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

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ABSTRACT

Eight lactating Italian Friesian cows were housed in individual respiration chambers in a repeated Latin square design to determine their dry matter intake (DMI) and their milk and methane production, as well as to collect the total feces and urine to determine the N and energy balances. Four diets, based on the following forages (% of dry matter, DM), were tested: corn silage (CS, 49.3), alfalfa silage (AS, 26.8), wheat silage (WS, 20.0), and a typical hay-based Parmigiano Reggiano cheese production diet (PR, 25.3 of both alfalfa and Italian ryegrass hay). The greatest DMI was observed for cows fed PR (23.4 vs. 20.7 kg/d, the average of the other 3 diets). The DM digestibility was lower for PR (64.5 vs. 71.7%, the average of the other diets). The highest ash-free neutral detergent fiber digestibility values were obtained for CS (50.7%) and AS (47.4%). In the present study, no differences in milk production were observed between diets, although PR showed a higher milk yield trend. The highest milk urea N concentration (mg/dL) was found for the cows fed the WS diet (13.8), and the lowest was observed for the cows fed AS (9.24). The highest milk urea N concentration for the cows fed WS was also correlated with the highest urinary N excretion (g/d), which was found for the cows fed that same diet (189 vs. 147 on average for the other diets). The protein digestibility was higher for the cows fed the CS and WS diets (on average 68.5%) than for the cows fed AS and PR (on average 57.0%); dietary soybean inclusion was higher for CS and WS than for AS and PR. The rumen fermentation pattern was affected by the diet; the cows fed the PR diet showed a higher rumen pH and decreased propionate production than those fed CS, due to the lower nonfiber carbohydrate content and higher ash-free neutral detergent fiber content of the PR diet than the CS diet. Feeding cows with PR diet increased the acetate:propionate ratio in comparison with the CS diet (3.30 vs. 2.44 for PR and CS, respectively). Cows fed the PR diet produced a greater daily amount of methane and had a greater methane energy loss (% of digestible energy intake) than those fed the CS diet (413 vs. 378 g/d and 8.67 vs. 7.70%), but no differences were observed when methane was expressed as grams per kilogram of DMI or grams per kilogram of milk. The PR diet resulted in a smaller net energy for lactation content than the CS diet (1.36 vs. 1.70 Mcal/kg of DM for the PR and CS diets, respectively). Overall, our research suggests that a satisfactory milk production can be attained by including different high-quality forages in balanced diets without any negative effect on milk production or on the methane emissions per kilogram of milk.
Key words: forage system, milk production, digestibility, methane, energy balance

INTRODUCTION

The concentrations of greenhouse gases (**GHG**) in the atmosphere have increased over the years and the rate of increase over the past century is unprecedented (Prentice et al., 2001). The livestock sector is responsible for about 14.5% of human-induced GHG emissions, with enteric methane being the single largest source (McAllister and Newbold, 2008; Gerber et al., 2013). The type and amount of forage used in ruminant diets have direct effects on enteric methane production, and mitigation strategies can be achieved by altering the rumen fermentation pattern (Benchaar et al., 2001). Forage maturity at harvest and the forage preservation method can be used to manipulate methane production in ruminants. The inclusion of early cut forages in a diet reduces methane production and improves OM and NDF digestibility compared with more mature forages (Brask et al., 2013). Furthermore, methane production was found to be lower for alfalfa used as silage rather than as hay (Benchaar et al., 2001). The production of methane could also be depressed by the use of legumes

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(e.g., alfalfa) instead of grass, due to the difference in their chemical composition (Benchaar et al., 2001). The on-farm production of forages and feeds may contribute to the mitigation of emissions from the dairy sector. For example, the dietary inclusion of high protein forages (e.g., alfalfa) can lead to a reduction of soybean meal (**SBM**) in the diet, a feed that involves a great environmental impact, mostly related to the change in land use. The intensity of agricultural practices may have a direct effect on emissions linked to feed production (Tabacco et al., 2018; Zucali et al., 2018), whereas the carbon sequestration potential of forage systems can indirectly mitigate the GHG emissions of the livestock sector (Soussana et al., 2010). Farming systems, based on permanent meadows or multiannual rotational grass and legume forages, might represent a significant GHG mitigation strategy, as they increase soil C sequestration in the OM of the soil (Stanley et al., 2018). On the other hand, annual crops, which require several external inputs and soil management practices for their growth (e.g., agrochemicals, synthetic nitrogen fertilizers, frequent plowing), reduce the soil C sequestration potential and may increase the consumption of direct and indirect energy (Soussana et al., 2010).

Water availability and the destination of milk for protected designation of origin (**PDO**) cheeses are the main drivers of the organization of forage systems in the Po Plain (Mantovi et al., 2015). The availability of water and the high soil fertility in the north of the Po River have historically favored the cultivation of corn (whole-plant silage and dry grain), which is recognized to produce a high DM yield per hectare and to be more suitable for an easy conservation by ensiling (Borreani et al., 2013; Gislon et al., 2020b) than permanent meadows and legume forage crops. An emerging forage system called dynamic forage system (Tabacco et al., 2018) is replacing the conventional system based on monocropped corn silage by reintroducing the use of legume forages and producing whole-ear silage, thereby producing high-quality forages and increasing farm protein self-sufficiency. South of the Po River, an area that is characterized by water scarcity, corn cultivation is less productive and has been replaced by winter cereals and multiannual legume crops, such as alfalfa, which are conserved as hay in the Parmigiano Reggiano PDO production area. Overall, the main target of dairy systems should be focused on feeding better quality diets to increase feed efficiency and lower the environmental impacts per kilogram of milk, namely methane emissions and N excretion, the sources of ammonia and nitrous oxide releases.

As different forage systems can have different environmental effects and implications on soil C sequestration, on the inclusion of SBM in the diet and on fiber digestibility and enteric methane production, the aim of the present study has been to provide data on the performance of animals fed diets characterized by different forages produced on commercial farms in Northern Italy. The hypothesis of this experiment is that including different high-quality forages in balanced diets with low soybean meal levels can lead to milk production similar to that achievable with conventional diets based on corn silage, without increasing the methane emission and N excretion per unit of product.

MATERIALS AND METHODS

The study was conducted at the Università degli Studi di Milano "Cascina Baciocca" Research Center at Cornaredo (Milan, Italy). All of the animal procedures were conducted with 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) under authorization 980/2017.

Cows, Experimental Design, and Methane Determination

Eight multiparous lactating Italian Friesian cows were used in a replicated 4×4 Latin square design. Each experimental period lasted 28 d: 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 a milk yield of 38.7 kg/d (SD: ±3.61).

The cows were fed the experimental TMR ad libitum twice daily. The animals had free access to drinking water. Orts were recorded daily, and the feeding rate was adjusted to obtain at least 5% of the supplied amount as orts (on an as-fed basis). During the adaptation periods, the animals were housed in individual tiestalls, which were equipped with rubber mattresses and bedded with straw. Each cow was weighed at the beginning and at the end of each experimental period. The cows spent the last 7 d of each experimental period in respiration chambers: the first 2 d to adapt to the chambers and the last 5 d for the sample collection. Four individual open-circuit respiration chambers were used to enable the measurement of CH_4, CO_2 emissions, and O_2 consumption. The chambers measured 3.6 m (length) × 2.4 m (width) × 2.3 m (height), and each contained a small pre-chamber for the personnel entrance, and wide glass walls to allow the cows to see each other and outside. Each respiration chamber was equipped with a feeder and contained a 2.5 m × 1.5 m stanchion that allowed the animal to stand or lie down. The air temperature in the chambers was maintained at 18 ± 1°C and a low negative pressure was maintained inside the chambers to prevent CH_4 losses produced by the cows. The 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). The air flux was on average maintained at 35 ± 1 m^3/h. The daily O_2 consumption and the CO_2 and CH_4 production were determined by measuring the volume of air circulating in the system in 24 h (and by referring to the standard temperature and pressure conditions) and multiplying this volume by the difference between the relative concentrations of the gases measured continuously in the ingoing and the outgoing air. The CH_4 and CO_2 concentrations were measured using an URAS 4 analyzer (Hartmann and Braun AG, Frankfurt am Main, Germany). The oxygen concentration was measured using a Magnos 6G analyzer (Hartmann and Braun AG). The gas concentrations were measured every 575 s, considering 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 were applied to account for the entrance of personnel.

The total heat production was determined using the Brouwer equation (Brouwer, 1965): heat production (kcal/d) = $3.866O_2 + 1.200CO_2 - 1.431N - 0.518CH_4$, where gas volumes (L/d) are expressed at standard conditions and N (g/d) is the urinary N. The ME requirement necessary for maintenance was assumed to be 115 kcal/metabolic BW (Van Es, 1978).

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

The cows were milked twice daily (0730 and 1830 h), and the milk production was recorded at each milking. Milk samples from individual cows were taken at each milking during the sample collection period and 2-bromo-2-nitropropan-1,3-diol was added to the milk as a preservative. Feces, TMR, and ort samples were dried in a ventilation oven at 55°C until constant weight. After drying, the samples were ground to 1 mm using a Fritsch mill (Pulverisette 19, Fritsch GmbH, Idar-Oberstein, Germany). A fresh feces subsample was used for N analysis. The N balance was determined by considering the N volatilized in the chamber, which was measured from the N concentration of the water condensed by the air conditioning system. This process involved collecting the total volume of condensed water in plastic canisters containing 20% sulfuric acid (vol/vol), which were placed inside of the chambers. The water volume was weighed daily, sampled to obtain a composite sample, and stored at −20°C for the subsequent ammonia N ($N\text{-}NH_3$) analysis.

Ruminal fluid was collected from cows after they left the chambers at the end of each experimental period. Ruminal liquid was taken 5 h after the morning feeding and samples were taken using an esophageal probe. To avoid saliva contamination, the first collected rumen sample (with the possible presence of saliva) was discarded. Approximately 0.6 L of rumen fluid was strained through 4 layers of cheesecloth. The pH was measured immediately after sampling, and 1 aliquot was stored at −20°C for subsequent VFA analysis.

Diets

The experimental treatments were based on the typical forage systems that have been identified as the most representative of the Po Plain (Northern Italy; Gislon et al., 2020b). The 4 dietary treatments were as follows: (1) a corn silage-based system (**CS**), which was considered representative of the most widespread intensive forage system in the Po Plain; (2) a forage system based on double-cropped corn (harvested as whole-ear silage), alfalfa (harvested as silage at an early growth stage), and Italian ryegrass (harvested as silage at an early growth stage; **AS**); (3) a forage system based on double-cropped corn (harvested as whole-ear silage) and winter cereal (harvested as silage, wheat in the present experiment; **WS**); and (4) a representative forage system of Parmigiano Reggiano cheese production (PDO), based on dried forages from alfalfa and permanent meadows (**PR**). The diets were formulated using the CNCPS model (version 6.5, Cornell University, Ithaca, NY) to provide a similar MP and energy concentration. Wrapped bales of TMR, prepared on 3 different commercial farms, using fodders produced directly on each farm, were used for the 3 silage-based diets. The TMR bales were made using an MP 2,000 compactor (Orkel, Fannrem, Norway). The PR diet was provided, as small TMR bales, by a feed compounder of the Parmigiano Reggiano area. The chemical composition of the forages and the VFA, lactic acid, and alcohol contents of the silages are reported in Tables 1 and 2, respectively.

Table 1. Chemical composition of the main forages included in the 4 experimental diets[1]

	Chemical composition[2] (% of DM unless noted)							
Item	DM (%)	Ash	CP	EE	aNDFom	ADFom	ADL	NFC
CS								
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								
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								
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								
Alfalfa hay	90.0	10.0	18.0	2.50	58.7	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]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

[2]EE = ether extract; aNDFom = NDF assayed with a heat-stable amylase and expressed exclusive of residual ash; ADFom = ADF expressed exclusive of residual ash; ADL = lignin content determined by solubilization of cellulose with sulfuric acid; NFC = 100 − (ash + CP + EE + aNDFom).

Chemical Analyses

The feed ingredients, TMR, orts, and feces were analyzed for chemical composition. The 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 International, 1995; method 945.15). The ash content was determined by incineration at 550°C overnight in a muffle furnace (AOAC International, 1995; method 942.05). The CP (N × 6.25) was determined according to the 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 sulfite, and expressed exclusive of residual insoluble ash (**aNDFom**). Acid detergent fiber (**ADFom**) and ADL, determined according to the method of Van Soest et al. (1991), were expressed exclusive of residual insoluble ash; lignin was determined by solubilization of cellulose with sulfuric acid. The NDF and ADF procedures were adapted for use in an Ankom200 fiber analyzer (Ankom Technology Corp., Fairport, NY). The ether extract was determined according to AOAC International (1995) method 920.29. The gross energy of the TMR, orts, feces, urine, and milk was determined using an adiabatic calorimeter (IKA 6000; IKA Werke GmbH and Co. KG, Staufen, Germany). The concentration of N in the acidified urine, in the condensed water collected in the chamber, in the fresh feces, and in composite milk samples was determined according to the Dumas method, using MAX N exceed.

The milk fat and lactose concentrations were determined using a Fourier transform infrared analyzer (MilkoScan FT6000; Foss Analytical A/S, Hillerod, Denmark). The MUN concentration was determined

Table 2. The pH and fermentative profiles of the silages used in the experimental diets[1] of the experiment

		Content (g/kg of DM)					
Item	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ethanol	1,2 Propandiol
CS							
Corn silage	3.60	64.3	22.2	0.0	0.0	15.1	10.2
AS							
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							
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]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage.

using a differential pH technique (method 14637; ISO, 2006). The ECM (3.5% fat and 3.2% protein) was calculated according to Tyrrell and Reid (1965).

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

A silage sample was divided into 2 subsamples. The first subsample was extracted for pH determination 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 subsample was extracted using a Stomacher blender for 4 min in 0.05 *M* sulfuric acid (H_2SO_4) at a 5:1 acid-to-sample material (fresh weight) ratio. A 40-mL aliquot of silage acid extract was filtered with a 0.20-μm syringe filter and used to quantify the fermentation products. The lactic and monocarboxylic acids (acetic, propionic, and butyric acids) were determined by means of 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 in an Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA).

Statistical Analysis

Statistical analysis was performed using the Mixed procedure of SAS, version 9.2 (SAS Institute, 2001). The data were analyzed with the following model:

$$Y_{ij(k)m} = \mu + S_m + C_{im} + P_{jm} + T_{(k)} + e_{ijm},$$

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 meter, with m = 1, 2; C_{im} represents the random effect of cow i within square meter, with i = 1, . . ., 4; P_{jm} represents the fixed effect of period j, with j = 1, . . ., 4 within square meter; $T_{(k)}$ represents the fixed effect of treatment k, with k = 1, . . ., 4; and e_{ijm} represents the residual error. Estimates of the least squares means are reported. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$ for all of the statistical analyses.

RESULTS

Forage and Diet Composition

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

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

The pH and fermentative profiles of the silages included in the diets are reported in Table 2. The corn silage had the highest lactic acid content (64.3 g/kg of DM), followed by the Italian ryegrass silage (58.3), and wheat silage (49.3). Moderate concentrations of butyric acid were detected in the alfalfa silage of both the AS and WS diets (10.1 and 4.9 g/kg of DM, respectively).

The ingredients and chemical composition of the 4 experimental diets are shown in Table 3. The diets were formulated to allow the maximum inclusion of forages. Corn grain meal was present in a higher proportion in the PR diet (22.8% of diet DM) than in the other treatments (12.1%, on average) because no forages with a high starch content or high-moisture ear corn were used. High-moisture ear corn was used in the AS and WS diets. Soybean meal inclusion was higher for CS and WS than for AS and PR. The aNDFom concentration (% of DM) was higher for the PR diet (36.6) and lower for the AS diet (27.1), with intermediate values for CS and WS (32.9%, on average).

Intake and Digestibility

The apparent total-tract digestibility of the nutrients and DMI are reported in Table 4. The DMI (kg/d) was higher ($P = 0.008$) for cows fed the PR diet (23.4) than for the other diets (20.7, on average). The lowest DM digestibility was observed for the PR diet (64.5%) and the highest for the CS diet (73.3%), and AS and WS diets showed intermediate values (70.9%, on average; $P < 0.001$). The CS and AS diets had the highest values of OM digestibility (75.1 and 74.7%, respectively), WS had intermediate an intermediate value (72.0%), and the PR diet had the lowest value (67.1%; $P < 0.001$). Significant differences ($P < 0.001$) between treatments were observed for CP digestibility, with the highest values attained by the CS and WS diets (69.0 and 67.9%, respectively) and the lowest by the AS and PR diets (58.4 and 55.6%, respectively). The aNDFom digest-

Table 3. Composition and chemical analysis of the 4 experimental diets

Item	Diet[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 soybean meal, 48% CP	15.7	8.1	0	9.0
Solvent soybean 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[3] (% of DM unless noted)				
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
Predicted ME content (Mcal/kg of DM)	2.63	2.43	2.55	2.50

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

[2]Mineral and vitamin supplements composition for the 3 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, 1,558 and 641 mg of Zn, 691 and 160 mg of Cu, 1,105 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, 1,000 and 1,400 IU of vitamin E.

[3]EE = ether extract; aNDFom = NDF assayed with a heat-stable amylase and expressed exclusive of residual ash; ADFom = ADF expressed exclusive of residual ash; NFC = 100 − (ash + CP + EE + aNDFom).

ibility was higher for the CS and AS diets (50.7 and 47.4%, respectively); the aNDFom digestibility of the PR diet (38.5%) was similar to that of the WS diet (40.5%; $P < 0.001$).

Milk Production and Composition

The milk production and composition data are presented in Table 5. Production (milk and ECM, kg/d)

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

Item	Diet[1]				SEM	*P*-value
	CS	AS	WS	PR		
DMI (kg/d)	20.3^{b}	20.9^{b}	20.9^{b}	23.4^{a}	0.70	0.008
Digestibility[2] (%)						
DM	73.3^{a}	71.4^{b}	70.3^{b}	64.5^{c}	0.90	<0.001
OM	75.1^{a}	74.7^{a}	72.0^{b}	67.1^{c}	0.91	<0.001
CP	69.0^{a}	58.4^{b}	67.9^{a}	55.6^{b}	1.86	<0.001
aNDFom	50.7^{a}	47.4^{a}	40.5^{b}	38.5^{b}	2.23	<0.001
ADFom	36.8	29.2	29.2	31.8	2.76	0.114

[a–c]Means in the same row with different superscript letters differ ($P < 0.05$).

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

[2]aNDFom = NDF assayed with a heat-stable amylase and expressed exclusive of residual ash; ADFom = ADF expressed exclusive of residual ash.

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

Item	Diet[1]				SEM	*P*-value
	CS	AS	WS	PR		
Production (kg/d)						
Milk	27.0	27.3	28.2	29.3	0.92	0.057
ECM[2]	30.5	31.4	33.1	32.7	1.00	0.122
Composition (%)						
Fat	4.38	4.60	4.71	4.26	0.18	0.172
CP	3.58	3.52	3.56	3.53	0.09	0.349
Lactose	5.02	5.03	5.06	5.09	0.03	0.075
Yield (kg/d)						
Fat	1.16	1.24	1.31	1.24	0.05	0.174
CP	0.96	0.95	1.00	1.03	0.04	0.052
Lactose	1.36	1.37	1.43	1.49	0.04	0.051
LS[3]	2.09	2.67	2.03	3.03	0.64	0.496
MUN (mg/dL)	11.8[b]	9.24[c]	13.8[a]	11.5[b]	0.18	<0.001
Acetone (mmol/L)	0.016	0.020	0.019	0.004	0.01	0.053
BHB (mmol/L)	0.03	0.05	0.03	0.03	0.01	0.093
Feed efficiency						
Milk/DMI	1.33	1.31	1.35	1.25	0.026	0.163
ECM/DMI	1.50	1.51	1.58	1.40	0.039	0.119

[a–c]Means in the same row with different superscript letters differ ($P < 0.05$).

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

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

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

was not affected by the diet, nor were the milk composition and yield in terms of fat, CP, and lactose. However, there was a tendency ($P = 0.057$) for cows fed the PR diet to show a higher milk production than those fed the other diets. We also found a slightly higher tendency ($P = 0.075$, $P = 0.052$, and $P = 0.051$) in the lactose concentration, CP, and lactose yield for cows fed the PR diet than those fed other diets. Feed efficiency (milk/DMI and ECM/DMI) was not statistically affected by the diet. Significant differences ($P < 0.001$) between diets were observed for the MUN concentration (mg/dL), which was the highest for WS (13.8 mg/dL) and the lowest for AS (9.24 mg/dL; $P < 0.05$).

Ruminal Fermentation Characteristics

The ruminal fermentation characteristics are reported in Table 6. The mean ruminal pH value was affected by the diet. The ruminal pH was significantly lower ($P = 0.020$) for cows fed the CS diet (6.23) than those fed the PR diet (6.60), with AS (6.47) and WS (6.43) showing intermediate values.

The total VFA concentration (mmol/L) was not affected by the treatment; however, there was a tendency ($P = 0.096$) for the CS diet to show the highest total VFA concentration (131).

The proportions of VFA were affected by the treatment. The acetate percentage was the smallest ($P = 0.021$) for AS (57.5%) and the highest for PR (62.7%), with WS and CS showing intermediate values; the butyrate content was lower in CS (12.6%) than in AS and PR (16.4 and 15.1, respectively), and was intermediate for WS (14.1; $P = 0.002$); isobutyric acid was lower for PR and higher for AS and WS, and was intermediate for CS. Isovaleric acid was lower in PR than in the other diets. Feeding cows with the PR diet increased the acetate:propionate ratio in comparison with CS (3.30 vs. 2.44 for PR and CS, respectively; $P < 0.05$).

Enteric Methane Production

The dietary effects related to rumen methanogenesis are reported in Table 7. Methane production (g/d) was higher ($P = 0.047$) for the cows fed the PR diet (413) than those fed the CS diet (378). The dietary treatment did not affect methane emissions in terms of enteric emissions related to intake or milk production. On average, the cows showed a methane production of 18.6 g/kg of DMI and 14.5 g/kg of milk. Differences were detected for the methane emission as percent of digestible energy (**DE**) intake, which was the highest for PR (8.67), intermediate for AS and WS (8.15 and 8.17, respectively), and the lowest for CS (7.70, $P = 0.018$).

Nitrogen Balance

The results concerning the N balance are presented in Table 8. Nitrogen intake was significantly lower ($P = 0.012$) for the CS diet (490 g/d) than for the others (on average 540 g/d). Fecal excretion (DM, kg/d) was lower

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

Item	Diet[1] CS	AS	WS	PR	SEM	P-value
pH	6.23[b]	6.47[ab]	6.43[ab]	6.60[a]	0.08	0.020
Total VFA (mmol/L)	131	109	104	100	10.8	0.096
VFA (mol/100 mol)						
Acetate	58.9[ab]	57.5[b]	60.0[ab]	62.7[a]	1.16	0.021
Propionate	24.6	21.8	21.5	19.1	1.33	0.056
Butyrate	12.6[c]	16.4[a]	14.1[bc]	15.1[ab]	0.56	0.002
Isobutyric acid	0.75[ab]	0.88[a]	0.94[a]	0.68[b]	0.05	0.011
n-Valeric acid	1.54	1.90	1.64	1.48	0.14	0.117
Isovaleric acid	1.64[a]	1.54[a]	1.82[a]	0.79[b]	0.16	<0.001
Acetate:propionate ratio	2.44[b]	2.71[ab]	2.83[ab]	3.30[a]	0.19	0.032

[a–c]Means in the same row with different superscript letters differ ($P < 0.05$).

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

for CS, but not different from AS ($P < 0.001$). Urinary excretion (kg/d) was higher for AS than for CS (25.0 and 22.8, respectively; $P = 0.046$).

Fecal N excretion (g/d) was higher ($P < 0.001$) for both the PR and AS diets (241 and 223, respectively), intermediate for WS (177), and lower for CS (152). Cows fed the WS treatment produced the largest amount of urinary N excretion (189 g/d, $P < 0.001$). Urinary N excretion, as a percentage of N intake, was lower for AS (24.9) than for CS (31.0) and WS (35.6, $P = 0.06$). The lowest manure N excretion value (g/d) was observed ($P < 0.001$) for the cows fed the CS diet (304); the PR diet had the largest value (397), but was not statistically different from WS (369). Dietary N utilization for milk protein synthesis (milk N excretion, % of N intake) differed between the CS (30.7) and AS (28.0) diets ($P = 0.026$). The N balance (N retained, % of N intake) for the cows fed the PR diet (−3.32) was lower than for the CS or AS diets ($P = 0.018$), but was not different from the WS diet (3.17).

Energy Balance

The results concerning the energy balance are presented in Table 9. The energy intake (Mcal/d) was greater ($P = 0.020$) for the cows fed PR (98.3) than for those fed CS and AS (88.1 and 88.3, respectively). Digestible energy (% of gross energy intake, **GEI**) was significantly lower ($P < 0.001$) for PR (64.7) than for the other diets; CS and AS showed the highest values (73.6 and 72.6, respectively) and WS was intermediate (70.9). The PR diet resulted in a greater ($P = 0.047$) methane energy loss (5.45 Mcal/d) than the CS diet (5.00 Mcal/d), but there were no differences when methane production was expressed as a percentage of the GEI. The ME (as a % of the GEI) was lower for the

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

Composition of methane	Diet[1] CS	AS	WS	PR	SEM	P-value
g/d	378[b]	396[ab]	396[ab]	413[a]	10.4	0.047
g/kg of DMI	18.6	19.0	19.0	17.8	0.59	0.547
g/kg of OM digested	26.8	28.3	28.7	28.9	0.74	0.126
% GE intake[2]	5.67	5.92	5.78	5.59	0.19	0.543
% DE intake[3]	7.70[b]	8.15[ab]	8.17[ab]	8.67[a]	0.21	0.018
g/kg of milk	14.4	14.8	14.4	14.2	0.56	0.582
g/kg of ECM[4]	12.5	12.7	12.1	12.7	0.37	0.389
g/kg of milk fat[4]	326	323	305	335	9.69	0.187
g/kg of milk protein[4]	400	421	404	403	20.8	0.505

[a,b]Means in the same row with different superscript letters differ ($P < 0.05$).

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

[2]GE = gross energy.

[3]DE = digestible energy.

[4]Milk yields, ECM, milk fat, and milk protein were measured over the collection period.

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

	Diet[1]					
Item	CS	AS	WS	PR	SEM	*P*-value
N intake (g/d)	490[b]	533[a]	542[a]	546[a]	17.4	0.012
Fecal excretion						
DM (kg/d)	5.44[c]	6.00[bc]	6.24[b]	8.23[a]	0.361	<0.001
Total N (g/d)	152[c]	223[a]	177[b]	241[a]	12.1	<0.001
Total N (% of N intake)	31.0[b]	41.6[a]	32.1[b]	44.4[a]	1.86	<0.001
Urinary excretion						
Urine (kg/d)	22.8[b]	25.0[a]	23.4[ab]	24.7[ab]	0.813	0.046
Total N (g/d)	152[b]	133[b]	189[a]	156[b]	9.43	<0.001
Total N (% of N intake)	31.0[a]	24.9[b]	35.6[a]	29.2[ab]	2.36	0.006
Manure excretion						
Total N (g/d)	304[c]	355[b]	369[ab]	397[a]	7.91	<0.001
Total N (% of N intake)	62.0[b]	66.6[ab]	67.7[ab]	73.7[a]	1.83	0.006
Milk excretion						
Total N (g/d)	150	149	156	162	5.55	0.052
Total N (% of N intake)	30.7[a]	28.0[b]	28.9[ab]	29.6[ab]	1.04	0.026
N balance						
N retained (g/d)	36.6[a]	28.6[a]	17.7[ab]	−13.8[b]	13.2	0.023
N retained (% of N intake)	7.23[a]	5.46[a]	3.17[ab]	−3.32[b]	2.47	0.018

[a–c]Means in the same row with different superscript letters differ ($P < 0.05$).

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

PR diet (56.7%, $P < 0.001$) than for the other diets. As far as the NE_L (Mcal/DM) energy content is concerned, the PR treatment was characterized by the lowest value (1.36, $P < 0.001$) and CS by the highest (1.70), but the latter was not significantly different from AS (1.57); WS (1.53) was different from both PR and CS.

DISCUSSION

The present study tested 4 diets as silages and hays, which included the most widespread forages grown in the Po plain area: corn silage, wheat silage, alfalfa, and Italian ryegrass, under the hypothesis that the presence

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

	Diet[1]					
Item	CS	AS	WS	PR	SEM	*P*-value
GEI[2] (Mcal/d)	88.1[b]	88.3[b]	90.7[ab]	98.3[a]	2.96	0.020
Fecal energy (Mcal/d)	23.4[c]	24.3[bc]	26.3[b]	34.8[a]	1.62	<0.001
Digestible energy (Mcal/d)	64.7	64.0	64.1	63.5	1.60	0.665
Urinary energy (Mcal/d)	2.49	2.46	2.44	2.24	0.14	0.622
Methane energy (Mcal/d)	5.00[b]	5.21[ab]	5.21[ab]	5.45[a]	0.14	0.047
ME (Mcal/d)	57.3	56.3	56.4	55.8	1.65	0.569
Heat production (Mcal/d)	29.9[b]	30.6[ab]	31.2[a]	30.8[ab]	0.72	0.019
Milk energy (Mcal/d)	21.4	22.1	23.4	23.0	0.70	0.111
Retained energy (Mcal/d)	5.88[a]	3.59[ab]	1.75[ab]	1.89[b]	1.27	0.031
Fecal energy (% of GEI)	26.4[c]	27.4[c]	29.1[b]	35.3[a]	0.98	<0.001
Digestible energy (% of GEI)	73.6[a]	72.6[a]	70.9[b]	64.7[c]	0.98	<0.001
Urinary energy (% of GEI)	2.84	2.78	2.71	2.32	0.19	0.328
Methane energy (% of GEI)	5.67	5.92	5.78	5.59	0.19	0.543
ME (% of GEI)	65.1[a]	63.9[ab]	62.2[b]	56.7[c]	0.86	<0.001
Heat production (% of GEI)	34.2	34.8	34.7	31.6	0.76	0.058
Milk energy (% of GEI)	24.4	25.1	25.7	23.5	0.68	0.274
Retained energy (% of GEI)	6.51[a]	3.98[ab]	1.49[ab]	1.51[b]	1.48	0.029
NE_L (Mcal/kg of DM)	1.70[a]	1.57[ab]	1.53[b]	1.36[c]	0.06	<0.001
kl[3]	0.63[a]	0.60[ab]	0.58[b]	0.59[ab]	0.02	0.037

[a–c]Means in the same row with different superscript letters differ ($P < 0.05$).

[1]Experimental diets: CS = corn silage; AS = alfalfa silage; WS = wheat silage; PR = Parmigiano Reggiano.

[2]GEI = gross energy intake.

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

of different high-quality forages in balanced diets can lead to a similar milk production without increasing methane emissions and N excretion per unit of product. The forages used in the trial were all produced locally on commercial farms and are representative of the most widespread forage systems in Northern Italy (Gislon et al., 2020b). The diets were formulated to meet ME and MP requirements, but some important differences in DMI, digestibility, rumen fermentative pattern, NE_L content, N excretion (g/d), and methane emissions (% DE intake) were observed. However, no differences were detected for the emissions per unit of product.

All of the diets were formulated to allow the maximum inclusion of forage. For example, the inclusion level of corn silage in the CS diet was 49.3% DM, which is a much higher value than the values (29.6 and 29.0) reported by Gislon et al. (2020a) and by Pirondini et al. (2012) for commercial dairy farms in the same area. The use of alfalfa silage is not very common in this area; that is, only 21.0% of the farms use it (Gislon et al., 2020a), and at a lower inclusion level than that used in the AS diet of the present study, which was ~27% on a DM basis. This suggested an opportunity to re-introduce legume forages into the cropping systems in this area and to increase farm protein self-sufficiency by producing high-quality forages. The inclusion level of whole cereal silage, on a DM basis of the WS diet (20%), fell between the lower level tested by Benchaar et al. (2014) for barley silage (27.2%) and the level (wheat silage, 10% of diet DM) tested by Harper et al. (2017); to the best of our knowledge, no studies have been conducted on the use of winter cereal silage in the diets of lactating cow in the study area. The PR diet was formulated according to the indications reported in the disciplinary codes for Parmigiano Reggiano cheese production, and for this reason it included a significant amount of hay in the TMR (50.6% of total DM).

DMI and Digestibility

The cows fed the PR diet had a significantly higher DMI; this agrees with the results of Brown et al. (1963), who reported an increase in DMI as the level of hay in the diet increased. The PR diet showed a lower digestibility for all of the main constituents. The lower digestibility values of the PR diet are mainly due to the higher fiber content of the diet and to the high ADL of the used alfalfa hay. Broderick (1995) showed higher values of apparent nutrient digestibility, together with lower DMI, for alfalfa silage when used as a replacement for alfalfa hay, which we also observed in the present study. This is also consistent with the studies of Colucci et al. (1982) and of de Souza et al. (2018), who reported that the digestibility of a diet is reduced as DMI increases.

Alfalfa, Italian ryegrass, and corn silages are the 3 most common forages fed to dairy cows in silage-based diets in Northern Italy, although the use of winter cereal silage is also increasing. The best results for aNDFom digestibility were obtained for the CS and AS diets. Therefore, the use of large amounts of alfalfa silage together with ryegrass silage may provide a valuable possibility to increase the proportion of feed ingredients produced on a farm and reduce the inclusion of soybean meal in the TMR. Interestingly, these forages are mainly used as hay in Northern Italy at a lower inclusion level than that used in the present study (Gislon et al. 2020a).

The diet based on wheat silage was characterized by a lower aNDFom digestibility than CS and AS, unlike what Harper et al. (2017) observed, but in agreement with the results of Benchaar et al. (2014) for barley silage as a replacement of corn silage. This difference can be explained by considering several factors, such as different inclusion levels or maturity at harvest; Arieli and Adin (1994), for example, showed that the in vivo NDF digestibility of cows fed wheat silage diets was 5 percentage points higher for cows fed a silage harvested at an earlier maturity stage than for a silage harvested at a later maturity.

One negative effect that has emerged in this study, related to the high proportion of alfalfa in the AS and PR diets, is a lower protein digestibility than the CS and WS diets, which were characterized by a higher amount of soybean meal. Therefore, as far as this aspect is concerned, soybean meal may be more favorable than alfalfa.

Milk Production and Composition

As far as milk production is concerned, the results of the present study did not point out any significant differences between diets, even though the cows offered the PR diet had a significantly greater DMI, which resulted in a tendency for a higher milk yield. Overall, feed efficiency (ECM/DMI) was only numerically lower for the PR diet. Broderick (1995) also reported a lower dairy efficiency for cows fed alfalfa as hay instead of silage. We found that the milk fat concentration was slightly lower for the cows fed the PR diet than the cows fed the other diets, probably because of a dilution effect due to the higher milk production for the PR diet. Under the experimental conditions of the current study, the smallest MUN was observed for the AS diet. The smaller MUN content of the cows fed the AS diet than those fed the CS diet agrees with other studies (Broderick, 1985; Benchaar et al., 2007) which reported a smaller MUN concentration when cows were fed alfalfa rather than corn silage as the sole source of forage. The cows fed the WS diet had the highest MUN content, a result that agrees with the findings of Harper et al. (2017), who found a greater MUN for cows fed wheat silage than for cows fed corn silage. However, the CS diet in Harper et al. (2017) was characterized by a lower CP content than their wheat diet, like in our experiment; the WS diet had a slightly higher CP concentration than the others. Moreover, in the present study, the WS diet resulted in a lower OM digestibility than the CS and AS diets, and it can therefore be speculated that there was less energy available for microbial protein synthesis at a rumen level, with a consequent decrease in the capturing of N (as AA or NH_3) by rumen microbes. In the present experiment, the WS diet had a relatively high inclusion of alfalfa silage, with a consequent probable high release of ammonia in the rumen. This high release was also correlated with the higher urinary N excretion (g/d) of the cows fed the WS diet.

Ruminal Fermentation Characteristics

The rumen fermentation pattern was also affected by the different chemical compositions of the diets; PR and CS differed the most. The rumen pH increased in the PR diet, which was characterized by a lower NFC content and higher aNDFom content than the CS diet. The propionate proportion was lower in the PR diet than the CS diet. In agreement with the findings of Broderick (1985), we found a higher ruminal pH, a lower propionate ruminal molar proportion, and a higher acetate-to-propionate ratio in the ruminal fluid of the cows fed alfalfa hay than in the ruminal fluid of the cows fed corn silage.

The rumen pH values were registered 5 h after the morning feeding; the nadir pH appears after this time interval and allows the differences in diets and possible subacidosis issues to be better understood. In this regard, despite the difference between the CS and PR diets, the pH values registered for each diet were adequate to support rumen bacteria growth and fermentation.

Replacing structural carbohydrates in the diet with nonstructural carbohydrates has resulted in notable modifications of the physical-chemical conditions and microbial populations of the rumen, such as the shift of VFA production from acetate toward propionate, which occurs with the development of starch-fermenting microbes (Martin et al., 2010). In agreement with this observation, the AS diet in the present study, which showed the smallest aNDFom concentration, resulted in a higher rumen acetate proportion than the PR diet, which was the diet with the highest fiber content.

Enteric Methane Production and Energy Balance

The cows fed the PR diet had a greater daily production of methane (g/d) than those fed the CS diet due to the ruminal fermentation profile (i.e., higher pH and higher acetate:propionate ratio) together with the increased DMI. Benchaar et al. (2001) found a greater daily methane production for cows fed hay than for cows fed silages. Despite this difference, no significant differences in methane production were observed between diets in terms of grams per kilogram of DMI or grams per kilogram of milk. Harper et al. (2017) did not detect any difference in the methane emissions of cows fed CS or WS diets. On the other hand, Hart et al. (2015) observed lower methane yields for cows fed a high corn silage ration than those fed a high grass silage ration, but the proportion of NFC (starch) and fiber in the diets in their experiment was more variable than in our study. As far as the overall methane energy losses (% of energy intake) are concerned, the results of the present study agree with those of Pirondini et al. (2015) and of Colombini et al. (2015), with methane produced by rumen fermentation accounting for 5 to 6% of the gross energy ingested by the cows. No difference between diets was found for the methane energy loss as a percentage of GEI, but the methane energy loss as a percentage of DE was lower for the cows fed the CS diet than the cows fed the PR diet. This may partially be related to the observed, previously described rumen fermentation pattern.

The energy balance results suggest a different utilization of energy, depending on the diet. In agreement with the digestibility results, the hay-based diet (PR) in the present study was characterized by the lowest digestible and ME (% of GEI), which overall resulted in a lower NE_L content for PR than for the other diets. The AS diet had a similar NE_L content to the CS diet, whereas the WS diet had an intermediate NE_L content between the CS and PR diets. The final ranking of the 4 diets suggest that the corn silage, which is rich in starch and with a fairly good NDF digestibility, supplied more NE_L than the other forages. Among the latter, the high-quality forages (the AS diet) showed similar values to the CS diet, and the wheat silage and hays provided less digestible fiber, and consequently less NE_L. The PR diet, which is a feeding system that is based totally on hays as the forage source, seemed less efficient than those based on silages, thus confirming the lower feeding value of hays than silages.

Nitrogen Balance

Any dietary strategy aimed at mitigating the methane emissions of dairy cows should consider the possible effect of N losses in manure, urinary N in particular, to ensure that the reduction in enteric methane emissions is not offset by an increase in nitrous oxide and ammonia emissions (Hassanat et al., 2017). Nitrous oxide is a powerful GHG, and ammonia, although not a GHG, is environmentally harmful because it favors environmental acidification and the formation of fine particulates, which pollute the air. In the present study, the urine volume was higher for the AS diet than for the CS diet. Other studies (Hristov and Broderick, 1996; Brito and Broderick, 2006) reported greater urine volumes for diets with higher dietary alfalfa silage proportions vs. corn silage. In agreement with the MUN concentration, the cows fed the AS diet showed a lower urinary N excretion (% of N intake) than those fed the CS and WS diets. These results were partially unexpected because a large proportion of CP is converted into NPN during ensiling, thus reducing the efficiency of CP utilization in lactating cows. Feeding carbohydrates that are more extensively fermented in the rumen may improve the utilization of alfalfa NPN through the stimulation of microbial protein synthesis. In this study, a consistent amount of high-moisture ear corn (28.6% of diet DM) was used in the AS diet and probably improved N use in the rumen, as confirmed from the MUN concentration and urinary N excretion (% N intake), which were significantly lower than for the CS and WS diets. Urinary N is largely represented by urea, and is therefore more rapidly nitrified with consequent nitrous oxide emissions (Eckard et al., 2010). Thus, urinary N is less desirable, and shifting N excretion from urine to feces may be useful (Brito and Broderick, 2007). The higher N urinary excretion (total N g/d) of the cows fed the WS diet than that of the other diets agreed with the results of Benchaar et al. (2014), who also observed an increase in N urinary excretion for a larger 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 concentrations than for the other diets.

CONCLUSIONS

This study has shown that, despite differences in several variables in the considered diets, no differences concerning methane production (per unit of product or per kilogram of DMI) have been observed between the diets that are commonly used in dairy feeding in Northern Italy. The use of high-quality forages, especially if preserved as silage rather than as hay, is a valuable strategy that can be adopted to increase the feeds produced on a farm, a strategy which is positively related to the development of an environmentally sustainable farming system. The use of a hay-based diet is interesting for the production of PDO cheese and, based on these results, the environmental impact of this feeding system is comparable with that of the other diets. The results of the present study show that corn silage can also have a high nutritive value in terms of fiber digestibility; however, the use of high-moisture ear corn, in combination with high-quality grass and legume silages, is a valuable alternative. Despite the similar milk production, the wheat silage diet showed a lower OM digestibility and a higher urinary N excretion than the AS diet. A long-term study would be useful to evaluate the maintenance of milk production over lactation when winter cereal silage is used as the main forage. Overall, on the basis of these results, an evaluation of the environmental impact of milk production should be performed that considers the entire milk production chain. Particular attention should be paid to the forage production system to identify the best dietary strategy to enhance the environmental sustainability of dairy farms in both agronomic and animal aspects.

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