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Effects of Dietary Starch Concentration on Milk Production, Nutrient Digestibility, and Methane Emissions in Mid-Lactation Dairy Cows

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Abstract: Our objective was to evaluate the effects of dietary starch concentration on milk production, nutrient digestibility, and methane emissions in lactating dairy cows. Thirty mid-lactation cows were randomly assigned to either a high-neutral-detergent-fiber, low-starch diet (LS; 20.2% starch) or a low-neutral-detergent-fiber, high-starch diet (HS; 25.2% starch) following a 3-week acclimation. The study lasted 8 weeks, with milk sampling and gas measurements conducted weekly during acclimation and at weeks 2, 4, 6, and 8. Blood and fecal samples were collected during acclimation and week 8. Compared with LS cows, HS cows produced 1.9 kg/d more energy-corrected milk (4.45% increase), with higher yields of true protein (+0.13 kg/day), lactose (+0.10 kg/day), and total solids (+0.24 kg/day). Dry matter and organic matter digestibility was 4.2 and 4.3% higher, respectively, in the HS group. The milk fatty acid (FA) profile differed, with LS cows having greater mixed FA content and HS cows showing higher de novo FA content and yield. Although methane production tended to be higher in HS cows (+25 g/day), methane yield decreased by 8.8%. Overall, the HS diet improved milk production, nutrient digestibility, and environmental efficiency by reducing methane yield in dairy cows.

Keywords: greenhouse gas; enteric methane; dietary starch; milk composition

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1. Introduction

The global population is projected to reach 9.7 billion by 2050 [1], presenting the major challenge of increasing food supply while minimizing environmental impact. Current production systems and consumption patterns have been deemed unsustainable [2]. Consequently, agriculture is at a critical juncture, needing to address both the demands of a growing population and its environmental footprint. In the livestock industry, particular attention has been given to reducing methane (CH_4) emissions due to the significant contribution of ruminants to anthropogenic CH_4 levels. Methane has a global warming potential 27 to 30 times greater than carbon dioxide (CO_2) over a 100 y horizon [3]. Furthermore, its shorter atmospheric lifespan makes it even more potent over a 20 y period, 84 to 86 times

that of CO_2. These factors suggest that targeting CH_4 could be a more effective strategy for short-term climate mitigation efforts [3].

Methanogenesis in ruminants not only poses environmental concerns but also represents a loss of gross dietary energy, reflecting suboptimal feed utilization [4]. This energy loss is substantial, ranging from 2 to 12% of gross energy intake, with an average of 5 to 6% in dairy cattle [5]. Therefore, researchers and dairy farmers are actively exploring methods to reduce on-farm enteric CH_4 emissions while enhancing cow efficiency [6]. The production of CH_4 is influenced by various dietary factors, including the type and quantity of feed, which affect the ruminal microbial population and alter hydrogen gas (H_2) utilization and overall fermentation patterns. Understanding nutrient profiles and optimal dietary inclusion levels is essential for reducing enteric CH_4 emissions. For instance, opting for starch over fiber and increasing the starch content in the concentrate portion of the diet are potential strategies for reducing ruminal CH_4 production [7]. This approach is particularly effective when concentrates are fed alongside a base diet of low-quality forage, further mitigating CH_4 emissions from cattle [8].

Starch is the main energy component in grains, playing a pivotal role as the primary source of glucogenic energy for high-producing dairy cows, serving as a fermentable substrate for rumen microorganisms, and driving microbial protein synthesis [9]. Understanding starch digestion is essential for optimizing metabolizable protein and energy supply, thereby enhancing dietary efficiency [10]. The fermentation of feed in the rumen produces volatile fatty acids, CO_2, and H_2. Methanogenic archaea utilize this H_2 to convert CO_2 into CH_4. Compared to dietary fiber, starch fermentation may decrease enteric CH_4 production because it generates more propionate, providing an alternative H_2 sink to methanogenesis [11]. Additionally, starch decreases rumen pH, creating an unfavorable environment for methanogens, protozoa, and cellulolytic bacteria. This acidic environment also hinders fiber digestibility and reduces H_2 availability for CH_4 production [12,13]. Moreover, unlike fiber and sugar, a substantial portion of starch may bypass rumen fermentation and undergo enzymatic digestion in the small intestine, contributing to the animal's energy supply without the associated losses from CH_4 production [14].

However, several factors, including starch source, inclusion level, and fermentation rate, can influence starch digestibility and, consequently, CH_4 production. Aguerre et al. [15] evaluated four diets with varying forage-to-concentrate ratios and starch levels ranging from 20.0 to 29.0%. They found that increasing starch content decreased CH_4 production, intensity, and yield without affecting dry matter intake (DMI) or milk yield [15]. Pirondini et al. [16] compared two starch levels (23.8 vs. 28.0%) by modifying concentrate composition while keeping forage inclusion constant and observed that the lower starch group had higher dry matter (DM) and organic matter (OM) digestibility, with no differences in CH_4 production, intensity, or yield. Hatew et al. [17] investigated various starch fermentation rates and inclusion levels, finding that rapidly fermenting starch and higher dietary starch levels reduced CH_4 yield. Additionally, higher starch inclusion decreased CH_4 production due to lower DMI [17]. The inconsistent findings across studies with dairy cows may be due to variations in starch levels between treatments, differences in the ingredient composition of basal diets, or discrepancies in DMI and production levels.

Measuring CH_4 emissions can be challenging, prompting the exploration of alternative methods for more practical and economical estimation. One promising approach for predicting CH_4 production in lactating dairy cows involves analyzing the concentration of specific fatty acids (FA) in milk [18]. Dijkstra et al. [19] observed a positive association between CH_4 production and the concentrations of C14:0 iso and C15:0 iso in milk, along with an inverse relationship with several trans-intermediates, particularly C18:1 *trans*-10 and *trans*-11. Similarly, Rico et al. [20] reported a negative correlation between CH_4 produc-

tion and various milk unsaturated FA with carbon chain lengths of 16, 18, 20, and 22. While this approach shows potential, further research is needed to validate these correlations.

The objective of this study was to evaluate the effects of two different levels of dietary starch inclusion on milk production, nutrient digestibility, and CH_4 emissions in mid-lactation dairy cows. We hypothesized that increasing starch concentration while decreasing fiber in the diet would lead to higher DMI, milk yield, milk true protein concentration, and OM digestibility while reducing CH_4 yield, CH_4 intensity, and fiber digestibility. The present investigation aims to expand our understanding of optimal feeding strategies to improve dairy production and mitigate CH_4 emissions on a global scale. Additionally, we aim to further elucidate the correlation between CH_4 production and specific milk FA.

2. Materials and Methods

2.1. Experimental Design

All experimental procedures were conducted in accordance with the Cornell University Institutional Animal Care and Use Committee (protocol no. 2022-0132). Thirty mid-lactation Holstein dairy cows averaging (± SD) 2.53 ± 1.78 lactations, 117 ± 24.9 d in milk, and 38.3 ± 9.13 kg of milk/d were enrolled in a study with a completely randomized design at the Cornell Dairy Research Center in Harford, NY, USA. Following a 3 wk acclimation to a tie-stall barn and training to GreenFeed units (C-Lock, Inc., Rapid City, SD, USA), cows were assigned to one of two treatment groups (15 cows/treatment) as follows: the high-neutral-detergent-fiber and low-starch diet (LS; 20.2% starch) or the low-neutral-detergent-fiber and high-starch diet (HS; 25.2% starch). Cows were balanced in energy-corrected milk (ECM) yield, d in milk, and parity at the time of assignment. Inclusion criteria included no active or 30 d previous case of mastitis. Diets consisted primarily of corn silage, triticale silage, ground corn, soybean meal, and soy hulls (Table 1). All diets were formulated using AMTS.Farm.Cattle(Pro) (Agricultural Modeling & Training Systems, LLC, Groton, NY, USA) to meet the requirements for metabolizable energy (ME) and protein of a 2nd lactation cow weighing 743 kg, consuming 26.0 kg/d of DMI, and producing 41.0 kg/d of milk with 4.10% fat and 3.40% true protein. Diets provided 1.14 g of methionine per Mcal of ME and maintained a lysine-to-methionine ratio of approximately 2.7:1. None of the diets contained probiotics, monensin, yeast, or yeast derivatives. Differences in starch levels were achieved by adjusting the forage and concentrate proportions. Concentrate mixes were provided by Purina Animal Nutrition (Trumansburg, NY, USA). Diets were mixed and delivered as total mixed rations (TMR) daily at ~0700 h, with the TMR amount adjusted daily to achieve 10% refusals. Cows were milked 3 times daily at 0600, 1400, and 2200 h. Barn temperature and humidity were monitored daily, with the temperature–humidity index calculated to assess the environmental conditions. Body weights (BW) and body condition scores (BCS; 1 to 5 point scale) [21] were measured weekly. Rumination was continuously recorded using Allflex collars (Allflex Livestock Intelligence Global, Madison, WI, USA) throughout the study [22].

Table 1. Ingredient and nutrient composition [% of dry matter (DM) unless otherwise noted] of the experimental diets.

Item	**Diet**		
Ingredient, %	Acclimation	Low Starch	High Starch
Corn silage	34.4	36.2	28.7
Triticale silage	25.4	28.4	21.9
Concentrate mix A [1]	40.2	-	-
Concentrate mix B [2]	-	35.4	-
Concentrate mix C [3]	-	-	49.4

Table 1. *Cont.*

Item	Diet		
Nutrient composition, %			
DM	37.0	33.9	39.1
Crude protein	15.2	16.3	16.2
aNDFom [4]	34.7	36.5	32.4
Acid detergent fiber	20.1	20.7	19.4
Starch	21.9	20.2	25.2
Sugar	2.71	2.57	3.40
Crude fat (ether extract)	3.88	4.19	4.53
Ash	7.22	7.93	7.68
Calcium	0.43	0.86	0.76
Phosphorus	0.40	0.42	0.42
Magnesium	0.30	0.31	0.32
Potassium	1.64	2.03	1.99
Sodium	0.25	0.35	0.32
Energy, Mcal/kg of DM			
Net energy$_{Lactation}$	1.66	1.62	1.71
Net energy$_{Maintenance}$	1.84	1.80	1.92
Metabolizable energy	2.78	2.73	2.87

[1] Concentrate mix A contains the following (as fed basis): 38.0% fine ground corn grain, 13.5% ground soybean hulls, 13.5% soybean meal, 10.9% cottonseed (Easiflo cottonseed, Cottonseed, LLC, Lacrosse, WI, USA), 8.85% rumen bypass soybean meal (SoyPlus; Landus Cooperative, Ames, IA, USA), 4.59% dextrose, 2.25% protein supplement (SPECTRUM AgriBlue; Perdue AgriBusiness, Binghampton, NY, USA), 1.53% sodium bicarbonate, 1.51% potassium carbonate (DCAD Plus; Arm & Hammer Animal Nutrition, Washington, NJ, USA), 1.08% limestone, 1.02% mineral premix (MIN-AD; Papillon Agricultural Company, Easton, MD, USA), 0.88% dicalcium phosphate, 0.78% salt, 0.62% vitamin mix (PAN Dairy VTM; Purina Animal Nutrition, Trumansburg, NY, USA), 0.41% lysine (USA Lysine; Kemin Industries, Inc, Des Moines, IA, USA), 0.27% magnesium oxide, 0.20% methionine (Smartamine M; Adisseo USA Inc, Alpharetta, GA, USA), and 0.11% selenium. [2] Concentrate mix B contains the following (as fed basis): 28.2% fine ground corn grain, 23.7% ground soybean hulls, 22.8% soybean meal, 13.4% rumen bypass soybean meal (SoyPlus; Landus Cooperative, Ames, IA, USA), 2.8% protein supplement (SPECTRUM AgriBlue; Perdue AgriBusiness, Binghampton, NY, USA), 2.06% sodium bicarbonate, 1.14% mineral premix (MIN-AD; Papillon Agricultural Company, Easton, MD, USA), 1.09% dried vegetable fat (Palmit 80; Global Agri-trade Corporation, Rancho Dominguez, CA, USA), 1.09% dicalcium phosphate, 1.03% limestone, 0.68% vitamin mix (PAN Dairy VTM; Purina Animal Nutrition, Trumansburg, NY, USA), 0.46% salt, 0.44% potassium carbonate (DCAD Plus; Arm & Hammer Animal Nutrition, Washington, NJ, USA), 0.38% lysine (USA Lysine; Kemin Industries, Inc, Des Moines, IA, USA), 0.32% magnesium oxide, 0.25% methionine (Smartamine M; Adisseo USA Inc, Alpharetta, GA, USA), and 0.16% selenium. [3] Concentrate mix C contains the following (as fed basis): 50.0% fine ground corn grain, 17.6% soybean meal, 15.8% ground soybean hulls, 7.37% rumen bypass soybean meal (SoyPlus; Landus Cooperative, Ames, IA, USA), 2.08% protein supplement (SPECTRUM AgriBlue; Perdue AgriBusiness, Binghampton, NY, USA), 1.21% sodium bicarbonate, 1.14% potassium carbonate (DCAD Plus; Arm & Hammer Animal Nutrition, Washington, NJ, USA, USA), 0.94% limestone, 0.76% mineral premix (MIN-AD; Papillon Agricultural Company, Easton, MD, USA), 0.72% dicalcium phosphate, 0.72% dried vegetable fat (Palmit 80; Global Agri-trade Corporation, Rancho Dominguez, CA, USA), 0.45% vitamin mix (PAN Dairy VTM; Purina Animal Nutrition, Trumansburg, NY, USA), 0.43% salt, 0.32% lysine (USA Lysine; Kemin Industries, Inc, Des Moines, IA, USA), 0.19% magnesium oxide, 0.18% methionine (Smartamine M; Adisseo USA Inc, Alpharetta, GA, USA), and 0.09% selenium. [4] Ash-free neutral detergent fiber.

2.2. Feed Sampling and Analyses

Samples of individual ingredients, TMR, and refusals were collected weekly, dried for 72 h at 55 °C in a forced-air oven (VWR Scientific) for DM determination, and ground to pass through a 1 mm screen using a Wiley mill (A. H. Thomas Co., Philadelphia, PA, USA). Ground samples of TMR were composited monthly and analyzed according to AOAC [23] methods for DM: 934.01, crude protein (CP): 990.03, ether extract (EE): 2003.05, ash: 942.05, starch [24], acid detergent fiber (ADF): 973.18, ash-free neutral detergent fiber (aNDFom) [25], and neutral detergent fiber after 240 h in vitro fermentation (iNDF) [26] by Cumberland Valley Analytical Services Inc. (Waynesboro, PA, USA). Additional TMR samples were analyzed weekly for particle size distribution using a Penn State Particle Separator [27]. Two samples of each diet were analyzed weekly for a total of 16 samples per diet over the study period.

2.3. Gaseous Emissions Measurements

Enteric CH_4, CO_2, and H_2 emissions were estimated using 3 GreenFeed units at 0200, 1000, and 1800 h, 3 d/wk (9 measurements/wk) during wk −1, 2, 4, 6, and 8. Prior to the start of the experiment, cows were acclimated to the GreenFeed units. However, 1 cow in the HS group did not consistently visit the unit, resulting in a subset of 14 cows for that treatment group. A custom-formulated pellet feed (Purina Animal Nutrition, LLC., Shoreview, MN, USA) composed primarily of grain, roughage, and molasses was used as bait (Table 2). Each sample collection lasted 5 to 7 min, with an additional 2 min for background measurements. To ensure consistent airflow, the air filters in the GreenFeed units were changed weekly. Additionally, a CO_2 recovery test was performed before the start of the study, during wk 3, and at the end of the study. In this recovery test, the air flux sensor was calibrated by releasing a known quantity of CO_2 into each system and comparing the amount released to the amount captured, achieving a CO_2 recovery rate of 99.8 ± 2.59% (n = 9).

Table 2. The ingredient and nutrient composition of bait feed used in the GreenFeed units.

Item	% of DM
Ingredient	
Wheat middlings	75.7
Ground soy hulls	8.06
Fine ground corn	5.00
Dehulled soymeal	5.00
Molasses	3.00
Calcium carbonate	2.58
Salt	0.50
Selenium 0.06%	0.07
Trace mineral premix [1]	0.05
Vitamin A, D, E premix [2]	0.04
Nutrient composition	
Dry matter	88.8
Crude protein	18.3
aNDFom [3]	38.9
Acid detergent fiber	17.0
Starch	20.3
Sugar	4.30
Crude fat (ether extract)	3.55
Ash	9.29
Calcium	1.36
Phosphorus	0.83
Magnesium	0.38
Sodium	0.18

[1] Contains 11.0% zinc, 8.20% manganese, 1.02% copper, 6500 PPM iron, 1400 PPM iodine, and 1400 PPM cobalt (Purina Dairy TM PMX; Purina Animal Nutrition, LLC., Shoreview, MN, USA). [2] Contains 12,000 kIU/lb of Vitamin A, 3000 kIU/lb of Vitamin D, and 75,000 kIU/lb of Vitamin E (Purina Animal Nutrition, LLC., Shoreview, MN, USA). [3] Ash-free neutral detergent fiber.

2.4. Milk Sampling and Analysis

Milk samples were collected 3 d/wk (9 milkings/wk) during wk -1, 2, 4, 6, and 8. The samples were stored in tubes containing the preservative 2-bromo-2-nitropropane-1,3-diol and stored at 4 °C for milk composition analysis within 5 d of collection. Samples were analyzed for fat, true protein, lactose, milk urea nitrogen, and total solid concentrations using Fourier transform infrared spectroscopy and somatic cell count (SCC) by flow cytometry (Dairy One, Ithaca, NY, USA). Milk samples for analysis of FA composition were composited based on milk fat yield to represent wk -1 and 8. Samples were centrifuged

at 17,800× *g* for 30 min at 4 °C, and fat cakes were collected and stored at −80 °C until lipid extraction. The total lipids from the fat cakes (320 ± 10 mg) were extracted using *n*-hexane/isopropanol (3:2, *v*/*v*) [28]. Gas–liquid chromatography (GC) analysis was conducted using a GC system-8890 (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector, autosampler, a split/spitless injector, and a CP-Sil 88 column (100 m × 0.25 mm internal diameter, 0.20 µm film thickness; Agilent, Technologies, Palo Alto, CA, USA). Hydrogen was used as the carrier gas at a flow rate of 1 mL/min and for the FID at 40 mL/min and nitrogen makeup gas at 30 mL/min. The injector and detector were maintained at 250 °C. The oven temperature program was as follows: an initial temperature of 80 °C held for 1 min, then increased to 215 °C at a rate of 2 °C/min and held for 21.5 min [29]. Each GC analysis involved injecting 1 µL of the sample with a 1:100 split ratio. Individual peaks were identified using reference standards (GLC reference standard 463, GLC reference standard 481-B, and octadecadienoic mixture # UC-59 M, Nu-Chek Prep Inc., Elysian, MN, USA). Short-chain FA methyl ester mass discrepancies were corrected using response factors published by Ulberth and Schrammel [30]. The concentrations of FA were determined on a mass basis using the molecular weight of each FA while correcting for glycerol [31].

2.5. Blood Sampling and Analyses

Blood samples were collected via venipuncture of the coccygeal vessels once weekly during wk −1 and 8. Upon collection, blood samples were immediately placed on ice for ~45 min, followed by centrifugation at 2171× *g* for 20 min at 4 °C. Plasma samples were stored at −80 °C until analysis. Plasma glucose concentrations (Autokit Glucose no. 997-03001, FUJIFILM Medical Systems USA, Lexington, MA, USA) were quantified in duplicate according to the manufacturer's instructions. Plasma insulin concentrations were measured using RIA (#PI-12K Porcine Insulin RIA Kit; EMD Millipore Corp., Burlingon, MA, USA) on an LKB-Wallac CliniGamma Counter (Beckman Coulter, Indianapolis, IN, USA). Intra- and inter-assay coefficients of variation were 4.79 and 1.65% and 4.53 and 2.96% for plasma glucose and insulin, respectively.

2.6. Feces Sampling and Analyses

Spot samples of feces were collected directly from the rectum or during voluntary defecation during wk −1 and 8. Samples were collected every 5 h for 3 consecutive d to account for diurnal changes. Approximately 200 g of fecal samples were obtained during each sampling point, transferred into 4 L bags to obtain a weekly composite sample for each cow, and stored at −20 °C until further processing. Samples were thawed at room temperature, placed in aluminum trays, and freeze-dried using a Virtis model 20SRC-X freeze-dryer (Gardiner, NY, USA); samples were kept at −40 °C for 3 h, followed by −20 °C for 3 h, 0 °C for 16 h, and 15 °C until dry. Then, samples were ground to pass through a 1 mm screen using a Wiley mill (A. H. Thomas Co., Philadelphia, PA, USA). Samples were shipped to Cumberland Valley Analytical Services, Inc. (Waynesboro, PA, USA), for analyses of DM, CP, EE, ash, starch, ADF, aNDFom, and iNDF as described above.

2.7. Calculations and Statistical Analyses

Yields of 3.5% fat-corrected milk (FCM), ECM, and milk components were calculated based on milk yields and component concentrations from each milking, summed for a daily total, and averaged for each collection period as follows: FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]) and ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.65 × kg of milk true protein)] [32]. Somatic cell score (SCS) was calculated from SCC using a logarithmic transformation, where SCS = $\log_2$ (SCC/100,000) + 3 [33]. Individual FA yields (g/d) were determined using milk fat yield and FA concentration to determine

yield on a mass basis, using the molecular weight of each FA while correcting for glycerol. Feed efficiency (FE) for milk yield, FCM, and ECM production was calculated as the ratio of milk yield, FCM, or ECM to DMI. Apparent total-tract digestibility was calculated using the following equation according to Hisadomi et al. [34]: digestibility (%) = 100 − {100 × [dietary iNDF content (%DM)/fecal iNDF content (%DM)] × [fecal nutrient content (%DM)/dietary nutrient content (%DM)]}. Gas emission samples collected weekly were summed to estimate total gas production for the sampling period. Emission intensity was analyzed by calculating gas production per kg of milk yield (g/kg milk yield) as well as per kg of energy-corrected milk (g/kg ECM) and fat-corrected milk (g/kg FCM). Gas yield was defined as the total gas production per kg of DMI (g/kg DMI) and per kg of organic matter intake (g/kg OMI). Enteric CH_4 emissions were expressed in CO_2-equivalent (CO_2-eq) terms using a 100-year global warming potential factor of 28. Percent differences were calculated using the formula $\{|a - b|/[(a + b) \div 2]\} \times 100$.

Statistical analyses were carried out using the mixed model procedure of SAS (v9.4, SAS Institute Inc., Cary, NC, USA) according to the following model:

$$Y_{ijk} = \mu + C_i + T_j + D_k + T_j \times D_k + PAR + pVar_i + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = overall mean effect for the measure; C_i = random effect of cow (i = 1 to 30); T_j = fixed effect of starch level (j = low or high); D_k = fixed effect of wk (l = 1 to 8); $T_j \times D_k$ = fixed effect of the interaction between starch level and wk; PAR = parity used as a covariate; $pVar_i$ = baseline measurement for each response variable used as a covariate; and e_{ijk} = the residual error. After assessing five distinct covariance structures (variance components, first-order autoregressive, unstructured, compound symmetry, and first-order ante-dependence), the most appropriate covariance structure for each variable in the repeated measures analysis was chosen. The selection process involved identifying the structure with the lowest Akaike's information criterion coefficient for subsequent analysis. By modeling the covariance structure, patterns that most effectively characterize the relationships between the repeated measures in the model were discerned. A post hoc Tukey test was employed for multiple comparisons to compare differences within each time point. The model evaluated production responses, blood metabolites, and gas measurements. The correlation between CH_4 production and individual milk FA concentration was assessed using Pearson correlations. To maintain a controlled false discovery rate at 5%, corrections for multiple comparisons were applied [35]. Only correlations falling within the acceptable range of this test were reported.

Observations were deemed as outliers if Studentized residuals > 3.0 or <−3.0. The normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values to ensure no violation of model assumptions. The least squares mean comparisons are reported using adjusted p-values. Results are expressed as least squares means ± standard error of the mean unless otherwise noted. Main effects were declared significant at $p \leq 0.05$ and trending towards significance at $0.05 < p \leq 0.15$.

3. Results

Cows fed the HS diet produced 2 kg more milk (40.6 vs. 38.6 kg/d; $p < 0.01$; Figure 1A) and consumed 4.2 kg more DM (28.6 vs. 24.4 kg/d; $p < 0.01$; Figure 1B) compared to those on the LS diet. However, FE was lower in HS cows (1.43 vs. 1.57; $p < 0.01$; Figure 1C), with a starch × wk interaction observed during wk 5, 6, 7, and 8 (Table 3). Additionally, HS cows had a greater BW (696 vs. 674 kg; $p < 0.01$) and tended to have a higher BCS (3.21 vs. 3.13; $p = 0.08$; Table 3) compared to LS cows. Particle size distribution in the HS TMR was 3.25 ± 0.60% for particles > 19.0 mm, 56.5 ± 2.50% for particles 8.0–19.0 mm, 12.8 ± 1.10%

for particles 3.18–8.0 mm, and 27.5 ± 2.50% for particles < 3.18 mm. In contrast, the LS TMR had 4.62 ± 0.83% of particles > 19.0 mm, 63.5 ± 2.52% of particles from 8.0 to 19.0 mm, 12.8 ± 0.60% of particles from 3.18 to 8.0 mm, and 19.1 ± 2.33% of particles < 3.18 mm). Cows on the HS diet had higher ECM yield (44.6 vs. 42.7 kg/d; $p = 0.04$); true protein content (3.47 vs. 3.27%; $p < 0.01$); and yields of true protein (1.36 vs. 1.23 kg/d; $p < 0.01$), lactose (1.95 vs. 1.85 kg/d; $p < 0.01$), and total solids (5.40 vs. 5.16 kg/d; $p < 0.01$; Table 3) compared to LS cows. However, LS cows tended to have a higher milk fat content (4.45 vs. 4.28%; $p = 0.09$; Table 3). Interactions between treatment × wk for DMI, milk, ECM and FCM yields, rumination, milk true protein content, milk fat yield, milk lactose yield, and FE (kg milk yield/kg DMI; kg ECM yield/kg DMI; kg FCM yield/kg DMI) are presented in Figures S1–S11. No differences were observed in plasma glucose concentrations ($p = 0.86$; Table 3), but HS cows tended to have higher plasma insulin concentrations compared to LS cows (1.61 vs. 1.31 ng/mL, $p = 0.12$; Table 3). Dietary starch content affected milk FA profile, with LS cows showing higher mixed FA content (35.2 vs. 32.8%; $p < 0.01$) and HS cows exhibiting greater de novo FA content and yield (22.0 vs. 23.6% and 362 vs. 405 g/d; $p < 0.01$). Concentrations and yields of C18:2 *cis*-9 and *cis*-12 were also greater in HS cows (1.41 vs. 1.75% and 190 vs. 206 g/d, respectively; $p \leq 0.02$; Table 4). A complete list of milk FA concentrations and yields is provided in Tables S1 and S2. Apparent total-tract DM and OM digestibility was lower in LS cows compared to HS cows (69.4 vs. 73.6% and 70.5 vs. 74.8%, respectively; $p < 0.01$; Table 5). Methane production tended to be lower for LS compared to HS cows (386 vs. 411 g/d, $p = 0.08$; Table 6; Figure 2A), showing a 6.27% difference. Cows on the LS diet also had lower CO_2-equivalent emissions per kg of fat produced (8.34 vs. 8.99 kg CO_2-eq/kg fat; $p = 0.02$) and lower CO_2 intensity in terms of FCM compared to HS cows (315 vs. 333 g CO_2/kg FCM; $p < 0.01$; Table 6). However, HS cows exhibited a reduced CH_4 yield compared to LS cows (14.6 vs. 16.0 g CH_4/kg DMI; $p = 0.03$; Table 6; Figure 2B), representing a 9.15% difference. Cows on the HS diet also had a lower CH_4 yield in terms of OMI (15.9 vs. 17.5 g CH_4/kg OMI; $p = 0.03$; Table 6), demonstrating a 9.58% difference. Additionally, a starch × week interaction was observed during wk 6 ($p = 0.08$; Table 6; Figure 2B). Methane production was negatively correlated with *anteiso* C15:0, C16:1 *trans*-9, C18:1 *cis*-9, and C20:1 *cis*-11 (−0.41, −0.43, −0.38, and −0.41, respectively; $p \leq 0.05$; Table 7).

Table 3. Effects of dietary starch concentration on productive performance, milk composition, and feed efficiency.

	Treatment			*p*-Value		
Variable	**LS**	**HS**	**SEM** [1]	**Treatment**	**Week**	**Treatment × Week**
Productive performance						
Milk yield, kg/d	38.6	40.6	0.40	<0.01	<0.01	<0.01
ECM [2], kg/d	42.7	44.6	0.61	0.04	0.39	0.01
3.5% FCM [3], kg/d	43.3	44.2	0.61	0.31	0.70	<0.01
Dry matter intake, kg/d	24.4	28.6	0.32	<0.01	<0.01	<0.01
Total dry matter intake [4], kg/d	26.8	31.0	0.31	<0.01	<0.01	<0.01
Energy intake, Mcal/d	40.6	47.8	0.58	<0.01	<0.01	<0.01
Energy balance, Mcal/d	−3.81	3.98	0.42	<0.01	<0.01	<0.01
Body weight, kg	674	696	3.31	<0.01	<0.01	0.31
Body condition score	3.13	3.21	0.03	0.08	0.23	0.47
Rumination, min/d	583	573	4.90	0.16	<0.01	0.02
Plasma glucose, mg/dL	63.8	64.5	2.87	0.86	-	-
Plasma insulin, ng/mL	1.31	1.61	0.13	0.12	-	-

Table 3. *Cont.*

	Treatment			*p*-Value		
Variable	**LS**	**HS**	**SEM** [1]	**Treatment**	**Week**	**Treatment × Week**
Milk composition, %						
Fat	4.45	4.28	0.07	0.09	0.19	0.11
True protein	3.27	3.47	0.02	<0.01	<0.01	<0.01
Lactose	4.91	4.92	0.01	0.29	0.02	0.15
Solids	13.6	13.6	0.08	0.85	<0.01	0.12
Somatic cell score [5]	1.21	1.40	0.12	0.27	0.08	0.07
Milk urea nitrogen, mg/dL	10.5	11.1	0.19	0.02	<0.01	0.15
Milk solids, kg/d						
Fat	1.63	1.66	0.03	0.52	<0.01	0.01
True protein	1.23	1.36	0.02	<0.01	<0.01	0.69
Lactose	1.85	1.95	0.02	0.01	0.20	0.02
Solids	5.16	5.40	0.06	0.01	0.11	0.17
Feed efficiency						
Milk yield/DMI	1.57	1.43	0.02	<0.01	<0.01	<0.01
Milk yield/Energy intake	0.95	0.86	0.01	<0.01	<0.01	<0.01
ECM/DMI	1.76	1.58	0.02	<0.01	<0.01	<0.01
ECM/Energy intake	1.07	0.94	0.01	<0.01	<0.01	<0.01
3.5% FCM/DMI	1.78	1.57	0.02	<0.01	<0.01	<0.01
3.5% FCM/Energy intake	1.08	0.93	0.01	<0.01	<0.01	<0.01

[1] Pooled standard error of the mean. [2] Energy-corrected milk = (0.327 × kg of milk yield) + (12.95 × kg of milk fat yield) + (7.65 × kg of milk true protein yield). [3] 3.5% fat-corrected milk = (0.4324 × kg of milk yield) + (16.216 × kg of milk fat yield). [4] Includes intake of pelletized bait feed. [5] Somatic cell score (cells × 10^3 mL^{-1}).

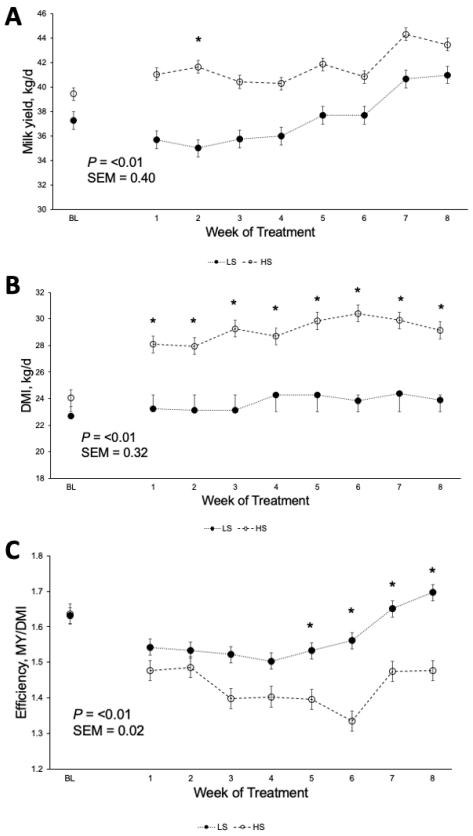

Figure 1. Effects of dietary starch concentration on (**A**) milk yield (MY), (**B**) dry matter intake (DMI), and (**C**) efficiency (MY/DMI). * Indicates a significant interaction ($p \leq 0.05$) between week and treatment.

Table 4. Effects of dietary starch concentration on milk fatty acid concentration (g/100 g of milk fat) and yield (g/d).

	Treatment			
Variable	**LS**	**HS**	**SEM** [1]	***p*-Value**
Fatty acid, g/100 g of milk fat				
C4:0	2.48	2.30	0.02	<0.01
C6:0	1.87	1.83	0.02	0.10
C8:0	1.23	1.27	0.01	<0.01
C10:0	3.21	3.54	0.03	<0.01
C12:0	3.85	4.48	0.06	<0.01
C14:0	11.2	11.9	0.16	<0.01
C15:0	1.21	1.35	0.04	0.02
Anteiso C15:0	0.42	0.39	<0.01	<0.01
C16:0	35.0	32.6	0.32	<0.01
C16:1 *trans*-9	0.23	0.24	<0.01	0.73
C18:0	6.63	6.43	0.12	0.25
C18:1 *cis*-9	12.0	11.9	0.18	0.89
C18:2 *cis*-9, *cis*-12	1.41	1.75	0.02	<0.01
C18:2 *cis*-9, *trans*-11 (CLA)	0.29	0.30	<0.01	0.25
C18:3 *cis*-9, *cis*-12, *cis*-15	0.27	0.29	0.01	0.18
C20:1 *cis*-11	0.09	0.09	<0.01	<0.01
C20:5 *cis-5*, *cis-8*, *cis*-11, *cis*-14, *cis*-17 (EPA)	0.03	0.03	<0.01	<0.01
C22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 (DPA)	0.05	0.06	<0.01	0.08
De novo [2]	22.0	23.6	0.19	<0.01
Mixed [3]	35.2	32.8	0.32	<0.01
Preformed [4]	27.7	28.1	0.27	0.35
Fatty acid, g/d				
C4:0	42.0	39.1	1.04	0.06
C6:0	31.8	31.1	0.71	0.50
C8:0	19.9	20.9	0.45	0.11
C10:0	51.7	58.1	1.22	<0.01
C12:0	63.5	77.4	1.82	<0.01
C14:0	184	201	4.18	<0.01
C15:0	20.7	23.0	0.83	0.06
Anteiso C15:0	7.15	6.63	0.23	0.12
C16:0	596	554	14.3	0.04
C16:1 *trans*-9	3.92	3.98	0.12	0.73
C18:0	107	111	2.72	0.34
C18:1 *cis*-9	190	206	4.41	0.02
C18:2 *cis*-9, *cis*-12	23.6	29.6	0.67	<0.01
C18:2 *cis*-9, *trans*-11 (CLA)	4.80	5.14	0.17	0.16
C18:3 *cis*-9, *cis*-12, *cis*-15	4.43	4.87	0.19	0.12
C20:1 *cis*-11	1.59	1.51	0.05	0.26
C20:5 *cis-5*, *cis-8*, *cis*-11, *cis*-14, *cis*-17 (EPA)	0.49	0.43	0.02	0.03
C22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 (DPA)	0.87	1.01	0.04	0.02
De novo [2]	362	405	7.94	<0.01
Mixed [3]	588	555	13.5	0.09
Preformed [4]	464	481	21.1	0.44

[1] Pooled standard error of the mean. [2] De novo fatty acids originated from mammary de novo synthesis (<16C). [3] Preformed fatty acids originated from extraction from plasma (>16C). [4] Mixed fatty acids originated from both sources (C16:0 plus C16:1 *cis*-9).

Table 5. Effects of dietary starch concentration on apparent total-tract digestibility.

Variable	Treatment			
	LS	HS	SEM [1]	*p*-Value
Intake				
Dry matter, kg/d	24.4	28.6	0.32	<0.01
Organic matter, kg/d	22.5	26.3	0.21	<0.01
Crude protein, kg/d	4.00	4.61	0.08	<0.01
aNDFom [2], kg/d	8.95	9.22	0.16	0.26
Starch, kg/d	4.99	7.19	0.12	<0.01
Acid-hydrolysis fat, g/d	1031	1291	21.4	<0.01
Apparent total-tract digestibility, %				
Dry matter	69.4	73.6	0.60	<0.01
Organic matter	70.5	74.8	0.56	<0.01
Crude protein	70.5	70.5	0.73	0.98
aNDFom	65.5	66.3	0.65	0.43
Starch	98.6	98.8	0.15	0.45
Acid-hydrolysis fat	60.2	61.4	0.80	0.34
Absorbed				
Dry matter, kg/d	16.7	21.2	0.35	<0.01
Organic matter, kg/d	15.5	19.9	0.34	<0.01
Crude protein, kg/d	2.74	3.31	0.05	<0.01
aNDFom, kg/d	5.75	6.18	0.10	0.01
Starch, kg/d	4.87	6.89	0.24	<0.01
Acid-hydrolysis fat, g/d	604	800	13.8	<0.01

[1] Pooled standard error of the mean. [2] Ash-free neutral detergent fiber.

Table 6. Effects of dietary starch concentration on enteric methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2) emissions.

	Treatment			*p*-Value		
Variable	LS	HS	SEM [1]	Treatment	Week	Treatment × Week
Gas production						
CH_4, g/d	386	411	9.70	0.08	0.13	0.68
CO_2, kg/d	13.6	14.9	0.21	0.12	0.79	0.88
H_2, g/d	0.85	0.91	0.05	0.37	0.01	0.35
Gas intensity						
CH_4, g/kg milk	10.5	10.7	0.31	0.55	0.30	0.39
CH_4, g/kg ECM	9.11	9.33	0.26	0.55	0.51	0.17
CH_4, g/kg FCM	9.00	9.39	0.27	0.32	0.35	0.14
CO_2, g/kg milk	361	370	4.45	0.20	0.01	0.05
CO_2, g/kg ECM	317	330	3.78	0.02	0.02	0.25
CO_2, g/kg FCM	315	333	4.13	<0.01	0.04	0.17
H_2, g/kg milk	0.02	0.02	<0.01	0.11	<0.01	0.43
H_2, g/kg ECM	0.02	0.02	<0.01	0.06	<0.01	0.15
H_2, g/kg FCM	0.02	0.02	<0.01	0.03	<0.01	0.13
Gas yield						
CH_4, g/kg DMI	16.0	14.6	0.41	0.03	<0.01	0.08
CH_4, g/kg OMI	17.5	15.9	0.45	0.03	0.02	0.24
CO_2, g/kg DMI	561	508	7.07	<0.01	<0.01	0.05
H_2, g/kg DMI	0.03	0.03	<0.01	0.75	<0.01	0.24

[1] Pooled standard error of the mean.

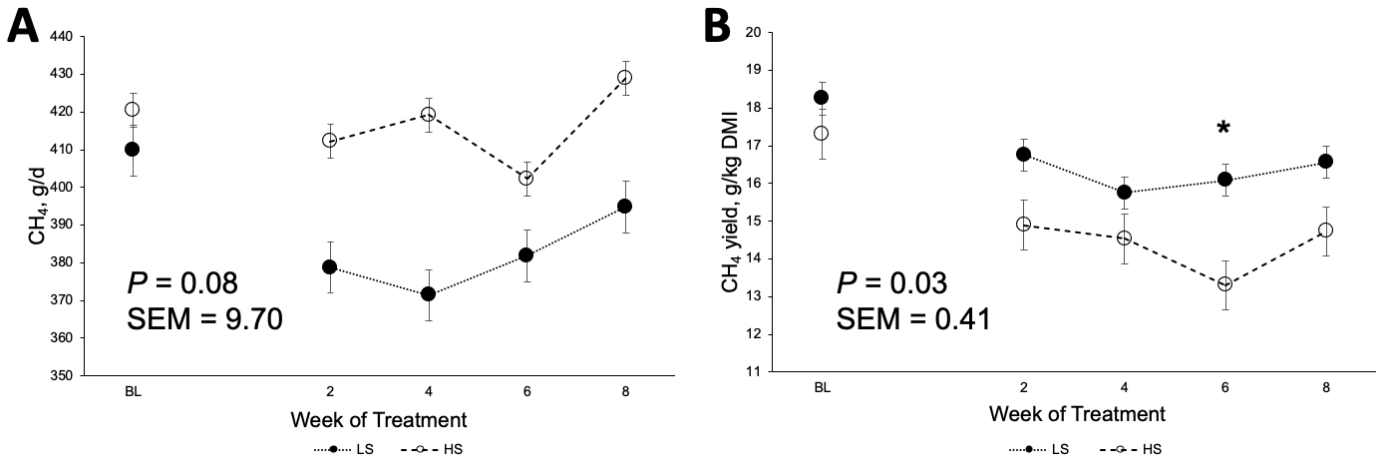

Figure 2. Effects of dietary starch concentration on (**A**) methane production (g CH_4/d) and (**B**) methane yield (g CH_4/kg DMI). * Indicates a significant interaction ($p \leq 0.05$) between week and treatment.

Table 7. Pearson correlation between milk fatty acid concentration (g/100 g of milk fat) and CH_4 production.

	CH_4, g/d	
Variable	**r**	***p*-Value**
C4:0	−0.04	0.89
C6:0	0.09	0.70
C8:0	0.22	0.38
C10:0	0.24	0.27
C12:0	0.18	0.43
C14:0	0.15	0.48
C15:0	−0.06	0.85
Anteiso C15:0	−0.41	0.03
C16:0	0.12	0.65
C16:1 *trans*-9	−0.43	0.03
C18:0	−0.09	0.70
C18:2 *cis*-9, *trans*-11 (CLA)	−0.38	0.05
C18:2 *cis*-9, *cis*-12	0.01	0.93
C18:2 *cis*-9, *trans*-11	0.18	0.43
C18:3 *cis*-9, *cis*-12, *cis*-15	−0.07	0.79
C20:1 *cis*-11	−0.41	0.03
C20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 (EPA)	−0.27	0.26
C22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 (DPA)	−0.31	0.14
De novo [1]	0.27	0.26
Mixed [2]	0.11	0.65
Preformed [3]	−0.32	0.13

[1] De novo fatty acids originated from mammary de novo synthesis (<16C). [2] Preformed fatty acids originated from extraction from plasma (>16C). [3] Mixed fatty acids originated from both sources (C16:0 plus C16:1 *cis*-9).

4. Discussion

Enteric CH_4 emissions significantly contribute to the environmental impact of the dairy industry. Research has shown that dietary carbohydrate composition can modulate rumen fermentation patterns and methanogenesis [36]. Increasing the starch proportion in dairy diets has been proposed as a strategy to reduce CH_4 emissions by favoring ruminal propionate production [11]. Since starch and fiber are the primary carbohydrate components, understanding how different inclusion levels influence CH_4 production has become a critical research focus. This study aimed to investigate the effects of dietary starch

concentration on milk production, nutrient digestibility, and CH_4 emissions in lactating dairy cows.

The reduced DMI observed in cows fed the LS diet is likely attributable to the higher forage content (i.e., aNDFom) and lower concentrate proportion. Forage contributes to greater physical gut fill, which can suppress DMI [37,38]. Consequently, LS cows consumed 4.2 kg less DM and produced 2 kg less milk than those on the HS diet. Compared to other components of the TMR, it has been demonstrated that the physical filling effect of a higher forage aNDFom concentration poses a more significant limitation to DMI as milk yield increases [39]. Additionally, high-producing cows often experience a decline in milk production when dietary starch concentrations are reduced [40]. Therefore, substituting concentrates with forage in the LS diet reduced the energy available to both rumen microbes and the host animal, leading to decreased milk production in LS cows. Feed efficiency in HS cows may have decreased due to a faster starch passage rate. Diets with a high concentrate-to-forage ratio can accelerate starch passage to the small intestine, which has a limited capacity for digesting large quantities of starch. This can lead to inefficient digestion and reduced overall FE [41,42]. As milk production increases, improvements in FE typically decline, partly due to reduced digestible energy associated with a high passage rate [43]. Conversely, lower DMI correlates to greater FE [44], and body tissue mobilization has been shown to enhance FE [45]. The negative energy balance in LS cows may have contributed to their observed increase in FE. Additionally, larger cows with higher BCS are genetically predisposed to lower FE [46], which aligns with our findings, as HS cows had greater BW and BCS.

Cows fed the HS diet had higher milk true protein and lactose content and greater true protein yield than LS cows, which is consistent with previous research [47–49]. This response in milk protein is likely due to higher DM and CP intake in HS cows, which may have enhanced microbial protein synthesis and ruminal propionate concentration [50]. Furthermore, HS cows tended to have higher plasma insulin concentrations, which is known to influence milk protein synthesis [51]. In contrast, the lower dietary starch content in the LS diet may have reduced microbial protein production, limiting the available protein pool for milk protein synthesis in LS cows [52]. The tendency for higher milk fat content in LS cows compared to HS cows was expected, as diets low in aNDFom and high in starch are known risk factors for milk fat depression [53]. This effect can be attributed to the improved buffering capacity of the LS diet, which had a higher proportion of aNDFom. This buffering helps maintain a higher pH in the rumen, reducing the incidence of milk fat depression [54]. The lower milk fat content in HS cows may also result from a dilution effect due to their higher milk yield compared to LS cows. Additionally, Reynolds et al. [55] associated reduced milk fat with elevated plasma insulin concentrations in cows consuming high-starch diets, as insulin decreases lipolysis and promotes lipogenesis in adipose tissue, decreasing the availability of FA for the mammary gland.

Dietary differences also affected nutrient digestibility. Starch is commonly used to increase the energy density of diets, enhance rumen fermentation, and improve OM digestibility. The lower apparent total-tract digestibility of DM and OM in cows fed the LS diet can be attributed to replacing non-fibrous carbohydrates (primarily from corn grain in the HS diet) with fibrous carbohydrates (primarily from corn silage and triticale silage), reducing overall nutrient digestibility. These findings align with Silvestre et al. [56], who compared a typical starch diet with a reduced-starch diet (24.8 vs. 18.4% starch). Organic matter digestibility was likely the primary driver of how effectively cows on the HS diet absorbed and utilized nutrients for milk production. Although diets rich in starch have been found to reduce fiber digestibility [57,58], this effect was not observed in our study. This may be due to the relatively small difference in starch concentration between diets (i.e.,

5%) and the fact that aNDFom content was above 32% in both diets, which likely allowed for rumen pH to remain high enough to support cellulolytic bacteria activity. Similarly, the study by Silvestre et al. [56], using comparable dietary starch concentrations, found no significant difference in aNDFom digestibility [56].

It must be noted that the source of starch, the grain type, and the degree of processing are critical factors influencing starch digestion in dairy cows. In this study, the HS diet utilized more finely ground corn, which is known for its rapid ruminal fermentation due to increased surface area, enhancing starch digestibility and microbial protein synthesis. This processing likely contributed to the improved energy-corrected milk yield and digestibility in the HS group. When comparing results across studies, it is essential to consider variations in starch source and processing, as coarser grinding or alternative grains may yield different fermentation dynamics and production responses.

Cows fed the LS diets had lower milk concentrations of C18:2 *cis*-9, *cis*-12, and de novo FA and lower yields of de novo FA. However, they showed a higher mixed FA content than HS cows. The observed decrease in DM and OM digestibility in cows fed the LS diets may have limited the availability of substrates necessary for de novo FA synthesis in the mammary gland. Milk FA have two distinct origins: those with fewer than 16 carbon atoms are produced through de novo synthesis in the mammary gland, while those with more than 16 carbon atoms are derived from plasma extraction. Fatty acids such as C16:0 and C16:1 *cis*-9 come from a mix of these two sources [59]. Given the significant decrease in de novo FA concentrations, the increase in mixed FA in LS cows is likely due to greater mobilization of body fat reserves as a result of their lower DMI and negative energy balance. The higher concentrations and yields of C18:2 *cis*-9, *cis*-12 in milk from HS cows were likely due to an increased intake of soybean meal, a dietary source of linoleic acid [60].

When accounting for variations in intake, cows on the HS diet had reduced CH_4 yield relative to both DMI and OMI compared to those on the LS cows. Similarly, Aguerre et al. [15] reported a consistent linear reduction in CH_4 yield, up to 19%, over a range of forage-to-concentrate ratios from 68:32 to 47:53. It is likely that the higher level of starch in the diet led to more efficient digestion, resulting in faster passage and a lesser extent of fermentation in the rumen. Likewise, Boadi and Wittenberg [61] demonstrated that CH_4 emissions per unit of OMI tend to decrease with increased diet digestibility. This aligns with our findings, as the higher digestibility of the HS diet led to lower CH_4 emissions per digested unit of OMI compared to the LS diet. Interestingly, Olijhoek et al. [62] observed CH_4 yield reductions of 27.2% and 13.8% for Holstein and Jersey cows, respectively, when the concentrate proportion in the diet increased from 32 to 61%. This suggests that increasing concentrate, and therefore starch, may be a more effective CH_4 mitigation strategy for Holstein than for Jersey cows.

However, this does not necessarily imply a reduction in total CH_4 production. The HS cows had greater overall DMI, providing more substrate for microbial fermentation. Although the CH_4 yield per unit of DMI and OMI was lower in HS cows, the LS cows tended to produce less absolute CH_4, emitting 25 g/d less. This result was expected, as the LS cows consumed 4.2 kg less DM than the HS cows, resulting in fewer substrates available for rumen microbes. It is well established that the primary driver of methanogenesis is feed intake above maintenance energy requirements [63,64]. Research has established a strong positive correlation between daily CH_4 production and the intake of forage-based diets, regardless of intake levels or forage type [65]. As such, the 6.27% difference in daily CH_4 production observed in the present study is likely due to the 15.9% difference in DMI rather than the starch content of the diets.

Incorporating more than 35% concentrate into dairy cow diets has been associated with reduced CH_4 production [66]. In the present study, concentrate levels were 35.4 and

49.4% for the LS and HS diets, respectively. The similar total CH_4 production observed in both groups could be attributed to both diets exceeding this threshold. Muñoz et al. [67] investigated the effects of two dietary concentrate levels (29 vs. 46% of diet DM) on CH_4 emissions in dairy cows and found that while the higher concentrate level increased total CH_4 production by 10.7%, it reduced CH_4 yield by 12.7%. Consistent with our study, CH_4 intensity remained unaffected. In contrast, Olijhoek et al. [62] compared concentrate levels of 32 vs. 61% and reported that the higher level decreased CH_4 production, intensity, and yield. The difference in starch concentration between their diets was 11.3%, which is larger than that between the LS and HS diets in our study, potentially explaining the different outcomes.

The negative correlation observed between CH_4 production and the FA *anteiso* C15:0, C16:1 *trans*-9, C18:1 *cis*-9, and C20:1 *cis*-11 is consistent with findings from previous studies [19,68,69]. This relationship can be explained by the role of rumen bacteria in utilizing H_2 for the biohydrogenation of unsaturated FA. As H_2 is consumed in this process, less is available for hydrogenotrophic methanogens, reducing CH_4 production [6]. Additionally, unsaturated FA can inhibit methanogenesis by exerting toxic effects on protozoa and cellulolytic bacteria [70]. Similarly, *anteiso* C15:0, predominantly produced by amylolytic bacteria [71], may promote increased H_2 consumption by enhancing propionate production, further limiting H_2 availability for methanogenesis.

A more substantial increase in starch concentration in the HS diet may have resulted in lower CH_4 production due to increased propionate production in the rumen, which would theoretically consume H_2, inhibiting methanogenesis. Additionally, high-starch diets have been shown to alter the rumen microbial composition, favoring propionate-producing bacteria [72]. A lower rumen pH resulting from a starch-rich diet also affects the growth of protozoa, methanogens, and cellulolytic bacteria [73]. However, a significant increase in starch inclusion could reduce DMI, as propionate stimulates hepatic oxidation, which signals satiety to the brain and decreases meal size [74]. If this occurs, the observed reduction in CH_4 production could be attributed to decreased DMI rather than shifts in fermentation pathways. For instance, Zang et al. [75] found that increasing dietary starch concentrations from 12.3 to 34.4% reduced DMI, leading to a 20% decrease in CH_4 production.

Targeting CH_4 yield rather than total production or intensity has been suggested as the most effective trait for breeding lower-emitting livestock. Reducing CH_4 yield can decrease individual emissions by altering rumen function, with minimal impact on productivity or BW [76]. However, while decreasing CH_4 yield is beneficial, caution is warranted when using high-starch diets, as excessive starch inclusion may negatively affect production and nutrient digestibility. Starch concentrations between 28 and 32% have been shown to lower rumen pH, increasing the risk of subacute ruminal acidosis and potentially compromising animal health and performance [77]. Additionally, environmental trade-offs must be considered, as higher dietary concentrate levels can lead to increased nitrogen losses [78,79] and greater water consumption [80], potentially exacerbating future water resource challenges. Therefore, balancing starch with other dietary components is essential for developing effective and sustainable feeding strategies.

5. Conclusions

Our findings demonstrate that increasing dietary starch concentration can improve milk production and diet digestibility while reducing CH_4 yield. This has important implications for dairy farming practices, especially in regions with limited access to advanced CH_4 mitigation technologies and feed additives. Future research should focus on determining the optimal starch concentration that maximizes energy-corrected milk yield while

minimizing CH_4 yield without increasing the risk of rumen acidosis or other health issues across dairy breeds. Once this threshold is established, the influence of seasonality and climate on starch levels should be examined to ensure the global applicability of this nutritional strategy. Moreover, investigating the long-term effects of high-starch diets across different lactation stages is crucial for understanding their broader impact on animal health and performance.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture15020211/s1, Table S1: Milk fatty acid concentration (g/100 g of milk fat) of LS and HS diets; Table S2: Milk fatty acid yield (g/d) of LS and HS diets; Figure S1: Effects of dietary starch concentration on milk yield; Figure S2: Effects of dietary starch concentration on energy-corrected milk yield; Figure S3: Effects of dietary starch concentration on 3.5% fat-corrected milk yield; Figure S4: Effects of dietary starch concentration on dry matter intake; Figure S5: Effects of dietary starch concentration on rumination; Figure S6: Effects of dietary starch concentration on milk protein contents; Figure S7: Effects of dietary starch concentration on milk fat yield; Figure S8: Effects of dietary starch concentration on milk lactose yield; Figure S9: Effects of dietary starch concentration on feed efficiency (kg milk yield/kg dry matter intake); Figure S10: Effects of dietary starch concentration on feed efficiency (kg energy-corrected milk yield/kg dry matter intake); Figure S11: Effects of dietary starch concentration on feed efficiency (kg 3.5% fat-corrected milk yield/kg dry matter intake).

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