate the
197 potential reduction in N and P excretions obtainable by the methods that will be discussed later.
198 The collected data were analysed to establish the descriptive parameters (mean and standard
199 deviation) and their statistical correlations (IBM SPSS, 2016).

200

201 **3. Results**

202 The farms selected for this study represent 4.6 and 2.5% of the Holstein Friesian plus Brown Swiss
203 cows and dairy cattle herds of the selected area, respectively. The average number of reared animals
204 per farm and their mean LW, as well as the average productive and reproductive indices of the
205 animal categories are shown in Tables 1 and 2, respectively. Only five of the sample farms had split
206 the LC into two groups: cows at the beginning of lactation (first 2-3 months of lactation) and other
207 lactating cows, with the 2 groups being fed different diets. The number of cows per group is not

208 reported in Table 1, but the subdivision was taken into account in the calculation of the nutrient
209 intake and requirements.

210 The feed sample composition referring to the DM content is shown in Table 3. The most common
211 feed were corn silage and permanent meadow hay (75% of the farms), corn meal (50% of the
212 farms), alfalfa hay (44% of the farms) and Italian ryegrass hay, soybean meal, wheat bran, cotton
213 seeds (31% of the farms). The other feed were collected in less than 20% of the farms. In all farms
214 the mineral supplementation was given within the feedstuffs and then was impossible to sampling
215 the supplement, but the phosphorous supplementation used was mineral dicalcium phosphate.

216 Table 4 shows the actual N and P feed contents and a comparison with literature data and puts in
217 evidence the amount of differences (as %) between such values. The nutritional values of the beet
218 molasses shows a wide variability compared to the literature data, which depends on the industrial
219 extraction process and subsequent concentration of sugar refining by-products; the literature data
220 should therefore only be considered indicative.

221 Tables 5 and 6 report the apparent balance and apparent efficiency for N and P and the
222 environmental release of NO_3 and P_2O_5 for the dairy cattle categories considered in this analysis. In
223 order to allow a comparison of the animal diets to be made, the study was referred only to four
224 categories (LC, DC, H and YH plus CA) combining the data of the different groups of LC in a
225 unique category. For the nutrients balance, the categories were reduced to three, combining LC with
226 DC data, because the balance referred to the whole year. All the data were elaborated to obtain the
227 values referring to the RU. The mean DM intake and standard deviation were 21.21 ± 1.26 ,
228 10.47 ± 0.90 , 8.54 ± 0.46 , 6.37 ± 0.47 (kg d^{-1}) for LC, DC, H and YH plus CA, respectively.

229 The statistical correlations between the N and P concentrations in the diets and the productive and
230 reproductive indices were verified and no correlations were found for the studied herds, except
231 between the N concentration of the LC diet and milk production ($R^2=0.813$; $P<0.01$) and the
232 number of somatic cells in milk ($R^2=0.558$; $P<0.05$).

233 The eutrophication potential and the reduction in the correlated risk obtainable with mitigation
234 strategies are shown in Table 7. On the basis of the farm characteristics shown in the current
235 investigation, the strategies that could be adopted to decrease N and P excretion are: i) routine
236 sampling of the feedstuffs, especially forages and by-products, to establish their nutrient content; ii)
237 splitting the cows into at least 3 groups (cows at the beginning of lactation, other lactating cows and
238 non-lactating ones); iii) adopting precision feeding for cows, but especially for replacement heifers.
239 In order to decrease N excretion, it is also possible to: i) reduce the CP content in the diet (a
240 reduction of 1 percentage point in the diet CP generally reduces the N excretion by 10%; CRPA,
241 2014a); ii) supplement the diet with essential amino acids; iii) synchronize the N, carbon chain and
242 energy availability over the entire digestive tract (rumen and intestine) on the basis of the protein
243 degradability. In order to decrease P excretion, it is possible to: i) accurately determine the
244 requirements of each group of animals on the basis of the most recent suggestions (Meschy, 2010),
245 which are lower than in the past, to avoid the recorded excess.
246 Considering this evidence, it was decided, as a minimum goal, to envisage the adoption of the
247 measures that could be applied immediately without difficulty by the farmers, but at the same time
248 those that give the best results. Therefore, it is possible to act, for N and P, by improving the
249 balance between nutrient intake and utilisation reducing the excesses adopting precision feeding
250 strategies, as routinely sampling and analysing the feed or considering the nutrient availability, and
251 in addition, but only for N, by generally reducing the CP content of the diet by at least 1%,
252 eventually supplementing the diet with amino acids if necessary (Tables 5 and 6). The latter action
253 has proved easily achieved, as can be seen from the results of the Life+ European project AQUA
254 (CRPA, 2014b). It is also important to consider the recent studies on P requirements that have been
255 revised downwards or given as available P (INRA, 2007; Meschy, 2010). The possibility to use
256 form of P mineral supplementation more easily available was not taken into account as common
257 strategy because the farms involved in the study already used a mineral form with a good
258 availability (dicalcium phosphate). The effects of these simple reduction strategies are shown in

259 Table 7 where the actual N and P release showed in the Tables 5 and 6 were reduced following the
260 suggested strategies and transformed in PO₄ equivalent to estimate the eutrophication potential.

261 Specific other strategies could be adopted in single farms but do not represent a common strategy
262 adoptable in all.

263 On the basis of these results, in particular for the reduction in the eutrophication potential per RU,
264 and considering that the number of RU in the western part of the Po Plain (Cuneo and Torino
265 provinces) was 40,402 (AIA, 2015), it is possible to estimate that the reduction in the potential
266 nutrient excretion would be equal to 5,693.5 metric T y⁻¹ or 1 metric T (km²)⁻¹ of Utilised
267 Agricultural Area per year of PO₄.

268

269 **4. Discussion**

270 The productive and reproductive indices of the 16 dairy farms investigated in this study are similar
271 to the mean values computed by the Italian Breeders Association (AIA, 2016). In fact, milk
272 production is an index of the productive and genetic level of the considered farms and the AIA
273 bulletin reports an average milk production of 31.07 kg d⁻¹ per cow for the considered breeds and for
274 the farms in the same area, that is, practically the same yield as the farms in this study (considering
275 the real milk yield, without correction for the fat content). The others indices reported in the bulletin
276 are also in agreement with this assertion; in fact, the milk fat (3.76%) and protein contents (3.38%),
277 calving-conception period (181 d), services per conception (2.83) and age at the first calving (26.4
278 month) are very near or even coincident with the indices calculated for the farms in this study. On
279 the basis of this evidence, the selected herds can be considered as representatives of the farms in
280 North-Western Italian plain.

281 The feed sample composition shows a higher DM variability for the silages than the other feeds,
282 and an apparent lower variability for the other determined parameters. The feed N and P contents
283 instead show interesting and large differences, compared to the literature data. Excluding the beet
284 molasses values, which, as previously mentioned, depend on the industrial extraction process and

285 on the subsequent concentration of the sugar-refining by-products, the N feed content differs by
286 between -41 and +23% from the literature data. Instead, the P differences, which varied from -23 to
287 +20%, according to the feed, were more limited, although always large. These results confirm the
288 importance of an accurate knowledge of the characteristics of the feed used in diets because the use
289 of balanced diets decreases the level of excretion of nutrients and in turn of their environmental
290 release.

291 The diet analysis has shown that the adopted DM intake satisfies the physiological requirements of
292 the different cattle categories, even though it could be slightly increased for H.

293 The observed differences in the N balance highlight a good balance in the diets for LC. Only a
294 slight deficit in CP supplementation can be observed, together with slightly more unbalanced diets
295 for DC. Such a result can be attributed to the approximation level in the diet evaluation. This
296 evaluation has determined the N supplementation in terms of CP, whereas diets, particularly those
297 of LC, are normally prepared on the basis of more precise parameters, *e.g.* digestible protein, rumen
298 degradable protein or protein solubility. However, the collected data confirm the good balance in
299 the diet of dairy cows, although the N balance shows that the level of utilization efficiency for these
300 animals (about 20%), considering the annual N balance (lactation plus dry period), is not excellent.
301 This efficiency is a little higher than the average N efficiency calculated in ERM AB-DLO (2002)
302 for large breeds (18%). In the intensive dairy farms of North Italy, Crovetto and Colombini (2010)
303 found that the N efficiency of cows, calculated as the ratio of N retained in milk and the N intake,
304 hence without considering the non-productive period (dry period), was between 27.9 and 28.7%,
305 according to the amount of milk produced, or 26.3 and 30.2% according to the starch/CP ratio in the
306 diet. This efficiency would surely be lower if the dry period were also considered. In fact,
307 Wilkerson *et al.* (1997) found an N efficiency of 31.1 and 27.3% in lactating cows producing 29
308 and 14 kg d⁻¹ of milk, respectively, but only 6.7% in non-lactating cows. In two experimental trials,
309 Crovetto and Colombini (2010) and Colombini *et al.* (2012) found an N efficiency (N in milk/N
310 intake) of between 22 and 32% and 25 and 26%, respectively. Other authors have calculated an N

311 efficiency (as the percentage of N in a feed that is recovered as N in the milk), which, according to
312 the diet and the number of parities, is between 29 and 31% (Nadeau *et al.*, 2007). The N efficiency
313 of dairy cows calculated by ADAS (2007), using the same balance model utilized in the present
314 trial, is 23.5% and therefore closer to those calculated for the investigated farms in this study.
315 As far as replacement animals are concerned, since calves and heifers need to grow for about 2 years
316 before they can produce milk, the H and YH plus CA show a low N utilization efficiency. In fact, H
317 and YH plus CA use most of the N intake for growth and do not retain as much of the additional N
318 absorbed as LC, which results in more N being excreted when additional N is absorbed (Nadeau *et*
319 *al.*, 2006). This suggests that H and YH have a lower demand than LC because they do not have to
320 restore the body protein that is mobilised in lactation, and an N excess in the feeds could therefore
321 lead to a greater excretion. On the other hand, the younger females (H and YH plus CA) have a higher
322 CP percentage of LW than LC (from 17% to 19% of LW for H and YH plus CA and 16% for LC;
323 National Research Council, 2003), but the N retention for growth has less efficiency than the N
324 utilised for milk production. These considerations justify the differences in N efficiency found for the
325 three cattle categories, and especially those between LC and the other categories. It is also possible
326 to suppose an increase in N efficiency in the investigated farms for the replacement females. As
327 regards the N efficiency rate that has been published in other papers, Stoumann Jensen and
328 Schjoerring (2011), in the European Nitrogen assessment, converting the data reported by CBS (2009)
329 to animals housed for 365 days per year, reported N efficiencies for Dutch livestock categories,
330 which, for dairy cattle fed high fodder maize diets, were 15.5, 6.6 and 26.3% for replacement females
331 under or over 1 year of age and lactating cows, respectively. Hill *et al.* (2007), in late-gestation dairy
332 Holstein heifers fed diets with varying forage contents, reported an N efficiency ranging between 10
333 and 19%. Wilkerson *et al.* (1997) found an N efficiency of 15.7% for growing and replacement cattle.
334 Consistently with literature, the proportion of N excreted decreased as the milk yield increased
335 (Crovetto *et al.*, 2010). In the studied herds, the absence of statistical correlations between the N
336 (and P) concentrations in the diet and the considered productive and reproductive indices suggests

337 that there may be room to reduce the contents of N (and P) in the diet without affecting the
338 performances of the animals.

339 Therefore, it is possible to consider increasing the N utilization efficiency with suitable feeding
340 strategies, such as a CP reduction and a contemporary supplementation of synthetic amino-acids in
341 the diet or with inorganic N (*e.g.* protected ureic nitrogen) with a gradual release to obtain the
342 contemporaneous availability of the carbon chain and of nitrogen in the protein synthesis.

343 The use of these strategies may raise the utilization efficiency of nitrogen to almost 35% in lactating
344 cows, but a dairy cow fed a well-balanced diet will on average convert about 30% of the N
345 consumed into protein (Chase, 2004).

346 The P balance showed a large excess in diet supply, especially for H. The apparent efficiency of RU
347 appeared to be low, even though the efficiency of the cows amounted to about 28%. This efficiency
348 confirms the mean efficiency found by other authors. Tamminga (1992) found a P intake/excretion
349 ratio of 75% (apparent P efficiency 25%) in Dutch dairy cows producing 6250 kg of milk per year,
350 but this efficiency was affected by milk production, which was lower than on the farms of the
351 present study. In fact, it is well known that P efficiency also depends on the productivity level and
352 consequently on the P diet concentration, as pointed out by Wu (2005), who, in cows fed diets
353 containing different amounts of P and sources of fibre, found an efficiency ratio of 24-44%.

354 Puggard *et al.* (2011) found a P apparent digestion (P feed – P faecal) of 26-36% in Holstein cows,
355 where the faecal P normally represented 98-99% of the total P excreted (faecal plus urinary).

356 Efficiency is normally lower for replacement cattle than for lactating cows, as affirmed by Nennich
357 *et al.* (2005), who found that heifers in late-gestation excrete 60 to 70% as much P as lactating
358 cows. These results were confirmed by Hill *et al.* (2007), who found a negative P balance with
359 depletion of body reserves in late-gestation dairy Holstein heifers. The differences in P efficiency
360 could also be imputable to the incomplete development of the digestive tract of the young animals
361 and to microbial equilibrium (*e.g.* a lower absorption or lower microbial phytase production), and to

362 the phosphorus metabolism of the cows, which is correlated to calcium bone storage and
363 mobilization.

364 It is also possible to consider increasing P utilization efficiency by adopting several strategies, and
365 the easiest ones to adopt for the farms considered in this research have already been described. In
366 order to estimate the reduction in excreted P, the easiest adoptable strategy was chosen that is, of
367 avoiding an unbalance and of paying attention to the most available mineral supplementation. In
368 fact, the P in animal diets is obtained from the P that is present in the basic feed ingredients and
369 from mineral supplementation. The feed ingredients, particularly forages, should be analysed
370 systematically to establish the exact P content, while it is very important to choose the forms of P
371 that are more easily available (monocalcium or dicalcium phosphate, monosodium phosphate, *etc.*)
372 for the mineral supplementation of the diet, and to reject the mineral supplementation with medium
373 (sodium triphosphate, defluorinated phosphate, *etc.*) or low (soft rock phosphate) availability
374 (Bleukx, 2005).

375 About the relationship between agroecosystems and natural environment, it is very difficult to
376 evaluate how the strategies proposed for the dairy farms could influence the natural environment
377 because in the considered area, part of the Piedmont region, the intensive dairy farms are located in
378 plain, area with a high level of anthropization, *i.e.* with a total conversion of open spaces,
379 landscapes and natural environments by human action. In Piedmont the natural environments are
380 mainly located in the hilly and mountainous area, therefore at a higher altitude than the farms
381 involved in the study, so there is no repercussion of eutrophication in the watersheds that are
382 located upstream of the considered area. Anyway some consideration could be done on the
383 protection of ground and surface water quality by preventing nitrates from livestock sources. In fact
384 on the basis of the proposed strategies it is possible estimate a reduction of N excretion of 15.10 N
385 kg year⁻¹ per RU. Considering that in Piedmont about 40% of the nitrates of livestock origin derives
386 by dairy farms (Anagrafe Agricola Regione Piemonte, 2011) the estimated value corresponds to a
387 reduction about 8% of the N of livestock origin in the Piedmont river basins. More difficult was

388 estimate this reduction for the P because no data were found about the phosphates release quantified
389 per sources. However in Piedmont this pollutant is important as well as the nitrates considering that
390 the phosphates, also if substantially firm in the period 2006-2013 (+1%), represent about 78% of the
391 agricultural P, while the nitrates of livestock origin, increased by 17% in the same period, represents
392 about 50% of the N of agricultural origin (Nappi *et al.*, 2016). So, a reduction of the nitrates and
393 phosphates could affect the water quality of the northern Adriatic Sea where the Piedmont rivers
394 flow together the Po, the main Italian river, and where, starting from the 70s of the last century, a
395 series of acute eutrophication phenomena occurred. In this closed sea basin the influence of the
396 anthropogenic nutrient input showed a dominant effect than the natural (climatic) causes (Degobbis
397 *et al.*, 2000), so a control of the input of livestock origin, one of the most important nutrient sources,
398 could have an important effect on preserving the marine ecosystem.

399 The reduction in the potential nutrient excretion, and therefore in the eutrophication risk, that can be
400 obtained with the proposed N and P reduction strategies should be considered the minimum target
401 that has to be reached by the farmers in the considered area. This reduction is not negligible,
402 considering that it is about 17% of the actual potential eutrophication risk derived from the local
403 dairy cattle sector, as the RU potential eutrophication reduction showed in Table 6.

404

405 **5. Conclusions**

406 This study has shown that it is possible to reduce pollution from dairy farms and in particular the
407 risk of water eutrophication. N supplementation has been found to be balanced for productive
408 animals, but in excess for replacement cattle, while the P surplus has been found in all the studied
409 farms. Therefore, it is necessary to pay more attention to each animal category reared on dairy
410 farms, and in particular to the diets of replacing animals, which should be formulated as closely as
411 possible to their requirements. The strategies suggested to decrease livestock pollution are simple:
412 reducing the N and P contents or supplementations in the diets to avoid overfeeding, and increasing

413 nutrient utilisation efficiency of the animals and at a herd level, without affecting the minimum
414 requirement and hence the productive and reproductive performance.

415 In short, the N and P balance method and the evaluation of nutrients efficiency could be useful tools
416 for livestock farms to estimate the risk of eutrophication from dairy farms, to control and reduce the
417 flow of these nutrients and to highlight the critical points of the production systems.

418

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422

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- 523

524 **Table 1.** Average number of reared animals per category and per farm and their mean live weight (mean value \pm
525 standard deviation).

	Number of animals (n)	Live weight (kg)
Total animals	217.8 \pm 98.3	n.r.
Total cows	116.4 \pm 54.0	656.3 \pm 22.5
Lactating cows	100.3 \pm 45.6	n.r.
Dry cows	16.1 \pm 9.0	n.r.
Heifer	49.4 \pm 22.0	426.7 \pm 25.3
Young heifers plus calves	48.5 \pm 23.6	254.7 \pm 35.9

526 n.r. = not recorded.

527

528 **Table 2.** Productive and reproductive indices (per year, mean value \pm standard deviation).

Productive indexes		Reproductive indexes	
Milk yield (kg cow ⁻¹ d ⁻¹)	31.19 \pm 3.91	Calving interval (d)	428.6 \pm 19.6
FCM ¹ yield (kg d ⁻¹)	30.29 \pm 3.38	Calving-conception period (d)	151.5 \pm 18.5
Milk fat (%)	3.82 \pm 0.19	Services per conception (n)	2.53 \pm 0.43
Milk crude protein (%)	3.38 \pm 0.19	Age at first calving (months)	25.44 \pm 1.32
Milk casein (%)	2.67 \pm 0.10	Calves per cow (n)	0.85 \pm 0.04
Milk urea (mg L ⁻¹)	26.07 \pm 3.60	Fertility rate (%)	40.59 \pm 6.56
Milk somatic cells (n mL ⁻¹)	219,130 \pm 56,028	Heifers per cow (n)	0.43 \pm 0.10
Milk bacterial count (CFU mL ⁻¹)	13,560 \pm 9,913	Young heifers and calves per cow (n)	0.42 \pm 0.05

529 ¹FCM = Fat Corrected Milk, corrected to 4% of fat.

530

531 **Table 3.** Feed compositions as a percentage of Dry Matter (% DM; mean value \pm standard deviation).

Feed	Samples (n)	DM ¹ (%)	Ash (% DM)	CP ² (% DM)	EE ³ (% DM)	NDF ⁴ (% DM)	ADF ⁵ (% DM)	ADL ⁶ (% DM)
Italian ryegrass hay	10	89.26 \pm 1.04	8.65 \pm 0.17	9.83 \pm 1.98	1.69 \pm 0.27	59.37 \pm 4.09	37.22 \pm 1.60	3.40 \pm 0.28
Fescue hay	6	88.60 \pm 1.01	11.90 \pm 0.42	12.70 \pm 0.39	3.50 \pm 0.08	66.50 \pm 4.28	36.50 \pm 1.58	3.20 \pm 0.21
Alfalfa hay	14	87.69 \pm 1.41	9.60 \pm 2.62	16.83 \pm 4.51	2.00 \pm 0.55	49.85 \pm 9.32	37.36 \pm 7.85	7.82 \pm 1.50
Permanent meadow hay	24	87.67 \pm 2.38	8.70 \pm 3.01	11.39 \pm 2.65	2.10 \pm 0.79	55.99 \pm 7.23	39.42 \pm 7.08	5.44 \pm 2.15
Corn silage	24	32.70 \pm 3.56	4.24 \pm 0.22	7.90 \pm 0.56	3.10 \pm 0.15	42.61 \pm 1.79	24.74 \pm 1.87	2.83 \pm 0.66
Italian ryegrass silage	6	31.93 \pm 3.97	9.15 \pm 1.91	10.57 \pm 0.93	2.33 \pm 0.58	57.97 \pm 2.70	36.70 \pm 0.26	5.55 \pm 0.64
Triticale silage	6	28.10 \pm 3.54	11.70 \pm 2.05	11.30 \pm 1.00	2.70 \pm 0.50	69.50 \pm 2.80	45.90 \pm 0.45	5.90 \pm 0.61
Corn meal	16	87.06 \pm 1.29	1.59 \pm 0.28	9.86 \pm 0.95	3.27 \pm 0.59	14.73 \pm 1.78	3.94 \pm 0.77	0.73 \pm 0.39
Wheat meal	6	89.5 \pm 0.14	2.85 \pm 0.07	14.75 \pm 0.07	1.48 \pm 0.04	18.05 \pm 0.07	3.50 \pm 0.98	0.60 \pm 0.11
Soybean meal	10	87.86 \pm 0.59	7.64 \pm 0.56	50.41 \pm 1.61	2.03 \pm 0.67	17.90 \pm 0.90	8.80 \pm 1.20	0.57 \pm 0.15
Wheat bran	10	87.66 \pm 1.01	4.60 \pm 0.37	17.68 \pm 0.82	2.30 \pm 0.01	40.00 \pm 2.95	10.70 \pm 1.08	3.10 \pm 0.31
Cotton seeds	10	91.24 \pm 1.31	4.88 \pm 0.22	24.79 \pm 2.95	23.47 \pm 2.96	38.23 \pm 3.67	26.48 \pm 3.51	8.40 \pm 1.19
Cereal straw	6	89.45 \pm 1.20	8.05 \pm 0.35	3.90 \pm 0.01	1.10 \pm 0.14	81.70 \pm 2.97	57.35 \pm 0.92	9.00 \pm 4.53
Beet pulp	6	88.52 \pm 0.46	6.48 \pm 2.02	10.40 \pm 0.99	0.90 \pm 0.11	60.01 \pm 2.36	29.80 \pm 2.75	4.40 \pm 0.51
Beet molasses	6	56.67 \pm 0.31	22.10 \pm 0.10	7.23 \pm 0.25	8.90 \pm 0.36	n.d.	n.d.	n.d.
Brewers grain	6	22.93 \pm 0.15	4.70 \pm 0.10	25.43 \pm 0.06	7.00 \pm 0.01	44.29 \pm 17.29	28.58 \pm 14.40	4.33 \pm 3.02
TMR Lactating Cows	32	53.07 \pm 4.28	5.38 \pm 0.34	15.83 \pm 0.75	4.42 \pm 0.54	35.29 \pm 1.12	20.07 \pm 0.73	3.23 \pm 1.14
TMR Dry Cows	32	64.24 \pm 3.80	5.98 \pm 0.37	10.82 \pm 1.55	2.35 \pm 0.48	51.93 \pm 3.67	29.29 \pm 5.60	3.37 \pm 0.97
TMR Heifers	32	64.23 \pm 4.63	5.78 \pm 0.38	12.21 \pm 0.58	2.49 \pm 0.48	50.91 \pm 2.70	30.86 \pm 1.82	3.51 \pm 1.06
TMR Young Heifers	18	64.59 \pm 3.84	5.86 \pm 0.34	13.64 \pm 0.84	2.40 \pm 0.54	48.37 \pm 3.43	28.92 \pm 2.20	3.25 \pm 0.94

532 ¹DM = Dry Matter.

533 ²CP = Crude Protein.

534 ³EE = Ether Extract.

535 ⁴NDF = Neutral Detergent Fibre.

536 ⁵ADF = Acid Detergent Fibre.

537 ⁶ADL = Acid Detergent Lignin.

538 n.d. = not determined.

539

540 **Table 4.** N and P of the feeds as a percentage of the actual Dry Matter (% DM) content compared with literature values
 541 (Cevolani, 2005; mean value \pm standard deviation).

Feed	Actual N content (% DM)	Literature N content (% DM)	Difference (%)	Actual P content (% DM)	Literature P content (% DM)	Difference (%)
Italian ryegrass hay	1.57 \pm 0.32	1.73	-10.2	0.28 \pm 0.04	0.28	0.0
Fescue hay	2.03 \pm 0.30	1.56	+23.2	0.23 \pm 0.04	0.23	0.0
Alfalfa hay	2.36 \pm 1.16	3.32	-40.7	0.26 \pm 0.09	0.30	-15.4
Permanent meadow hay	1.82 \pm 0.42	1.79	+1.7	0.28 \pm 0.05	0.32	-14.3
Corn silage	1.26 \pm 0.09	1.34	-6.4	0.22 \pm 0.03	0.27	-22.7
Italian ryegrass silage	1.69 \pm 1.49	1.87	-10.7	0.28 \pm 0.04	0.36	-28.6
Triticale silage	1.81 \pm 0.10	n.a.	-	0.42 \pm 0.05	n.a.	-
Corn meal	1.58 \pm 0.15	1.50	+5.1	0.30 \pm 0.04	0.23	+23.3
Wheat meal	2.37 \pm 0.01	1.94	+18.1	0.46 \pm 0.01	0.37	+19.6
Soybean meal	8.06 \pm 0.26	8.26	-2.5	0.72 \pm 0.03	0.71	+1.4
Wheat bran	2.83 \pm 0.13	2.72	+8.7	0.95 \pm 0.02	1.13	-18.9
Cotton seeds	3.97 \pm 0.47	3.74	+5.8	0.67 \pm 0.06	0.70	-4.5
Cereal straw	0.62 \pm 0.05	0.54	+12.9	0.10 \pm 0.01	0.12	-20
Beet pulp	1.66 \pm 0.16	1.78	-7.2	0.10 \pm 0.01	0.10	0.0
Beet molasses	1.16 \pm 0.04	2.32	-100.0	0.24 \pm 0.02	0.03	+87.5
Brewers grain	4.07 \pm 0.17	4.13	-1.5	0.58 \pm 0.01	0.60	-3.4

542 n.a. = not available.

543

544 **Table 5.** N apparent balance and NO₃ environmental release (mean value ± standard deviation).

	LC ¹	DC ²	H ³	YH ⁴ and CA ⁵	RU ⁶
CP in diet (% DM)	16.17±1.26	11.01±1.73	13.58±1.40	13.70±1.95	14.12±1.51
CP requirements (g d ⁻¹)	3533.9±302.0	1094.0±13.9	1153.3±53.7	744.0±17.1	3624.1±327.8
CP in feed (g d ⁻¹)	3494.1±300.0	1166.7±230.6	1162.2±145.6	872.4±126.7	3781.5±326.5
CP balance (%)	-1.75±6.00	6.56±20.66	0.65±12.90	17.23±17.26	4.6±6.0
N intake (kg y ⁻¹)	186.15±21.78		21.72±2.24	21.93±2.90	233.9±23.0
N retention (kg y ⁻¹)	37.65±5.14		4.74±0.74	3.41±0.97	41.3±5.5
N excretion (kg y ⁻¹)	148.50±19.18		62.14±8.53	47.54±7.61	192.6±21.0
N apparent efficiency (%)	20.32±2.51		8.58±1.67	6.95±2.67	17.7±2.0
N losses (kg y ⁻¹)	41.58±5.37		17.40±2.39	13.31±2.13	53.27±5.44
NO ₃ release (kg y ⁻¹)	657.43±84.93		275.71±37.76	210.47±33.71	842.31±85.99
NO ₃ excess (kg y ⁻¹)	-13.10±64.47		1.69±37.27	33.03±33.18	0.45±68.86

545 ¹LC = Lactating Cows.

546 ²DC = Dry Cows.

547 ³H = Heifers.

548 ⁴YH = Young Heifers.

549 ⁵CA = Calves.

550 ⁶RU = Replacement Unit.

551

552 **Table 6.** P apparent balance and P₂O₅ environmental release (mean value ± standard deviation).

	LC ¹	DC ²	H ³	YH ⁴ and CA ⁵	RU ⁶
P in diet (% DM)	0.38±0.03	0.35±0.03	0.19±0.01	0.25±0.01	0.29±0.03
P requirements (g d ⁻¹)	80.14±8.22	36.06±0.94	15.23±1.04	15.93±1.18	65.58±5.95
P in feed (g d ⁻¹)	93.09±9.70	43.26±10.26	31.61±8.28	23.59±4.51	91.20±12.96
P balance (%)	17.16±16.19	19.99±28.21	106.98±51.28	49.14±31.86	39.11±15.55
P intake (kg y ⁻¹)	30.68±3.33		11.54±3.02	8.61±1.65	36.43±4.02
P retention (kg y ⁻¹)	8.56±1.82		1.97±1.21	0.87±1.12	7.23±1.94
P excretion (kg y ⁻¹)	22.13±2.90		9.57±2.88	7.74±2.17	29.21±4.04
P apparent efficiency (%)	27.90±5.73		17.22±11.52	10.82±14.46	19.94±5.71
P ₂ O ₅ release (kg y ⁻¹)	50.70±6.63		21.93±6.60	17.74±4.97	66.93±9.25
P ₂ O ₅ excess (kg y ⁻¹)	7.44±5.93		13.70±6.65	6.41±4.01	16.44±8.87

553 ¹LC = Lactating Cows.

554 ²DC = Dry Cows.

555 ³H = Heifers.

556 ⁴YH = Young Heifers.

557 ⁵CA = Calves.

558 ⁶RU = Replacement Unit.

559

560 **Table 7.** Eutrophication potential and reduction in the correlated risk obtainable by adopting mitigation strategies as
 561 precision feeding and reduction of CP content of the diet by at least 1% (mean value \pm standard deviation).

	LC ¹ and DC ²	H ³	YH ⁴ and CA ⁵	RU ⁶
Actual N net release (kg y ⁻¹)	106.92 \pm 13.81	44.74 \pm 6.14	34.23 \pm 5.48	138.68 \pm 15.10
Actual NH ₃ release (kg y ⁻¹)	50.56 \pm 6.53	21.16 \pm 2.90	16.19 \pm 2.59	65.57 \pm 7.14
Actual P release (kg y ⁻¹)	22.13 \pm 2.90	9.57 \pm 2.88	7.74 \pm 2.17	29.21 \pm 4.04
Eutrophication potential (PO ₄ kg y ⁻¹)	129.89 \pm 14.28	54.80 \pm 9.79	37.82 \pm 16.64	168.93 \pm 14.81
Obtainable N net release (kg y ⁻¹)	98.56 \pm 13.40	41.15 \pm 6.01	31.55 \pm 5.40	127.81 \pm 14.64
Obtainable NH ₃ release (kg y ⁻¹)	46.61 \pm 6.34	19.46 \pm 2.84	14.92 \pm 2.55	60.43 \pm 6.92
Obtainable P release (kg y ⁻¹)	16.26 \pm 2.91	3.90 \pm 1.01	4.33 \pm 1.84	22.21 \pm 3.32
Eutrophication potential (PO ₄ kg y ⁻¹)	106.86 \pm 13.20	35.10 \pm 2.44	33.29 \pm 3.56	140.92 \pm 12.79
Potential Eutrophication reduction (%)	17.73	35.95	11.97	16.59

562 ¹LC = Lactating Cows.

563 ²DC = Dry Cows.

564 ³H = Heifers.

565 ⁴YH = Young Heifers.

566 ⁵CA = Calves.

567 ⁶RU = Replacement Unit.

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