1	A quantitative description of the effect of breed, first calving age and feeding strategy
2	on dairy systems enteric methane emission
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ABSTRACT

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This work aimed to merge the results of long-term experiment and quantify dairy cows 30 and replacement heifer's enteric methane (CH₄) emission and integrate this information at 31 32 the dairy system. From 2006 to 2015, at INRA dairy research farm, an experiment was conducted to measure, over successive lactations, the effect of breed (Holstein, Ho; 33 Normande, No) and first calving age (two or three years old) on the performance of 34 distinct types of cows in two feeding strategies (FS; High or Low FS). The proportion and 35 36 composition of feedstuffs used on each FS were measured and it was possible to estimate 37 the heifers and dairy cows total dry matter (DM) intake and enteric CH₄. The enteric CH₄ was quantified by feeding level (DM intake per % BW), proportion of total intake 38 concentrate and digestible organic matter (OM) in the total tract. All results obtained were 39 integrated and represent eight dairy systems combining two breeds, two first calving age, 40 two feeding strategies. Each dairy system sold the same amount of milk volume (400 t). 41 Daily enteric CH₄ was related to DM intake. Dairy cows CH₄/head per year varied from 42 123 to 158 kg. On average, the relationship between enteric CH₄ and DM intake was 21.5 43 g/kg DM intake. The enteric CH₄ intensity (CH₄ per kg of milk yield) decreased as dairy 44 cow's productivity improved. The number of cows and heifers required for the same milk 45 sold in different systems varied considerably. The heifers replacement rate and first 46 47 calving age had a strong effect on the number of dairy cows and replacements heifers, and consequently on enteric CH₄ emission. On average, the Holstein herd emitted 2.5 t/year of 48 49 enteric CH₄ less than the Normande herd. Reducing first calving age on average decreased dairy systems enteric CH₄ by 2.2 t per year. Dairy system annual enteric CH₄ emission 50 increased from 13.1 t to 17.3 t in moving from the High to Low FS. Our results at the 51 dairy system level represent an opportunity to develop mitigation strategies. Feeding 52

strategy, breed, first calving age and replacement rate showed potential to reduce enteric

CH₄ emissions mainly by modifying the number of dairy cows and replacements heifers in

55 the herd.

56 Key words: dairy cow, greenhouse gas emission, mitigation strategies, replacement rate

1. Introduction

Livestock sector contribute to methane (CH₄) emission in developing and developed countries (FAO, 2010). The majority, about 46% of dairy cattle's total greenhouse gas (GHG) emission results from CH₄ emitted from enteric fermentation (Gerber et al., 2013). The volume of CH₄ produced by individual ruminants is dependent on the substrate available for ruminal fermentation, the total dry matter (DM) intake and composition of feed intake (Hristov et al., 2013a; Knapp et al., 2014).

Over the years, many studies have been conducted to assess the effect diet composition has on enteric CH₄ production. These studies have assessed the effect of tropical or temperate grasses and legumes (Archimède et al., 2011; Wims et al., 2010), forage-to-concentrate ratios (Aguerre et al., 2011; Muñoz et al., 2015), forage conserved supplementation (Hassanat et al., 2013; Lettat et al., 2013) and feed additives (Alves et al., 2017; Cottle et al., 2011; Doreau et al., 2018) on dairy cattle enteric CH₄ and proposed feeding management and nutrition mitigation strategies.

In dairy farms, the enteric CH₄ represents 36 and 46% of total GHG in confinement and grass-based systems, respectively (O'Brien et al., 2012). In general, the enteric CH₄ intensity (CH₄ emitted *per* output product) is lower in mixed dairy systems than in grass-based systems (FAO, 2010; Gerber et al., 2013). A clear majority of these results are explained by feed efficiency, genetic merit, reproductive performance and feeding strategy

applied in each dairy system (Belflower et al., 2012; O'Brien et al., 2014). As mentioned already, the enteric CH₄ is a product from feed digestion and the mitigation strategies have largely focused on establishing CH₄ mitigation approaches based on nutrition, feeding, and modifications of rumen function. However, others approaches must be discussed at herd and dairy production system, including herd productivity, dairy heifers and reproductive management. Additionally, few studies have quantified the effect of first calving age, breed and heifers replacement rate strategies at dairy system on enteric CH₄ emission (Garnsworthy, 2004; Knapp et al., 2014).

The biggest difficulties on farms using conventional methods to measure the enteric CH₄ are related to long-term data evaluation and the cost of measuring individual animal's enteric CH₄ emission (Hristov et al., 2018). Improvements of the equations used to quantify total and enteric CH₄ emission have shown that it is possible to conduct the evaluations at the dairy system (Appuhamy et al., 2016; Niu et al., 2018).

This study aims to quantify the dairy cows and heifers enteric CH₄ emission and integrate the effects of different grass-based feeding strategies, breeds (Holstein or Normande), ages at first calving (two or three years old) and heifers replacement rates on enteric CH₄ emission at the dairy system.

2. Materials and methods

The enteric CH₄ emissions from Holstein and Normande dairy cows under different grass-based feeding strategies, first calving ages and heifers replacement rates were quantified using Sauvant and Nozière (2015) equation from data generated from a ten-year study. This long-term study investigated, throughout successive lactations, the adaptability of diverse types of cows (breed, age at first calving) on feeding strategies. The impact of

individual management factors on enteric CH_4 emission was described in eight dairy systems with the same annual milk production.

2.1 Experimental design and dataset

From 2006 to 2015, a breed × feeding strategy (FS) experiment was conducted at INRA dairy research farm in Le Pin-au-Haras (latitude 48.72, longitude 0.18; Normandy, France). In short, Holstein and Normande two- or three-year-old dairy cows at first calving, were equally distributed within two FS grass-based every year. At the beginning of the experiment, systems were balanced for parity, calving date, breeding index for milk yield, fat and protein content, body condition score (BCS), body weight (BW). Cows remained in their feeding group until culling (i.e. not pregnant, health problem or death). Replacement primiparous were balanced for age at first calving, breeding index, BCS and BW. The herd was managed under a compact breeding season (13-weeks). This permitted experimental cows to calve over the first three months of the year. The yearly calendar of the experimental design is described in Fig. 1.

The FS were denominated as High FS and Low FS. In the winter, cows in High FS were fed *ad libitum* with a total mixed ration composed of 55% maize silage, 15% dehydrated alfalfa and 30% winter concentrate (% of DM), and during the grazing period, supplemented with 4 kg of grazing concentrate. For the same period, cows in Low FS received an *ad libitum* mixture of 50% grass silage and 50% haylage (% of DM), and during grazing period, no concentrate was offered. Concentrates contained soybean, barley, beet pulp, wheat, maize and salt and minerals. From July to November, when grass growth was insufficient to fulfill the animal demand, cows received maize silage or grass

silage supplementation, in High FS and Low FS. During the dry period, cows were fed *ad libitum* with grass silage and hay only.

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The cows were milked twice daily at 06:30 A.M and 4:00 P.M. Individual milk yields were recorded by flow meters (Metatron, Westfalia, Germany). Milk samples were collected three times a week, both a.m. and p.m. and analyzed for fat and protein content using an infrared analyzer (MilkoScanTM, Foss Electric, Hillerad, Denmark). Bodyweight (BW) was measured regularly once a week. During the winter period, the amounts of forage and concentrate offered and refused in each FS were weighed every day to obtain the intake quantities. During the grazing season, the intake of concentrate and minerals by each cow were recorded daily using an automatic feeder. In situations where was necessary the conserved forage uses; the forage conserved intake was measured by the difference between the offered and refused in each FS every day. The grass sward consisted of permanent and perennial ryegrass pastures, either pure or associated with white clover. A sample of each feed offered was collected once a week, and a grass sample at each passage to a new paddock. The grass disappearance approach was used to quantify grass DM intake. All feed samples were dried and conserved for chemical composition analysis. The typical chemical composition and nutritive value of feedstuffs from experimental years are presented in Table 1.

Nulliparous heifers were separated into two FS depending on calving age, two (Fig. 2.) or three years old (Fig. 3.). The objective of the FS for heifers expected to calve at 2 years was to have higher daily weight gain than the FS for heifers calving at 3 years. Heifers were weighed at birth and then fortnightly. All forages and concentrates offered to heifers were collected regularly to determine their chemical composition and nutritive value. Intake of individual heifers was estimated from BW and intake capacity (INRA,

2018). The BW and DM intake were recorded from birth to calving age and averaged by trimesters.

2.1.1 Animals and calculations

A total of 589 lactations from 297 cows (Holstein = 146; Normande = 151) were recorded throughout the experiment, which corresponds to 258 Holsteins lactations and 331 Normande lactations. The lactation rank was divided between primiparous (Lactation 1), and multiparous cows (Lactation 2 and Lactation 3+). Over the experiment, 41% of the 589 lactations were primiparous.

The DM intake of supplements was calculated daily based on the difference between what was offered and refusals. At grazing, the grass disappearance approach was used to quantify grass DM intake. This approach was applied at the paddock level and calculated intake from pre- and post- grass height and grass density measurements as described by Delaby et al. (2001). The total DM intake was measured daily by group during all the experimental period, while the individual total DM intake was estimated considering the individual intake capacity (IC) as proposed by INRA (2018). The intake capacity is based on the influence of both animal and environment factors, the equation takes into an account the body weight (BW), the potential milk yield (MY_{Pot}), the stage of lactation and pregnancy, and the age of the animals and body condition score (BCS) (INRA, 2018).

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$$IC = [14.25 + (0.015 \times (BW - 600)) + (0.11 \times MY_{Pot}) + ((2.5 - BCS))] \times Ind_IC_{lact}$$
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$$\times Ind_IC_{gest} \times Ind_IC_{mat} \times Ind_IC_{PDI}(Eq.1)$$

Where, Ind_IC _{lact, gest, mat, PDI} are the index of intake capacity due to lactation, gestation, maturity and truly digestible protein (PDI).

Individual total DM intake (kg DM/day) = Individual Intake Capacity \times (Total DM intake of the group / Total of Intake Capacity of the group) (Eq. 2)

For each cow, their lactation was composed of twenty-six periods (2-weeks period), being four periods dry and twenty-two periods milking. Individual milk yield, fat and protein milk concentration, BW and total intake averages were grouped by 2-week periods.

The effects of experimental factors (breed, lactation, feeding strategy, and age at first calving) on animal performance (milk production, fat and protein milk concentration, BW and total DM intake) were assessed using the following linear mixed model:

$$\begin{split} & Y_{ijklmn} = \mu \, + \, year_i \, + \, first \, \, calving \, \, age_j \, + \, lactation \, \, rank_k \, + \, breed_l \, + \, FS_m \, + \, calving \, \end{split}$$
 $period_n \, + \, lactation \, \, rank_k \, \times \, breed_l \, + \, lactation \, \, rank_k \, \times \, FS_m \, + \, breed_l \, \times \, FS_m \, + \, lactation \, \, rank_k \, \times \, S_m \, + \, breed_l \, + \, br$

where Y_{ijklmn} was milk yield, fat and protein concentration, total DM intake or BW for the ijklmn observation, μ was the mean of the variable of interest, year_i was the fixed effect of experimental year (i = 2006 to 2015), first calving age_j was the fixed effect of calving age (j = two or three years old), lactation rank_k was the fixed effect of lactation (k = 1, 2, or \geq 3), breed₁ was the fixed effect of breed (l = Holstein or Normande cows), FS_m was the fixed effect of feeding system (m = high or low), calving period_n was the fixed effect of

possible 2-week calving period (n = 1 to 6), and e_{ijklmn} the random residual effect. All linear mixed model analyses were performed using the *lmer* procedure of the R statistical package (R Core Team, 2016).

2.3 Individual enteric CH₄ emission calculations

Daily enteric CH₄ from heifers and dairy cows in different FS was quantified for all experimental years using estimated DM intakes and the measured chemical compositions of feedstuffs. According to the linear coefficients from the model equations described earlier, the DM intake was estimated for 144 distinct types of cows combining breed (2), feeding strategy (2), age at first calving (2), lactation rank (3) and 2-weeks period of possible calving (6). For each type of cow, daily enteric CH₄ was estimated for twenty-six 2-week periods (lactation plus dry period). For heifers, CH₄ estimation was performed during trimesters.

Initially, enteric CH₄ emission was estimated as a function of organic matter (OM) digestibility intake, as proposed by Sauvant and Nozière (2015):

CH₄, g/kg DOM =
$$45.42 - 6.66$$
 FL + 0.75 FL² + 19.65 PCO – 35 PCO² – 2.69 FL × PCO (Eq. 3)

where, DOM is the digestible OM in the total tract; FL is the feeding level (DM intake per % BW); and PCO is the proportion of total intake concentrate.

The OM intake and diet OM digestibility were calculated by INRA (2018), where they were corrected by feeding level, proportion of concentrate in the total diet, and rumen protein balance (INRA, 2018). Both, OM intake and diet OM digestibility were quantified

for dairy cows and heifers across all experimental years (2006 – 2015). After that, it was possible to estimate daily enteric CH₄ (g/day):

 CH_4 , g/day = CH_4 , g/kg $DOM \times OM$ intake $\times OM$ digestibility (Eq. 4)

Total enteric CH₄ was calculated for fifty-two weeks (twenty-six 2-weeks periods) for dairy cows, and from birth to age at first calving (two or three years old) for heifers. The quantity of CH₄ emitted *per* year was the unit used for heifers and cows.

2.4 Dairy systems enteric CH₄ calculations

The factors studied during the experimentation were integrated to estimate the enteric CH₄ from eight different dairy systems (two breeds, two feeding strategies and two ages at first calving). This was calculated by integrating individual enteric CH₄ from dairy cows for the lactation and dry period with enteric CH₄ from heifers for the birth to calving period.

The results obtained *per* 2-weeks period were aggregated by year for each type of cow (144). The proportion of cows in each lactation rank (rank 1, 2 and \geq 3) was estimated according to the herd demography for each system (Table 2). The primiparous proportion was used as the heifer replacement rate and, consequently, estimated the number of replacement heifers. For each system, the total enteric CH₄ was estimated considering the same annual herd milk yield. The quantity of milk sold was set at 400 t. This corresponded to an annual milk yield of approximately 434,800 kg milk. Approximately, eight percent of the milk production was considered unsold (health problems as mastitis, calf rearing). The herd for this production was presented as cows, heifer and total animals (cows plus

replacement heifers). One livestock unit (LU) was equivalent to a dairy cow with a milk yield of 6,000 kg/year. The corresponding LU values for heifers 0-1, 1-2 and 2-3 years old were 0.3, 0.6 and 0.9 LU, respectively.

At the end, the effect of different replacement rates on enteric CH₄ was estimated from the observed reproductive performance and the herd demography (i.e. proportion of cows in each lactation ranks) for each studied system. The total enteric CH₄ emission from dairy cows and heifers for replacement rates 25, 30, 35, 40 and 45% were quantified for dairy systems producing the same quantity of milk (400 t sold). The simulations accounted for interactions between management options (replacement rate, FS, first calving age, breed). The potential effect of these options on enteric CH₄ emission was estimated following the linear model:

$$Y_{iikl} = \mu + breed_i + first calving age_i + FS_k + replacement rate_l + e_{iikl}$$

where Y was the total enteric CH₄ emission for the ijkl observation, μ was the mean of enteric CH₄, breed_i was the fixed effect of breed (i = Holstein or Normande cows), first calving age_j was the fixed effect of calving age (j = two or three years old), FS_k was the fixed effect of feeding system (m = high or low), replacement rate_l was the fixed effect of replacement rate (l = 25 to 45), and e_{ijkl} the random residual effect. The analyses were performed using R statistical package (R Core Team, 2016).

3. Results

3.1 Feeding strategies description

The different FS used for nulliparous replacement heifers calving for the first time at two or three years had average daily gain (ADG) of 780 and 620 g/day, respectively. The dietary composition (i.e. proportion of each feedstuff) of heifer's total intake was not similar for the different calving ages or feed strategies (Table 3). The grazed grass proportion of the diet was 50% of the total DM for heifers calving at 3 years and 30% for heifers calving a year earlier. The total DM intake from birth to calving was on average 3,435 kg greater for heifers calving at three-years-old compared to two-years-old, regardless of breed.

During the years of experimentation two feeding strategies were used to compare different breed across successive lactations. The Low FS represents a system with lower inputs and a strong self-sufficiency (0.55 ha/cow), based almost entirely on grazed grass (66% of total diet, Table 4) and conserved forage (31%). The High FS corresponds to a high producing system *per* animal and *per* hectare (0.35 ha/cow at grazing) due to the concentrate and conserved forage supplementation. Grazed grass represented 44% of total DM diet for HS, while the conserved forage and concentrate supplementation proportion were 34 and 22%, respectively. On average, the dairy cows in High FS had a total DM intake 800 kg/year greater than those on the Low FS.

3.2 Intake, Milk yield and Milk solids

The averages observed across successive lactations for Holstein and Normande performance across two feeding strategies and two ages at first calving is presented in Table 5. The total DM intake was 577 kg DM/year greater in Holstein cows (6,748 kg DM/year) when compared to Normande cows (6,171 kg DM/year) and was more pronounced for the High FS (+ 676 kg DM/year). The effect of age at first calving on the

total DM intake was similar for breed and feeding strategy. Three-year-old cows at first calving had a 9.5% greater intake than two-year-old cows at first calving. The total DM intake increased as cows progressed through their initial lactations and then stabilized. The intake difference between two- or three-year-old cows at first calving was only 3% in lactation 3+.

The Holstein dairy cows had a higher milk yield (+ 1,852 kg milk/year) and milk solids (+ 94 kg/year) than the Normande cows. Independent of lactation number, milk yield and milk solids were greater in High FS when compared to Low FS, averaging 2,109 kg milk/year and 148 kg milk solids/year. As expected, the largest milk yield and milk solids difference between two- or three-year-old heifers at first calving was in lactation 1 (746 kg of milk yield and 58 kg of milk solids). This difference decreased in lactation 2 (436 kg of milk yield and 34 kg of milk solids) and lactation 3+ (126 kg of milk yield and 6 kg of milk solids). The Normande two-year-old cows at first calving were more sensitive to FS than Holstein cows. On Low FS the milk yield was 11% and 8% less in Normande and Holstein cows, respectively, when compared to three-year-old cows at first calving.

3.3 Individual enteric CH4 emission

There was an important effect of age at first calving on the total enteric CH₄ in heifers (Table 6). When considering the period from birth to calving, independently of breed, three-year-old heifers at first calving enteric CH₄ was 84 kg greater than the two-year-old heifers at first calving. For dairy cows, the average daily enteric CH₄ of the Normande and Holstein breeds during the experimentation were 389 and 374 g/day, respectively.

Dairy cows on the High FS emitted 410 g CH₄/day and those receiving Low FS emitted 353 g CH₄/day, which resulted in a 21 kg increase in enteric CH₄ *per* year for the High FS when compared to the Low FS (Table 7). Three-year-old dairy cows at first calving emitted 11.0 and 7.5 kg of CH₄ *per* year more in High and Low FS, respectively, when compared to the two-year-old heifers at first calving. The lactation rank influenced the relationship between CH₄ and milk yield or milk solids yield. Dairy cows in lactation 3+ emitted less enteric CH₄ *per* unit of output (g/kg of milk yield and milk solids) regardless of the breed, feeding strategy and age at first calving.

3.4 Dairy system enteric CH₄ emission

Because of the short breeding period, the replacement rate for all of the systems studied was high (Table 8). The replacement rate ranged from 37% for High FS with Normande cows calving for the first time at two years to 60% for Low FS with Holsteins calving for the first time at three years. Generally, the replacement rate was greater for systems with Holstein than Normande dairy cows (55% vs. 44%, respectively), and in Low FS than High FS (52% vs. 45%, respectively). For an expected milk sale of 400,000 kg/year, the dairy systems with Holstein three-year-old dairy cows at first calving receiving High FS required the least cows and the systems with the Normande two-year-old dairy cows at first calving receiving Low FS required the most (50 and 102 cows, respectively). The number of heifers and total herd was greater for systems with three-year-old cows at first calving and Low FS, independently of breed.

The dairy system annual enteric CH₄ emission was mostly influenced by FS and first calving age. The system with two-year-old dairy cows at first calving on High FS had less enteric CH₄ emission (11.8 t of CH₄ *per* year) than the system with three-year-old

cows at first calving on Low FS (18.2 t of CH₄ *per* year). The enteric CH₄ intensity was expressed *per* cow and total herd. When expressed *per* cow, the CH₄ intensity ranged from 17.3 to 27.9 g CH₄/kg of milk and from 24.6 to 44.2 g CH₄/kg of milk at herd. There was a large effect of breed and FS on CH₄ intensity in the studied systems. Nonetheless, the enteric CH₄ emission *per* kg DM intake was similar between different cows (21.5 g/kg DM intake) and herds (22.3 g/kg DM intake).

The system with Normande three-year-old dairy cows at first calving receiving Low FS and with a 45% replacement rate showed the greatest enteric CH₄ emission (19.0 t/year), while Holstein two-year-old dairy cows at first calving receiving High FS, and with a 25% replacement rate showed the lowest enteric CH₄ emission (8.8 t/year) when compared to other systems (Table 9). The difference between the two extremes systems total enteric CH₄ emission shows the max reduction potential for systems emissions was 54% (Fig.4). The segmentation between factors indicates that breed was the most impacting factor, reducing enteric CH₄ emission by 17.1%, followed by feeding strategy (-14.8%). The first calving age effect is nearly half of the two previous (-8.7% for two-year-old cows at first calving). Decreasing the replacement rate by 10% reduced CH₄ emission by 1,290 kg (equivalent to a 13.6% difference between the two extreme systems).

4. Discussion

The CH₄ emission quantified during this work was based firstly on the extensive description of diet and DM consumed by heifers and dairy cows and secondly using an integrating approach to performance data collected during ten years of experimentation. The equation proposed by Sauvant and Nozière (2015) allowed the calculation of the

individual enteric CH₄ emission on dairy cows during unproductive and productive periods from the diet description, and notably the OM digestibility of the different diets used.

Measurements of enteric CH₄ emission from growing heifers are scarce and very variable (Cottle et al., 2011; Morrison et al., 2017). According to IPCC (2006), the emission factor (kg CH₄/head *per* year) for growing heifers in Western Europe is 57 kg CH₄ *per* year. Recently, Jiao et al. (2014) reported that grazing Holstein heifers have an emission factor of 36.2 kg CH₄ from birth to 12 months of age, and 64.3 kg CH₄ from 13 to 24 months. In the present study, two-year-old heifers calving emitted on average 57 kg of CH₄ *per* year, whereas from birth to 12 months of age the emission factor was 31 kg of CH₄ *per* year and, from 12 to 24 months of age the factor was 75.5 kg of CH₄ *per* year. This variation may be due to the feeding system used and the age of growing heifers.

The daily enteric CH₄ observed from cows was similar to other European studies (Appuhamy et al., 2016) with grass-based FS (Muñoz et al., 2015; O'Neill et al., 2012; Robertson and Waghorn, 2002). The emission factor was greater than that reported by IPCC (2006) and Vermorel et al. (2008). This result may be associated with the total period used to estimate the enteric CH₄. In French dairy systems, the observed emission factor was 118 kg of CH₄ *per* year in dairy cows with milk yield of 6,300 kg/year during a period of 47 weeks (Vermorel et al., 2008). In our study, the annual milk yield was 6,470 kg producing an emission factor of 139 kg of CH₄ *per* year for 52 weeks (lactation and dry period).

4.1 Effect of feeding strategy on enteric methane emission

As expected, the individual enteric CH₄ was driven by DM intake. This relationship has been well discussed in the literature (Beauchemin et al., 2008; Benchaar et al., 2001;

Knapp et al., 2014; Martin et al., 2010). In growing heifers, our data allows for a description of enteric CH₄ from birth to calving and reports both the breed and first calving age effects on CH₄ emission. Excluding the effect of breed, three-year-old heifers at first calving emitted more enteric CH₄ than heifers calving at two years old. From birth to calving, 50% of enteric CH₄ was emitted between 24 and 36 months for 3 year old heifers. This result was directly influenced by the higher duration of unproductive period, greater DM intake and growth during the last year (Jiao et al., 2014; Morrison et al., 2017). In this view, the enteric CH₄ *per* ADG in two and three-year-old heifers at first calving were 208 and 304 g CH₄/ADG, respectively. Morrison et al. (2017) showed that heifers up to 23 months of age emitted 252 g CH₄ per kg of body weight. However, the CH₄ emission *per* ADG in the last year of heifers with expected first calving at three years old was on average 446 g CH₄/ADG. This result may be, at least partially, explained by the higher DM intake and slower growth of this animal over this period.

The FS had an evident effect on an annual enteric CH₄ emission. Dairy cows in High FS showed greater DM intake and enteric CH₄ production than Low FS. The CH₄ emission *per* unit of DM intake (g CH₄ / kg DM intake) ranged from 20.6 to 22.7 and averaged 21.5. These values agree with the variation proposed by other authors (Berndt et al., 2014; Dijkstra et al., 2011). Normally, the use of concentrate and maize silage supplementation can reduce the enteric CH₄ relative to DM intake due to changes in fermented substrate (Beauchemin et al., 2008). Replacing structural carbohydrates from forages by non-structural carbohydrates, results in large modifications on rumen physic-chemical conditions and microbial populations, and thus alters CH₄ production (Hassanat et al., 2013; Martin et al., 2010). Moreover, it is known that the grazing grass of greater OM digestibility can result in less CH₄ emitted *per* kg of DM intake when compared to

pastures with less OM digestibility (O'Neill et al., 2011; Wims et al., 2010). In our work, the close values of this relationship between FS can be associated to three factors. First, the low concentrate proportion, second maize silage was used in winter only for High FS and third the high nutritive value of grass grazed.

4.2 Effect of managements factors on dairy system enteric methane emission

Once the enteric CH₄ estimated for each type of cows and heifers studied at INRA dairy research farm was analyzed, the objective was to integrate the enteric CH₄ at the dairy system. It was possible to simulate the total enteric CH₄ emission (productive and unproductive periods) from eight different dairy systems aiming to sell the same amount of milk. The different systems were used to evaluate the effect breed, first calving age, FS and heifers replacement rate combinations on total enteric CH₄ emission. As discussed previously, there was a direct effect of FS on enteric CH₄. The dairy system simulations indicated three factors were associated with CH₄, namely the milk yield potential, first calving age and the replacement rate. These factors directly influenced the number of cows and replacements heifers required for a fixed amount of milk sales and thus affected enteric CH₄.

Improving animal productivity is widely used as a greenhouse gas mitigation strategy. In principle the strategy, reduces the number animals required for the same product output and thus reduces GHG emissions and the environmental footprint (Hristov et al., 2013b). Our research showed that a dairy system with Holstein cows receiving High FS had an annual milk yield of 8,400 kg per cow and required 52 cows to sell 400 t of milk. In contrast, the same FS required 70 Normande cows due to the lower annual milk yield of 6,215 kg per cow. Consequently, the Normande emitted 25% more annual enteric

CH₄. This breed also emitted more enteric CH₄ in the Low FS, but the difference reduced to 18%. Thus, these findings clearly imply that the selection of more productive animals and the use of a FS that allows the expression of the genetic merit of cows reduce emissions. This result is consistent with that showed by O'Brien et al. (2014), where the enteric CH₄ (kg of CO₂-eq/t of energy corrected milk) was 13% less in a high-performing confinement than in grass-based system. These differences in emissions were largely due to variation in feed efficiency i.e. milk yield *per* unit of intake. Improving feed efficiency mitigates enteric CH₄ emissions and tends to improve financial returns (Gerber et al., 2011; Yan et al., 2010).

The compact calving season and the different ability of the cows to be in calf (Bedere et al., 2017) resulted in high replacement rates for the different systems. The effect of fertility on CH₄ emission was previously studied by Garnsworthy (2004). Improving herd fertility influences the heifer replacement number for a fixed herd and reduced CH₄ emission by up to 24%. According to our observations, decreasing the first calving age to two years old was possible and reduced emission by 2.2 t of CH₄/year. This was largely due to the replacement rate (Table 9). It is important to highlight that we simulated the replacement rate improvements in each dairy system studied. Considering the extreme situations, improving the replacement rate from 45 to 25% reduced enteric CH₄ emission from dairy systems with first calving age of two and three years old by 16.4 and 20%, respectively. The additivity of replacement heifer numbers and unproductive periods were the key factors influencing total enteric CH₄ emissions for each dairy system. Similar to previous reports, fewer replacement animals are needed in herds with better reproduction rates, resulting in a direct effect on CH₄ emissions (Crosson et al., 2011; Knapp et al., 2014).

The difference in enteric CH₄ emission between the two extreme simulated systems was more than double (Table 9; Fig. 4.). These results clearly show the strong mitigation potential when the animal and feed factors in the current study are considered simultaneously. Globally, the individual mitigation strategies are applied in an integrated way at a system (Bell et al., 2011). Strategies that aim to improve animal productivity have the potential to reducer CH₄ by 20 to 30 % (Boadi et al., 2004; Knapp et al., 2014) with feeding, nutrition, and genetic selection strategies representing 15% and 19%, respectively, of the reduction of CH₄ *per* energy-corrected milk (Knapp et al., 2014). The management factors in the dairy system studied, breed, FS, replacement rate and first calving age act directly on dairy cow productivity, feed efficiency and unproductive periods. When all these factors were analyzed, breed had the greatest potential to reduce enteric CH₄ (17.1%) followed by FS (14.8%).

5. Conclusion

The initial description of system studied for ten-years confirmed the major individual influences of DM intake and feed efficiency on enteric CH₄ emission. At the dairy system, breed and feeding strategy affect the CH₄ emission, especially when expressed *per* kg of milk produced. The enteric CH₄ emission was linked to the individual performance and it was reduced on high genetic merit cows and high feeding strategies. Within a breed and feeding system, combining the lower replacement rate and the two-year-old at first calving represent an interesting opportunity to reduce the enteric CH₄ emission mainly due to the influence on the herd size. Reducing replacement rate and age at first calving will have also a positive influence on the farm incomes due to the feeding costs reduction associated. In this way, economic and environmental impacts are

481	positively combined. These results can serve as the basis for quantitative enteric CH ₄
482	emission discussions and as a reference to a general approach for quantifying total
483	greenhouse gas emissions from dairy systems.
484	
485	Acknowledgements
486	This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de
487	Nível Superior - Brasil (CAPES) - Finance Code 001. This project (n°16-60-C0004) was
488	also financially supported by ADEME (France) and integrated in the research program
489	REACCTIF. The authors would like to thank the two native English speakers for their
490	important contribution to improve the quality of English writing.
491	
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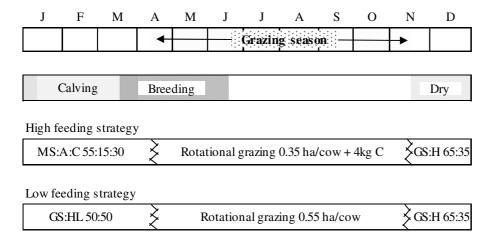


Fig. 1. Yearly calendar experimental design of the feeding strategies experiment conducted in the INRA dairy research farm Le Pin-au-Haras. Grazing season, breeding season, high feeding strategy and low feeding strategy. MS, maize silage; A, dehydrated alfalfa pellets; C, concentrate; GS, grass silage; HL, haylage; H, hay).

Table 1Chemical composition and nutritive value of feedstuffs offered during experimental period (average 2006 – 2015).

			2		Grass	Haylage	Concentrate	
	High FS	Low FS	silage	alfafa	silage	Tiujiuge	Winter	Grazing
DM, % fresh weight	20.7	21.8	32	91	30	67	88	89
		(Chemical	composition,	g/kg Dl	М		
OM	889	889	960	862	901	902	900	948
CP	178	170	70	214	129	142	276	157
NDF	505	505	415	348	481	531	155	192
ADF	249	248	225	233	286	281	78	85
			Λ	lutritive value	,1			
OMd, %	75	74	73	62	73	71	86	85
UFL, /kg DM	0.89	0.88	0.91	0.70	0.89	0.83	1.08	1.13
PDI, g/kg DM	95	92	68	109	65	94	163	133

FS, feeding system; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; OMd; organic matter digestibility; UFL, energy feed unit equivalent to 1700 kcal of net energy for lactation; PDI, protein digestible in the intestine.

¹INRA (2018).

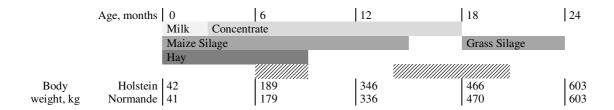


Fig. 2. Feeding strategy used in Holstein and Normande heifers with first calving age of two years during experiment conducted in INRA experimental farm Le Pin-au-Haras. Hatched period: grazing period.

	Age, months	0	6	12	18	24	30	36
		Milk	Concentrate					
		Maize	Silage	GS or H		GS or H	GS	
		Hay		l				
								_
Body	Holstein	41	180	302	407	491	601	700
weight, kg	Normande	42	169	287	395	482	604	721

Fig. 3. Feeding strategy used in Holstein and Normande heifers with first calving age of three years during experiment conducted in INRA experimental farm Le Pin-au-Haras. GS, grass silage; H, hay. Hatched period: grazing period.

Table 2Distribution of lactation rank (%) according to breed, feeding strategy and first calving age (2006 – 2015).

		Hol	stein		Normande				
	Hi	High		High Low		High		Low	
First calving age	2	3	2	3	2	3	2	3	
Lactation 1	51	46	60	55	37	45	47	46	
Lactation 2	27	32	22	30	31	28	32	25	
Lactation 3+	22	22	18	15	32	27	21	29	

Table 3 Dry matter intake estimation and the dietary composition of the total intake of Holstein and Normande nulliparous heifers with first calving age of two or three years old.

	Hols	stein	Norn	nande
First calving age, year	2	3	2	3
Total DM intake, kg				
0-12 months	1480	1321	1379	1257
12 – 24 months	3423	2914	3357	2835
24 - 36 months	-	4072	-	4110
Total ¹	4903	8307	4736	8202
Feedstuff,				
Grass grazed	0.30	0.50	0.31	0.50
Maize silage	0.31	0.06	0.30	0.05
Grass silage	0.26	0.39	0.27	0.39
Hay	0.03	0.02	0.03	0.01
Concentrate + minerals and vitamins	0.09	0.04	0.10	0.04

DM, Dry matter.

¹ Total DM intake from birth to calving.

Table 4Total intake and the dietary composition in proportion of the total intake of dairy cows for High and Low feeding strategies during lactation and dry period.

	High feeding strategy	Low feeding strategy
Total DM intake, kg/year	6860	6060
Lactation		
Grass grazed	0.44	0.66
Maize silage	0.23	-
Dehydrated alfafa pellets	0.03	-
Grass silage	0.08	0.24
Haylage	-	0.07
Concentrates + minerals and vitamins	0.22	0.03
Dry period		
Grass silage	0.65	0.65
Hay	0.35	0.35

DM, Dry matter.

Table 5 Total intake, milk yield, milk solids and feed efficiency of Holstein and Normande dairy cows according to feeding strategies, first calving age and lactation rank (average 2006 - 2015).

		Hol	stein			Normande					
•	High		L	ow	Hi	gh	Low				
First calving age, year	2	3	2	3	2	3	2	3			
Total intake, kg/year											
Lactation 1	6108	6765	5425	5941	5466	6114	4981	5487			
Lactation 2	7133	7555	6263	6544	6441	6854	5769	6040			
Lactation 3+	7658	7956	6738	6894	6978	7266	6256	6402			
	6966	7425	6142	6460	6295	6745	5669	5976			
Milk yield, kg/year											
Lactation 1	7145	7764	4820	5640	4945	5618	3644	4518			
Lactation 2	8798	9107	5948	6457	6327	6690	4501	5065			
Lactation 3+	9714	9712	6723	6922	7211	7263	5244	5497			
	8553	8861	5830	6340	6161	6524	4463	5027			
Milk solids, kg/year											
Lactation 1	484	536	324	382	364	421	264	328			
Lactation 2	595	624	411	445	471	503	339	379			
Lactation 3+	664	664	473	481	539	542	399	412			
	581	608	403	436	458	489	334	373			
Feed effiency ¹											
Lactation 1	1.17	1.15	0.89	0.95	0.90	0.92	0.73	0.82			
Lactation 2	1.23	1.21	0.95	0.99	0.98	0.98	0.78	0.84			
Lactation 3+	1.27	1.22	1.00	1.00	1.03	1.00	0.84	0.86			
	1.22	1.19	0.95	0.98	0.97	0.96	0.78	0.84			
Feed effiency ²											
Lactation 1	79.2	79.3	59.8	64.3	66.7	68.8	53.0	59.8			
Lactation 2	83.5	82.6	65.6	68.0	73.2	73.4	58.7	62.7			
Lactation 3+	86.7	83.4	70.3	69.8	77.2	74.6	63.7	64.4			
	83.1	81.8	65.2	67.4	72.4	72.3	58.5	62.3			
Body weight, kg											
Lactation 1	573	654	532	610	614	686	558	628			
Lactation 2	634	686	586	635	688	732	626	667			
Lactation 3+	695	727	632	661	758	781	680	700			
	634	689	583	635	687	733	621	665			

¹Feed efficiency = kg milk yield/kg DM intake. ²Feed efficiency = kg milk solids/kg DM intake.

Table 6 Accumulated enteric methane emission of Holstein and Normande nulliparous heifers with first calving age of two or three years old (average 2006 - 2015).

	Hols	stein	Normande	
First calving age, year	2	3	2	3
Accumulated CH ₄ emission, kg				
0-12 months	34	30	31	29
12 – 24 months	83	70	81	68
24 – 36 months	-	100	-	101
Total ¹	117	200	112	198
CH ₄ , g/kg of DM intake				
0-12 months	22.9	22.9	22.6	22.8
12 – 24 months	24.2	23.9	24.1	23.8
24 – 36 months	-	24.7	-	24.7
Total	23.5	23.8	23.4	23.8
CH ₄ , g/kg of ADG ²				
0-12 months	110	114	104	115
12 – 24 months	317	364	299	341
24 – 36 months	-	474	-	418
Total	214	317	202	292

¹ Accumulated methane emission from birth to calving. ² Average daily gain.

 $\begin{tabular}{ll} \textbf{Table 7} \\ \textbf{Enteric methane emission of Holstein and Normande dairy cows according to feeding strategies, first calving age and lactation parity (average 2006 - 2015). \\ \end{tabular}$

		Hol	stein			Normande				
	Hi	gh	Lo	ow	Hi	gh	Lo	ow		
First calving age, year	2	3	2	3	2	3	2	3		
CH ₄ , g/d										
Lactation 1	357	401	310	345	339	381	297	329		
Lactation 2	409	438	354	374	394	420	341	359		
Lactation 3+	443	462	381	393	430	447	370	380		
	403	434	348	371	388	416	336	356		
CH4, kg/year										
Lactation 1	130	146	113	126	124	139	108	120		
Lactation 2	149	160	129	137	144	153	124	131		
Lactation 3+	162	169	139	143	157	163	135	139		
	147	158	127	135	141	152	123	130		
CH4, g/kg of DM int	take									
Lactation 1	21.3	21.6	20.9	21.2	22.6	22.7	21.7	21.9		
Lactation 2	20.9	21.1	20.6	20.9	22.3	22.4	21.6	21.7		
Lactation 3+	21.1	21.2	20.6	20.8	22.5	22.4	21.6	21.7		
	21.1	21.3	20.7	20.9	22.5	22.5	21.6	21.8		
CH ₄ , g/kg of OM dig	estible									
Lactation 1	32.7	33.0	33.0	33.2	34.1	34.2	33.9	34.0		
Lactation 2	32.3	32.5	32.6	32.8	33.8	33.8	33.6	33.7		
Lactation 3+	32.5	32.6	32.6	32.7	33.9	33.9	33.6	33.6		
	32.5	32.7	32.7	32.9	33.9	34.0	33.7	33.8		
CH ₄ , g/kg of milk										
Lactation 1	18.2	18.8	23.5	22.3	25.0	24.7	29.7	26.6		
Lactation 2	17.0	17.5	21.7	21.1	22.7	22.9	27.7	25.9		
Lactation 3+	16.7	17.4	20.7	20.7	21.8	22.4	25.8	25.2		
	17.2	17.9	21.8	21.3	23.0	23.3	27.5	25.9		
CH ₄ , g/kg of milk so	lids									
Lactation 1	269	273	349	330	339	330	410	366		
Lactation 2	251	256	314	307	305	305	368	346		
Lactation 3+	244	254	294	298	291	301	339	337		
	253	260	315	310	309	311	367	349		

OM, organic matter.

Table 8Estimate dry matter intake, milk yield, milk solid, and total enteric methane emission in Holstein and Normande herd with total milk sales of 400 ton *per* year from different dairy systems.

		Hols	tein			Norn	nande	
	Hi	gh	Lo	ow	Hi	gh	Lo	ow
First calving age, year	2	3	2	3	2	3	2	3
Replacement rate	51	46	60	55	37	45	47	46
Number of								
Dairy cows	53	50	80	71	71	68	102	88
Heifers, LU ¹	24	42	43	71	24	55	43	73
Total, LU	88	104	119	143	96	126	129	153
Milk yield, kg/cow/year	8168	8632	5430	6098	6067	6364	4266	4951
Milk solids, kg/cow/year	604	647	405	455	494	519	345	399
Total DM intake, kg								
Dairy cows	6723	7277	5842	6261	6244	6625	5499	5889
Total herd	5580	5393	5910	5422	5940	5613	6104	5567
CH ₄ , ton/year								
Dairy cows	7.53	7.79	9.69	9.37	9.98	10.16	12.11	11.24
Total herd	10.71	12.42	15.31	17.21	12.93	16.22	17.48	19.20
Per LU, kg/year	122	119	129	120	135	129	136	126
CH4, g/kg DM intake								
Dairy cows	21.1	21.3	20.7	21.0	22.4	22.5	21.6	21.7
Total herd	21.8	22.2	21.8	22.2	22.7	22.9	22.2	22.5
CH ₄ , g/kg MY								
Dairy cows	17.3	17.9	22.3	21.6	23.0	23.4	27.9	25.9
Total herd	24.6	28.6	35.2	39.6	29.8	37.3	40.2	44.2
CH ₄ , g/kg milk solids								
Dairy cows	234	239	299	289	283	286	344	321
Total herd	333	382	472	531	367	457	496	548

LU, Livestock Unit; DM, dry matter; MY, milk yield.

Table 9Number of cows required, replacements heifers required and total enteric methane emission for dairy systems selling of 400 t of milk/year and calving cows for the first time at 2 or 3 years old. The dairy systems were simulated using 5 different replacement rates.

-	Holstein				Normande				
	Н	igh	Lo	Low		High		Low	
First calving age, year	2	3	2	3	2	3	2	3	
Replacement rate	Number of cows								
25	49	48	72	67	68	65	93	84	
30	50	48	73	68	69	66	95	85	
35	51	49	74	68	70	66	96	86	
40	52	50	76	69	72	67	99	87	
45	52	50	77	70	73	68	101	88	
	Heifers replacement, LU								
25	11	22	16	30	15	29	21	38	
30	13	26	20	37	19	35	26	46	
35	15	30	23	42	21	41	30	53	
40	19	36	27	50	26	49	36	63	
45	21	41	31	57	30	55	41	71	
	Total CH ₄ emission, t								
25	8.82	10.07	11.40	12.52	11.69	13.17	14.30	15.29	
30	9.16	10.61	11.93	13.26	12.19	13.91	14.97	16.20	
35	9.45	11.05	12.36	13.87	12.60	14.51	15.54	16.95	
40	9.88	11.72	13.02	14.80	13.22	15.44	16.39	18.08	
45	10.25	12.29	13.59	15.58	13.75	16.22	17.11	19.04	
		CH ₄ reduction by changing replacement rate to 25 %							
30	3.8	5.1	4.4	5.6	4.0	5.3	4.5	5.6	
35	6.7	8.9	7.8	9.8	7.2	9.2	8.0	9.8	
40	10.8	14.1	12.4	15.4	11.5	14.7	12.7	15.4	
45	14.0	18.1	16.1	19.7	15.0	18.8	16.4	19.7	

LU, Livestock Unit

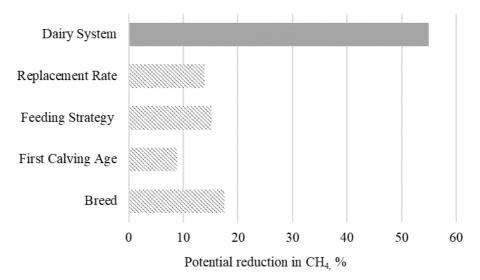


Fig. 4. The effect of replacement rate, feeding strategy, first calving age and breed on enteric methane emission (t/year) from two dairy systems.