UNIVERSITÀ DEGLI STUDI DI MILANO

PhD School “Agriculture, Environment and Bioenergy”
XXXII Cycle

Faculty of Agricultural and Food Sciences
Department of Agricultural and Environmental Sciences. Production, Landscape, Agroenergy

GREENHOUSE GAS EMISSIONS RELATED TO MILK PRODUCTION OF DAIRY COWS

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A.A. 2018-2019

Abstract

With global emissions estimated at 7.1 Gt CO_2 eq per annum, livestock represents 14.5% of all human-induced emissions and it is considered to be the largest source of greenhouse gas (GHG) emissions from the agricultural sector. However, livestock can contribute to convert nutrients from plant biomass into animal-sourced foods, which are rich in essential macro and micronutrients in the form of milk and meat, thereby utilizing resources that cannot otherwise be consumed by humans. Livestock also contributes to global food security and poverty reduction, providing regular income to producers. To achieve a sustainable supply of animal origin food, farmers need, therefore, to identify strategies, in terms of livestock management and feeding, forage systems and feed growing practices, that make the best use of available resources and minimize the potential environmental impact.

The studies of the PhD thesis were mainly developed inside the Life project "Forage4Climate", a four years project, aimed at demonstrating that forage systems connected to milk production can promote climate change mitigation.

The aim of the PhD thesis was the evaluation of GHG emission, related to dairy cattle milk production. Specific aims were:

- to identify and evaluate the most common forage systems adopted in dairy cow farms in the Po plain, selecting the systems that can improve milk production and soil carbon (C) sequestration reducing emissions per kg of milk;
- to evaluate commercial diets related to these different forage systems, in order to directly assess their digestibility, milk and methane (CH_4) production;
- to identify, through a survey analysis, the main ingredients used in the total mixed ration (TMR) of high producing lactating cows, in order to assess the best diet composition that can lead to high feed efficiency (FE) and low global warming potential (GWP) at commercial farms scale;
- in a future perspective of circular economy, to study the exploitation of different inedible human by-products as growing substrates for *Hermetia Illucens* larvae, in order to substitute soybean meal (SBM) in the livestock diets with insect proteins.

A total of 46 dairy cattle farms in Lombardy, Piedmont and Emilia-Romagna were visited, in order to map the main forage systems adopted in each area and to characterize them for GHG emission related to milk production (FPCM, fat and protein corrected milk), and soil organic C stock. The evaluation of environmental impact, in terms of GWP, related to the different forage systems was carried out though a Life Cycle Assessment (LCA) method, using the Software SIMAPRO. Six forage systems based on different forages were identified. The main results in terms of GHG per unit milk were:

- CONV - Conventional corn silage system: 1.37 kg CO_2 eq/kg FPCM (SD 0.26)
- HQFS - High quality forage system: 1.18 kg CO_2 eq/kg FPCM (SD 0.13)
- WICE - Winter cereal silage system: 1.44 kg CO_2 eq/kg FPCM (SD 0.43)
- MIXED - Mixed less intensive system: 1.36 kg CO_2 eq/kg FPCM (SD 0.26)
- PR FRESH- Hay and fresh forage system for Parmigiano Reggiano PDO cheese production: 1.51 kg CO_2 eq/kg FPCM (SD 0.23)
- PR DRY- Hay system for Parmigiano Reggiano PDO cheese production: 1.36 kg CO_2 eq/kg FPCM (SD 0.19).

The HQFS system registered the lowest value for GWP, mainly due to the higher milk production per cow (daily FPCM/head). More intensive systems, such as HQFS, confirmed that milk production per cow is negatively related to the impact per kilogram of product, as highlighted also by a PROC GLM analysis. The HQFS system also resulted to be more sustainable, in terms of feed self-sufficiency, as it provided a high amount of dry matter (DM) per hectare, consisting of high digestible forages. Despite the lowest value for GWP, the forage system identified as HQFS showed the lowest organic C soil density: 5.6 kg/m^2 (SD 1.1). On the contrary, PR FRESH showed the highest value in terms of organic C density in the soil: 9.7 kg/m^2 (SD 2.2), compared with an average of 6.7 kg/m^2 (SD 0.88) for the other systems. Further investigations are needed to consider environmental sustainability over a wider spectrum.

Enteric CH_4 was the main contributor to GWP for all forage systems: on average 45.6% (SD 3.89). For this reason, an *in vivo* evaluation of CH_4 and milk production of lactating dairy cows fed four different diets, obtained from the forage systems identified, was performed. Also digestibility of the diets, energy and nitrogen (N) balance were assessed. Four pairs of Italian Friesian lactating cows were used in a repeated Latin Square design, using individual open circuit respiration chambers to determine dry matter intake (DMI), milk production and CH_4 emission and to allow total faeces and urine collection for the determination of N and energy balances. Four diets, based on the following main forages, were tested: corn silage (49.3% DM; CS), alfalfa silage (26.8% DM; AS), wheat silage (20.0% DM; WS), hay-based diet (25.3% DM of both alfalfa and Italian ryegrass hays; PR) typical of the area of Parmigiano Reggiano cheese production. Feeding cows with PR diet significantly increased DMI (23.4 kg/d; P=0.006), compared with the others (on average, 20.7 kg/d), while this diet resulted to be the least digestible (e.g. DM digestibility=64.9 *vs* 71.7% of the other

diets, on average). This is probably the reason why, despite higher DMI of cows fed PR diet, the animals did not show higher production, both in terms of milk (kg/d) and energy corrected milk (ECM; kg/d), compared with the other treatments.

The urea N concentration was higher in milk of cows fed WS diet (13.8) and lower for cows fed AS diet (9.24). This was also correlated to the highest urinary N excretion (g/d) for cows fed WS diet (189.5 *vs* 147.0 on average for the other diets). The protein digestibility was higher for cows fed CS and WS diets (on average 68.5%) than for cows fed AS and PR (on average 57.0%); the dietary soybean inclusion was higher for CS and WS than AS and PR. The higher values for aNDFom digestibility were obtained for CS (50.7%) and AS (47.4%) diets.

The rumen fermentation pattern was affected by diet; in particular PR diet, characterized by a lower content of NFC and a higher content of aNDFom as compared to CS diet, determined a higher rumen pH and decreased propionate production as compared to CS. Feeding cows with PR diet increased the acetate:propionate ratio in comparison with CS (3.30 *vs* 2.44 for PR and CS, respectively). Ruminal environment characteristics (i.e higher pH and higher acetate: propionate ratio), together with increased DMI, led cows fed PR DRY diet to have greater (P=0.046) daily production of CH_4 (413.4 g/d), compared to those fed CONV diet (378.2 g/d). However, no differences were observed when CH_4 was expressed as g/kg DMI or g/kg milk.

Hay based diet (PR) was characterized by the lowest digestible and metabolizable energy contents which overall determined a lower NEL content for PR than CS diet (1.36 vs 1.70 Mcal/kg DM respectively for PR and CS diets).

In order to meet the high demand of nutrients needed to assure high milk production, in addition to fodder a lot of concentrates are also used in dairy cows' TMR. A survey analysis conducted in commercial farms was performed to evaluate the GWP of different lactating cow TMR and to identify the best dietary strategies to increase the FE and to reduce the enteric CH_4 emission. A total of 171 dairy herds were selected: data about DMI, lactating cows TMR composition, milk production and composition were provided by farmers. Diet GWP (kg CO_2 eq) was calculated as sum of GWP of each ingredient considering inputs needed at field level, feed processing and transport. For SBM, land use change was included in the assessment. Enteric CH_4 production (g/d) was estimated using the equation of Hristov et al. (2013) in order to calculate CH_4 emission for kg of FPCM. The dataset was analysed by GLM and logistic analysis using SAS 9.4.

The results of frequency distribution showed that there was a wide variation among farms for the GWP of TMR: approximately 25% of the surveyed farms showed a diet GWP of 15 kg CO_2 eq, 20% of 13 kg CO_2 eq and 16.7% of 17 kg CO_2 eq. The variation among farms is due to the feed used. Among feed, SBM had the highest correlation with the GWP of the TMR with the following equation: TMR GWP (kg $CO_{2\,eq}$) = 2.49*kg SBM + 6.9 (r^2=0.547). Moreover, an inclusion of SBM >15% of diet DM did not result in higher milk production with respect to a lower inclusion (≤15%). Average daily milk production of cows was 29.8 (SD 4.83) kg with a fat and protein content (%) of 3.86 (SD 0.22) and 3.40 (SD 0.14), respectively. The average value of DMI (kg/d) of lactating cows was 22.3 (SD 2.23). The logistic analysis demonstrated that a level of corn silage ≤ 30% on diet DM was associated with higher FE. Almost 50% percent of the farms had an average value of 15.0 g CH_4/kg FPCM and about 30% a value of 12.5 g CH_4/kg FPCM. The results demonstrated that a lower enteric CH_4 production was related to inclusion (% on diet DM) of less than 12% of alfalfa hay and more than 30% of corn silage. Diets with more than 34% of NDF determined higher CH_4 production (≥14.0 g/kg FPCM) compared with diets with lower NDF content. On the contrary, a lower enteric CH_4 production (<14.0 g/kg FPCM) was related to diets characterized by more than 1.61 NEl (Mcal/kg) and more than 4% of ether extract.

The variability in the GWP of TMR shows a significant potential to reduce both the GWP of the diet through a correct choice and inclusion level in the ration of the ingredients (mainly SBM) and the possibility to decrease CH_4 enteric emission associated to milk production.

Looking forward, in order to evaluate the opportunity of alternative protein sources in the cow diet, to reduce SBM, waste production, and competition between animals and human for crops, a study on the effects of different by-products for *Hermetia illucens* rearing on the chemical composition of larvae and their environmental impact was conducted, even if, according to the European legislation, today the use of insects as feed source is not possible in ruminants. Regarding climate change, okara and brewer's grains were the most promising substrates: 0.197 and 0.228 kg CO_2 eq/kg of larvae fresh weight, respectively.

Results from these studies show the importance of adopting a holistic approach for the assessment of GHG emission from milk production. Therefore, any strategy aimed at mitigating CH_4 emission of dairy cows must also take into account the possible effect on the other GHGs, as well as the effect on C sequestration. Based on the studies, it could also be worth evaluating novel feed as a new and useful solution for mitigation of GHG emission related to milk production.

The thesis highlights essential differences among forage systems and among feed ingredients of cow ration, confirming that there is room for improvement in sustainability of milk production. These issues should be taken into consideration by farmers, technicians and policy makers, considering that sustainability of livestock production will be one of the priorities for humankind in next future.

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1. Introduction

Feeding the world's poor is one of the most important challenges of the present days, as human population grows and put increasing efforts on natural resources. Livestock has an important role to play, as it provides high-quality protein to consumers and regular income to producers (FAO, 2011). In particular, the dairy sector contributes to global food security and poverty reduction, through the supply of dairy products (FAO and GDP, 2018). Globally, in the period 1960-2000 milk production has increased (nearly doubled), as well as meat and egg productions have more than trebled and increased by nearly four times, respectively. Livestock production is growing rapidly, particularly in developing countries (Speedy, 2003).

The rise in livestock production is due to an increasing demand for animal products, even if the pattern of consumption is very uneven across the world. According to data reported by Speedy (2003), globally, milk consumption is about 46 kg/capita/y. However, milk consumption ranges between 0.6 kg/capita/y (Dem Republic of Congo) and 242 kg/capita/y (Albania). In Italy, consumption of milk is about 46 kg/capita/y (Speedy, 2003).

At the same time, the world is showing a change in the global climate that will impact on local and global agriculture, which led to the Paris Agreement, between the members of United Nations Framework Convention on Climate Change (UNFCCC), concerning the reduction of greenhouse gas (GHG) emission, starting from 2020. To achieve sustainable animal food supply, indeed, livestock producers need to identify forage systems, feed growing, harvesting practices and livestock management that make the best use of available resources and minimize the potential environmental impact (Capper et al., 2009).

1.1 Climate change

According to Allen et al. (2018), the period 2006–2015 was 0.87°C (with a 66-100% confidence interval of 0.75°C–0.99°C) warmer than the pre-industrial level (1850–1900).

The Intergovernmental Panel on Climate Change (IPCC) has traditionally defined changes in observed global mean surface temperature (GMST) as a weighted average of near-surface temperature changes over land and sea surface temperature changes over the oceans. Warming is not observed spatially and seasonally uniform: an increase in GMST is associated with warming substantially greater in many land regions and lower in most ocean regions (Collins et al., 2013). However, at regional level, from 1901 to 2012, in almost the whole globe the surface, temperature has increased (IPCC, 2013; Figure 1).

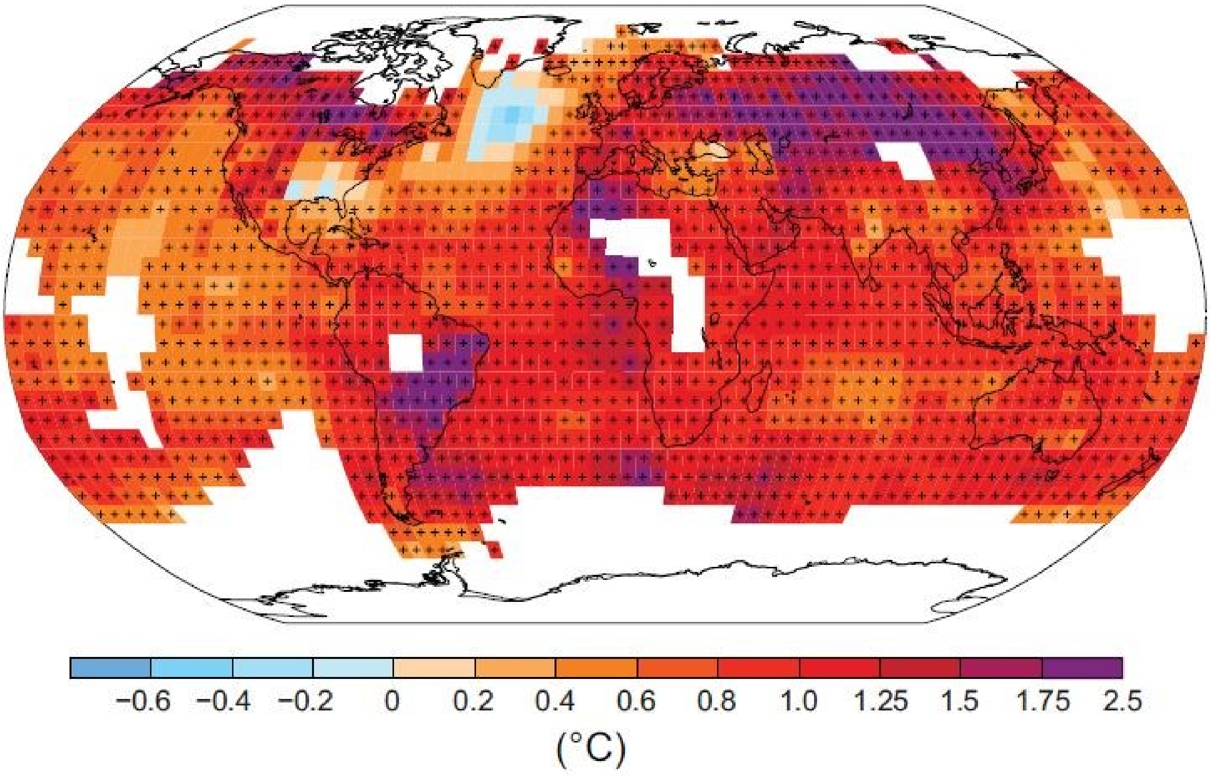

Figure 1. Observed change in surface temperature in the period 1901-2012 (IPCC, 2013).

Total warming refers to the actual temperature change, while human-induced warming refers to the component of that warming that is attributable to human activities: in the absence of strong natural forcing (i.e. due to changes in solar or volcanic activities), the difference between the two is small (Allen et al., 2018). Several authors have shown that the warming observed since the middle part of the 20th century can be attributed almost entirely to human influence (Jones et al., 2013; Ribes and Terray, 2013), meaning increased greenhouse effect, due to higher GHG concentration in the atmosphere (related to human activities). Haustein et al. (2017) give a 5-95% confidence interval for human induced warming in 2017, over the period 1850-79, of +0.87°C to +1.22° C (with a best estimate of +1.01°C), compared to the corresponding natural externally-driven change of - 0.01° C ± 0.03° C. They also estimate a human-induced warming trend over the past 20 years of +0.16° C (+0.12° C to +0.32° C) per decade. Essentially, all the observed warming since 1850-79 seems to be anthropogenic.

The theory emerging from these type of simulations is known as the *Anthropogenic Global Warming Theory* (Scafetta, 2013). The *Anthropogenic Global Warming Theory* may be erroneous because of large uncertainties in the forcings (in particular, the aerosol forcing) and in the equilibrium climate sensitivity to radiative forcing (Scafetta, 2013). The models used by IPCC (2007), general circulation models (GCMs), claim that a doubling of CO_2 atmospheric concentration induces a warming between 2° and 4.5° C: global warming, indeed, would be caused by further substantial rise of greenhouse effect, due to higher amount of GHGs in the atmosphere. The greenhouse effect is a natural phenomenon, essential for life on earth, but, according to *Anthropogenic Global Warming Theory*, increasing of GHG concentration (anthropic) in the atmosphere has led to increased greenhouse effect and consequently global warming. The equilibrium climate sensitivity derives from the direct CO_2 greenhouse warming effect plus the warming contribution of its climatic feedbacks (e.g., water vapor feedback). The calculated climate sensitivity value is a simple by-product of the physical mechanism and of the parameters implemented in the GCMs (e.g. those related to water vapor feedback): if the modelled feedback mechanisms are erroneous, then the modelled climate sensitivity would be inaccurate (Scafetta, 2013). Lindzen and Choi (2011) pointed out that observational data indicate that positive and negative climate feedbacks to CO_2 variations compensate each other, leaving a net equilibrium climate sensitivity to CO_2 doubling between 0.5° C and 1.3° C. These findings question the existence of a strong GCMs global water vapor feedback to anthropogenic GHGs, suggesting that GCMs overestimate the climatic effect of the anthropogenic GHG forcing (Scafetta, 2013; Lindzen and Choi, 2011).

Climatic Research Unit (University of East Anglia) and Hadley Centre (UK Met Office) developed a global temperature dataset (2012), that reveals periodicities in warming and cooling periods: in particular, a natural oscillation explains the 1850-1880, 1910-1940 and 1970-2000 warming periods, the 1880-1910 and 1940-1970 cooling periods and the post 2000 GMST plateau. This oscillation appears to be a combination of long soli-lunar tidal oscillations, solar and heliospheric oscillations (driven mostly by Jupiter and Saturn movements). The models used by IPCC (2007) GCMs found not able to reconstruct this variability (Scafetta, 2013). Ljungqvist (2010) estimations of the last 2000 years of extra-tropical Northern Hemisphere (30-90° N) decadal mean temperature variations are presented in Figure 2.

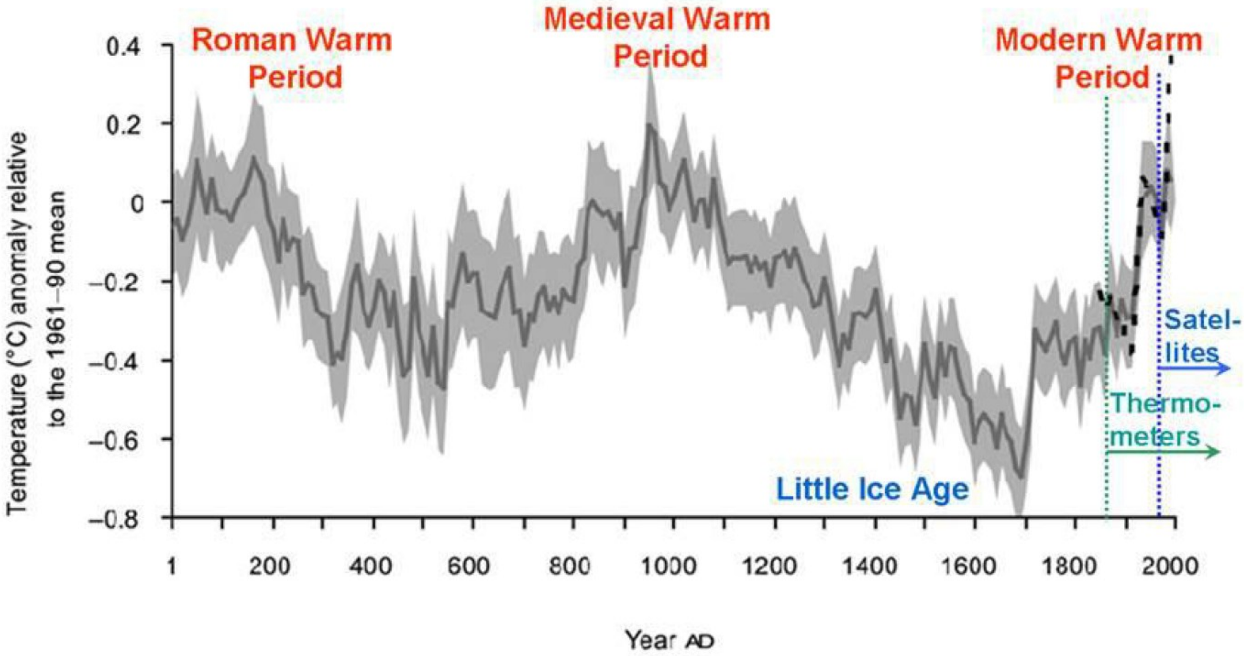

Figure 2. A new reconstruction of temperature variability in the extra-tropical Northern Hemisphere during the last two millenia (Ljungqvist, 2010).

This reconstruction shows manifestations of quasi-millennial climatic cycles represented by: Roman Warm Period, Dark Age Cold Period, Medieval Warm Period, Little Ice Age and the twentieth-century warming. Substantial parts of the Roman Warm Period, from the first to the third centuries, and the Medieval Warm Period, from the ninth to the thirteen centuries, seems to have equalled or exceeded the AD 1961-1990 mean temperature level in the extra-tropical Northern Hemisphere. According to these hypotheses, about 50% of the 0.5° C global surface warming observed from 1970 to 2000 was due to natural oscillations of the climate system (Scafetta, 2013).

This disagreement about causes of climate change is also reflected in a national survey of American Meteorological Society (AMS) member views on climate change, conducted by George Mason University and AMS, with National Science Foundation funding (Maibach et al., 2016). Almost all the AMS members (96%) think that climate change is happening (Figure 3).

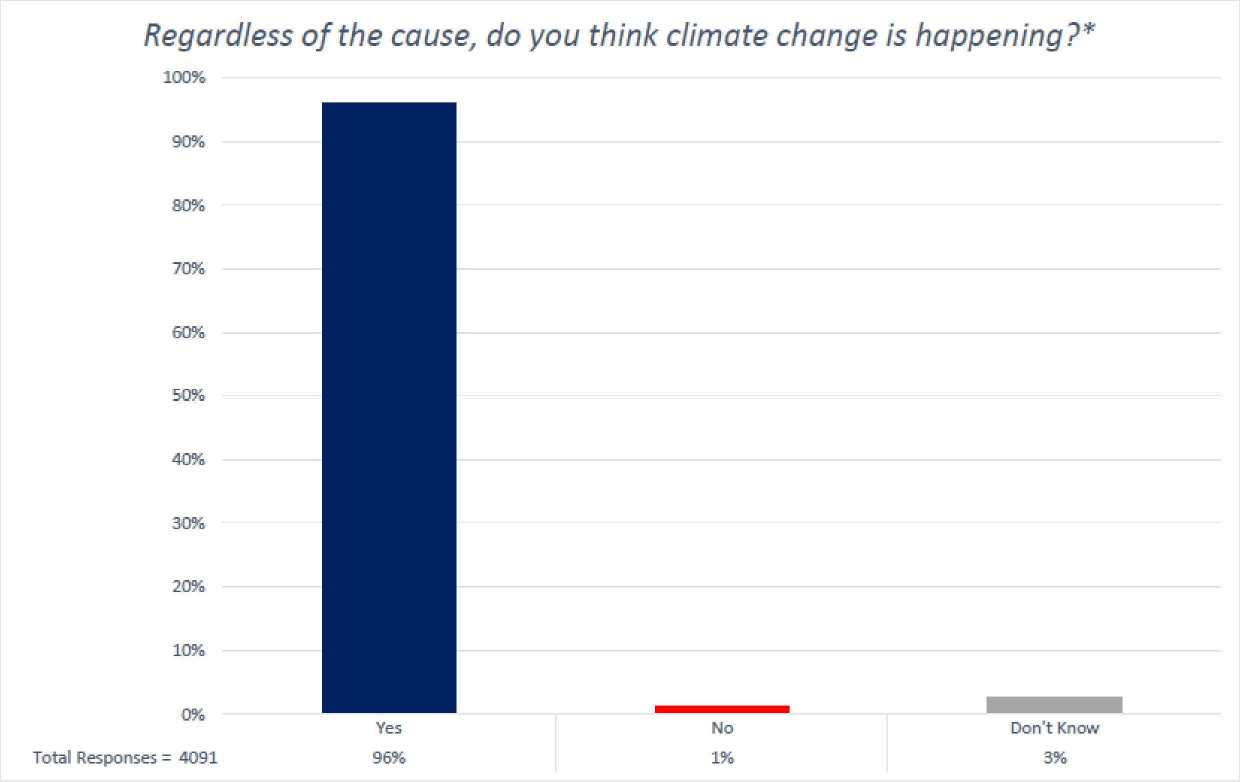

Figure 3. Results of a survey on American Meteorological Society members about climate change (Maibach et al., 2016). *Question was preceded by this statement: “Please read the following information: The American Meteorological Society (AMS) defines climate change as: any systematic change in the long-term statistics of climate elements (such as temperature, pressure, or winds) sustained over several decades or longer. Climate change may be due to: natural external forcings, such as changes in solar emission or slow changes in the earth’s orbital elements; natural internal processes of the climate system; or anthropogenic forcing.”

Nearly three out of every four AMS members (74%) think the local climate in their area has changed in the past 50 years as a result of climate change, while one in ten (11%) think it hasn’t and a nearly one in six say they don’t know (15%; Maibach et al., 2016).

About the causes, only 29% of the interviewees says that climate change over the past 50 years has been caused entirely by human activity, 38% attribute it mostly to human activity, while 14% equally to human activity and natural events (Maibach et al., 2016; Figure 4).

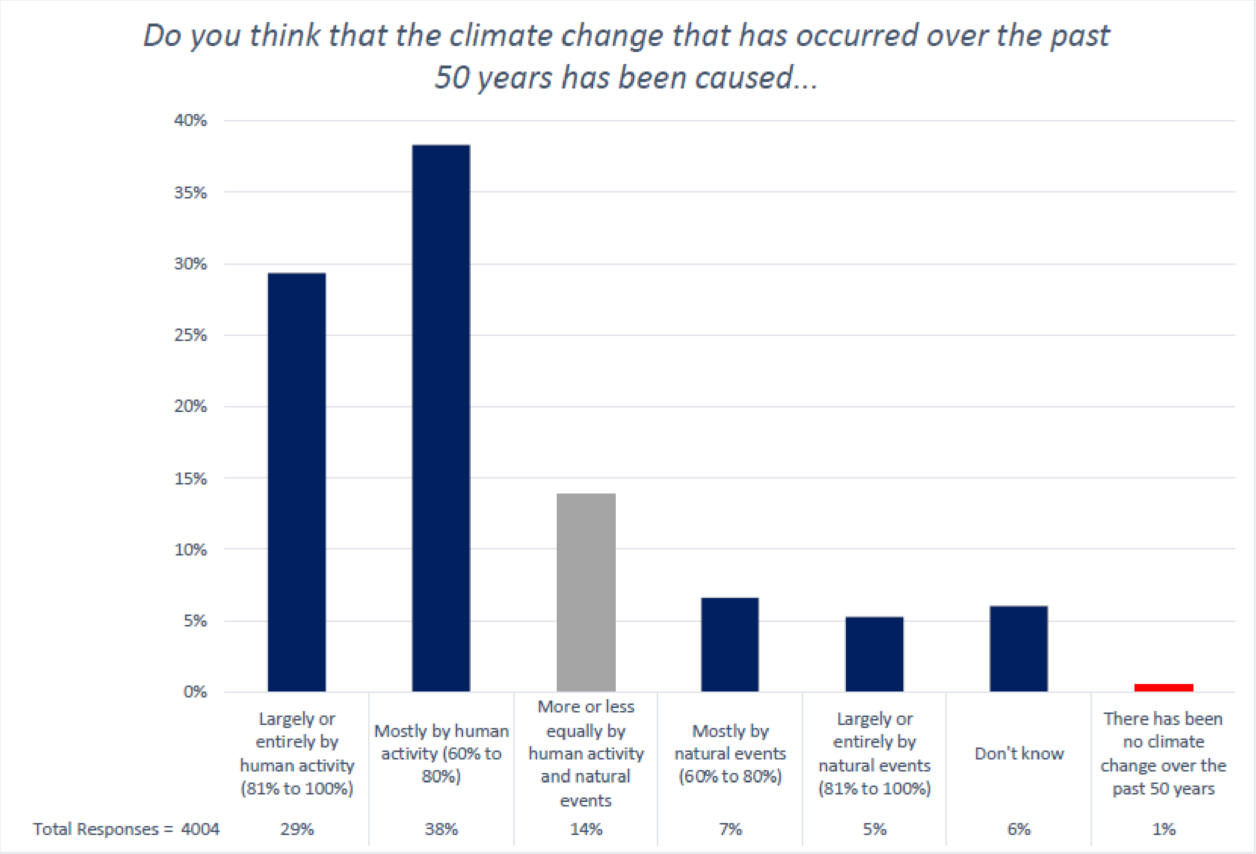

Figure 4. Results of a survey on American Meteorological Society members about climate change (Maibach et al., 2016).

Other key indicators of climate change have been found: compared to the pre-industrial period, even oceans level has increased; the ice caps of Greenland and Antarctica and ices all over the world have lost their mass and the spring snow cover in the northern hemisphere has decreased (IPCC, 2013).

1.2 Greenhouse gases

Although among the scientific community there is no agreement concerning anthropogenic contribution to the warming over the last decade (i.e. increase of the natural greenhouse effect), global GHG atmospheric concentration has increased. The present atmospheric CO_2 concentration has not been exceeded during the past 420,000 years, and likely not during the past 20 million years. The rate of increase over the past century is unprecedented, at least during the past 20,000 years (Prentice et al., 2001). Global monthly mean CO_2 concentration has reached around 410 ppm (February 2019), while in February 2018 it was around 407 ppm (NOAA Earth System Research Laboratory, 2019; Figure 5).

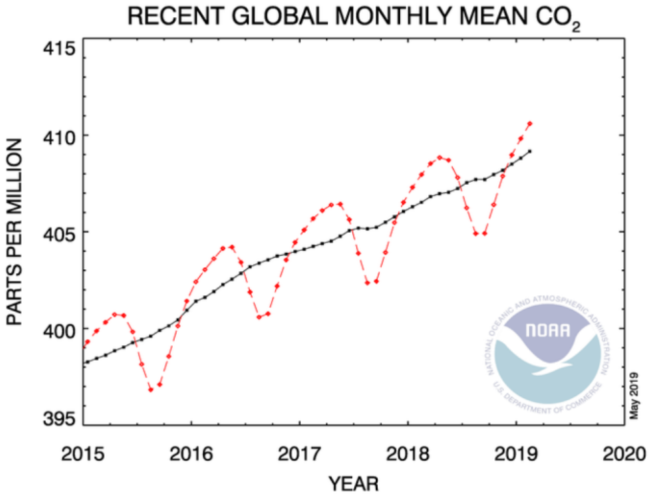

Figure 5. Recent monthly mean carbon dioxide globally averaged over marine surface sites (NOAA Earth System Research Laboratory, 2019). The dashed red line with diamond symbols represents the monthly mean values, centered on the middle of each month. The black line with the square symbols represents the same, after correction for the average seasonal cycle.

Gases that trap heat in the atmosphere are called GHGs: as it is shown in Figure 6, the main GHGs in the atmosphere are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gases (F-gases). There are four main categories of F-gases: hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), sulphur hexafluoride (SF_6) and nitrogen trifluoride (NF_3; EPA, 2019).

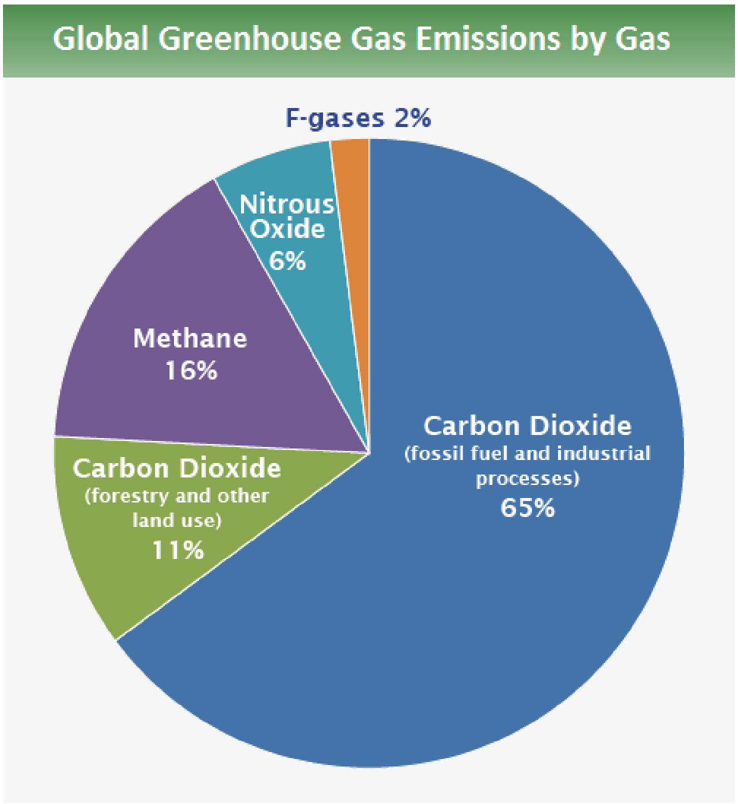

Figure 6. Global GHG emission in 2010 (IPCC, 2014).

Except for F-gases, GHGs have both natural and anthropological sources. In particular, CO_2 is the primary GHG emitted through human activities: in 2017, CO_2 accounted for about 81.6% of all U.S. anthropogenic GHG emissions (EPA, 2019). Globally, 50-65% and about 40% of total CH_4 and N_2O emissions, respectively, come from human activities (EPA, 2019). Figure 7 shows economic activities that lead to globally GHG production.

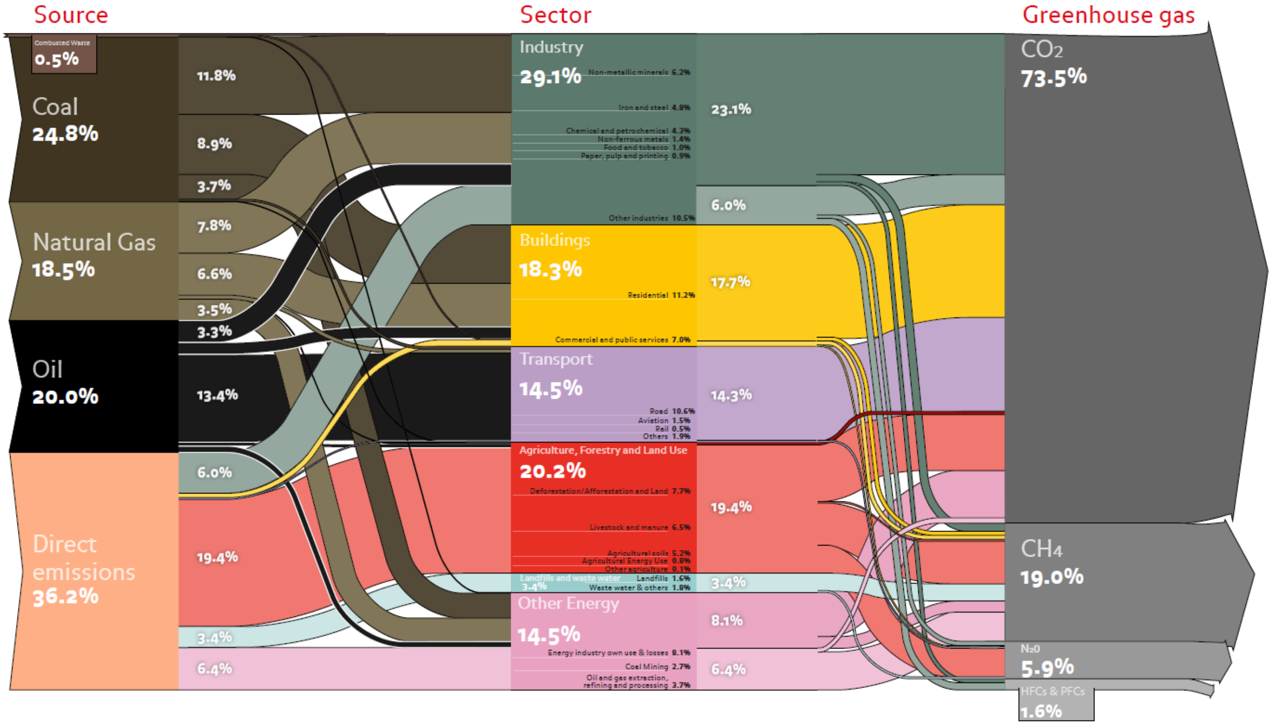

Figure 7. Global GHG anthropogenic emission by sector, with CH_4 and N_2O on a CO_2 equivalent (CO_2 eq) basis (Ecofys, 2013).

As can be seen from Figure 7, globally, GHG emission is mainly related to industry sector, followed by agriculture and buildings (29.1, 20.2 and 18.3%, respectively; Ecofys, 2013).

At national level, for the year 2017, the greatest part of the total GHG emissions is to be attributed to the energy sector, with a percentage of 80.9%, followed by industrial processes and product use and agriculture, accounting for 7.7% and 7.2%, respectively. Waste contributes with 4.3% to total emissions (ISPRA, 2019). Total national GHG emissions and removals, including Land Use, Land Use Change and Forestry (LULUCF) activities, are shown in Figure 8, subdivided by sector. As will be explained below in details, CO_2 emissions and removals are affected by changes in land-use and management activities, as well as because of forestry activities and disturbances: LULUCF activities.

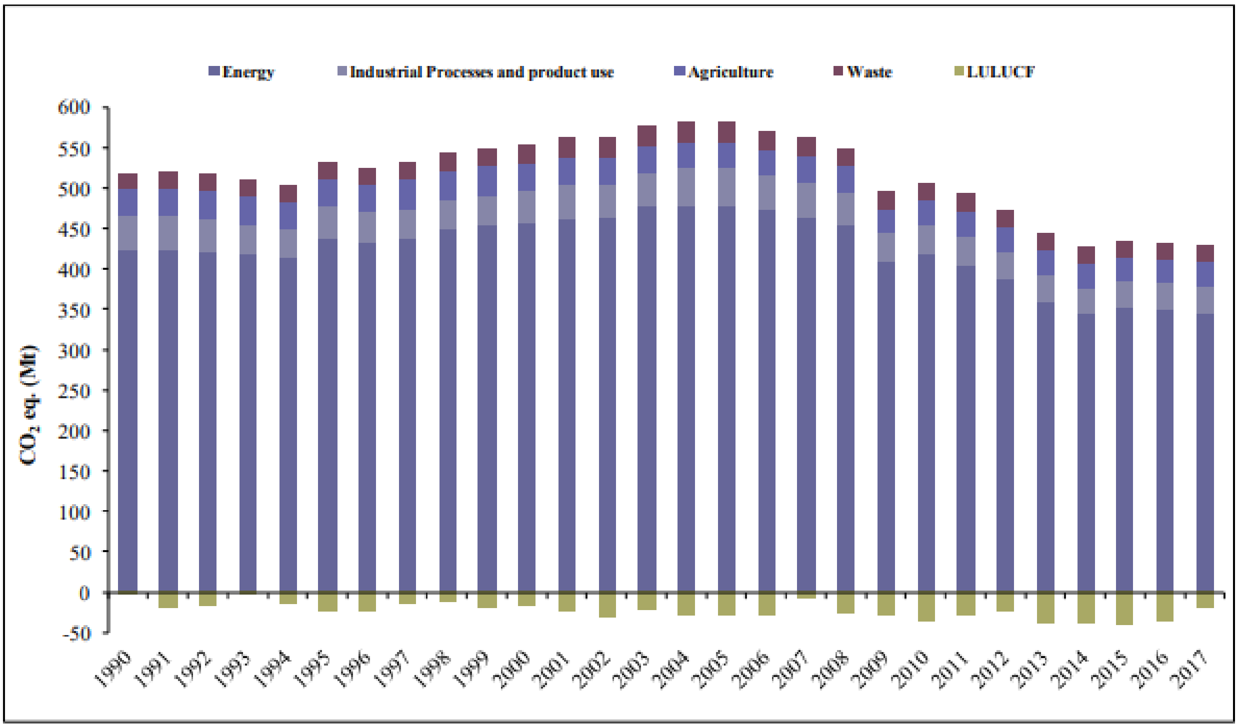

Figure 8. Greenhouse gas emissions and removals from 1990 to 2017 by sector, in Italy (Mt CO_2 eq; ISPRA 2019). LULUCF means Land Use, Land Use Change and Forestry.

According to ISPRA (2019), national greenhouse gas emissions (in CO_2 eq, excluding emissions and removals from LULUCF) decreased by 17.4% between 1990 and 2017 (from 518 to 428 millions of CO_2 eq tons; Figure 9). The most important GHG, CO_2, which accounted for 81.6% of total emissions in 2017, showed a decrease by 20.6% between 1990 and 2017. CH_4 and N_2O emissions were equal to 10.3% and 4.2%, respectively, of the total CO_2 eq GHG emissions, in 2017. Both gases showed a decrease from 1990 to 2017, 9.1% and 31.8% for CH_4 and N_2O, respectively. Other GHGs, HFCs, PFCs, SF_6 and NF_3, ranged from 0.01% to 3.6% of total emissions (Figure 9; ISPRA, 2019).

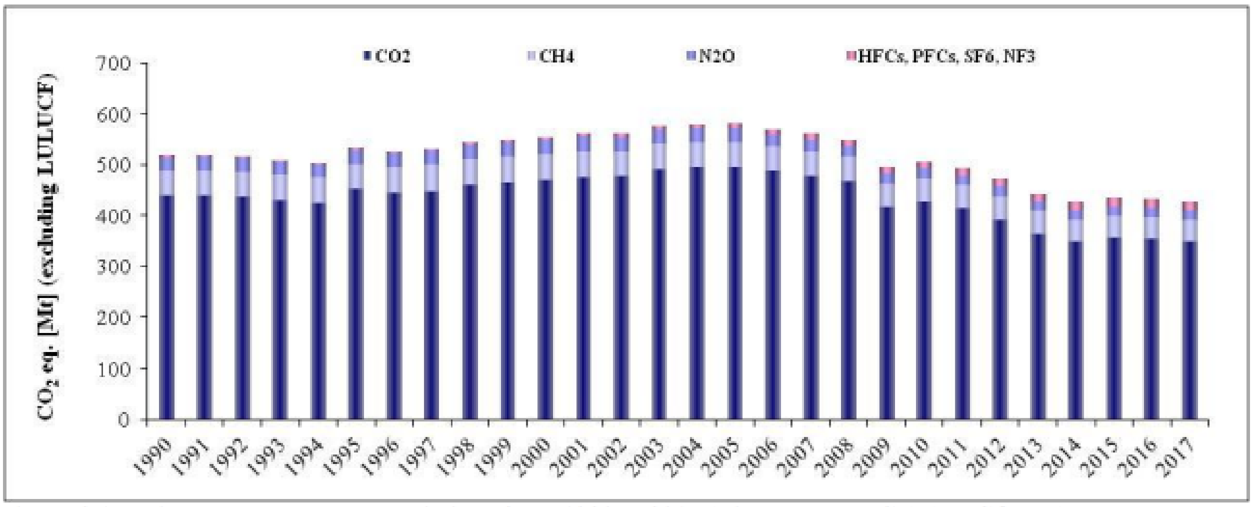

Figure 9. National greenhouse gas emissions from 1990 to 2017 (without LULUCF, Mt CO_2 eq; ISPRA, 2019). LULUCF means Land Use, Land Use Change and Forestry.

GHGs fall into two broad categories in terms of their impact on global temperature: long-lived GHGs, and short-lived GHGs. Emissions of long-lived greenhouse gases such as CO_2 and N_2O have a very persistent impact on radiative forcing, lasting from hundreds of thousands of years to over a century, respectively (Myhre et al., 2013). Atmospheric CO_2 is part of the global carbon (C) cycle, and therefore its fate is a complex function of geochemical and biological processes. Some of the excess carbon dioxide will be absorbed quickly (e.g. by the ocean surface), but some will remain in the atmosphere for thousands of years, due in part to the very slow process by which carbon is transferred to ocean sediments (EPA, 2019). On the contrary, the radiative forcing impact of short-lived GHGs, such as methane, persists for at most a decade (IPCC, 2018). Thus, warming impact related to long-lived GHGs depends primarily on the total cumulative amount emitted over the past century, while warming impact related to short-lived GHGs depends primarily on current and recent annual emission rates (Myhre et al., 2013). In addition, each GHG has a different strength in the absorption of infrared radiation emitted by the Earth (EPA, 2019).

With the aim of comparing the effects and determining the contribution of each gas to the greenhouse effect, four reference indexes have been developed: Absolute Global Warming Potential (AGWP), Global Warming Potential (GWP), Absolute Global Temperature Potential (AGTP) and Global Temperature Potential (GTP).

The AGWP is the time integrated radiative forcing of the climate system following a pulse emission of a gas over a specified time horizon. Standard time horizons for AGWP are 20, 100 and 500 years (Reisinger et al., 2010).

GWP is a measure of how much energy the emissions of 1 ton of a gas will absorb over a given period of time, relative to the emissions of 1 ton of CO_2 (Equation (1)). The larger the GWP, the more that a given gas warms the Earth compared to CO_2 over that time period: the time period usually used for GWPs is 100 years (EPA, 2019).

$$GWP_{gas} = \frac{\mathrm{AGWP_{gas}}}{\mathrm{AGWP}_{CO_2}} \quad (1).$$

According to Solomon et al. (2007), CH_4 and N_2O have a GWP (100 years) 25 and 298 times of that of CO_2, respectively. HFCs have a GWP up to 14,800; PFCs 7,390-12,200; NF_3 17,200 and SF_6 22,800.

Following Shine et al. (2005), the AGTP is defined as the increase in global annual mean surface temperature after a specific time horizon following an emissions pulse.

GTP of a GHG is defined as the ratio of its AGTP with this for CO_2 (Reisinger et al., 2010; Equation (2)).

$$GTP_{gas} = \frac{\mathrm{AGTP_{gas}}}{\mathrm{AGTP}_{CO_2}} \quad (2).$$

1.3 Livestock and dairy sector contribution to anthropogenic GHG emission

In 2006, with the report “Livestock’s long shadow”, Food and Agriculture Organization of the United Nations (FAO) defined the livestock sector as a major player in the overall anthropogenic GHG emission, responsible for 18% of GHG emissions, measured in CO_2 eq: this was a higher share than transport. The news gave rise to criticism and perplexity against livestock sector, but nevertheless some researchers disputed results presented in the FAO report published in 2006 (Glatzle, 2014; Pitesky et al., 2009). In particular, “Livestock’s long shadow” report was criticized for the use of Life Cycle Assessment (LCA) only for data related to livestock and not for transport. Transportation figures used in FAO report (2006), indeed, were direct emissions, associated mainly with combustion during transportation and not including indirect emissions associated with the transportation or oil industries (i.e., manufacturing of vehicles, resource extraction, etc.). Nevertheless, the report assessed livestock holistically, from a direct and indirect perspective. A comparison between livestock production versus transportation, with one (livestock) assessment based on a complex LCA and the other (transportation) without LCA, was, therefore, contested by Pitesky et al. (2009). In 2013, therefore, with a new report (“Tackling climate change trough livestock”), FAO stated that, with global emissions estimated at 7.1 Gt CO_2 eq (per annum, for the 2005 reference period), livestock represents 14.5% of all human-induced emissions.

Anyhow, livestock is considered to be the largest source of GHG emissions from agricultural sector (EPA, 2009).

About 44% of the livestock sector’s emissions are in the form of CH_4. The remaining part is equally shared between N_2O (29%) and CO_2 (27%; Gerber et al., 2013).

Globally, according to IPCC (2007), livestock production chains emit:

- 2 Gt CO_2 eq of CO_2 per annum, 5% of total anthropogenic CO_2 emissions
- 3.1 Gt CO_2 eq of CH_4 per annum, 44% of total anthropogenic CH_4 emissions
- 2 Gt CO_2 eq of N_2O per annum, 53% of total anthropogenic N_2O emissions.

Emissions of HFCs are considered marginal on a global scale (Gerber et al., 2013).

At national level, for agriculture, emissions refer mainly to CH_4 and N_2O levels, which account for 64.0% and 34.6% of the sectoral total, respectively; CO_2, on the other hand, shares only 1.4% of the total (ISPRA, 2019).

Nationally, for the agriculture sector, the trend of GHGs from 1990 to 2017 shows a decrease of 11.4% (Figure 10). Main drivers behind these downward trends are the reduction in the number of animals, especially cattle in the whole period and the use of nitrogen fertilizers, mainly due to the European Common Agricultural Policy measures. In addition, there has been a significant increase in the recovery of the biogas produced from animal manure and used in the energy sector for the production of electricity and combined electricity and heat production in the last years, thus contributing to the reduction of total emissions (ISPRA, 2019).

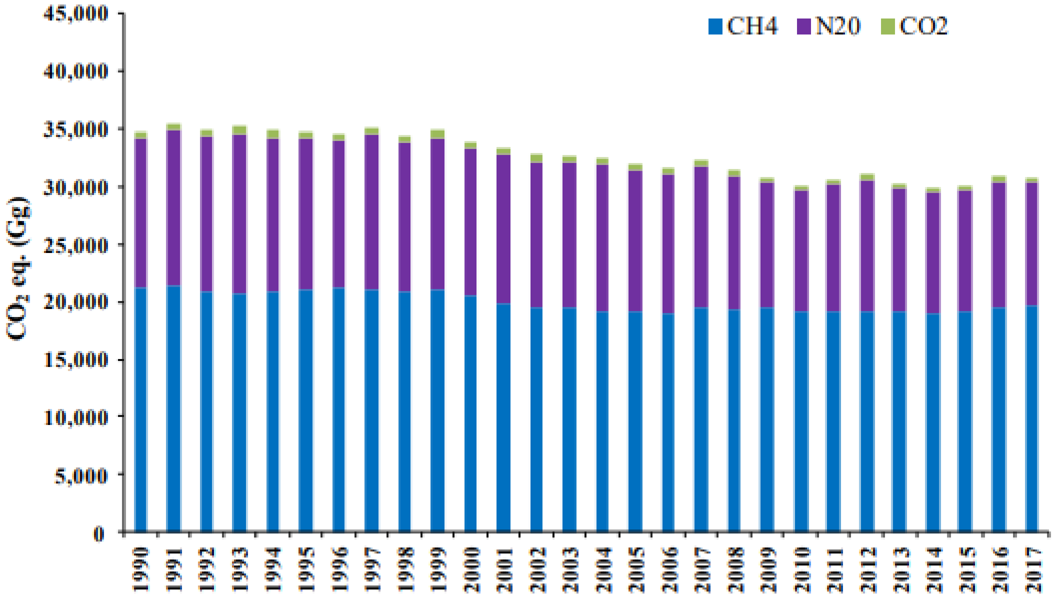

Figure 10. Trend of GHG emissions for the Italian agriculture sector, from 1990 to 2017 (Gg CO_2 eq; ISPRA, 2019).

The decrease observed in the total emissions (-11.4%) is mostly due to the decrease of CH_4 emissions from enteric fermentation (- 8.2%), which account for 46.2% of sectoral emissions and to the decrease of N_2O from agricultural soils (- 16.8%), which accounts for 27.2% of sectoral emissions (ISPRA, 2019).

According to GLEAM model (Global Livestock Environmental Assessment Model; developed by FAO in 2017), globally, cattle are the main contributor to the livestock sector's emissions, with about 5.0 Gt CO_2 eq (Figure 11). Beef cattle (producing meat and non-edible outputs) and dairy cattle (producing both meat and milk, in addition to non-edible outputs) generate similar amounts of GHG emissions: 2495 and 2128 million tonnes CO_2 eq, respectively (Gerber et al., 2013). However, average emission intensities are 46.2 kg CO_2 eq per kg of carcass weight for beef and 2.8 kg CO_2 eq per kg of Fat and protein Corrected Milk (FPCM; Gerber et al., 2013). Beef production, indeed, accounts for 2.9 Gt (41% of total sector emissions), while emissions from milk production account for 1.4 Gt (20% of total sector emissions). Pigs, poultry, buffaloes and small ruminants have much lower emission levels, each representing between 7 and 11% of sector emissions (Gerber et al., 2013; Figure 11).

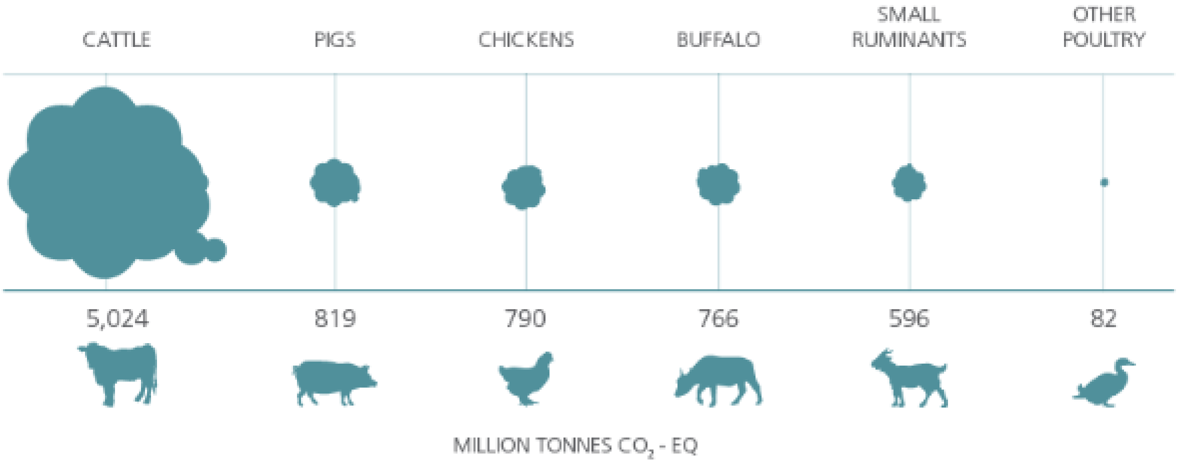

Figure 11. Global estimates of emissions by species. It includes emissions attributed to edible products and to other goods and services, such as draught power and wool. Beef cattle produce meat and non-edible outputs. Dairy cattle produce milk and meat as well as non-edible outputs (GLEAM model; FAO, 2017).

When emissions are expressed on a protein basis, beef is the commodity with the highest emission intensity (amount of GHGs emitted per unit of output produced), with an average of over 300 kg CO_2 eq per kg of protein, followed by meat and milk from small ruminants, with averages of 165 and 112 kg CO_2 eq per kg of protein, respectively. Cow milk (FPCM), chicken products and pork have lower global average emission intensities, all below 100 kg CO_2 eq per kg of edible protein (Figure 12; Gerber et al., 2013).

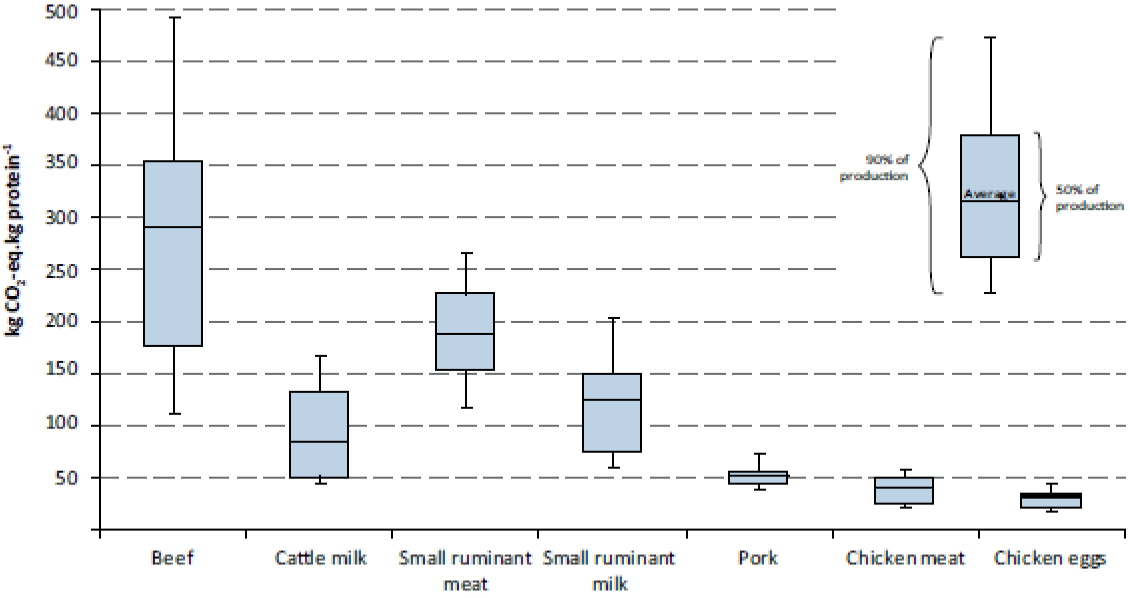

Figure 12. Global emission intensities by commodity (Gerber et al., 2013).

Considering dairy cattle sector, enteric fermentation is the main source of emissions from cattle. Related emissions amount to 1.1 Gt, representing 46.5% of the total emissions in dairy supply chain (Figure 13; Gerber et al., 2013).

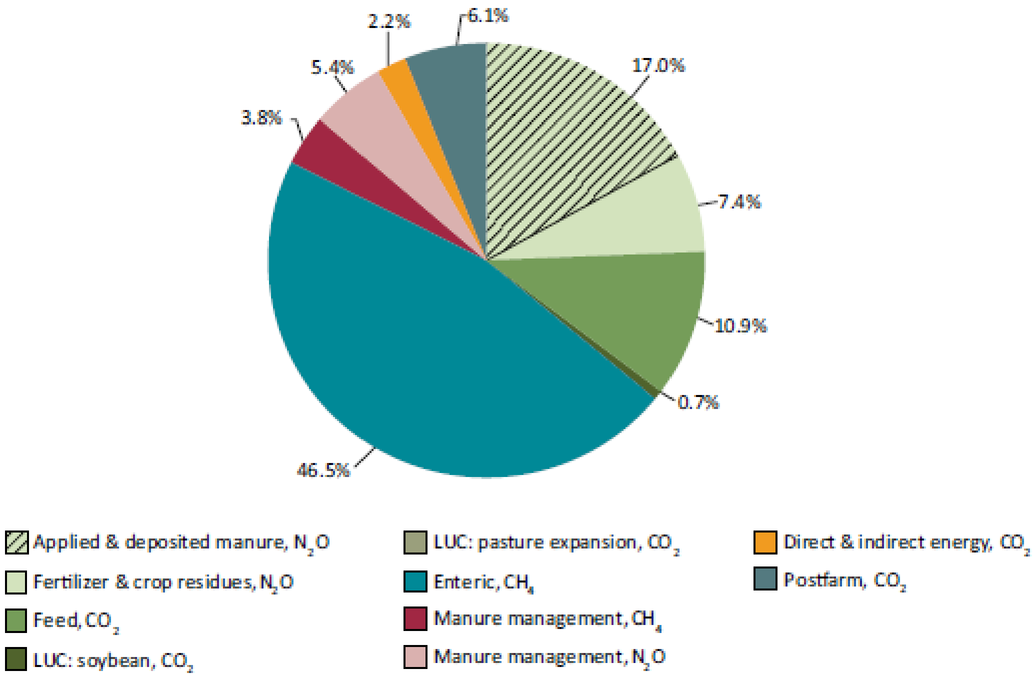

Figure 13. Global emissions from cattle milk chain, by category of emissions (Gerber et al., 2013).

Feed emissions (including emissions from pasture management) is the second largest category of emissions, contributing about 36 % to milk emissions (Figure 13). Emissions from feed production is mostly related to nitrous oxide, mainly originating from soil fertilization for growing feed crops. About 0.7% of feed emissions are related to land use change (LUC) for soybean cultivation (CO_2), while LUC for pasture expansion is not considered. Carbon dioxide emissions from energy use in feed supply chains represent about 11% of overall emissions. Emissions from energy consumption on farm (CO_2) contribute only for 2.2%. In this graph, also post-farm emissions (milk processing and transport, CO_2) are considered, accounting for 6.1% (Figure 13; Gerber et al., 2013). It is important to consider also indirect GHG emissions generated by farm activity, through the use of farm inputs (fertilizers, feed, pesticides) that do not belong to the agriculture sector, but are covered by other sectors such as industry (for the synthesis and packaging of inorganic N fertilizers and pesticides) and transport (transport of fertilizers, pesticides and feed). Emissions from electricity and fuel use are included in the buildings and transport sector, respectively (Soussana et al., 2010). Manure management involves emission of CH_4 (3.8%) and N_2O (5.4%) (Figure 13; Gerber et al., 2013).

In ruminant production, there is a strong relationship between productivity and emission intensity up to a relatively high level of productivity: emission intensity decreases as yield increases.

Gerber et al. (2011) demonstrated this relationship for milk, illustrating how differences in productivity explain the variation in emission intensity between countries. There is, indeed, a difference in emission intensities between regions: generally, emission intensity of milk production is lowest in developed dairy regions (ranging between 1.3 to 1.4 kg CO_2 eq per kg FPCM, in 2015), while developing dairy regions such as South Asia, Sub-Saharan Africa, West Asia and North Africa have higher emission intensities (ranging between 4.1 to 6.7 kg CO_2 eq per kg FPCM, in 2015). Large variations in emission intensity was also found within the same regions (FAO and GDP, Global Dairy Platform, 2018).

Figure 14 highlights the strong correlation between output per cow and emission intensity per unit of product produced.

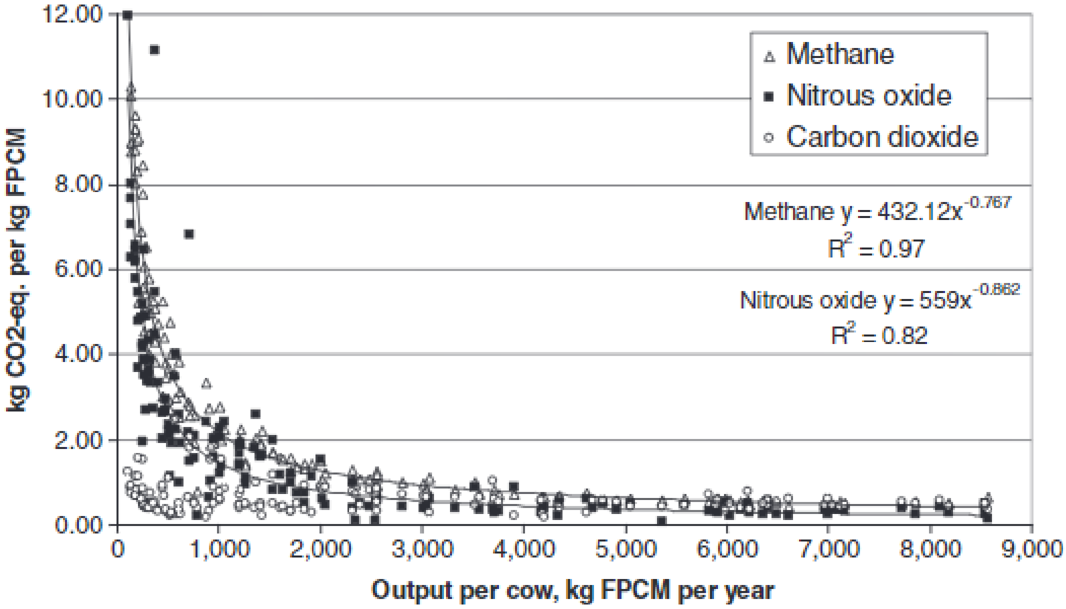

Figure 14. Relationship between methane, nitrous oxide and carbon dioxide emissions and output per cow (Gerber et al., 2011).

High-yielding animals, producing more milk per lactation, generally exhibit lower emission intensities, firstly because emissions are spread over more units of milk, thus diluting emissions relative to the maintenance requirements of the animals. As shown in Figure 15, the maintenance energy requirement does not change as a function of production, while the daily energy requirement increases as milk yield increases, thereby reducing the proportion of total energy used for maintenance. The total energy requirement per kg of milk produced is therefore reduced: a cow producing 7 kg/d requires 2.2 Mcal/kg of milk, whereas a cow yielding 29 kg/d needs only 1.1 Mcal/kg of milk (Capper et al., 2009).

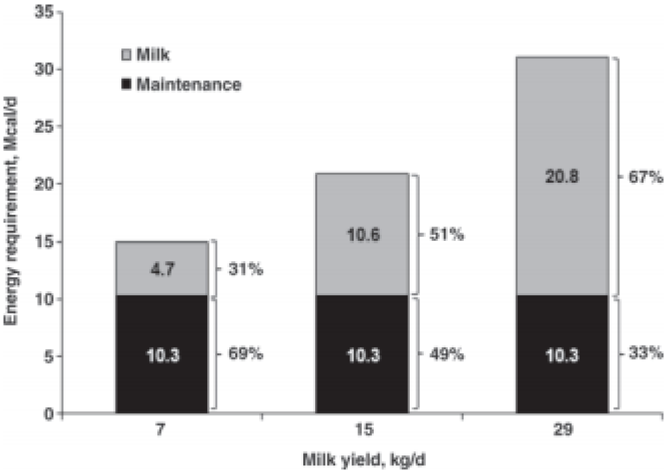

Figure 15. The dilution of maintenance effect by increasing milk production in a lactating dairy cow (650 kg of BW, 3.69% milk fat; Capper et al., 2009).

Secondly, productivity gains are usually achieved through improved practices and technologies which also contribute to increase efficiency in feed conversion ratio and consequently to emissions reduction, such as high quality feed and high performance animal genetics. Lastly productivity gains are generally achieved through herd management, animal health and reproduction practices that increase the proportion of resources utilized for productive purposes rather than simply being used to maintain the animals. This results in a reduced standing biomass (both in lactating and in replacement herds) per unit of milk produced. The impact per unit of milk is therefore reduced at both the individual cow and dairy herd level (Gerber et al., 2013).

In FAO and GDP study (2018) is shown that the dairy cattle sector's GHG emissions have increased by 18% between 2005 and 2015, because overall milk production has grown substantially by 30% (almost 666.5 billion kg of milk was produced globally in 2015; Figures 16-17).

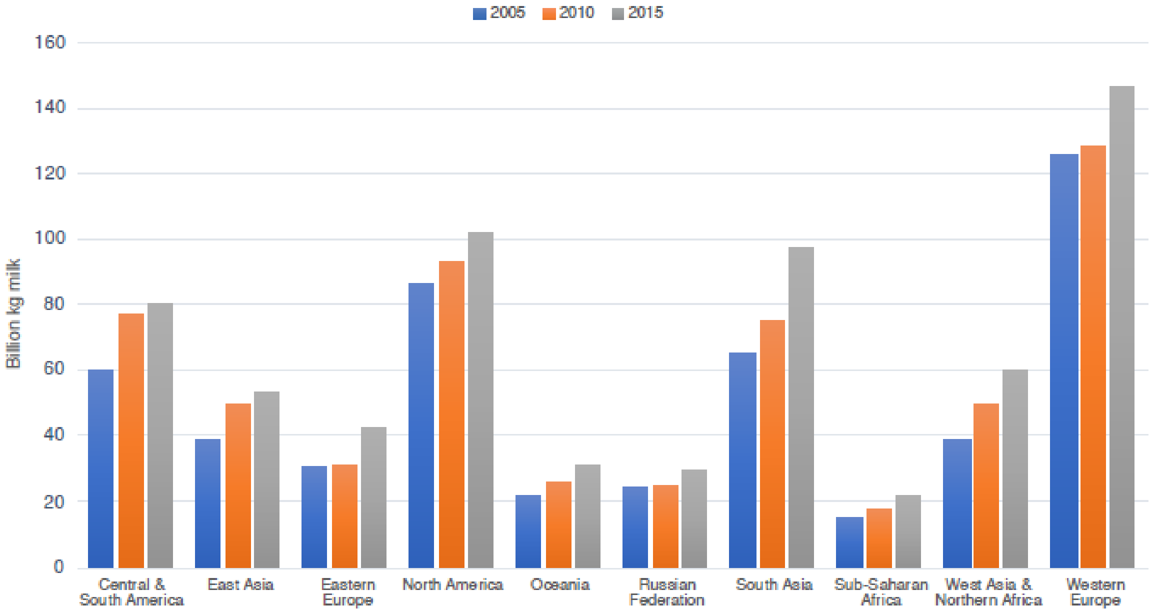

Figure16. Milk production by region in 2005, 2010 and 2015 (FAO and GDP,2018).

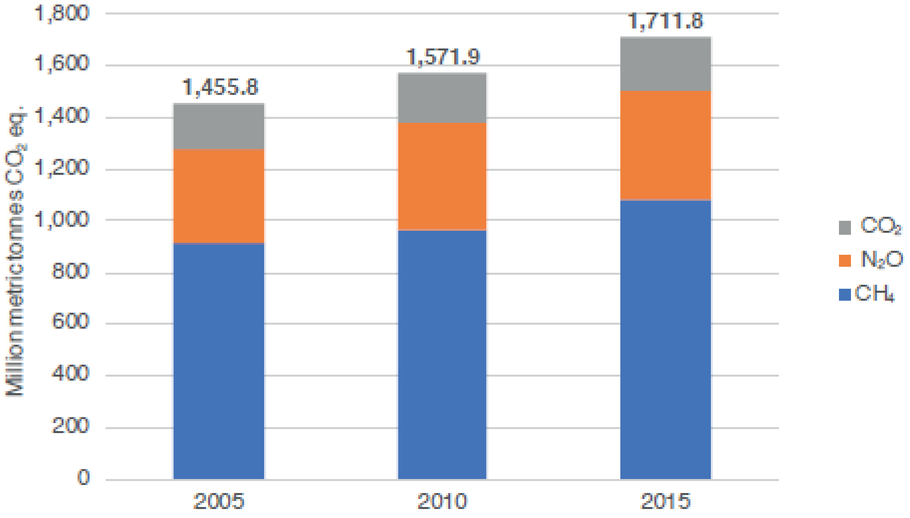

Figure 17. Absolute emissions from dairy cattle sector in 2005, 2010 and 2015 (million metric tonnes, CO_2 eq; FAO and GDP,2018).

While total emissions have increased, however, dairy farming has become more efficient resulting in declining emission intensities per unit of product. Emission intensities (GHG per kilogram of milk), indeed, have declined by almost 11% over the period 2005-2015. These declines are recorded in all regions reflecting continued improvements in on-farm efficiency (Figure 18; FAO and GDP, 2018).

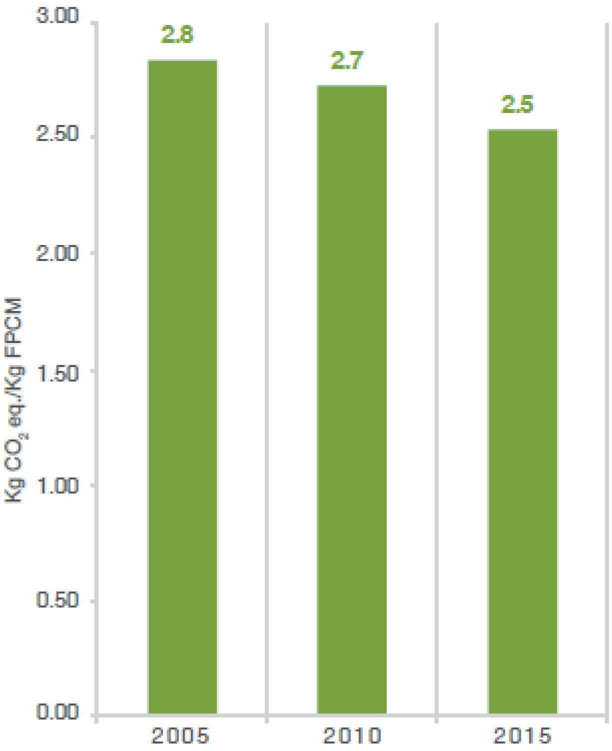

Figure 18. Average emission intensity of milk in 2005, 2010 and 2015 (FAO and GDP,2018).

1.4 Evaluation of Global Warming Potential related to milk production through Life Cycle Assessment method

In order to quantify GHG emissions, the resources used and other environmental impacts of cattle husbandry, and to highlight goal conflicts when improvements are sought, a holistic analytical approach should be undertaken. Life Cycle Assessment is the prevailing framework for environmental assessment of products and production systems that is widely used for this purpose (Roer et al., 2013). The main environmental effect quantified in LCA studies on dairy systems, indeed, is the Global Warming Potential effect (GWP), besides acidifying and eutrophic effects on watercourses, and the utilization of resources such as land and non-renewable energy during the production of milk (O'Brien et al., 2012). Global warming potential is also defined as carbon footprint of a product and it only includes the climate impact category: the product carbon footprint, indeed, is the sum of the GHGs emitted throughout the life cycle of a product (IDF, 2015). However, the simultaneous evaluation of different impact categories, allowed by the LCA method, ensures to assess consequences of mitigation strategies in a wide sense. Some mitigation strategies for one impact category, therefore, could have, at the same time, negative effects on another one.

The LCA method is a standardized procedure, that allows to record, quantify and assess the environmental damages related to a product or a service, within a very specific context, that should be determined *a priori*. This approach can be defined as integrated approach, thus it considers also steps before and after the procedure in question (Striegel, 2000). The LCA method describes, qualitatively and quantitatively, for a process or a service, emissions and refuses released into the environment, besides its requirement of energy and materials (Berlin, 2002).

The basic idea of the method LCA, indeed, is to record all the fluxes of material and energy connected to a product, a process or a service: one acts, therefore, in a *from cradle to grave* point of view.

The structure of the LCA method is defined by the international standards ISO 14040's (ISO, 2006) and it's arranged in following steps (Figure 19; Curran, 2008).

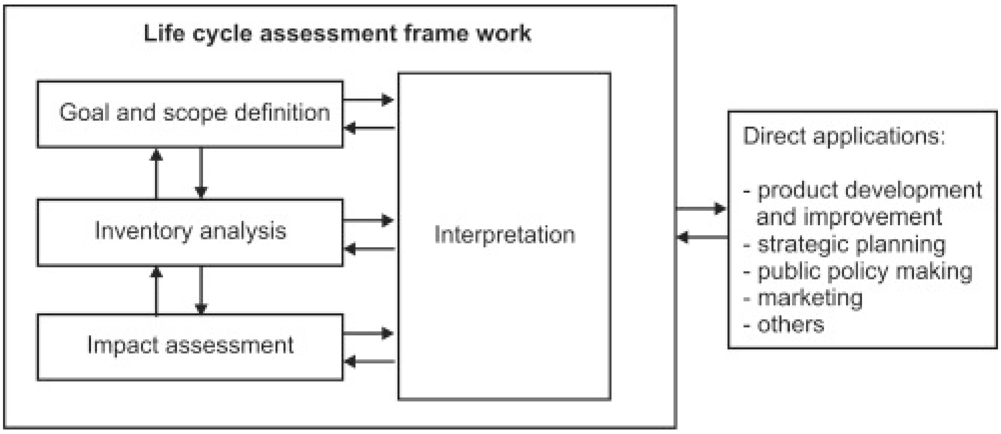

Figure 19. The four working stages of the LCA under the ISO guidelines (Curran, 2008).

A Life Cycle Assessment analysis starts with a clear declaration of the aim of the study; the system and the system boundary must be defined, which means that the processes included and those excluded from the study are stated (Figure 20). The system boundary is largely dependent on the goal of the study: specified system boundary for dairy productions could be dairy farm, a dairy plant or the entire dairy production system (Figure 20; IDF, 2015).

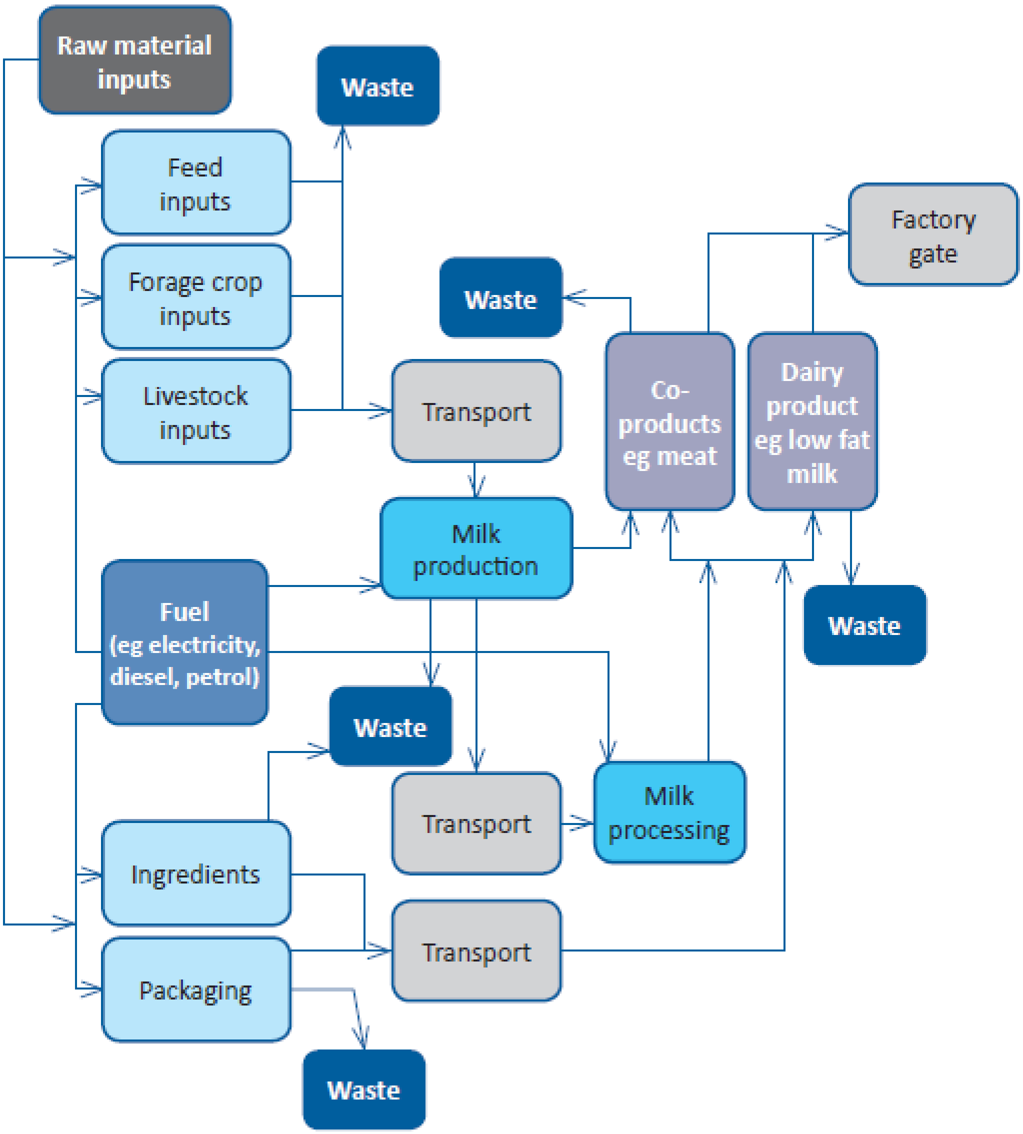

Figure 20. Example of system boundaries for milk production with low fat milk as an end product (IDF, 2015).

A proper Functional Unit (FU) must be selected. The FU is the unit to which are referred all inputs and outputs of the study. The FU is chosen to represent the function of the system; therefore, it must be selected in accordance with the goal and scope. For dairy products, in the international bibliography the most widespread FU are a kg of fresh milk or, even more, a kg of energy corrected milk (ECM, 4% fat, 3.3% protein; Hagemann et al., 2012) or FPCM (IDF, 2015; Cederberg et al, 2000; Flysjo et al., 2014; Guerci et al., 2013). ECM can be calculated as follows (Equation (3); Hagemann et al., 2012):

$$ECM = Milk\ production\ \times (0.383\ \times Fat\ \% + 0.242\ \times Protein\ \% + 0.7832)/3.1138 \text{ (3).}$$

FPCM can be calculated as follows (Equation (4); IDF, 2015):

$$FPCM\ (kg/yr) = Milk\ production\ (kg/yr) \times [0.1226\ \times\ Fat\ \% + 0.0776\ \times True\ Protein\ \% + 0.2534] \text{ (4).}$$

Next step is the choice of the allocation method: all the impact categories can be allocated through different methods. The amount of the environmental impact is, therefore, divided between the product analysed (i.e. the FU) and the other co-products of the process. The allocation is, therefore, the method used for determining the environmental impact of each product, when several products are involved in the same process (Berlin, 2002). For the dairy farm system, indeed, where the main focus is on production of milk, the meat generated from surplus calves and culled dairy cows is an important co-product. It is, therefore, necessary to determine total emission and to allocate them between milk and meat (IDF, 2015). Different allocation opportunities are available. In the last few years the most widespread allocation methods found in the international bibliography are: economic allocation method, allocation on the basis of mass or on the basis of the energy content of the products (Cederberg et al., 2000; Flysjo et al., 2014; Guerci et al., 2013). Even more, for dairy products in particular, the International Dairy Federation (IDF) suggests to use a common allocation method, in order to make all studies comparable: the global dairy sector, indeed, has been at the forefront in aligning carbon footprint calculations (IDF, 2015). This physical allocation method is based on the use of feed energy by the dairy animals and the physiological feed requirements of the animals to produce milk and meat (Equation (5)):

$$AF_{milk} = 1 - 6.04 \times \frac{M_{meat}}{M_{milk}} \text{ (5).}$$

In which:

AF is the allocation factor for milk;

M_{meat} is the sum of live weight of all animals sold

M_{milk} is the sum of FPCM.

A typical value for $\frac{M_{meat}}{M_{milk}}$ is 0.02, yielding an allocation of 12% to meat and 88% to milk (IDF, 2015).

The choice of the allocation method is one the main drivers that influence the final result in a LCA study (Cederberg e Stadig, 2003; Feitz et al., 2007).

The second phase of the LCA method is the inventory analysis, in which a flux model of the process considered is realised, as faithful as possible to reality (Figure 20; Sanfilippo e Ruggeri, 2009). The flux model includes data concerning inputs and outputs of resources, energy, transport emissions in air and water for all the activities within the system boundaries (Berlin, 2002). In order to realize this flux model, it’s necessary to carry out a detailed survey, in order to collect primary data. The inventory analysis can also be completed with the help of dedicated databases (e.g. Ecoinvent, Agri-footprint), that are available in the most used Software for LCA studies (e.g. Simapro; Blonk Consultants, 2014; Frishknecht et al., 2007).

Following the inventory analysis, there is the real assessment of the environmental impact (Figure 20): in particular, results are ordered and awarded to specific impact categories (Berlin, 2002), such as, the most widespread, GWP, measured in CO_2 eq. Different characterisation methods are available: for agricultural and livestock systems International Reference Life Cycle Data System (ILCD) and ReCiPe are the most applied (ILCD Handbook, 2011; Goedkoop et al., 2008). Within the characterisation method, for each impact category, different characterisation factors allow the conversion of the inputs and outputs in the environmental impact: this makes it difficult to compare studies adopting different characterisation method, mainly because each method expresses impact categories through different unit of measure.

The last phase is the interpretation of the results obtained (Figure 20), that have to be analysed in order to highlight critical points among the process and to assess mitigation strategies, that represents the final aim of each environmental impact study.

1.5 Enteric methane emission

As it has been said, it is very well known that in ruminant production systems, enteric CH_4 is the most important GHG emitted at farm scale (Ogino et al., 2007). In addition, enteric fermentation is also the second major source (17%, while the first major source are wetlands, 30%) of global CH_4 emission, including natural and anthropogenic origin (Figure 21; EPA, 2010 and EPA, 2011a).

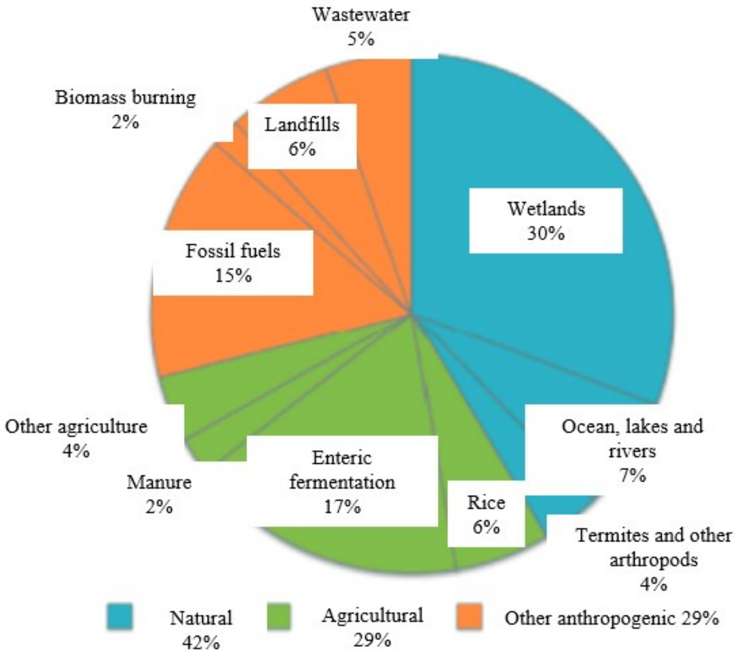

Figure 21. Estimated proportion of global CH_4 emissions from natural and anthropogenic sources (EPA ,2010 and EPA, 2011a).

Globally, countries with the largest livestock-associated enteric CH_4 emissions are Brazil, China, India, Europe and United States (Figure 22; EPA, 2011a). In the United States, 95% of enteric CH_4 arises from ruminant livestock (EPA, 2011b): this proportion can be assumed for other countries, although the contributions from beef versus dairy operations will vary.

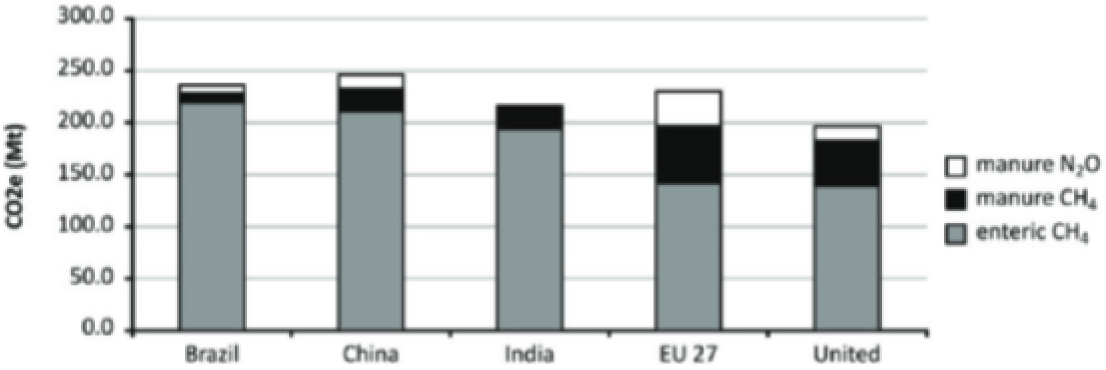

Figure 22. The 5 countries and regions with the largest livestock-associated enteric CH_4 emissions on a million-metric-tonne (Mt) of CO_2 eq basis. Manure CH_4 is emitted by storage systems where anaerobic fermentation occurs. Manure CH_4 and N_2O can be from either ruminant or non-ruminant livestock operations (EPA, 2011a). EU 27 = European Union countries.

In Italy, in 2017, CH_4 emissions from enteric fermentation were 569.26 Gg, which represents 72.3% of CH_4 emissions for the agriculture sector (72.7% in 1990) and 32.5% for national CH_4 emissions excluding LULUCF (32.1% in 1990). Methane emissions from this source consist mainly of cattle emissions: dairy cattle (263.55 Gg) and non-dairy cattle (196.84 Gg). These two sub-categories represented 46.3% (47.4% in 1990) and 34.6% (37.6% in 1990) of total enteric fermentation emissions, respectively (ISPRA 2019).

Enteric fermentations occur in anaerobic conditions, mainly in the rumen of cattle (87%), by microbial population (Murray et al., 1976). Primary digestive microorganism (bacteria, protozoa and fungi) hydrolyze proteins, starch and plant cell wall polymers into amino acids and sugars. Sugars are then fermented to volatile fatty acids (VFA), hydrogen (H_2) and CO_2, by primary and secondary digestive microorganism (Boadi et al., 2004). The most important VFA produced are: acetate, propionate and butyrate and they are absorbed through ruminal wall and used by the animal as source of energy (Bittante et al., 2012). Conversely, H_2 does not accumulate in the rumen, but as soon as produced, is used by methanogenic archaea, a microbial group distinct from Eubacteria, to reduce CO_2 into CH_4, according to the following equation ((6); Martin et al., 2010):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (6).$$

The level of enteric methane emission is mainly determined by the level of feed intake: Benchaar et al. (2001) reported an increase in CH_4 (Mcal d^{-1}) with increasing DMI, irrespectively of the diet type. However, an increase in feeding level induces higher passage rate, reducing the extent of microbial access to organic matter, which in turns could reduce the extent and rate of ruminal fermentation (Mathison et al., 1998).

Besides feed intake, enteric methane emission is due to the composition of the diet (Beauchemin et al., 2008; Boadi et al., 2004; Martin et al., 2010). In particular, NDF and EE are the main dietary drivers influencing the availability of H_2, the main substrate for CH_4 formation. Body weight and milk fat percentage also have an important role in predicting methane emissions from enteric fermentations.

Moraes et al. (2014), developed methane emission prediction equations ((7), (8), (9), (10)) of distinct cattle categories. In particular:

- Lactating cows: $CH_4 = -9.311\ (1.060) + 0.042\ (0.001) \times GEI + 0.094\ (0.014) \times NDF - 0.381\ (0.092) \times EE + 0.008\ (0.001) \times BW + 1.621\ (0.119) \times MF$(7).
- Non lactating cows: $CH_4 = 2.880\ (0.200) + 0.053\ (0.001) \times GEI - 0.190\ (0.049) \times EE$ (8).
- Heifers: $CH_4 = -1.487\ (0.318) + 0.046\ (0.001) \times GEI + 0.032\ (0.005) \times NDF + 0.006\ (0.0007) \times BW$ (9).
- Steers: $CH_4 = -0.221\ (0.151) + 0.048\ (0.001) \times GEI + 0.005\ (0.0005) \times BW$ (10).

Equations are presented as parameter means and standard deviation in parenthesis, where:

$GEI = Gross\ energy\ intake\ \ (MJ/d)$

$NDF = Dietary\ neutral\ detergent\ fiber\ proportion\ (\%\ of\ dry\ matter)$

$EE = Dietary\ ether\ extract\ proportion\ (\%\ of\ dry\ matter)$

$BW = Body\ weight\ (Kg)$

$MF = Milk\ fat\ (\%)$

In order to assess CH_4 produced through enteric fermentation, it is possible to use several estimation equations, or to make *in vitro* analysis, or to make *in vivo* trials in respiration chambers.

Methane emissions can be considered as methane production (g/day), methane yield (g/kg dry matter intake) or methane intensity (g/kg milk). In Aguerre et al. (2011), indeed, increasing forage:concentrate ratio from 47:53 to 68:32 increased CH_4 emission from 538 to 648 g/cow per day, from 25.9 to 31.9 g/kg dry matter intake and from 14.0 to 17.8 g/kg of milk.

In Arndt et al. (2015), in the same way, cow fed diet with increasing alfalfa silage:corn silage ratio of 20:80, 40:60, 60:40 and 80:20 showed a methane production of 697, 743, 729, 683 g/cow per day, respectively. They showed a methane yield of 26.6, 28.1, 27.5 and 25.7 g/kg dry matter intake, increasing the amount of alfalfa in the diet, while methane intensities were, for cows fed increasing amount of alfalfa silage in the diet, 18.5, 19.1, 18.7 and 18.0 g /kg FPCM.

1.6 Ration composition and its relationship with GHG emission

1.6.1 Forages

Forages are the main component of lactating dairy cows diet: they represent from 30% to 80% of the dietary dry matter (DM; Gallo et al., 2013). Fodder systems are strictly related to the environment and farming system. In particular, in the Po plain of Northern Italy, the main forages fed to dairy cattle are whole plant corn silage, alfalfa as silage or hay and cool-season crops (silage or hay). Another forage quite widespread in the Po plain is meadow hay, especially in those farms that produce milk for Parmigiano Reggiano Protected Denomination of Origin (PDO) cheese.

Forages are variable in terms of chemical composition and nutritive values (Cherney, 2000); in Gallo et al. (2013), a study was conducted on the chemical analysis of the most common Northern Italian fodder. The crude protein resulted to be on average 166 g/kg DM (SD 31) for alfalfa hay and 80 g/kg DM (SD 19) for grass hay. The lowest value came out with the corn silage that had a crude protein content of 76 g/kg DM (SD 7). On the contrary, corn silage had the highest value of non fibre carbohydrates, 408 g/kg DM (SD 46), while alfalfa and grass hay had a non fibre carbohydrate content of 274 g/kg DM (SD 74) and 194 g/kg DM (SD 59), respectively. In particular, corn silage showed a starch content of 288 g/kg DM (SD 43). Because of its high starch content, indeed, corn silage is considered a good source of ruminally fermentable carbohydrates (Brito and Broderick, 2006; Dhiman et al., 1997). Due to its energy value, in addition to a high agronomic yield, whole plant corn silage is largely used in the Po plain. Moreover, the environmental conditions of Northern Italy are particularly favourable to the production of corn silage (Zucali et al., 2018). According to Pirondini et al. (2012), for commercial dairy farms of the Po plain, therefore, it represents about 29.0% of the diet (on DM). Also the fibre content of forages is very different: grass hay had a value of 625 g/kg DM (SD 53), alfalfa hay 494 g/kg DM (SD 74) and corn silage 453 g/kg DM (SD 46) of neutral detergent fiber (Gallo et al., 2013).

1.6.2 Concentrates

In order to meet the high demand of nutrients needed to assure high milk production, a lot of concentrates are also used in dairy cows' diet. High moisture ear corn and corn grain meal usually provide the animals with non-structural carbohydrates, contributing to meet their energy requirements.

The availability of carbohydrates for ruminal microbes and the host animal are affected by grain source and processing (Ekinci and Broderick, 1997). Because of its starch granule structure (McAllister et al., 1993), corn starch is not extensively degraded in the rumen: early harvesting for ensiling as high moisture corn is one of the successful method to increase its digestibility (Hale, 1973). Compared to corn grain meal, indeed, high moisture ear corn, due to the fermentation process, has a greater starch rumen degradability and, as a consequence, a greater digestibility (McCaffree and Merril, 1968; Clark and Harshbarger, 1972). Indeed, cows fed high moisture ear corn show higher milk yield, compared to those fed corn grain meal (De Brabander et al., 1992; Clark et al., 1973). Soybean meal (SBM) is the most widespread protein source; according to Groff and Wu (2005), it seems that milk production benefits until 17.0% of diet protein content, but additional increasing in protein percentage has no further effect, unless production is exceptionally high.

1.6.3 Diet composition and methane emissions

As it has been previously mentioned, diet composition is, first of all, strictly related to enteric methane production. The level of enteric methane emission, indeed, is mainly determined by feed intake, followed by composition of the diet and type of forage preservation (Boadi et al., 2004).

Concerning forage source in the diet, according to the international bibliography, it is very well known that grain silages involves less CH_4 emissions compared to grass silages. In Hassanat et al. (2013), it was demonstrated that feeding dairy cows with corn silage despite alfalfa silage involves less CH_4 emissions (in terms of g/kg of DMI). The high starch content of corn silage, compared to perennial grass or legume silage, therefore, tends to shift ruminal fermentation patterns towards the formation of more propionate and less acetate, which lowers hydrogen availability for methanogens that use hydrogen and carbon dioxide to produce CH_4 (Little et al., 2017). In addition, grain silages are readily digestible and increase animal intake and performance. By increasing voluntary intake, forage crops such as corn silage can reduce the ruminal residence time of feed (increasing turnover), hence restricting ruminal fermentation and promoting post ruminal digestion (Beauchemin et al., 2008). When corn silage replaces grass silage, moreover, the increase in voluntary intake, combined with the increase in the energetically more efficient post ruminal digestion, improves animal performance, lowering CH_4 emissions per unit of product (O`Mara et al., 1998). Even the type of forage preservation has an effect on enteric methane production (Boadi et al., 2004). Daily methane production, indeed, was reported to be lower when cows were fed ensiled forages rather than dried ones (Benchaar et al., 2001). Diets of higher yielding animals usually contain more concentrates and less roughage and, hence, have a higher digestibility that contributes to reduce enteric methane production (Capper et al., 2009). Replacing structural carbohydrates from forages in the diet with non-structural carbohydrates contained in most energy-rich concentrates, indeed, is associated with increases in feed intake, higher rates of ruminal fermentation, accelerated feed turnover and a shift of VFA production from acetate towards propionate. This results in lower CH_4 production (Martin et al., 2010). Diets characterized by a high content of NDF, conversely, involves higher methane production at enteric level: roughage based diets favor acetate production and increase CH_4 per unit of fermentable organic matter (Johnson and Johnson, 1995).

A lower enteric methane production results, also, to be related to diets containing more than 4% (%DM) of ether extract. Dietary fat, indeed, seems to act as reducers of ruminal methanogenesis, without affecting other ruminal parameters (Martin et al., 2010). Dietary lipid reduces CH_4 emissions by biohydrogenation of unsaturated fatty acids, enhancing propionate production and inhibiting protozoa (Johnson and Johnson, 1995).

1.6.4 Diet composition and other GHG emissions

Besides enteric methane emission, diet composition is also linked to CO_2 and N_2O losses in the air.

Feed production processes, both on-farm and out of the farm, and transport, indeed, are considered to be, in ruminant production systems, responsible of a high amount of GHG emissions as well (Ogino et al. 2007). According to FAO and GDP (2018), emissions (CO_2 and N_2O) from feed production, processing and transport account for 29.4% of the total emission from dairy sector.

Considering forage sources, corn silage implies a greater energy CO_2 emission (per hectare), that can be attributed to cropping practises, including use of fertilizers and herbicide, compared to a grassland cultivation, for example alfalfa silage (Little et al., 2017). Planting perennial forages such as alfalfa (or meadow), in addition, may reduce agricultural GHGs emissions by sequestering CO_2 in agricultural soils

(Jarecki and Lal, 2003), as explained below. The lower crude protein content of corn silage, furthermore, necessitate supplementing corn silage-based diets with protein sources (e.g. soybeans), adding CO_2 emissions (Little et al., 2017), mostly related to LUC, but also to feed production processes. Using concentrates, indeed, can reduce CH_4 emissions from enteric fermentations, particularly in the case of energy concentrates, but this could have limited advantages. Increased dietary concentrates may sometimes increase total net emissions, as more grain must to be grown, processed and transported, leading to increase use of pesticides, fertilisers and additional sources of emissions associated with production and transportation infrastructure (Beauchemin et al., 2008) and change of land use. Ogino et al. (2007) reported that production and transport of concentrates produce 2.1 times as much CO_2 as that of roughage per unit of total digestible nutrient.

Some perennial crop such as legumes (e.g. alfalfa) may increase soil cropping direct N_2O, because of their greater N input from crop residues (Little et al., 2017). In addition, increasing soil aeration with tillage, as for annual crop (e.g. corn), may significantly reduce N_2O emissions, formed in the soil through nitrification and denitrification (Pinto et al., 2004; Eckard et al., 2010). In terms of nitrogen utilization, corn silage also promotes lower urinary and fecal N losses (Hassanat et al., 2013), compared to the other forage sources and this would result in lower N volatilization from manure (i.e. N_2O emissions; Dijkstra et al., 2011; Little at al., 2017). The increase in volatile solids content of the manure related to corn silage, however, may also lead to increase CH_4 emissions from manure storage (Hassanat et al., 2013).

1.7 Carbon soil sequestration and land use change in milk production system

1.7.1 Carbon soil sequestration

Soil respiration is the primary path by which CO_2 fixed by plants returns to the atmosphere. The total global emission of CO_2 from soils is recognized as one of the largest fluxes in the global carbon cycle (Figure 23) and small changes in the global environment could have a large effect on the concentration of CO_2 in the atmosphere (Schlesinger and Andrews, 2000).

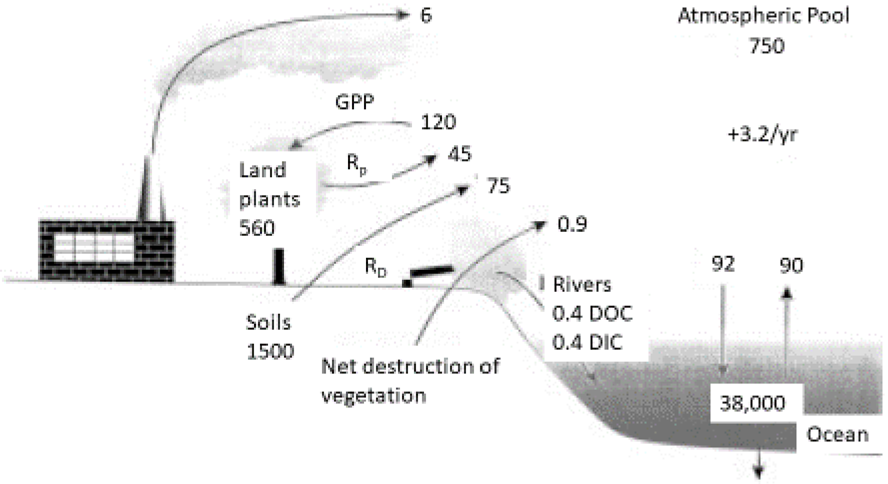

Figure 23. The global carbon cycle. All pools are expressed in units of 10^{15} gC and all fluxes in units of 10^{15} gC/yr, averaged for the 1980s (Schlesinger and Andrews, 2000).

Human activities, indeed, are altering the natural carbon cycle by adding more CO_2 to the atmosphere, by influencing the ability of natural *sinks* (natural reservoir that stores C accumulated over an indefinite period of time, e.g. forests) to remove CO_2 from the atmosphere (process of C sequestration), and by influencing the ability of soils to store carbon (EPA, 2019). Soil C sequestration is considered to be the mechanism responsible for most of the GHG mitigation potential in the agriculture and livestock sector (Soussana et al., 2010). The world's soils, in fact, are estimated to contain 1500 Gt of soil organic carbon (SOC), roughly double the amount of C in the atmosphere (750 Gt; Schlesinger and Andrews, 2000). Permanent grasslands in the European Union represent a *sink* of 3.1 ± 18.8 million tonnes C per year (11.4 ± 69.0 million tonnes CO_2 eq per year), equivalent to 3% (± 18 %) of the yearly emissions of the ruminant sector in the European Union (Gerber et al., 2013). Thus, crops are usually considered as potential *sink* of carbon dioxide, because CO_2 is fixed into carbohydrate via photosynthesis (Hopkins and Del Prado, 2007). Any practice that increases this photosynthetic input of C and that slows the return of stored C in the atmosphere, will, thereby, increase C stock (amount of C sequestered from the atmosphere), will increase the sequestration of C, or will build C *sinks* (Smith et al., 2007). Practices such as conservation tillage, the use of cover crops, and erosion control, therefore, can promote C storage in the soil (Pacala and Socolow, 2004). Conservation tillage sequesters the equivalent of about 0.3 t C ha^{-1} $year^{-1}$ (Baker et al., 2007). On the contrary, when soils are disturbed through cultivation, their content of organic matter declines. This decline is seen because conditions for decomposition (i.e. soil aeration and moisture content) are often improved when soils are disturbed, leading to greater rates of soil respiration. In addition, soil respiration increases with increasing temperature, thus, as the planet warms, the area of temperature-limited decomposition should decline, and soils increasingly should become a *source* of CO_2 to the atmosphere (Schlesinger and Andrews, 2000). While changes in soil carbon can affect the carbon footprint of agricultural systems, they are rarely included in GHG analysis of dairy products because of the complexity and lack of the consensus about the methodology (Little et al., 2017).

Perennial vegetation exhibits net C assimilation (through photosynthesis) for a much greater portion of growing period (longer presence in the field), compared to annual cropping (Baker et al., 2007, Figure 24). That will lead to have higher amount of C stored in the soil with perennial vegetation, compared to annual crops.

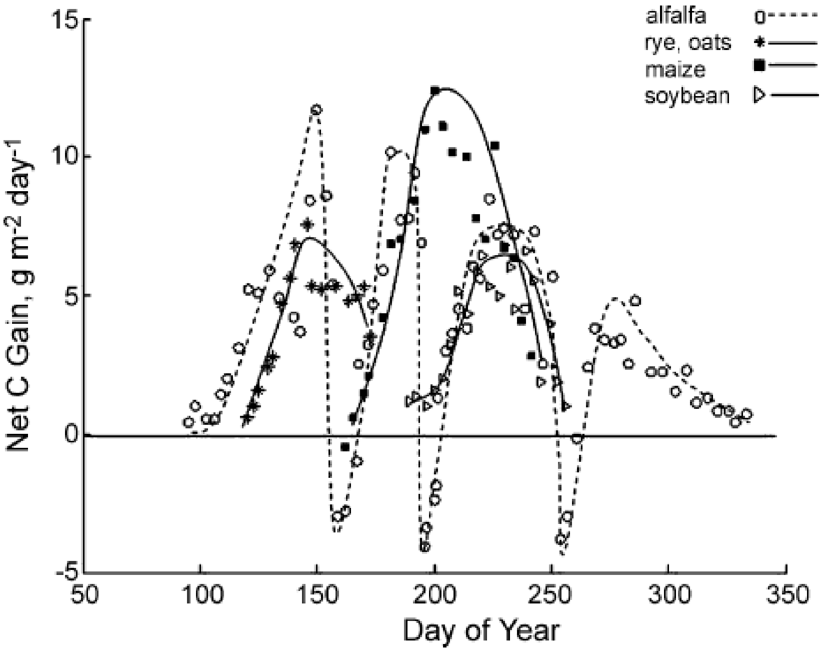

Fig. 24. Measurements of daily net carbon gain for selected crops in Minnesota. Alfalfa is the sole perennial of the group; its growing season, or C assimilation period, exceeds that of each annual crop by 100 days or more. (The three periods during the growing season when alfalfa exhibits net loss of C are periods of regrowth following hay harvest, Baker et al., 2007).

The cumulative effect on SOC of perennial vegetation versus annual cropping is evident in the data collected in Russia by Mikhailova et al. (2000; Figure 25).

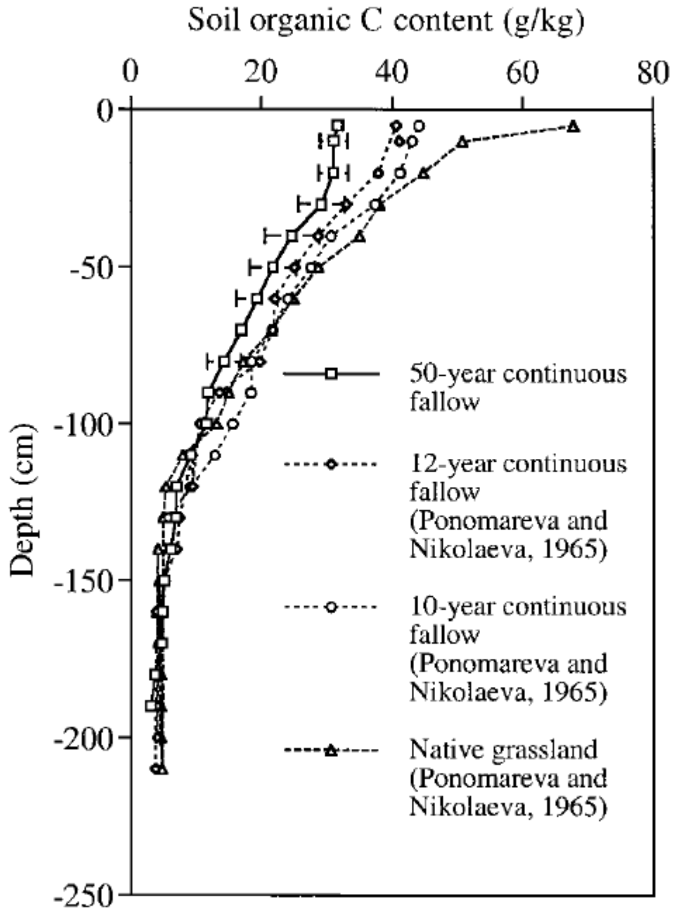

Figure 25. Changes in SOC and total N with time of plowing and depth (Mikhailova et al., 2000).

1.7.2 Land use change

When forest or natural grassland is converted to cropland, up to one-half of the soil C is lost, primarily because annual tilling increases the rate of decomposition, by aerating undecomposed organic matter: about 55 Gt C has been lost historically in this way (Pacala and Socolow, 2004). As a protein sources for animal nutrition, SBM is the first widely available (71%, USDA, 2017), but it has a great environmental impact, mostly related to LUC that this crop carries with itself. Deforestation phenomena are taking place in all the South of America, mostly in the Amazon forest, mainly located in Brazil but also in some forest areas in Argentina, Paraguay, Uruguay and Chile. Europe and China are the main importers of South American soybean and, according to ASSALZOO (2018), the dependence of Italy on foreign countries oscillates, depending on the years, from 85% to more than 90%; the leading suppliers for Italy are Argentina and Brazil. Especially during the last few months, attention has more turned towards Brazilian Amazon deforestation, due to the installation of Jair Bolsonaro as Brasilian President. The president is building his success denying the climate change and assuming projects of mining operations in the Amazon forest (Blunck, 2019). Although pasture expansion remains by far the primary direct cause of Amazonian deforestation, also increased mechanized crop production (i.e. soybean) must be viewed as a driver of deforestation, even if new fields replace only existing pastures or savannah lands (Arima et al., 2011). In particular, in some Brasilian states (e.g. Mato Grosso), the increase in soybean crop in regions previously used for pasture, may have led to a displacement of pastures further north into the forested areas, causing indirect deforestation there. Therefore, soybean cultivation may still be one of the major causes of deforestation in Brazilian Amazon (Barona et al., 2010). LUC is estimated to contribute 9.2% to livestock sector overall GHG emissions (6% from pasture expansion, with the rest from feed crop expansion). While relatively limited when averaged globally and over all species, LUC emissions are significantly higher for some specific supply chains and regions: they amount to 15% for beef production (linked to pasture expansion) and 21% in chicken meat production (linked to soybean expansion; Gerber et al., 2013).

The uncertain role of soybean production has brought to have, in the international bibliography, a high debate on how to account for emissions from LUC and there is still no current shared consensus (Flysjo et al., 2011). According to IPCC (2006), LUC emissions estimated for Argentina ranged between 0.3 and 4.2 kg CO_2 eq per kg of soybean and it ranged between 3.0 and 7.7 kg CO_2 eq per kg of soybean produced in Brazil. Leip et al. (2010), due to the lack of knowledge of the origin of the converted land, investigated three scenarios to span possible outcomes. In scenario I all additional cropland is assumed to come from grassland and savannas. Scenario II applies a more likely mix of transition probabilities (grassland, shrub land and forests) and scenario III is considered as a maximum emission scenario, with a high share of carbon-rich forest in the original land cover. This method of calculation resulted in three different weighted LUC-factors for imported soybeans from non-European countries in kg CO_2 per kg product: 0.371 according to the first scenario, 1.684 for the second one and 7.912 for the third scenario. In addition, some authors assume that all land occupation is associated with some GHG emissions from LUC, irrespective of where in the world the land occupation takes place. The central argument for the method in Audsley et al. (2009) is that all demand for agricultural land contributes to commodity and land prices, and, therefore, contributes to LUC. Thus, total LUC emissions attributable to agriculture are divided by total commercial agricultural land area, resulting in a LUC factor of 1.4 t CO_2 eq per ha. The method suggested by Schmidt et al. (2011) means that all GHG emissions, as a result of LUC during one year, are distributed on all activities occupying land. This method is under development and LUC factors will change in the future, but the principle that all land occupation is responsible for LUC (deforestation) will remain.

1.8 Mitigation strategies for GHG emissions related to milk production

World population is expected to grow to 9.6 billion in 2050 (Gerber et al., 2013).

As a consequence, a huge increase in the demand of animal production is foreseen in the next decades: food and water security will be one of the priorities for humankind in the 21th century. Over the same period the world will experience a change in the global climate that will impact on local and global agriculture (Nardone et al., 2010). It seems to be clear, indeed, that agricultural and livestock sector have to keep on provide food security in a global point of view, while reducing GHG emission: according to Allen et al. (2018), indeed, we are faced with the need of limiting global temperature rise to 1.5° C, warming relative to pre-industrial levels. This could be reached through different pathways (Figure 26; Allen et al. 2018).

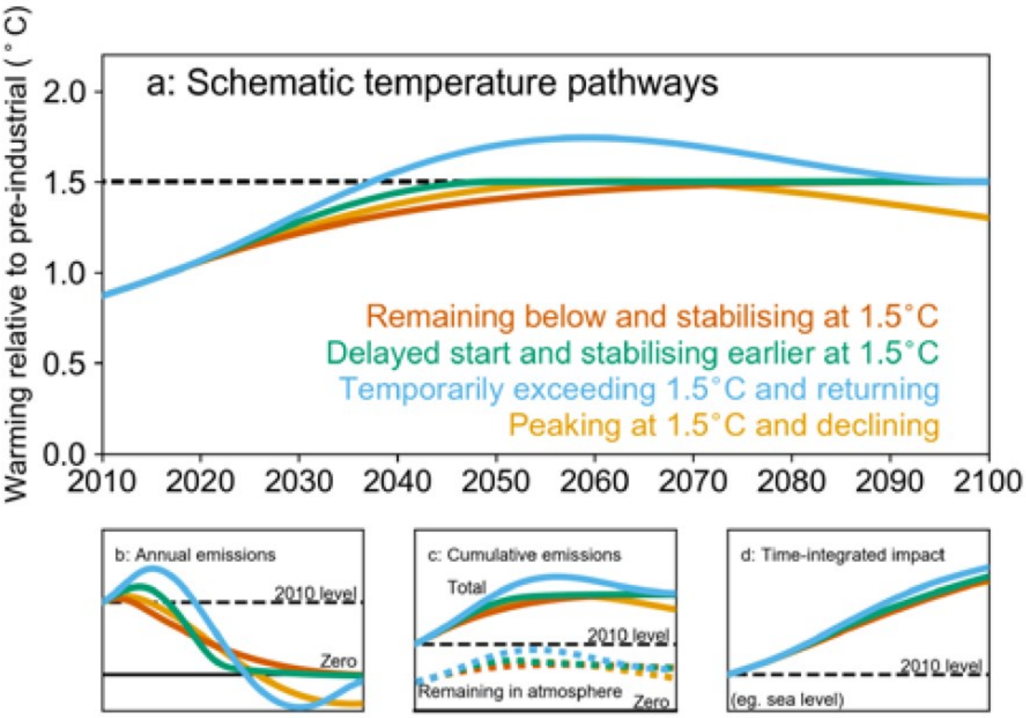

Figure 26. Different 1.5°C pathways1: Schematic illustration of the relationship between (a) global mean surface temperature (GMST) change; (b) annual rates of CO_2 emissions, assuming constant fractional contribution of non-CO_2 forcing to total human-induced warming; (c) total cumulative CO_2 emissions (solid lines) and the fraction remaining in the atmosphere (dashed lines; these also indicates changes in atmospheric CO_2 concentrations); and (d) a time-integrated impact, such as sea level rise, that continues to increase even after GMST has stabilized. Colours indicate different 1.5°C pathways. Brown: GMST remaining below and stabilizing at 1.5°C in 2100; Green: a delayed start but faster emission reductions pathway with GMST remaining below and reaching 1.5°C earlier; Blue: a pathway temporarily exceeding 1.5°C, with temperatures reduced to 1.5°C by net negative CO_2 emissions after temperatures peak; and Yellow: a pathway peaking at 1.5°C and subsequently declining (Allen et al., 2018).

The global livestock sector contributes a significant share to anthropogenic GHG emissions, but it can also deliver a significant share of the necessary mitigation effort (Gerber et al., 2013). FAO has developed a GLEAM model (2017), which estimates mitigation potential based on the wide gap in emission intensities that exists on a global and regional scale and within production systems and agro-ecological regions. The estimation for mitigation is around 33%, or about 2.5 Gt CO_2 eq, with respect to the baseline scenario. As it's shown in Figure 27, results of the model arise from the assumption that producers in a given system, region and agro-ecological zone were to apply the practices of the 10th percentile of producers with the lowest emissions intensities, while maintaining constant output.

Figure 27. Mitigation potential of the global livestock sector. The mitigation potential estimate excludes changes between farming systems and assumes the overall output remains constant (GLEAM model, FAO, 2017).

According to Smith et al. (2007), measures for mitigating GHG emissions from agricultural ecosystem include cropland management, grazing land management, management of organic soils restoration of degraded lands, livestock management, manure management and bioenergy.

Croplands, because they are often intensively managed, offer many opportunities to impose practises that reduce net emissions of GHGs. Mitigation practises in cropland management include agronomic practices and tillage management, nutrient management and LUC. Improved agronomic practices that increase yields and generate higher inputs of residue C can lead to increased soil C storage (Follett, 2001). In particular, those with perennial crops or conservation tillage (e.g. minimum tillage), which allocate more C below the ground, are considered good mitigation practises. Indeed, soil carbon sequestration (enhanced *sink*) is considered the mechanism responsible for most of the GHG mitigation in the agricultural sector (Soussana et al., 2010). By 1995, conservation tillage practices had been adopted on 110 million ha of the World's hectares of cropland (Pacala and Socolow, 2004). Conversion of all croplands to conservation tillage could sequester 25 Gt of C over the next 50 years, marking it as one of the key global strategies for stabilizing atmospheric CO_2 concentrations (Pacala and Socolow, 2004). Conservation tillage, indeed, sequesters the equivalent of 0.3 t of C ha^{-1} year^{-1} (Baker et al., 2007). Other agronomic practices are those that provide temporary vegetative cover between agricultural crops. These "catch" or "cover" crops add C to soils (Freibauer et al. 2004) and may also extract plant-available N unused by the preceding crop, thereby reducing N_2O emissions.

Adding nitrogen fertilizers can also increase SOC storage (even if there are different effects, depending on climate, soil and management; Alvarez, 2005), but the benefits from N fertilizer can be offset by higher emissions of N_2O from soils (Gregorich et al., 2005). A good mitigation strategy in that way could be the use of rotations with legume crops (Izaurralde et al., 2001), which reduce inputs of N, though legume-derived nitrogen can be a source of N_2O (Rochette and Janzen, 2005). Emissions can also be reduced by adopting less intensive cropping systems, which reduce reliance on pesticides and other inputs and therefore the GHG cost of their production (Paustian et al., 2004). In order to enhance soil carbon sequestration, conservative assumptions lead to the conclusion that a mitigation strategy for reducing GHG emission would be available from reducing tropical deforestation and from the management of temperate and tropical forests. This could be done if the current rate of cutting of primary tropical forest were reduced to zero over 50 years. Alternatively, it could be reforested or afforested approximately 250 million hectares in the tropics or 400 million hectares in the temperate zone (current areas of tropical and temperate forests are 1500 and 700 million ha, respectively). Another way would be created by establishing approximately 300 million ha of plantations on non-forested land (Pacala and Socolow, 2004). The growing of pasture, the maintenance of other farmland vegetation (e.g. hedges, trees, scrubs) and the accumulation of carbon as organic matter in grasslands, therefore, can all contribute to the temporary removal and, in some cases, to the long-term sequestration of CO_2 from the atmosphere (Hopkins and Del Prado, 2007). Areas maintained under well managed permanent grassland, as pasture or rangelands, indeed, constitute potential carbon sinks (Conant et al., 2001). Management techniques of pastures have evolved to increase forage production for livestock and, at the same time, increasing C stock in the soil (Figure 28; Conant et al., 2001).

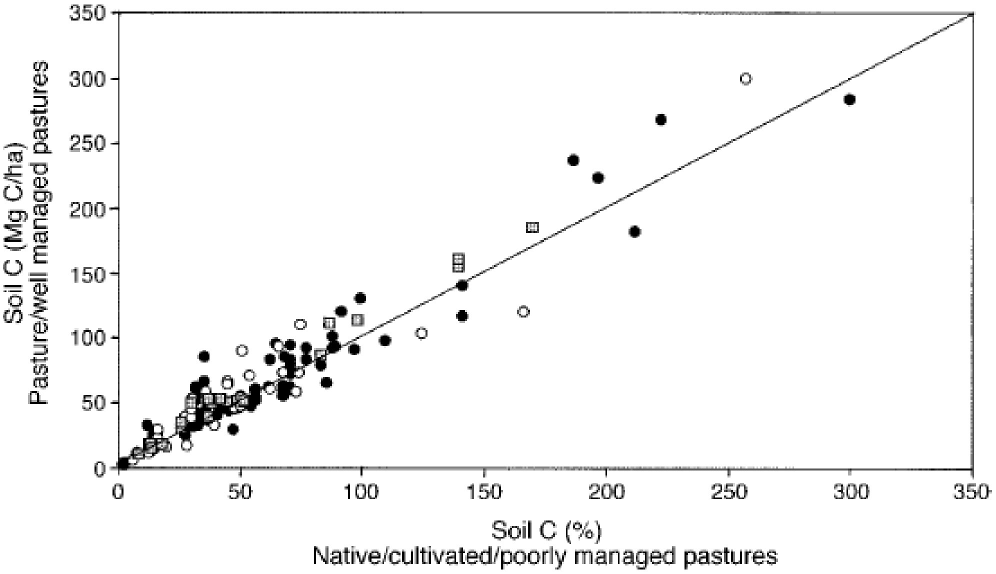

Figure 28. Soil carbon content for improved vs. unimproved pastures (closed circles), or grassland vs. cultivated fields (gray squares) or native vegetation (open circles). Soil carbon content increased for points above the line and decreased for points below the line. All data are in Mg C/ha (Conant et al., 2001).

Improved management includes suitable fertilization, appropriate irrigation, introduction of earthworms, intensive grazing management and sowing of favourable forage grasses and legumes (Conant et al., 2001). Application of animal manures on grazing lands, as well as on croplands, can release significant amounts of N_2O, in addition to notably amounts of N_2O and CH_4 during storage (Smith et al., 2007). In this sense, the most efficient way to reduce GHG emission during collection and storage of excreta is fast removal of manure from the housing system, in addition to a cool storage and covering the source (Clemens et al., 2001). These suitable application techniques of animal manure can be considered as an example of “end of pipe” mitigation strategies. This “end of pipe” measures reduce GHG emissions with the amounts of C and N excreted by the animal (Clemens et al., 2001).

Measures to reduce GHGs may be also “preventive”. Preventive measures reduce the carbon and nitrogen in- and output, resulting in lower GHG production (Clemens et al., 2001). A “preventive” strategy may be an optimised diet aiming to reduce CH_4 enteric emission. Optimised dietary interventions can be used to directly control methane production from ruminants: methane emissions from ruminants can be reduced by 9 to 40% depending on the nature of the intervention (Benchaar et al., 2001). Strategies for reducing enteric CH_4 include supplementing diets with feed additives and dietary fats, offering diets high in starch content, improving forage quality and changing forage type (e.g., increase corn silage and increase legumes forages as partial replacement of SBM; Little et al., 2017). Overall, intensifying production per dairy cow is suggested to be a visible measure to reduce emissions per unit of product: while improving animal productivity results in increased GHG emissions per animal, the high milk response rate results in a trend of decreasing net emissions per kilogram of milk (Gerber et al., 2011). Emission intensities, expressed as GHGs per kilogram of milk, have declined by almost 11% over the period 2005-2015, reflecting constant improvements in on-farm efficiency achieved via improved animal productivity and better management (FAO and GDP, 2018). GHG emission intensity, indeed, resulted to be inversely related to productivity, reflecting the strong effect of increased efficiency and dilution of emissions across a larger volume of milk (Gerber et al., 2011). Looking forward, there is still chance to work in terms of mitigation strategies of environmental impact. The use of by-products as feed, indeed, affords considerable advantages, such as the reduction of waste production, a reduction in competition between animals and human for crops, and the possible reduction of feeding costs. A possible alternative exploitation of by-products is represented by their use as a rearing substrate for insects, constituting an interesting example of a sustainable circular economy (Diener et al., 2011; Jucker et al., 2017; Meneguz et al., 2018). In this context, organic by-products can be enhanced to produce a valuable insect biomass, which is rich in protein and fat, for the animal feed industry (Tschirner and Simon, 2015; Makkar et al., 2014; Barragan-Fonseca, et al., 2007). In this regard, the use of insects as animal feed, due to its high protein content could, above all, reduce SBM purchased, that, as has been mentioned several times, involves LUC for its growing. According to the European legislation, today the use of insects as feed source is possible in the aquaculture sector (European regulation, 2017/893), as regards ruminants, therefore, further insights are still needed.

2. Aim of the study

The theme of the PhD project was mainly developed inside the Life project “Forage 4 Climate”, a four years project, with the aim of demonstrating that forage systems connected with milk production can promote climate change mitigation.

The aim of the PhD thesis was the evaluation of greenhouse gas emission, related to milk production. Specific aims were:

- to identify and evaluate the most common forage systems adopted in dairy cow farms in the Po plain, to select the systems that can improve milk production and C sequestration and can achieve the best results as emissions;
- to evaluate commercial diets related to these different systems, in order to directly assess their digestibility, milk and methane production;
- to identify the main ingredients used in the TMR of high producing lactating cows, through a survey analysis, in order to assess the best diet composition that can lead to high feed efficiency and low GWP at commercial farms scale;
- in a future perspective of circular economy, to study the exploitation of different inedible human by-products as growing substrates for *Hermetia Illucens* larvae, in order to partially substitute soybean meal (SBM) in the livestock diets with insect proteins.

3. Author contribution to the papers/manuscripts attached in the thesis

The contribution of Giulia Gislon to the papers included in this PhD Thesis was as follows:

- **Forage systems and sustainability of milk production: feed self-sufficiency, environmental impacts and soil carbon stocks:** collection of primary data at the farms, evaluation of the environmental impact related to milk production through the Life Cycle Assessment method and writing the paper, in collaboration with other coauthors.
- **Milk production, methane emission, nitrogen and energy balance of cows fed diets based on different forage systems:** management of the *in vivo trial*, collection of sampling, data processing and writing of the paper, in collaboration with other coauthors.
- **Looking for high productive and sustainable diets for lactating cows: a survey in Italy:** data processing, evaluation of the environmental impact related to feed ingredients and writing the paper, in collaboration with other coauthors.
- **Rearing of *Hermetia illucens* (Black soldier fly) on different by-products: influence on growth, waste reduction and environmental impact:** evaluation of CH_4 production and environmental impact of the larvae and writing the paper, in collaboration with other coauthors.

4. Forage systems and sustainability of milk production

Short introduction - Contribution of the dairy sector to global warming and GHG emissions is a concern for the public opinion, dairy producers and policy makers. A holistic approach for the contribution of dairy sector to GHGs, therefore, is needed. In particular, Life Cycle Assessment method is a useful methodology that allows to evaluate simultaneously several environmental impacts and can be adopted for comparing the environmental sustainability of different forage systems related to milk production. Moreover, the soil organic carbon (SOC) density and soil organic matter (SOM) allow to consider the possible mitigation effect of carbon sink.

Forage systems and sustainability of milk production: feed self-sufficiency, environmental impacts and soil carbon stocks

Submitted to Journal of Cleaner Production, under review

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Highlights

- Forage systems for optimizing milk production, C stock and environmental sustainability
- LCA evaluation of milk production was assessed on 46 farms
- 6 forage systems based on different forage quality and preservation were identified
- More intensive system provides high feed self-sufficiency and milk production per cow
- Intensive system was the best for environmental sustainability but with low C stock

Abstract

Farm sustainable intensification is an important strategy to reduce environmental impacts, which can be obtained in particular through improvements in the milk production per cow, as well as by increasing forage productivity. Farming systems that are mainly based on multi-annual/perennial forages may increase soil C sequestration, which is a significant GHG mitigation strategy. The aim of the present study has been to identify the forage systems related to dairy farms that are able to maximize feed production and to improve feed efficiency and C sequestration, in order to achieve the best environmental sustainability result. A group of 46 dairy farms was selected to represent the most widespread forage systems in Northern Italy. The identified forage systems were: CONV, conventional corn silage system; MIXED, low intensity mixed system;

HQFS, high quality forage system; WICE, winter cereal silage system; PR DRY, hay system for Parmigiano Reggiano PDO cheese production and PR-FRESH, hay and fresh forage system for Parmigiano Reggiano PDO cheese production.

Primary data were collected to carry out a life cycle assessment (LCA). Soil samples of representative crops of the forage systems were collected to determine several soil characteristics, and in particular the soil organic carbon (SOC) density and soil organic matter (SOM). The WICE, CONV and HQFS forage systems provided the greatest amount of DM per hectare, whereas the PR FRESH system showed the highest soil C density. The LCA results showed that the HQFS system registered the lowest values for all the impact categories (except for land occupation and the use of non-renewable resources), mainly due to the higher individual milk production (FPCM/head/day). More intensive systems, such as HQFS, demonstrated that the milk production per cow is negatively related to the impact per kilogram of product, as highlighted also by PROC GLM analysis. The HQFS system also resulted to be more sustainable, in terms of feed self-sufficiency, as it provided a high amount of DM per hectare, consisting of high digestible forages. However, as far as this forage system is concerned, there is still room to strenghten environmental sustainability, since HQFS also showed the lowest C soil density. Further investigations are needed to consider environmental sustainability over a wider spectrum.

Keywords: forage systems, milk production efficiency, life cycle assessment, C stock

1. Introduction

According to projections made with the CAPRI (Common Agricultural Policy Regionalised Impact) model, by 2025, dairy cattle will produce around 30% of the total agriculture greenhouse gas (GHG) emissions in EU-28 (European Commission, 2015). This will pose several environmental challenges, such as eutrophication, acidification and biodiversity losses (Mottet et al., 2017). However, at the same time, livestock can help to transfer and convert proteins from plant biomass into animal-sourced foods, which are rich in essential macro and micronutrients in the form of milk and meat, thereby utilizing resources that otherwise cannot be consumed by humans, and globally contributing 18% and 25% of food energy and protein, respectively (Steinfeld et al., 2006). Moreover, ruminants can use forages because of their unique digestive system and, unlike monogastric animals, reduce the competition for food used by humans and maintain/enhance various ecosystem services associated with forage cropping (Guyader et al., 2016; Tabacco et al., 2018).

It is clear that the agricultural and livestock sectors have to continue providing food security from a global point of view, while reducing the environmental impact. To achieve a sustainable food supply, agricultural producers need to identify cropping/forage systems, as well as feed growing/harvesting and livestock management practises that make the best use of the available resources and minimize the potential environmental impact (Capper et al., 2009). According to the literature, farm sustainable intensification has been identified as a relevant strategy to achieve a reduction in the environmental impact (Tilman et al., 2002; Balmford et al., 2018). Some characteristics related to intensive farms, such as milk production per cow, are inversely related to the impact per kilogram of product (Bava et al., 2014). The mitigation effect of enhancing individual milk production is due to the fact that emissions are spread over more units of milk, thus the emissions related to the maintenance requirements of the animals are diluted. The maintenance energy requirement does not change as a function of production, but the daily energy requirement increases as the milk yield increases, thereby reducing the proportion of total energy used for maintenance (Capper et al., 2009). A good strategy to increase the intensity of milk production, while reducing environmental impacts, is to improve the net primary production of the Utilized Agricultural Area (UAA) that serves the dairy farm; this can be achieved by correctly choosing crops and their sequence/rotation, increasing the efficiency of feed harvesting and conservation, and relying more on multi-annual and permanent grasslands, while taking advantage of the inherent internal resources of the system (synergisms, nutrient cycling, natural pest control and soil water; Liebman et al., 2008). An increase in forage system productivity reduces the amount of imported feed, and it also has a clear potential for mitigating yield-scaled farm GHG emissions (Doltra et al., 2018). Furthermore, increasing the on-farm production of highly digestible forages (with high protein concentrations, such as legume forages cut at early stages), apart from reducing the agricultural intensity of the cultivation processes (Tabacco et al., 2018), could also reduce the inclusion of soybean meal, which is mainly imported to Europe from South America, in the diet (Peyraud et al., 2009). Although there is still a lively debate on the role of soybean production and how to account for emissions from Land Use Change (LUC) (Audsley et al., 2009; Gerber et al., 2010; Leip et al., 2010; Shmidt et al., 2011), it is well known that this protein source involves additional GHG emissions attributable to deforestation. Moreover, producing high quality forages can help to improve the digestibility of cow rations, thus reducing methane emissions from rumen fermentation, which is positively related to the fibre content, and enhancing the feed conversion ratio, with a reduction in environmental costs related to the feed production per kg of milk (Beauchemin et al., 2008; Guyader et al., 2016).

Farming systems, based mainly on permanent meadows or multi-annual rotational grass and legume forages, may act as active carbon (C) *sinks* and might represent a significant GHG mitigation strategy, by increasing soil C sequestration in the soil organic matter (Stanley et al., 2018). Soil organic matter is generated from the dynamic abiotic and biotic processing of plant and animal detritus, and it represents the balance of inputs versus losses via such pathways as mineralization and leaching (Campbell and Paustian, 2015). Thus, the management of C sequestration requires increasing the C inputs and/or decreasing decomposition (Paustian et al., 1997). In confinement dairy farms, manure and slurry are collected, stored and applied to croplands to recycle nutrients back through feed production. Livestock manure is applied annually to specific crops once or twice per rotation cycle and, even though a part of the manure C is decomposed by soil microorganisms and the soil C accrual rates decrease over time as stocks approach a new equilibrium, part of it remains in the soil and thus contributes to long term C storage (Zavattaro et al., 2017). The maintenance of soil organic matter (SOM) may at least be regarded as a mitigation option and as a policy target to satisfy international food security and climate objectives (European Commission, 2011). Through the maintenance of SOM, many soil properties, including the soil structure, nutrient availability and soil health, are protected as a resource base for food production, soil life conservation and carbon storage (Chenu et al., 2019).

The present study has investigated the main forage systems used in dairy farms in Northern Italy, with the aim of characterizing the C content of the soils, of evaluating on farm feed production potential and, to assess their effectiveness in improving system efficiency. The aim was also to assess the effectiveness of the different forage systems in achieving the best results in terms of mitigation of emissions and environmental impacts per kg of milk produced.

2. Materials and methods

In this study, 46 dairy farms were selected from among the most widespread forage systems in Northern Italy. All the farms were located in the plain area (Po plain) of the Lombardy, Piedmont and Emilia Romagna regions, and the animals were bred in intensive confined livestock holdings, with no pasture.

2.1 Forage dairy systems

The forage systems, which were identified by a panel of experts involved the activities of the Forage4Climate project (LIFE15 CCM/IT/000039), were selected to be representative of dairy farms operating in this area. The forage systems are described hereafter.

2.1.1 Conventional system (CONV; 10 farms)

This forage system relies mainly on mono-cropped corn (harvested as whole crop silage and/or dry grain) and represents the most widespread intensive forage system in the Po plain. Part of the UAA is dedicated to permanent meadows or Italian ryegrass (harvested at a late stage of maturity as hay or silage), double cropped with corn. Forage legumes are grown less extensively, but just to satisfy the Ecological Focus Area requirements of the CAP greening measures (European Commission, 2013).

2.1.2 Mixed system (MIXED; 10 farms)

This forage system is less intensive than the previous one and is based on different proportions of crops: corn (for whole crop silage, dry grain or whole ear silage), permanent meadows and grass and legume forages (harvested as hay and/or silage). Crop rotation is adopted over almost all of the UAA. This group includes some organic farms and some rainfed farms.

2.1.3 High quality forages system (HQFS; 6 farms)

This forage system includes grass and legume forages for high quality silages with the aim of improving self-sufficiency in terms of energy and protein. The forage system is based on double cropped corn (mainly harvested as whole ear silage) and Italian ryegrass (harvested at an early stage of growth as silage). Alfalfa and permanent meadows (harvested as silage at an early stage of growth) are grown on 25-50% of the UAA. Crop rotation is practiced over almost all of the UAA.

2.1.4 Winter cereal silage system (WICE; 7 farms)

This forage system is based on mono-cropped corn (harvested as whole crop silage, whole ear silage and/or dry grain) and winter cereals for whole crop silage, double cropped with corn, over more than 40% of the UAA. Rotational grass forages and permanent meadows are also cultivated, but to a lesser extent. Forage legumes are grown just to satisfy the Ecological Focus Area requirements of the CAP greening measures (European Commission, 2013).

2.1.5 Hay forage for Parmigiano Reggiano Protected Designation of Origin (PDO) cheese production (PR DRY; 7 farms)

This forage system is based on alfalfa and permanent meadows conserved as hay. Winter cereals and sorghum (or corn in irrigated areas) are grown for dry grain, albeit less extensively. Double cropping is not practiced.

2.1.6 Hay and fresh forages for Parmigiano Reggiano PDO cheese production (PR-FRESH; 6 farms)

This forage system is less intensive than the previous one (PR DRY) and is based on permanent meadows and alfalfa conserved as hay and/or fed as fresh forages. Double cropping is not practiced. This group includes organic farms.

The average proportion of each crop on farm UAA and its final utilization is reported in Figure 1, for each considered cropping system. Each of the considered cropping system has its own particular characteristics: CONV farms grow corn over more than 60% of the UAA; MIXED farms grow a balanced proportion of crops over the UAA; HQFS farms grow alfalfa and Italian ryegrass over more than 50% of the UAA; WICE farms adopt winter cereal-corn double cropping over more than 40% of the UAA; PR DRY and PR FRESH grow alfalfa and permanent meadows over more than 80% of the UAA, respectively.

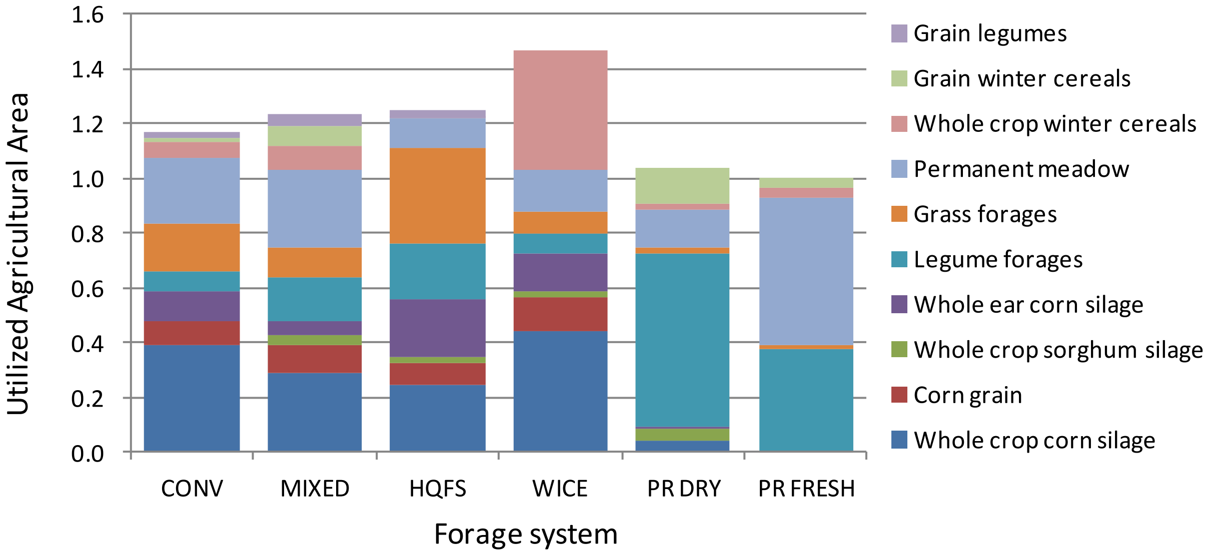

Figure 1. Average proportion of crops over the UAA in the six studied cropping systems. Values above 1 represent the proportion of the double cropped area. Abbreviations: CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production.

2.2 Farm soil survey

Two to 4 crops considered representative of the related forage system were selected for each farm, and soil samples for each crop (depth of 0.3 m) were analyzed in order to determine: the soil organic carbon (SOC) density, SOM, total nitrogen content, exchangeable potassium, available phosphorus, cation exchange capacity (CEC), pH and soil texture. A questionnaire was filled out for each field/crop where soil sampling was performed in order to obtain information about the adopted fertilization, crop rotation and yields over the last five years. A pool of soil samples was chosen to be representative of the agricultural management practices adopted on each farm and to be representative of the majority (more than 80%) of the UAA of the studied farms. The characteristics of the soil were all determined according to the methods described by MIPAF (1999). When primary data were not available, the soil bulk density was determined utilizing pedo-transfer functions (PTF) from the literature (Arrouays et al., 2012; Walter et al., 2016; Al-Shammary et al., 2018).

The SOC densities were calculated, at a depth of 0.30 m, as follows (1):

$$SOC\ density = [SOC_i \times BD_i \times D_i \times (1 - CF_i)] \quad (1)$$

where:

$SOC\ density$ = total amount of SOC per unit area (kg C/m^2);

SOC_i = SOC content in layer i (g/kg);

BD_i = bulk density of layer i (Mg/m^3);

D_i = thickness of layer i (m);

CF_i = fraction of coarse fragment volumes > 2 mm in layer i ($0 \leq CF_i < 1$).

2.3 Life Cycle Assessment

2.3.1 Goal and scope definition

The goal of this LCA study was to quantify the environmental impact of milk production on the studied forage systems.

2.3.2 Functional units, allocation and system boundary

The considered functional unit was 1 kg of Fat and Protein Corrected Milk (FPCM, 4.0% fat and 3.3% protein), calculated as suggested by the International Dairy Federation (IDF, 2015).

Allocation between milk (FPCM) and meat was calculated through a physical method (IDF, 2015), based on the use of feed energy by the dairy animals and the physiological feed requirements of the animals to produce milk and meat, as follows:

$$AF_{milk} = 1 - 6.04 \times BMR$$

In which:

AF is the allocation factor for milk;

BMR is the ratio kg_{meat}/kg_{milk}; where: M_{meat} is the sum of live weight of all animals sold

M_{milk} is the sum of FPCM.

A typical value for BMR is 0.02 kg_{meat}/kg_{milk}, yielding an allocation of 12% to meat and 88% to milk (IDF, 2015).

An attributional approach, which considered f*rom cradle to farm gate* system boundaries, was adopted. All the inputs (e.g. off farm feeds and bedding, machinery, fuel, lubricants, electricity, organic and mineral fertilizers, pesticides, plastics and water) and outputs (i.e. emissions to the air, soil and water, milk and meat) involved in the productive process were considered within the system boundaries (Figure 2).

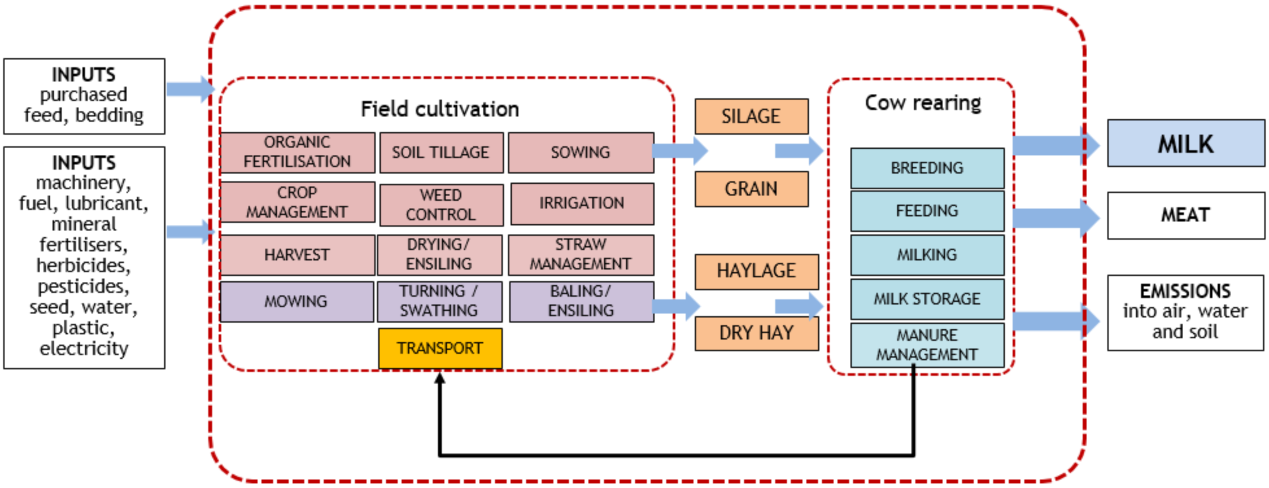

Figure 2. System boundaries considered for the life cycle assessment.

2.3.3 Inventory data collection

In order to collect information about their adopted management practices, the farmers were interviewed directly about several aspects of their farming system. Information about the cropping system, feed crops and their dry matter (DM) yields, the herd composition, manure management, feed rations, purchased forages, concentrates and mineral-vitamin, milk production and composition were collected, with the help of questionnaires and the registered data available on each farm. All the inventory data collected on the farms (input and output included in the milk production) and some of the most important traits of the herds and farm efficiency are shown in Table 1, divided on the basis of the forage system. Farm structures and their construction, maintenance and disposal of the milking parlours were excluded from the system boundaries. All the DM yields (t) per year of each crop grown on each farm were quantified and then added together, and the total obtained DM was divided by the total farm UAA to obtain an average DM yield per hectare for each forage system. The livestock units (LU) were calculated according to Eurostat (2013).

Table 1. Inventory data for each forage system

Parameter	Unit	CONV			MIXED			HQFS			WICE			PR DRY			PR FRESH		
		Mean	min	max	**Mean**	min	max	**Mean**	min	max	**Mean**	min	max	**Mean**	min	max	**Mean**	min	max
Farm traits																			
UAA	*ha*	**97**	42	175	**71**	29	195	**91**	59	146	**71**	29	213	**445**	110	946	**85**	32	241
Cows total	*n*	**218**	109	376	**128**	53	260	**199**	62	385	**237**	66	855	**627**	327	953	**164**	73	435
Dry cows	*%*	**14**	8	17	**15**	9	18	**14**	11	19	**14**	12	22	**14**	10	15	**17**	15	18
Replacement rate	*%*	**38**	25	54	**26**	3	47	**34**	31	38	**36**	15	44	**36**	25	41	**29**	16	44
Forage:concentrate ratio[a]	*n*	**1.04**	0.71	1.47	**1.32**	0.68	3.46	**1.11**	0.78	1.57	**0.96**	0.45	1.35	**0.72**	0.58	1.03	**1.51**	1.03	2.70
Stocking rate	*LU/ha*	**3.99**	2.90	6.26	**4.04**	2.25	9.18	**3.41**	1.58	4.58	**4.86**	3.62	6.71	**2.62**	1.49	4.92	**3.49**	2.54	4.66
Dry matter intake	*kg DM/d*	**23.9**	21.6	29.8	**20.8**	13.8	24.7	**22.2**	21.2	22.7	**23.4**	22.8	24.2	**23.9**	20.5	26.5	**23.8**	20.1	27.9
Feed efficiency	*kg milk/kg DMI*	**1.36**	1.21	1.56	**1.30**	1.04	1.45	**1.40**	1.27	1.51	**1.24**	0.88	1.43	**1.25**	1.14	1.64	**1.14**	0.86	1.46
Production intensity	*t FPCM/ha*	**23.1**	15.8	39.1	**16.4**	10.0	31.7	**20.7**	8.0	27.2	**28.6**	17.9	40.9	**15.4**	9.2	31.2	**16.0**	12.9	22.6
Farm input																			
Purchased forages	*t DM/year*	**248**	0	718	**67**	0	315	**183**	37	319	**154**	0	424	**792**	0	5010	**54**	0	300
Purchased concentrates	*t DM/year*	**794**	266	2389	**316**	54	792	**479**	176	906	**814**	133	3106	**2686**	1495	6037	**560**	38	1756
Feed self-sufficiency[b]	*% DMI*	**62.6**	46.4	77.0	**72.1**	41.5	87.6	**68.7**	61.0	79.5	**59.3**	45.7	80.1	**54.3**	31.7	83.7	**62.8**	49.1	77.4
Electricity	*kWh 10^3/year*	**111**	44	272	**72**	5	259	**110**	50	221	**109**	32	335	**320**	21	801	**87**	44	191
Diesel fuel	*l 10^3/year*	**48**	24	125	**34**	10	107	**42**	18	75	**43**	5	157	**125**	23	479	**19**	10	48
N chemical fertilizer	*kg N/year*	**8308**	0	20286	**3608**	0	9867	**6403**	474	10950	**6229**	1636	15123	**7339**	0	19898	**25**	0	94
P chemical fertilizer	*kg P/year*	**2063**	0	9363	**634**	0	2894	**1349**	0	3595	**416**	0	2102	**1511**	0	10573	**0**	0	0
Farm output																			
Annual milk production	*t FPCM/year*	**2070**	1124	3279	**1030**	305	1934	**2015**	498	4115	**2292**	480	8579	**5792**	3373	8680	**1314**	490	3394
Milk protein	*%*	**3.39**	3.29	3.57	**3.39**	3.09	3.55	**3.44**	3.19	3.66	**3.43**	3.20	3.55	**3.47**	3.35	3.60	**3.30**	3.21	3.38
Individual milk production[c]	*kg FPCM/d*	**30.9**	25.4	36.4	**25.8**	18.2	31.0	**30.9**	29.0	32.7	**29.0**	18.4	34.3	**28.3**	26.2	31.6	**25.4**	21.9	29.9
Meat production	*t/year*	**63**	31	121	**28**	2	57	**58**	19	124	**75**	11	288	**158**	70	256	**34**	13	84

Abbreviations: DM, dry matter; DMI, DM intake; FPCM, fat protein corrected milk; LU, livestock unit; N, nitrogen; P, phosphorus; CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production.

[a] in the lactating cows ration

[b] calculated as: (DM self produced / DM self produced + DM purchased) X 100

[c] considering the whole herd, as average of each farm

2.3.4 Estimation of the on-farm emissions

The chemical composition of the feed rations (used to estimate the emissions) was calculated for all the animal categories, using CPM-Dairy Beta V 3.0.7bs (Tedeschi et al., 2008). The enteric methane emissions from livestock were calculated using the equations proposed by Moraes et al. (2014). The methane emissions from manure storage were estimated using the Tier 2 method suggested by the Intergovernmental Panel on Climate Change (IPCC, 2006a). In the current study, animal nitrogen excretion was estimated as proposed by the IPCC (2006a) Tier 2 method, considering the nitrogen intake (on the basis of the CP% of the diet) minus the nitrogen retained by the animals and excreted with milk. The dinitrogen monoxide (N_2O) emissions from manure storage were considered to have occurred in both direct and indirect form, and they were both estimated using the Tier 2 method from IPCC (2006a). The direct and indirect N_2O losses from fertilizer application were estimated following the Tier 2 and Tier 1 methods suggested by IPCC (2006b), respectively; the amount of nitrogen applied to the soils from synthetic fertilizers and from manure (slurry and solid) were accounted for in the estimation.

The ammonia (NH_3) and nitrogen oxide (NOx) emissions that occur during animal housing, manure storage and spreading were estimated according to the method proposed by the European Environment Agency (EEA, 2009) on the basis of the total amount of nitrogen excreted by the animals. The Tier 2 method uses a mass flow approach, based on the concept of a flow of total ammonia nitrogen through the manure management systems.

The NH_3-N and NOx emission factors were specific of each manure type (slurry or solid) and each manure handling step and were considered as a proportion of the total ammonia nitrogen (EEA, 2009a). The NH_3 and NOx emitted during manure spreading and the application of synthetic fertilizers were estimated according to the EEA (2009b) guidelines. The amount of leached nitrogen was estimated using the IPCC (2006b) model (Table 2). In order to estimate the PO_4^{3-} emissions, the amount of phosphorus lost, in dissolved form, to the surface water (run-off) and leached was considered, as proposed by Nemecek and Kägi (2007).

Detailed information about the estimation of the emissions is reported in Guerci et al. (2013) and Bava et al. (2014). The background data for the production of seeds, raw materials, diesel fuel, fertilizers, pesticides, tractors and agricultural machines (equipment and self-propelled machines), as well as for transport, were obtained from the Ecoinvent Database V.3 (Ecoinvent, 2015) and Agri-footprint Database.

2.3.5 Life Cycle Impact Assessment

Different characterization factors were used to translate the inventory data into the potential environmental impact, using the SimaPro V 8.3 software tool (Goedkoop et al., 2013). Climate change (kg CO_2 eq), acidification (molc H^+ eq), terrestrial (molc N eq) and marine (g N eq) eutrophication, freshwater ecotoxicity (CTUe) and particulate matter (g $PM_{2.5}$ eq) were calculated through ILCD 2011 Midpoint V 1.03 (Wolf et al., 2012). Land occupation (m^2) was estimated using the Ecological Footprint V 1.01 (2009) method, while the energy use from non-renewable fossils (MJ) was calculated using Cumulative Energy Demand V 1.08 (2010).

2.3.6 Statistical analysis

The whole dataset, including data on the farm characteristics, feed production, environmental impact evaluation and soil characteristics, was analysed using the Statistical Package for Social Science (v 24.0, SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were performed (mean, min, max) and an ANOVA model (post-hoc test- Tukey) was used to test the effect of the forage system on DM yield (t/ha) and C density (kg/m^2). According to the international bibliography (Kristensen et al., 2011), results are presented by simple means, minima and maxima in order to give as much information as possible about the background for the aggregated results.

The effect of farm characteristics on climate change was tested by a variance test using PROC GLM of SAS ver 9.2 (SAS Institute, 2001), with climate change as the only dependent variable.

The complex relations between the variables used to describe climate change were analysed with PROC FACTOR (SAS, 2001, ver 9.2), in order to identify factors highly correlated. Variables with communality estimates higher than 0.7 were maintained in the analysis (Sharma, 1996) and tested using PROC GLM (SAS, 2001, ver 9.2).

3. Results

3.1 Feed production

Figure 3 reports, for each forage system, the contribution of each crop to the average DM yield on 1 ha of the farm UAA. The WICE, CONV and HQFS forage systems provided the highest amounts of DM per hectare (even though the results were not statistically different from the MIXED and PR FRESH systems), with more than 15 t DM/ha produced annually, whereas PR DRY showed the lowest value, which was less than 9 t DM/ha per year (result not statistically different from MIXED or PR FRESH). Corn silage provided more than half of the DM produced on the CONV and WICE system farms, whereas almost all the DM produced on the PR DRY and PR FRESH system farms was from alfalfa and permanent meadows.

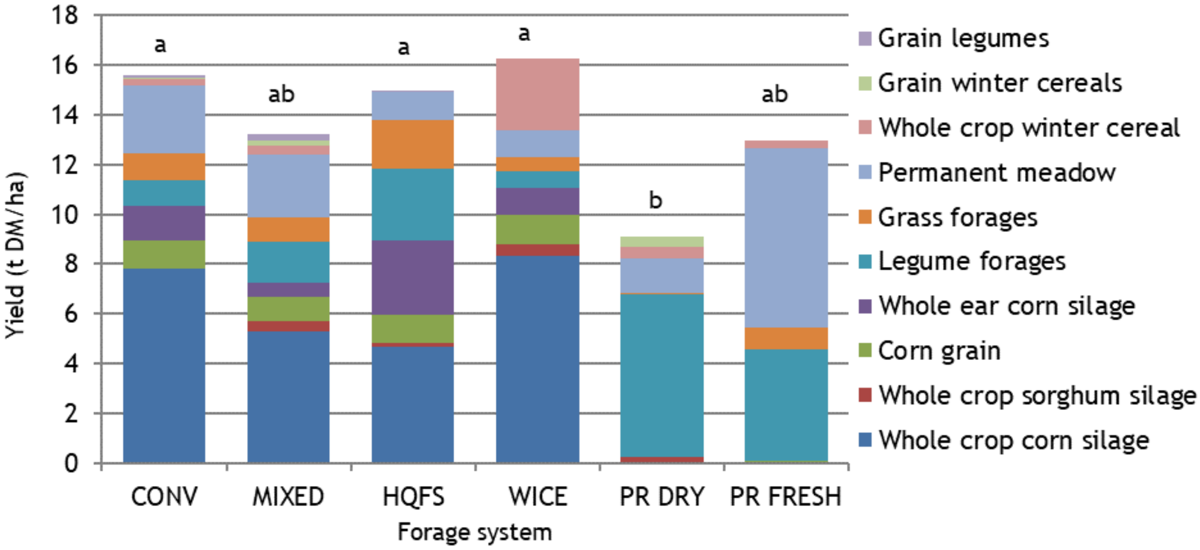

Figure 3. Contribution of each crop to the average DM yield, referring to 1 ha of UAA, for each of the studied systems.

Abbreviations: CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production. Bars with different superscripts are significantly different (P<0.05).

The studied forage systems also differed among each other as far as the methods adopted for feed conservation are concerned: the HQFS system conserved more than 90% of the home-grown feeds by ensiling, whereas PR DRY and PR FRESH conserved almost all the feeds in a dry state (i.e. all the forages by haymaking); the CONV, WICE and MIXED systems adopted the ensiling technique for more than 70% of the feeds produced on the farms (Figure 4).

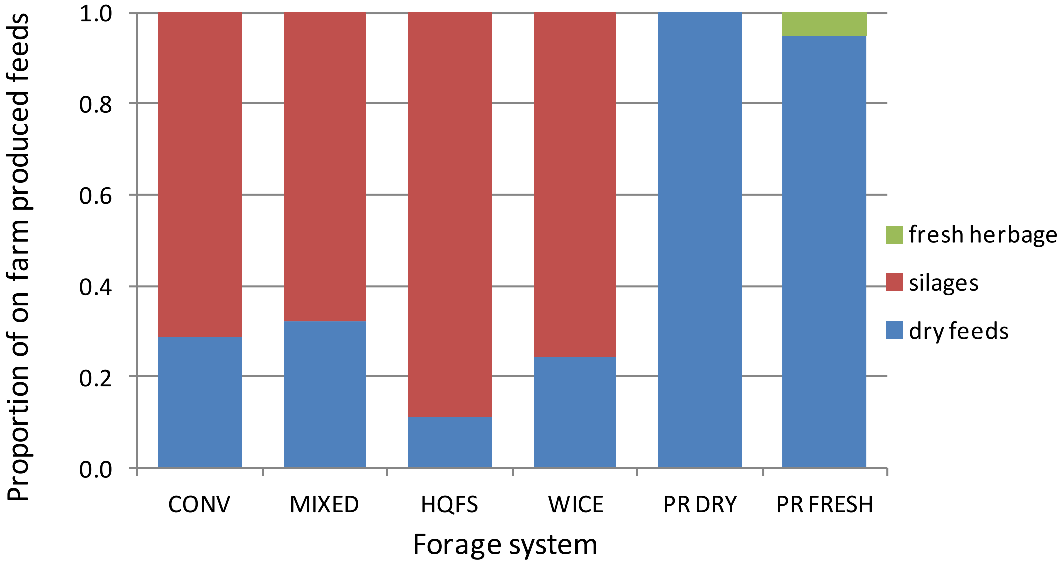

Figure 4. Proportion of the on farm produced feeds conserved as silage or dry feeds/forages or consumed as fresh herbage.

Abbreviations: CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production.

3.2 Organic C densities

Table 2 reports the main soil characteristics of each of the studied cropping systems. The pH was higher than 7.0 in the PR DRY and PR FRESH systems, around 6.0 in the CONV and HQFS systems, and intermediate for the WICE and MIXED systems. The organic matter and total nitrogen contents were higher for the PR FRESH and PR DRY systems, whereas they showed similar values for the other cropping systems. The C/N ratio did not show a large variability over the cropping systems, and ranged from 6.8 to 8.5. Moreover, the P levels were high on all the farms, and homogeneous over the groups, except for the PR DRY farms, which showed lower values. Exchangeable K was very high, with the highest values being observed in the PR DRY and PR FRESH soils.

Table 2. Average soil characteristics of the studied forage systems.

Parameter	Unit	CONV	MIXED	HQFS	WICE	PR DRY	PR FRESH
		Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
		min max	min max	min max	min max	min max	min max
pH		**6.0**	**6.5**	**6.1**	**6.6**	**7.5**	**7.3**
		5.5 7.0	5.8 7.2	5.1 6.8	5.9 7.8	7.3 7.9	7.2 7.7
Organic matter	(%)	**2.63**	**2.86**	**2.33**	**3.12**	**3.60**	**4.52**
		1.76 4.13	1.87 6.18	1.74 3.17	1.77 5.69	2.24 6.00	2.82 6.13
N	(%)	**0.18**	**0.22**	**0.20**	**0.23**	**0.28**	**0.34**
		0.12 0.26	0.15 0.48	0.15 0.27	0.14 0.46	0.13 0.44	0.19 0.50
C/N		**8.5**	**7.6**	**6.8**	**8.0**	**7.7**	**7.9**
		7.0 11.1	6.3 9.5	5.4 8.2	7.4 9.5	6.9 9.7	7.1 8.7
P	(ppm)	**70**	**71**	**62**	**71**	**51**	**73**
		44 110	35 175	46 82	45 125	22 79	44 135
K	(ppm)	**231**	**230**	**177**	**185**	**519**	**472**
		66 555	97 589	100 266	161 238	227 696	351 678

Abbreviations: CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production.

The organic C stored in the first 0.30 m of the soil for each forage system is reported as the C density (kg C/m^2) in Figure 5. The PR FRESH system showed the highest C density, whereas CONV and HQFS showed the lowest values (no statistical differences from the MIXED, WICE and PR DRY systems).

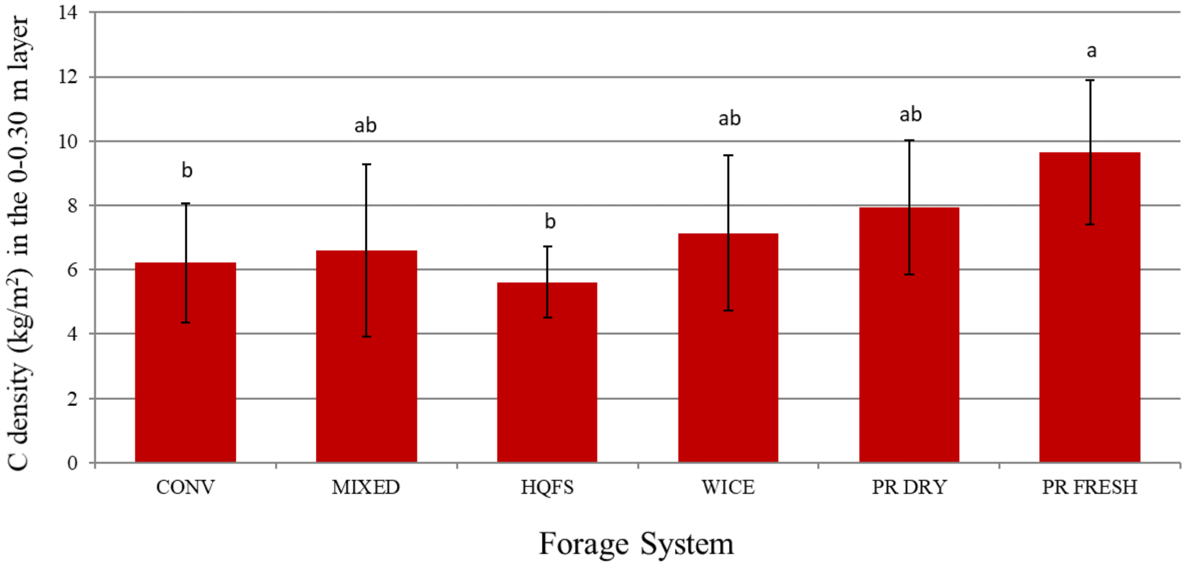

Figure 5. Average organic carbon density observed in the soils (0-30 cm layer) of the studied forage systems.
Abbreviations: CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production.
Bars with different superscripts are significantly different ($P<0.05$).

3.3 Environmental impact

The environmental impacts of milk production, related to the different forage systems (obtained from the LCA analysis), are shown in Table 3.

Table 3. Environmental impact of milk production for the considered forage systems, expressed per kg FPCM

Parameter	Unit	CONV	MIXED	HQFS	WICE	PR DRY	PR FRESH
		Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
		min max	min max	min max	min max	min max	min max
Climate change	*kg CO$_2$ eq*	**1.37**	**1.36**	**1.18**	**1.44**	**1.36**	**1.51**
		1.00 1.85	1.13 1.84	0.96 1.36	1.00 2.32	0.98 1.77	1.16 1.86
Acidification	*molc H+ eq*	**0.03**	**0.03**	**0.02**	**0.03**	**0.04**	**0.04**
		0.01 0.04	0.02 0.04	0.02 0.03	0.02 0.04	0.03 0.04	0.04 0.05
Terrestrial eutrophication	*molc N eq*	**0.14**	**0.13**	**0.11**	**0.14**	**0.16**	**0.19**
		0.06 0.19	0.10 0.17	0.08 0.13	0.09 0.18	0.13 0.19	0.16 0.24
Marine eutrophication	*g N eq*	**8.48**	**8.83**	**7.52**	**9.80**	**9.55**	**9.88**
		2.52 13.36	6.17 13.14	5.71 8.69	7.42 14.62	7.37 11.51	7.23 12.95
Fresh-water ecotoxicity	*CTUe*	**1.53**	**1.57**	**1.43**	**1.86**	**1.83**	**1.69**
		0.73 2.31	0.70 2.51	1.23 1.59	1.06 3.04	0.67 2.80	0.40 2.23
Particulate matter	*g PM$_{2.5}$ eq*	**0.76**	**0.72**	**0.61**	**0.78**	**0.88**	**1.03**
		0.36 1.04	0.55 0.88	0.47 0.70	0.50 1.02	0.75 1.05	0.87 1.27
Land occupation	*m^2*	**2.17**	**2.85**	**2.26**	**2.41**	**2.97**	**2.70**
		0.99 3.28	2.00 5.56	1.72 3.52	1.44 4.10	2.36 3.58	1.69 3.17
Non-renewable, fossil	*MJ*	**3.17**	**3.98**	**3.23**	**3.94**	**3.94**	**3.74**
		1.78 5.99	1.82 6.05	2.18 3.87	2.66 5.54	1.94 6.94	1.11 5.27

Abbreviations: CONV: Conventional corn silage system; MIXED: mixed less intensive system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production.

The HQFS system showed the lowest value for all the impact categories, except for land occupation and the use of non-renewable resources (Table 3). As far as climate change (kg CO$_2$ eq) is concerned, the HQFS system showed a value of 1.18, while the others on average showed a value of 1.41. HQFS reported values of 0.11 and 7.52, respectively, for terrestrial (molc N eq) and marine (g N eq) eutrophication. The lowest fresh water ecotoxicity (CTUe) value was shown for the HQFS system: 1.43, in comparison with the average value of 1.70 for the other systems. As for the particulate matter (g PM$_{2.5}$ eq), the HQFS system showed a value of 0.61, while the other systems showed an average value of 0.83. The environmental impact was the lowest, in terms of land occupation (m^2), for the CONV system (2.17 vs. 2.64, the average value for the other systems). The CONV system also resulted to be less impacting for the use of non-renewable resources (MJ) than the other systems. The most impacting forage system was PR FRESH, which showed the highest values for all the categories, except for freshwater ecotoxicity, land and non-renewable resource use. As far as fresh water ecotoxicity (CTUe) is concerned, WICE was the most impacting forage system, with a value of 1.86 (Table 3). The PR DRY system showed the highest occupation of land (2.97 m^2), while the MIXED one showed the highest use of non-renewable resources (3.98 MJ).

The contribution to climate change of the single gases (kg CO$_2$ eq/kg FPCM) and their sources are reported in Figure 6.

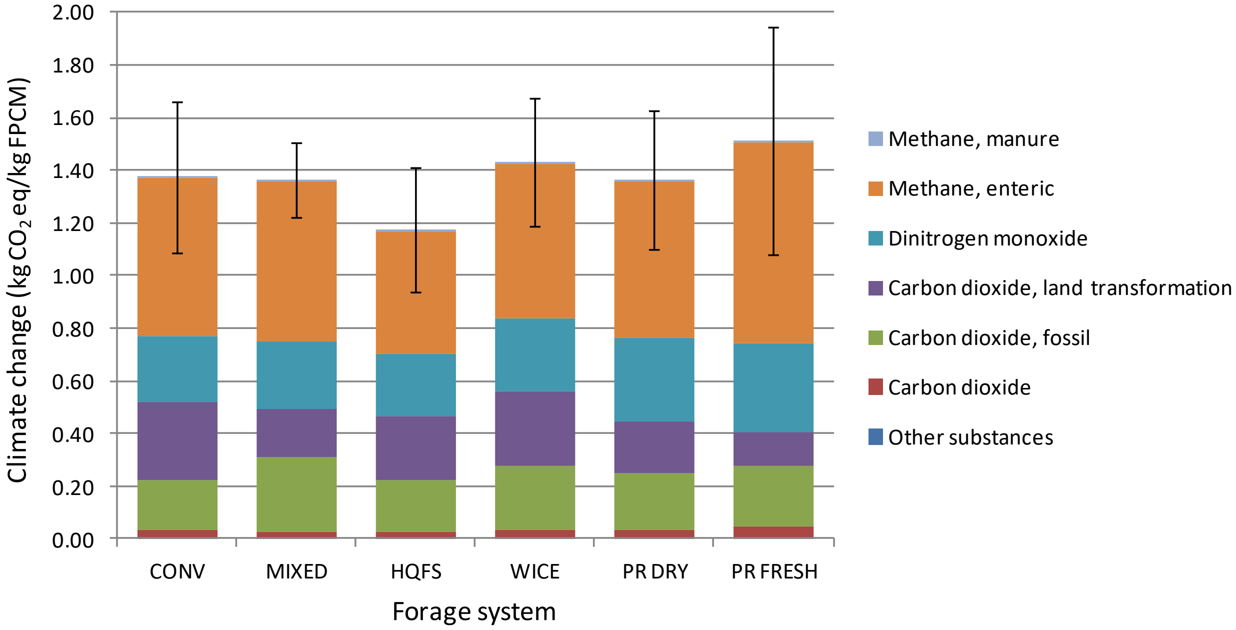

Figure 6. Contribution of the single gases to climate change, expressed as kg CO_2 eq per kg of Fat Protein Corrected Milk (FPCM).

Abbreviations: CONV: Conventional corn silage system; MIXED: low intensity mixed system; HFQS: High quality forage system; WICE: Winter cereal silage system; PR DRY: Hay system for Parmigiano Reggiano PDO cheese production; PR-FRESH: Hay and fresh forage system for Parmigiano Reggiano PDO cheese production. The error bars show the standard deviations.

Enteric fermentation was the main source of emissions for all the forage systems, with average values ranging from 0.47 kg CO_2 eq/kg FPCM for the HQFS system to 0.76 kg CO_2 eq/kg FPCM for the PR FRESH system (Figure 6). Enteric methane accounted for the majority of the total GHG emissions across the forage system groups (from 39.8% to 50.6%). The second major source of emission was CO_2, due to land transformation for the CONV, HQFS and WICE systems, with values of 0.30, 0.24 and 0.28 kg CO_2 eq/kg FPCM, respectively (accounting for 21.6, 20.7 and 19.5% of the total GHG emissions). The second major source of GHG emissions in the PR DRY and PR FRESH systems was N_2O, which accounted for 23.4 and 22.0% of the total emissions, with values of 0.32 and 0.33 kg CO_2 eq/kg FPCM, respectively. The major contribution to the overall GHG emissions in the MIXED system, other than enteric methane, came from CO_2 from fossil fuel consumption, with a value of 0.28 kg CO_2 eq/kg FPCM (which accounted for 20.8% of the total emissions).

Factors influencing climate change (kg CO_2 eq/kg FPCM) are reported in Table 4.

Table 4. Effect on emission per kg of FPCM of different farm characteristics.

Parameter	*Unit*	Estimate	SE	t Value	Pr > |t|
DMI lactating cows	*kg/cow/d*	0.08	0.03	2.51	0.02
Feed efficiency	*kg milk/kg DMI*	0.72	0.59	1.21	0.24
Forage:concentrate ratio	*n*	-0.14	0.08	-1.8	0.08
Grass hey	*%DMI*	0.00	0.00	0.17	0.87
Commercial feed	*%DMI*	0.01	0.00	1.71	0.10
Land	*ha*	0.00	0.00	-1.43	0.16
Permanent meadow	*%*	0.01	0.01	0.68	0.50
Alfalfa	*% land*	0.01	0.01	0.84	0.41
Meadow	*% land*	-0.01	0.01	-0.93	0.36
Cornsilage	*%land*	0.00	0.00	0.82	0.42
Stocking rate	*LU/ha*	0.02	0.03	0.67	0.51
Individual milk production	*kg FPCM/day*	-0.06	0.03	-2.49	0.02
Milk production per hectare	*Kg FPCM/ha*	0.00	0.00	-1.66	0.11
Low DM yield	*t DM/ha≤13*	-0.11	0.12	-0.92	0.36
Medium DM yield	*13<t DM/ha≤16*	0.05	0.10	0.46	0.65
High DM yield	*t DM/ha>16*	0.00	.	.	.

Climate change is significantly dependent on DMI of lactating cows (kg/cow/d) and Individual milk production (kg FPCM/day; inversely proportional). A tendency was detected for forage:concentrate ratio (inversely proportional) and commercial feed included in the ration.

The analysis revealed that different level of DM yield is not significantly related to climate change. Therefore, farms with low DM yield showed an average value of climate change of 1.29 kg CO_2 eq/kg FPCM, the ones with medium DM yield 1.44 kg CO_2 eq/kg FPCM, while farms with high DM yield reported on average 1.40 kg CO_2 eq/kg FPCM (difference not statistically significant).

4. Discussion

4.1 Environmental impact

The aim of the study was to characterize different forage systems adopted on intensive dairy farms in the Po plain in Northern Italy and assess whether feed conservation and dairy management could affect the environmental performance of the farm feed production potential, as obtained from an LCA analysis.

The dairy-forage systems analysed in this study encompass a wide range of agricultural intensities, in terms of milk production intensities (t/ha) and livestock stocking rates (LU/ha), ranging from high intensive systems, such as the WICE system (28 t FPCM/ha, 4.86 LU/ha) to moderately intensive systems, like the MIXED, PR DRY and PR FRESH systems (16.4, 15.4, 16.0 t FPCM/ha, and 4.04, 2.62, 3.49 LU/ha, respectively). The analysed systems also differed according to the level of cow performances, in terms of individual milk production (kg FPCM/cow/day) and feed efficiency (kg milk/kg DMI), ranging from less intensive (the MIXED and PR FRESH systems) to more intensive ones (the CONV and HQFS systems). The higher individual milk production of the farms in the HQFS system has led to a lower environmental impact for almost all the impact categories. Particularly, has also explained by the PROC GLM analysis, climate change resulted to be inversely proportional to Individual milk production. This result is in accordance with results from the international literature (Capper et al., 2009; Bava et al., 2014), which demonstrate that milk production per cow is negatively related to the impacts per kilogram of product. The HQFS farms showed better production performances and this was probably due to the inclusion of forages with high digestibility (ensiled alfalfa and Italian ryegrass harvested at an early stage of growth) and whole ear corn silage instead of whole plant corn silage in the diet (Brito and Broderick, 2006; Randby et al., 2012). Diets containing high digestible silages have been shown to reduce GHG intensity by about 17%, compared to diets containing forages with lower digestibility (Guyader et al., 2017). The PROC GLM analysis revealed, in fact, that higher DM yield itself, doesn't mean necessarily lower environmental impact, but also quality of the forages is important to reduce climate change, even though feed efficiency resulted to be not significantly related to GHG emissions. Higher DM yield in the field, in fact, could also mean higher use of inputs (e.g. pest control and chemical fertilizers), therefore despite higher DM yield is not necessary to have a reduction in terms of climate change. On the other hand, the highest environmental impact related to PR FRESH, for almost all the impact categories, is mainly related to the low individual milk production. Considering climate change, also the higher DMI, probably contributed to increase environmental impact, as confirmed by analysis through GLM procedure. Higher DMI, in fact, involves higher daily methane emission (g/d; Benchaar et al., 2014). PR FRESH system, showed the highest GHG emissions, despite the high forage:concentrate ratio, inversely related to climate change, as resulted from PROC GLM analysis. The PR DRY system showed the highest value for land occupation, which could be related to the lower average DM yield per hectare observed for this system. In the PR DRY and PR FRESH systems, almost all the feeds produced on the farms are based on forage from alfalfa and permanent meadows, harvested through haymaking, which is characterized by high DM and quality losses and by low efficiency of the overall harvesting system (Tabacco et al., 2018). This could also explain the high amounts of concentrates purchased in these systems (464 kg of concentrate feed per tonne of FPCM, compared to an average value of 321 kg/t FPCM for the other systems). For the same reasons, the PR DRY system showed a lower feed self-sufficiency, which is directly related to the environmental sustainability concerning land occupation, than the other analysed systems. This result is in accordance with the result of Cederberg (2004), who reported that more intensive systems have less total land occupation than more extensive ones. Land use is an important aspect in terms of environmental impact, since the total area dedicated to feed crops amounts to 33% of the total arable land at the world level (Steinfeld et al., 2006; Mottet et al., 2017). The high level of purchased concentrates, together with the high UAA and low feed self-sufficiency of PR DRY has led the system to also have high values for freshwater ecotoxicity. Like the PR DRY system, the WICE system is characterized by a high rate of purchased concentrates, together with a low feed self-sufficiency, while the double cropping of winter cereal and corn in the same year probably contribute to a high utilization of agrochemicals and synthetic fertilizers, which could partially explain the high freshwater ecotoxicity. Less intensive farms, including organic farms and some rainfed farms, were classified within the MIXED forage system. This system showed a higher environmental impact concerning the use of non-renewable fossil energy. The main reason for this result is probably due to the lower crop yield of their cropping systems. Improving the high quality forage productivity of cropping systems that serve dairy farms has shown a considerable potential for mitigating yield-scaled GHG emissions on farms, in order to reduce the external dependence on feeds for the animals. Commercial feed included in the rations, indeed, showed a tendency to be related to climate change, such as forage:concentrate ratio (inversely proportional).The more extensive and less productive farms show the highest potential to improve their environmental sustainability, which may be achieved through an increase in the milk yield, with enhanced forage productivity (Doltra et al., 2018). The high contribution to climate change of enteric fermentation, which was detected for all the forage systems, has been confirmed by several authors in the international literature (Ogino et al., 2007; Doltra et al., 2018). The high value of enteric fermentation shown by PR FRESH is probably mainly related to the extensive inclusion of forages characterized by low fibre digestibility in the diet, which also results in a reduced individual milk production.

4.2 Organic C densities

Another aim of the study was to characterize the C stock in the 0-0.30 m soil layer of each forage system, in order to collect information about the potential of additional C sequestration attainable by applying targeted mitigation practices. For example, by raising dairy cows on cropping systems that use more forages, some of the enteric methane emissions could be offset by preserving/enhancing the soil C reserves, thereby withholding carbon dioxide from the air (Guyader et al., 2016). Furthermore, well managed cropping systems, based on legume forages, may reduce the use of synthetic nitrogen fertilizers, thereby increasing manure efficiency and making it possible to take advantage of the benefits provided by nitrogen-fixing plants that are capable of taking up large amounts of nitrogen from the system (Russelle et al., 2001), thus curtailing nitrous oxide emissions (Tilman et al., 2002; Guyader et al., 2016). The data from our survey are just the result of an audit conducted on a particular day to record the quantity of C present at that particular time (Powlson et al., 2011): it provides no information on the trends, or whether the C stock is increasing or decreasing, but it could be related to the particular cropping management practices of each studied cropping system to obtain management indications for future applications. The values of C density observed were in the range of values (5 to 8 kg/m^2) reported for other European countries at the same soil reference level (Minasny et al., 2017), with relatively high values for the PR DRY and PR FRESH systems, which, in many cases, exceeded 8 kg/m^2. The high values observed in the present study for these two systems (PR DRY and PR FRESH) are in agreement with results from the literature, since a cropping system based mainly on multi-annual/perennial crops generally increases the belowground C inputs (by reducing soil disturbance and increasing the functional diversity of crop rotations), and this leads to a higher C storage in the first layer of soil than in a system based only on arable crops (King and Blesh, 2018). The potential of additional C sequestration for these two cropping systems (PR DRY and PR FRESH) remains uncertain, because it was not possible to know how far they are from equilibrium. Nevertheless, many studies have indicated that well-managed grasslands continue to sequester C, if the inputs (such as the crop residues, manure or improved primary production) are maintained at a certain level, even to a lower rate of C accumulation, for a long period of time (Powlson et al., 2011). A good mitigation option for these two systems would at least be to maintain the C stocks that already exist. It could be hypothesized that the other studied systems are further from C saturation and they would accumulate C faster than soils closer to saturation, if the right agricultural practices were applied, or continue to be applied in the future. There is a considerable opportunity for improvements in the C sequestering ability of these systems, which could be attained through different agronomic practices, such as leaving more crop residues (for example, stalks after the ensiling of whole ear corn), an increased use of multi-annual or legume crops in rotation, a partial change from annual to perennial crops, the adoption of conservation tillage, and, where not limited by nitrate regulations or an excess of nutrients (P and N) in the soils, an increased use of manure in substitution of chemical fertilizers (Minasny et al., 2017). If adopted, these practices, other than promoting the storage of soil C, may have the potential of expanding the environmental benefits of these cropping systems even further by enhancing other ecosystem services, such as improving soil health, enhancing water quality, conserving biodiversity, and providing a wildlife habitat (Guyader et al., 2016).

Further studies, including multi-year researches and system-based approaches, such as LCA analysis, are needed to evaluate the effects of these management practices applied to forage-based cropping systems that serve milk production with the aim of tuning each practice according to the local lands to achieve the greatest net benefits, while maintaining a high output of milk and furnishing other environmental benefits, such as reduced overall GHG emissions.

5. Conclusion

The present study has taken into account dairy-forage systems characterized by different levels of agricultural intensities and cow performances. More intensive systems, such as HQFS, which are characterized by high individual milk production, showed a low environmental impact for almost all of the impact categories, thus confirming that milk production is negatively related to the impacts per unit of product. The HQFS system also resulted to be more sustainable, in terms of feed self-sufficiency, as it provides a high amount of DM per hectare, consisting of high digestible silages. However, there are still grounds for strengthening the environmental sustainability of this forage system, as HQFS showed the lowest C soil density. Therefore, further investigations on how to consider environmental sustainability over a wider spectrum are still needed.

Acknowledgements

The authors thank the farmers who took part in the research for their collaboration and data providing.

Funding

The study was part of the project Forage4Climate (LIFE15 CCM/IT/000039) supported by EU commission.

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5. Methane emission and forage systems

Short introduction - Since enteric CH_4 emission during digestion is the major contributor to GHGs related to milk production, the *in vivo* measurement of methane production in lactating cows fed with diets based on different forages can be an important contribution for identifying the best mitigation strategies for GWP of milk production. An *in vivo* experimental study, in open respiration chambers under controlled conditions, was, therefore, performed to compare methane emission and milk production of lactating dairy cows fed four different diets, based on four of the six forage systems identified in the previous study (Forage systems and sustainability of milk production: feed self-sufficiency, environmental impacts and soil carbon stocks). Nitrogen volatilization, during manure storage and application to the soil (N_2O), is also a major source of GHGs. For this reason, also nitrogen partitioning was evaluated.

Milk production, methane emission, nitrogen and energy balance of cows fed diets based on different forage systems

Submitted to Journal of Dairy Science

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Interpretive summary

Forage system and milk and methane production in cows

The study determined milk production, digestibility and methane emission of cows fed diets characterized by a different forage basis. The dietary forage did not affect milk production and methane production per kg of milk or per kg of dry matter intake. Digestibility was lower for hay based diet which was characterized by a lower energy value. The use of high-quality grass silages can reduce the amount of the purchased feeds, such as soybean meal, which overall increases the environmental sustainability of the dairy farm.

ABSTRACT

The objective of the present study was to evaluate milk production, digestibility and energy and nitrogen balance of cows fed diets with a different forage basis, reflecting the different forage production systems of Po plain. Four pairs of Italian Friesian lactating cows were used in a repeated Latin Square design, using individual respiration chambers to determine dry matter intake, milk and methane production and to allow total faeces and urine collection for the determination of N and energy balances. Four diets, based on the following main forages (% DM), were tested: corn silage (49.3) (CS), alfalfa silage (26.8) (AS), wheat silage (20.0) (WC), hay-based diet (25.3 of both alfalfa and Italian ryegrass hays) typical of the area of Parmigiano Reggiano cheese production (PR).

Dry matter intake resulted higher for cows fed PR diet than for the other diets (23.4 *vs* 20.7 kg/d on average). Digestibility of DM was lower for PR diet (DMd=64.9 *vs* 71.7% of the other diets, on average). The higher values for aNDFom digestibility were obtained for CS (50.7%) and AS (47.4%) diets. Considering milk production, the results of the present study did not underline any difference among diets. The urea N concentration was higher in milk of cows fed WS (13.8) diet and lower for cows fed AS diet (9.24). This was also correlated to the highest urinary N excretion (g/d) for cows fed WS diet (189.5 *vs* 147.0 on average for the other diets). The protein digestibility was higher for cows fed CS and WS diets (on average 68.5%) than for cows fed AS and PR (on average 57.0%); the dietary soybean inclusion was higher for CS and WS than AS and PR.

The rumen fermentation pattern was affected by diet; particularly PR diet, characterized by a lower content of NFC and a higher content of aNDFom as compared to CS diet, determined a higher rumen pH and decreased propionate production as compared to CS. Feeding cows with PR diet increased acetate:propionate ratio in comparison with CS (3.30 vs 2.44 for PR and CS, respectively).

Cows fed PR diet had a greater daily production of CH_4 compared to those fed CS diet (413.4 *vs* 378.2 g/d) but no differences were observed when CH_4 was expressed as g/kg DMI or g/kg milk.

Hay based diet (PR) was characterized by the lowest digestible and metabolizable energy contents which overall determined a lower NEL content for PR than CS diet (1.36 vs 1.70 Mcal/kg DM respectively for PR and CS diets).

Key words: forage system, milk production, digestibility, methane, energy balance

INTRODUCTION

Greenhouse gases (GHG) atmospheric concentration has increased over the years and the rate of increase over the past century is unprecedented (Prentice et al., 2001). Among the scientific community, there is no agreement on drivers of recent global warming increase, for instance the anthropogenic role ranges from ~50% (Scafetta, 2013) to ~98% (IPCC, 2013). Considering the dairy sector, GHG emission has increased by 18% between 2005 and 2015, because overall milk production has grown by 30%; however, since dairy farming has become more efficient, the emission per unit of product has decreased (FAO and GDP, 2018). With emissions estimated at 7.1 Gt (gigatonnes) of CO_2-eq per annum, the livestock sector is responsible for ~14.5% of human-induced GHG emissions, with enteric CH_4, as a by-product of anaerobic fermentation produced in the digestive tract by *Archaea* microorganisms, being the single largest source (Gerber et al., 2013; McAllister and Newbold, 2008).

Forage type and the amount utilized in ruminant diets have a direct effect on enteric CH_4 production, and in this regard, mitigation strategies can be achieved by altering rumen fermentation pattern (Benchaar et al., 2001). Forage maturity at harvest and the method of forage preservation can be used to manipulate CH_4 production in ruminants: including early cut forage in the diet greatly reduced the production of CH_4 compared to more mature forage (Brask et al., 2013); furthermore, CH_4 production was lower with alfalfa silage rather than with alfalfa hay (Benchaar et al., 2001). Methane production could also be depressed by the utilization of legume (e.g. alfalfa) instead of grass, due to the difference in their chemical composition (Benchaar et al., 2001). Temperate grasses (C3) tend to be more digestible than tropical grasses (C4) due to their lower NDF content and lower lignification, and produce less CH_4 per unit of intake (Archimède et al., 2011).

Utilization of slowly degradable rather than rapidly degradable starch, can also be a strategy to reduce CH_4 emissions from ruminants; for example, corn starch is less rapidly fermented by ruminal microorganism than barley starch (McAllister et al., 1993). In addition, increasing animal productivity through the diet can lower CH_4 emissions per unit of product: feeding better quality diets to increase milk production per cow will dilute the CH_4 emission associated with maintenance energy requirement and metabolism (Knapp et al., 2014).

It has also to be underlined that on-farm production of forages and feeds could contribute to directly and indirectly mitigate emissions from the dairy sector. For example, the dietary inclusion of high protein forages such as alfalfa can lead to a reduction of soybean meal (SBM) in the diet. Among protein sources for animal nutrition, SBM is the first widely available (71% of total protein availability, USDA, 2017), but it has a great environmental impact, mostly related to the land use change (LUC) for its production. Moreover, when forest is converted to cropland, part of the soil carbon (C) is lost, since tilling increases the rate of decomposition, by aerating undecomposed organic matter (Pacala and Socolow, 2004).

Intensity of agricultural practices could directly affect emission linked to feed production (Tabacco et al., 2018; Zucali et al., 2018), whereas C sequestration potential by forage systems can indirectly mitigate the greenhouse gas emissions of the livestock sector (Soussana et al., 2010). Hence, any practice that increases the photosynthetic input of C into the soil and slows the return of stored soil C into the atmosphere, will increase C sequestration (Smith et al., 2007). Practices such as conservation tillage, use of cover crops, rotation with legume crops, increase of double cropping in the same year and best management of livestock manures, all can promote C storage in the soil (Pacala and Socolow, 2004). Farming systems, based mainly on permanent meadows or multi-annual rotational grass and legume forages, may act as active C sinks and might represent a significant GHG mitigation strategy, by increasing soil C sequestration in the soil organic matter (Stanley et al., 2018). By contrast, annual mono-cropping crops, which require for their growth several external input and soil management practices (e.g. agrochemicals, synthetic nitrogen fertilizers, frequent ploughing) reduce the potential of soil C sequestration (Soussana et al., 2010). In a recent survey in Northern Italy (Tabacco et al., 2019), forage systems based on permanent meadows showed an organic soil C content in the 0-0.30 m layer of 23.3 g/kg, whereas forage systems, mainly based on mono-cropped corn for silage, showed an average value of 15.3 g/kg. However, both forage systems have the potential of mitigating livestock emission by maintaining/increasing their C sequestration capacity by the application of the right agricultural production practices.

Since different forage systems can have different environmental impact in terms of soil C sequestration capacity, inclusion of SBM in the diet, and enteric CH_4 production, the aim of this study was to evaluate milk production efficiency, digestibility, N and energy balance and CH_4 emission of dairy cows fed diets with a different forage basis. The evaluated forages were produced from different forage systems selected among the most widespread forage systems in Northern Italy. The present study aims to provide *in vivo* animal performance data, utilizing forages produced on commercial farms which represent management and conservation techniques commonly adopted at the farm scale, and which can be useful for farmers to choose the best forage system.

MATERIALS AND METHODS

The study was conducted at the Research Center "Cascina Baciocca" at Cornaredo (Milan) of the Università degli Studi di Milano (Italy). Animal procedures were conducted under the approval of the University of

Milan Ethics Committee for animal use and care and in accordance with the guidelines of the Italian law on animal welfare for experimental animals (Italian Ministry of Health, 2014), with the authorization 980/2017.

Cows, Experimental Design and Diets

Eight multiparous lactating Italian Friesian cows were used in a replicated 4x4 Latin Square design. Each experimental period lasted 28 days: 23 d of diet adaptation and 5 d of sample collection. At the start of the trial, the cows averaged 127 DIM (SD ±19.6), with an average BW of 608 kg and milk yield of 38.7 kg/d (SD ±3.61). The experimental treatments were based on typical forage systems identified as the most representative of the Po plain (Northern Italy). The 4 dietary treatments were: (1) **CS**: corn silage based system, representative of the most widespread intensive forage system for the Po plain; (2) **AS**: forage system based on double cropped corn (harvested as whole ear silage) and Italian ryegrass (harvested at an early stage of growth as silage) and alfalfa (harvested as silage at an early stage of growth); (3) **WS**: forage system based on double cropped corn (harvested as whole ear silage) and winter cereal (harvested as silage, wheat in the present experiment); (4) **PR:** forage system for Parmigiano Reggiano cheese production (protected designation of origin), based on dried forages from alfalfa and permanent meadows. Diets were formulated using the CNCPS model (version 6.5, Cornell University, Ithaca, NY) to provide the same metabolizable protein and energy concentration. For the three diets based on silage, wrapped bales of TMR were made in 3 different commercial farms, using fodders produced directly in the farm. The TMR bales were made using a MP 2000 compactor (Orkel, Fannrem, Norway). The PR diet was provided by Parmigiano Reggiano consortium, as small bales of TMR. The chemical composition of forages and the VFA, lactic acid and alcohols contents of silages are reported in Tables 1 and 2, respectively.

Cows were fed ad libitum the experimental TMR twice daily. The animals had free access to drinkable water. Orts were recorded daily and the feeding rate was adjusted to yield orts on the basis of at least 5% of the amount supplied (on as fed basis). During the adaptation periods, the animals were housed in individual tie-stalls, fitted with rubber mattresses and bedded with straw. Each cow was weighed at the beginning and at the end of each experimental period. Two days before the sample collection periods, the animals were moved into 4 individual open-circuit respiration chambers to enable the measurement of CH_4 and CO_2 emissions and O_2 consumption. The chambers measured 3.6 (length) x 2.4 (width) x 2.3 (height) m, and were equipped with a small pre-chamber for the entrance of the personnel, and wide glass walls to allow the cows to see each other and outside. Cows entered in the chambers 2 d before the start of the measurements as further adaptation to chamber environment. Each respiration chamber, equipped with a feeder, contained a 2.5 x 1.5 m stanchion that allowed the animal to stand or to lie down. Air temperature into the chambers was maintained at 18±1°C and a low negative pressure was maintained inside the chambers to prevent losses of the CH_4 produced by the cows. Air flow through the chambers was measured using a diaphragm flow-meter (PH 20/335 G 25, 40 m^3/h, Sacofgas, Città di Castello, Perugia, Italy). On average, the air flux was maintained at 35±1 m^3/h. Daily oxygen consumption and carbon dioxide and methane productions were determined measuring the volume of air circulated in the system in 24 h (and referred to standard temperature and pressure conditions) and multiplying it by the difference between the relative concentrations of the gases measured continuously in the ingoing and the outgoing air. Methane and carbon dioxide concentration were measured using an URAS 4 analyzer (Hartmann & Braun AG, Frankfurt am Main, Germany). Oxygen concentration was measured using Magnos 6G analizer (Hartmann & Braun AG, Frankfurt am Main, Germany). Gases concentration was measured every 575 s, using 105 s of air change and 10 s of O_2, CO_2, and CH_4 determination for each chamber and the external air, for a total of 150 observations/d for each gas and each cow. Corrections for personnel entrance were applied taking into account the increased chamber volume (chamber + pre-chamber) at every opening of the pre-chamber.

Total heat production (HP) was determined using Brouwer's (1965) equation: HP (kcal/d) = $3.866\ O_2 + 1.200\ CO_2 - 1.431\ N - 0.518\ CH_4$, where gas volumes (L/d) are expressed at standard conditions and N (g/d) is the urinary nitrogen. The requirement for metabolizable energy for maintenance was assumed to be 115 kcal/$BW^{0.75}$ (van Es, 1978).

Urine and feces were separately collected daily as follows: cows were fitted with Foley urinary catheters (model 1855H24, C. R. Bard Inc., Covington, GA) and urine was collected into a plastic bin containing sulphuric acid (20% vol/vol) to maintain pH below 2.5 and to prevent ammonia losses. Feces left the chamber through openings on the floor in the back of the stanchion and were collected in tanks located underneath the floor of the chambers, as reported by Colombini et al. (2012). Feces and urine were weighed daily, sampled (2% of the total weight) and pooled per cow, during each collection period.

Cows were milked twice daily (7:30 and 6:30 p.m.) and milk production was recorded at each milking by weight. Individual milk samples were taken for each milking and cow during the sample collection period and 2-bromo-2-nitropropan-1,3-diol was added as preservative. Feces, TMR and ort samples were dried in a ventilation oven at 55°C until constant weight. After drying, the samples were ground at 1 mm, using a Fritsch mill (Pulverisette 19, Fritsch GmbH, Idar-Oberstein, Germany). A fresh feces subsample was used for N analysis. Nitrogen balance was determined considering also the N volatilized in the chamber, measured from the N concentration of the water condensed by the air conditioning system. Specifically, the total volume of condensed water was collected in plastic canisters placed inside the chambers and containing sulfuric acid 20% (vol/vol). The water volume was daily weighed, sampled to obtain a composite sample, and stored at −20°C for the subsequent ammonia nitrogen (N-NH_3) analysis.

Ruminal fluid was collected from cows after leaving the chambers at the end of each experimental period. Ruminal liquid was taken five hours after the a.m. feeding and samples were taken through an esophageal probe. Approximately 0.6 L of rumen fluid were strained through 4 layers of cheesecloth. The pH was measured immediately after sampling and one aliquot was stored at -20°C for subsequent VFA analysis.

Chemical analyses

Feed ingredients, TMR, orts and feces were analysed for chemical composition. DM was determined by oven drying at 55°C until constant weight. Analytical DM was determined by drying in a ventilated oven at 100°C overnight (AOAC, 1995; method 945.15). Ash content was determined by incineration at 550°C overnight in a muffle furnace (AOAC, 1995; method 942.05). Crude protein (N × 6.25) was determined according to Dumas method using MAX N exceed (Elementar Analysensystem GmbH, Langenselbold, Germany). The concentration of fiber was determined as described by Mertens (2002), with the inclusion of heat-stable α-amylase and sodium sulphite, and expressed exclusive of residual insoluble ash (aNDFom). Acid detergent fiber (ADFom) and lignin, determined according to the method of Van Soest et al. (1991), were expressed exclusive of residual insoluble ash; particularly, lignin was determined by solubilization of cellulose with sulphuric acid (lignin (sa)). The NDF and ADF procedures were adapted for use in an ANKOM200 fiber analyzer (Ankom Technology Corp., Fairport, NY). The EE was determined according to the method of AOAC (1995; method 920.29). Gross energy was determined using an adiabatic calorimeter (IKA 6000; IKA Werke GmbH and Co. KG, Staufen, Germany). The concentration of N in acidified urine, condensed water collected in the chamber, fresh feces and composite milk samples was determined according to Dumas method by MAX N exceed (Elementar Analysensystem GmbH, Langenselbold, Germany).

Milk Fat, and lactose concentrations were determined using a Fourier transform infrared analyser (MilkoScan FT6000; Foss Analytical A/S, Hillerod, Denmark). MUN concentration was determined by using a differential pH technique (method 14637; ISO, 2006).

Rumen samples were analysed for VFA using an Agilent 3000A micro GC gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) according to Pirondini et al. (2012).

A silage sample was divided in two sub-samples. The first sub-sample was extracted for pH, using a Stomacher blender (Seward Ltd, Worthing, UK), for 4 min in distilled water at a 9:1 water-to-sample material (fresh weight) ratio. The second sub-sample was extracted, using a Stomacher blender, for 4 min in H_2SO_4 0.05 mol/L at a 5:1 acid-to-sample material (fresh weight) ratio. An aliquot of 40 ml of silage acid extract was filtered with a 0.20-μm syringe filter and used for quantification of the fermentation products. The lactic and monocarboxylic acids (acetic, propionic and butyric acids) were determined, by means of high performance liquid chromatography (HPLC), in the acid extract (Canale et al., 1984). Ethanol and 1,2-propanediol were determined by means of HPLC, coupled to a refractive index detector, on an Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA).

Statistical Analysis

Statistical analysis was performed using the Mixed procedure of SAS ver 9.2 (SAS Institute, 2001). Data were analysed with the following model:

$$Y_{ij(k)m} = \mu + Sm + C(S)_{im} + P_j + T_{(k)} + e_{ij(k)m},$$

where $Y_{ij(k)m}$ represents the dependent variable calculated as the mean of the daily measurements during each sampling period ij(k)m; μ is the overall mean; S_m represents the fixed effect of square m, with m = 1, 2; $C(S)_{im}$ represents the random effect of cow i within square m, with i = 1,..,4; P_j represents the fixed effect of period j, with j = 1, ..,4; and $T_{(k)}$ represents the fixed effect of treatment k, with k = 1,..,4; $e_{ij(k)m}$ represents the residual error. Least squares means estimates are reported. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$.

RESULTS

Forages and diet composition

The forages used in the different TMR were: corn silage, Italian ryegrass hay and silage, alfalfa hay and silage and wheat silage. The chemical composition of the forages is reported in Table 1.

Table 1. Chemical composition of the main forages included in the four experimental diets[1] (CS, AS, WC, PR)

	Chemical composition (%DM)							
	DM (%)	Ash	CP	EE	aNDFom[2]	ADFom[3]	Lignin (sa)[4]	NFC[5]
CS [1]								
Corn silage	38.3	4.30	7.29	3.19	41.0	24.3	2.92	44.2
Italian ryegrass hay	89.0	7.00	13.3	3.00	60.0	36.0	5.35	16.7
AS [1]								
Alfalfa silage	44.6	16.2	21.4	4.40	40.0	29.3	6.85	16.0
Italian ryegrass silage	44.5	11.7	8.63	3.31	55.0	35.3	5.08	21.4
WS[1]								
Alfalfa hay (mixed hay)	87.0	10.0	13.0	3.00	56.5	38.2	6.27	17.5
Wheat silage	26.9	7.50	9.84	3.34	62.7	37.8	5.39	16.6
Alfalfa silage	53.0	9.05	21.5	4.40	40.0	31.5	6.82	25.1
PR[1]								
Alfalfa hay	90.0	10.0	18.0	2.50	43.8	34.2	7.26	25.7
Italian ryegrass hay	90.0	10.5	7.99	3.00	61.3	42.0	6.24	17.2

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

[2] aNDFom: NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

[3] ADFom: ADF expressed exclusive of residual ash.

[4] Lignin (sa): Lignin determined by solubilization of cellulose with sulphuric acid.

[5] NFC= 100 - (Ash + CP + EE + aNDFom).

As previously described, the TMR bales were prepared in different farms with different forages, and this explains the differences in terms of chemical composition within the same forage category. Unexpectedly, ash content was very high in the alfalfa silage of AS diet (16.2% on DM). In terms of CP content (% on DM), alfalfa was the forage with the highest value; the values were higher for silages (21.5, on average) than hays (15.5, on average).

Forages were characterized by a wide variability in fiber concentrations. Corn and alfalfa silages had the lower concentrations of aNDFom, but they were characterized by a different lignin (sa) content which was lower for corn silage (2.92% on DM) than alfalfa silages (on average 6.82% on DM). Italian ryegrass hays and wheat silage were characterized by the highest aNDFom concentrations. Corn silage, as expected, had the highest NFC content (44.2% on DM).

The pH and fermentative profiles of the silages included in the diets are reported in Table 2.

Table 2. The pH and fermentative profiles of the silages used in the diets CS, AS, and WS of the experiment.

	Content (g/kg DM)						
	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ethanol	1,2 Propandiol
CS[1]							
Corn silage	3.60	64.3	22.2	0.0	0.0	15.1	10.2
AS[1]							
Alfalfa silage	4.97	33.4	28.6	1.7	10.1	4.7	1.6
Italian ryegrass silage	4.28	58.3	27.3	0.0	0.0	3.5	4.4
High moisture corn	3.96	19.2	5.9	0.0	0.0	3.2	0.3
WS[1]							
Wheat silage	4.26	49.3	43.5	4.3	4.9	13.8	2.7
Alfalfa silage	5.21	13.8	39.9	0.2	1.4	0.0	0.0
High moisture corn	3.76	35.6	11.8	0.0	0.0	9.4	0.7

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

Corn silage had the highest lactic acid content (64.3 g/kg DM) followed by Italian ryegrass silage (58.3) and wheat silage (49.3). Moderate concentrations of butyric acid were detected in alfalfa silage of both AS and WS diets (10.1 and 4.9 g/kg DM, respectively).

Ingredients and chemical composition of the 4 experimental diets are shown in Table 3.

Table 3. Composition and chemical analysis of the four experimental diets

	Diets[1]			
	CS	AS	WS	PR
Composition (% of DM)				
Corn silage	49.3	0	0	0
Alfalfa silage	0	26.8	10.4	0
Italian ryegrass silage	0	19.1	0	0
Italian ryegrass hay	17.3	0	0	25.3
Alfalfa hay	0	0	10.6	25.3
Wheat silage	0	0	20.0	0
High moisture ear corn	0	28.6	29.1	0
Corn grain	12.1	11.4	12.7	22.8
Solvent soy bean meal 48% CP	15.7	8.1	0	9.0
Solvent soy bean meal 44% CP	0	0	12.7	0
Corn gluten feed dry	0	0	0	4.4
Corn grain flaked	0	0	0	8.6
Sugarcane	3.0	3.5	1.9	2.2
Mineral and vitamin supplement[2]	2.5	2.5	2.5	2.4
Rumen-protected methionine	0.03	0.03	0.03	0.03
Chemical analysis (% of DM)				
DM (%)	53.0	54.6	51.2	89.6
OM	92.4	89.6	91.9	92.1
Ash	7.51	10.3	8.12	8.00
CP	15.0	15.3	15.7	14.3
EE	2.34	2.87	2.83	2.52
aNDFom	32.8	27.1	33.7	36.6
ADFom	22.0	22.7	23.8	27.7
NFC	41.2	44.3	38.6	38.5
Diet energy content, Mcal/DM				
Metabolizable energy	2.63	2.43	2.55	2.70

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

[2] Mineral and vitamin supplements composition for the three silage based diets (CS, AS, WS) and PR diet, respectively: 37.4 and 19.4% calcium carbonate, 24.0 and 12.9% sodium bicarbonate, 14.2 and 9.8% sodium chloride, 12.2 and 11.3% magnesium oxide, 8.2 and 4.3% dicalcium phosphate, 5.0 and 5.2 microminerals and vitamins, 0 and 37.1% wheat bran. Provided (per kg): 870 and 918 mg of Fe, 1558 and 641 mg of Zn, 691 and 160 mg of Cu, 1105 and 822 mg of Mn, 26 and 19 mg of I, 14 and 12 mg of Se, 400 and 224 kIU of vitamin A, 60 and 36.4 kIU of vitamin D, 1000 and 1400 IU of vitamin E.

As described in the previous section, the diets were characterized by a different forage basis and a different inclusion level of the main forages used. Particularly, the main forages were included in the diets with the following levels (% of total DM): corn silage (49.3) was the main forage included in the CS diet, alfalfa (26.9) and Italian ryegrass (19.2) silages were the forages of AS diet; alfalfa (25.2) and Italian ryegrass (25.2) hays were the forages of PR diet; wheat silage (20.0) and alfalfa hay (10.6) and silage (10.4) were the forages of WC diet. In terms of concentrates, AS and WS diets included high moisture ear corn (on average 28.9% of total DM), while corn grain meal and SBM were used in all diets. However, the inclusion of SBM was different according to the diet, ranging from 8.10 to 15.7 (% of DM) for AS and CS, respectively.

The experimental diets contained (on DM basis) similar concentrations of OM (91.5% ± 1.26), CP (15.1% ± 0.59) and EE (2.6% ± 0.25). The aNDFom content was highest for PR (36.6% on DM), intermediate for CS (32.8) and WS (33.7) and lowest for AS (27.1%). The ADFom was highest for PR (27.7% on DM) and lowest for CS (22.0%). The NFC content was slightly higher for CS and AS diets (42.8% on DM ± 2.20) and lower for WS and PR diets (38.6% ± 0.07).

Intake and digestibility

Dry matter intake (DMI) and apparent total tract digestibility of nutrients are reported in Table 4.

Table 4. Intake and total tract apparent digestibility of nutrients of lactating cows fed diets based on different forage.

	Diets[1]					
	CS	AS	WS	PR	SE	P
DMI[2] (kg/d)	20.3^{b}	20.9^{b}	20.9^{b}	23.4^{a}	0.770	0.006
Digestibility (%)						
DM	73.3^{a}	71.4^{ab}	70.3^{b}	64.9^{c}	1.06	<0.001
OM	75.1^{a}	74.7^{a}	72.0^{b}	67.1^{c}	1.08	<0.001
CP	69.0^{a}	58.4^{b}	67.9^{a}	55.6^{b}	1.74	<0.001
aNDFom	50.7^{a}	47.4^{ab}	40.5^{bc}	38.5^{c}	2.52	0.001
ADFom	36.8	29.2	29.2	31.8	3.07	0.183

$^{a\text{-}c}$ Means in the same row with different superscripts differ (P<0.05).

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

[2] DMI= dry matter intake.

Dry matter intake (kg/d) was higher (P=0.006) for cows fed PR diet (23.4), compared with the others (on average, 20.7). Digestibility of DM resulted to be the lowest for PR diet (64.9%), and higher for CS (73.3%) but not different than AS (71.4%) (P<0.001). CS and AS diets showed the highest values of OM digestibility (75.1 and 74.7%), WS was intermediate (72.0%) while PR diet had the lowest value (67.1%, P<0.001). Significant difference (P<0.001) among treatments occurred for CP digestibility, with the highest values attained by CS and WS diets (69.0 and 67.9%, respectively) and the lowest by AS and PR diets (58.4 and 55.6%, respectively). aNDFom digestibility was higher for CS and AS diets (50.7 and 47.4%, respectively), although AS was not different from WS (40.5%). aNDFom digestibility of diet PR (38.5%) was similar to that of diet WC.

Milk production and composition

Data of milk production and composition are presented in Table 5.

Table 5. Milk production and milk composition of lactating cows fed diets based on different forage systems.

	Diets[1]					
	CS	AS	WS	PR	SE	P
Production (kg/d)						
Milk	27.0	27.3	28.2	29.3	1.04	0.167
ECM[2]	30.5	31.4	33.1	32.7	1.01	0.148
Composition (%)						
Fat	4.38	4.60	4.71	4.26	0.18	0.100
CP	3.58	3.52	3.56	3.53	0.08	0.364
Lactose	5.02	5.03	5.06	5.09	0.03	0.093
Yield (kg/d)						
Fat	1.164	1.236	1.313	1.236	0.045	0.131
CP	0.959	0.952	0.998	1.03	0.040	0.080
Lactose	1.358	1.372	1.431	1.491	0.051	0.135
LS[3]	2.09	2.67	2.03	3.03	0.630	0.432
MUN[4] (mg/dl)	11.8^b	9.24^c	13.8^a	11.5^b	0.397	<0.001
Acetone (mmol/l)	0.016	0.020	0.019	0.004	0.008	0.259
BHB[5] (mmol/l)	0.03	0.05	0.03	0.03	0.011	0.582
Feed efficiency						
Milk/DMI	1.33	1.31	1.35	1.25	0.030	0.188
ECM/DMI	1.50	1.51	1.58	1.40	0.040	0.070

$^{a\text{-}c}$ Means in the same row with different superscripts differ (P<0.05).

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

[2] ECM (3.5% fat and 3.2% protein) according to Tyrrell and Reid (1965).

[3] LS= linear score, logaritmic transformation of SCC.

[4] MUN= milk urea nitrogen.

[5] BHB= ß-hydroxybutyrate.

Production (milk and energy-corrected milk, kg/d) was not affected by the diet, as well as milk composition and yield in terms of fat, CP and lactose. However, there was a tendency (P=0.100) for milk fat concentration to be slightly lower in cows fed PR diet. On the contrary, there was a tendency (P=0.093 and P= 0.080) for lactose concentration and CP yield to be slightly higher in cows fed PR treatment. Feed efficiency (milk/DMI and ECM/DMI) was not statistically affected by treatment, although there was a tendency (P=0.07) for a lower feed efficiency, expressed as ECM/DMI, for cows fed PR diet (1.40 for PR *vs* an average value of 1.53 for the other treatments). Significant differences (P<0.001) among treatments occurred for MUN concentration (mg/dl) which was highest for WS and lowest for AS (13.8 and 9.24, respectively for WS and AS, P<0.05).

Ruminal fermentation characteristics

Ruminal fermentation characteristics are reported in Table 6.

Table 6. Ruminal pH, total VFA, VFA molar proportion of ruminal fluid of lactating dairy cows fed diets based on different forage systems.

	Diets[1]					
	CS	AS	WS	PR	SE	P
pH	6.23^{b}	6.47^{a}	6.43^{ab}	6.60^{a}	0.075	0.007
Total VFA (mmol/L)	131.0	109.4	103.8	100.1	10.5	0.073
VFA (mole/100 mole)						
Acetate	58.9^{b}	57.5^{b}	60.0^{ab}	62.7^{a}	1.21	0.020
Propionate	24.6^{a}	21.8^{ab}	21.5^{ab}	19.1^{b}	1.18	0.018
Butyrate	12.6^{b}	16.4^{a}	14.1^{ab}	15.1^{ab}	0.640	0.003
Isobutyric acid	0.75^{b}	0.88^{ab}	0.94^{a}	0.68^{c}	0.041	0.001
n-Valeric acid	1.54	1.90	1.64	1.48	0.123	0.081
Isovaleric acid	1.64^{a}	1.54^{a}	1.82^{a}	0.79^{b}	0.170	<0.001
Acetate: propionate ratio	2.44^{b}	2.71^{ab}	2.83^{ab}	3.30^{a}	0.178	0.013

[a-c] Means in the same row with different superscripts differ (P<0.05).

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

Mean ruminal pH value was affected by the diet. The ruminal pH was significantly lower (P=0.007) for cows fed CS diet (6.23), compared with PR (6.60) and AS (6.47) treatment; WS (6.43) was intermediate.

Total VFA concentration (mmol/L) was not affected by treatment, but proportions of VFA were affected by treatment. Particularly, acetate percentage was lower for CS and AS (on average 58.2%) than PR (62.7%) with WC intermediate (P>0.05); propionate content was higher in CS (24.6%) than PR (19.1%) (P<0.05) and not different among AS, WS and PR; butryrate was lower in CS (12.6%) than in AS (16.4%) (P<0.05); isovaleric acid was lower in PR as compared with the other diets. Feeding cows with PR diet increased acetate:propionate ratio in comparison with CS (3.30 vs 2.44 for PR and CS, respectively; P<0.05).

Enteric Methane Production

Dietary effects related to rumen methanogenesis are reported in Table 7.

Table 7. Methane production of lactating dairy cows fed diets based on different forage systems.

	Diets[1]					
	CS	AS	WS	PR	SE	P
CH_4						
g/d	378.2^{b}	395.8^{ab}	396.3^{ab}	413.4^{a}	8.89	0.046
g/kg of DMI	18.6	19.0	19.0	17.8	0.712	0.516
g/kg of OM digested	26.8	28.3	28.7	28.9	0.929	0.338
% GE intake[2]	5.67	5.92	5.78	5.59	0.223	0.621
% DE intake[3]	7.70	8.15	8.17	8.67	0.242	0.120
g/kg of milk	14.4	14.8	14.4	14.2	0.617	0.741
g/kg of ECM[4]	12.5	12.7	12.1	12.7	0.390	0.529
g/kg of milk fat[4]	326.5	322.7	305.1	335.2	9.08	0.107
g/kg of milk protein[4]	400.1	420.6	403.7	402.5	21.9	0.613

[a-c] Means in the same row with different superscripts differ (P<0.05).

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

[2] GE= gross energy.

[3]DE= digestible energy.
[4]yields of milk, ECM, milk fat and milk protein measured over collection days.

Methane production (g/d) was higher (P=0.046) for cows fed PR diet (413) compared to those fed CS diet (378). Dietary treatment did not affect CH_4 emission, in terms of enteric emission related to intake or milk production: on average, cows showed a methane production of 18.6 g/kg of DMI and 14.5 g/kg of milk.

Nitrogen Balance

Results concerning nitrogen balance are presented in Table 8.

Table 8. Nitrogen balance of lactating dairy cows fed diets based on different forage systems.

	Diets[1]					
	CS	AS	WS	PR	SE	P
N intake (g/d)	499.4^{b}	533.2^{ab}	541.9^{ab}	546.0^{a}	19.4	0.003
Fecal excretion						
DM (kg/d)	5.44^{b}	6.00^{b}	6.24^{b}	8.23^{a}	0.400	<0.001
Total N (g/d)	151.9^{c}	222.6^{a}	176.8^{b}	241.3^{a}	12.4	<0.001
Total N (% of N intake)	31.0^{b}	41.6^{a}	32.1^{b}	44.4^{a}	1.74	<0.001
Urinary excretion						
Urine (kg/d)	22.8^{b}	25.0^{a}	23.4^{ab}	24.7^{ab}	0.859	0.004
Total N (g/d)	151.6^{b}	132.9^{b}	189.5^{a}	156.5^{b}	9.57	<0.001
Total N (% of N intake)	31.0^{ab}	24.9^{c}	35.6^{a}	29.2^{bc}	2.12	<0.001
Manure excretion						
Total N (g/d)	303.5^{c}	355.4^{b}	369.2^{ab}	397.2^{a}	9.80	<0.001
Total N (% of N intake)	62.0^{b}	66.6^{b}	67.7^{ab}	73.7^{a}	1.61	<0.001
Milk excretion						
Total N (g/d)	150.3	149.2	156.4	161.8	6.12	0.082
Total N (% of N intake)	30.7^{a}	28.0^{b}	28.9^{ab}	29.6^{ab}	1.09	0.002
N balance						
N retained (g/d)	36.6^{a}	28.6^{a}	17.7^{ab}	-13.8^{b}	12.3	0.005
N retained (% of N intake)	7.23^{a}	5.46^{a}	3.17^{ab}	-3.32^{b}	2.29	0.004

[a-c] Means in the same row with different superscripts differ (P<0.05).
[1]Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

N intake was significantly higher (P=0.003) for PR diet, compared to CS diet (546 versus 499 g/d, Table 8). Fecal N excretion (g/d) resulted to be significantly higher (P<0.001) both for PR and AS diets (241 and 223), in comparison with the other two treatments, while the highest value for N urinary excretion (g/d) was registered for WS treatment (190, P<0.001). Urinary N excretion as percentage of N intake was lower for AS (24.9) as compared to CS (31.0) and WS (35.6) (P<0.05). Manure N excretion (g/d) was the lowest (P<0.001) for cows fed CS diet (304) and PR diet had the highest value (397), but not statistically different from WC (369). Dietary N utilization for milk protein synthesis (milk N excretion, % of N intake) resulted different among CS (30.7) and AS (28.0) diets (P=0.002). Finally, N balance (N retained, % of N intake) was the lowest (-3.32) for cows fed PR diet, although not significantly different from WS treatment (3.17) (P=0.004).

Energy Balance

Results concerning energy balance are presented in Table 9.

Table 9. Energy balance of lactating dairy cows fed diets based on different forage systems.

	Diets[1]					
	CS	AS	WS	PR	SE	P
Gross Energy Intake (GEI) (Mcal/d)	88.1^{b}	88.3^{b}	90.7^{ab}	98.3^{a}	3.27	0.002
Fecal Energy (Mcal/d)	23.4^{b}	24.3^{b}	26.3^{b}	34.8^{a}	1.82	<0.001
Digestible Energy(Mcal/d)	64.7	64.0	64.1	63.5	1.99	0.939
Urinary Energy (Mcal/d)	2.49	2.46	2.44	2.24	0.122	0.464
Methane Energy (Mcal/d)	5.00^{b}	5.21^{ab}	5.21^{ab}	5.45^{a}	0.132	0.005
Metabolizable Energy (Mcal/d)	57.3	56.3	56.4	55.8	1.93	0.900
Heat production (Mcal/d)	29.9	30.6	31.2	30.8	0.750	0.121
Milk Energy (Mcal/d)	21.4	22.1	23.4	23.0	0.705	0.134
Retained Energy (Mcal/d)	5.88	3.59	1.75	1.89	1.71	0.225
Fecal Energy (%GEI)	26.4^{c}	27.4^{bc}	29.1^{b}	35.3^{a}	1.19	<0.001
Digestible Energy (%GEI)	73.6^{a}	72.6^{ab}	70.9^{b}	64.7^{c}	1.19	<0.001
Urinary Energy (%GEI)	2.84	2.78	2.71	2.32	0.178	0.190
Methane Energy (%GEI)	5.67	5.92	5.78	5.59	0.223	0.621
Metabolizable Energy (%GEI)	65.1^{a}	63.9^{a}	62.2^{a}	56.7^{b}	1.09	<0.001
Heat production (%GEI)	34.2	34.8	34.7	31.6	0.856	0.052
Milk Energy (%GEI)	24.4	25.1	25.7	23.5	0.769	0.195
Retained Energy (%GEI)	6.51	3.98	1.49	1.51	2.00	0.215
NEL	1.70^{a}	1.57^{ab}	1.53^{ab}	1.36^{b}	0.294	0.014
Kl[2]	0.626	0.601	0.585	0.589	0.019	0.298

$^{a\text{-}c}$ Means in the same row with different superscripts differ (P<0.05).

[1] Diets, CS: corn silage diet; AS: alfalfa silage diet; WS: wheat silage diet; PR: parmigiano reggiano diet

[2] kl = milk energy/(ME − 110 kcal/$BW^{0.75}$), where milk energy and ME are expressed as kcal/$BW^{0.75}$, metabolizable energy for maintenance was assumed to be 110 kcal/$BW^{0.75}$ (van Es, 1978).

Energy intake was higher (P=0.002) for cows fed PR and WS diet (98.3 and 90.7 Mcal/d). However, WS was not statistically different than CS and AS. Digestible energy (% Gross Energy intake) was significantly lower (P<0.001) for PR (64.7) than the other treatments; CS and AS had the highest values but AS was not different from WS (P>0.05). PR diet also showed higher (P=0.0046) methane energy loss (5.45 Mcal/d), compared with CS diet (5.00 Mcal/d), but the difference disappeared when expressed as percentage of the gross energy intake. Metabolizable energy (as % of the gross energy intake) was the lowest for PR diet (56.7%, P<0.001) among all the diets. Concerning NEL (Mcal/DM) energy content, PR treatment was lower (P=0.014) than CS (1.36 and 1.70) with AS (1.57) and WS (1.53) diets intermediate and not different from CS and PR.

DISCUSSION

Forages and diet composition

One of the main objective of the present study was to evaluate milk production and methane emission of cows fed diets based on different forages, locally produced in commercial farms and representative of the forage systems serving dairy farms most widespread in northern Italy. For these reasons, the chemical composition of the same forages (alfalfa and Italian ryegrass) used in the experimental diets was different although representative of actual Northern Italy forage production systems. In the Po plain (Northern Italy), the main forages fed to dairy cattle are corn silage, alfalfa (silage or hay), cool-season crops (silage or hay) and winter cereal silages. A study conducted by Zucali et al. (2018) in 134 farms located in the same area of the present study showed that corn for whole plant silage was the crop most frequently grown as single (83% of the farms) or double crop (54%) followed by permanent grass hay (80%) and alfalfa (hay or silage, 58%). Concerning corn silage, Gislon et al. (2019, unpublished data) showed that corn silage was used in significant amounts representing, on average, 29.6 % of the diet (on DM). This value is similar to the data (29.0 %) reported by Pirondini et al. (2012) for commercial dairy farms of the Po plain and both values are lower than that of CS diet in the present study. On the other hand, the use of alfalfa silage is less common (21.0% of farms according to Gislon 2019, unpublished) and with lower inclusion level than that used in the present study. Considering the diet WS, the inclusion level on DM used in the present study (20 %) was intermediate between the lower level tested by Benchaar et al. (2014) for barley silage (27.2 %) and the level (wheat silage10% on diet DM) tested by Harper et al. (2017). Overall, in the present study, all the diets were formulated to allow the maximum inclusion level of forages.

Overall, chemical composition of corn silage, alfalfa hay and grass hay is comparable with the values reported by Gallo et al. (2013) for the most common Northern Italian forage sources. Chemical composition of alfalfa silages used in WS and AS diets, is comparable with the values reported in the literature (Broderick, 1985; Kammes et al., 2012), except for ash concentration. Particularly, alfalfa silage used in the AS diet was characterized by a high ash content, higher than that used in the WS diet. This is probably due to soil contamination during harvesting for the alfalfa silage used in AS diet. The alfalfa and ryegrass hays and silages used in PR and AS diets respectively, were comparable in terms of aNDFom concentration, and the observed differences, such as a higher CP content for silage than hay, depend mainly on the conservation method.

Dry matter intake and digestibility

Considering the cow performances when fed the different diets, a remarkably lower digestibility was observed when cows were fed PR diet. The lowest digestibility values for PR diet are mainly due to the high fiber content of the diet, and to the high lignin of the alfalfa hay used, as confirmed by digestibility evaluation (48 h), using *in vitro* technique (unpublished values). Similarly, Broderick (1995) showed higher values of apparent nutrient digestibility, together with lower DMI, for alfalfa silage in replacement of alfalfa hay. Cows fed PR diet had also a greater DMI and in agreement with our findings, Brown et al. (1963) reported an increase in DMI as the level of hay in the diet increased. According to the international bibliography (Colucci et al., 1982; de Souza et al., 2018) digestibility of the diet is reduced as DMI increases and this can be also related to the lower digestibility of PR diet.

Considering the silage based diets, alfalfa, Italian ryegrass and corn silages are the 3 most common forages fed to dairy cows in Northern Italy, although the use of winter cereal silage is also increasing. The best results for NDF digestibility were obtained for CS and AS diets, hence the use of high amount of alfalfa silage, together with ryegrass silage can be considered a good mitigation option; furthermore, this dietary management strategy can reduce the inclusion of soybean meal in the TMR. However, according to a study conducted in Northern Italy (Gislon et al., unpublished data), these forages are used mainly as hay and with an inclusion level lower than that utilized in the present study. The diet based on wheat silage was characterized by a lower NFD digestibility as compared to CS and AS, differently than what observed by Harper et al. (2017) but in agreement with the results of Benchaar et al. (2014) for barley silage as replacement of corn silage. The difference can be explained by several factors such as: different inclusion levels or maturity at harvest; for example, Arieli and Adin (1994) showed that *in vivo* NDFD of cows fed wheat silage diets was 5 points percentage higher for cows fed a silage harvested at an earlier maturity stage than late maturity.

One negative effect observed in this study related to the high proportion of alfalfa in the AS and PR diets was the lower protein digestibility, as compared to CS and WS diets which were characterized by a higher amount of soybean meal. Therefore, in this view, soybean meal seems to be more favorable compared to alfalfa.

Milk production and composition

Considering milk production, the results of the present study did not underline any difference among diets, hence the higher DMI of cows fed PR diet did not significantly increase milk production, in agreement with the results of Broderick (1995). However, a tendency for a lower ECM/DMI, was observed for cows fed PR diet, due to the lower digestibility of this diet. Also Broderick (1995) reported lower dairy efficiency for cows fed alfalfa as hay, instead of silage. It has also to be underlined that there was a tendency for milk fat concentration to be slightly lower in cows fed PR diet, compared to cows fed the other treatments, probably for the dilution effect due to the numerically higher milk production with PR diet. Under the experimental conditions of the current study, the lower MUN was registered for AS diet. The lower urea milk content of cows fed AS diet than CS is in agreement with other studies (Benchaar et al., 2007; Broderick, 1985), who reported a higher MUN concentration when cows were fed corn silage in comparison with alfalfa silage as the sole source of forage. The highest urea N concentration was higher in milk of cows fed WS diet in agreement with the results of Harper et al. (2017) which showed a higher MUN for cows fed wheat silage rather than corn silage. In Harper et al. (2017), the CS diet was characterized by a lower CP content than wheat diet, such as in our experiment with WS diet having a slightly higher CP concentration than the others. Moreover, the diet WS resulted in a lower OM digestibility than CS and AS diets, hence, it can be speculated that at rumen level there was less energy available for microbial protein synthesis with a consequent decrease in capture of N (as AA or NH_3) by rumen microbes. It has also to be underlined that diet WS had a relatively high inclusion of alfalfa silage with a consequent probable high release of NH_3 in the rumen. This was also correlated to the highest urinary N excretion (g/d) for cows fed WS diet. Reflecting also MUN concentration, branched-chain VFA (iso-valeric and iso-butyric) in the rumen was higher for WS. Higher value of branched-chain VFA in the rumen is related to an increased AA deamination, involving higher level of MUN, while a decline in ruminal branched-chain VFA molar proportion is an indication of an inhibition of the deamination process and increased capture of N (as AA or NH_3) by rumen microbes (Hristov and Jouany, 2005).

Ruminal fermentation characteristics

The rumen fermentation pattern was also affected by the different chemical composition between diets; particularly PR diet, characterized by lower content of NFC and higher content of aNDFom as compared to CS diet, increased rumen pH and decreased propionate production. Replacing structural carbohydrates in the diet with non-structural carbohydrates results in large modifications of rumen physical-chemical conditions and microbial populations, such as the shift of VFA production from acetate towards propionate, that occurs with the development of starch-fermenting microbes (Martin et al., 2010). High starch forages also promote lower pH values and a decrease in protozoa numbers (Lettat et al., 2013). In agreement with our findings, Broderick (1985) found higher ruminal pH, less propionate ruminal molar proportion and higher acetate to propionate ratio in cows fed alfalfa hay, compared to cows fed corn silage. Although not statistically significant (P=0.120), CH_4 energy loss as percentage of digestible energy, was lower for cow fed CS diet than the other treatments, and this can be partially related to the observed rumen fermentation pattern.

Enteric methane production and energy balance

Ruminal fermentation profile (i.e higher pH and higher acetate:propionate ratio), together with increased DMI, lead cows fed PR diet to have greater daily production of CH_4 (g/d), compared to those fed CS diet. Also Benchaar et al. (2001) showed higher daily methane production for cows fed hay, compared to cows fed silages. Despite this difference, no significant differences on methane production in terms of g/kg of DMI were observed among diets. Similarly, Harper et al. (2017) did not detect any difference on methane emissions for cows fed CS or WS diets. On the other hand, Hart et al. (2015) observed a lower CH_4 emissions, when expressed per kg DMI, for cows fed high corn silage ration in comparison with high grass silage ration, but in their experiment the proportion of NFC (starch) and fiber in diets was more variable than in our study. Concerning overall methane energy losses (% of energy intake), the results of the present study are in agreement with Pirondini et al. (2015) and Colombini et al. (2015) with methane produced by rumen fermentation accounting for 5-6% of the gross energy ingested by the cow.

The energy balance results underlined a different utilization of energy depending on the diet. In agreement with the digestibility results, in the present study, hay based diet (PR) was characterized by the lowest digestible and metabolizable energy, which overall determined a lower NEL content for PR than CS diet making this feeding system totally based on hays as forage source, less practicable than that based on silages and confirming the lower feeding value of hays as compared to silages.

Nitrogen balance

Any dietary strategy aiming to mitigate CH_4 emission of dairy cows must also take into account the possible effect on N losses in manure, in particular urinary N, to ensure that the reduction of enteric CH_4 emission is not counterbalanced by increased N_2O and NH_3 emissions (Hassanat et al., 2017): N_2O is a powerful GHG and ammonia, although it is not a GHG, is environmentally harmful as it favors acidification and fine air particulate. In the present study, urine volume was higher for AS than CS diets. Other studies (Brito and Broderick, 2006b; Hristov and Broderick, 1996) reported greater urine volumes on diets with higher dietary alfalfa silage proportion vs. corn silage. In agreement with MUN concentration, cows fed AS diet had a lower urinary N excretion (% N intake) than CS and WS diets. These results were partially unexpected since upon ensiling, a large proportion of the CP in hay-crop silages is converted to NPN, thus reducing the efficiency of CP utilization in lactating cows. Feeding carbohydrates that are more extensively fermented in the rumen may improve utilization of the alfalfa NPN through stimulation of microbial protein synthesis. In this study, a consistent amount of high moisture ear corn (28.6% on diet DM) was used in AS diet which probably improved N use in the rumen as confirmed by MUN concentration and urinary N excretion (% N-intake) although N efficiency was slightly lower as compared to CS. Urinary N is largely represented by urea and it is therefore more rapidly nitrified involving N_2O emissions (Eckard et al., 2010). Thus, urinary N is less desirable, and shifting N excretion from urine to feces is useful (Brito and Broderick, 2007). The higher N urinary excretion (total N g/d) for cows fed WS diet, compared with the other treatments, is in accordance with Benchaar et al. (2014), who also observed an increase in N urinary excretion with higher amount of winter cereal (i.e. barley silage) in the diet. These increased urinary N losses are probably related to a reduced N utilization in the rumen as confirmed by the higher amount of MUN and rumen branched-chain VFA (i.e. isovaleric acid and isobutyric) concentrations for cows fed WS diet.

CONCLUSION

The study showed that, in terms of methane production per unit of product or per kg of DMI, no differences occurred among the diets which are commonly used in Northern Italy dairy feeding. Particularly, the use of good-quality grass silages can reduce the amount of the purchased feeds without negative implications on the quantitative and qualitative milk production and without any decrease in OM digestibility.

On the other hand, the diet based on hays determined greater daily enteric CH_4 emission (g/d), compared to the CS diet. In addition, a diet related to an extensive forage system based on hays (i.e. PR) could involve greater N_2O emission from manure, due to its higher N manure excretion, compared to the CS one. Cows fed PR diet also shows the tendency to be less efficient, in terms of ECM/DMI, compared with the other treatments, typical of more intensive systems.

Given the obtained results, the evaluation of the environmental impact of milk production considering the whole milk production chain, in order to consider all the aspects related to GHG emission, should be performed.

ACKNOWLEDGMENT

The study was part of the project Forage4Climate (LIFE15 CCM/IT/000039) supported by EU commission.

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6. Sustainability of dairy cattle diets

In addition to forages, in order to meet the high demand of nutrients needed for high milk production, a lot of concentrate feed are used in dairy cow diet. The assessment of the effects on GHG emissions of different feed ingredients and different level of inclusion in the ration can give interesting contributions for increasing production efficiency and reducing carbon footprint of milk production. A study was, therefore, performed starting from a wide survey on composition of lactating cow rations in 170 dairy farms from the PO plain.

Looking for high productive and sustainable diets for lactating cows: a survey in Italy

Submitted to Journal of Dairy Science, under review

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Interpretive Summary

Dairy cows diet productive and sustainable

Increase of greenhouse gases concentration in the atmosphere is considered to be due to anthropogenic activities, including livestock production. Dairy cows diet manipulation can improve animal productive efficiency and reduce greenhouse gases emissions, in particular methane, increasing the sustainability of milk production. The study revealed that a low enteric CH_4 production is related to a dietary inclusion of alfalfa hay lower than 12% and inclusion of corn silage higher than 30%, although feed efficiency decreased. The variability of diets composition suggests that is possible to reduce greenhouse gas emissions of the diet through a correct choice of the ingredients and their amounts.

High productive and sustainable diet

Abstract

The aim of the present study was to evaluate, by a survey analysis conducted in commercial farms, the global warming potential (GWP) of different lactating cow TMR and to identify the best dietary strategies to increase the feed efficiency (FE) and to reduce the enteric CH_4 emission.

A total of 171 dairy herds were selected: data about DMI (dry matter intake), lactating cows TMR composition, milk production and composition were provided by farmers. Diet GWP (kg CO_2 eq) was calculated as sum of GWP (kg CO_2 eq) of each ingredient included, considering inputs needed at field level, feed processing and transport. For soybean solvent meal, land use change was included in the assessment. Enteric methane (CH_4) production (g/d) was estimated using the equation of Hristov et al. (2013) in order to calculate CH_4 emission for kg of fat and protein corrected milk (FPCM). The dataset was analysed by GLM and logistic analysis using SAS 9.4.

The results of frequency distribution showed that there was a wide variation among farms for the GWP (kg $CO_{2\ eq}$) of TMR: approximately 25% of the surveyed farms showed a diet GWP of 15 kg CO_2 eq, 20% of 13 kg CO_2 eq and 16.7% of 17 kg CO_2 eq. The variation among farms is due to the feed used. Among feed, soybean meal (SBM) had the highest correlation with the GWP (kg CO_2 eq) of the TMR with the following equation: TMR GWP (kg $CO_{2\ eq}$) = 2.49*kg SBM + 6.9 (r^2=0.547). Moreover, an inclusion of SBM >15% of diet DM did not result in higher milk production than with a lower inclusion (≤15%).

Average daily milk production of cows was 29.8 (SD 4.83) kg with a fat and protein content (%) of 3.86 (SD 0.22) and 3.40 (SD 0.14), respectively. The average value of DMI (kg/d) of lactating cows was 22.3 (SD 2.23). The logistic analysis demonstrated that a level of corn silage ≤ 30% on diet DM was associated with higher FE.

Almost 50% percent of the farms had an average value of 15.0 g CH_4/ kg FPCM and about 30% of farms of 12.5 g CH_4/ kg FPCM. The results demonstrated that a lower enteric CH_4 production was related to inclusion (% on diet DM) of less than 12% of alfalfa hay and more than 30% of corn silage. Diets with more than 34% of NDF determined higher CH_4 production (≥14.0 g/kg FPCM) compared with diets with lower NDF content. On the contrary, a lower enteric CH_4 production (<14.0 g/kg FPCM) was related to diets characterized by more than 1.61 NEl (Mcal/kg) and more than 4% of ether extract.

The variability in the TMR GWP shows a significant potential in reducing both the GWP of the diet through a correct choice and dietary inclusion level of the ingredients (mainly SBM) and the possibility to decrease methane enteric emission associated to milk production at commercial scale.

Key Words: diet composition, feed efficiency, global warming potential, enteric methane emission.

INTRODUCTION

Key indicators of climate change (e.g. increased global average temperatures, changes in patterns of precipitation, increased cloud cover and more frequent floods) are widely accepted to be due to the radiative forcing effects of increased concentrations of greenhouse gases (GHG) in the atmosphere.

Deforestation in the tropics and consequent land use change (LUC) for agricultural activity is also a factor contributing to the increase emissions of GHG in the atmosphere (Hopkins et al., 2007).

The increase in the concentration of GHG in the atmosphere is considered mainly related to anthropogenic activities among which agriculture and livestock production plays an important role.

In particular, using a global life cycle assessment approach, in 2015 dairy production system was estimated to have emitted 1,711.8 million tonnes of CO_2 eq, which represents about 3.2% of the total anthropogenic emissions (FAO, 2019). Dairy sector contributes to GHG emissions through emissions related to enteric fermentation, manure management and crop cultivation for feed at farm and outside of the farm (Hassanat et al., 2017; FAO, 2019). Methane emissions, in particular, are by far the largest contributor to the GHG emission profile of the cattle dairy sector, accounting for about 58.5% of total emissions (FAO, 2019). The level of enteric methane emission is mainly determined by feed intake, composition of the diet, forage preservation and to the rate of fermentation of the different carbohydrate sources in the rumen (Boadi et al., 2004). Strategies for reducing enteric CH_4 include supplementing diets with feed additives (e.g. plant extracts as condensed tannins, saponins and essential oils) and dietary fats, offering diets rich in starch, improving forage quality and changing the forage type used in the TMR (e.g., increase corn silage as replacement of grass) (Little et al., 2017). Forages are among the main component of dairy cow diets: they represent from 30% to 80% of the dietary dry matter (DM) (Gallo et al., 2013). In the Po plain (Northern

Italy), the main forages fed to dairy cattle are corn silage, alfalfa (silage or hay) and cool-season crops (silage or hay). A study conducted by Zucali et al. (2018) in 134 farms located in the same area of the present study showed that corn for whole plant silage was the crop most frequently grown as single (83% of the farms) or double crop (54%) followed by permanent grass hay (80%) and alfalfa (hay or silage, 58%). Being a forage so widespread in the area and also used in high amount in lactating cow diets, it would be useful to evaluate the optimum corn silage level to be included in the diet in order to optimize milk production and decrease methane emission for kg of FPCM. Overall, the potential impact of all the main ingredients included in the TMR should be considered; for example, as reported by Evans (2018), the choice of forage type such as maize silage is an easy strategy to employ when CH_4 mitigation is solely considered but needs to be questioned when full associated GHG production is taken into account. Feed production, both on-farm (mainly forages) and out of the farm (mainly concentrates), and transport are considered to be responsible of a significant amount of GHG emission as well: for example, Bava et al. (2014) showed that the emissions related to the production of concentrated feed is about the 20% of total GHG impact for milk production. Following emissions from enteric fermentations (accounting for 46%), therefore, feed emissions represent the second largest category of emissions, contributing for about 36 % to the total emissions in dairy supply chain (Gerber et al., 2013).

To the best of our knowledge, no studies nor feeding models have investigated/implemented the evaluation of global warming potential (GWP) associated to the use of different ingredient in the diet for lactating cows. As a matter of fact, GHG emission from animal feeding is not limited to enteric and manure methane emission but also to the $CO_{2\ eq}$ associated to the production of the different feeds. Therefore, aims of the present work were 1) to quantify the GWP of different lactating cow TMR commonly used in intensive farms and to identify the best feed ingredient composition to decrease the GWP; 2) to determine the best dietary strategies to increase the feed efficiency (FE) and to reduce the enteric CH_4 emission at commercial farm scale.

MATERIALS AND METHODS

Farm data collection

A representative sample of 171 Italian Holstein dairy herds was selected, in Lombardy region (North Italy). Farms were chosen among milk producers that participated in milk national quality control program, consisting of individual milk samples collection (every 40 days) and analysis by the extension service lab. Milk was analyzed for fat and protein (MilkoScan FT6000; Foss Analytical A/S, Hillerød, Denmark) in the dairy service extension lab. The average number of cows was 156 (SD 116) while the utilized agricultural area (UAA) was, on average, 84.4 hectares (SD 82.3); all the farms used TMR as diet supply. Each farm was visited once for collecting data, provided by farmers, about estimated dry matter intake (DMI), lactating cows TMR ingredient composition and chemical analysis, milk production and composition (fat and protein content). Particularly, for each farm the amount of homegrown forages and concentrates (commercial mixed feed and raw materials both homegrown and purchased, depending by the farm) used in the diets was registered. For some farms, which used commercial mixed feeds, the exact amount of each ingredient used in the mix was not declared by producers; hence, for these farms, the mix formula was estimated through CNCPS model (version 6.1; Cornell University, Ithaca, NY; Tylutki et al., 2008), based on the declared chemical composition of the mix and on the basis of the raw materials included in the formula which were listed by the producers in a rank based on a predominance according to weight.

Chemical composition of the TMR (ash, crude protein, ether extract, neutral detergent fiber and non fiber carbohydrates), obtained through chemical or Near InfraRed (NIR) analysis, was provided by farmers.

Milk production was transformed in Fat and Protein Corrected Milk (FPCM), according to International Dairy Federation (IDF, 2015) equation. The equation was the following:

FPCM (kg)= Milk production (kg)*(0.1226*fat (%)*0.0776*protein (%)+0.2534).

Feed and diet GWP calculation

The GWP (kg CO_2 eq) of individual daily TMR was calculated as the sum of GWP of each feed ingredient. The GWP of forages (corn silage, meadow hay, alfalfa and Italian ryegrass hay and silage) was derived from a study performed in the same geographical area by Zucali et al. (2018).

The GWP of concentrates and raw materials was obtained from Ecoinvent (2013), Eco-Alim (2015) and Agri-footprint (Blonk Consultants, 2014) databases. These databases provide documented process data about environmental impact of thousands of products.

For each ingredient of diets, GWP (kg CO_2 eq /kg DM) was calculated considering inputs needed at field level (e.g. fossil fuel, seeds, fertilizers, pesticides, agricultural machines), feed processing (e.g. drying, ensiling) and national and transnational transport. For solvent soy bean meal (SBM), direct LUC was included in the assessment. Different LUC methods result in significantly different output: in this study the value reported by the Agri-footprint database (Soybean, at farm/BR Economic, Blonk Consultants, 2014) was used, considering 3.5 kg of CO_2 eq /kg SBM, for SBM coming from South America. For all purchased SBM, an amount of 20% coming from Italy and an amount of 80% coming from South America were considered (ASSALZOO, 2018). For all farms, diet GWP was calculated as sum of GWP of each ingredient included in the TMR.

Enteric methane production

The published equations summarized by Appuhamy et al. (2016), to estimate CH_4 produced by lactating cows, were tested on a dataset of *in vivo* trials, conducted on lactating cows fed diets based on different forages produced in the same area of the study (Colombini et al., 2015; Pirondini et al., 2015). The equation of Hristov et al. (2013) resulted in the highest r^2 value (0.880; RMSE: 15.3) between predicted and observed values, hence it was used to estimate enteric methane production as follows:

CH_4 (g/d) = 2.54 + 19.14 x DMI

Statistical analysis

Complete dataset was analysed using SAS 9.4 (2012); some descriptive statistic procedures as frequency (FREQ), distribution (CHART), simple regression (REG) and means (MEAN) were performed.

The relationship between TMR characteristics (ingredients and chemical composition) and estimated enteric methane emission was evaluated through two different multiple correspondence analyses (Proc CORRESP) to report a two-dimensional graphical representation of the rows and columns of a contingency table. Data were analyzed by Proc GLM to test the influence of SBM (≤10%; 10-15% and >15%), corn silage (≤30% and >30%) and corn grain meal (≤20% and >20%) inclusions and of NDF (≤34.0 and >34.0 % on DM) and energy (≤1.61 Mcal/kg DM and >1.61 Mcal/kg DM) contents on ration characteristics and milk production.

Multivariate logistic analyses were performed by LOGISTIC procedure to identify the main management factors associated to high level of FE (> 1.30 kg FPCM/kg DMI). The threshold was chosen based on the mean of the two variable in the whole dataset. The logistic regression analyses examined all the possible interactions among variables. Variables or combinations of variables (interaction terms) were excluded through a stepwise backward multiple regression method based on a 20% significance level. The results of the analyses built a final model including variables (risk factors) that were significantly associated with FE (>1.3 kg FPCM/kg DMI). The final models were described in terms of odds ratios, 95% confidence intervals.

RESULTS AND DISCUSSION

Diet composition

All the diets adopted by the farmers were formulated to satisfy nutrient requirements for milk production according to the Cornell Net Carbohydrate and Protein System ver 6.5 as described by Van Amburgh et al. (2015). Average chemical composition of TMR is in Table 1.

Table 1. Average nutrient composition (DM basis) of the lactating cow rations fed in the surveyed dairy farms

	Unit	Mean	SD	Min	Max
CP	%	15.7	1.09	10.0	18.4
NDF	%	33.9	3.44	26.3	45.6
ADF	%	20.7	2.82	11.4	30.0
NFC	%	39.8	3.35	29.8	49.9
Starch	%	27.2	2.99	14.2	35.7
EE	%	3.89	1.11	0.71	8.13
Ash	%	7.28	1.58	4.37	13.1
NEl	Mcal/kg	1.61	0.14	1.19	1.96

Considering the main forages used in the TMR, corn silage was used in 92% of the surveyed farms (Figure 1).

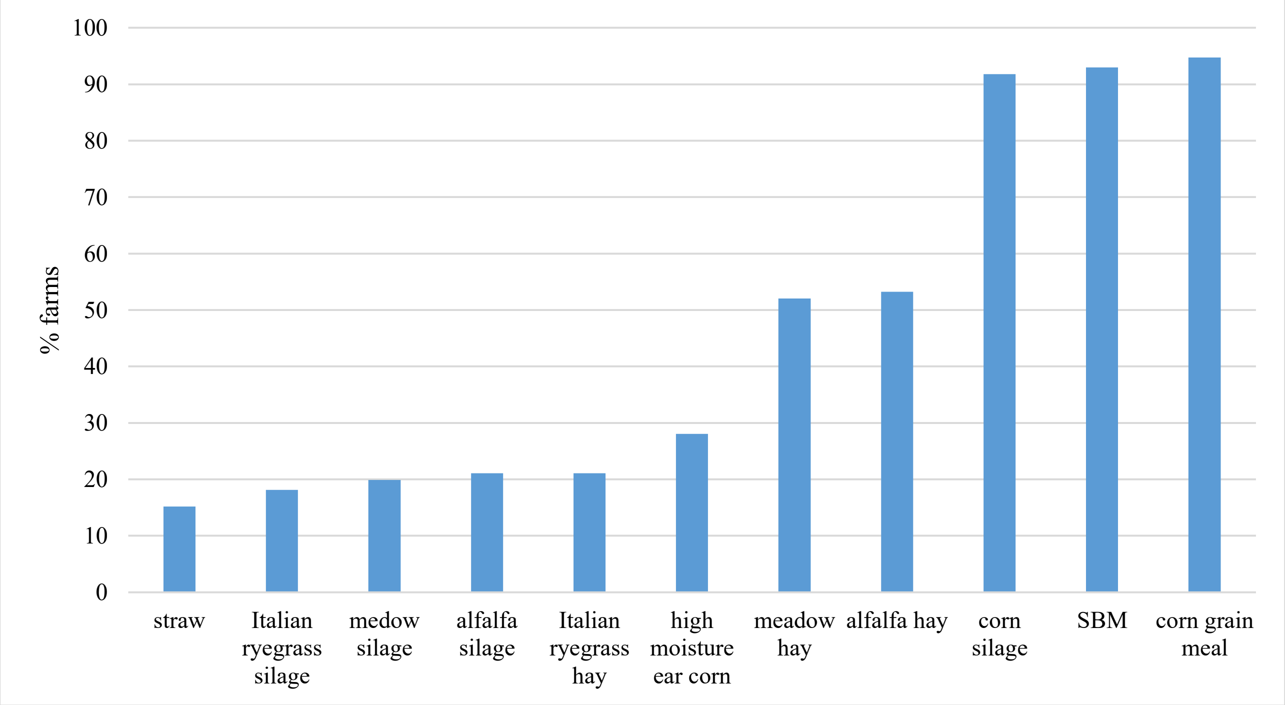

Figure 1. Utilization of main ingredients (% of farms which use the feed) for lactating cow diet formulation

The main reasons for such a high use of corn silage in the Po plain are high agronomic yield and high energy value mainly for its starch content. Because of its high starch content corn silage is considered a good source of ruminally fermentable carbohydrates (Brito and Broderick, 2006; Dhiman et al., 1997).

As reported in Figure 2, corn silage was used in significant amounts representing, on average, 29.6 (SD 11.1) % of the diet (on DM). This value is similar to the data (29.0 %) reported by Pirondini et al. (2012) for commercial dairy farms of the Po plain and much higher than the value (15 %) reported by Arriaga et al. (2009) in the Basque Country. This confirms that Northern Italy environmental conditions are favourable to the production of corn silage, as also reported by Zucali et al. (2018).

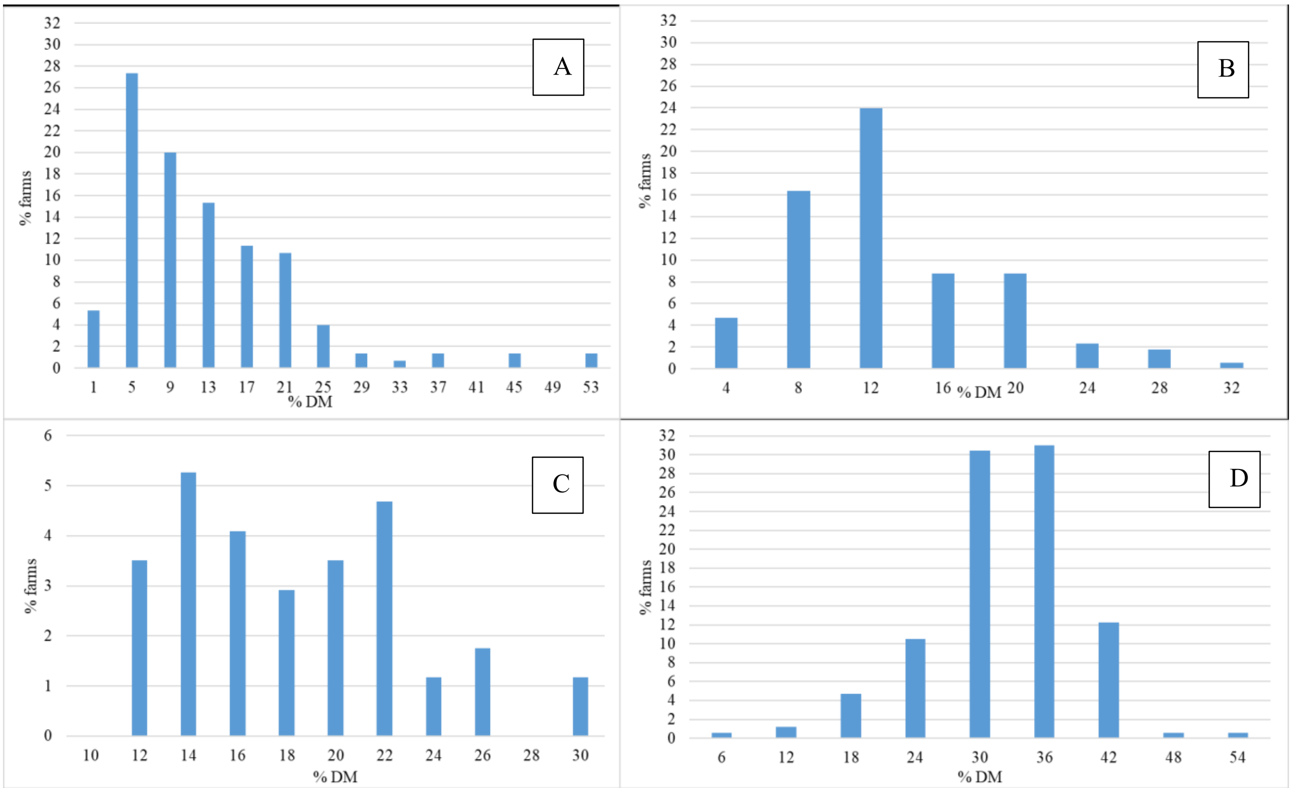

Figure 2. Level of inclusion (% DM) in the lactating cow diet of the main ingredients in the studied farms. A) Italian ryegrass and meadow hay; B) alfalfa hay; C) high moisture ear corn; D) corn silage

The other forages used in TMR were: Italian ryegrass, meadow and alfalfa hays; they were used in 21%, 52% and 53% of the farms, respectively (Figure 1). The average inclusion (% of DM) was 9.69 (SD 8.56) for the Italian ryegrass hay, 11.4 (SD 8.00) for the meadow hay and 13.0(SD 5.79) in the case of the alfalfa hay. On the other hand, these same forages preserved as silage were used in fewer farms: Italian ryegrass silage was present in 18% of the farms, meadow silage in 19% and alfalfa silage in 21% (Figure 1).

Considering concentrates, SBM was used in 93% of the farms (Figure 1). However, the amounts used in the ration (% DM) were different: particularly, in 28% of the farms SBM was lower/equal than 10%, in 46% of farms was in the range between 10 to 15% and in the 26% of farms was more than 15%. These differences resulted in different (P=0.003) crude protein content (% DM) of the diets: 15.3, 15.9, and 16.0%, respectively. However, variation in inclusion level of SBM did not significantly affect (P=0.470) milk production that was 29.03, 29.9 and 29.2 kg FPCM for the three groups of farms, respectively. Therefore, it seems that for the feeding systems of northern Italy, high inclusion (>15% on diet DM) of SBM in the diet is not necessary linked to an improvement in milk production. Similarly, Colmenero and Broderick (2006) obtained the best results for fat corrected milk yield (36.7 kg/d) with an inclusion of 9.2% of SBM on diet DM, in addition to 25% of alfalfa silage.

Corn grain meal was used in more than 95% of the surveyed farms, compared with high moisture ear corn that was used only in the 28% of the farms, with an inclusion in the diet (% DM) of 13.7 (SD 8.98) and 5.23 (SD 8.81), respectively (Figure 1). The availability of carbohydrates for ruminal microbes and the host animal are affected by grain source and processing (Ekinci and Broderick, 1997). Compared to corn grain meal high moisture ear corn, due to the fermentation process, has a greater starch rumen degradability and, as a consequence, a greater digestibility (McCaffree and Merril, 1968; Clark and Harshbarger, 1972). Cows fed high moisture ear corn show higher milk yield, compared to those fed corn grain meal (De Brabander et al., 1992; Clark et al., 1973). Therefore, the use of high moisture ear corn may be increased in dairy farms, due to its promising results in terms of milk production and to the earlier harvest stage than the harvest for the production of corn dry meal.

Diet composition and GWP

Diet GWP corresponds to an average of 0.61(SD 0.15) kg CO_2 eq/kg DM with an average value of 13.7 (SD 3.71) kg CO_2 eq for the total daily administered TMR (Figure 3 and 4).

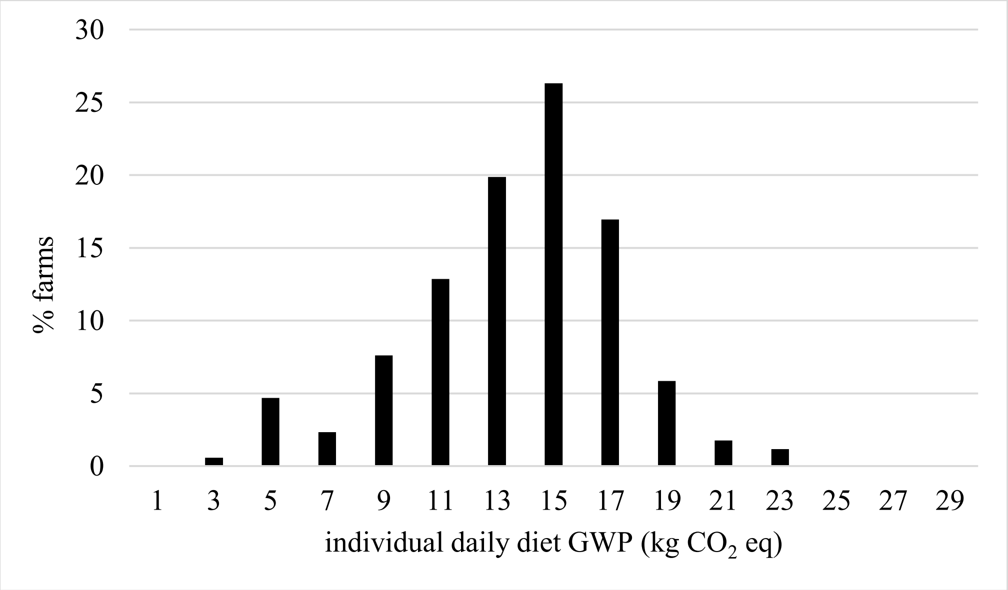

Figure 3. Frequency distribution (% of farms) of daily diet GWP (kg CO_2 eq) in the studied farms

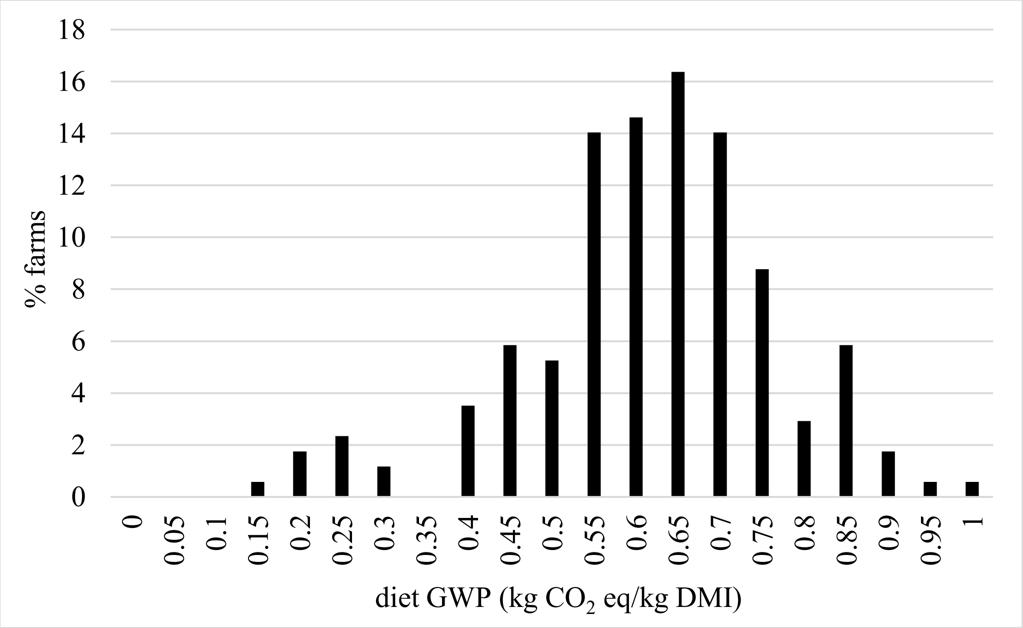

Figure 4. Frequency distribution (% of farms) of diet GWP for kg of diet DM (kg CO_2 eq/kg DM) in the studied farms

However, there was a wide variation among farms for these values: more than 26.3% of the surveyed farms shows an individual daily diet GWP of 15 kg CO_2 eq, followed by 20% of the farms showing a GWP value of 13 kg CO_2 eq and more than 17.7% of farms with 17 kg CO_2 eq. These reported values are the ones with the highest frequency; however, it has to be emphasized that approximately 28% of farms were characterized by a low GWP potential (<11 kg CO_2 eq), whilst, on the other hand, a smaller percentage of farms (8.74%) was characterized by a value >17 kg CO_2 eq.

The variation among farms for TMR GWP is due to the feed ingredients used and to the feed production and transport which are considered to be responsible of high amount of GHG emissions (Ogino et al. 2007). According to FAO (2019) emissions (CO_2 and N_2O) from feed production, processing and transport account for 29.4% of the total emission from dairy sector.

The variability of GWP among diets is directly related to their feed composition and to the GWP of each feed. For example, using concentrates to lower CH_4 emissions, in ruminants, could have limited advantages because increased dietary concentrates may sometime increase total net emissions. The use of great amount of concentrates requires huge inputs for growing, processing and transport, leading to increase use of pesticides, fertilisers and additional ancillary sources of emissions associated with production and transportation infrastructure (Beauchemin et al., 2008) and change of land use. Ogino et al. (2007) reported that production and transport of concentrates for animals feeding produce 2.1 times as much CO_2 as that of roughage per unit of total digestible nutrient.

Considering forage ingredients, as previously reported corn silage was the main forage source of the studied TMR. However, compared to alfalfa silage, corn silage implies a greater CO_2 emission due to increased energy demand for its production, that can be attributed to cropping practises, including use of fertilizers, lime and herbicide (Little et al., 2017). Planting perennial forages such as alfalfa, in addition, may reduce agricultural GHG emissions by sequestering CO_2 in agricultural soils (Jarecki and Lal, 2003). While changes in soil carbon can affect the carbon footprint of agricultural systems, they are rarely included in GHG analysis of dairy products because of the complexity and lack of the consensus about the methodology (Little et al., 2017). Even in this study, carbon soil sequestration was not taken into account in evaluating the environmental impact of different feed sources; considering this aspect, the results obtained may change. As previously reported, soybean meal was used in 92% of surveyed farms in order to satisfy the protein requirements of cows. Use of protein sources like soybean meal, that in most cases comes from outside of the farm, mostly South America, implies an addition in CO_2 emission related especially to transport and to LUC. In this study the GWP value for SBM was 3.09 kg CO_2 eq/kg DM (Agri-footprint, 2014 modified) and included the environmental impact of 80% of SBM imported from South America and 20% of SBM produced locally because Italy is not self-sufficient for this production. As expected, as it is shown in Figure 5, there is a linear correspondence between the increasing of the daily diet GWP (kg CO_2 eq) and the increasing of the amount of SBM in the ration.

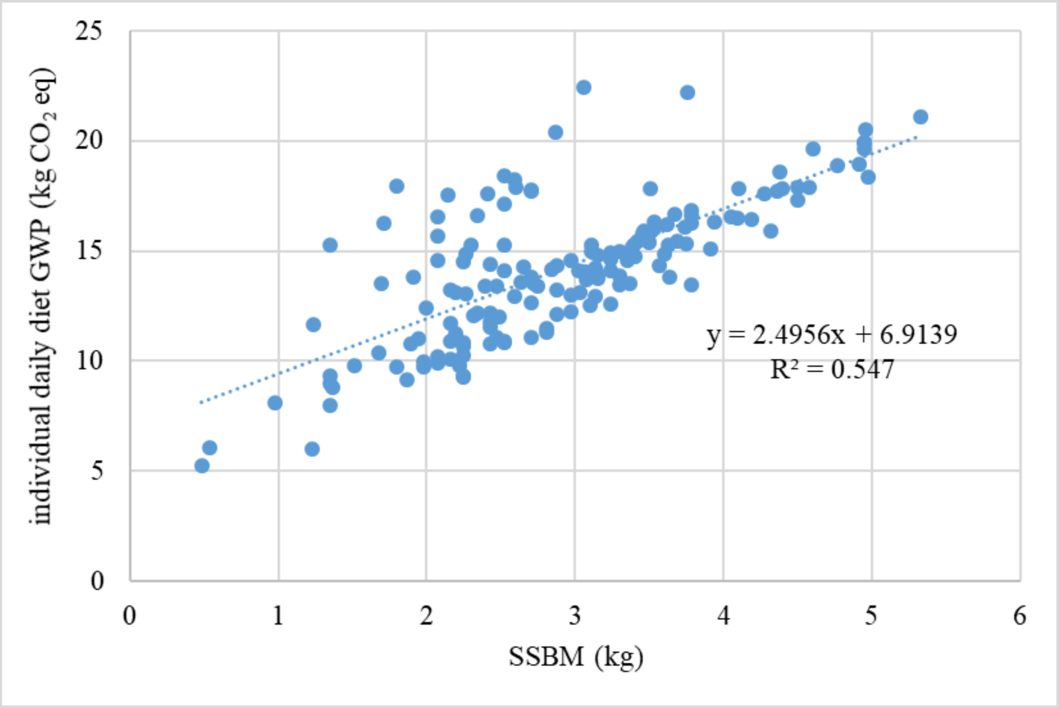

Figure 5. Relationship between dietary soybean meal amount and daily GWP of the diet in the studied farms

Although pasture expansion remains by far the primary direct cause of Amazonian deforestation, also the increasing of mechanized crop production such as soybean must be viewed as a driver of deforestation, even if new fields replace only existing pastures or savannah lands (Arima et al., 2011). In some Brasilian states such as Mato Grosso the increase in soybean crop in regions previously used for pasture, may have led to a displacement of pastures further north into the forested areas, causing indirect deforestation there. Therefore, soybean cultivation may still be one of the major causes of deforestation in the Legal Amazon (Arima et al., 2011; Barona et al., 2010; Lapola et al., 2010).

Diet composition and feed efficiency

The knowledge of feed efficiency at herd level is an important aspect that should be evaluated in order to increase farm profitability. In this regard the choice of ingredients used to formulate TMR can strongly affect FE values. For example, as reported by Phuong et al. (2013) fibre, concentrate proportion and digestible energy are the common nutritional factors affecting feed efficiency. For this reason, in the study, a relationship between diet composition and FE was studied through a logistic analysis at commercial farm scale. Average milk production of the surveyed farms was 29.8 (SD 4.83) with a fat and protein content (%) of 3.86 (SD 0.22) and 3.40 (SD 0.14), respectively.

In this study, FE was affected by dietary amount (% on DM TMR) of corn silage, other forage sources and corn grain meal (Table 2). In particular, a level of corn silage ≤ 30% of DMI, associated with the inclusion in the diet of other forage sources (e.g. alfalfa and grass hay), means a higher FE (Table 2).

Table 2. Logistic analysis of feed sources related to high feed efficiency level (>1.30 kg FPCM/kg DMI).

Effect	Odds ratio estimate	95% confidence limits		*P*
Corn silage inclusion: < 30% vs >30% DM	2.35	1.14	4.85	0.021
Corn grain meal inclusion: <20% vs >20% DM	2.10	1.11	3.97	0.022
Forages excluding corn silage inclusion: <20% vs >20% DM	2.03	1.00	4.13	0.051

This result is in accordance with other studies, which demonstrated that a mix of different forage sources leads to an optimum balance in terms of energy and protein supply for milk production, compared with either corn silage or another forage as the sole component of the forage amount (Brito and Broderick, 2006; Dhiman and Satter, 1997; O' Mara et al., 1998).

As described previously, corn silage is the main ingredient of lactating cow TMR in the Po plain; however, the results of the present study confirm that there is a threshold level (approximately ≤30%) that should be taken in account in lactating cows diet formulation. Farms with less than 30% of corn silage included in the diet (%DM) showed an average milk FPCM production of 30.2 kg/d, whereas those with more than 30% of the inclusion had an average milk FPCM production of 29.0 kg/d (P=0.099, for the difference in milk production according to corn silage inclusion).

The results shown in Table 2 also pointed out that a FE > 1.30 is linked to an inclusion in the diet of corn silage lower than 30%, corn grain meal lower than 20% and forages excluding corn silage lower than 20%, as results by the odds ratio levels higher than 1. Hence, an excessive increase of corn grain meal in the diet is not related to any productive advantage for the animals. In the surveyed farms, therefore, FPCM production was on average 30.1 (SD 4.82) kg/d for the group with less than 20% (% DM) of corn grain meal, whereas it was 28.8 (SD 4.39) kg/d for those farms with more than 20% of corn grain meal in the diet (% DM) (P=0.073). According to Powell et al. (2016), feeding more corn grain meal does not significantly impact milk production (kg FPCM/cow per day). Similarly, a review based on a meta-analysis approach to evaluate the effect of dietary starch on lactation performance by dairy cows (Ferraretto et al., 2013), underlined that milk fat content decreased as dietary starch content increased and that milk fat depression in high-starch diets is likely related to greater starch and lower NDF intakes (Jenkins and McGuire, 2006).

Diet composition and enteric methane emission

The study was conducted on 171 commercial farms hence it was not possible determining CH_4 emission by a direct measurement. The level of enteric methane emission is mainly determined by feed intake and by the composition of the diet (Beauchemin et al., 2008; Boadi et al., 2004; Martin et al., 2010) and for this reason, in the present study, methane emission was estimated according to DMI using the equation of Hristov et al. (2013). The average value of DMI of lactating cows measured by the farmers involved in the study was 22.3 (SD 2.23) kg/d. The results are reported as grams of methane/kg milk production and composition (FPCM). Differently than methane, FPCM was directly measured by farmers and with this regard the obtained results can be applied on a territorial scale to rank farms and dietary strategies to evaluate CH_4 emission/ kg FPCM.

As it is shown in Figure 6, most of the considered farms (almost 50% percent of the farms investigated) had an average value of 15.0 g CH_4/ kg FPCM, followed by about 30% of farms with a value of enteric methane production of 12.5 g CH_4/ kg FPCM. These values are in line with results obtained in several trials conducted using the respiration chambers (Benchaar et al., 2014; Hassanat et al., 2017; Hassanat et al., 2013).

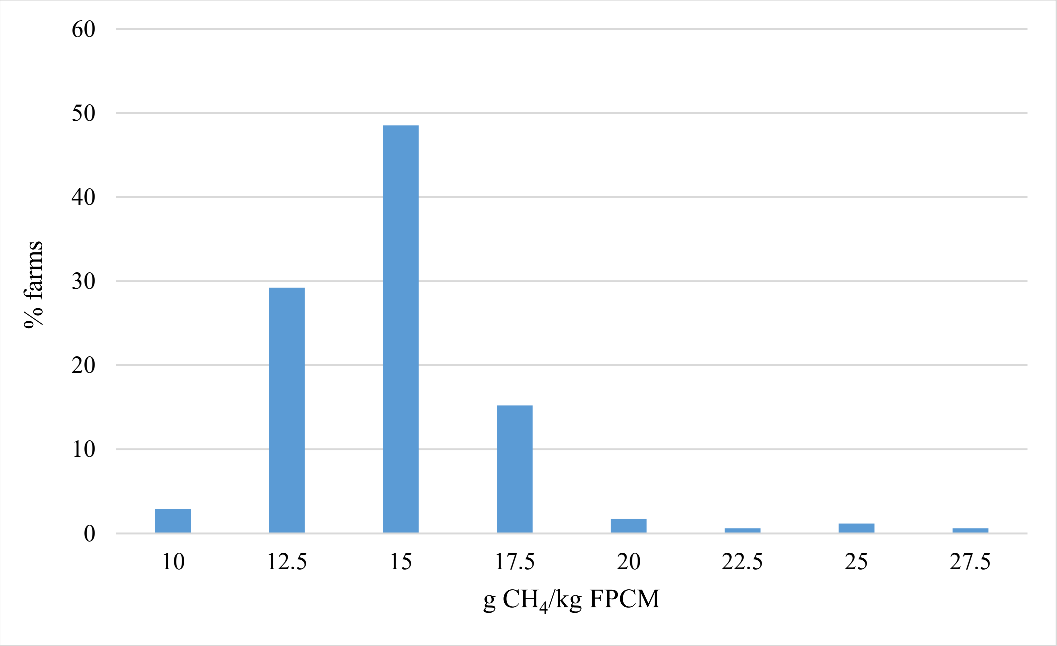

Figure 6. Frequency distribution (% of farms) of estimated enteric methane production per unit of fat and protein corrected milk in the studied farms

The wide variability among commercial farms for the emissions shows that for a significant number of farms, it is possible to decrease the emissions applying appropriate dietary strategies. For example, as it is shown in Figure 7, a low enteric CH_4 (≤ 14 g CH_4/ kg FPCM) production was related to the inclusion of less than 12% of alfalfa hay in the diet and more than 30% of corn silage and less than 20% of other forage sources excluding corn silage (i.e. Italian rye grass and permanent meadow). Hassanat et al. (2013) demonstrated that feeding dairy cows with corn silage as total replacement of alfalfa silage involves less CH_4 emissions (in terms of g/kg of DMI). Similarly, Aguerre et al. (2011) reported a higher CH_4 emission per kg of both milk and energy corrected milk with increased forage proportion in the diet in agreement with the results of the present study.

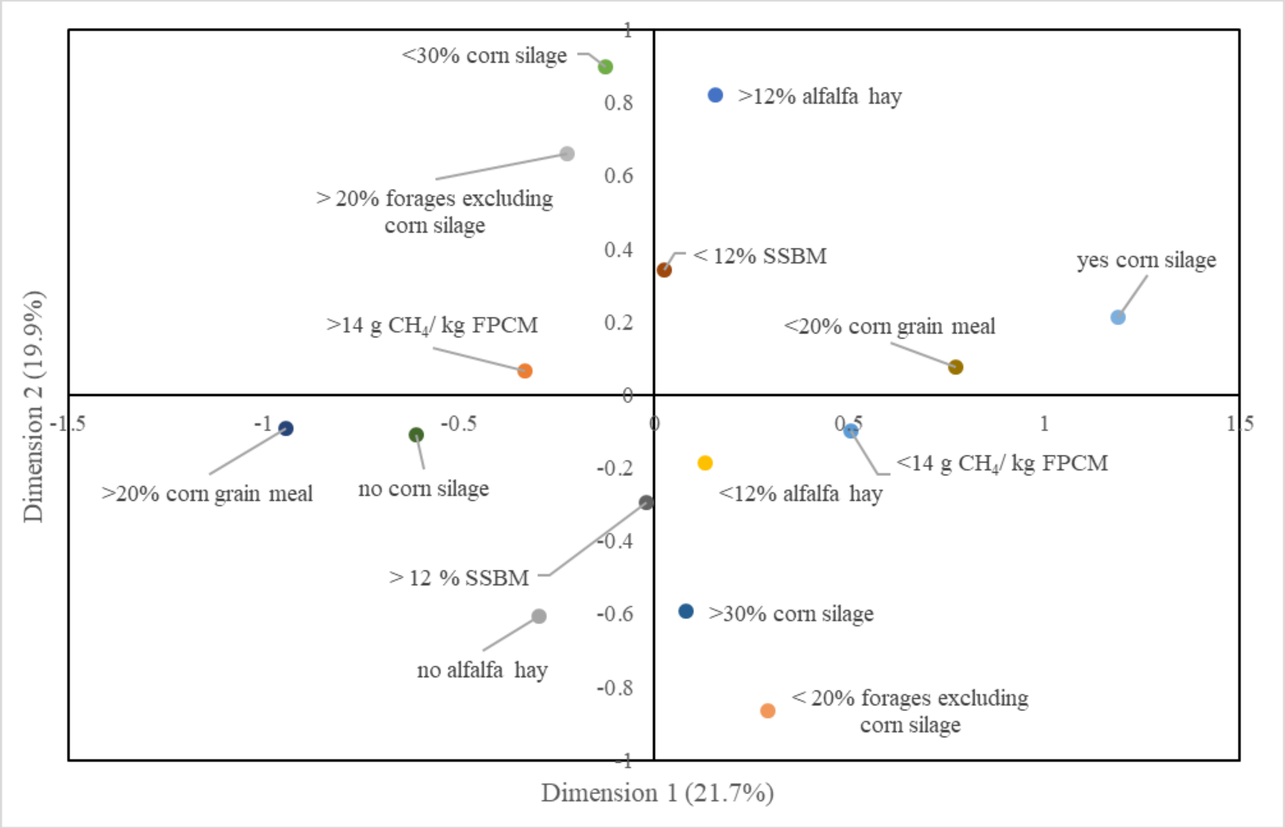

Figure 7. Correspondence analysis for methane production per kg of fat and protein corrected milk and feed inclusions (on DM basis) in the diet.

Overall the inclusion of corn silage in the diet (about 30% dietary DM) seems to be positive related to a lower methane emission however, as shown previously, the amount of corn silage used in the rations should not reduce the FE. This agrees with the results of van Gastelen et al. (2015) who showed a lower methane emission by cows fed diets with corn silage instead of grass silage, although the calculated FE was lower for cows fed diets based on corn silage as sole forage source. The increase in the dietary corn silage proportion is generally associated to some changes in the rumen, such as lower pH and acetate:propionate ratio and reduced fiber digestibility (Hassanat et al., 2013), which can negatively affect cow performance.

Diets characterized by a high content of neutral detergent fibre (NDF), conversely, involve higher methane production at enteric level. Roughage based diets favour acetate production and increase CH_4 per unit of fermentable organic matter (Johnson and Johnson, 1995). As it is presented in Figure 8, diets with more than 34% of neutral detergent fibre (NDF) (% DM) involve an enteric methane production higher than 14 g/kg FPCM. In the surveyed farms, cows fed diets with a lower NDF dietary concentration (≤34.0%) produced approximately +15.0% of FPCM (31.6 kg/d) as compared with cows fed diets with higher NDF (>34.0%) which produced 27.2 kg FPCM/d and the difference was significant ($P<0.001$). On the other hand, DMI intake was on average about +5% for cows fed diet with NDF ≤34.0 than for cow fed diets with NDF>34.0 (22.8 vs 21.7 kg/d; $P=0.002$ respectively for low NDF and high NDF diets) hence cows fed diets with a high NDF content were overall less efficient resulting in a higher production of CH_4/kg FPCM.

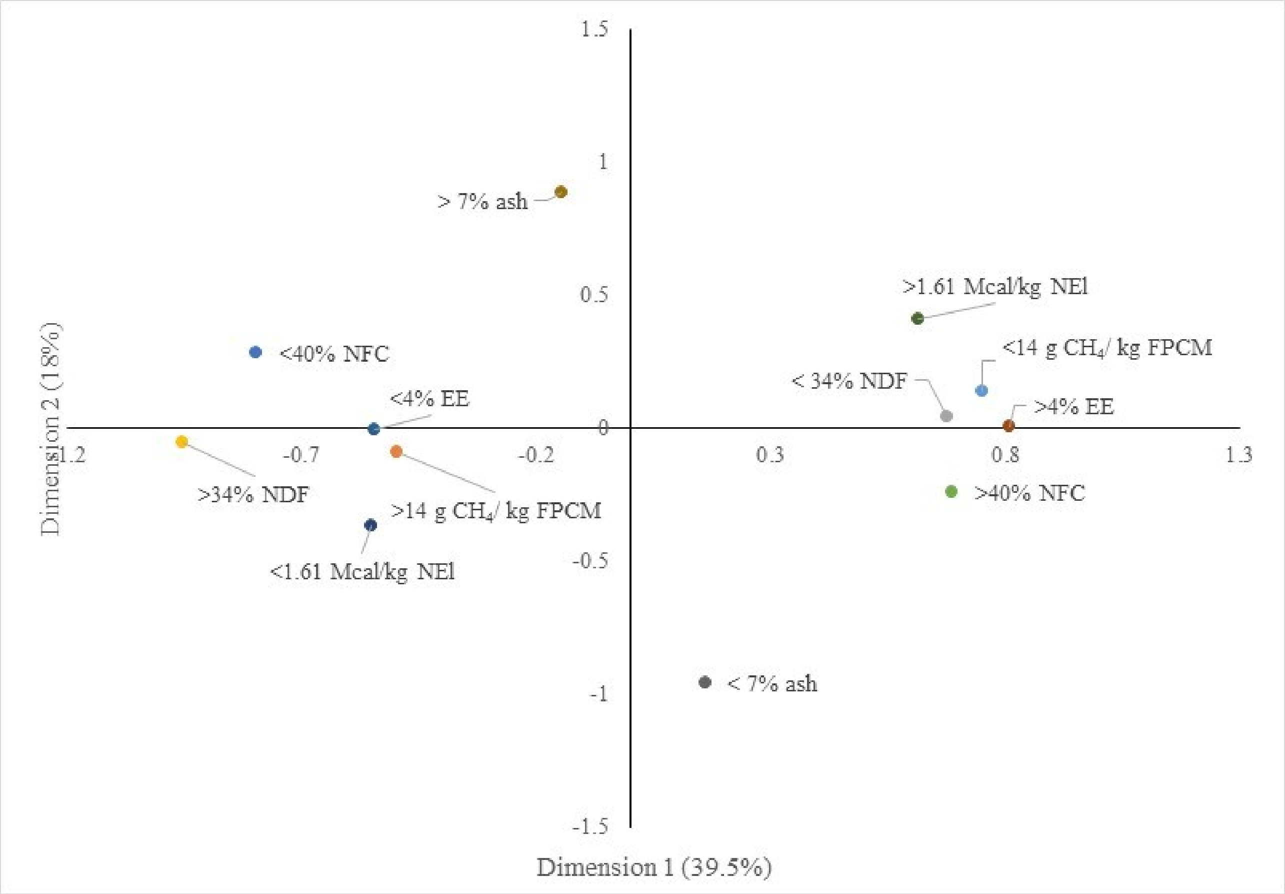

Figure 8. Correspondence analysis for methane production per kg of fat and protein corrected milk and chemical composition and energy content (on DM basis) of the diet.

Similarly, as it is shown in Figure 8, diets characterized by more than 1.61 NEl (Mcal/kg) tend to have less than 14 g CH_4/kg FPCM. The diets high in energy contents were formulated with a slightly higher concentrate inclusion (48.9 vs 46.4% on DM respectively for high and low energy diets; P=0.03) and consequently higher FPCM production (30.9 vs 28.2 kg/d; P<0.001). Replacing structural carbohydrates from forages in the diet with non-structural carbohydrates contained in most energy-rich concentrates is associated with increases in feed intake, higher rates of ruminal fermentation, accelerated feed turnover and a shift of volatile fatty acid production from acetate towards propionate and this results in lower CH_4 production (Johnson et al., 1996; Hegarty and Gerdes, 1998; Martin et al., 2010).

Similarly, a lower enteric methane production was also related to diets containing more than 4.0% (% DM) of ether extract (EE) (Figure 8) which increases the dietary energy content. Moreover, dietary fat seems to act as reducers of ruminal methanogenesis without affecting other ruminal parameters (Martin et al., 2010). With this regard a survey of Giger-Reverdin et al. (2003) demonstrated that an increase of 1% of dietary EE decreased the methane production of 0.68 l/kg DM of feed. Similarly, considering methane/FPCM, Knapp et al. (2014) showed (by meta-analysis) that increasing EE content in the ration reduced CH_4/energy corrected milk and it appears that this is achieved by dilution of the fermentable carbohydrates in the DMI and potentially reduced DMI (Knapp et al. 2014). The threshold value of EE of 4% on DM has been chosen to divide the farms into two groups numerically equivalent. The data obtained indicate that increasing the EE content of the diet above 4% on DM is a simple but effective strategy for about half of the farms to reduce the methane emission/ kg of FPCM.

CONCLUSIONS

The results of the survey revealed that corn silage, corn grain meal and soybean meal are the most popular ingredients included in the total mixed ration for lactating cows in Northern Italy, due to the high yield of corn and the high availability of the protein of soybean meal. However, the use of these feeds generates some concerns. An excessive inclusion of corn silage (>30% on DM) or corn grain meal (>20% on DM) in the diet is not related to higher milk yield and has a negative effect on feed efficiency. A high inclusion of soybean meal (>15%) is also not related to a higher FPCM, but nevertheless increases the GWP of the total mixed ration. On the other hand, it is important to consider that a high inclusion of roughages in the diet determined a high enteric methane production that contributes to increase the GWP of the diets. The variability in the total mixed ration GWP obtained in this study suggests that - through a correct choice of the ingredients and their dietary proportion and obviously respecting dairy cow nutrient requirements - it is possible to reduce the GWP of the diet and, consequently, of milk production. This suggestion could be also useful for the formulation of heifers and calves diets, considering their important contribution to GHG emissions. In this view other studies should be promoted. In a future perspective, when a balanced diet is formulated, it is important to include also a GWP evaluation of both the feed and of the entire total mixed ration, in order to contribute to the overall sustainability of livestock production.

ACKNOWLEDGMENT

The study was part of the project Forage4Climate (LIFE15 CCM/IT/000039) supported by EU commission.

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7. Alternative protein sources for animal feeding

Short introduction - As emerged from the previous study (Looking for high productive and sustainable diets for lactating cows: a survey in Italy) soybean meal is the main protein source in dairy cow diets and involves high GHG emission, mostly related to land use change. As a consequence, an increasing interest has been developing in the recent years about alternative protein sources for animal feeding. In particular insect-derived protein feed seems to be promising in terms of nutritive characteristics and environmental sustainability. In a perspective of circular economy, a study on the effects of different feed or by-products for insect rearing on the chemical composition of larvae and their environmental impact was performed through in vitro methodology and Life Cycle Assessment.

Rearing of *Hermetia illucens* (Black soldier fly) on different by-products: influence on growth, waste reduction and environmental impact

Published on Animals, 9(6), 289

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Abstract

In the last few years, great attention has been paid to the valorisation of organic by-products for the production of a valuable insect biomass rich in protein and fat sources for animal feed industry. The aim of the study was to exploit three inedible human by-products as growing substrates for *H. illucens* (Black Soldier Fly-BSF) larvae: okara, maize distiller and brewers' spent grains mixed with trub (later called brewer's grain) and a commercial hen diet, used as a control. The study focused on: growth performance of larvae; production of methane and other gases by BSF larvae and environmental burden caused by larvae production, using Life Cycle Assessment (LCA). Chemical composition of substrates differed, okara had highest values of CP (39.2 % on DM) and EE (17.2% on DM) while brewer's grains showed the highest fiber content, and conditioned larvae growth and biomass conversion ability. In particular larvae fed on hen diet and maize distiller exhibited the highest final weight (2.29±0.20 and 1.97±0.14, respectively). Okara was better converted in biomass, larvae grown on this substrate showed the highest waste reduction index and efficiency of conversion of the ingested food indexes (4.9 and 0.36 respectively), probably induced by the high protein content of okara. The results of the present study show that BSF larvae, grown on all substrates, did not produce any detectable trace of CH_4. LCA evaluation showed that larvae production (expressed both as kg of larvae and kg of protein) on hen diet resulted the most impactful for the most of environmental categories, caused by the inclusion of soybean meal in the diet, but the results were slightly lower if compared with soybean seed production. Feed production activities resulted the main contribute to the environmental impact (from 60 to 99%). In order to compare larvae production obtained on all substrates, an environmental impact was attribute to okara and brewer's grain throughout a substitution method, the best sustainable product resulted the larvae grown on maize distiller. It is necessary to deepen the knowledge and methods to attribute an environmental weight to by-products. Only considering this crucial point is possible to better evaluate the environmental benefit of producing insects as novel feed or/and food.

Keywords: Black soldier fly, LCA, animal feeding, larval development, bioconversion, by-products

1. Introduction

By-products are considered as incidental or secondary products resulting from a production process, the primary aim of which is not the production of the item itself. Most of by-products derive from agri-food industry and are largely used for livestock feeding due to their interesting nutritive value. The most widespread is the soybean meal but other interesting products are used such as sunflower meal, bran and cotton seed, all of them are usually characterized by very high price. The advantages of these feed utilization are the reduction of waste production that can cause environmental pollution, the reduce in competition between animals and human for crops and sometimes the reduction of feeding costs.

A possible alternative exploitation of by-products in agriculture, is represented by their use as rearing substrate for insects. Bioconversion of by-products by insects constitutes a new approach and an interesting example of sustainable circular economy (Newton et al., 2005; Diener et al., 2011; Jucker et al., 2017; Meneguz et al., 2018). In this context, organic by-products can be valorised for the production of a valuable insect biomass rich in protein and fat sources for animal feed industry (Tschirner and Simon, 2015; Makkar et al., 2014; Barragan-Fonseca et al., 2017) or for biodiesel production (Manzano-Agugliaro et al, 2012; Leong et al., 2016; Surendra et al., 2016).

Among the widely availability of by-products from food industry, some of them show interesting characteristics such as high protein and energy contents that meet the insect nutritive requirements. Among these, okara (soy pulp or fiber obtained from tofu production), wet brewers' spent grains (from beer industry) and maize distiller's grains (from spirit or ethanol production) represent a possible substrate for insects rearing. Okara and wet brewers' spent grains are considered as waste from food industry and their disposal has a high cost.

Okara availability has been increasing throughout the world due to an increase production of soybean foodstuff and the dumping of okara has become a problem to be solved due to its contamination to the environment (Li et al., 2013). Similarly, brewer's spent grain forms up to 85 % of by-products of the brewing industry (Mussatto et al., 2006) and in the EU, about 3.4 million tons of brewers' spent grains are generated annually in beer production (Repro, 2006).

Larvae of *Hermetia illucens* (Diptera: Stratiomyidae), also known as the Black soldier fly (BSF), are able to grow on a wide range of organic material, ranging from fruits and vegetables to animal remains and manure (Newton, 2005; Diener et al., 2011; Nguyen et al., 2015; Spranghers et al., 2017; Jucker et al., 2017). BSF larvae are capable of extracting energy from all kinds of organic waste to produce protein and lipids useful to be used as animal feed (Barroso et al., 2014; Makkar et al., 2014; De Marco et al., 2015). From previous studies, larvae have an average content of 45.2% crude protein and 31.4% fat, however, their fat and protein composition can vary as function of growing substrate characteristics (Makkar et al., 2014; Nguyen et al., 2015; Jucker et al., 2017).

Another advantage of BSF is that, differently from many pests that consume waste, BSF larvae do not carry bacteria or diseases and are capable of inactivating *Escherichia coli* and *Salmonella* (Erickson et al., 2004). In addition to its nutrient-producing capacity, BSF can greatly contribute to the reduction of waste volume up to 44 to 99% as shown by Nguyen et al. (2015).

Recent studies, where by-products are used as substrates for insect growth (Halloran et al., 2016; Smetana et al., 2016; Salomone et al., 2017), underlined that the environmental load of feed takes more than half of the impact of insect production. From Life Cycle Assessment (LCA) point of view, the inclusion of a by-product, often without economic value, in another production process could cause some methodological problems, thus there isn't scientific consensus to assign an environmental load to by-products. In many studies by-products or food waste assume zero burden (Bacenetti et al., 2015; Salomone et al., 2017), as the authors considered only the environmental load of transport of by-products or waste in the secondary process. On the other hand, if residues are continuously considered burden-free by default, no matter the system from which they arose, materials (by-products) from systems causing high environmental impact can be premiered for their environmental performance when compared to other resources (Olofsson and Borjesson, 2018). The problem could usually be solved throughout an allocation strategy of environmental impact between the principle products and by-products based on mass or economic or biophysical approach, but in many cases this approach is not possible for food by-products, for example they could not have an economic value or their mass is higher than the primary products (for example trub from beer production). Another possibility, suggested by many authors (Eriksson et al., 2015; Salemdeeb et al., 2017; Olofsson and Borjesson, 2018), is the substitution, where the by-products are considered to replace other comparable products. This solution was applied by Eriksson et al. (2015) where vegetable waste was assumed to replace oats (based on energy content) in feed pig.

The aim of the study was to exploit three inedible human by-products as growing substrates for BSF larvae. Okara, maize distiller and brewers' spent grains mixed with trub (later called brewer's grain) were tested as growing feed for BSF production at experimental scale. A commercial hen diet was used as a control. The study focused on: 1) growth performance of larvae; 2) production of methane and other gases by BSF larvae; 3) environmental burden, with a LCA approach, of larvae production grown on tested substrates, including also the environmental impact of by-products.

2. Materials and methods

2.1 Growth performance

Larvae used in the current study were provided from a BSF colony previously founded from wild specimens collected in Lombardy (Northern Italy) (45°19'54''N; 9°05'58''E) (Jucker et al., 2017).

The following rearing substrates were tested: okara, maize distiller, brewer's grains and hen diet. For each experimental diet, 1000 2-day-old larvae were placed in a plastic container (5 l) covered with a mesh netting and stored in climate chamber (T 25°±0.5°C, RH 60±0.5%, photoperiod 12:12 L:D). Substrates were tested in triplicates. Each feeding was provided *ad libitum*. In order to study larval growth performance, 10 larvae of each replicate were weighted every three days with an analytical balance (SartoriusCP64, Germany).

After weighing, larvae were replaced in their container. Each trial ended when 40% of the larvae in each container reached prepupal stage (indicated by the darker colour of the larvae). Thus, prepupae were removed daily from the container and counted. At the end of each trial, the number of alive prepupae was registered in order to know the larval survival.
To calculate the larval growth rate, the method suggested by Leong et al. (2016) was used:

- Larval growth rate (GR) = (final larval average weight-initial larval average weight)/number of days of the trial.

Conversion efficiency
To evaluate larval efficiency to consume and metabolize the growing substrates, the total final biomass (larvae+pupae) and the residual substrates were weighted. Waste reduction index (WRI) and the efficiency of conversion of the digested food (ECD) were calculated for the determination of the waste consumed by the larvae and the conversion efficiency of the substrates into valuable biomass. The following indexes, based on dry weight, were calculated, as in Leong et al. (2016):

- Waste reduction index (WRI) = [W-R/W]/days of trial (d) x 100

Where W = total amount of diet provided; R = residual of the diet

- Efficiency of conversion of the ingested food (ECD) = B/(W-R)

Where B = total larval+pupal biomass (g); W= total amount of diet provided; R = residual of the diet.

2.2 Substrate composition and larvae composition analysis

Larvae and substrate samples were freeze dried. After freeze-drying, the substrates were ground through a 1 mm screen (Pulverisette 19, Fritsch) whilst larvae were ground by pestle. Larvae were analysed for the concentrations of dry matter (DM) (method 945.15; AOAC International, 2005), ash (method 942.05; AOAC International, 2005), crude protein (CP) (method 984.13; AOAC International, 2005) and ether extract (EE) (method 920.29; AOAC International, 1995). Substrates were also analysed for neutral detergent fiber (NDF) with the addition of α-amylase according to Mertens, (2002) and using the Ankom 200 fiber apparatus (Ankom Technology Corp., Fairport, NY). Non fibrous carbohydrates (NFC) were calculated as follows:
NFC= 100-Ash-CP-EE-NDF.

2.3 Environmental impact evaluation

Goal and scope definition, functional unit selection
The environmental impact of *H. illucens* larvae production, on three different food by-products and on hen diet, was evaluated using a life cycle assessment method. Maize distiller grain, brewer's grains and okara were obtained directly from food industry while hen diet was purchased from the market. All of them were obtained directly from food industry while hen diet was purchased from market.
Since larvae can be used as feed for livestock animals, different functional units (FU) were used in order to underline the different aspects of feed utilization by animals for supporting livestock productions. The FUs used were 1 kg of larvae (fresh wet weight), 1 kg of protein content of larvae and 1 kg of fat content of

larvae, expressed as ether extract. This also allowed to compare larvae with other feed used in livestock sector.

System boundary

The system boundary (Figure 1) included the development of BSF from eggs to prepupae. Adults and eggs production were excluded from the study for their negligible impact (Salomone et al., 2017). Energy, water and substrates production were considered. Larvae manure obtained after separation of larvae from the rearing substrate was analysed for N, K and P composition. For N analysis method 984.13 (AOAC International, 2005) was applied. Total P and K contents were determined by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion (EPA, 1998) of the samples. Manure was considered as avoided fertilizer in the environmental impact calculation.

Inventory data collection

Primary data on insect growth, feed consumption and water consumption were obtained directly from the experimental rearing.

The emissions related to substrates were considered as follows:

- for hen diet, considering all the singles ingredients of the diet; the majority of ingredients came from European countries and were quantified using data from Ecoinvent V3 and Agri-footprint (Blonk Consultants, 2014) databases. Protein feeds, mainly soybean meal, originated from Brazil. Direct land use change (LUC) for soybean meal and soybean oil productions was considered in the assessment using the value reported by the Agri-footprint database (Soybean, at farm/BR Economic, Blonk Consultants, 2014);
- for maize distiller from ethanol, the value proposed by Ecoinvent V3 database was used with economic allocation (2.4%), it was assumed that it had been produced in Italy (50%) and Germany (50%);
- for brewer's grains mixed with trub and for okara zero environmental impact was assumed because they did not actually have economic value. In order to analyse the possible effects of different allocation choices on by-products environmental impacts assessment, a sensitivity analysis was performed, as explained below.

Methane emission and gas production

An *in vitro* method derived by evaluation of feed for ruminants using a semiautomatic pressure system (Theodorou et al., 1994) was adapted for this purpose. Twenty 4th instar BSF larvae with a weight between 80 and 100 mg were positioned in duplicate into serum bottles with the addition *ad libitum* of the experimental diets (substrates). For each sample two blanks (i.e. substrates without larvae) were added. Headspace pressure was recorded after 24 hours of incubation using a digital manometer (model 840082, Sper Scientific, Scottsdale, AZ, USA). The gas pressure data recorded after 24 h were converted to moles of gas using the ideal gas law ($n=p*(V/R*T)$, where: n: gas produced (mol), p: pressure (kPa), V: headspace volume in bottles (L), R: gas constant (8.314 $L*kPa*K^{-1}*mol^{-1}$, T: temperature (K)), converted to millilitres of gas. A fixed-volume sample of gas was also collected for subsequent methane analysis using gas-tight syringes fitted with needles through the bottle top. The gas composition of the headspace was determined by micro GC gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). An external standard mixture of CO_2 and CH_4 was used for instrument calibration.

Impact Assessment

Within the life cycle impact assessment, SimaPro 8 software (Pre Consultant, 2014) was used to estimate the environmental impact of the tested systems.

The following impact categories were considered for evaluation: Climate change, Ozone depletion, Human toxicity, Cancer effects Human toxicity, Non cancer effects, Particulate matter, Ionizing radiation HH, Ionizing radiation E (interim), Photochemical ozone formation, Acidification, Terrestrial eutrophication, Freshwater eutrophication, Marine eutrophication, Freshwater ecotoxicity, Land use, Water resource depletion, Mineral and fossil renewable resource depletion. The characterization factors considered were those from ILCD 2011 Midpoint V1.03.

Sensitivity analysis

Sensitivity analysis was performed to investigate the variability of environmental impacts of larvae production due to the variation of the environmental impact associated to two by-products substrates (brewer' grains and okara) that does not have an environmental load evaluation in the main databases. Two approaches were used:

1) the substitution of the whole amount of each by-product with soybean meal, which is the main protein feed for animal production;
2) the substitution of the whole amount of each by-product with sunflower meal, which is a protein feed source produced in the European area.

Statistical analysis

Statistical analyses were performed by SPSS® Statistic (Version 24 for Windows, SPSS Inc Chicago, IL, USA). Data recorded on time of larval development, final larval weight, larval survival and conversion efficiency of BSF on the four substrates were compared by one-way analysis of variance (ANOVA). Tukey-Kramer's Honestly Significant Difference (HSD) multiple comparisons test was applied for mean separation ($P<0.05$) between tested diets, where significant differences occurred.

3. Results and discussion

3.1 Substrate chemical composition

Chemical composition of experimental substrates is in table 1. Substrates were characterized by a different composition in terms of ash with hen diet, having the highest value (13.5% on DM) as compared to other treatments (on average 4.55% on DM). A wide variability for CP and EE concentrations was also observed. Particularly, okara was characterized by the highest values of CP (39.2 % on DM) and EE (17.2% on DM) as compared to the other substrates, hen and brewer's grains were characterized by the lowest concentrations of CP and EE. Brewer's grains substrate was characterized also by the highest fiber content. Tschirner and Simon (2015) showed that the nutrient composition of growing substrates has a great influence on critical production factors like total larvae yield and individual larvae body weight with the best production results achieved with a mixture of middlings (CP 22% on DM) whereas the use of dry sugar beet pulp (high in fiber, NDF: 15.6% on DM of crude fiber) and dried distiller grains (high in protein: 31.2% on DM) as substrates led to significantly more unfavourable results. However, as shown by Meneguz et al. (2018), BSF larvae are able to bioconvert wastes and by-products characterized by high fiber content. This is probably due to the presence in the intestine of bacteria able to degrade cellulose. This is very important because by-products characterized by high fiber content could be positively converted in a high protein sources which could be used for animal feeding.

Table 1. Ash, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and non-fibrous carbohydrates (NFC) concentrations (% on DM) of the experimental substrates

	Ash	CP	EE	NDF	NFC
Hen diet	13.5	17.0	4.00	15.7	49.8
Okara	4.13	39.2	17.2	32.0	7.47
Maize distillers	5.40	29.5	11.1	36.7	17.3
Brewer's grains	4.13	15.8	2.89	53.6	11.2

3.2 Performance of larval growth and bio-reduction of the substrates

In table 2 the influence of the rearing substrates on the larval development, survival and conversion efficiency are reported. All the substrates tested allowed the BSF larval growth and development, but different feeding diets influenced the final weight reached by larvae. Larvae fed on hen diet and maize distiller exhibited the higher final weight (2.29±0.20 and 1.97±0.14, respectively), and were statistically different from okara and brewer's grain (F=21.85; dF= 3, 8; P<0.05). Moreover, larvae grew on maize distiller and hen diet required less days to reach prepupal stage (16±0.58 and 15±0.58 days, respectively). Only larvae on brewer's grains significantly differed from the others requiring more days to reach prepupal instar (22±0.58 days) (F=29.00; dF=3, 8; P<0.05). The growth rate index was calculated on larvae fed with the four diets. The weight gained per day (gd^{-1}) was highest when larvae were fed with maize distiller (0.0056±0.0001 gd^{-1}), followed by larvae on the control diet. Larvae grown on okara and brewer's grains showed a smaller weight increment, significantly lower than the larvae on others two substrates.

Larval mortality was not affected by the rearing substrates (F=4.005; dF= 3, 8; P>0.05). All by-products in fact allowed a high survival with a total mean of 91.2±4.11%. Only maize distiller showed a higher larval mortality of 17%, but no significant differences were observed. In general, larval mortality was very low and in the range reported by several authors on a wide variety of rearing substrates (Oonincx et al., 2015; Jucker et al., 2017; Chia et al., 2018).

In order to acquire data on the BSF larval capability to reduce the waste and their efficiency to convert in biomass, the WRI and ECD indexes were calculated. Statistical differences were observed for both indexes on larvae grown on the different rearing substrates (WRI: F=37.32; df=3, 8; P<0.05; ECD: F=7.76; df=3, 8; P<0.05). As shown in table 2, WRI indexes ranged from a minimum of 3.01± 0.06 in brewer's grain to a maximum of 4.90±0.07 in okara. Larvae reared on maize distiller and brewer's grain were statistically different from okara and hen diet, with a lower capability to reduce the substrates. Considering the ECD index, only larvae grown on okara significantly differed from the others, showing a higher index (0.36±0.02) thus corresponding to a major attitude to convert the digested food.

The rearing substrates significantly impacted the final larval weight and developmental time, as reporter in other papers. Results obtained in this study was comparable to those reported in previous research, also if few information is available on the three tested by-products. In particular, Meneguz et al. (2018) and Chia et al. (2018) reared BSF larvae on brewery by-products obtaining similar results in terms of larval mortality, length of larval development and bioreduction. Also results obtained by ur Rehman et al. (2017) on okara, were similar to those obtained in this paper.

Egert et al. (2003) demonstrated that CH_4 production from insects derives from bacterial fermentation by methanobacteriaceae in the hindgut. The results of the present study show that BSF larvae did not produce any detectable trace of CH_4 similarly to the results of Perednia et al. (2017). On the other hand, Oonincx et al. (2015) showed that for insects only representatives of cockroaches, termites, and scarab beetles produce CH_4 in agreement with Hackstein and Stumm (1994).

Table 2. Performance of larval growth and bioreduction of the substrates.

Substrate	Larval weight (g) (n=10) (fresh weight)	Larval survival (%)	WRI	ECD	GR
Hen diet	2.29±0.20 b	97.53±1.86	4.46±0.36 b	0.27±0.02 a	0.0051±0.0007 b
Maize Distillers	1.97±0.14 b	73.00±11.92	3.22±0.21 a	0.27±0.02 a	0.0056±0.0001 b
Okara	1.38±0.06 a	98.5±0.84	4.90±0.07 b	0.36±0.02 b	0.0021±0.0.000 a
Brewer's grain	0.98±0.01 a	95.87±1.51	3.01±0.06 a	0.25±0.01 a	0.0014±0.0000 a

WRI = Waste reduction index; ECD = Efficiency of conversion of the ingested food; GR= growth rate index (g d^{-1})

3.3 Larvae chemical composition

Larvae chemical composition in terms of ash, CP and EE is reported in table 3. The average concentrations of ash, CP and EE were: 7.50, 52.9 and 27.4% on DM. The potential use of BSF larvae in animal feeding is mainly due to the high CP content which makes them a possible alternative to soybean meal. In this study, the CP content of larvae was higher than that reported by Sánchez-Muros et al. (2014); however, as reported in recent review (Wang and Shelomi, 2017), the protein and fat compositions of BSF depend on the diet. For example, Oonincx et al. (2015) showed that CP content of BSF larvae was higher when they were reared on a high protein and fat diet than to low protein diets. All the diets tested in the present study were characterized by a considerable high CP contents (from 15.8 to 39.2% on DM), which in turn can have positively affected the CP content of larvae. There were also some differences in ash and EE concentrations of larvae which were affected by rearing substrate. Particularly, ash content was higher for larvae grown on hen diet (11.7%) as compared to other treatments (on average 6.09% on DM). On the other hand, ether extract was higher for larvae grown on okara and maize distillers as compared to hen and brewer's grains diets. This agrees with the results of Spranghers et al. (2017) which showed that EE and ash contents of BSF were significantly affected by the rearing substrate; in according with our results, larvae reared on energy dense substrates turn into prepupae with a high fat content.

Table 3. Ash, crude protein (CP) and ether extract (EE) concentrations (% on DM) of the larvae grown on the experimental substrates.

	Ash	CP	EE
Hen diet	11.7	52.8	25.1
Okara	5.91	51.2	31.2
Maize distillers	4.94	53.4	29.9
Brewer’s grains	7.41	54.1	23.2

Environmental impacts

Environmental impact evaluation of the production of 1 kg of larvae is showed in table 4. In these results for okara and brewer’s grains none environmental impacts were attribute to their productions, this is the main reason of the very low impact categories values of these two larval production systems.

Larvae production on hen diet resulted the most impactful for the most of environmental categories, for example for climate change, acidification, eutrophication (terrestrial and marine), and land use. The main gas contributor to climate change of larvae production using hen diet was CO_2 due to land transformation (53%), caused by the inclusion of soybean meal in the diet, the second one (28%) was CO_2 used as input for the crop production (e.g. maize) or for feed processing (drying and milling).

Though hen diet production system resulted impactful, comparing the climate change due to this production of larvae with the production of 1 kg of soybean seed (CP 39.6% and EE 21.4%), the insect production on hen diet, resulted slightly lower than soybean seed production. As reported by Agri-footprint database, climate change due to the production of 1 kg of soybean seed (whole or roasted) in Brazil can be between 4 and 4.7 CO_2 eq for each kg of product.

As suggested by van Huis and Oonincx (2017), high feed conversion efficiency of the insects is one of the main reasons why insects are considered as potentially sustainable sources of animal protein. Feed conversion efficiency is also the main driver that influence the environmental sustainability of animal production. For example, some authors (Cesari et al., 2017; Cesari et al., 2018; Bava et al., 2017) showed a close relation between feed conversion and environmental impact in monogastric meat production. However, high efficiency requires optimal diets and therefore knowledge of the nutritional requirements of insect species needs to be established. Moreover, a high feed conversion determined a high insect yield, but, as underlined by Smetana et al., (2016), a higher efficiency in term of insect yield (and protein content) was achieved via the use of feed with good nutritional quality (e.g. rye meal, soybean meal), but in this case insect production was associated with high environmental impacts.

Table 4. Environmental impact of larvae grown on different substrates (kg of larvae fresh weight)

Impact category		Hen diet	Maize Distillers	Okara	Brewer's grains
Climate change	kg CO2 eq	3.704	1.882	0.197	0.228
Ozone depletion	g CFC-11 eq	1.33E-07	3.16E-07	2.99E-08	4.32E-08
Human toxicity, cancer effects	CTUh	8.06E-08	5.92E-08	6.96E-09	1.01E-08
Human toxicity, non-cancer effects	CTUh	4.54E-06	2.71E-07	2.13E-08	3.08E-08
Particulate matter	kg PM2.5 eq	1.01E-03	3.16E-04	9.74E-05	1.31E-04
Ionizing radiation HH	kBq U235 eq	0.102	0.304	0.006	0.009
Ionizing radiation E (interim)	CTUe	3.28E-07	9.41E-07	1.97E-08	2.85E-08
Photochemical ozone formation	kg NMVOC eq	0.0061	0.0030	0.0005	0.0006
Acidification	molc H+ eq	0.0325	0.0036	0.0011	0.0012
Terrestrial eutrophication	molc N eq	0.1377	0.0076	0.0005	-0.0005
Freshwater eutrophication	kg P eq	2.60E-04	4.30E-04	6.30E-05	9.10E-05
Marine eutrophication	kg N eq	2.51E-02	2.23E-03	1.50E-04	1.60E-04
Freshwater ecotoxicity	CTUe	8.393	4.430	0.562	0.812
Land use	kg C deficit	62.76	3.303	0.374	0.540
Water resource depletion	m3 water eq	0.587	0.662	0.248	0.358
Mineral, fossil & ren resource depletion	kg Sb eq	1.76E-05	6.04E-06	-6.14E-07	-1.40E-06

Figure 2 shows the activities that contributed to environmental impact of larvae production, only for hen diet and maize distiller systems, because in these systems an environmental weight has been assigned to the substrate used. For both systems, feed production activities resulted the main contribute to the environmental impact (from 60 to 99% of the impact depending of the different impact categories and substrates), as reported by other authors (Salomone et al., 2017; van Huis and Oonincx, 2017). Because of the high impact of feed production, becomes urgent to assign an environmental load to the substrate production, even when it is a by-product; this subject is faced in the following paragraph using sensitive analyses.

Considering that all over the world larvae production is now studied as alternative feed or/and food, it is useful to evaluate their environmental impact using as functional unit protein and fat content. Figure 3 compares climate change generate for the production of 1 kg of protein deriving from insect larvae, grown on the experimental substrates, and from two vegetable feed (sunflower and soybean meal). Sunflower and soybean meal are two by-products largely used as feed in animal production systems (both monogastrics and ruminants) due to their high crude protein content; their environmental impact is allocated between oil and meal, usually following an economic allocation.

Most of the soybean meal used in Europe (about 80%) comes from South America, for this reason the recent international opinion is to assign a significant part of the CO_2 emissions due to Land Use Change (LUC), for this reason, and for the transoceanic transport, the climate change associated to soybean meal production is higher than the one assigned to sunflower meal. Sunflower meal can be considered a local sources of protein (38.5% protein on dry matter). The lowest Climate change for the production of 1 kg of protein was achieved using sunflower meal.

Looking at the climate change generated for the production of 1 kg of lipid (figure 4), results suggest that the production of 1 kg of lipid is generally more impactful than the production of 1 kg of protein, mainly because often feed used for animal diets are by-products and the main quantity of fat goes in the main product, the vegetable oil, used for human nutrition or biodiesel (environmental data for vegetable oil mix and rapeseed meal were obtained from Ecoinvent database). Furthermore, as previously described, substrates were characterized by a different EE content (hen diet and brewer’s grain had the lower values) which affect the EE content of larvae, especially of larvae reared on hen diet and on brewer’s grain.

In the present study climate change and marine eutrophication (per kg of lipid) resulted higher using insects (grown on hen diet) than using rapeseed meal (0.23 vs 0.25 kg N eq for BSF on hen diet and rapeseed meal, respectively). Other authors (Salomone et al., 2017) found that, when compared with an alternative source of protein such as soymeal or alternative source of lipids such as rapeseed, the BSF system entails a higher climate change and a higher eutrophication. On the contrary, significant benefits were found connected to acidification, land use and water resource depletion.

3.4 Sensitive analyses

The allocation choices for by-products have important effects on their environmental load and on the ‘secondary’ systems associated to them, as insect’s production.

In the present study, two substituting approaches were used in order to assign an environmental load to the by-products used: okara and brewer’s grains. Substituting the quantity of substrates used in each system with a quantity of other protein sources, on the bases of the protein content, is indicated by Olofsson and Borjesson, (2018). So an alternative environmental loads to the BSF larvae production grown on the two by-products were assigned. In this study two protein feed were used: soybean meal and sunflower meal.

As shown in figure 5, climate change and water resource depletion resulted higher when soybean meal was used as substrate substitution, this is due to LUC load for climate change category and to the high water requirement compared to sunflower crop production. For marine eutrophication and acidification categories, sunflower meal substitution resulted more impactful, due to ammonia and nitrate emission during crop production.

Moreover, the results obtained from the sensitive analysis showed that environmental impact and in particular climate change of larvae grown on okara and brewer’s grain (as substitution of soybean meal) were higher than one of larvae grown on hen diet and maize distillers. Similar or lower results were obtained for BSF on the two by-products (in each substitution) for marine eutrophication and water resource depletion.

The sensitive analyses applied make possible the comparison among different larvae production, in this way the best sustainable product resulted the larvae grown on maize distiller which showed the lowest value for the most impact categories considered.

4. Conclusions

The use of by-products for insect production seems to be promising as all substrates allowed the larval growth resulting in larvae characterized by a high CP content. It should be underlined that all the substrates used were characterized by a high quality in terms of CP and EE content (particularly for okara and maize distillers) which allowed a high conversion efficiency. The composition of substrate affected the quality of larvae, demanding further study to evaluate the diets requirements of BSF also in relationship to the quality of products.

Maize distiller seems the most promising substrate in term of sustainability as the impact was expressed of quantity of larvae, if an environmental contribution based to protein content was applied okara and brewer's grains production.

Considering that the substrate production gives an important contribution to the environmental impact of larvae production, it is necessary to deepen the knowledge and methods to attribute an environmental weight to by-products. Only considering this crucial point is possible to better evaluate the environmental benefit of producing insects as novel feed or/and food.

Authors contributions

All authors contributed equally to the study and to the compilation of the paper.

Acknowledgements

This work was supported by the grants of the University of Milan Project “PROINSECT - Proteine da insetti per l’alimentazione animale: allevamento, valore nutritivo e sostenibilità ambientale” and “PROENTO - Insects: an alternative source of proteins”.

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4. General conclusion

Results from these studies show that it is important to adopt a holistic approach for the assessment of GHG emissions from milk production. Any strategy aiming to mitigate, for example, CH_4 emission of dairy cows, therefore, must also take into account the possible effect on the other greenhouse gases, as well as the effect on C sequestration. What stands out from the thesis is also that it could be interesting to evaluate novel feed as a new and useful solution for mitigation of GHG emissions related to milk production.

The present thesis took into account dairy-forage systems characterized by different levels of intensification, in terms of agricultural intensities and cow performances. More intensive systems involved lower GHG emissions, confirming that milk production per cow is negatively related to the impact per unit of product. These intensive systems also resulted to be more sustainable in terms of feed self-sufficiency. However, there are still grounds for strengthening the environmental sustainability of these forage systems, as they showed a low organic C soil density. Therefore, further investigations on how to consider environmental sustainability over a wider spectrum are still needed.

The study also showed that, under controlled conditions, no differences in terms of enteric CH_4 production per unit of product occur among cows fed diets based on forage systems with different levels of intensification. However, if it is true that more extensive forage systems could lead to mitigate environmental impact of milk production, through C soil sequestration, animals fed diets coming from extensive forage systems produce higher amount of daily CH_4 (g/d) from enteric fermentations. In addition, diets related to extensive fodder systems could involve greater N_2O emission from manure, due to higher N manure excretion, compared to the more intensive ones.

Results of the survey in the Northern Italy revealed that the most popular ingredients included in the diets for lactating cows in the Po plain area are corn silage, corn grain meal and soybean meal. However, the study showed that the use of these feed in the ration formulation needs particular care. An excessive inclusion in the diet of corn silage or corn grain meal is not related to higher milk yield and has a negative effect on feed efficiency, while a high inclusion of SBM does not improve milk production, but increases the GWP of the TMR. On the other hand, it is important to consider that a high inclusion of roughages in the diet determined a high enteric methane production that contributes to increase the GWP of the diets. The variability in the total mixed ration GWP (expressed per unit of milk) suggests that is possible to reduce GWP of the diet and, consequently, of milk production, through a correct choice of the ingredients and their dietary proportion.

Looking forward, in order to reduce SBM purchased as protein source in the cow diet, waste production, and competition between animals and human for crops, trials on growing of *Hermetia Illucens* were conducted on different by-products, even if, according to the European legislation, today the use of insects as feed source is not yet possible in ruminants. The study revealed that okara and brewer's grains seem the most promising substrates in terms of Global Warming Potential. Considering that the substrate production gives an important contribution to the environmental impact of larvae production, it is necessary to deepen the knowledge and methods to attribute an environmental weight to by-products. Only considering this crucial point is possible to better evaluate the environmental benefit of producing insects as novel feed.

In conclusion the thesis shows up essential differences among forage systems and among feed ingredients of cows' diet, confirming that there is room for improvement in sustainability of milk production. These are issues which should be taken into consideration by farmers, technicians and policy makers, considering that sustainability of livestock production will be one of the priorities for humankind in next future.

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