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1. Background and objectives

This deliverable reports the activities and results regarding two strategies tested at farm level. The first consisted in the assessment of feeding strategies and precision feeding tools of ruminants concerning the gaseous emissions (from manure) and the effect on manure characteristics. The second consisted in the evaluation of different managing strategies of the bedding on the gaseous emissions and the effect on manure characteristics.

1.1. Background

Animal production systems are important and complex sources of greenhouse gas (GHG) emissions, notably methane (CH4), nitrous oxide (N2O) and carbon dioxide (CO2). The GHG emissions of cattle are nearly 65% of the livestock sector's emissions: 4.6 gigatones CO2-eq, being 2.9 and 1.4 gigatones CO2-eq from meat and milk production, respectively (Gerber et al., 2013). Entheric fermentation is the main source of emissions from cattle: 1.1 gigaton CO2-eq, which accounts for 46% and 43% of the total emissions in the dairy and beef supply chains respectively. Feed-related emissions, including emissions from pasture handling, are the second most important category of emissions, contributing about 36% of emissions from milk and meat production. In this category, emissions of N2O from mostly fertilisation of feeds predominate (Gerber et al., 2013).

Emissions in the livestock sector can be reduced by reducing the intensity of emissions, by reducing the consumption of resources (particularly food) in production, or by combining the two. In this context, food efficiency emerges as being of greater relevance because of its impact on the environmental footprint of the livestock industry and because of the wide range of resources between human feed, animal feed and biofuels.

Manipulation of diet and feed additives have been considered the main methods for mitigating enteric CH4 production. In general, their effectiveness is estimated to be low to medium in absolute emissions, but some of these options can achieve considerably lower emission intensity levels by improving feed efficiency and animal productivity. Diets also affect manure emissions by modifying the content of manure: the composition of the rations and additives influence the form and amount of nitrogen in urine and faeces, as well as the amount of organic matter fermentable in faeces. CH4 emissions from manure can be effectively controlled by shortening the storage duration, ensuring aerobic conditions, or capturing the biogas emitted under anaerobic conditions. However, it is much more difficult to prevent direct and indirect N2O emissions after nitrogen is excreted. Techniques that avoid emissions during the initial phases of management conserve nitrogen in manure, which is often released in later phases. Thus, effective mitigation of nitrogen losses (i.e., as ammonia NH3) is often offset by nitrogen losses in other forms (i.e., as N2O or NO3). These transfer effects must be considered when designing mitigation practices (see Annex A).

Nitrogen excretion from livestock production contributes to environmental pollution (Castillo et al., 2000). The loss of nitrogen from urine and manure in form of NH3 emitted to the atmosphere or deposition can produce soil acidification, air quality degradation, biodiversity loss and eutrophication. Ammonia 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) or in nitrate accumulation in groundwater resulting in less water availability (Postigo et al., 2021).

Therefore, there is pressure on the livestock industry to adjust diets to animal requirements avoiding nutrients overfeeding, and to mitigate ammonia and greenhouse emissions. The protein and amino acid content of diets is optimized, resulting in relatively lower amounts of nitrogen excreted in faeces and urines and thereby reducing N2O emission per kg of milk. Gerber et al. (2011) found a significant

relationship between milk production per cow and total greenhouse gas emissions per kilogram of milk.

Dairy cows need different amounts of protein depending on their milking performance and physiological status. However, because cows are fed in groups, and cows consume different amounts of dried matter (DM), nutritionists formulate rations using percentages rather than amounts. Feeing dairy cows in mid-lactation between 16.5-17% crude protein (CP) is sufficient to maintain their production levels (Olmos Colmenero and Broderick, 2006; Groff, 2005). As lactation advances, CP recommendations decrease below these levels (Law et al., 2009) and dairy cows in late lactation fed 16.5% CP may increase urinary and faecal N excretion and reduce profits. Barros et al. (2017) evaluated CP levels in diets of late-lactation cows and N use efficiency improved at 14.4% CP levels, but not lower than that, since lower CP levels had greater negative impacts on milk yield and efficiency.

1.2. Objectives

To evaluate the impact of precision feeding technology (that adjust individual dairy cow nutrient requirements), in comparison with conventional feeding (a unique diet for all the animal independently of their stage of lactation), on dairy cow performance, feed efficiency and N balance (including excreted urine and faeces, and their storage).

To evaluate the impact of type of bedding materials during animal housing and yard operations on GHG emissions (including NH3), as well as the animal performance, behaviour, and cleanliness.

2. Materials and methods

2.1. Precision and conventional feeding

IRTA assessed the precision feeding system in its experimental facility (EVAM), a dairy cow farm located in Monells (Girona). The technical implementation of the precision feeding at IRTA facility is summarized in Annex B. Two studies were performed within the Circular Agronomics project (study 1 and 2) to evaluate the impact of precision feeding systems on performance, N balance and emissions from manure (Table 1) in 2019 November and 2021 May.

Each study included 28 dairy cows (11 primiparous and 17 multiparous) Holstein dairy cows (723 ± 3.1 kg of BW; 34.5 ± 1.69 kg/d of milk; 159 ± 17.7 DIM) that were blocked by parity and DIM, and randomly assigned to a conventional (CONV) feeding scheme based on a unique TMR or to a precision feeding scheme (PREC) for a 21 day period. The CONV group was offered a TMR (1.63 Mcal/kg DM, 16.5% CP), and PREC cows were fed a partial mixed ration (PMR; 1.59 Mcal/kg DM, 13.5% CP) and a concentrate feed supplement twice daily in the milking parlor, which contained different proportions of soybean meal, corn, and wheat middlings according to animal estimated needs above the PMR consumption. Daily needs[1] were calculated using NRC equations (2001) with a rolling average of performance data (milk yield and quality, and body weight) from ten preceding days, and subtracting the nutrients consumed from the PMR computed also using a rolling average from ten preceding days. Daily TMR and PMR intake, milk yield and quality were daily recorded. The energy corrected milk (ECM) was calculated following NRC (2001), and the feed efficiency was computed as the of ratio milk or ECM to total DMI, and the body weight (BW) change was calculated as the difference between the average BW in the week before the study and in the last week of study.

[1] For the precision feeding, the algorithm to evaluate dairy cow requires the information collected in the farm (milk yield and composition, daily body weight, dry matter, and nutrients intake) to finally obtain the amount of the 3 different feeds (soybean, a mixture of wheat and corn meal, and wheat middlings) to be offered in the milking parlour was implemented successfully before this study (Annex B).

Table 1. Summary of feeding studies 1 and 2.

	Study feeding 1		Study feeding 2	
Feeding strategy	CONVENTIONAL	PRECISION	CONVENTIONAL	PRECISION
Season	November 2019		May 2021	
Animals	24	28	27	30
Nutrient composition, as DM basis				
NE_L, Mcal/kg	1.63	1.59	1.59	1.53
DM, %	60.5	60.7	53.4	53.1
CP, %	16.4	13.9	16.5	13.7
NDF, %	38.9	43.1	32.2	35.0
Fat, %	4.7	4.9	3.0	3.1
Sampling				
DM intake	x	x	x	x
Milk yield and composition	x	x	x	x
Body weight	x	x	x	x
N balance	x	x	-	-
Manure evolution	x	x	x	x

For N balance, 26 animals (blocked by parity and DIM) from Study 1 were selected. During the last 3 days of the experiment, feed, faecal and urine spot samples were collected at different times along the day. On these 3 consecutive days, TMR offer, and refusals samples were collected and composited for the measurement period for further nutrient composition analysis. Samples were homogenized, for further analysis of N content in feed, urine, and faeces. Faecal spot (about 300 g) samples from the rectum and urine spot (about 250 mL) samples obtained by massaging the perivaginal area were collected from 19 to 21 d of study, at different time hours (on day 1 at 08:00, 14:00, 20:00, 02:00 h; on day 2 at 10:00, 16:00, 22:00, 04:00, and on day 3 at 12:00, 18:00, 00:00, 06:00 h).

Faecal samples were dried at 60ºC for 4 days, grounded, and further composited on a dry weight basis by cow. The composite samples were analysed for dry matter (DM), total N content (APHA 2005), neutral detergent fibre (NDF), and acid insoluble ash (AIA) as internal marker (Sales and Janssens, 2003). TMR samples were composited and analysed for DM, CP, NDF, and AIA. Total tract apparent digestibility was calculated based on AIA concentration in mixed rations and faeces. Aliquots of 30 mL of urine were immediately acidified with 0.1 M sulphuric acid, diluted 1:5.7, and stored at -20ºC. These samples were composited by cow and analysed for total N (APHA 2005). 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, based on urine N concentrations, used to estimate total urinary N excretion. N efficiency was calculated as the proportion of N excreted in milk of the total N intake.

For the storage of manure collected in feeding studies 1 and 2, 0.6 m3 of a mixture of bedding & manure of PREC and CONV pens was collected and stored in open air containers (2 replicates per manure type, 4 containers). These containers have holes in all walls allowed a passive aeration of piles and the drainage of leachates (dimensions of containers 1.11x 0.91x0.43 m) (Figure 1). All containers were covered, avoiding rainfall, and kept at ambient temperature during the storage.

For the feeding study 1, manure from CONV and PREC pens were store without stirring for 30 days (2019 July). In that period, 3 sampling campaigns were carried out (beginning, middle and final stage of the storage). For the feeding study 2, the storage lasted 47 days (2021 May-July). In this case, 4

storage options, CONV manure with and without stirring, PREC manure with and without stirring, were evaluated. From day 14th, when a natural crust at the upper layer became significant, 2 replicates (1 per treatment) were stirred just before sampling, whereas the two others were kept without stirring.

Periodic sampling campaigns were done to monitor the pH, total C and total N (TC, TN), total ammonia nitrogen (TAN), volatile fatty acids (VFA), total and volatile solids content (TS, VS) of stored manures, following APHA methods (2005). Also, the GHG and NH3 emissions per container was monitored. The gas samples for the determination of the GHG and the NH3 concentration on quiescent surfaces (containers) were collected with a Lindvall hood (model CSD30; Olfasense GmbH) (Martínez et al. 2004; Richter and Frechen, 2009; Prenafeta-Boldú et al., 2015; Torrellas et al., 2018). The samples for NH3 determination were taken by bubbling the 3-L of air into 20 mL acid solution (sulphuric acid 10%) tubes, and subsequent quantification by UV-VIS spectrometry at the laboratory according to the NIOSH 6015 method (Eller and Cassinelli, 1994). A 30-mL samples of air were collected in 12.5 mL vacutainer-type tubes (Labco Ltd., Buckinghamsire, UK) for GHG measurement. The analysis of GHG was done by gas chromatography (model Thermo Trace 2000, Thermo Finnigan Scientific) equipped with a flame ionization detector and an electron capture detector (model 7820A, Agilent). The measurement of the NH3 concentration were also done in-situ with a portable sensor equipped with an electrochemical detector with a sensitivity range of 0.5 to 150 ppm v/v (model GX-6000, RKI Instruments).

Statistical analysis

Performance data of both studies were analysed together. The experimental unit for the statistical analysis was pen. Data were analysed with a mixed-effects model including the fixed effects of feeding system, DIM category (early or late), day of study, and their 2 -way interaction plus the random effect of pen, study, and cow within pen (to account for the dependence of animals within pen), and lactation number during the study as covariates. Time entered the model as a repeated measure using the autoregressive order 1 or compound symmetry according to the lowest Bayesian information criterion. Initial data and milk composition were analysed with a mixed-effects model including the fixed effects of feeding system, DIM category (early or late), and their 2 -way interaction plus the random effect of pen and study, and lactation number during the study as covariate. N balance in Study 1 were analysed with an analysis of variances considering feeding system as fixed effect, and lactation number and average DIM as covariates. Significance was declared at $P< 0.05$ and tendencies at $P\leq 0.10$.

2.2. Bedding materials

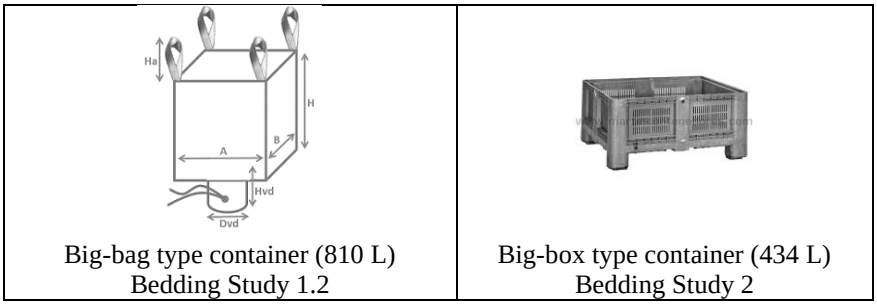
Three studies were conducted regarding bedding materials. Bedding study 1 was done on 2018 September - 2019 January. It was divided into two parts: bedding study 1.1 and 1.2. Then, a second bedding study was done on 2019 April-September. Finally, the bedding study 3 was performed in 2020 November – 2021 May.

The bedding study 1.1 was focused on the effect of cleaning frequency (once a week vs once every 2 weeks) and straw bedding length (long vs chopped) in fattening bulls (performance, behaviour, pen, and animal cleanliness). A total of 20 Holstein bulls (beef weight (BW) of 422 ± 4.2 kg and age of 292 ± 0.5 days) were allocated in individual pens (2.90 x 1.97 m) and distributed randomly (depending on initial BW) to 4 treatments that followed a 2×2 factorial design, being the cleaning frequency (once a week vs once every 2 weeks) and the straw bedding length (long vs chopped; both at a rate of 2.1 kg-straw/animal·d) as the studied factors. Concentrate and straw were recorded daily, while BW was recorded biweekly, for 56 days. Weekly, before bedding addition and cleaning of pens, the animal behaviour, and both animal and pen cleanliness scoring were recorded. Data were analysed with an analysis of variance or a Chi-Square.

T**he bedding study 1.2** was focused on beef animals' performance, behaviour and cleanliness raised under commercial conditions, but also the evolution of stored manure (composition evolution) was assessed. In this case, a total of 119 Holstein bulls (BW of 440 ± 5.6 kg and age of 250 ± 0.2 days) were allocated in 6 pens (6x12 m; 3 pens per treatment; 19-20 bulls/pen). Each pen was assigned to one of the two treatments: bedding with long or chopped straw, both at a rate of 1.9 kg-straw/animal·day.

Each pen had 1 drinker, 1 separate straw feeder, and 1 single-space feeder with lateral protections where concentrate was offered. Concentrate intake was recorded daily, and BW was registered fortnightly for 84 days. Animals were slaughtered after 84 days of study (6 periods of 14 days), HCW and carcass quality were recorded. Pens were cleaned once every 2 weeks and, in the week in-between, only 250 kg-straw/pen was added. The cleanliness of animals and pens was recorded twice weekly, but only records of 3 days after were analysed (as in the other records no differences among treatments were observed). Data were analysed using a mixed-effects model with repeated measures and categorical data with a Chi-Square.

During the storage of manure, and after the last cleaning, 2 piles per treatment (long or chopped straw manures) were built-up, with 130 kg-manure/pile. Four big-bag type containers (dimensions 0.9x0.9x1 m) with 5 holes (2 cm diameter) in the bottom (corners and centre) for leachate withdrawal were used to hold each pile (Figure 1). These containers were passive aerated and placed inside the farm in a dry and covered place, avoiding rainfall. These piles simulated the storage of the manure for 12 weeks at ambient temperature. Every 2 weeks, each pile was sampled to record pH and determine total C, total N, TAN, VFA, TS, VS evolution. Also, at day 0 and day 84 (week 12) of storage, each pile was weighed.



Big-bag type container (810 L)
Bedding Study 1.2

Big-box type container (434 L)
Bedding Study 2

Figure 1. Containers for manure storage during study 1.2 (left) and study 2 (right) with bedding materials.

The bedding study 2 was focused on the effect of long straw bedding in beef-animals (performance, behaviour, pen, and animal cleanliness). The long straw manure and acidification were combined to assess its effect on stored manure evolution (chemical composition; greenhouse gases (GHG) and ammonia (NH3) emissions). In this study with a total of 114 Holstein bulls (BW of 457 ± 3.77 kg and age of 296 ± 0.8 days) were housed in pens (6 x12 m; 3 pens/treatment; 19 bulls/pen). A total of 114 animals were allocated in 6 pens, and each pen was assigned to on the two treatments: added amount of long straw bedding at a rate of 1.87 or 3.75 kg/animal·day, labelled as "normal" or "extra", respectively. Pens were cleaned once every week and, in the in-between week only 250 or 500 kg of long straw/pen was added depending on treatment. Animal and pen cleanliness were recorded before cleaning once every week. Each pen had 1 drinker, 1 separate straw feeder, and 1 single-space feeder with lateral protections where concentrate was offered. Concentrate intake was recorded daily, and BW at day 0 and day 22. Animals were slaughtered after 22 days of study (mean of 28 days), HCW and carcass quality were recorded. Data were analysed using a model with treatment as fixed effect and initial BW as covariable and categorical data with a Chi-Square.

During the storage of manure, 8 piles were built-up, with 130 kg-manure/pile and 2 replicates/treatment. The treatments were four: manure with normal quantity of long straw acidified or not (labelled as "normal" and "normal +AA", respectively); manure with extra quantity of long straw acidified or not (labelled as "extra" and "extra +AA", respectively). The initial acidification of each manure type was done dosing 0.06 L of acetic acid (richness 80%) per kg of manure. Eight big-box type containers (dimensions 1.11x0.91x 0.43 m; Figure 1) with holes in all walls allowed a passive aeration of piles and the drainage of leachates. All containers were covered, avoiding rainfall. These piles simulated the storage of the manure for 11 weeks at ambient temperature. Every 2 weeks, each pile was sampled to record pH and determine total C, total N, TAN, VFA, TS, VS, as well as samples for GHG and NH3 determination were collected. Also, at day 0 and day 79 (week 11) of storage, each pile was weighed.

Table 2. Summary of bedding materials studies 1 and 2.

	Bedding study 1.1	Bedding study 1.2	Bedding study 2
Feeding strategy	Conventional (concentrate and straw) (1)		
Bedding	cleaning freq // straw length	bedding with long or chopped straw	long straw bedding // storage and acidification
Season	2018 September-2019 January	2018 September-2019 January	2019 April-September
Animals	20	119	114
Sampling			
Intake (DM, water)	x	x	x
Carcass quality	x	x	x
Body weight	x	x	x
Behaviour	x	x	x
Manure evolution	-	x	x

Notes. (1) Commercial beef farms. (2) Experimental dairy farm (IRTA).

The **bedding study 3** was focused on assessing the management options of bedding and manure, applying solid-liquid separation and solar drying of manure from conventional fed dairy cows. For this, similar quantities of manure and beds of CONV pens (P) was collected at IRTA dairy farm, as well as the solid fraction of the same manure (FS). These 2 materials were homogenized and 60 kg per material was distributed in 4 small-scale greenhouse-type solar driers (Figure C1; Annex C). Acetic acid (richness 80%) was added in 2 of the 4 solar driers to adjust the initial pH to 5.5 (0.006 L-acid/kg-manure). Therefore, the drying of P with and without acid (PA, P) and of FS with and without acid (FSA, FS) were monitored for 47 days. This assay was done in duplicate.

The evolution of temperature (ambient, inside each drier) and relative humidity, as well as GHG and NH3 emission were registered weekly. The gas samples for the determination of the GHG and NH3 were collected directly (ambient air, outlet air of the drier and biofilter). A 30-mL samples at ambient temperature were taken for GHG determination in 12.5 mL tubes (vacutainer; Labco Ltd., Buckinghamsire, UK). The analysis of GHG was done by gas chromatography (model Thermo Trace 2000, Thermo Finnigan Scientific) equipped with a flame ionization detector and an electron capture detector (model 7820A, Agilent). The measurement of the NH3 concentration were also done in-situ with a portable sensor equipped with an electrochemical detector with a sensitivity range of 0.5 to 150 ppm v/v (model GX-6000, RKI Instruments). Initial and final samples of each material were weighted and analysed (pH, total C, total N, total ammonia nitrogen, volatile fatty acids (VFA), total and volatile solids content) (APHA methods 2005).

3. Results and discussion

3.1. Precision and conventional feeding

In total, around 100 dairy cows at different lactation stage for a period of 21 days were studied in two feeding studies. Animals following both feeding systems had similar milk yield (Figure 3), milk fat and protein content, and total DMI. However, CP intake was greater ($P < 0.05$) in CONV than in PREC cows (4.26 vs 3.70 ± 0.170, respectively, kg/d), and improvements ($P < 0.05$) in feed efficiency (1.47 vs 1.39 ± 0.170, respectively) and efficiency of N utilization (0.32 vs 0.29 ± 0.005, respectively) were observed in PREC compared with CONV fed cows. Total daily N urine excretion (197 vs 142 ± 11.7, respectively, g/d) and milk urea concentration (130 vs 77 ± 14.5, respectively, mg/dL) were greater in CONV than in PREC fed cows. The main outputs regarding precision feeding were as follows (Table 3):

- did not negatively affect BW change
- had a 2-kg numerical decrease in milk yield
- increased fat percentage in milk
- did not change protein percentage in milk
- did not change energy-corrected milk (Figure 3)
- decreased milk urea concentration
- decreased total DM intake at some specific days of the study (Figure 3)
- decreased protein intake, but not NDF
- did no affect feed efficiency
- decreased urinary total N excretion (Table 3)

Table 3. Digestibility and Nitrogen balance of dairy cows fed under a conventional (CONV) or a precision (PREC) feeding system during the 3-day sampling period (from 19 to 21 d of study).

Parameter	Feeding system[1]		SEM	P-values[2]
	CONV	PREC		FS
Apparent digestibility, %				
DM	61.2	62.0	1.34	0.68
NDF	43.5	47.3	2.07	0.21
N balance				
N intake, g/d	657	606	32.5	0.28
Milk protein N, g/d	191	182	11.5	0.58
Urinary total N, g/d	197	141	12.6	0.004
Faecal N, g/d	304	305	16.8	0.97
N efficiency	0.29	0.30	0.012	0.69
As a proportion of N intake, %				
Milk N	29.3	30.0	1.21	0.70
Urine N	30.7	23.9	2.20	0.04
Faecal N	46.8	50.3	1.70	0.15
Plasma urea, mg/dL	18.1	15.4	1.35	0.17

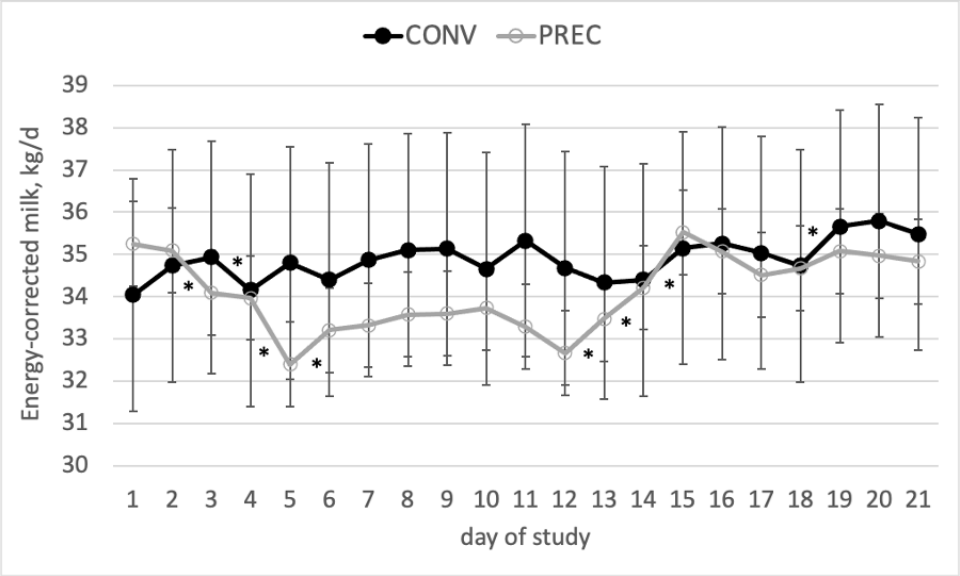


Figure 3. Evolution of energy corrected milk of dairy cows fed under a conventional (CONV) or a precision (PREC) feeding system. The asterisks denote differences in the evolution between days within treatments.

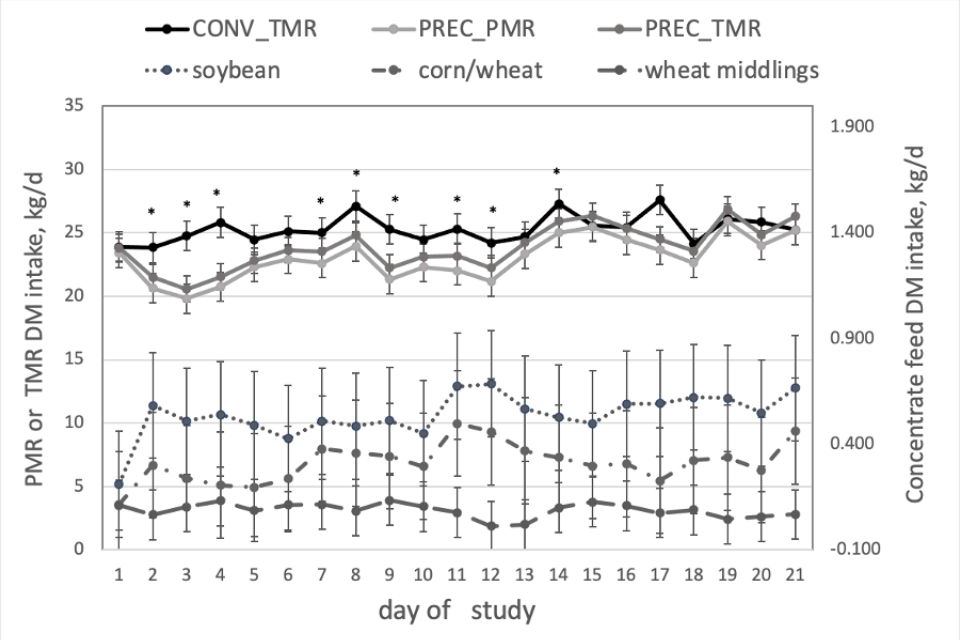
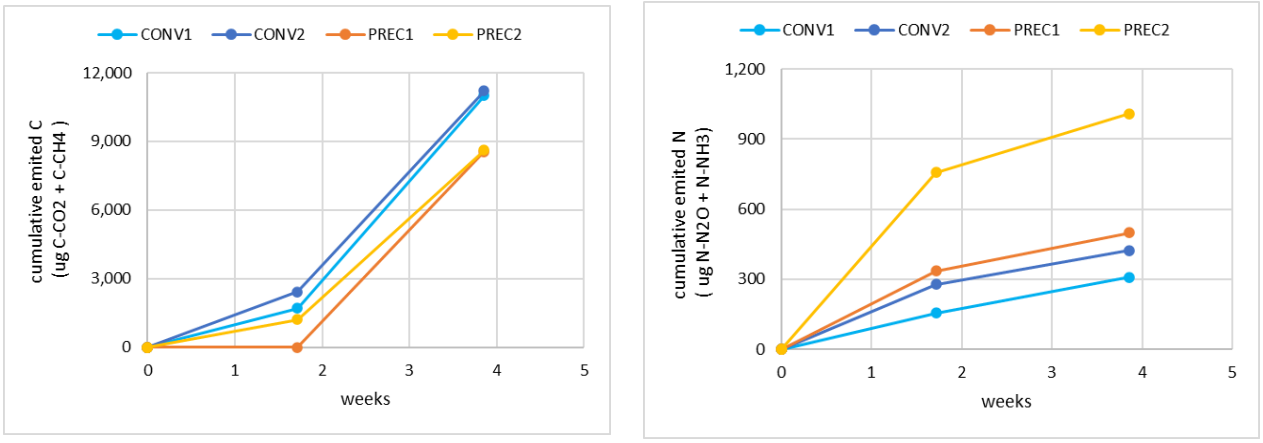
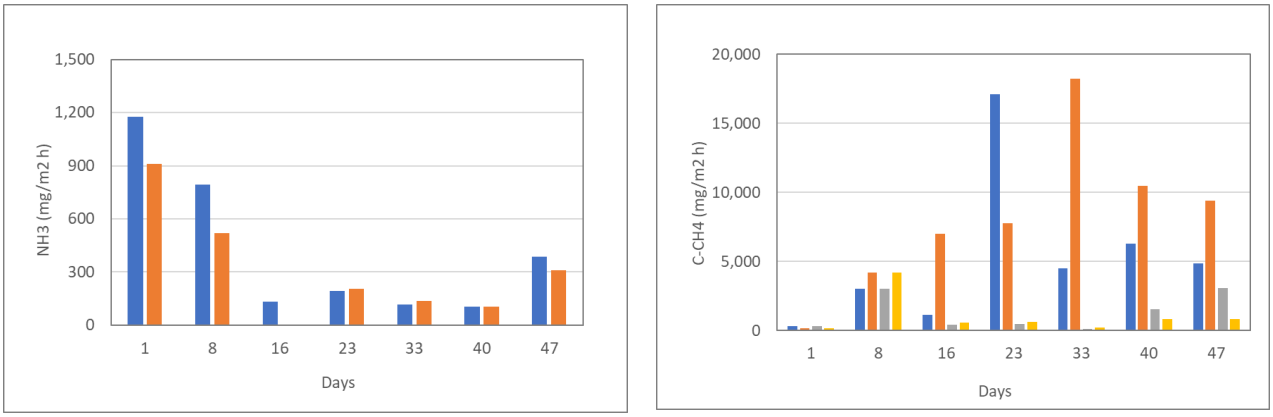


Figure 4. Evolution of total or partial mixed ration (TMR or PMR), and concentrate feed supplemented in the milking parlour of dairy cows fed under a conventional (CONV) or a precision (PREC) feeding system. The asterisks denote differences between TMR of CONV fed cows and PMR plus concentrate feed of PREC fed cows.



(a) (b)

Figure 5. Manure storage in feeding study 1. (a) Cumulative carbon-related emission (C-CH4+C-CO2). (b) Cumulative nitrogen-related emission (N-NH3+N-N2O). Colours: orange, yellow, replicates of stored PREC manure; light and dark blues, replicates of stored CONV manures.



(a) (b)

Figure 6. Manure storage in feeding study 2. (a) Ammonia emissions from CONV and PREC in stirred manure storages. Colours: blue, manure from CONV; orange, manure from PREC. (b) Methane emissions from CONV and PREC in stirred and non-stirred manure storages. Colours: blue, stirred manure from CONV; grey, non-stirred manure from CONV; orange, stirred manure from PREC; yellow, non-stirred manure from PREC.

The results after the **storage of manure from feeding study 1** showed slightly differences in fresh collected manures in terms of TS content (TS of 14.1 and 15.4% wet weight for CONV and PREC manures, respectively), TN (3.95 and 4.22 %TS for CONV and PREC manures, respectively) or TAN (1.52 and 1.22 %TS for CONV and PREC manures, respectively). At the end of the storage (20 days or 4 weeks), a natural cover or crust was formed, of dried materials, that prevented the evaporation of moisture, with different effects in each container depending on the manure (in average, the humidity loss of 1±2 and 1±0% for CONV and PREC manures, respectively).

Regarding the initial total carbon (TC-initial), the CONV manure lost 6±2% TC-initial, while the PREC manure lost 10±10% TC-initial. This TC loss could be explained by an anaerobic manure degradation during storage, producing CH4 and CO2 emissions, or due to the release of the already contained methane in the raw manure before the crust was formed. As shown in Figure 5, cumulative

C-related emissions (CH4+CO2) were higher in CONV manure storage whereas N-related emissions were higher in PREC manure storage. The initial total N (TN-initial) lost at the end of the storage was 1±4 and 21±12 %TN-initial for CONV and PREC manures, respectively.

Therefore, the evaluation of the **storage of manures from feeding study 2** was focussed on the crust effect. Similarly, again the bedding&manure mixtures were collected and stored in containers, but in this case 2 containers (one per manure type PREC or CONV) were stirred in day 14th to break the natural crust formed in the previous days (from 0 to 14th) before the sampling.

For the stirred storage, NH3 emissions in containers with PREC manure were on average 23% lower than in containers with CONV manure, especially during the first 3 weeks of storage (Figure 6a). Besides, the TAN content was on average 19% lower in all PREC manures along the storage. In this study, among the emissions evolution, tested feeding strategies reduced both the total daily N urine excretion and the milk urea concentration by 28% and 41%, respectively, with PREC (14.2% Crude Protein (CP)) in comparison to CONV (16.4% CP). Reduced CP in animal diets could decrease the amount of N in manure: because of a higher incorporation of N into a stable state as microbial protein, the ratio of TAN to total N in manure decreased, and consequently NH3 emissions declined (Sajeev et al. 2018).

The CH4 emissions (Figure 6b) increased up to 54% in PREC stirred storages. This might be because, low CP levels can reduce fibre digestibility, providing an additional carbon source (undigested fibre) in the manure for methanogenesis, generating a CH4 emission during the manure storage (Sajeev et al. 2018). However, when no mixing was applied, the natural crust drastically reduces CH4 emissions for both feeding systems, by 81% on average (Figure 6b). The same trend was observed for CO2 emissions; CO2 emissions of PREC manures increased by 41%. Nevertheless, CO2 emissions were reduced by 78% when de natural crust was formed. N2O emissions significantly increased as the surface crust appeared, since crust creates both aerobic and anaerobic zones, providing suitable conditions for the nitrification and denitrification processes responsible for N2O emissions. Additionally, external factors such as moisture content of the crust, manure storage time, and weather conditions during storage may also influence the emission pattern of N2O (Sajeev et al. 2018). In any case, the greater N loss were due to NH3 emissions.

3.2. Management of bedding materials

In the bedding study 1.1, the effect of bedding or acidification on methane and N emission, performance, behaviour, dirtiness, and carcass quality in beef- in vivo. In this study, the concentrate (8.9 ± 0.37 kg/d) and straw intake (0.9 ± 0.13 kg/d) did not differ among treatments, however ADG and efficiency were lesser (interaction $P < 0.05$) when bulls were bedded with long straw and cleaned every 2 weeks (1.38 ± 0.095 kg/d and 0.14 ± 0.007 kg/kg, respectively) compared with the other treatments (1.66 ± 0.095 kg/d and 0.17 ± 0.007 kg/kg, respectively). When bulls were cleaned every 2 weeks compared with once a week, ruminating decreased ($P < 0.01$, 2.53 vs 2.10 ± 0.067 times every 15 min), self-grooming increased ($P < 0.01$, 0.84 vs 1.59 ± 0.067 times every 15 min), and stereotypies tended to increase ($P = 0.06$, 0.09 vs 0.23 ± 0.020 times every 15 min).

Animals bedded in long straw and cleaned every 2 weeks were dirtier, followed by the animals bedded with chopped straw and cleaned every 2 weeks, bedded in long straw cleaned once a week, and the cleanest pens and bulls were the once that were bedded in chopped straw and cleaned once a week. Bedding the animals in long straw and cleaning them once every 2 weeks impairs performance, animal welfare (rumination, stereotypies) and cleanliness of the animal and the pen.

Therefore, **in the bedding study 1.2,** beef animals were raised under commercial conditions, but also manure storage evolution (evolution and gaseous emissions) was assessed. In this study, the

concentrate (8.9 ± 0.37 kg/d), straw intake (0.9 ± 0.13 kg/d), ADG (1.46 ± 0.082 kg/d), efficiency (0.21 ± 0.013 kg/kg), carcass yield (253 ± 1.8 kg) did not differ among treatments. However, bulls with long straw bedding had a greater ($P < 0.05$) percentage of carcasses scored as "3" carcass fatness (94.0 vs 80.4% for long and chopped straw, respectively). Eating behaviour (number of meals, meal size, meal duration eating rate) or animal behaviour (social, agonistic, or sexual behaviours) did also not differ among treatments. Pens of animals bedded with long straw had 47% dryness and with a deep layer of straw (score "1"), compared with the 17% recorded in pens with chopped straw; and differences became more evident at the end of the study (interaction treatment by time $P < 0.01$). Pens of animals bedded with long straw had 8% of the pen dry and with a thin layer of straw (score "2"), compared with the 30% recorded in pens with chopped straw; and differences became more evident at the end of the study (interaction treatment by time $P < 0.01$). In both treatments, around 40% of the pen was scored as "4" (wet and thin layer of straw). Despite some interactions between treatment and time were observed in pen cleanliness scoring, animal cleanliness scoring did not differ among treatments and over 80% of the animals were classified less than score "3" (less than 25% of the body surface covered with manure).

During the storage, the main registered changes occurred before week 4, except the pH that decreased till week 12 (from 8.2 to 7.5). The total solids of manure from chopped bedding slightly increased throughout the storage period (from 209 ±0.0 to 248±8.6 g/kg) whereas in long straw total solids increased from 284 ±0.0 to 550±0.0 g/kg after 4 weeks and remained constant thereafter until week 12; therefore, the humidity loss was 5% throughout the storage period in manure of chopped bedding and was around 37% in manure of long bedding. Total ammonia N (TAN) also evolved differently among bedding treatments: the TAN loss after 12 weeks was 26% and 34% of the corresponding initial TAN in chopped and long bedding manures, respectively.

The volatile solids content decreased more quickly in chopped than in long bedding manure (Figure 7); chopped and long bedding manures needed 4 and 6 weeks to stabilise its volatile solids from 88% to 80% total solids. Although the initial C/N ratio was greater in chopped bedding manure at day zero (21 gC/gN) than that of the manure with long straw bedding (13 gC/gN), in both treatments the C/N ratio decreased till 11-12 gC/gN after week 4.

In the bedding study 2, the impact of the quantity of long straw bedding on the performance of fattening bulls, behaviour, pen & animal cleanliness, as well as acidification of the manure on the evolution of GHG emissions and manure storage. The concentrate (9.0 ± 0.27 kg/d), ADG (1.88 ± 0.007 kg/d), or efficiency (0.21 ± 0.009 kg/kg), carcass yield (252 ± 1.9 kg) did not differ among treatments. Animals with extra straw bedding ate less straw at the feeder ($P < 0.05$), compared with animals with normal straw bedding (0.79 vs 1.79 ± 0.282 kg/d). However, bulls with extra straw bedding tended ($P = 0.07$) to have a greater percentage of carcasses scored as "O" carcass (33.9 vs 28.1% for extra and normal straw, respectively). Eating behaviour (number of meals, meal size, meal duration eating rate) did not differ among treatments except for meal duration; bulls bedded with extra straw had a reduced meal duration compared with bulls bedded with normal straw (9.4 vs 10.5 ± 0.30 min per meal for extra vs normal, respectively). Before cleaning, no differences in animal cleanliness were observed between treatments, over 90% of the animals were classified with score "2" (10-25% of the body surface covered with manure). In both treatments, 100% of the pen was scored as "4" (wet and thin layer of straw).

Data from storage step of bedding study 2 indicated that some N loss occurred during storage, probably caused by bacterial activity. Manure acidification in long straw bedding could prevent this N loss (as N-NH3 emission). The estimated acetic acid content in the beginning of the storage in "normal +AA" and "extra +AA" manures was 49 g/kg, but this value was not reached even during the first 2 weeks (Figure 8).

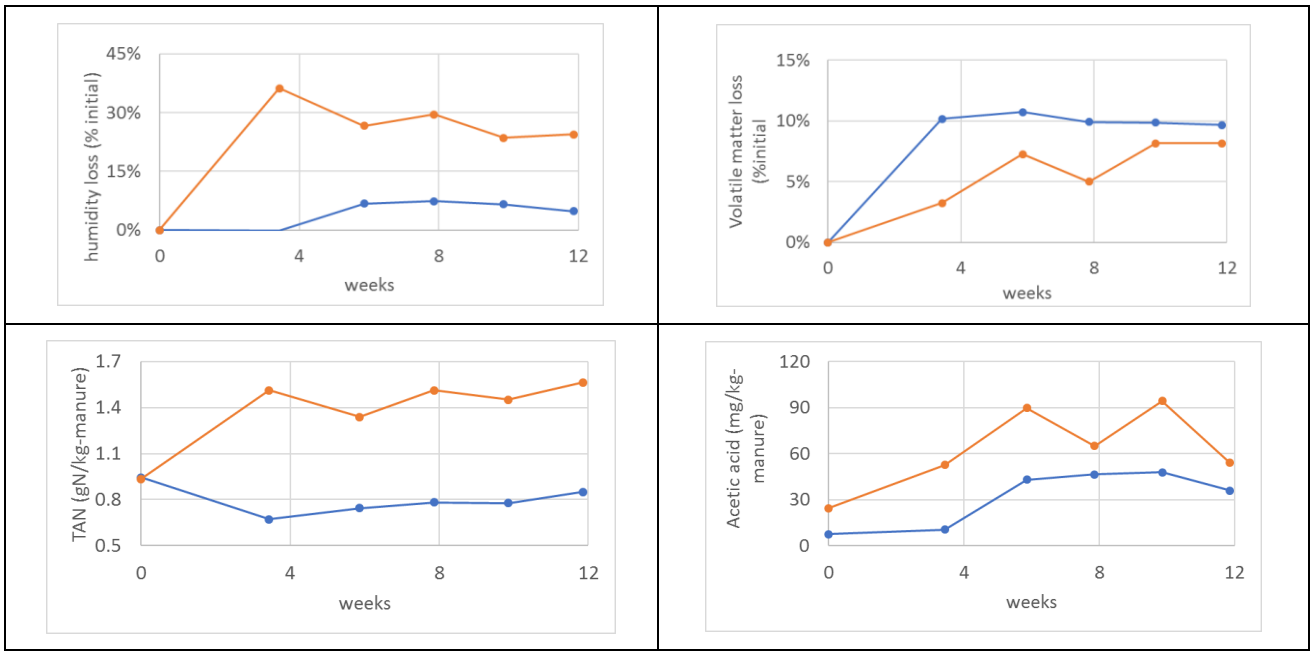


Figure 7. Evolution of parameters during manure storage of bedding study 1.2. (a) Humidity loss. (b) Volatile solids loss. (c) total ammonia nitrogen (TAN) content of the manure. (d) Acetic acid content of the manure. Symbols: blue, "chopped" manure; orange, "long" manure. Note: Lines denote the average value two replicates per treatment.

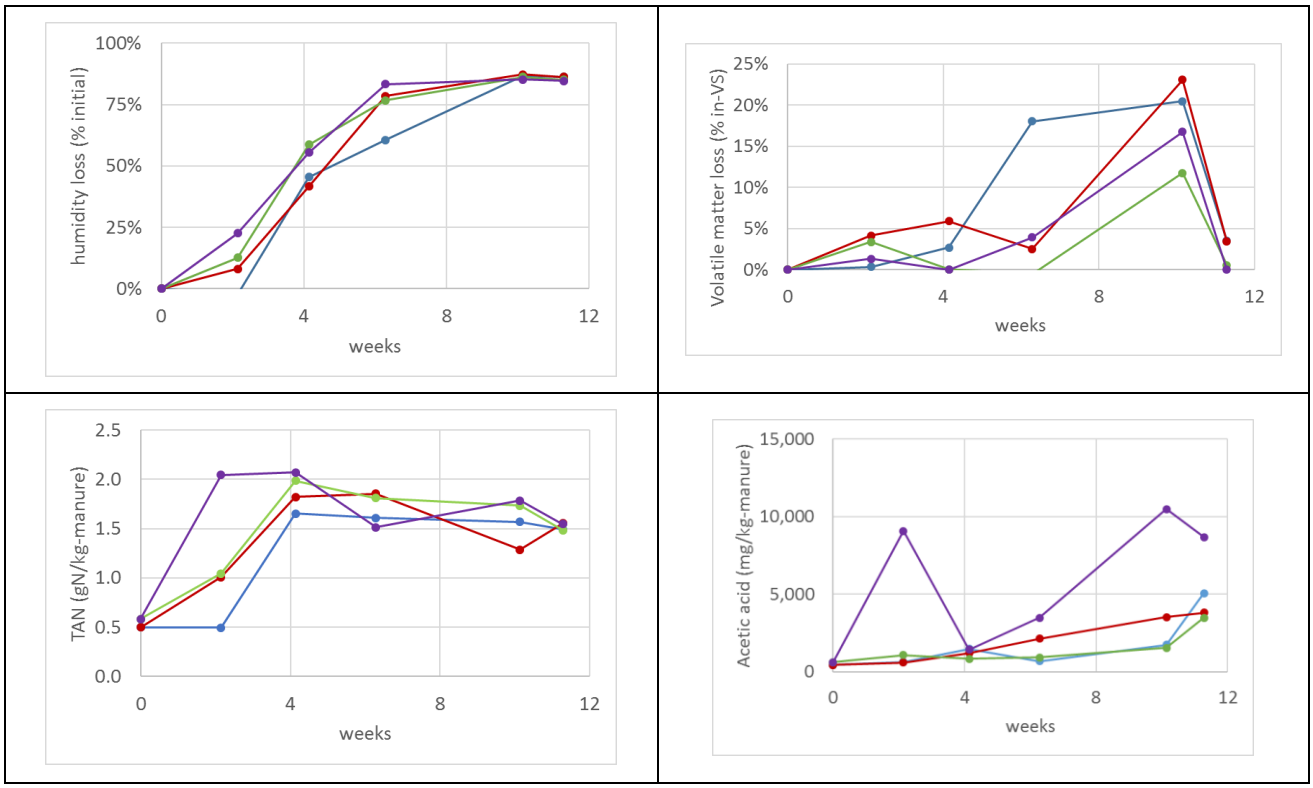


Figure 8. Evolution of parameters during manure storage of bedding study 2. (a) Humidity loss. (b) Volatile solids loss. (c) total ammonia nitrogen (TAN) content of the manure. (d) Acetic acid content of the manure. Symbols: blue, "extra" manure; red, "extra +AA" manure; green, "normal" manure; violet, "normal +AA" manure. Note: Lines denote the average value two replicates per treatment.

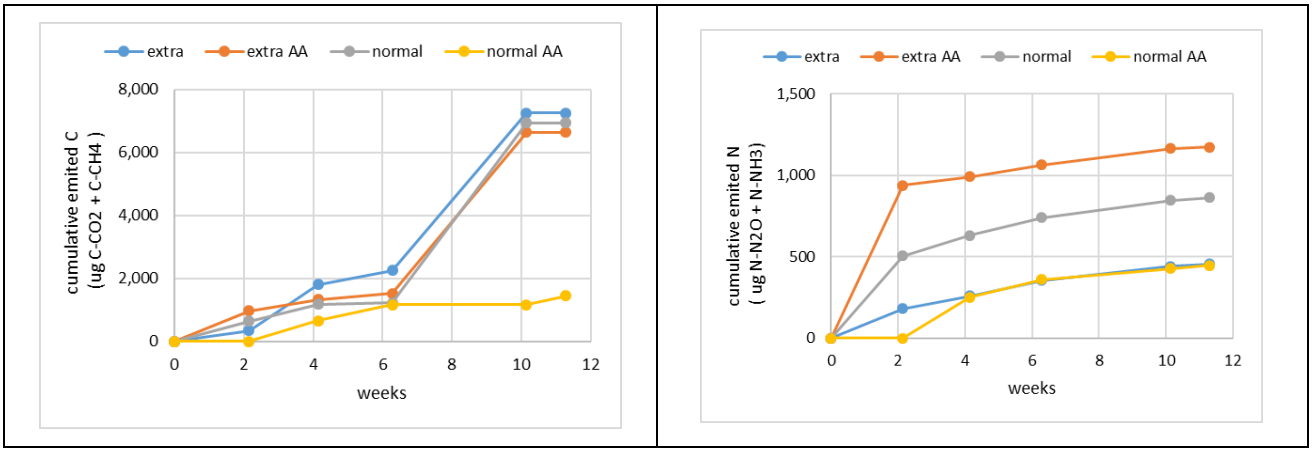


Figure 9. Evolution of GHG and NH3 emissions during manure storage of bedding study 2. (a) Cumulative emission of C-CO2 plus C-CH4. (b) Cumulative emission of N-N2O plus N-NH3. Symbols: blue, "extra" manure; red, "extra +AA" manure; green, "normal" manure; violet, "normal +AA" manure. Note: Lines denote the average value two replicates per treatment.

In the bedding study 2, a quick drainage of the dosed acid through the wall holes of containers might explain this result. Nevertheless, clearly a higher acid content was found in "normal + AA" manure in parallel with a clear lower cumulative gaseous emission GHG and NH3 (Figure 9). Besides, the acidification of "extra" manure was negative, since higher both gaseous emissions were registered in comparison with the-no acidified manure. The expected effect of acidification was the retention of ammonia in the manure matrix; this was only partially achieved in the first two weeks, based on TAN content (5% vs 8% initial TN in "extra" manures without and with acid, respectively; 5% vs 13% initial TN in "normal" manures without and with acid, respectively).

The addition of the "extra" quantity of bedding straw delayed the rate of humidity loss (almost 4 weeks), but finally both "extra" and "normal" manures attained similar dryness of 89.8, 89.8, 90.5 and 90.2% for "extra", "extra +AA", "normal" and "normal +AA" manures, respectively. Like in study 1.2, main changes occurred before week 4. The average values showed a higher volatile solid loss in "extra" manure (VS loss of 20%) than in "normal" manure (VS loss of 10%). As mentioned before, "extra" manure retained more TAN (between 7 and 33% more TAN content in "extra" than in "normal" manure, depending on the week). The C/N ratio almost was rather stable in "normal" and "normal +AA" manures (between 15-16), while the ratio in "extra" and "extra +AA" manures slightly increased till the end of the storage (from 12 to 18). Therefore, the amount of straw bedding increased the C/N ratio of the manure.

Finally, as a summary of these 2 bedding studies, there were no benefits to increasing the supply of straw (apart from increasing costs) at the production level. It should be noted, however, that the period studied was very short. A great moisture loss, up to 80%, comparing studies 1 and 2. There are several reasons; the first explanation may be the time of year in which the studies were conducted (study 1, in fall and study 2, in summer). Second, the storage-aeration system: there was no ventilation in any case, but the drainage system of the big-bags may have been worse than that of the boxes: the boxes had the 4 side walls with holes through which more air could circulate. The usage of chopped straw increased the density and humidity of manures, also disturbing its management. At the storage level, the increase in the straw quantity did not lead to a significant increase in the C/N ratio (Table 4), probably because manure and urine have a significant impact on the composition and after a week, the effect of the extra

straw was diminished. The acidification of straw, with normal straw supply, was the most efficient method to reduce GHG and NH3 emissions (Table 4), but the acidification of manure with more straw had the opposite effect: N-related emissions were higher.

Table 4. Comparison of results of bedding materials studies. All percentages are expressed regarding the results of the usual quantity ("normal") and straw type ("long"). Abbreviations: +AA, acetic acid addition only once at the beginning of the storage; nm, not measured.

Bedding study	1	2	2	2
Straw quantity per pen Straw conditioning	normal (1) chopped	normal +AA long (1)	extra long (1)	extra +AA long (1)
Apparent density (kg/m3)	70%	-12%	-12%	-18%
Humidity (%)	5%	-87%	-87%	-87%
VS or organic matter (%TS)	-9%	2%	-4%	-4%
TC (%TS)	6%	6%	-2%	4%
TN (%TS)	21%	-8%	6%	-18%
TAN (%TN-initial)	-14%	6%	-18%	-15%
C/N ratio	-13%	15%	-8%	27%
Cum. Emitted C (mg C-CO2+C-CH4)	nm	-60%	5%	-4%
Cum. Emitted N (mg N-N2O+N-NH3)	nm	-58%	-55%	49%

Notes. (1) business as usual (BAU).

4. Annex A - Mitigation Options

Summary of mitigation strategies for the 3 greenhouse gases.

Methane

- Dietary measures. Quantity of methane produced is strongly influenced by the form, quality, and composition of feed. Feeding strategies likely to lower methane emissions include:
    - **Altering and improving diet for higher animal productivity**. Feeding increased levels of starch, feeding supplementary dietary fat, and reducing the proportion of fibre in the diet are examples of potential methane reduction strategies. In the case of diet changes, one should be aware of possible trade-offs caused by land use change or by changes of **the nitrogen content in the diet**.
    - Forage selection and management. Increasing forage quality combined with the management of stocking rates and rotational grazing strategies have been demonstrated to reduce enteric methane emissions.
    - Use of feed additives. Additives can manipulate rumen microflora populations to induce a stable and modified rumen fermentation with lower emissions. Some of the additives are not permitted in the European Union, because they are considered medicine. Research on additives is still ongoing.
- Herd management for increased animal productivity. Management systems designed for high milk output per cow will tend to result in lower emissions per unit of milk produced. In contrast, more extensive systems require more animals to produce a given quantity of milk-- resulting in higher methane output per litre of milk. The opportunities to reduce methane emissions by increased animal productivity are larger in the extensive systems compared to the intensive systems with already high milk production levels per cow.
- **Manure management and treatment.** Changes to manure handling practices including use of anaerobic digesters can improve energy efficiency as well as reduce methane output. Helpful manure-management techniques include **frequent and complete removal of manure from indoor storage**, deep cooling of manure, and **management of bedding** and manure heaps to avoid anaerobic conditions.

Nitrous Oxide

The most important sources of nitrous oxide emissions on dairy farms are application of mineral and organic fertilizer as well as manure deposition and spreading by grazing stock. Options to reduce nitrous oxide emissions from dairy systems include:

- **Dietary manipulation to increase efficiency**. Avoiding excess N in the diet and/or making dietary N more absorbable reduces N excretion.
- **Manure management techniques**. Methods such as anaerobic digestion indirectly reduce N2O emissions when slurry is applied to land by decreasing the available N content. **Increasing manure storage time and covering manure storage structures, also help**.
- Grazing management methods. Reduced stocking and minimized grazing periods—which reduce compaction through grazing--increase soil aeration and are likely to result in lower emissions.
- Manure application techniques to increase N use efficiency. Optimizing methods and timing of applications using rapid incorporation; use of injection methods; use of chemical nitrification

inhibitors, better N use from fertilizers and manure through synchronization of N release with plant growth are all options for lowering emissions.

- Housing system and management. Options for mitigating emissions include more frequent removal from housing floors and changing housing systems. Animal housing and manure stores of straw-based systems result in higher N2O emissions than anaerobic slurry-based systems.

Carbon dioxide

Carbon dioxide emissions are linked to energy and resource use. Major sources of carbon dioxide emissions from the dairy chain are related to land use and land-use change, energy use on the farm, and post-farm processing and distribution of milk and dairy products.

- Increasing carbon storage. Opportunities to increase carbon storage within dairy farming systems include:
    - agricultural intensification to reduce the land needed for production. This can decrease the rate of land-use change or halt this process at all.
    - restoring soil carbon by improving soil management techniques.
    - improved grassland management; and
    - changing from highly intensive, short duration pastures to more permanent grasslands, as well as reduced tillage, can also increase carbon sequestration.
- **Increasing energy efficiency along the dairy food chain**. Energy efficiency can be improved in milking parlours and milk processing plants.
- Digestion of manure to produce heat and electricity will also contribute to lower fossil fuel energy use and CO2 emissions.
- Renewable energy may have a large role to play on farms and in processing as well.

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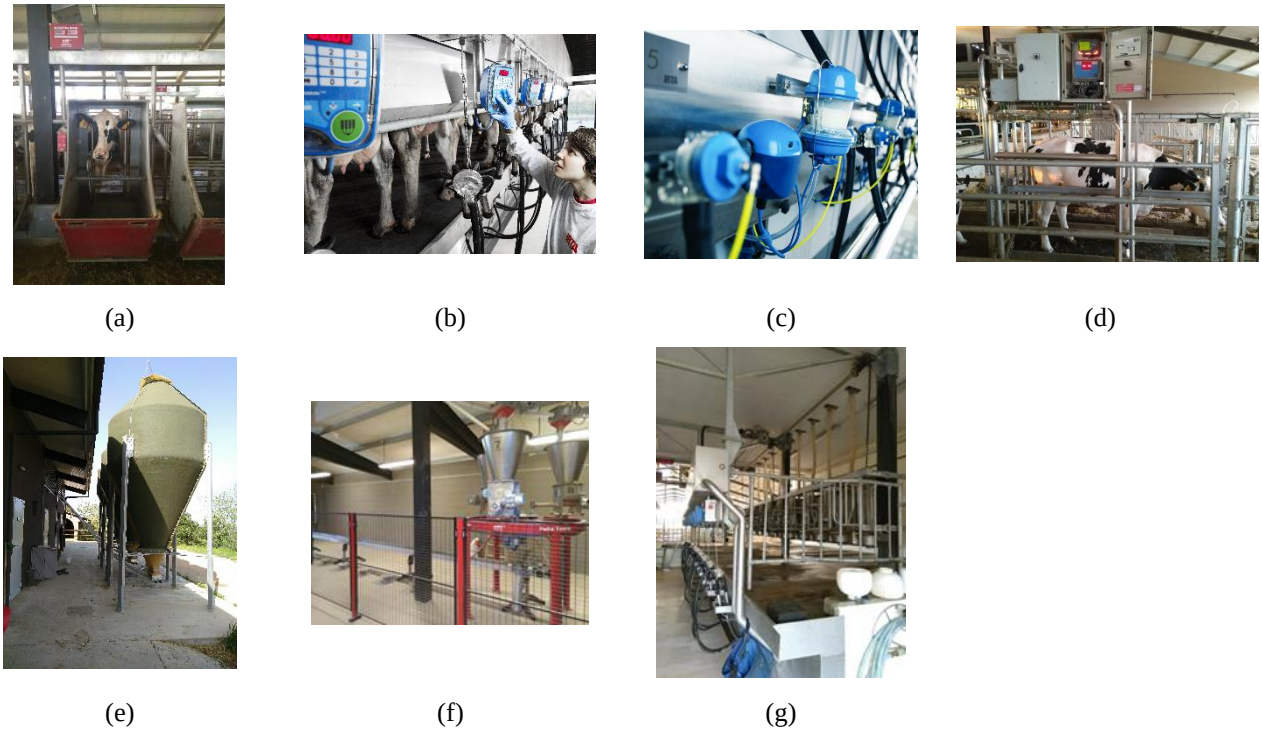
FAO, 2010. Greenhouse Gas Emissions from the Dairy Sector: A Life Cycle Assessment. Food and Agriculture Organization, Rome, Italy. Available at: http://www.fao.org/3/k7930e/k7930e00.pdf

5. Annex B - Technical requirements for the precision feeding strategy at IRTA dairy farm

The implementation of a precision feeding system requires several devices to collect animal information. At IRTA facility (EVAM, Monells, Girona, Spain), the following information was obtained using several technological tools (Figure B1):

1) Total mixed ration intake (using MooFeeder, MooSystems, Cortes, Spain). This parameter is a challenge to obtain in conventional farms, but some devices have appeared in the market, which might estimate with limited accuracy individual feed intake. (i.e., cow's head movements).
2) Milk yield (AfiMilk, Afikim Ltd., Kibbutz Afikim, Israel) and milk quality (AfiLab system, Afikim Ltd., Kibbutz Afikim, Israel). This technology is already used at commercial levels to detect decreases in milk yield and quality, and to detect health problems like mastitis, ketosis, or uncertain health problems. It also helps to take decisions.
3) Body weight using AfiMilk SortWeight system (Afikim Ltd., Kibbutz, Israel).
4) Animal records: days in milk, days in pregnancy, lactation number. This information allows to estimate nutrient requirements using guides for this purpose (NRC 2001; INRA 2018).

To feed dairy cows under a precision feeding system: (i) offer a low nutrient diet (a partial mixed ration, PMR) in the feed bins to meet low-producing milk cows nutrient needs; (ii) use the animal information to estimate its nutrient needs; (iii) record individual DM intake in the feed bunk and calculate individual nutrient intake; and (iv) if an animal does not cover its needs with the PMR, deliver extra feeds in the milking parlour to adjust nutrients to its needs. At IRTA facility, a system which allows feeding animals in the milking parlour, in a robot, or in a feeding station located in the pen, is also needed. At IRTA dairy cow farm, three silos (with different feeds: soybean meal, corn, and wheat middlings) are connected to the individual feeders in the milking parlour (Figure B1).



(a) (b) (c) (d)

(e) (f) (g)

Figure B1. (a) Feed bin scales to deliver PMR. (b) Milking parlour. (c) Online NIR for milk quality. (d) Animal scale for body weight. (e) Concentrate feed silos for milking parlour. (f) Feed delivery system in the milking parlour. (g) Feeders in the milking parlour.

6. Annex C - Experimental set-up of small scale solar drying system

At IRTA facility (Torre Marimon, Caldes de Montbui, Barcelona), 4 small-scale solar drying systems (Figure C1) were set up to perform the drying of bedding&manure or solid fraction of bedding&manure, with or without acidification. Each system included a greenhouse-type drier, a biofilter, an air blower that displaced air out of the drier towards its biofilter, a relative humidity (RH) probe and two temperature sensors connected to a datalogger.

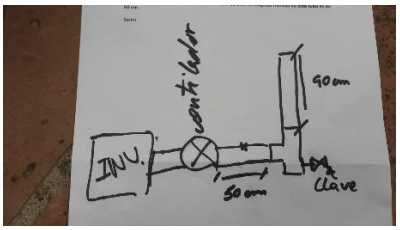
Four transparent greenhouses, made of aluminium and polycarbonate (180x51x51 cm) with a total volume of 413 L, were modified installing a grill for the air inlet and a connection with the tube to the fan (air outlet). Each greenhouse was mounted on a concrete floor. To provided further thermal isolation and physical protection from the ground, a geotextile was placed in the lower area, inside each greenhouse.

Two sensors, one to monitor temperature and relative humidity was placed inside and at the middle of the headspace of each greenhouse. An under floor sensor temperature was buried in the material, near the geotextile. The operation of the air blower was controlled by the humidity signals (switch on if RH of 85%; switch off RH of 60%). Also, an outdoor humidity and temperature sensor was installed (ambient air). All sensors were connected to a datalogger (data measurements were recorded every 15 min) and controller (controller SmartStruxure^{TM} and datalogger AS-Bundle, Schneider Electric.).

Each biofilter bed (7 L of packed volume) consisted of a mixture of mature compost and pine bark (mass ratio compost: pine bark of 1:5) and was encased in a PVC tube (10 cm of diameter; 9 L of and total volume). The biofilter was periodically irrigated with tap water, to wet the packed bed, and the excess water was withdrawn through a bottom valve.



Images of the experimental set-up



Scheme: greenhouse, air blower, biofilter



Aluminium - polycarbonate greenhouse



WebStation image.
Monitoring and control system
(relative humidity and temperature)

Figure C1. Experimental set-up of study 3.

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