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Effectiveness of precision feeding in reducing N excretion in dairy cattle

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ABSTRACT

Two periods enrolling 56 (milk yield 36.2 ± 7.91 kg/d) and 58 (milk yield 32.4 ± 4.99 kg/d) grouped-fed dairy cows were performed to evaluate nutrient adjustment and N emissions of a precision feeding strategy. Pens blocked by parity were randomly assigned to a conventional (CONV) or to a precision feeding scheme (PREC) for a 21-d period. The CONV group was offered a total mixed ration (TMR, 6.82 and 6.65 MJ of NEl/kg of DM, in period 1 and 2, respectively, and 165 g of CP/kg of DM in both periods; whereas PREC cows were fed a partial mixed ration (PMR, 6.65 and 6.40 MJ of NEl/kg of DM, 135 and 137 g of CP/kg of DM, in period 1 and 2, respectively) and a concentrate feed supplemented twice daily in the milking parlour, which contained different quantities of soybean meal, corn meal, and wheat middling's according to estimated nutritional needs of each cow above those supplied by the consumption of PMR. Individual daily nutritional needs and nutrients consumed from the PMR were calculated using a 10-d rolling average of performance data (milk yield and concentration of its components, and BW daily recorded in both periods). A N balance using urine and fecal spot sampling during the last 3 d of the study was performed in period 1, and stored manure gaseous emissions (ammonia, methane, nitrous oxide, and carbon dioxide) were measured for 2 wk in period 2. After 2 wk of adaptation to the diet, 82 cows homogeneously distributed in 4 DIM categories: early DIM (< 81), mid-early DIM (81–150), mid-late DIM (151–220), late DIM (> 220) were used to assess how energy and protein requirements were adjusted using both feeding system. Dairy cows in both feeding systems were energetically overfed, and CONV cows tended to be more CP overfed in mid-late and late DIM cows than PREC fed cows. Total daily N urine excretion, and milk N urea concentration were greater in CONV than in PREC cows. There were no differences in ammonia and nitrous oxide emissions from the manure storage between PREC and CONV cows; however, methane and carbon dioxide emissions from manure increased by 55% and 15%, respectively in PREC fed cows. Precision feeding system based on preceding average daily milk yield and composition can

Abbreviations: AIA, acid insoluble ashes; CONV, conventional feeding; DIM, days in milk; DMI, dry matter intake; ECM, energy-corrected milk; ENU, efficiency of Nitrogen utilization; GHG, greenhouse gases; LN, lactation number; PMR, partial mixed ration; PREC, precision feeding; SCC, somatic cell counts; TAN, total ammonia Nitrogen; TC, total Carbon; TMR, total mixed ration; TN, total Nitrogen.

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reduce N excretion without affecting short-term milking performance but increasing C gaseous emissions from manure.

1. Introduction

Excretion of N from livestock production contributes to environmental pollution. Losses of N from urine and manure as ammonia can be oxidized to nitrous oxide, one of the greenhouse gases (GHG) that contributes to global warming and stratospheric ozone depletion (Hokestra et al., 2020; Zhang et al., 2020). Moreover, ammonia can leach to the soil and groundwater leading to soil acidification (Zhang et al., 2020), nitrate accumulation in groundwater (Postigo et al., 2021), and ammonia emission to the atmosphere. An important fraction of ammonia emission is produced during manure storage (Kupper et al., 2020). At the top storage layer, where air and manure interface, ammonia may disperse into the atmosphere depending, among other aspects, on manure temperature and air speed (VanderZaag et al., 2015).

Dairy cows need different amounts of protein depending on their milking performance and physiological status. However, under most commercial situations, cows are fed ad libitum in groups without being able to adjust individual nutrient supply. For this reason, nutritionists formulate diets using percentages rather than amounts. Typically, dairy rations for mid-lactation cows contain 165–170 g of CP/kg of DM (Groff, 2005; Olmos Colmenero and Broderick, 2006). As lactation advances, protein requirements decrease, and feeding 165 g of CP/kg of DM diets dairy to cows in late lactation may increase urinary and faecal N excretion and reduce profits (Law et al., 2009). Barros et al. (2017) reported that efficiency of N utilization (milk protein N yield /N intake; ENU) improved when feeding 144 g of CP/kg of DM diets to late-lactation cows, but lower CP levels had negative impacts on milk yield and efficiency.

Table 1
Ingredient and nutrient composition of the conventional (CONV) and the precision feeding (PREC) partial mixed ration (PMR) fed in Studies 1 and 2.

	Study1		Study2	
Item	CONV-PMR	PREC-PMR	CONV-PMR	PREC-PMR
Ingredient (DM basis), kg				
Alfalfa hay	3.34	3.34		-
Rye grass silage	1.58	1.58		-
Rye grass hay	1.74	1.74		-
Barley silage	3.25	3.25		-
Barley straw	0.74	0.74		-
Wheat silage	-	-	3.76	3.76
Corn silage	-	-	3.70	3.70
Alfalfa silage	-	-	3.49	3.49
Oat hay	-	-	1.75	1.75
Corn meal	7.93	7.49	5.27	4.84
Wheat meal	0.45	-	1.41	0.97
Canola meal	1.81	1.81	-	-
Soybean meal	2.34	0.55	2.75	1.00
Soybean hulls	-	-	1.17	1.17
Wheat middlings	0.89	0.45	0.82	0.82
Hydrogenated vegetable fat	0.42	0.42	-	-
Calcium carbonate	0.08	0.08	0.14	0.14
Magnesium oxide	0.03	0.03	0.04	0.04
Salt	0.08	0.08	0.05	0.05
Vitamin minerals premix[a]	0.04	0.04	0.05	0.05
Nutrient composition				
DM, g/kg of feed[b]	605	607	534	531
NEl, MJ/kg of DM[b]	6.82	6.65	6.65	6.40
CP, g/kg of DM[b]	164	139	165	137
RDP, g/kg of DM[b]	107	88	108	89
RUP, g/kg of DM[b]	57	51	57	48
Metabolizable protein, g/kg of DM[b]	109	93	111	92
Digestible Lys, g/kg of MP[b]	53.3	45.3	49.8	41.3
Digestible Met, g/kg of MP[b]	20.4	17.4	20.1	16.7
NFC, g/kg of DM[c]	334	315	419	415
aNDF, g/kg of DM[b]	389	431	322	350
ADF, g/kg of DM[b]	234	218	198	217
Fat, g/kg of DM	47	49	30	31
Ash, g/kg of DM	66	66	64	66

[a] 14.6% Ca, 0.03% Na, 4.48% Mg, 2250,000 UI/kg Vitamin A, 8,8000 mg/kg Vitamin E, 665,000 UI/kg Vitamin D3, 40 mg/kg Co, 30,000 mg/kg Zn, 150 mg/kg Se, 20,000 mg/kg Fe, 250 mg/kg I, 30,000 mg/kg Mn, 5000 mg/kg Cu, 1500 mg/kg Butylhydroxytoluene, 279,949 mg/kg sepiolite.
[b] DM: dry matter, NEl: Net Energy for lactation, CP: crude protein, RUP: rumen degradable protein, RDP: rumen undegradable protein, MP: metabolized protein estimated using NRC (2001), aNDF: amylase treated neutral detergent fiber, ADF: acid detergent fiber.
[c] Non-fiber carbohydrate calculated as 100 – (CP+aNDF+fat+ash).

Considering the potential environmental impact of feeding excessive amounts of CP to dairy cows along with the increasing demand for feed and food globally, there is an opportunity to adjust nutrient supply to cows to improve the efficiency of utilization of natural sources. Although cows are managed and fed in groups, each animal has its own requirements according to their stage of lactation, productivity, milk composition, and physiological state. Precision feeding strategies may allow matching nutrients supply with animal requirements to improve animal productivity while reducing environmental pollution and production costs (van Empel et al., 2016). Nowadays, the existence of several on-farm technologies that allow automatic collection of data such as concentrate intake, milk yield and composition, BW or animal behaviour may facilitate the application of precision feeding strategies. For example, Fisher et al. (2020) proposed to restrict feed intake to less efficient dairy cows using individual total mixed ration feeders, and several authors (Bach and Cabrera, 2017; Moore et al., 2020) have proposed to modulate the amount concentrate feed supplemented in automatic milking systems depending on milking performance of individual animals.

In a preliminary study, we observed that concentrate feed supplementation in the milking parlour according to milk yield and quality and mixed ration intake can reduce N urine excretion (Terré et al., 2020). Therefore, we hypothesized that feeding dairy cows a partial mixed ration (PMR) in the feed bunk and a mix of concentrate feeds supplement in the milking parlour according to individual needs estimated based on individual 10-d rolling average of milking performance and individual PMR intake would optimize the use of dietary N and reduce manure ammonia emissions. Thus, the objective of the present study was to evaluate the effects of a precision feeding strategy on N balance and gaseous emissions during manure storage, and how nutrients (protein and energy) were adjusted at different stages of lactation.

2. Material and methods

Dairy cows were managed under the supervision of IRTA technicians and with the approval from the Animal Care Committee of the Government of Catalonia (authorization code 10640). Animals were housed at IRTA dairy farm (EVAM, Monells, Spain). The study consisted of two 21-day periods separated by 15 months (November 2019 and May 2021).

2.1. Animals and treatments

A total of 20 primiparous and 36 multiparous Holstein cows (729 $\pm$ 69.9 kg of BW; 36.2 $\pm$ 7.91 kg/d of milk; 155 $\pm$ 89.3 DIM; 2.1 $\pm$ 1.02 lactations, mean $\pm$ SD) in the first period, and a total of 29 primiparous and 29 multiparous Holstein dairy cows (708 $\pm$ 79.2 kg of BW; 32.4 $\pm$ 4.99 kg/d of milk; 160 $\pm$ 59.7 DIM; 2.0 $\pm$ 1.23 lactations, mean $\pm$ SD) in the second period with good initial health status were enrolled in a 21-day study (16 days of adaptation to the feeding system, and 5 days of measurements and nutrient calculations). Cows were blocked by parity (primiparous or multiparous) within feeding treatment and distributed in four free-stall pens (2 pens per feeding treatment). Animals were milked twice daily in a parallel milking parlour. Milk yield and fat and protein concentrations were recorded using electronic milk meters (AfiMilk, Afikim Ltd., Kibbutz Afikim, Israel) and an on-line system to determine milk components concentrations (AfiLab system, Afikim Ltd., Kibbutz Afikim, Israel). After each milking, all cows were weighed using AfiMilk SortWeight system (Afikim Ltd., Kibbutz, Israel). Each pen was equipped with 20 cubicles bedded with a mixture of compost and sawdust, 4 water troughs, and 15 electronic feed bins (MooFeeder, MooSystems, Cortes, Spain) to record individual feed intake. Animals in each of the 2 pens per period were fed a conventional (CONV) TMR (6.82 MJ of NEl//kg of DM, 165 g of CP/kg of DM in the first period; 6.65 MJ of NEl//kg of DM, 165 g of CP/kg of DM in the second period, Table 1), and animals in the other 2 pens per period were fed under a precision feeding strategy (PREC). This consisted of feeding a PMR with lower energy and CP content than the CONV one (6.65 MJ of NEl//kg of DM, 135 g of CP/kg of DM in the first period; 6.40 MJ of NEl//kg of DM, 137 g of CP/kg of DM in the second period, Table 1) and a combination of 3 feeds (soybean, 458 g of CP, 136 g of NDF, 30.7 g of fat, 69.8 g of ash per kg of DM; a mix of 50% wheat and 50% corn, 101 g of CP, 126 g of NDF, 36.0 g of fat, 15.2 g of ashes per kg of DM; wheat middling's, 180 g of CP, 457 g of NDF, 5.2 g of fat, 53.8 g of ash per kg of DM) in the milking parlour (supplement). These 3 ingredients were stored separately in 3 different silos that were connected to individual feeders at each milking place of the milking parlour. Automatically, on a daily basis, individual data were stored in a file that an algorithm, developed using Python (van Rossum and Drake, 1995) as programming language, used to calculate, using NRC (2001) equations, the energy and CP needs for every individual cow using a 10-d rolling average of performance data recorded in our facilities (milk yield, milk fat and protein composition, and BW) from ten preceding days (estimated needs). Then, a 10-d rolling average daily consumption of nutrients from the PMR was computed considering individual DMI and the estimated nutrient composition of the diet. Then, it was subtracted from the estimated needs to determine the amount of nutrients needed to be supplemented (if any) in the milking parlour. The algorithm determined the amount of soybean, mix of wheat and corn, and wheat middling's to deliver in the milking parlour according to the nutrient composition of the feeds available, and the estimated needs above the precision PMR nutrient consumption. The algorithm consisted of linear programming optimization that found the optimum combination of the 3 ingredients complying with constraints on a minimum supply of energy, CP, and NDF within the limit of 3000 g per day of concentrate fed in the parlour. Cows in PREC treatment not needing supplementation were offered 150 g of wheat middling's per milking to ensure that all cows had some feed offered while milking. The final amount of each ingredient was split in two feedings during each milking (at 0800 and 1900 h), meanwhile both, PMR and TMR were delivered in the feed bins twice daily while the animals were in the milking parlour.

2.2. Sampling and Measurements

Individual feed intake was monitored daily using MooFeeders (MooSystems, Cortes, Spain). Both PMR and feed ingredients composing the concentrate offered in the parlour were sampled weekly and composited for the 3-wk period for subsequent nutrient composition analysis. Samples were frozen at − 20ºC to subsequently determine DM (method 934.01), N (method 984.13), ether extract (method 920.39), and ash (method 942.05) following AOAC International (2000) and for aNDF according to Van Soest et al. (1991) using sodium sulphite and heat-stable amylase. Non-fibre carbohydrates were calculated as 100 minus CP, aNDF, fat, and ash (NRC, 2001). For all animals involved in the study, energy-corrected milk (ECM) was calculated using the equation (Bernard, 1997): (0.3246 × milk) + (12.86 × kg fat) + (7.04 × kg protein) using AfiLab system data, and feed efficiency was computed as the of ratio of ECM to total dry matter intake (DMI). Furthermore, milk samples of all animals enrolled in the study were obtained at day 19 of study in tubes containing 2-bromo-2-nitropropane-1,3-diol, which were analysed for urea, using infrared spectroscopy (MilkoScan™ 7; Foss Iberia S.A., Barcelona, Spain).

From the first group of animals, a set of 26 cows homogeneously distributed by lactation number (LN) and DIM (5 primiparous and 8 multiparous averaging 183 ± 60.4 DIM vs 6 primiparous and 7 multiparous averaging 171 ± 75.8 DIM; in CONV vs PREC fed cows, mean ± SD) in both treatments were selected to perform a N balance. Faecal spot samples (about 300 g) from the rectum and urine spot samples (about 250 mL) obtained by massaging the perivaginal area were collected from d 19–21 of study, at different times: on day 1 at 0800, 1400, 2000, 0200 h; on day 2 at 1000, 1600, 2200, 0400, and on day 3 at 1200, 1800, 0000, 0600 h. On these 3 consecutive days, samples of both PMR offers were collected and composited for further nutrient composition analysis. Faecal samples from the dairy cows were dried at 60ºC for 4 d, ground at 1 mm, and further composited on a dry weight basis by cow. Composite samples were analysed for DM, total N (TN) content (Standard methods for the Examination of Water and Wastewater, APHA 2005 using Kjeldahl digester and distillation unit from Buchi®, Switzerland), aNDF, and acid insoluble ash (AIA), which was used as an internal marker to estimate faecal output (Sales and Janssens, 2003). Samples of both PMR were composited and analysed for DM, CP, aNDF, and AIA. Total tract apparent digestibility was calculated based on AIA concentration in the rations and faeces. Aliquots of 30 mL of urine were immediately acidified with 0.1 M sulfuric acid, diluted 1:5.7, and stored at − 20ºC. These samples were composited by cow and analysed for TN (APHA 2005, using Kjeldahl digester and distillation unit from Buchi®, Switzerland). A 15-mL aliquot was used to determine urine creatinine (Jaffe Method using Olympus System Reagent®, Beckman Coulter®, Ireland). Daily urine excretion was estimated assuming a urinary creatinine excretion of 29 mg/kg of BW/d (Valadares et al., 1999), and then, combined with urine N concentrations, used to estimate total urinary N excretion. Efficiency of N utilization was calculated as the proportion of N secreted in milk relative to total N intake. Furthermore, milk samples from the 26 cows selected for the N balance were collected in tubes containing 2-bromo-2-nitropropane-1,3-diol on days 19, 20, and 21 of study and composited for the morning and afternoon milking according to milk weights and analysed for fat, CP, lactose, total solids non-fat, urea, and SCC using infrared spectroscopy as previously described. Lastly, blood samples (10 mL) were collected in ethylene diamine tetra acetic tubes (Vacutainer, Becton Dickinson, Madrid Spain) from the coccygeal vessels 5 h after the morning feeding on d 21. They were centrifuged at 1500 x g for 10 min and frozen at − 20 ºC for further plasma urea determination following the L-glutamate dehydrogenase method (Talke and Schubert, 1965) (Olympus System Reagent®, Beckman Coulter®, Irlanda). Change in BW, in these cows, was calculated as the difference between average BW the week before the study begun and the last week of study.

Lastly, the last week of the study, manure scrapers were stopped overnight to accumulate manure in the pens. Next morning, the scrappers were activated to bring all the manure accumulated at the end of the alley to collect two plastic boxes of 0.675 m^3 (600 L capacity; internal dimensions: 91×111×62 cm) each per treatment to simulate manure ponds (supplemental material). Boxes were manually filled to the top with 0.6 m^3 of manure directly from the pen floor (the manure sample for the CONV was a mixture of the two pens in CONV, and the manure sample for PREC was a mixture of the manure from the two pens in PREC), and they were kept at ambient temperature. Then, on a weekly basis, the evolution of manure composition and GHG and ammonia emissions were monitored during manure storage for 14 days.

Manure samples were collected weekly and analysed following the Standard methods (APHA, 2005) to determine DM content (2540 G), total ammonia N (TAN) (4500-NH3-C) and pH, directly measured in homogenized samples using a pH-meter (4500-H+) (pH basic 20, Crison, Spain). Total carbon (TC) and TN were determined by using an elemental analyzer LECO® (Leco Corporation, Michigan, USA) (ISO 13878: Soil Quality - Determination of Total Nitrogen Content by Dry Combustion). Gaseous emissions were collected weekly using a dynamic chamber coupled to an ammonia sensor Riken Keiki GX-6000 (RKI Analytical Instruments GmbH, Bad Homburg vor der Höhe, Germany) for ammonia emissions. Vacuum tubes, Labco Exetainer® (Labco Limited, Lampeter, United Kingdom) were used to collect 30 mL of gas for the GHG emissions. Methane, carbon dioxide and nitrous oxide were analysed as described in Torrellas et al. (2018), sealing the area between the surface and the air, avoiding gas losses, and creating a controlled airflow of 0.2–0.3 m/s following EPA recommendations (EPA, 2001). Emission fluxes (g/m^2h) at 25ºC of ammonia and GHG were calculated by multiplying the gas concentration (mg/m^3) by the dynamic chamber internal flux (30 m^3/m^2h), the flow (m^3/m^2h) emitted per unit area of the chamber (m^2). The total gas emission after 14 d was calculated by the trapezoidal method of integration (Levy et al., 2017; Dalby et al., 2022).

2.3. Calculations

To assess how cows in CONV and PREC adjusted daily to their energy and protein requirements according to their stage of lactation, cows were classified in 4 DIM categories: early DIM (< 81), mid-early DIM (81−150), mid-late DIM (151−220), late DIM (> 220). Then, 82 animals (156 ± 66.0 DIM, 2.0 ± 1.19 lactations, 34.0 ± 6.13 kg/d, 707 ± 69.5 kg of BW distributed as follow n = 5 cows/treatment in early DIM, n = 17 cows/treatment in mid-early DIM, n = 11 cows/treatment in mid-late DIM, n = 8 cows/treatment in late DIM, and the same number of cows per study within DIM category) from both treatments homogeneously distributed within DIM category and period were selected to evaluate the algorithm for precision feeding by calculating total daily energy and protein intake for the last 5 d of the study, and the proportion of nutrients consumed above or below their estimated requirements for their actual production level using NRC (2001) equations.

2.4. Statistical analysis

All data were analysed using the SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Comparisons with $P < 0.05$ were considered significant, whereas comparisons with $0.05 \leq P < 0.10$ were presented as tendencies. The experimental unit for the statistical analysis was cow because treatments were applied at the cow level. Performance and nutrient evaluation (nutrient requirements, TMR and PMR nutrient intake, and proportion of nutrients overconsumed) data were analysed with a mixed-effects model that included the fixed effects of feeding system, lactation stage (early, mid-early, mid-late, and late DIM), day of study (from 17 to 21), and their 2-way and 3-way interactions plus the random effect of pen, study, and cow within pen (to account for the dependence of

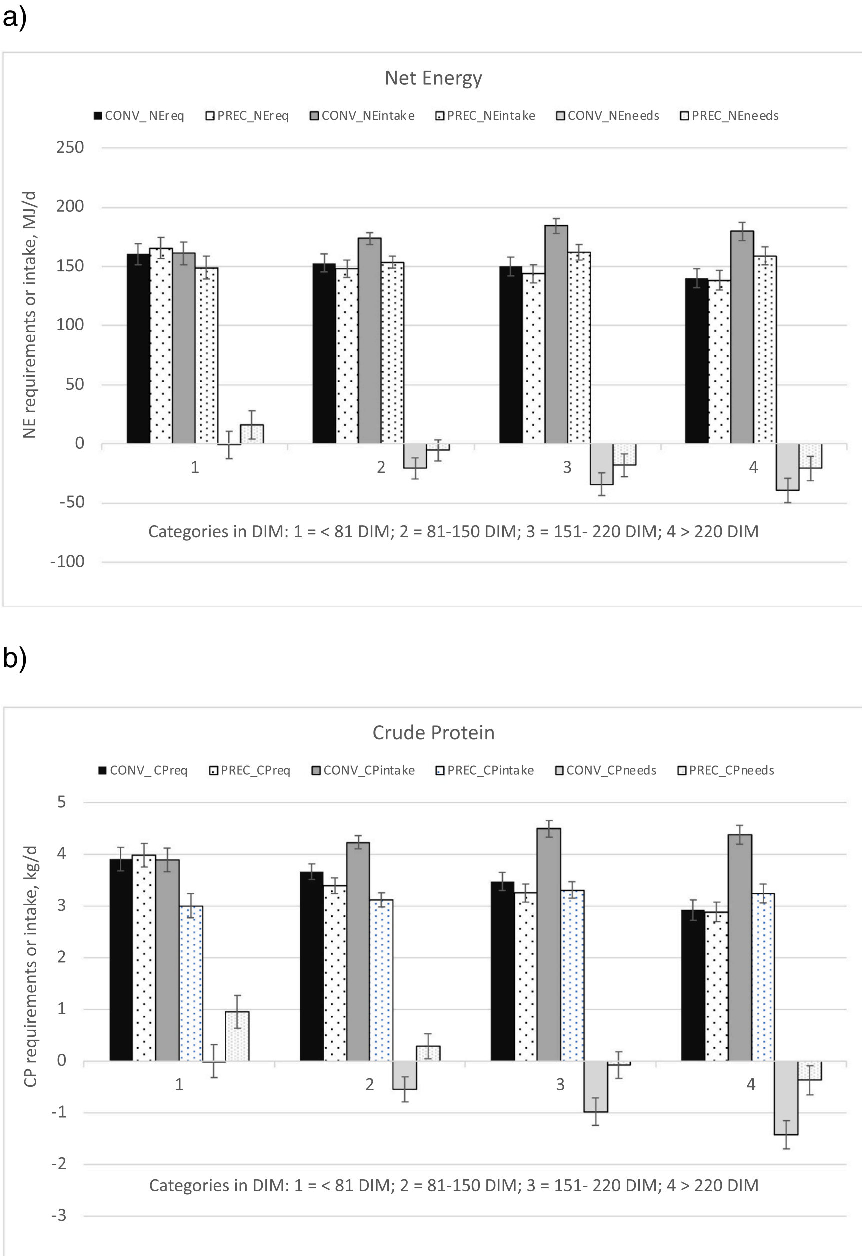

Fig. 1. Least square means of net energy (1a) and crude protein (1b) requirements (NEreq and CPreq), intake (NEintake and CPintake) and needs (NEneeds and CPneeds) of dairy cows fed under a conventional (CONV, solid fill) or a precision (PREC, pattern fill) feeding system using performance data of the previous 10 rolling days and NRC (2001) equations, and categorized in 4 DIM categories (data from periods 1 and 2).

animals within pen), parity (primiparous or multiparous) as a block. Day entered in the model as a repeated measure using an autoregressive order 1 or compound symmetry variance-covariance matrix according to the lowest Bayesian information criterion. The 3-way interaction was not significant for any parameter, and it was removed from the final model. The model used was:

$$Y_{ijklmno} = \mu + C_i\,(P_j) + P_j + S_k + L_l + T_m + M_n + D_o + (T_m \times M_n) + (M_n \times D_o) + (T_m \times D_o) + (T_m \times M_n \times D_o) + e_{ijklmno}$$

where Y_{ijklmn} is the dependent variable, μ is the overall mean, C_i (P_j) is the cow within pen, P_j is the pen, S_k is the study, L_l is the lactation number, T_m is the treatment (CONV or PREC), M_n is the categorical DIM, D_o (k = 17 − 21) is the day of study, ($T_m \times M_n$) is the interaction between treatment and categorical DIM, ($M_n \times D_o$) is the interaction between categorical DIM and day, ($T_m \times D_o$) is the interaction between treatment and day, ($T_m \times M_n \times D_o$) is the interaction between treatment, categorical DIM and day, and e_{ijklmn} is the residual error. Pen, study, and cow within pen were random effects, lactation number a block, and the other parameters were fixed effects.

Data pertaining to the N balance in Study 1, were analysed with an analysis of variance considering the feeding system as fixed effects, parity as a block, and pen as a random effect. The model used was:

$$Y_{ijklm} = \mu + P_i + L_j + T_k + e_{ijk},$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, P_i is the pen, L_j is the lactation number, T_k is the treatment (CONV or PREC), and e_{ijk} is the residual error. Pen was the random effect, lactation number a block, and feeding treatment was fixed effect.

Gaseous emissions from boxes in Study 2 were analysed with a mixed-effects model that accounted for the fixed effects of feeding system, day of sampling, and their interaction plus the random effect of the box. Day entered the model as a repeated measure using the autoregressive order 1 or compound symmetry variance-covariance matrix according to the lowest Bayesian information criterion. Herein, the experimental unit was box (n = 2).

The model used was:

$$Y_{ijk} = \mu + B_i + T_j + D_k + (T_j \times D_k) + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, B_i is the box, T_j is the treatment (CONV or PREC), D_k is the day of sampling, $T_j \times D_k$ is the interaction between treatment and the day of sampling, and e_{ijk} is the residual error. Box was a random effect, and the other parameters were fixed effects.

3. Results

From the initial dataset of 114 animals, a group of 26 cows from period 1 were selected to perform a N balance, another group of 82 cows from both periods were selected for the evaluation of nutrient adjustment and performance responses. All 114 animals were considered for the evaluation of manure emissions in period 2.

3.1. Diets

Ingredient composition of the diets was different in both periods because they were conducted in different seasons (Table 1), and forage availability was different. Dietary DM, aNDF, ADF, and fat concentrations were slightly greater in period 1 than in period 2, but

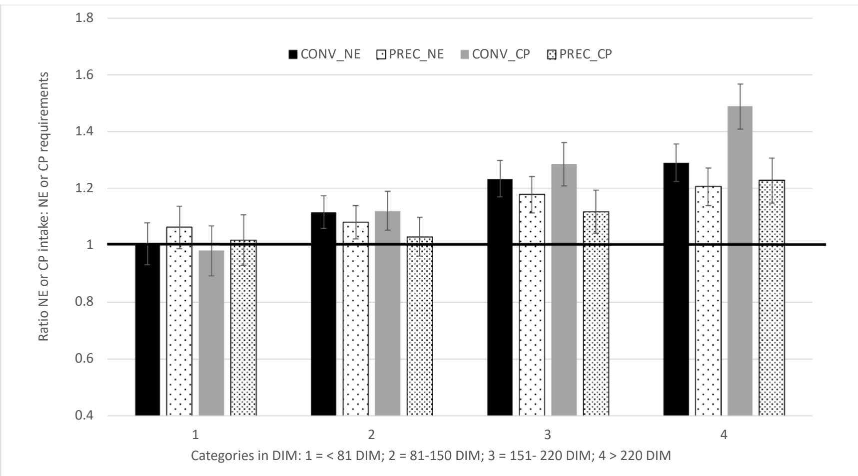

Fig. 2. Least square means of the proportion of net energy (NE) and crude protein (CP) overfed in cows fed under a conventional (CONV, solid fill) or a precision (PREC, pattern fill) feeding system using performance data of the previous 10 rolling days and NRC (2001) equations, and categorized in 4 DIM categories (data from periods 1 and 2).

CP content was kept similar in both studies.

3.2. Nutrient adjustment and performance

Estimated energy (Fig. 1a) and protein (Fig. 1b) requirements decreased ($P < 0.001$) with DIM categories and increased with day of study, and there were slight differences ($P < 0.05$) between days and treatments. Estimated energy requirements increased in CONV cows from 20 to 21 d, and estimated CP requirements increased from 17 to 18 d, and from 20 to 21 d of study; whereas estimated energy requirements PREC fed cows were similar during the last 5 d of study, but estimated CP requirements increased with time. Neither energy nor CP estimated requirements differed between treatments within DIM categories. As expected, energy and CP intake (Fig. 1a and 1b) from the TMR and PMR was greater ($P < 0.001$) in CONV than in PREC cows and there were slight differences ($P < 0.001$) in the daily variation between treatments and DIM categories. Cows in both treatments and DIM categories covered their estimated energy and CP requirements solely with consumption of TMR and PMR, except for PREC cows in the early DIM category that required a supplement of 16.4 extra MJ of NEl/d, and 955 g of CP/d, and PREC cows in mid-early DIM category that needed 287 extra g of CP/d (Figs. 1a and 1b). Both CONV and PREC cows overconsumed energy without differences between DIM categories and treatments (Fig. 2), and with slight variations ($P < 0.05$) between days of study. However, CONV cows consumed more ($P < 0.05$) CP above their requirements than that that consumed by PREC cows, and these differences tended ($P = 0.09$) to be greater in cows with mid-late and late DIM (Fig. 2).

Although it was not the main objective herein, due to the short duration of the study, cows produced similar amounts of milk in both feeding treatments, and as expected it progressively decreased ($P < 0.01$) as DIM category increased. Milk components (fat and protein) did not differ between feeding treatments, and both increased ($P < 0.01$) by DIM category. Milk fat content in PREC cows tended ($P = 0.09$) to be similar whereas in CONV fed cows tended to increase at mid-early and mid-late DIM (Table 2). On the other hand, milk N urea was lower ($P < 0.05$) in PREC than in CONV cows from early-mid DIM to late DIM, whereas it did not differ in cows with early DIM between feeding treatments. Total DM intake was similar in both feeding schemes, but CP intake was lower ($P < 0.05$) in PREC cows with more than 81 DIM in comparison with CONV cows (Table 2). Dietary energy density of the total diet, considering feed supplements in the milking parlour, was lower ($P < 0.001$) and aNDF content greater ($P < 0.001$) in PREC than in CONV, but it did not differ across DIM categories within treatment. However, dietary CP content decreased ($P < 0.05$) within DIM category in PREC and it was constant in CONV.

3.3. N balance and diet digestibility

During the 3 d of N balance duration, N intake and N concentration in milk and faeces were similar in both feeding regimes, but N in urine was greater ($P < 0.05$) in CONV than in PREC cows (Table 3). Digestibility of DM and apparent CP digestibility were not affected by feeding regime, but aNDF digestibility was greater ($P < 0.05$) in PREC than in CONV cows. Lastly, plasma urea concentration was unaffected by feeding strategy.

3.4. Manure emissions

Manure of PREC cows had lower ($P < 0.05$) pH and ammonia N concentration than manure from CONV cows. Although TC and

Table 2

Least square means of milk yield and composition, and nutrients intake of dairy cows fed under a conventional (CONV) or a precision (PREC) feeding system during a period of 5 days, after 16 days of adaptation (period 1 and 2).

	Treatments[a]			P-values[b]		
	CONV	PREC	SEM[c]	FS	SL	FSxSL
Cows, n	41	41	-	-	-	-
Body weight, kg	713	708	12.5	0.76	< 0.001	0.95
Total DM intake, kg/d	26.0	25.7	0.64	0.71	0.10	0.19
Total CP intake, kg/d	4.25	3.72	0.132	0.002	0.15	0.03
Diet CP, g/kg of DM	164	145	1.4	< 0.001	0.007	0.018
Diet aNDF, g/kg of DM	356	379	35.8	< 0.001	0.05	0.31
Diet NEl, MJ/kg of DM	6.74	6.57	0.100	< 0.001	0.21	0.12
Milk yield, kg/d	34.0	33.8	1.70	0.90	< 0.001	0.62
ECM, kg/d[d]	35.4	35.4	2.61	0.98	< 0.001	0.53
Milk fat, g/kg[e]	37.7	38.1	2.54	0.59	< 0.001	0.09
Milk protein, g/kg[e]	34.3	33.9	0.44	0.34	< 0.001	0.97
Milk N urea, mg/dL	10.8	6.8	25.05	< 0.001	0.73	0.02
Feed efficiency	1.41	1.40	0.129	0.84	< 0.001	0.70

[a] CONV = cows fed a PMR in the feed bin scales; PREC = cows fed a PMR plus a concentrate feed supplementation in the milking parlour
[b] FS = effect of the feeding system; SL = effect of stage of lactation; FSxSL = effect of the interaction of feeding treatment with stage of lactation
[c] Standard error of the mean
[d] Energy corrected milk calculated as (0.3246 × milk) + (12.86 × kg fat) + (7.04 × kg protein) (Bernard, 1997)
[e] Milk fat and protein components content of daily measured using the AfiLab system.

Table 3
Least square means of digestibility and nitrogen balance of dairy cows fed under a conventional (CONV) or a precision (PREC) feeding system (days 19–21 of period 1).

	Treatments[a]			P-value[b]
	CONV	PREC	SEM[c]	FS
Cows, n	13	13		
Apparent digestibility, g/kg				
DM[d]	605	618	10.7	0.42
aNDF[d]	426	469	17.2	0.08
CP[d]	481	450	13.4	0.12
N balance,				
N intake, g/d	636	636	56.1	0.99
Milk protein N, g/d	189	182	11.5	0.64
Urinary total N, g/d	196	140	12.8	0.006
Faecal N, g/d	305	308	19.9	0.91
N retained, g/d	-51.4	-0.4	35.1	0.32
BW change, kg[d]	-12	-3	7.3	0.35
As a proportion of N intake, g/kg				
Milk N	298	295	12.4	0.85
Urine N	312	226	32.0	0.07
Faecal N	484	498	13.5	0.46
Plasma urea, mg/dL	18.2	15.5	1.45	0.19

[a] CONV = cows fed a PMR in the feed bin scales; PREC = cows fed a PMR plus a concentrate feed supplementation in the milking parlour
[b] FS = effect of the feeding system
[c] Standard error of the mean
[d] DM: dry matter, aNDF: amylase treated neutral detergent fiber, CP: crude protein, BW: body weight.

ADF concentration were similar in both treatments, the ratio C to N tended to be greater ($P < 0.10$) in the manure from PREC than in that from CONV cows (Table 4). Ammonia emissions decreased throughout sampling days, and although they did not differ between both feeding systems (Table 4), they were numerically reduced by 20% in PREC in comparison to CONV manure. In contrast, carbon dioxide and methane emissions increased ($P < 0.05$) in PREC manure, by 14% for carbon dioxide and 55% for methane compared with CONV manure (Table 4). The emissions of carbon dioxide and methane emissions from manure in PREC increased throughout the 14 d of monitoring, in contrast to CONV manure, which carbon dioxide and methane emissions started to decrease by 14 d (Fig. 3). Nitrous oxide emissions tended ($P = 0.06$) to be lower in PREC compared with CONV manure at 14 d of sampling (11%).

4. Discussion

Differences observed in milk components in the different lactation stage categories such as an increase in fat, protein in greater DIM

Table 4
Least square means of manure characterization and gas emission production (g/m^2 h) under conventional (CONV) and precision (PREC) feeding treatments for 14 days using manure from period 2.

	Feeding system[a]			P-values[b]		
	CONV	PREC	SEM[c]	FS	time	FSxtime
Number of boxes	2	2				
Manure characterization						
pH	7.45	7.17	0.045	0.01	0.05	0.02
Conductivity	4.07	3.23	0.882	0.54	0.06	0.71
Total Solid content (g/kg)	136	137	6.5	0.89	0.03	0.89
Volatile Solid content (g/kg)	107	108	4.6	0.85	0.03	0.89
Total ammonia, g/kg[d]	13.4	10.2	0.46	0.005	0.002	0.55
Total N, g/kg[d]	45.3	38.7	1.4	0.08	0.002	0.008
Total C, g/kg[d]	505	476	22.2	0.45	0.77	0.42
Ratio C/N	11.2	12.3	0.28	0.10	0.16	0.19
aNDF, g/kg[d]	514	413	37.3	0.13	0.17	0.76
ADF, g/kg[d]	374	339	21.2	0.30	0.81	0.74
Gas emissions, g/m[b]h						
Ammonia	0.75	0.51	0.112	0.24	0.006	0.85
Carbon dioxide	16.9	26.8	1.92	0.02	0.004	0.010
Methane	1.59	3.94	0.512	0.03	0.015	0.041
Nitrous oxide	28.1	24.4	1.21	0.10	< 0.001	0.06

[a] CONV = cows fed a PMR in the feed bin scales; PREC = cows fed a PMR plus a concentrate feed supplementation in the milking parlour
[b] FS = effect of the feeding system; time = effect of day of study; FSxtime = effect of the interaction between feeding system with day of study
[c] Standard error of the mean
[d] as dry weight

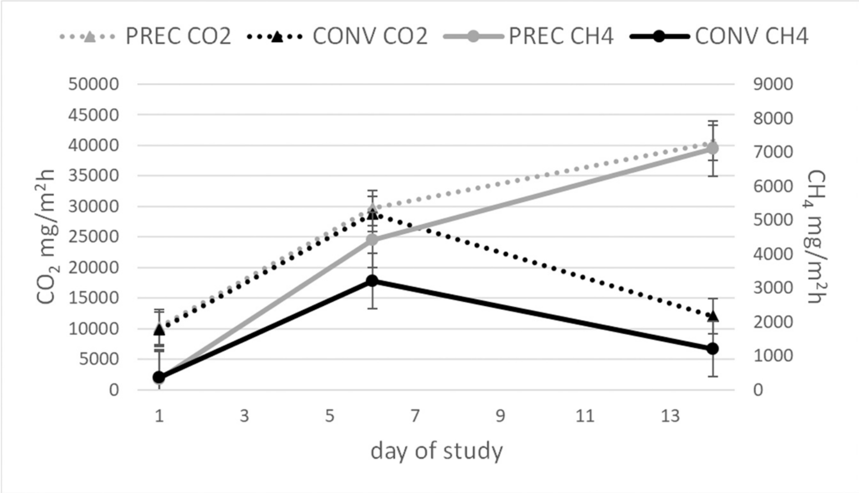

Fig. 3. Least square means and standard error of the mean of the evolution of methane (right axis) and carbon dioxide (left axis) emissions from manure of cows fed under a conventional (CONV) or a precision (PREC) feeding system during 2-week storage (period 2).

cows are widely described in the literature (Linn, 1988), and the main interest in splitting data herein in DIM categories was to determine whether a precision feeding system would have the same capacity to modulate nutrient supply according to stage of lactation and thus elicit a similar response in performance throughout all phases of lactation.

Cows fed in CONV feeding system overconsumed CP except those with less than 81 DIM. However, PREC cows consumed the amount of CP needed to satisfy their estimated requirements in cows < 150 DIM, but PREC cows with > 150 DIM overconsumed CP. Cows in CONV received a fixed CP concentration in their diet, whereas PREC cows were offered a range between 133 and 167 g of CP/kg of DM. In general, PREC cows consumed less CP than CONV cows, but PREC and CONV cows used in the N balance had similar N intake, probably because the selected cows for N balance averaged 161 DIM. Olmos Colmenero and Broderick (2006) evaluated different dietary CP levels (from 135 to 194 g of CP/kg of DM) and reported no changes in production traits, but they also reported a decrease in milk urea N (MUN) as CP levels in the diet decreased. However, Law et al. (2009) described performance benefits when cows were fed 173 g of CP/kg of DM (compared with 144 and 114 g of CP/kg of DM) during the first half of lactation, but they did not observe detrimental effects on production when CP was decreased to 144 g of CP/kg of DM during the second half of lactation. The low MUN observed in PREC cows from cows with more than 81 DIM suggests that these cows had a limited dietary CP supply, and this fact might limit long-term milking performance as observed in Reynolds et al. (2016). Our study covered a relatively short period of time because the main objectives were to evaluate the precision feeding system, but Reynolds et al. (2016) conducted a study to assess long-term effects of dietary protein concentration (140, 160, 180 g of CP/kg of DM) over 3 consecutive lactations. Their results indicate that low protein diets (i.e., 140 g of CP/kg of DM) are more efficient, but they have detrimental effects on milk yield. This suggests a need to evaluate this precision feeding system in long-term conditions to properly assess performance data.

Estimated energy needs were met with both diets except for early DIM cows in PREC, who needed some feed supplements to fully satisfy them. However, in general, cows overconsumed energy, even in PREC cows, in part, because when supplementing for CP using soybean pellets, cows were also supplemented for energy. However, CONV cows were fed a constant of 6.82 or 6.65 MJ of NEl/kg in period 1 and 2, respectively, in contrast to PREC cows that consumed varying amounts of supplements in the milking parlour resulted in a range in energy density of the total DM consumed between 6.82 and 6.40 MJ of NEl/kg. Maltz et al. (2013) proposed to feed energy by adjusting caloric density of the diet between 1.59 and 1.68 NEl weekly for 3–19 wk postpartum, and they succeeded in improving dairy cow performance.

The equations used to estimate the protein and energy requirements of PREC cows, decreased protein and NE concentration in the diet, and increased aNDF dietary concentrations in PREC cows. This allowed to reduce urine N excretion, but also to improve aNDF digestibility. Although the AIA recovery rate was not calculated in the present study, the ratio AIA in faeces: PMR was similar in both diets (2.44 vs 2.46 $\pm$ 0.086 in CONV and PREC fed cows, respectively). Lee and Hristov (2013) calculated recovery rate for high-producing dairy cows fed two different CP diets, and they obtained similar AIA recovery rate for both diets (86.9%). However, the ratio AIA faeces: PMR was greater in low than in high CP diet, and it resulted in greater apparent nutrient digestibilities in low than in high CP diets. In our study, we could envisage a similar impact of AIA recovery rate in both feeding systems. In the present study, nutrient digestibility was lower compared with the literature (Martins et al., 2022; Hynes et al., 2016), which was probably due to an overestimation of fecal output when using AIA as an internal marker (Morris et al., 2018). This fact has probably affected N fecal output, overestimating its value, and it may explain why N balance of cows are negative, while calculation suggest an excess of protein in most of the animals. Rius et al. (2010) observed a maximal ENU when a high energy diet was combined with a low protein diet. In contrast, herein, PREC fed cows did not improve ENU. The lack of improvement might be due to the use of milk total protein instead of milk true protein in N balance calculations as done in Rius et al. (2010) study. Although milk NPN was not measured in our study, we analysed MUN, and it was greater in CONV than in PREC cows. Assuming that MUN is the main contributor to milk NPN (dePeters and

Ferguson, 1992) and both measures are correlated (Ruska and Jonkud, 2014), we could envisage more NPN concentration in CONV than in PREC cows. Both, NPN composition and milk NPN yield was increased in late lactation cows fed 165 vs 120 g of CP/kg of DM diets (Cantalapiedra-Hijar et al., 2014). When using the equation proposed by dePeters and Ferguson (1992) based on blood urea N and milk yield to predict NPN yield, ENU herein resulted in 19.5% and 19.1% in CONV and PREC fed cows, respectively. These values were lower than the ones reported in Table 4, but they followed the same trend. When N excretion in faeces, urine, and milk were subtracted to N intake, the negative N balance was numerically worse in CONV than in PREC cows, which was in line with the numerical BW loss observed in both feeding treatments. The difference in urinary N output between both diets was expected since almost all N ingested in excess of requirements is excreted in urine (Castillo et al., 2000; Cantalapiedra-Hijar et al., 2014). Olmos Colmenero and Broderick (2006) also reported similar percentage of urinary N excreted as dietary CP increased. The reduction in N excreted in urine represents a potential reduction of footprint and ammonia emissions and a potential reduction in feed costs (Hynes et al., 2016). Although manure storage emissions were evaluated in Study 2 taking manure directly from the floor of the pen, and N balance was performed in Study 1, the lower total N concentration in the boxes of manure of cows fed PREC than in those on CONV, also indicates a reduction of N in the manure from PREC cows, which resulted in a numerical reduction of ammonia gas and tendency to increase nitrous oxide emissions. The N in urine is rapidly hydrolysed to ammonia and then transformed to nitrous oxide via nitrification-denitrification process (Dijkstra et al., 2011). As reported by Mohankumar Sajeev et al. (2018), the reduction of dietary CP could represent an ammonia emission decrease by 42 ± 21%. Besides, the reduction of CP in manure also affects methane emissions (Mohankumar Sajeev et al., 2018; Montalvo et al., 2013). These authors described an increase in methane emissions of 20 ± 30% by each percentage-point reduction in dietary CP. In our study, PREC cows consumed a diet with a 2% points lower CP than CONV, and the manure methane emissions increased 27.5% increase per every percentual-point difference in dietary CP in PREC. This could be explained by an increase in the concentration of carbohydrates of diets with low protein concentration and a decrease in fibre digestion, which tend to increase manure methane production (Nampoothiri et al., 2015). Furthermore, the dimensions of the box used for manure storage and its depth could also influence gas emissions. The minimum depth recommended for lagoons is 2.5 m, being 2.5 – 6 m typical values (Pfost et al., 2000). As the depth increases, the surface can be reduced, so less emissions can be released. Leytem et al. (2017) described methane emissions from different studies ranging from 125 mg/m^{2}h to 8458 mg/m^{2}h, but depth was not a factor affecting the emissions. Temperature and wind were the main ones. McGinn et al. (2008) described ammonia emissions in dairy cow manure stored in lagoons over summer from 3600 to 8600 mg/m^{2}h depending on the time of the day, while Leytem et al. (2018) reported an annual ammonia emission media in lagoons from 50 to 179 mg/m^{2}h with variations depending on the season (temperature, rain and wind involved), the fraction stored (liquid or solid) and the formation of a natural crust (Grant and Boehm, 2020).

Nutrient manure characterization of CONV and PREC fed cows resulted in a similar C, aNDF, and ADF content for both treatments, and only the ratio C to N slightly favoured PREC manure to produce more methane (Dobre et al., 2014). Furthermore, in Study 1, PREC cows showed an improvement of fibre digestibility, which might suggest a more cellulolytic microbiome that may also enhance biogas production (Sinha et al., 2021). Methane and carbon dioxide emission peak for CONV manure was found by day 6, and suddenly a reduction of emissions occurred. This fact can be explained by the formation of a superficial solid air-dried crust which may affect gas transportation, as it decreases superficial water content and thus, the decrease of liquid-air (atmosphere) exchanges (Aguerre et al., 2012; Misselbrook et al., 2005; VanderZaag et al., 2008). Although there were differences in methane emission between PREC and CONV manure, both values were within the range of emission (3–25 g methane/m^{2}h) proposed by di Petra et al. (2019) during the first 2 wk of storage. In our study, we did not measure enteric emissions, but a prediction of the methane emission factor (Y_m) was performed in cows in Study 1, in which diet digestibility was assessed, using the equation described by Jaurena et al. (2015). No differences between feeding treatment were observed in predicted methane emissions (3.19 and 3.49 ± 0.155% GE in CONV and PREC cows, respectively). Therefore, we can only infer herein that manure from PREC cows generates more methane emissions during storage than the manure from CONV cows. Thus, if manure is used as a substrate for biogas production or if methane is recovered from covered ponds, precision feeding can be considered as a good alternative, not only to reduce ammonia emissions from manure storages and land application, but also to produce more biogas during the anaerobic fermentation, thus reducing GHG emissions to the atmosphere.

5. Conclusion

In conclusion, adjusting total dietary N supply based on individual animal requirements can be an effective strategy to reduce N excretion. Further research is envisaged to evaluate long-term effect of this proposed precision feeding. In addition, a potential reduction of ammonia emissions and an increase of GHG emissions (methane and carbon dioxide) from manure of cows fed using a precision feeding strategy could be expected. This increase of methane production during the storage of manure from cows fed using a precision strategy, opens a new possibility towards the integration of animal feeding systems and manure valorisation technologies to increase the sustainability of the dairy sector.

CRediT authorship contribution statement

Lluis Morey: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Alex Bach:** Conceptualization, Methodology, Software, Writing – review & editing. **Daniel Sabrià:** Resources, Investigation. **Víctor Riau:** Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Belén Fernández:** Writing – review & editing, Supervision. **Marta Terré:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

None.

Data Availability

The data that support the findings of this study are available from the corresponding author (M. Terré), upon reasonable request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2023.115722.

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