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Use of Increasing Levels of Low-Quality Forage in Dairy Cows' Diets to Regulate Enteric Methane Production in Subtropical Regions

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Abstract: Dairy cows are the highest daily and annual methane (CH_4) producers among all cattle categories. So, the present study aimed to evaluate the effect of increasing supplementation levels of a low-quality forage on dry matter intake (DMI), DM digestibility (DMD), milk production, enteric CH_4 emission, gross energy, and protein partitioning in Holstein cows. In total, eight cows (112 ± 38 days postpartum; mean ± s.d.) were randomly assigned to 4 treatments composed of 4 dietary neutral detergent fibre (NDF) inclusion levels (40.2% (control), 43.3%, 46.5%, and 50.5%) in a 4 × 4 repeated Latin square experimental design. The cows were fed corn + alfalfa silage and a concentrate (60:40 forage:concentrate ratio). To increase the contents of low-quality NDF, part of the silage was replaced with maize stover (MSTV). The CH_4 production was measured in an open-circuit respiration chamber. The DMI increased significantly and linearly ($p < 0.05$) with increasing levels of MSTV. However, the CH_4 yield decreased ($p < 0.0001$) as the NDF level increased (32.1, 28.1, 23.1, and 21.3 CH_4 L/kg DMI, respectively). DMD decreased as NDF levels in the diet increased ($p < 0.0001$). The NDF digestibility (DNDF) explained the better ($p < 0.0001$) CH_4 production response than DMD. It was concluded that low-quality forages can be used to regulate CH_4 production in subtropical and tropical climate regions.

Keywords: fibre; methane; digestibility; dairy cattle; methane yield; methane intensity

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1. Introduction

Modern dairy cows are bigger and consume more feed, which results in higher enteric CH_4 emissions per cow than other cattle categories. The amount of CH_4 produced (kg or L) by cows is influenced by different factors like diet [1–3], DMI, animal characteristics [4], and the environment [5]. The fibre concentration in diets is one of the main factors associated with CH_4 daily production and CH_4 yield because, typically, the higher fibre content of forages is associated with higher CH_4 emissions [6]. The importance of fibre as a predictor of CH_4 production was documented for the first time by [7] with their empirical model (r^2 = 0.67) that uses the content of the different fibre fractions as predictor variables: CH_4 (Mcal/d) = 0.814 + 0.122 * soluble residue (kg fed) + 0.415 * hemicellulose (kg fed) + 0.633 * cellulose (kg fed), being that the structural carbohydrate cellulose was the most significant contributor to CH_4 emission in the new model. Likewise, these authors presented a second model that correlated the total daily CH_4 production to the amount of each fibre fraction in the diet that was apparently digested as (r^2 = 0.73): CH_4 (Mcal/d) = 0.439 + 0.273 soluble residues (kg digested) + 0.512 * hemicellulose (kg digested) + 1.393 * cellulose (kg digested), where again cellulose was the most significant contributor to CH_4 emission. Later studies used the digestibility of the dietary fibre content to explain and predict CH_4 formation and emission in sheep [8], dairy cattle [9], and beef cattle [10]. Meanwhile, the former studies assumed that a significant fibre content in the diet was associated with increased energy loss in CH_4, regardless of the fibre's digestibility [11]. Based on this early assumption, it was generally agreed that cattle in tropical regions produce more CH_4 than cattle in temperate regions because the forages and grasses that form the basal diet of cattle in these parts of the world contain high amounts of fibre [12–14]. However, recent measurements of enteric CH_4 emissions on dual-purpose cattle conducted in open-circuit respiration chambers in the tropical regions of Mexico indicate that the CH_4 conversion factor or Ym factor can be as low as 4.9% in cattle fed with low-quality (50% DMD and >70% NDF) tropical grasses [3,15,16]. The Ym factor is defined as the percentage of the animal's gross energy intake (GEi), which is lost in the form of CH_4.

Similar CH_4 conversion factors (Ym values, mean = 6.4, min = 2.6, max = 15) for beef cattle were reported by [17], who compiled a dataset of 1100 individual observations from individual beef cattle heads fed on high-forage diets in Latin America and the Caribbean. These results contrast with the Ym factor of 8.7% reported by [18] for dairy cattle fed a good-quality TMR (DMD = 68% and NDF = 34%) in central Mexico's highland subtropical climate regions. So, it is suggested that the digestibility of the diet in dairy cattle plays a critical role in CH_4 production in these warmer regions. Extant CH_4 prediction models [7,19–21] assumed lower CH_4 emissions in temperate climate regions than in the tropics. However, these models were developed using individual CH_4 emissions generated from experiments where the experimental diets had a low to moderate fibre content ranging from 25% to 35%. Furthermore, model development was conducted using temperate climate forages characterised by high digestibility, low to medium NDF, and low acid detergent fibre (ADF) contents. For example, Kreuzer et al. [22] and Holter and Young [23] used ryegrass, whereas [24] used *Holcus lantus* and *Phalaris aquatic* in temperate climate Australia, [25] used ryegrass in New Zealand, and [26] used a diet composed of 91% concentrate diets in Canada. Therefore, the predictive capacity of these models may be compromised when tropical forages are used because of the critical role of fibre and its digestibility on CH_4 production. For example, [23] observed that dietary ADF had twice as much impact as body live weight (BLW) and dietary CP, and three times as much impact as NDF digestibility on CH_4 output.

Furthermore, according to the former authors, the Ym factor declined with increasing BW, and the CP and ADF contents in the diet increased as the digestibility of NDF increased, whereas the digestible energy content of dietary DM was related positively to CH_4 output (r = 0.45). Therefore, fibre digestibility in diets is critical in CH_4 production, as Moe and Tyrrel [7] stated initially. They found that CH_4 energy production could be predicted (r^2 = 0.74) from intakes of digested soluble residue, cellulose, and hemicellulose. Similarly, [23] found a negative relationship between dietary ADF percentage and CH_4 production from GE (r = −0.50), suggesting that the negative effect of lignin was more significant than the potentially digestible cellulose effect. This early finding indicates that low-quality fibre can change the CH_4 production of dairy cattle in subtropical and tropical climate regions.

Therefore, the objective of the present study was to evaluate the effect of increasing supplementation levels of NDF in a low-quality forage on CH_4 production, DMI, DMD, milk production, milk composition, energy, and protein intake partitioning in lactating Holstein dairy cows, and to discuss its potential use to regulate CH_4 production in dairy cattle production in subtropical climate regions.

2. Materials and Methods

2.1. Location

The present study was conducted at the Laboratory for Research on Livestock, Environment and Renewable Energy of the Faculty of Veterinary Medicine and Animal Science, Universidad Autónoma del Estado de Mexico, located in Toluca, State of Mexico at 19, 24′15″ N, and 99, 41′06″ W. The University Committee on Animal Welfare and Research Ethics approved the experimental protocol DC2018/2-8. Before the start of the experiment, all cows were dewormed with ivermectin and were found to be clinically healthy.

2.2. Characteristics of Experimental Animals and Treatments

Eight first-calving Holstein cows with an average BLW of 443 ± 28 kg and a mean milk yield of 15.8 ± 3.2 kg/day were used. The cows were randomly distributed in a 4 × 4 × 2 Latin Square experimental design, where four treatments with increasing levels of NDF from MSTV were evaluated: treatment A = 40.1% NDF, treatment B = 43.3% NDF, treatment C = 46.4% NDF, and treatment D = 50.4% NDF. Treatment A was the control treatment with no MSTV. All treatments were prepared daily, and each cow received each treatment in turn, once in each of the four experimental periods. They had ad libitum access to the diet and water at all times.

2.3. Measurements on Animals

The experiment lasted 126 days; the first 30 days were used to adapt animals to the experimental diets and procedures in the respiration chamber (RC). The RC was of the head-box type and was equipped with a metabolic crate, automatic drinker, and a trough [18], so the animals had permanent access to feed and water ad libitum during their time at the chamber. Each animal adapted well to the RC, and their DMI was unaffected during the assays. Each animal visited the chamber for seven days during the adaptation period. The remaining 96 days were divided into four experimental periods of 24 days each. Fourteen days of each experimental period were used for adaptation to the assigned treatment diet, and the last seven days were used to conduct measurements on animals.

The CH_4 production was measured for 48 h on each cow. So, eight days were necessary to measure CH_4 in all cows. Cows were milked twice daily at 6:00 a.m. and 15:00 h, and milk yield was weighed daily during the entire 24-day period. Milk composition was measured daily during the measuring period. A Lactichek™-01 milk analyser (Rapi Read, Page & Pedersen International Ltd., Hopkinton, MA, USA) was used to measure fat, lactose, protein, and non-fat milk solids. The BLW was measured with a livestock scale (model WIM-LP7510, Wim Systems, Shanghai, China), once at the beginning and once at the end of each experimental period after the morning milking.

Methane Production Measurement

The CH_4 production was measured using a dual-wavelength infrared optical bench CH_4 analyser (model: MA-10, range 0–10% and high resolution 0.0001% to 0.01%, Sable Systems International, Las Vegas, NV, USA), with barometric pressure compensated for and not sensitive to flow rate changes, which eliminated errors caused by ambient pressure variation. Every assay started at 10:00 h; the mass flow generator (Model FK 500, Sable Systems International, Las Vegas, NV, USA) was set at 480 L/min, the analyser was set to measure CH_4 concentration every second, and the chamber was closed. The CH_4 emissions were measured for 48 h. The data from the CH_4 analyser were recorded and transferred to the computer in real time. The data were stored in an Excel spreadsheet to calculate daily CH_4 emissions. Before the beginning of the experiment, a CH_4 recovery test was conducted as described by [27] for the types of chambers used in the present experiment, and a 100% ± 2% recovery rate was found. The cows were removed from the chamber for milking and returned to the chamber to complete the measurement. The diet was weighed daily before the beginning of the assay, and all animals received the same amount at 9:00 h and 16:00 h. The next morning, the orts were removed and weighed to calculate the DMI. During the time inside the chamber, urine and faeces were collected. A device was fixed around the vulva of the cows to collect urine, and it remained attached for a 24 h period. The daily faeces production was collected in the metabolic crate tray and weighed daily. A sample of approximately 1 kg of faeces was separated and kept frozen until laboratory analysis. Diet samples were collected and kept in a freezer until laboratory analysis. Four assays were completed in each experimental period.

2.4. Sample Collection and Laboratory Analysis of Feed and Faeces Samples

Before the laboratory analyses, the diet and faecal composite samples (10% of a pool from all samples) were dried in a forced-air oven at 60 °C for 72 h and grounded to pass through a 1 mm sieve. The DM content was determined using the method n° 7.007 of AOAC (1980). The crude protein (CP) content was determined using the Kjeldahl method (n° 7.007 of AOAC 1980) for nitrogen determination, and the result was multiplied by 6.25 to estimate the CP content. The NDF and ADF contents were determined using the method of [28]; heat-stable α-amylase was used for the NDF analyses of concentrate and faeces samples. Ligning (LIG) content was determined using the method of [29]. The ashes (ASH) content was determined using a furnace oven at 530 °C (method 942.05, AOAC 2000), and the percentage of organic matter (OM) was determined as OM % = $((DM_{sample} - ASH)/DM_{sample}) * 100$. The faeces' and feeds' gross energy (GE) content was determined with an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA). The urine volume produced during 24 h was measured, and a sample was collected. We added 10% sulphuric acid to the urine samples to prevent nitrogen volatilisation and then froze them for later N analysis using the Kjeldahl method. The GE content of urine (GEu) was calculated assuming that 1 g of N in urine is equivalent to 9 kJ, as in [30].

The diet's digestible energy intake (DEi) was calculated by subtracting the energy loss in faeces (GEf) from the GEi. Similarly, the metabolisable energy in the diet was calculated as ME, MJ/day = GEi − (GEf + GEu + CH_4) [31]. Finally, the daily CH_4 production was converted to energy by assuming that 1 g of CH_4 equals 55.22 kJ of the diet's gross energy, as in [30]. The Ym factor was calculated according to the Tier 2 level method for national inventories calculation, as in [32]. This calculation is based on the quotient of the energy lost in the form of CH_4 per animal per day by the total gross energy intake of the same animal per day.

2.5. Analysis of Results

The results for the DMI, digestibility, energy and protein balance, milk yield, milk composition, and CH_4 production were analysed using an analysis of variance for a replicated Latin square experimental design, as in Equation (1).

$$Y_{ijkl} = \mu + A_{i(l)} + T_j + P_k + S_l + \xi_{ijkl} \tag{1}$$

where Y_{ijkl} is the response variable of the *i*th animal (*i* = 1, 2, 3, 4), nested in the lth square (l = 1, 2) that received the *j*th treatment (*j* = 1, 2, 3, 4) during the *k*th period (*k* = 1, 2, 3, 4), where μ is the overall mean of all observations, $A_{i(l)}$ is the random effect of the experimental animal nested in the lth square, T_j is the fixed effect of the treatment, P_k is the fixed effect of the period, S_l is the fixed effect of the square, and ξ_{ijkl} is the random error component. The residuals and random effects were assumed to be independent and normally distributed with a mean of zero and constant variance. Post hoc pairwise comparisons were conducted using Tukey's HSD test when significant differences between means ($p < 0.05$) were observed. The PROC MIXED procedure of the SAS statistical software 9.4 (SAS Inst. Inc., Cary, NC, USA) was used for the analysis.

3. Results

The ingredients and chemical constituents of the experimental diets are presented in Table 1. It can be observed that the forage:concentrate ratio of treatment A was 65:35, the forage was composed of maize + alfalfa silage only, and the increasing levels of NDF for treatments B, C, and D were achieved by replacing the silage with MSTV finely chopped at 8 mm size.

Table 1. Ingredients and chemical composition of the experimental treatment diets, basal diet, and forages offered to lactating Holstein cows.

	Treatments g/kg DM					
Ingredients	**A**	**B**	**C**	**D**		
Maize + alfalfa silage	600.5	520.4	447.3	376.9		
Maize straw	-	80.1	153.2	223.6		
Ground maize	185.8	185.8	185.8	185.8		
Soja bean meal	85.9	85.9	85.9	85.9		
Canola meal	56.2	56.2	56.2	56.2		
Wheat bran	64.8	64.8	64.8	64.8		
Minerals and vitamins additive	6.8	6.8	6.8	6.8		
	Chemical composition (mean ± SD)				Maize stover	Maize + alfalfa silage
DM (%)	54.1 ± 0.3	55 ± 2.1	56.3 ± 1.2	60.3 ± 0.7	89.8	36.7
OM (g/kg DM)	915.4 ± 18	927.1 ± 14	923.8 ± 12	923.2 ± 12	93.5	89.0
CP (g/kg DM)	162 ± 2.1	159.8 ± 15	158.8 ± 1.7	158.1 ± 2.3	6.0	10.8
NDF (g/kg DM)	401.8 ± 12	433.4 ± 3.7	464.8 + 3.9	504.7 ± 8.8	75.4	52.7
ADF (g/kg DM)	244.1 ± 6.1	282.1 ± 6.4	310.5 ± 7.2	347.3 ± 19	54.7	39.0
LIG (g/kg DM)	31.7 ± 8.1	39.4 ± 4.8	52.9 ± 6.9	58.8 ± 9.2	12.3	10.2
CEL (g/kg DM)	213.1 ± 16.4	242.6 ± 7.8	257.6 ± 8.5	288.4 ± 24.4	207	28.8
HEM (g/kg DM)	157 ± 15	151.4 ± 10	154.3 ± 9	157.5 ± 18	84	12.7
NFC (g/kg DM)	326.9 ± 16	310.4 ± 14	281.2 ± 29	240.4 ± 34	-	-
EE (g/kg DM)	24.6 ± 3.8	23.4 ± 3.3	19.8 ± 0.9	20 ± 1.0	-	-
GE (MJ/kg DM)	17.07 ± 0.4	16.95 ± 0.5	16.97 ± 0.6	16.61 ± 0.9	12.0	16.7

3.1. Voluntary Dry Matter Intake

The results in Table 1 suggest that the increasing levels of MSTV in the treatments augmented the LIG content in diets by 3.9, 5.3, and 5.9% for treatments B, C, and D, respectively. Similarly, the NDF content increased by 25% between treatment A and treatment D, whereas ADF content increased by 42% for the same treatments. The DM, EE, and GE contents did not change between treatment diets. The effect of increasing levels of MSTV's fibre in experimental diets on BLW, $BW^{0.75}$, DMI, DMD, digestibility of GE (DGE), fibre fractions intake, and GEi is in Table 2. It can be observed that the NDFi, ADFi, LIGi, and CELi increased as the inclusion level of MSTV in the diet increased ($p \leq 0.0008$, <0.0003, <0.0001, and <0.001, respectively) without a significant adverse effect on OMi, CPi, HEMi, NFCi, and GEi ($p > 0.05$). In contrast, the DMI significantly increased by 2.2 kg DM/d in treatment D compared to control treatment A ($p < 0.05$) without any effect on the animals' BLW ($p < 0.05$). However, all the digestibility variables dropped linearly with increasing levels of MSTV, as in Table 2 for DMD ($p < 0.0001$), DOM ($p < 0.002$), DGE ($p < 0.0001$), fibre fractions ($p < 0.002$), and digestibility of the crude protein (DCP) ($p < 0.01$).

3.2. Energy and Protein Balance

The results in Table 3 illustrate the effect of increasing levels of MSTV in treatments on energy and protein balance in the experimental dairy cows. It is observed that the daily GEi increased linearly with increasing levels of MSTV, but no significant effect was observed ($p > 0.05$). However, the energy loss in faeces (GEf) increased linearly and significantly ($p < 0.001$) with increasing levels of MSTV. This reduction was accompanied by a decline in the DMD and DOM from 73.6% and 72.8% in treatment A to 57.9% and 58.5% in treatment D, respectively (Table 2). The metabolisability of the diet (MEi:GEi), known as *qm* value, also declined significantly from 0.64 in treatment A to 0.5 in treatment D because of the increasing GEf. The rest of the energy balance was not affected by increasing levels of

MSTV. On the other hand, the protein balance was not affected by any of the treatments, although there is a declining trend with increasing levels of MSTV (Table 4).

Table 2. Effect of increasing levels of maize stover in treatment diets on body live weight (kg), metabolic body weight, dry matter intake (kg/day), nutrient intake (kg/day), gross energy intake (MJ/day), and digestibility (%) of nutrients for the four treatments of lactating Holstein cows.

	Treatments				SEM	*p* Value	Contrast		
	A	B	C	D			Lineal	Cuadr.	Cub.
BLW	441.1	440.8	431.2	447.5	10.1	NS	NS	NS	NS
BW $^{0.75}$	96.2	96.1	94.5	97.1	1.9	NS	NS	NS	NS
Intake, DM kg/day									
DMI	13.4	14.3	15.4	15.6	0.81	0.05	0.016	NS	NS
DMI, %LW	2.9	3.2	3.5	3.5	0.19	0.06	0.017	NS	NS
OMi	11.8	13.3	13.9	14.4	0.73	0.07	0.017	NS	NS
CPi	2.5	2.4	2.3	2.1	0.13	NS	0.026	NS	NS
NDFi	5.1 ^{c}	6.2 ^{bc}	7.1 ^{ab}	7.9 ^{a}	0.36	0.0008	0.0001	NS	NS
ADFi	3.1 ^{c}	4.0 ^{bc}	4.7 ^{ab}	5.4 ^{a}	0.27	0.0003	<0.0001	NS	NS
LIGi	0.41 ^{b}	0.58 ^{b}	0.83 ^{a}	0.9 ^{a}	0.05	<0.0001	<0.0001	NS	NS
CELi	2.7 ^{c}	3.4 ^{bc}	3.9 ^{ab}	4.5 ^{a}	0.24	0.001	0.0001	NS	NS
HEMi	2.0	2.1	2.4	2.4	0.14	NS	0.02	NS	NS
NFCi	4.2	4.4	4.4	3.7	0.24	NS	NS	NS	NS
GEi, MJ/d	219.9	241.8	262.2	261.5	14.1	NS	0.03	NS	NS
Digestibility, %									
DMD	73.6 ^{a}	68.3 ^{b}	62.2 ^{c}	57.9 ^{d}	1.00	<0.0001	<0.0001	NS	NS
DGE	75.4 ^{a}	69.2 ^{ab}	62.5 ^{bc}	58.6 ^{c}	1.85	0.0001	<0.0001	NS	NS
DOM	72.8 ^{a}	67.4 ^{ab}	61.4 ^{bc}	58.5 ^{c}	2.15	0.002	0.0003	NS	NS
DNDF	48.3 ^{a}	46.2 ^{ab}	44.2 ^{b}	41.6 ^{c}	0.50	<0.0001	<0.0001	0.06	NS
DADF	49.1 ^{a}	41.5 ^{b}	36.3 ^{c}	30.4 ^{d}	0.44	<0.0001	<0.0001	0.07	NS
DCP	70.3 ^{a}	69.5 ^{ab}	68.9 ^{ab}	68.7 ^{b}	0.33	0.01	0.002	NS	NS
DDMi	9.5	9.8	9.7	9.1	0.5	NS	NS	NS	NS
DEi, MJ/d	167.3	167.9	165.3	152.4	9.3	NS	NS	NS	NS

Different lowercase superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). BW = body live weight (kg), BW $^{0.75}$ = metabolic body weight, DMI = dry matter intake, DMI, %LW = DMI as a percentage of body weight, Omi = organic matter intake, Cpi = crude protein intake, NDFi = neutral detergent fibre intake, ADFi = acid detergent fibre intake, LIGi = ligning intake, CELi = cellulose intake, HEMi = hemicellulose intake, NFCi = non-fibre carbohydrate intake, Gei = gross energy intake MJ/day, DMD = DM digestibility, DGE = digestibility of the GE, DOM = OM digestibility, DNDF = NDF digestibility, DADF ADF digestibility, DCP = crude protein digestibility, DDMi = digestibility of DM intake, and Dei = digestible energy intake (MJ/day).

Table 3. Effect of increasing levels of maize stover in treatment diets on energy and protein balance in the experimental Holstein dairy cows.

	Treatments				SEM	*p* Value	Contrasts		
	A	B	C	D			Lineal	Cuadr.	Cub.
Energy (MJ/day)									
GEi	219.9	241.8	262.2	261.5	14.1	NS	0.03	NS	NS
GEf	54.3 ^{a}	74.0 ^{ab}	98.6 ^{bc}	109.9 ^{c}	7.2	0.0007	<0.0001	NS	NS
DEi	167.3	167.9	165.3	152.4	9.3	NS	NS	NS	NS
GEu	9.40	10.2	10.8	11.0	0.4	0.09	0.02	NS	NS
MEi	141.7	141.7	140.5	128.4	8.2	NS	NS	NS	NS
MEi:Gei (qm)	0.64 ^{a}	0.58 ^{ab}	0.53 ^{bc}	0.50 ^{c}	0.018	0.0006	<0.0001	NS	NS
MEi:DEi	0.84	0.84	0.85	0.84	0.005	NS	NS	NS	NS

Table 3. *Cont.*

	Treatments				SEM	*p* Value	Contrasts		
	A	B	C	D			Lineal	Cuadr.	Cub.
			Protein (kg or g/d)						
CPi (kg/d)	2.50	2.48	2.32	2.21	0.13	NS	0.025	NS	NS
Nf (g/d)	110.1	112.4	118.5	119.2	7.50	NS	0.02	NS	NS
DCPi (kg/d)	1.7	1.7	1.6	1.4	0.08	NS	0.03	NS	NS
Nu (g/d)	159.0	169.9	179.2	181.3	11.2	NS	NS	NS	NS
MPi (Kg/d)	0.75	0.78	0.66	0.57	0.11	0.09	0.01	NS	NS
MPi:CPi	0.30	0.32	0.29	0.27	0.07	NS	NS	NS	NS
MPi:DPCi	0.43	0.46	0.42	0.40	0.05	NS	NS	NS	NS

Different lowercase superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). GEi = gross energy intake, GEf = gross energy in faeces, DEi = digestible energy intake, GEu = gross energy in urine, MEi = metabolisable energy intake, CPi = crude protein intake, Nf = N loss in faeces, DCPi = digestible crude protein intake, Nu = N loss in urine, and MPi = metabolisable protein intake.

Table 4. Effect of increasing levels of maize stover in treatment diets on CH_4 production, CH_4 yield, and CH_4 intensity of production by lactating Holstein cows.

	Treatments				EEM	*p* Value	Contrast		
	A	B	C	D			Lineal	Cuadr.	Cub.
CH_4 L/day	409	405	381	390	21.3	NS	NS	NS	NS
CH_4 L/kg DM	32.1 [a]	28.1 [b]	23.1 [c]	21.2 [d]	0.4	<0.0001	<0.0001	NS	NS
Ym	7.4 [a]	6.6 [b]	5.3 [c]	5.0 [d]	0.09	<0.0001	<0.0001	NS	NS
CH_4 L/kg milk	28.9	30	20.2	21.2	3.8	NS	0.07	NS	NS

Different lowercase superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). *Ym* = methane conversion factor, the energy in the form of CH_4 as a percentage of GEi.

3.3. Methane Emission

The results in Table 4 illustrate the effect of increasing levels of MSTV in treatment diets on CH_4 production, CH_4 yield, Ym factor, and CH_4 emission intensity (CH_4 L/kg milk). No differences were observed between the treatments for daily CH_4 production and emission intensity ($p > 0.05$). In contrast, the CH_4 yield (CH_4 L/kg DMI) and the Ym factor declined significantly ($p < 0.0001$) with increasing levels of MSTV; the yield declined by 32.5% between the control treatment and treatment D, whereas the Ym factor dropped by 34% for the same treatments.

3.4. Milk Production and Milk Quality

In Table 5, the effect of increasing levels of MSTV in treatment diets on milk yield and composition can be seen. The energy-corrected milk is also presented in Table 5 [33]. It can be observed that daily milk yield was not affected by treatments ($p > 0.05$) despite the significant reduction in DMD, DGE, DOM, and DNDF ($p < 0.001$). However, the milk fat concentration (%) increased linearly (lineal = 0.05) with increasing levels of MSTV ($p < 0.03$), which was accompanied by a larger NDFi from 5.1 kg DM/day in treatment A to 7.9 kg DM/day in treatment D (Table 2). In contrast, the CP content in milk showed a declining trend with increasing levels of fibre from MSTV (lineal = 0.08).

Table 5. Effect of increasing levels of maize stover in treatment diets on milk yield (kg/day) and milk composition (%) on lactating Holstein cows.

	Treatments				SEM	*p* Value	Contrast		
	A	**B**	**C**	**D**			**Lineal**	**Cuadr.**	**Cub.**
				Yield, kg/d					
Milk yield	16.1	17.9	16.9	17.1	2.3	NS	NS	NS	NS
ECM	16.9	17.2	19.5	19.8	1.8	NS	0.06	NS	NS
Fat content	0.56	0.66	0.62	0.67	0.09	0.09	NS	NS	NS
Protein content	0.54	0.60	0.58	0.59	0.08	NS	NS	NS	NS
				Composition, %					
Fat content	3.6 ^{b}	3.6 ^{b}	3.69 ^{ab}	3.9 ^{a}	0.04	0.03	0.01	NS	NS
Protein content	3.5	3.4	3.42	3.4	0.04	0.09	0.08	NS	NS
NFS	8.6	8.8	8.76	8.6	0.15	0.07	NS	0.02	NS
FEC	1.2	1.1	1.1	1.1	0.11	NS	NS	NS	NS
N conversion	0.26	0.25	0.25	0.24	0.02	NS	NS	NS	NS

Different lowercase superscript letters within rows indicate statistically significant differences ($p \leq 0.05$). ECM = Energy corrected milk = 0.3246 × milk (kg/day) + 13.86 × fat (kg/day) + 7.04 × protein (kg/day). NFS = non-fat solids. FEC = milk (kg)/DMI (kg, DM). N conversion = protein (kg)/CPi (kg).

4. Discussion

We aimed to evaluate the effect of replacing variable amounts of good-quality fibre in alfalfa + maize silage with low-quality fibre from MSTV (up to 25% of the NDF in silage) on dairy cows' CH_4 production, DMI, digestibility, energy partitioning, protein balance, and milk yield. We expected to observe a reduction in voluntary DMI and CH_4 production, particularly in treatment D, because it is generally agreed that including low-quality and low-energy density forages in dairy cows' diets reduces voluntary DMI and, thus, CH_4 production [34]. Maize stover is a typical low-quality forage, high in fibre (NDF ≥ 700 g/kg DM, ADL ≥ 389 g/kg DM), low in CP (<62 g/kg DM), and low in digestibility (DMD = 48%) [35]. Considerable evidence indicates that voluntary DMI is limited by the capacity of the reticulo-rumen and the rate of disappearance of digesta from this organ when cows are fed forages with moderate to high content of structural carbohydrates [36,37]. This is because the rate of disappearance of digesta depends on the rate of passage and rate of absorption, and both rates are lower in low-quality forages than in high-quality ones [38]. While we agree with previous assumptions, our results in Table 2 indicate that voluntary DMI did not decrease; on the contrary, it increased but was accompanied by a reduction in digestibility, CH_4 yield, Ym factor, and no change in milk yield.

So, we hypothesised that experimental cows increased their voluntary DMI to compensate for a reduction in MEi supply from diets as the MSTV level increased. This assumption is supported by the decline in the qm value observed in the experimental diets, which passed from 0.64 in treatment A to 0.5 in treatment D (Table 3). So, cows likely ate more to maintain the MEi necessary to support milk yield without significant change (Table 5). The BLW was unaffected by treatments (Table 2), suggesting that preserving constant milk yield was not at the expense of the body's fat reserves. We believe the small particle size of MSTV used in the present work was critical in allowing increased voluntary DMI across all experimental treatments. It also helped that MSTV formed only a small part of the forage diet. For example, [39,40] demonstrated that the chemical composition of the fibre in forages cannot be considered the only factor in determining voluntary DMI; other factors, like particle size of the forage fed, play a critical role. When the forage particle size is small (<9 mm), the DMI is less affected than when the forage is fed coarse. Likewise, according to [41], reducing the particle size of forages to less than 10 mm improves feed intake, helping cows cope with less energy-dense feeds.

Furthermore, [41] concluded that a reduction in forage particle size in high-forage diets could be a measure to improve energy intake, performance, and, hence, forage utilization. Therefore, it is possible to assume that the particle size of the MSTV of 8 mm used in the present work explains the increased DMI to compensate for the lower energy content of experimental treatments. A compensatory DMI was also reported by [41] in lactating cows fed on a diet based on MSTV and rice straw compared with a diet based on alfalfa hay as a forage source.

Methane emission: the CH_4 yield and Ym factors observed in the present work are within the range reported by other authors. For example, in a meta-analysis conducted for tropical and subtropical regions in Brazil and India, the Ym factor ranged from 5% to 7.4%, and the yield varied from 21 to 31 L CH_4/kg DMI [19]. The CH_4 yield observed in treatment A (control) is also close to the average value of 28 L CH_4/kg DM reported by [42] from the analysis of an intercontinental database of 2566 individual observations of dairy cows. The Ym value for treatments with the highest levels of MSTV (C and D) is closer to the 5.2 reported by [16] for tropical regions in Mexico. On the other hand, the decline in the size of the Ym factor can be explained by the increment in the DMI and GEi, both associated with the increasing levels of MSTV in experimental treatments but without changes in total daily CH_4 production. Larger DMIs + GEi are associated with smaller Ym factors because this factor represents the amount of GEi lost as methane.

Meanwhile, the yield (CH_4 L/kg DMI) is calculated by dividing the total CH_4 production by the daily DMI. So, the reduction in CH_4 yield was associated with a decrease in diet digestibility (DMD and DOM) and a higher DMI in treatments with MSTV. In contrast, the largest yield with the highest digestibility was observed in the control treatment. A similar response was reported by [43] but in sheep, where increased CH_4 yield was observed with increased DOM. Methane is a byproduct of fermentation and degradation of the DM in the rumen, so a reduction in the digestibility and structural carbohydrates yielded less degraded substrate and CH_4 produced per unit of DM consumed by cows [43]. For example, [44] measured CH_4 production in two grasses, *Dicanthium aristatum* hay (55% de DNDF) and *Chloris gayana* hay (69% de DNDF), and found that both daily CH_4 production and CH_4 yield were lower in *Dicanthium aristatum* than in *Chloris gayana* (158 vs. 360 L CH_4/day and 44 vs. 51 L/kg MSI, respectively). This finding aligns with our work, where the lowest CH_4 yields were associated with the lowest DNFD and DADF in treatments C and D (Table 2).

Milk production and milk quality: in the present study, the milk yield was not affected by treatments; however, fat content in milk was significantly higher in treatment D compared to the rest of the treatment diets. This effect was associated with the highest content of NDF in treatment D because high-fibre diets produce a more significant concentration of volatile fatty acids in the rumen, which are precursors of lipogenesis in the mammary gland tissue, like butyric and acetic acids [45].

5. Conclusions

The use of small to moderate amounts of finely chopped MSTV in dairy cows' diets reduces the quality and energy value of the diet, in terms of its digestibility, because of the high fibre content of MSTV. This increment in fibre did not affect the milk yield and body weight; however, it negatively affected the CH_4 yield and the amount of GEi lost as CH_4, suggesting that low-quality fibre from forages like MSTV could be used to regulate CH_4 emission or as a mitigation strategy in dairy cows in subtropical climate regions.

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